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PHYSIOLOGICAL RESPONSE, BLOOD PARAMETERS, AND REPRODUCTIVE PERFORMANCE OF BUFFALO COWS TREATED WITH ZINC METHIONINE AND NIACIN UNDER COOLING SYSTEM DURING SUMMER SEASON IN EGYPT

Khalifa, H. H.[1] ; A. M. Ashour[1] ; M. M. Youssef[2] and M. M. Ghoneim[2]

[1]dept. of Animal Production, Fac. Agric., Al-Azhar Univ., Nasr City, Cairo, Egypt

[2]Animal Production Res. Inst., Dokki, Giza, Egypt.

ABSTRACT

Thirty Egyptian healthy female buffalo cows were used in this study. The experimental animals were divided into two similar groups (15 in each group). control (G_1, non-cooling) and cooling group (G_2). Each group was divided into three sub-groups (5 in each),control, supplemented with zinc methionine or niacin.Source of cooling water was the main concrete tank of the farm where temperature of the water ranged between 19-21 C0 at time of cooling. Results show that skin and rectal temperature degrees, and respiration rate decreased under cooling system. Cooling system decreased ($P<0.05$) haemoglobin concentration and packed cell volume (PCV %), while the effect of feed supplements was not significant. Zinc and Niacin supplements increased ($P<0.05$) total protein concentration in blood plasma only under cooling system. Albumin and glucose concentrations in blood plasma were not significantly effected. Blood urea nitrogen concentration in blood plasma decreased ($P<0.05$) in feed supplements under cooling groups. All groups of under cooling system showed shorter days open compared with those under non-cooling system, being the highest with Zn and Niacin supplementats. Zn and Niacin supplementation increased ($P<0.05$) conception rate under cooling system than those in the under non-cooling system.

Keywords: Buffalo cows, THI, haematology, blood biochemicals, Days open.

INTRODUCTION

Effects of the ambient temperature on cow performance have been measured by establishing critical body temperature of the cow (Berman *et al.*, 1985; Johnson, 1987 and Igono *et al.*, 1992), an equivalent temperature index incorporating temperature, humidity, and air velocity (Baeta *et al.*, 1987), and temperature humidity index (THI), which incorporate combined effects of temperature and relative humidity (NOAA, 1976). Johnson *et al.* (1963) reported that milk yield exhibited significant declines when maximum THI reached 77. The later research determined that the critical values for minimum, mean and maximum THI were 64, 72 and 76, respectively (Igono *et al.*, 1992). Also, the respiratory rate can be used as an indicator of heat stress (Gaughan *et al.*, 2000). In this respect, Bianca (1971) described fast and shallow respiration in the first phase and slightly slower but deeper respiration in the second phase of the heat stressed animals.

Zinc plays significant role in different enzyme systems needed in nucleic acid metabolism, protein synthesis and carbohydrate metabolism (Chester, 1997). Kegley and Silzeli (1999) observed increased average daily gain in calves supplemented with 25 or 360 mg Zn/ kg/-1 to the basal diet marginally deficient in Zn (21 or 33 mg Zn/ kg/1 diet). Marginal deficiency of zinc in ruminants has been reported in many parts of the tropical countries and thus needs supplementation in their diet.

Cooling can alleviate the heat stress and can improve thermal balance and both productive and reproductive performances of dairy cows (Lee *et al.*, 1999). Omar *et al.* (1996) found that red blood cell number and haematocrite value reduced by cooling, while white blood cell number increased. However, Abdel-Same and Ibrahim (1992) described a significant rise in haemoglobin concentration and haematocrite value. Lee *et al.* (1976) described a significant decrease in haematocrite value in dairy cows exposed to high temperature. Cooling by sprinkling prevented marked changes in red blood picture, only non-significant increase in haematocrite value occurred at the beginning of cooling. Some articles (Koubková *et al.*, 2002), however, reported that cooling of heat stressed dairy cows increased blood hemoglobin concentration (Aboulnaga *et al.*, 1989 and Abdel-Samee and Ibrahim, 1992).

Shams (2008) found that concentrations of total proteins and globulin were higher significantly ($P<0.05$) in blood plasma of cows supplemented with zinc methionine 5 or 10 g than that of control cwos. However, the concentration of albumin and albumin to globulin ratio was not affected significantly by zinc methionine supplementation. Conception rate declined from 61 to 45% when rectal temperature 12 h post breeding increased 1°C (Ulberg and Burfening, 1967). Jordan *et al.* (2002) evaluated season of calving and its influence on conception rate at a timed AI first service in a herd with a 60-d voluntary waiting period, cows calving in the summer had the lowest first-service conception rate. Cooling procedures used on farms today have not been successful to markedly improve fertility and conception rate of lactating cows during summer and is still appreciably below that of winter (Hansen, 1997).

The objectives of the present work to study the physiological response of short-term cooling and dietary supplementation of Zn and Niacin on alleviation of the heat stress of lactating buffaloes.

MATERIALS AND METHODS

This study was carried on Mehallet Mousa Experimental Station, located in the north of Nile delta (90 km south of the Mediterranean coast, latitude 31° 15 N and longitude 31° 45 E). The station is belonging to the Animal Production Research Institute (APRI), Ministry of Agriculture. The experimental work was conducted during the period from June to September 2009.

Animals:

Thirty Egyptian healthy lactating buffalo cows were used in this study. The experimental animals were divided into two main groups (each of 15 group) kept under non-cooling and cooling systems. Each group was further divided into three subgroups (5 animals in each). Buffalo cows in the 1st group were fed the control diet, while those in the 2nd and 3rd groups were fed diet supplemented with zinc methionine (5 g/head) and Niacin (5 g/head), respectively.

All experimental buffalo cows were at the beginning of the lactation period. Live body weight (LBW) of buffalo cows ranged between 508 and 552.8 kg and parity ranged between 2-5 lactations.

Experimental procedures:

Managemental and feeding system:

The experimental buffalo cows were kept in an open yard with 75% shed all the day, where animal shed was made from roof of 3.60 m height. Animals were kept restrained under sheds from the morning (7 a.m.) till the evening (4 p.m.), thereafter they were kept loose in the shed during the rest of the day. All buffalo cows were hand milked twice daily at 8 a.m. and 4 p.m., throughout the lactation period. Milk production was recorded daily and body weight of all animals was recorded on monthly basis.

The experimental animals were fed in groups according to their LBW and reproductive stage. Animals were fed on concentrate feed mixture (CFM) along with Egyptian clover hay (*Trifolium alexandrinum*) and wheat straw according to APRI allowances (1997) as shown in Table (1). Concentrated feed mixture (CFM) was given twice at 8 a.m. and 4 p.m., wheat straw was offered once daily at 9 a.m., whereas clover hay was offered at 11 p.m. The experimental animals were allowed to drink water three times a day from water troughs.

Water cooling system was conducted alternatively and repeatedly among the three treated subgroups of buffaloes. Daily cooling of the treated animals took place within four hours starting at 12 to 4 p.m. (i.e. 80 minutes water cooling allowed for each subgroup per day). The source of cooling water was the main concrete tank of the farm where temperature of the water ranged between 19-21 C^0 at time of cooling.

Table (1): Chemical compositions of feed ingredients (on DM basis).

Item	Feed ingredients			
	CFM	Clover hay	Rice straw	Yellow corn
DM	89.9	89.0	92.5	88.0
OM	88.7	85.4	83.9	98.6
CP	16.1	14.2	3.7	7.7
EE	4.8	0.8	1.3	3.8
CF	18.4	32.9	36.4	2.3
NFE	49.4	37.5	42.5	84.8
ASH	11.3	14.6	16.1	1.4

Meteorological data

Average minimum and maximum air temperature in centigrade and relative humidity were recorded during the experimental period. The combined effect of both air temperature and relative humidity was expressed in a single figure namely, temperature-humidity index (THI). The index was calculated according to the formula given by Livestock and Poultry Heat Stress Indices, Agricultural Engineering Technology Guide, Clemson University, Clemson, Sc. 29634, USA as the following:

$THI = db\,^{0}F - (0.55 - 0.55\,RH)\,(db\,^{0}F - 58.00)$ where

$db\,^{0}F$ = dry bulb temperature in fahrenheit and RH = relative humidity.

Adaptive responses:

Individual skin temperature (ST), rectal temperature (RT) and respiration rate (RR) were measured just before time of sprinkling (at 12 p.m.) and once again after cooling program (at 4 p.m.).

Rectal temperature (RT) was measured by a clinical thermometer which, inserted to almost its full length into the animal rectum for two minutes. The thermometer was carefully introduced close to the rectal wall and rectal temperature reading was recorded in centigrade (C°).

Skin temperature (ST) was measured by attaching a skin thermometer on the upper internal part of the rear legs for two minutes. Respiration rate (RR) was determined by visual observation of the flank movements until reasonable consistency was obtained. One complete movement (in ward and out ward) of the flank was taken as one cycle. Counts of RR were estimated using stop watch.

Blood sampling and analysis:

Blood samples were individually collected from each animal at the end of experiment at 8 a.m. via jugular vein puncture into a heparinized glass tubes. Fresh blood samples were taken to determine packed cell volume (PCV %) and haemoglobin concentration. While, blood samples were centrifuged for plasma separation, which was stored at -20°C until analysis. Blood plasma was used for the determination of the following blood constituents: total protein, albumin, urea nitrogen and total lipids and glucose, Ready made diagnostic kits were used for plasma analysis according to the procedure outlined by the manufacturer.

Reproductive measurements:

One month after delivery, lactating buffaloes were kept under observation twice daily at 8 a.m. and 3 p.m. for heat detection by using a fertile bull. Mating procedure was conducted naturally after 12 hours of heat detection. The following parameters were estimated as a reproductive performance of the buffalo cows: Conception rate (%), days open and calving interval (day). Date of conception was performed by rectal palpation on day 60 post-insemination

Statistical analysis

Statistical analysis was carried out using the statistical package of SAS (1988). The significant differences among means in factorial analysis were tested using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Thermo-humidity index:

Data in Table (2) revealed that buffalo cows in all groups were exposed to heat stress during the experimental period, being moderate (THI = 73 – 74.89) during July and September and very sever (THI over 79.81) during August.

Table (2): Average values of ambient temperature (C°) and relative humidity (RH %) during different months of the experimental period.

Item	Experimental month:		
	July	August	September
Ambient temperature (C°):			
Maximum	32.2±1.3	34.8±1.4	33.3±1.23
Minimum.	17.0±2.3	19.6±2.4	15.01±2.07
Relative humidity (RH %):			
Maximum	90.5±6.5	93.0±8.4	77.4±5.28
Minimum.	60.4±7.4	58.0±6.4	47.0±3.74
Temperature humidity index (THI):			
THI	73.23±7.4	79.81±4.6	74.89±6.4

Such results indicated that buffalo cows have been exposed to heat stress through summer months, in particular during August as reported by Abdel-Khalek (2000) on Friesian bulls and Abu El-Hamd *et al.* (2007) on Friesian cows kept at the same conditions in Egypt.

Degree of temperature affects the levels of heat stress to which animals are exposed (Fuquay, 1981). Five environmental factors influence effective temperature: air temperature, humidity, air movement, solar radiation, and precipitation (Igono, *et al.*, 1992). There are many approaches to quantify heat stress, from complex formulas (Igono *et al.*, 1992 and Linville and Pardue, 1992) to simpler methods such as the temperature humidity index (THI). THI can be used to estimate the effect of heat stress on production (Ravagnolo *et al.*, 2000).

Thermoregulatory responses:

Results presented in Table (3) reveled that skin temperature (ST) was not affected by cooling system, but ST insignificantly decreased from 39.29 to 36.75°C for the control group by cooling system. The dietary supplementation of Zn or Niacin significantly ($P<0.05$) decreased ST under cooling system not non-cooling system.

On the other hand, cooling system was more effective on reducing respiration rate (RR), regardless the dietary supplementation. Under cooling or non-cooling system, dietary supplementation failed to affect on RR. However, rectal temperature was not affected significantly by cooling system or dietary supplementation.

Table (3): Physiological **response of buffalo cows as affected by dietary Zn methionine or niacin feed supplementation under cooling System.**

Item	Cool system			Non-cool system			MSE
	Control	**Zn**	**Niacin**	**Control**	**Zn**	**Niacin**	
kin temperature(C°)	36.75^{c}	35.33^{d}	35.47^{d}	39.29^{a}	37.95^{b}	39.41^{a}	0.03
Rectal temperature(C	37.98^{b}	37.91^{b}	37.94^{b}	38.19^{a}	38.08^{a}	38.11^{a}	0.03
spiration rate/min	22.52^{b}	23.33^{b}	22.28^{b}	25.16^{a}	25.45^{a}	25.86^{a}	0.22

$^{A, b\ and\ c}$**: Means having different superscripts within the same row are significantly different at P<0.05.**

Similar trends of changes in body temperature as affected by cooling or dietary supplementation. were reported on Friesian calves (Fawzy *et al.*, 1998 and Abu El-Hamd, 2000) and on Friesian bulls (Fawzy and Rabie, 1996 and Abdel-Khalek, 2000). Exposing cows to heat stress during summer months caused disturbances in animal body thermoregulation, resulting in marked increase (P<0.05) in RT and RR (Abdel-Samee, 1995 and 1997) and Marai *et al.* (1997). The observed trend of decrease in treatment groups than in the control group was similar to that obtained by Darwish *et al.* (1972) and Salem *et al.* (1984) in cattle and Abu El-Hamd (2000) in Friesian calves. These responses have a deleterious effect on physiologic status of the cow (West, 2003).

Haematological response:

Data in Table (4) show that the effect of cooling system on haemoglobin concentration and packed cell volume (PCV %) significantly(P<0.01), decreased, while the effect of feed supplements was not significant. As affected by cooling system the difference in PCV % of buffalo cows significantly deceased under cooling system (32.64%) than under non – cooling system (35.53).

Table (4): Haematological and bichemical parameters of buffalo cows as affected by dietary supplementation and cooling system.

Item	Cool system			Non-cool system			MSE
	Control	**Zn**	**Niacin**	**Control**	**Zn**	**Niacin**	
Haematological parameters:							
Haemoglobin g/nl	13.13^{b}	13.39^{b}	13.70^{b}	14.25^{a}	14.20^{a}	14.20^{a}	0.33
PCV %	31.61^{b}	30.62^{b}	32.82^{b}	36.22^{a}	37.62^{a}	36.82^{a}	1.37
Biochemical parameters:							
Total protein, g/100 ml	6.62^{b}	7.63^{a}	8.20^{a}	6.25^{b}	7.27^{a}	6.43^{b}	0.22
Albumin, g/100 ml	3.22	3.51	3.41	2.95	3.15	3.18	0.19
Urea-N,mg/dl	32.17^{a}	24.17^{b}	25.23^{b}	33.28^{a}	32.88^{a}	33.28^{a}	1.28
Total lipids mg/dl	477.75$_{a}$	506.7$_{a}$	405.6b	348.1c	388.4b	337.4c	18
Glucose, mg/dl	52.60	56.41	57.75	49.23	57.24	59.53	4.5

$^{a\ and\ b}$ **means within the row same with different superscript are significantly different at P<0.05.**

Similar results were reported by Lee *et al.* (1999), who found that cooling can alleviate the heat stress and can improve thermal balance and both productive and reproductive performances of dairy cows. Omar *et al.* (1996) found that red blood cell number and PCV % reduced by cooling. However, Abdel-Samee and Ibrahim (1992) observed a significant rise in hemoglobin concentration and hematocrit value. However, Toharmat and Kume (1997) did not find any significant differences between haematocrit value and haemoglobin concentration in hot and cool weather. In further work, however, Toharmat *et al.* (1998) found an increase in haematocrit values and haemoglobin concentration in summer. Lee *et al.* (1976) reported a significant decrease of PCV % in dairy cows exposed to high temperature. As result of a rise in erythrocyte destruction. Hemodilution effect could also participate here, because more water was transported in circulatory system for evaporative cooling. Under heat stress, cooling by sprinkling prevented marked changes in red blood picture, only non-significant increase in hematocrite value occurred at the beginning of cooling. Koubková *et al.* (2002) reported that cooling of heat stressed dairy cows increased blood haemoglobin concentration (Aboulnaga *et al.*, 1989; Abdel-Samee and Ibrahim, 1992).

Blood biochemical response:

Results in Table (4) revealed that Zn and Niacin supplements significantly ($P<0.05$) increased total protein concentration in blood plasma of buffalo cows only under cool system. However, the effect of cooling system was not significant. As affected by cooling system and dietary supplements the differences in albumin and glucose concentrations in blood plasma of buffalo cows were not significant. This finding may indicant that dietary Naicin had beneficial effect on total protein concentration only with cooling.

Bharadwaj and Sengupt (1999) in early lactation found that the average level of plasma glucose of buffalo cows did not differ significantly under different feeding treatments. Krober *et al.* (2001) found no significant differences in plasma glucose concentration and albumin with supplementation of methionine in cows. Kholif and Kholif (2003) in early lactation goats fed protected methionine found no significant differences albumin and globulin concentration as affected by supplementation of methionine.

Results in Table (4) revealed that Zn Meth and Niacin supplements under cool system significantly ($P<0.05$) decreased urea-N concentration in blood plasma than those of the control group of buffalo cows. However, concentration of urea-N in buffalo cows fed Zn Meth or Niacin diet were not differ significantly. Similar results were noted by Dinn *et al.* (1998), who reported that blood urea nitrogen concentration decreased ($P<0.05$) with supplementation of rumen protected methionine and lysine. Bharadwaj and Sengupt (1999) in early lactation found that the average plasma urea nitrogen levels of buffalo cows was found to be significantly lower in protected protein fed group as compared to other treatment groups.

Reproductive performance:

All groups of buffalo cows under cooling system showed shorter days open (DO) compared with those under non-cooling system, being the highest with Zn and Niacin supplementation. This may indicate beneficial effects of dietary supplementation rather than cooling system on decreasing days open.

Table (5): Days open, calving interval and conception rate during postpartum of buffalo cows as affected by dietary methionine or niacin supplementation and cooling System.

Item	G1 (Cooled)			G2 (Non-cooled)		
	Control (Ranged)	Zn (Ranged)	Niacin (Ranged)	Control (Ranged)	Zn (Ranged)	Niacin (Range)
Days open, day (Ranged) (Mean)	108-122 115	93-117 105	92-118 105.3	136	127-147 137.4	101-135 117.5
Calving interval, day (Ranged) (Mean)	418-432 425	406-430 418	402-428 415.3	446	440-461 450.4	411-444 427.5
Conception rate, %	40	100	80	20	60	60

In association with decreasing days open, calving interval decreased under cooling system than under non-cooling system (Table 5).

Results revealed that Zn and Niacin supplementation increased conception rate of buffalo cows under cooling system than those in the under non-cooling system. Conception rate of buffalo cows fed Zn and Niacin diets showed the highest values under cooling system. This finding may indicant that Zn and Niacin supplementation had beneficial effect on conception rate with cooling. Cooling procedures used on farms today have not been successful to markedly improve fertility and conception rate of lactating cows during summer and is still appreciably below that of winter (Hansen, 1997). Conception rate declined from 61 to 45% when rectal temperature 12 h post breeding increased 1°C. Furthermore, cattle with rectal temperatures of 40°C as a result of exposure to 32.2°C ambient temperature for 72 h after insemination had conception rates of 0% compared with a conception rate of 48% when rectal temperature was 38.5°C for cows in an ambient temperature of 21.1°C (Ulberg and Burfening, 1967). Cartmill *et al.* (2001) reported that whenever the THI was ≥72, fewer cows were detected in estrus and CR was lower. Also, Ingraham *et al.* (1976) reported that CR declined from 66 to 35% when the THI increased from 68 to 78 the second day before breeding. Jordan *et al.* (2002) evaluated season of calving and its influence on CR at a timed AI first service in a herd with a 60-d voluntary waiting period, cows calving in the summer had the lowest first-service CR. This reduction in fertility may subsequently result in more days open than cows calving in other seasons. Moore *et al.* (1992) reported that days open was higher for cows calving in Mississippi in July than for those calving in August or September.

CONCLUSION

The current study concluded that short-term cooling and dietary supplementation of Zn and Niacin on alleviation of the heat stress could be physiological response and reproductive performance could be improved for lactating buffalo cows

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الاستجابة الفسيولوجية وخصائص الدم والكفاءة التناسلية فى الجاموس المعامل غذائيا بالزنك مثيونين والنياسين تحت نظم تبريد أثناء موسم الصيف فى مصر

هشام حسين خليفه[1] ، عبدالله محمد عاشور[1] ، محمد محمد يوسف[2] و مسعود محمد غنيم[2].

[1]قسم الإنتاج الحيوانى _كلية الزراعة - جامعة الأزهر - مدينة نصر - القاهرة.
[2]معهد بحوث الإنتاج الحيوانى ، مصر - الجيزة- الدقي .

استخدم في هذه الدراسة ثلاثون جاموسة حلابة من الجاموس المصرى قسمت إلى ٦ مجموعات متشابهه الثلاث مجموعات الأولى وضعت تحت نظام تبريد بنظام الرش بالماء والثلاث مجاميع الأخرى تحت نظام عادى من ظروف حرارية. وتحت كل نظام تبريد كانت الثلاث مجموعات تغذى على عليقة كنترول ، عليقة مضاف إليها زنك مثيونين ٥جم / حيوان وعليقة مضاف إليها نياسين ٥ جم / حيوان ، على الترتيب.

- أظهرت النتائج انخفاض درجة حرارة الجلد و الجسم ومعدل التنفس في الجاموس الموجود تحت نظام تبريد فقط.
- أظهرت مجموعة الحيوانات الموجودة تحت نظام تبريد انخفاض فى تركيز الهيموجلوبين والنسبة المئوية لحجم الخلايا بينما الإضافات الغذائية لم يكن لها تأثير معنوي.
- أدت إضافة كل من الزنك مثيوثين وَالنياسين إلي زيادة تركيز البروتين الكلي في بلازما الدم في الجاموس الموجود تحت نظام تبريد. لم يتغير تركيز كل من الألبيومين والجلوكوز بالإضافة الغذائية أو التبريد.
- انخفض تركيز يوريا النتروجين معنويا في بلازما الدم في المجموعات الإضافة تحت نظام التبريد .
- أظهرت كل المجموعات الموجودة تحت نظام التبريد القصيرالمده فترة الأيام المفتوحه المقارنة بالمجموعات الموجودة تحت نظم غير مبردة وخصوصا عند إضافة الزنك أوالنياسين.
- أظهرت كل المجموعاتالمغذاه على الزنك أوالنياسين الموجودة تحت نظام زيادة معدل الإخصاب بالمقارنة بالمجموعات الموجودة تحت نظم غير مبردة سواء بإضافة أو غير إضافة .

نستنتج من هذه الدراسة أن استخدام نظام التبريد مع أضافة الذنك والنياسين اثناء الإجهاد الحراري أدى إلي تحسين الصفات الفسيولوجية والكفائة التناسلية في الجاموس الحلاب.

قام بتحكيم البحث

أ.د / عبد الخالق السيد عبد الخالق	**كلية الزراعة – جامعة المنصورة**
أ.د / سعيد احمد محمود	**كلية الزراعة – جامعة كفر الشيخ**