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Effect of Hot and Humid Conditions on Cortisol Levels in Lactating Dairy Cows

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تأثير الأحوال الحارة والرطبة على مستويات الكورتيزل في الأبقارالمدرة للحليب

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خلاصة: تم مزامنة دورة الشبق في ١٤ من أبقار الهولستين الحلوب غير الحوامل ووضعها عشوائياً في حظائر مزودة بمراوح أو بغير مراوح لدراسة تأثير التهوية القسرية علىمعدلات تركيز هرمون الكورتيزول. بعد فترة تلائم قدرها ٢١ يوما، تم وضع أنابيب لجمع الدم من الوريد وتم حقن مادة شبيه معامل النمو (ليوتلايز، صنع شركة أبجون ، كالامنزو). كانت كل الحيوانات في مرحلة الجسم الأصفر عند الحقن وذلك من الملاحظات السابقة لدورة الشبق وعن طريق الجس. بعد حقن البيتا بي جي إف تو تم جمع عينات دم كل ست ساعات من الساعة صفر إلى الساعة ٣٦، وكل أربع ساعات من الساعة ٣٦ إلى الساعة ٨٨، وثلاث مرات أسبوعيا لمدة ثلاثة أسابيع بعد ذلك، وتم تحليل عينات الدم لتحديد نسبة الكورتيزول. كما تم قياس درجات الحرارة من الشرج و أخذ بيانات عناصر المناخ، وكان معدل درجة حرارة الشرج أقل (٣٩,١ درجة مئوية) في الأبقار المزودة بمراوح منها في تلك غير المزودة بمراوح (٣٩,٥ درجة مئوية)، ولم يلاحظ أن هناك نمط ليلي لإفراز الكورتيزول ولم تكن هناك فروق معنوية بين المجموعات المختلفة.

ABSTRACT : Fourteen lactating, non-pregnant Holstein cows were heat synchronized for estrus and assigned randomly to pens in a freestall barn with (Fan) or without (Control) a fan to observe the effect of forced ventilation on cortisol concentrations. After a 21-day adjustment period, jugular cannulae were inserted for blood sampling before PGF2α injection. All animals were in their luteal phase at the time of injection as determined by previous observations for estrus and palpation. After PGF2α, blood samples were collected at 6-h intervals from 0 to 36h, 4-h intervals from 36 to 88h and 3 times weekly for three weeks thereafter. Blood samples were assayed for cortisol. Daily rectal temperatures and ambient data were recorded. Average daily rectal temperatures were lower ($P<0.05$) in the Fan (39.1°C) than in the Control (39.5°C). Cortisol did not show a diurnal pattern and the values did not differ ($P>0.05$) between treatment groups.

Heat stressed animals undergo changes like elevated rectal temperature, depressed appetite (McDowell, 1972), reduced milk production (Fuquay, 1981) and low reproductive efficiency (Badinga *et al,* 1985). Heat stress has also been reported to affect the reproductive behaviour and efficiency of animals possibly through the alteration of the reproductive hormones (Roman-Ponce *et al,* 1981).

The change in the body temperature has been listed as an indicator of heat stress, which directly or indirectly may change the hormonal profile of the animals causing a silent and missed periods of estrous, prolong or shortened estrous cycles (Fuquay *et al,* 1970), and early embryonic mortality (Ulberg and Burfening, 1967). Thermal stress resulted in a sharp increase in plasma cortisol levels (Stott and Robinson, 1970; Lefcourt *et al,* (1989). In agreement with these results are data from Wise *et al,* (1988a) who reported elevated levels of serum cortisol concentrations in stressed cows compared to cows maintained under artificial cooling.

Previous studies completed at Mississippi State (Younas *et al,* 1993) has shown an improved estrual response (71%) for cows under cooling by fans as compared to those under heat-stress (33%). In order to confirm the estrual response, the objective of this study was to determine the effect of cooling through forced ventilation on plasma concentrations of cortisol under estrous-synchronized animals in the hot and humid summer conditions of Mississippi.

Materials and Methods

DESIGN OF EXPERIMENT: The experiment was conducted using fourteen non-pregnant, normally cycling lactating Holsteins cows (for a complete estrous cycle) during the month of July-August at the Bearden Dairy Research Center at Mississippi State University, Mississippi, U.S.A. All animals were checked for a normal estrous cycle from previous records, by palpation and from blood progesterone levels. The cows were housed in a section of a freestall barn, having insulated roof with peak ventilation, with or without overhead ceiling fans.

Employing the completely randomize design, the animals were assigned to their respective treatment pens

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with (Fan) or without an overhead fan (Control). Animals were allowed a 21-day adjustment period before intravenous catheters were inserted (Angiocath, 14 gauge x 5 1/4 in, Deseret Medical, Inc, Sandy, UT, U.S.A.) in their jugular veins. On the same evening at 1800h the first blood sample was drawn and 25 mg of Prostaglandin F2α (Lutalyse, The Upjohn Co. Kalamazoo, MI, U.S.A.) was injected to each cow. All were judged to be in their luteal phase of their cycles based on previous observations and palpation of ovaries. After PGF2α injection, blood samples were collected at 6-h intervals from 0 to 36h, 4-h intervals from 36 to 88h and 3 times weekly for three weeks thereafter.

ENVIRONMENTAL DATA: The ambient temperature and relative humidity data in the research barns was collected by using a Hygrothermograph (Model H 311, Weather Measure Corporation, Sacramento, CA, U.S.A.). Animal comfort indices were determined by calculating Temperature-Humidity Index (THI) using the formula: THI = Temperature −0.55 (1-Relative Humidity) (Temperature − 14) as described by Lutgens and Tarbuck (1982). Rectal temperatures were taken before each bleeding by a high-speed electronic rectal thermometer (M211, GLA Agricultural Electronics, San Luis Obispo, CA, U.S.A.).

BLOOD COLLECTION: A 10-ml blood sample was drawn after discarding the first 2 to 3 ml from the catheters containing anti-coagulant. Sodium citrate (3.5%) as an anti-coagulant was used to fill the catheters between the bleedings. Blood collection tubes (Vacutainer 6840, Becton Dickinson, Rutherford, NJ, U.S.A.) were used for the collection of samples. Immediately after sampling, they were placed in an insulated box containing crushed ice. Samples were taken to the laboratory, refrigerated for few hours and centrifuged (Model CRU-5000, Damon/IEC Division, Needham Heights, MA, U.S.A.) for 20 min. at 1200 x g at 4°C. The serum was then pipetted off, placed in labeled vials and frozen at −20°C until the time of assay.

CORTISOL ASSAY: For cortisol determination, a commercial kit (Animal Cortisol Radioimmunoassay; Cambridge Medical Technology Corporation, 575 Middlesex Turnpike, Billerica, MA, U.S.A.) was used. The kit primarily developed for in vitro diagnostic testing of cortisol, has been validated for serum and plasma levels of cortisol (Cox *et al,* 1986). Radioactive cortisol tracer ^{125}I labeled in buffer, containing bovine serum albumin and a displacing agent (4.5 μCi ^{125}I, 110 ml/vial) was used. Before the start of the assay, the standards were tested and parallelism was observed and samples were assayed in duplicate. With the exception of total count tubes, the liquid was aspirated and radioactivity was counted for one minute in Gamma Counter (Model 4/200, Micromedic Systems, Inc., Horsham, PA, U.S.A.). Counts per minute were merged in the RIA program and the values for the samples were calculated from log-logit value procedure. The average sensitivity of the cortisol assays was 0.93 ng/ml. The intraassay coefficient of variation was 5.45%, while the interassay coefficient of variation was 5.88%.

STATISTICAL ANALYSIS: Cortisol values were analyzed using general lineal model procedures (SAS^R, 1990) as a split-plot design in time within treatments. The effects of fan group were tested with error term in animals within fan group. Split-plot analysis over time was used to analyze the rectal temperature and THI data. The Student-Newman-Keuls tests (SAS^R, 1990) were used for mean comparison where significant differences were observed.

Results and Discussion

AMBIENT TEMPERATURE AND HUMIDITY: Ambient temperature in research barns ranged from 22.2 to 36.1°C, relative humidity from 45 to 100% and THI from 21.9 to 32.8 during the experiment. Mean values for ambient temperature, relative humidity and THI are shown in Figure 1.

The mean diurnal ambient temperature ranged from 25.8 ± 0.3°C (0400h) to 31.2 ± 0.4°C (1600h) and relative humidity from 70.2 ± 2.3% to 94.6 ± 0.7% at 1600h and 2400h, respectively. Temperature-humidity indices ranged from 25.5 ± 0.3 (0400h) to 27.4 ± 0.4 (1600h) in the experimental barns. Ambient temperature rose from 0800 until 1600h with relative humidity dropping during that period. Relative

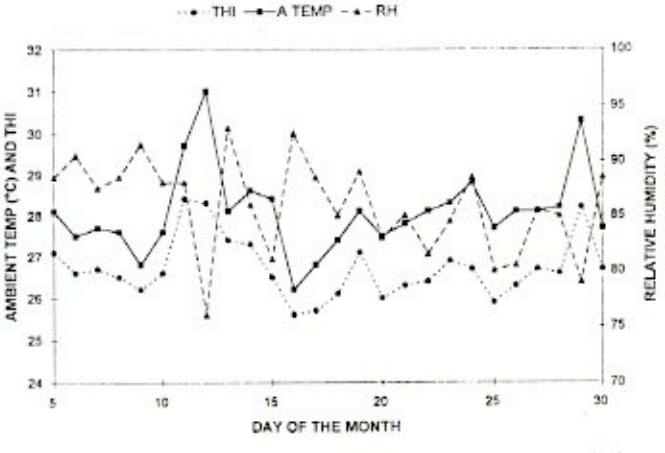

Figure 1. Average daily temperature (ATEMP), relative humidity (RH) and temperature-humidity-index (THI) during the experimental period

humidity increased at night when ambient temperatures were dropped. Temperature-humidity indices increased slowly from 0400h to 1600h and then dropped slowly for the remainder period at night.

Higher relative humidity accentuates the effect of high ambient temperatures by impairing the heat loss system of the animals (Fuquay, 1981). Some researchers have reported that animals suffer more heat-stress during the afternoon because of high THI (Stott and Williams, 1962; Vincent, 1972). The resulting index is based on the fact that values in excess of 25 indicate that most animals will feel uncomfortable, while a THI between 15 and 20 is considered within the comfort zone (Maunder, 1970). The THI in our study had a diurnal range of 22.0 to 32.8 with all points above the comfort zone (Table 1). Stott and Williams (1962) stated that the increase in relative humidity is the important factor in lowering the breeding efficiency in August in Arizona compared to other months with even higher temperatures.

RECTAL TEMPERATURE: Diurnal rectal temperatures during the first 88h ranged from 38.9 to 39.7°C in the Control group and 38.6 to 39.4°C in the Fan group. Diurnal rectal temperatures tended to be lower in early mornings but higher at 1400 and 1800h. The diurnal rectal temperatures were lower in the Fan group and they cooled down faster at night than the Control cows. Rectal temperatures recorded at all 1400h observations with standard error of means (SEM) are shown in Figure 2, which indicates that rectal temperatures in the Control were significantly higher ($P < 0.05$) than the Fan for the entire experiment.

The forced ventilation provided by the ceiling fans decreased the rectal temperature (0.37°C) in the Fan group as compared to the Control. The lower rectal temperatures for the fan group in this study are similar to those reported earlier for lactating dairy cows under fans (Gonzalez *et al*, 1981;Younas *et al*, 1989) and were more pronounced than those observed with simple cross ventilation (Fuquay *et al*, 1979).

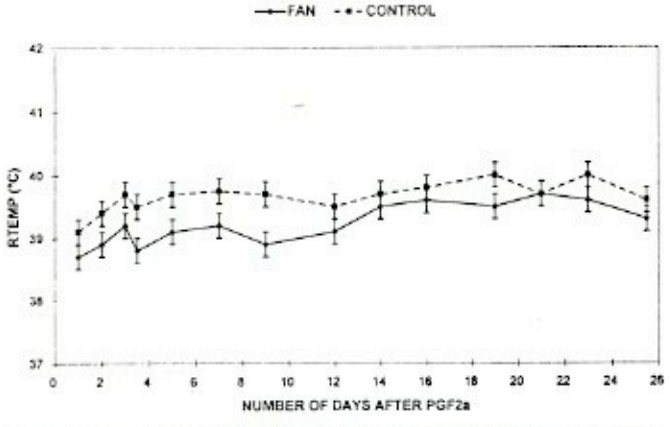

Figure 2. Average rectal temperatures (RTEMP) of Fan and Control group animals taken at 1400 h daily

TABLE 1

Average daily ambient temperature (ATEMP), relative-humidity (RH) and temperature-humidity-index (THI) during the experimental period

Date	d	ATEMP (°C)	RH (%)	THI
5	1	28.11 ± 0.85	87.80 ± 4.36	27.09 ± 0.50
6	2	27.50 ± 0.97	89.83 ± 4.91	26.62 ± 0.52
7	3	27.69 ± 0.88	87.17 ± 3.30	26.69 ± 0.73
8	4	27.59 ± 0.87	87.83 ± 5.20	26.58 ± 0.52
9	5	26.85 ± 0.57	91.33 ± 1.50	26.22 ± 0.44
10	6	27.69 ± 1.12	87.67 ± 3.34	26.66 ± 0.78
11	7	29.72 ± 1.62	87.67 ± 5.04	28.48 ± 1.22
12	8	31.02 ± 1.74	75.83 ± 8.14	28.37 ± 0.77
13	9	28.06 ± 1.16	92.83 ± 2.75	27.49 ± 1.11
14	10	28.70 ± 1.26	86.00 ± 4.70	27.42 ± 0.77
15	11	28.33 ± 1.09	80.83 ± 7.56	26.63 ± 0.54
16	12	26.20 ± 0.17	92.33 ± 1.50	25.69 ± 0.15
17	13	26.76 ± 1.14	88.50 ± 3.64	25.85 ± 0.80
18	14	27.41 ± 1.25	85.00 ± 3.81	26.26 ± 1.07
19	15	28.15 ± 0.75	89.00 ± 4.40	27.21 ± 0.39
20	16	28.33 ± 1.32	83.17 ± 5.42	26.18 ± 0.89
21	17	26.20 ± 1.28	83.67 ± 5.61	26.50 ± 1.03
22	18	26.76 ± 0.79	81.50 ± 6.44	26.61 ± 0.47
23	19	28.15 ± 1.01	84.50 ± 3.91	27.01 ± 0.63
24	20	28.33 ± 1.31	88.50 ± 4.60	27.85 ± 0.85
25	21	27.69 ± 1.10	79.83 ± 4.67	26.05 ± 0.69
26	22	28.08 ± 1.08	80.33 ± 8.16	26.40 ± 0.78
27	23	28.06 ± 0.93	85.67 ± 6.18	26.90 ± 0.90
28	24	28.15 ± 1.27	85.33 ± 5.57	26.87 ± 0.91
29	25	30.28 ± 0.99	80.00 ± 6.19	28.33 ± 0.41
30	26	27.78 ± 1.28	88.33 ± 4.41	26.83 ± 0.87

Higher rectal temperatures have been reported for dairy cows near midnight than during midday. Minimum rather than maximum ambient temperatures are more important as hormonal indicators of physiological stress (Fuquay *et al*, 1979; Clemmer, 1977).

CORTISOL: Average serum cortisol values are presented in Figure 3. Serum cortisol concentrations for the Fan and Control groups, were 6.60 ± 0.58 ng/ml and 5.53 ± 0.59 ng/ml, respectively. No significant differences were observed in serum cortisol concentrations in the Fan and Control groups ($P > 0.05$). Cortisol values showed no diurnal pattern and fluctuated considerably throughout the study. A small increase was observed in some animals toward the end of the bleeding schedule; however, this was not seen in mean values. The increase near the end could be due to the tail bleeding through the coccygeal artery

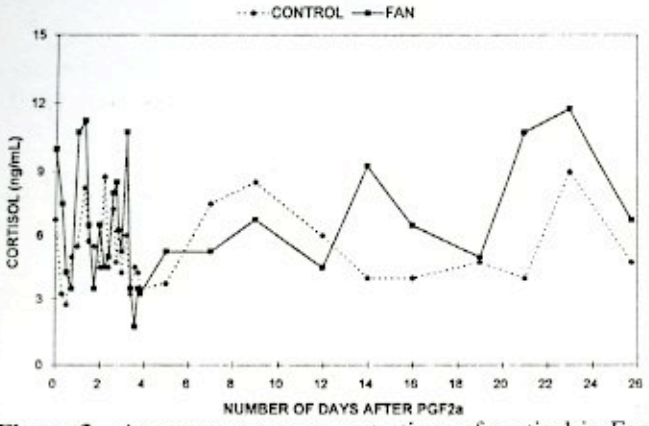

Figure 3. Average serum concentrations of cortisol in Fan and Control groups.

after cannulae were removed.

Under varying physiological conditions it is difficult to accurately assess adrenal function due to variability in basal glucocorticoids levels (Gwazdauskas and Vinson, 1979). However, Abilay and Johnson (1983) in a study of Guernsey heifers, whose estrous cycles were syncronized under two controlled conditions (18.2°C, 55% RH and 33.5°C, 55% RH), reported decreased values of mean plasma cortisol. Decreased cortisol levels have also been reported by Stott and Williams (1962); Abilay *et al,* (1975); and Roman-Ponce *et al,* (1981). These decreases have been implicated as causative hormonal agents in which heat stress has negative influence on reproductive efficiency.

Wise *et al,* (1988 a & b) reported contrasting results in two experiments. In the first study, they observed higher serum cortisol concentrations in heat stressed cows as compared to those maintained under cooling. Yet in another study, they reported no difference in serum cortisol values between cooled and control cows. No difference in cortisol levels due to heat stress have been reported earlier (Gwazdauskas *et al,* 1973). Stott and Robinson (1970) reported a short-lived surge in cortisol and progesterone followed by a return to basal values. These studies suggest that heat stress does not appear to evoke the classic-induced cortisol response and, therefore, may not be directly involved in the etiology of the impaired reproductive efficiency that is seen during the heat stress.

Cortisol levels in lactating and non-lactating Holsteins under Pakistani summer conditions from June to October have also been explored (Imtiaz Hussain et al. 1992). Higher mean cortisol values were observed in June (6.4 ng/ml) and except for August there were a decreasing trend ($P<0.05$) in the following months with a minimum mean serum cortisol in October (1.6 ng/ml). During August there was a significant rise ($P<0.05$) in serum cortisol (4.2 ± 0.1 ng/ml). These results are parallel to those of Stott *et al,* (1972) who have reported decreased cortisol levels in August.

Higher cortisol in June might be due to the bleeding technique which improved later or cows became habituated to the technique. The mid summer surge in cortisol during August might have been due to weather change because of rainfall and higher humidity associated with monsoon. This could indicate that cows respond emotionally to this stressor, given the evidence that emotional stress evokes such a response (Christison and Johnson, 1972).

Conclusions

Diurnal as well as overall rectal temperatures were lowered due to forced ventilation but no difference was observed in serum cortisol values. Hyperthermia have been reported to affect the concentrations of hormones in peripheral circulation. The lack of effect in this study on the values of cortisol may indicate that these concentrations may remains unchanged in hot and humid areas. However, similar research efforts are needed to further explore these physiological norms and hormonal parameters.

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