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Impact of heat stress on estrus expression and follicle size in estrus under field conditions in dairy cows

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ABSTRACT

The objective of this study was to investigate the impact of heat stress on the expression of estrus signs and follicular diameter at the day of estrus in dairy cows under farm conditions. Cows reported in estrus (i.e., by an automated activity monitoring system or by the herd manager) were examined by a veterinarian. Uterine contractility and the largest diameter of all ovarian structures was determined by transrectal palpation and ultrasonography, respectively. The amount of estrus discharge, mounting traces and the color of the vaginal mucosa were determined in an external examination and scored on 3-point scales. Blood samples were obtained for analysis of serum progesterone concentrations. Cows with a high uterine contractility and high amount of estrus discharge were 4.05 and 1.72 times more likely to have an estrus follicle (large, presumptive preovulatory follicle $\geq$ 12 mm) than cows expressing low amount of the estrus sign, respectively. The likelihood for a pink vaginal mucosa, clear stringy estrus discharge and mounting traces at the cows back decreased continuously with increasing temperature-humidity index (**THI**) at the day of estrus. The likelihood for a serum progesterone concentration < 1 ng/ml at the day of estrus decreased continuously with increasing THI $\geq$74. Follicular size decreased 0.1 mm for each incremental THI point at the day of estrus. The results of this study indicate, that heat stress at the day of estrus significantly reduces the intensity of external estrus signs and the size of estrus follicle decreases with increasing THI.

1. Introduction

Reproductive performance of dairy cows is negatively affected by heat stress in several ways. Higher body temperature under heat stress conditions alters standing and lying behavior of dairy cows [1] which has been described to decrease mounting and standing activity of cows in estrus [2,3]. Therefore heat stress leads to reduced estrus detection rates when using automated activity monitoring systems as well as in visual observation resulting in a decrease in pregnancy rate [4]. The use of visual observation for estrus detection offers the advantage that the color of the vaginal mucosa and the presence of vaginal discharge [5,6] may also be used as reference for estrus. Despite these additional options estrus detection rates of visual observation are decreased under heat stress conditions [4]. Previous studies assumed, that heat stress compromises the endometrial function and secretory activity of the uterus [7] what might lead to smaller amount of transparent vaginal discharge, coloration of the vaginal mucosa and uterine contractility. The ability of herdsmen and veterinarians to interpret the signs of estrus correctly and to detect cows in estrus accurately is essential and significantly affects resulting pregnancy rate [4]. Interestingly, there is a lack of science-based information about the relationship of follicle size and expression of estrus signs under heat stress conditions in dairy cows.

Furthermore, heat stress can have major effects on follicle quality and hormonal patterns in dairy cows. These include smaller follicular size and suppressed dominance of the large follicle under estrus [7]. Hormonal imbalances include decreased serum estradiol concentration [8], decreased plasma concentration of LH and alterations in progesterone secretion [7,8]. Results of previous studies indicate, that heat stress appears to impair follicular development and quality of estrus follicles. In a study of Badinga et al. [9] when cows were either assigned to shade (n = 11) or no shade (n = 12) dominant follicles of cows exposed to shade were bigger (16.4 vs 14.5 mm) compared to follicles of cows without access to shade [9].

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Similarly, in a study conducted in a climate laboratory, cows under thermoneutral conditions (n = 11, 19 °C ambient temperature, 60% relative humidity) had a dominant follicle that was either equivalent or greater than that of heat stressed cows (n = 11, 29 °C, 60%) [8]. The evidence of these study results, however, is limited because of the study design (i.e., artificial climate conditions), limited sample size, and lacking analyses of the relationship between the follicular diameter and THI at the day of estrus. The THI, as a function of ambient temperature and relative humidity is the most widespread indicator of heat stress in dairy cows and can be used as heat stress indicator in the tropical or subtropical [10,11] as well as in the temperate climate [12,13]. Furthermore, the calculation of critical THI-thresholds enables an objective comparison of heat stress conditions and resulting physiological responses of dairy cows [14,15].

To our knowledge, there is no field study investigating the impact of different THI-thresholds on expression of estrus signs in dairy cows. Therefore, the objective of this study was to investigate the impact of heat stress on the expression of estrus signs and follicular diameter at the day of estrus in dairy cows under field conditions.

2. Material and methods

2.1. Design of the barn

The study was conducted on a commercial dairy farm in Brandenburg, Germany from March 2012 to November 2012. The herd consisted of 676 Holstein-Jersey mix dairy cows with an average milk production of 9699 kg (4.0% fat, 3.4% protein). The barn was positioned in a NW-SE orientation (52° 15′ N, 13° 22′ E) with open ventilation and a mechanical fan-system. Five fans were installed above the stalls and controlled manually by the farm manager. All cows were housed in a free-stall facility with concrete floor and beds equipped with straw. On average 90 cows were housed inside the study pen. Group composition was dynamic with cows entering at DIM 54 to 60 and leaving the study pen after pregnancy confirmation. Cows were fed a partial mixed ration consisting of 54.3% corn silage, 25.4% haylage and 20.3% concentrate mineral mix on a DM basis. Concentrate was administered based on individual milk yield through 2 computer feeders inside the barn. The rations were formulated to meet or exceed the requirements of the National Research Council [16]. Lactating cows were milked twice daily at 0800 and 1700.

2.2. Reproductive management

The voluntary waiting period was set at 54 d postpartum. Between 47 and 53 d cows were examined by one veterinarian before inclusion into the study. The uterus and ovarian structures were examined by rectal palpation and ultrasonography. Ultrasonography was performed using a portable ultrasound scanner (Easiscan bovine, BCF Technology, Livingston, Scotland) equipped with a 4.5–8.5 MHz rectal transducer. The largest diameter of existing corpora lutea were measured in a frozen image with the electronic caliper function [17]. Body condition was scored using a 5-point scale with 0.5 increments (BCS) [18], and locomotion was scored using a 5-point scale [19]. The following inclusion criteria were set: no pathological findings of the uterus (e.g., purulent discharge, adhesions) by rectal palpation and rectal ultrasonography, no luteal or follicular cysts with a diameter ≥ 25 mm [20,21], a locomotion score ≤ 3 and a BCS ≥ 2.5. If these criteria were not fulfilled, the cow was not included. Seven d later all cows without a corpus luteum in the first examination were reexamined by rectal ultrasonography. Cows without a corpus luteum ≥10 mm on at least one ovary in the second examination were defined as acyclic and also excluded from the study.

The observation period for estrus detection started at 54 days postpartum. An automated activity monitoring system (Heatime, SCR Engineers Ltd. Netanya, Israel) and visual observation were used to detect estrus. Visual estrus detection was performed by the herd manager twice a day between 0900 and 1000 and between 1600 and 1700 for approximately 30 min each. The herd manager defined a cow in estrus based on primary (i.e., cows standing to be mounted) and secondary signs of estrus (i.e., high activity, mounting other cows, transparent vaginal discharge, cows sniffing, licking or resting the chin on the rump of other cows).

Cows reported in estrus (i.e., by the automated activity monitoring system or by the herd manager) were examined by transrectal palpation and ultrasonography by a veterinarian. Uterine contractility was scored using a 3-point scale (low, medium, and high contractility) [22] and the largest diameter of all ovarian structures (i.e., corpora lutea, follicles, luteal and follicular cysts) was measured by ultrasonography. In an external examination the amount of estrus discharge, mounting traces and the color of the vaginal mucosa was determined before the transrectal palpation. The amount of estrus discharge was scored using 3-point scale (no visible mucus, low amount of clear stringy mucus, high amount of clear stringy mucus). The amount of mounting traces at the cows back was scored using a 3-point scale (no visible traces, low amount of traces, high amount of traces). The color of vaginal mucosa was scored using a 3-point scale (pale, light pink, red). In addition blood samples were obtained by puncture of the vena coccygea mediana for analysis of serum progesterone concentrations. Samples were centrifuged (10 min, 1000 × g), and serum was stored in three aliquots at −20 °C until progesterone analysis. Serum progesterone concentration was determined in an external laboratory by the DRG Progesterone Chemiluminescence Immunoassay Kit (DRG Instruments GmbH, Germany).

Cows detected in estrus by the automated activity monitoring system or the herd veterinarian were inseminated by 2 technicians within 12 h after detection of estrus. Pregnancy diagnoses were performed 35–45 d after the day of breeding with transrectal ultrasonography. Cows with unclear pregnancy diagnosis were reexamined 2 weeks later.

2.3. Climate data

Ambient temperature and relative humidity within the experimental barn were recorded using a Tinytag Plus II logger (Gemini loggers Ltd, Chichester, UK) secured at a beam 2 m from the ground within the barn. This logger measured ambient temperature from -25 to +85 °C with an accuracy of ±0.3 °C and a resolution of 0.01 °C and relative humidity from 0 to 100% with an accuracy of ±3% and a resolution of 0.3%. These data were recorded hourly. Ambient temperature (AT) and relative humidity (RH) data were used to calculate the THI according to the equation reported by the NRC [23]: $THI = (1.8 \times AT + 32) - ((0.55 - 0.0055 \times RH) \times (1.8 \times AT - 26))$.

2.4. Statistical analyses

Data from the onsite climate logger and from the examination protocols were exported into Excel spreadsheets (Office 2013, Microsoft Deutschland GmbH, Munich, Germany) and statistical analysis was performed using SPSS for Windows (Version 22.0, SPSS Inc. IBM, Ehningen, Germany). Hourly measures of ambient temperature, relative humidity and resulting THI were used to calculate daily means. These values were dichotomized (i.e., above or below threshold) for THI-increments ranging from 49 to 79 to identify thresholds. Descriptive statistics of continuous variables