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Influence of heat stress on the reproductive performance of dairy cows in the moderate climate of the temperate latitude

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Den Kühen

“In theory, theory and practice are the same. In practice, they are not.”

(Albert Einstein)

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1 INTRODUCTION

Heat stress in dairy cows can lead to substantial economic losses in summer months due to decreased milk yield and impaired reproductive performance (West, 2003). Therefore, this topic generated a great deal of interest and research activities in the last years. Furthermore, changes in the moderate climates in the temperate latitudes have been anticipated (Menzel et al., 2006, Alcamo, 2007), which might increase the magnitude and duration of heat stress periods.

Most of the studies investigating heat stress in dairy livestock have been conducted in tropical or subtropical areas because the negative effects are obvious in these climates. However, not much is known about the impact of heat stress on dairy cows in the moderate climates in the temperate latitudes although in summer months extreme temperatures can occur (Alcamo, 2007).

The overall objectives of this study were to determine the incidence of heat stress in the dairy livestock in the moderate climate and to examine the impact on the reproductive performance of dairy cows.

1.1 Measurement of heat stress in dairy livestock

In most of the studies investigating heat stress in dairy cows it is common practice to obtain the climate data from a meteorological station located in the vicinity of the study sites, but the value of information from weather stations to augment dairy records is not known (Ravagnolo et al., 2000). Cows release high amounts of heat and humidity via convection, conduction and radiation to the environment (Silanikove, 2000) during hot climate conditions which can influence the environmental conditions inside a dairy farm considerably (Berman et al., 1985, Robinson et al., 1986). Recently, it was demonstrated that there is a trend in the dairy industry toward fewer and larger dairy farms housing more cows under one roof (Winsten et al., 2010) which might increase the risk of suboptimal climate conditions. Additionally, there is often heat released by radiation from machinery located in the barns as well as humidity through cleaning processes. Obviously, all these factors can lead to considerably different climate conditions in dairy livestock compared to those of a meteorological station recording outdoor data.

The objective of my first study was to compare the climate conditions of dairy freestall facilities with the climate recorded from the closest official meteorological stations.

The results of this study were recently published in the Journal of Dairy Science (impact factor 2.55):

L. K. Schüller, O. Burfeind and W. Heuwieser. 2013. Short communication: Comparison of ambient temperature, relative humidity, and temperature-humidity index between on farm measurement and official meteorological data. 96:7731-7738.

1.2 Impact of heat stress on conception rate in dairy cows

Heat stress can have major effects on fertility and reproductive performance in lactating dairy cows (Hansen and Arechiga, 1999). These include compromised physiological function of the reproductive tract (Wolfenson et al., 2000), disturbances in hormonal balance (Wilson et al., 1998a, Wilson et al., 1998b, Wolfenson et al., 2000), decreased oocyte quality (Ferreira et al., 2011, Gendelman and Roth, 2012) as well as decreased embryo development (De Rensis and Scaramuzzi, 2003, Gendelman et al., 2010, Silva et al., 2013) and embryo survival (Wolfenson et al., 2000). These processes lead to a decrease in conception rate in the subtropical areas during the hot season ranging between 20 and 30% compared to the winter season (De Rensis et al., 2002). Therefore, heat stress is one impacting factor responsible for extensive economic losses to the dairy industry (Collier et al., 2006).

Heat stress in dairy cows can be calculated with the temperature-humidity index (THI) (Kendall and Webster, 2009) combining the ambient temperature and the relative humidity. To investigate the impact of heat stress on the reproductive performance of dairy cows, the determination of THI-thresholds for this relationship is mandatory. In previous studies, conducted in the subtropical areas, THI-thresholds of ≥ 70 (Ravagnolo, 2002) and ≥ 72 (Morton et al., 2007) were described for the influence of heat stress on conception rate in dairy cows. However, the impact of heat stress on the conception rate in dairy cows in the moderate climate is not known and a THI-threshold for this relationship has not been described so far.

Therefore, the objective of this study was to examine the effects of heat stress on the conception rate of dairy cows in the moderate climates of the temperate latitudes and to determine a threshold for this relationship.

The results of this study were recently published in Theriogenology (impact factor 1.84):

L. K. Schüller, O. Burfeind and W. Heuwieser. 2014. Impact of heat stress on conception rate of dairy cows in the moderate climate considering different temperature-humidity index thresholds, periods relative to breeding, and heat load indices. Theriogenology. 81:1050-1057.

The two papers are presented in the format outlined in the guide for authors of the respective journal.

1.3 Efficiency of different breeding programs under heat stress

As a result of impaired reproductive performance and less efficient detection of estrus during heat stress, timed artificial insemination breeding programs have become popular because of their predictability and their likelihood of producing a greater proportion of total pregnancies in the herd (Stevenson, 2005). The Ovsynch program is probably the most popular breeding program employed (Stevenson, 2005). In a meta-analysis (Rabiee et al., 2005) it was shown that there is little or no significant improvement in pregnancy rates using the Ovsynch program over other breeding programs and the costs of labor and hormone administration should be considered when selecting Ovsynch for routine use. Irrespective of these observations, heat stress has led to an increased use of timed artificial insemination during summer months to improve the conception rate (Collier et al., 2006) because management limitations induced by heat stress on estrus detection are eliminated (De la Sota et al., 1998, DeJarnette et al., 2001). However, there is not only a dearth of information to compare a timed artificial breeding program with a conventional reproductive program during heat stress (Burke et al., 1996) but also a lack of controlled field data evaluating reproductive performance of cows that implement such breeding programs (Jobst et al., 2000). Furthermore, reproductive efficiency in dairy cows is decreasing worldwide. Therefore, methods for timed insemination of dairy cattle still need to be optimized (Lucy, 2001).

Therefore, the objectives of a third study were to examine the effect of heat stress on reproductive performance of dairy cows considering natural and artificial insemination breeding programs. These data have not been published yet and are presented in section 4 “Additional unpublished work”.

2 PUBLICATION I

Short communication: Comparison of ambient temperature, relative humidity, and temperature-humidity index between on-farm measurements and official meteorological data

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3 PUBLICATION II

Impact of heat stress on conception rate of dairy cows in the moderate climate considering different temperature–humidity index thresholds, periods relative to breeding, and heat load indices

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3.1 Abstract

The objectives of this retrospective study were to investigate the relationship between temperature-humidity index (THI) and conception rate (CR) of lactating dairy cows, to estimate a threshold for this relationship and to identify periods of exposure to heat stress relative to breeding in an area of moderate climate. In addition, we compared 3 different heat load indices related to CR: mean THI, maximum THI and number of hours above the mean THI threshold. The THI threshold for the influence of heat stress on CR was 73. It was statistically chosen based on the observed relationship between the mean THI at the day of breeding and the resulting CR. Negative effects of heat stress, however, were already apparent at lower levels of THI and 1 h of mean THI ≥ 73 decreased CR significantly. The CR of lactating dairy cows was negatively affected by heat stress both before and after the day of breeding. The greatest negative impact of heat stress on CR was observed 21 to 1 d before breeding. When the mean THI was ≥ 73 in this period, CR decreased from 31% to 12%. Compared to the average maximum THI and the total number of hours above a threshold of ≥ 9 h, the mean THI was the most sensitive heat load index relating to CR. These results indicate that the CR of dairy cows raised in the moderate climates is highly affected by heat stress.

3.2 Key words

dairy cow, heat stress, conception rate, moderate climate

3.3 Introduction

In the last 60 years conception rate (CR) in high-yielding dairy cows decreased from 55% to 35% worldwide and is suggested to further decrease in Germany [1-3]. One important factor for decreasing reproductive efficiency in dairy cows is heat stress which may reduce CR up to 23% [4]. High-yielding cows are particularly affected by heat stress because the heat tolerance decreases with increasing milk yield and dry matter intake [5,6]. As milk yield of dairy cows is expected to further increase [7,8], the negative impact of heat stress will become more important. There is a trend in the dairy industry toward fewer and larger dairy farms housing more cows under one roof [9] which might increase the risk of suboptimal climate conditions [10,11].

Heat stress can have major effects on fertility and embryonic survival in lactating dairy cows [12]. These include compromised endometrial function and secretory activity, smaller follicular size and suppressed dominance of the large follicle [13]. Disturbances in hormonal balance include decreased serum estradiol concentration [14,15], decreased plasma concentration of LH and decreased progesterone secretion [13]. Furthermore, oocyte quality

[16,17], embryo development [18-20] and embryo survival [13] are impaired by heat stress. These processes lead to a decrease in CR in the subtropical areas during the hot season at 90d and 135d postpartum (33% and 62%) ranging between 20 and 30% compared to the winter season (46% and 76%) [21]. Thus, heat stress is one impacting factor responsible for extensive economic losses to the dairy industry [22].

Morton et al. [23] estimated that heat stress defined as a daily maximum temperature-humidity index (THI) of ≥ 72 from d 35 before to d 6 after the day of breeding decreases CR of lactating dairy cows by around 30 percentage points relative to days of breeding in which there was no heat load from d 35 before to da 6 after the day of breeding. The majority of studies about heat stress in dairy cattle were conducted in tropical or subtropical areas (e.g. Florida, Mexico, Southwest of USA) because the negative effects are obvious in these climates. However, the average THI in the moderate climates in the temperate latitudes (e.g. Central Europe, Northern US and Canada) can also reach the threshold of 72 during summer months. Most recently we demonstrated that the THI threshold of 72 was reached on 162 of 756 experimental days inside a commercial dairy barn in Germany. This observation highlighted the importance of heat stress even in the moderate climates [24].

Heat stress in the period around the day of breeding was consistently associated with reduced CR [4,23]. Furthermore, negative effects of heat stress have been identified from 42 d before to 40 d after insemination [25]. The mechanisms by which heat stress impairs conception considering for specific periods, however, remain unclear [23,26].

Therefore the objective of the current study was to examine the effects of heat stress on reproductive performance of dairy cows in the moderate climates of the temperate latitudes. Specifically we set out 1) to investigate the relationship between THI and CR of lactating dairy cows, 2) to determine a critical threshold of THI on the day of breeding for CR and 3) to identify periods of exposure relative to the day of breeding during which heat stress is most closely associated with impaired CR. In addition, we assessed whether the mean THI is more closely associated with CR than the maximum THI or the total number of hours above the mean THI threshold in certain periods of exposure relative to day of breeding.

3.4 Materials and methods

3.4.1 Herd and barn

The retrospective study was conducted on a commercial dairy farm located in Sachsen-Anhalt, Germany from May 2010 to October 2012. The herd consisted of 1,150 Holstein dairy cows with an average milk production of 10,345 kg (4.0% fat, 3.3% protein). The barn was positioned in a NE-SW orientation with open ventilation and a mechanical fan-system. Sixty fans were installed above the stalls and controlled manually by the farm manager. All cows were housed in a free-stall facility with slatted floors and beds equipped with rubber mats.

Group composition was dynamic with cows entering and leaving the experiment depending on their calving dates. Cows were fed a TMR. The rations were formulated to meet or exceed the requirements of the NRC [27]. Lactating cows were milked 3 times a day at around 6 AM, 2 PM and 10 PM.

3.4.2 Reproductive management

The voluntary waiting period was set at 49 d postpartum. Between 35 and 49 d cows received an initial injection of 25 mg PGF2α (Dinoprost, Dinolytic, Zoetis Deutschland GmbH, Berlin, Germany) and a second injection of 25 mg PGF2α 2 weeks later to regress the corpora lutea. Cows that showed estrus after the second injection of PGF2α were artificially inseminated. Pedometers (Milkline, Gariga di Podenzano, Italy) and visual observation were used to detect estrus. Cows that did not show estrus after the second injection of PGF2α were examined and treated with an Ovsynch program initiated 12 d later. On this day, treated cows received an initial injection of 100 µg of GnRH (Gonadorelin, Gonavet Veyx, Veyx-Pharma GmbH; Schwarzenborn, Germany). Seven d later at the same time, cows received 25 mg PGF2α to regress the corpora lutea and 48 h later, a second injection of 100 µg of GnRH to induce ovulation of the dominant follicle. The AI was performed 16 to 17 h after the second injection of GnRH. Cows that showed estrus after the injection of GnRH of the Ovsynch program were inseminated prematurely. Cows with a corpus luteum at the time of examination (i.e. after the second injection of PGF2α) were treated with a modified Ovsynch program. Cows received 25 mg PGF2α to regress the corpora lutea and 48 h later an injection of 100 µg of GnRH to induce ovulation of the dominant follicle. The AI was performed 16 to 17 h after the injection of GnRH. Inseminations at detected estrus were performed within 12 h after detection of estrus. AI and inseminations at detected estrus were performed with frozen-thawed or fresh semen. Cows after caesarean section, with adhesions of the uterus and repeat breeder cows in third or higher lactation received natural service by the bull. Pregnancy diagnoses were performed 34 d after the day of breeding with transrectal ultrasonography by the herd veterinarian. Cows that were not pregnant and did not have a CL were reassigned to the Ovsynch protocol. Cows that were observed in estrus > 10 d after AI were assumed nonpregnant and re-inseminated.

3.4.3 Data collection

Ambient temperature (AT) and relative humidity (RH) within the experimental barn were recorded using a Tinytag Plus II logger (Gemini loggers Ltd, Chichester, UK) secured at a beam 3 m from the ground. These loggers measured AT from - 25 to + 85 °C with an accuracy of ± 0.3 °C and a resolution of 0.01 °C and RH from 0 to 100% with an accuracy of ± 3% and

a resolution of 0.3%. These data were recorded hourly. Ambient temperature and RH data were used to calculate the THI according to the equation reported by
Kendall et al. [28]: THI = (1.8 x AT + 32) - ((0.55 - 0.0055 x RH) x (1.8 x AT - 26)).
Cow ID, breeding dates and results of pregnancy diagnoses were obtained from computerized herd records managed using HerdeW (Version 5.5, Software Projektierungs- und Handels GmbH, Aschare, Germany).

3.4.4 Statistical analyses

Data from the onsite climate loggers and from HerdeW were exported to Excel spreadsheets (Office 2010, Microsoft Deutschland GmbH, Munich, Germany) and analyzed using SPSS for Windows (Version 19.0, SPSS Inc., IBM, Ehningen, Germany). Cold and hot months during the study period were determined by visual observation of monthly means. Furthermore we determined the months with minimum one day with a mean THI ≥ 72 as hot months because this THI threshold is commonly accepted as threshold for heat stress [29]. Daily mean and daily maximum AT, RH and THI were calculated from hourly measures. These values were dichotomized (i.e. above or below threshold) for THI-increments ranging from 41 to 79 to identify thresholds. Furthermore hours per day above the THI thresholds from 41 to 79 were determined.

Three statistical analyses were conducted to investigate associations between THI and CR. These analyses of CR were conducted regardless of different types of breeding programs and inseminations to estimate the general influence of heat stress on the overall CR of the total herd. In analysis 1, the association between THI at the day of breeding and CR was determined by Pearson´s correlation. The CR for each THI threshold from 41 to 79 was calculated from herd reproductive data. The association of each THI threshold from 41 to 79 and CR was evaluated in a univariable logistic regression model. Odds ratios including 95% confidence intervals (CI95) were reported for all thresholds and significance was set at $P < 0.05$. Furthermore the association between hours above determined THI threshold at the day of breeding and CR was determined by Pearson´s correlation. The CR for each threshold from 1 h to 24 h above a certain THI threshold at the day of breeding was calculated from herd reproductive data. The relation of number of hours above determined THI threshold at the day of breeding and the CR was evaluated in a univariable logistic regression model. Odds ratios, including CI95, were reported for all THI thresholds and significance was set at $P < 0.05$.

In analysis 2, the relationship of 3 different heat load indices and CR was determined and compared. Coefficients of correlation between mean THI (THImean), maximum THI (THImax) and maximum number of hours above a certain threshold of THI (THIhours) at the day of breeding were determined. The relationship of each heat load index and the CR was evaluated in a univariable logistic regression model. Odds ratios, including CI95, were reported for all heat load indices and significance was set at $P < 0.05$.

In analysis 3, the relationships of 7 different periods of heat stress relative to the day of breeding and the CR were determined and compared. Period 1 was determined from d 42 to d 1 before breeding because it is known that the follicle which ovulates at the day of breeding emerges as an antral follicle 42 d before ovulation [30], and the oocyte competence depressed by heat stress is not restored until at least 2 to 3 estrous cycles [31]. Period 2 was determined from d 21 to d 1 before day of breeding because there is evidence that heat stress beginning on d 1 of the previous estrus cycle leads to reduced number of follicles, reduced concentration of estradiol in the follicles and an earlier emergence of the dominant follicle [32,33]. Period 3 was determined from d 2 to d 1 before the day of breeding because the average THI during 2 d prior to breeding was related to CR [34]. Period 4 was determined at the day of breeding because heat stress around the day of breeding was consistently associated with reduced CR [4,23]. Period 5 was determined from d 1 to d 3 after the day of breeding because in a previous study not a single cow that was exposed to heat stress (n = 23) in this period got pregnant [35]. Period 6 was determined from d 1 to d 21 after the day of breeding because implantation of the fetus takes place between d 19 to d 21 after fertilization and the peri-implantation period is critical in the life of the embryo [36]. Period 7 was determined from d 1 to d 31 after the day of breeding because at 31 d of age the fetus has gone through most of the critical events of its development that are susceptible to heat stress [37]. The relation of each period to the CR was evaluated in a univariable logistic regression model. Odds ratios including CI95 were reported for all periods of exposure and significance was set at $P < 0.05$.

For all variables, one class of each variable was considered as the reference and an odds ratio significantly higher (or lower) than 1 for any other class of this variable was indicative of an increased (or decreased) risk for pregnancy when compared to the class used as reference.

3.5 Results

The dataset contained 7,252 breeding records and associated pregnancy diagnoses from 1,707 lactating dairy cows. The overall CR obtained was 31.0%. Minimum, maximum, and mean AT during the study period were 2.3°C, 29.8°C and 16.0 ± 5.5 °C, respectively. Minimum, maximum, and mean RH during the study period were 48.9%, 96.1% and 76.0 ± 8.2%, respectively. Minimum, maximum, and mean THI during the study period were 40.8, 79.9 and 60.4 ± 8.5, respectively. The THI showed seasonal fluctuations with lowest monthly means during cold climate conditions (October to April) and highest monthly means during hot climate conditions (May to September, Figure 1).

3.5.1 Analysis 1

Forty different THI thresholds were determined ranging from THI ≥ 41 to THI ≥ 79. The CR for each THI threshold from 41 to 79 was calculated from herd reproductive data. The greatest CR was observed at a mean daily THI < 41 (30.4%, n = 12) and the lowest at a mean daily THI ≥ 78 (10.5%, n = 15). Overall, coefficient of correlation between THI at the day of breeding and CR was r = -0.49 (n = 7,252 inseminations; P < 0.01). In the univariable logistic regression models, the effect of THI thresholds from THI ≥ 56 to THI ≥ 76 on CR remained significant (Table 1). Odds ratios decreased as THI increased, indicating a negative effect of increasing THI on CR (Table 1).

The threshold for the influence of mean THI at the day of breeding on CR was determined by the relationship described graphically and the B slope resulting from the univariable logistic regression model. An increase in the THI threshold was associated with a continuous decline in the CR. The fitted line between THI threshold and CR was approximately linear above THI threshold of 73 (Figure 2) indicating that the fitted effect of each incremental THI threshold was constant and additive [23]. In the univariable logistic regression B slope started to decrease considerably at the THI threshold of 73 (Table 1). Therefore the THI threshold of 73 was used for further calculations.

Furthermore, 24 different durations of THI ≥ 73 at the day of breeding (i.e. 0 to 24 hr) were considered. The greatest CR (31.90%, n = 6,018) was observed when cows were not exposed to THI ≥ 73 at the day of breeding (i.e. 0 h per day with THI ≥ 73) and the lowest at 17 h per day with THI ≥ 73 (16.17%, n = 235). The coefficient of correlation between h per day with THI ≥ 73 and CR was r = -0.06 (n = 7,252 inseminations; $P < 0.01$). In the univariable logistic regression models, the effect of all durations from 0 to 24 h per day with THI ≥ 73 on CR remained significant (Table 2). Odds ratios decreased with increasing hours per day with THI ≥ 73 indicating a negative effect on CR (Table 2).

The threshold for hours per day with THI ≥ 73 used for further calculations was chosen based on the relationship between CR and hours per day with THI ≥ 73 at the day of breeding. Lower CR were observed for ≥ 9 h per day with THI ≥ 73. The fitted line was approximately linear among this threshold of 9 h per day with THI ≥ 73 (Figure 3), so over this range, the fitted effect of each incremental threshold for hours per day with THI ≥ 73 was constant and additive [23]. In the univariable logistic regression the B slope started to decrease at the threshold of 9 h per day with THI ≥ 73 (Table 2).

3.5.2 Analysis 2

Coefficients of correlation were 0.56, 0.84, and 0.66 between THImean and THImax, between THImean and THIhour, and between THImax and THIhour (n = 7,252; $P < 0.01$). In the univariable logistic regression models, all 3 heat load indices showed a significant effect on CR (Table 3). Cows experiencing a mean THI ≥ 73 at the day of breeding were 39% less likely to get pregnant than those experiencing a mean THI < 73. Cows experiencing a maximal THI ≥ 73 at the day of breeding were 22% less likely to get pregnant than those experiencing a maximal THI < 73. Cows experiencing 9 h or more THI ≥ 73 at the day of breeding were 26% less likely to get pregnant than those experiencing less than 9 h with THI ≥ 73 (n = 7252; $P < 0.01$, Table 3). THImean was chosen as heat load index used for further calculations because it had the lowest odds ratio for the association of CR and mean THI at the day of breeding.

3.5.3 Analysis 3

In the univariable logistic regression models, all 7 periods of heat stress showed a significant effect on CR (Table 4). Cows experiencing a mean THI ≥ 73 during the period from d 21 to d 1 before breeding showed the lowest odds ratio and were 61% less likely to get pregnant than those experiencing a mean THI < 73 during this period. Cows experiencing a mean THI ≥ 73 during the period from d 42 to d 1 after the day of breeding showed the highest odds ratio and were only 31% less likely to get pregnant than those experiencing a mean THI < 73 during this period (Table 4).

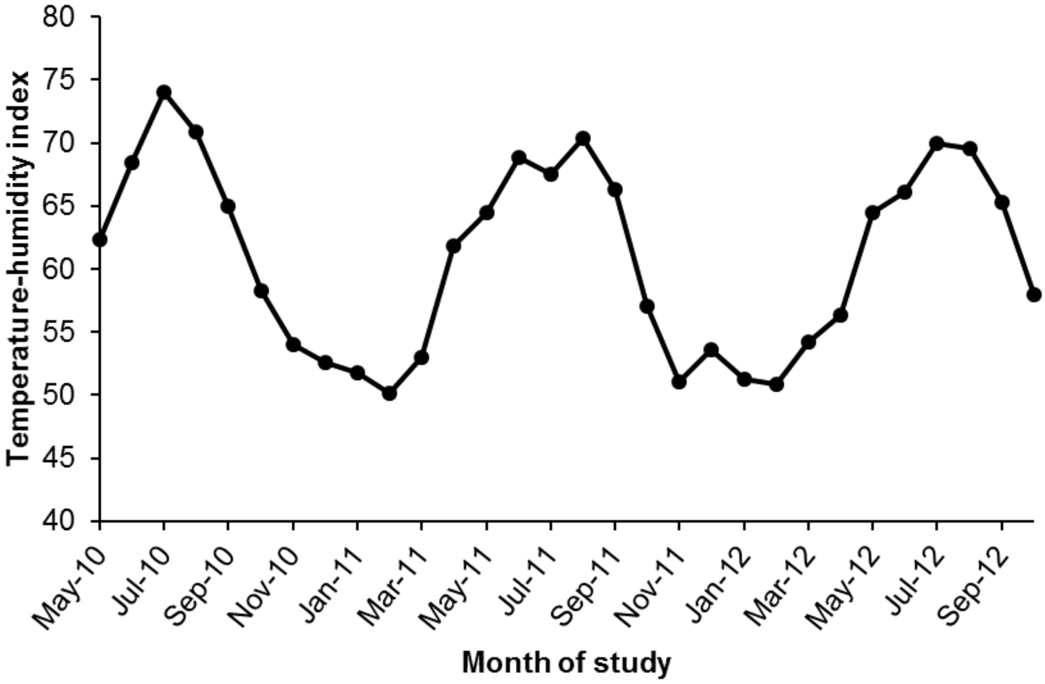

Figure 1. Mean monthly temperature-humidity index in the breeding pen from May 2010 to October 2012 (n = 909 experimental days).

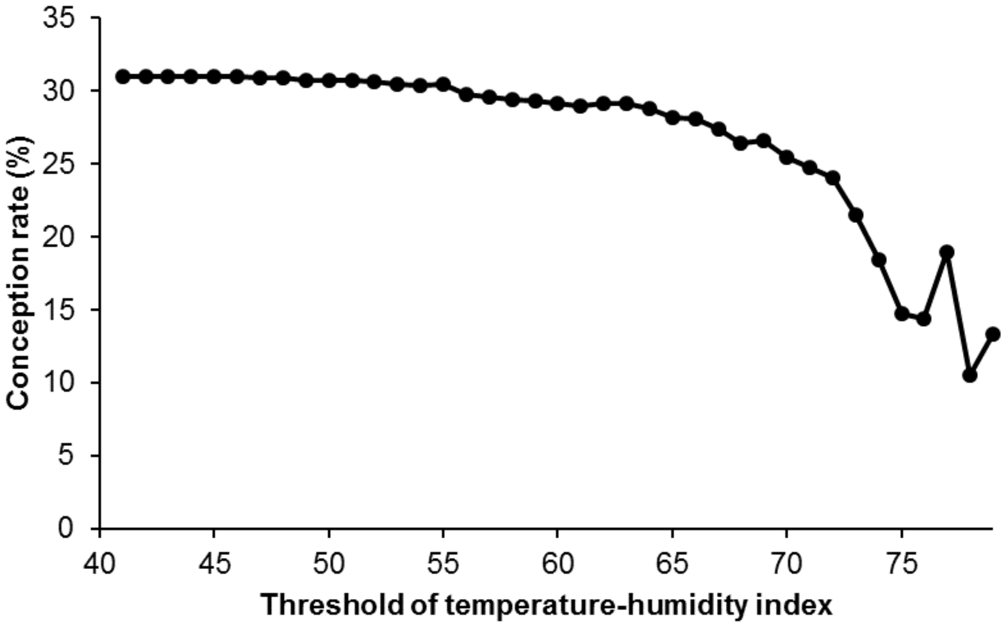

Figure 2. Conception rate of cows (n = 7,252) exposed to heat stress at the day of breeding considering thresholds of temperature-humidity index from 41 to 79.

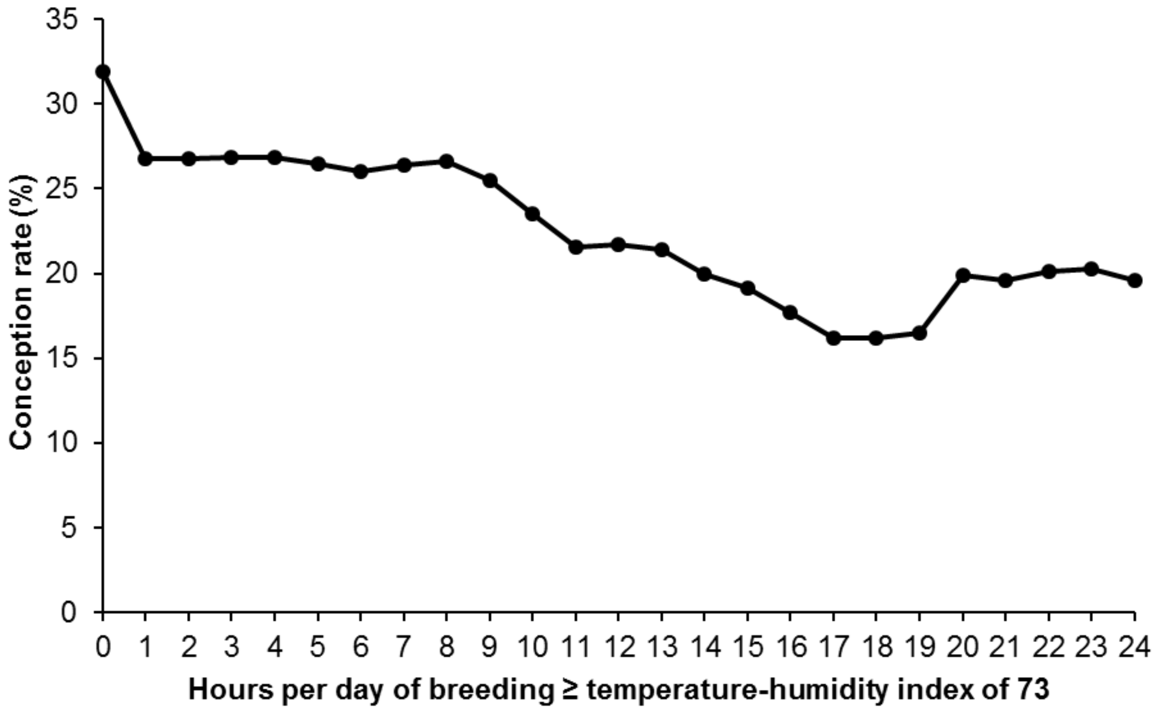

Figure 3. Relationship between conception rate and hours per day of breeding with an temperature-humidity-index ≥ 73.

Table 1. Results of univariable logistic regression models of mean THI thresholds associated with conception rate in lactating dairy cows (n = 7,252 inseminations).

THI threshold	B slope	Odds ratio	95% Confidence interval	n ≥ threshold	*P*	Conception rate (%)
55	-0.08	0.92	0.83-1.02	4837	0.12	30.43
56	-0.15	0.86	0.77-0.95	4547	< 0.01	29.78
57	-0.17	0.84	0.76-0.93	4361	< 0.01	29.58
58	-0.17	0.84	0.76-0.93	4096	< 0.01	29.39
59	-0.17	0.84	0.76-0.93	3863	< 0.01	29.30
60	-0.17	0.84	0.76-0.93	3658	< 0.01	29.17
61	-0.19	0.83	0.75-0.92	3497	< 0.01	28.97
62	-0.17	0.85	0.77-0.94	3361	< 0.01	29.13
63	-0.16	0.85	0.77-0.94	3213	< 0.01	29.13
64	-0.18	0.84	0.75-0.93	2935	< 0.01	28.76
65	-0.21	0.81	0.73-0.90	2599	< 0.01	28.20
66	-0.20	0.82	0.73-0.91	2245	< 0.01	28.06
67	-0.24	0.79	0.70-0.89	1882	< 0.01	27.36
68	-0.28	0.75	0.66-0.85	1603	< 0.01	26.45
69	-0.26	0.77	0.67-0.88	1379	< 0.01	26.61
70	-0.32	0.73	0.63-0.85	1005	< 0.01	25.47
71	-0.35	0.71	0.60-0.84	799	< 0.01	24.78
72	-0.38	0.68	0.56-0.82	666	< 0.01	24.02
73	-0.49	0.61	0.49-0.77	439	< 0.01	21.50
74	-0.72	0.49	0.37-0.65	326	< 0.01	18.40
75	-0.98	0.38	0.26-0.54	230	< 0.01	14.78
76	-1.00	0.36	0.24-0.55	195	< 0.01	14.36
77	-0.66	0.52	0.27-1.00	58	0.05	18.97

Table 2. Results of univariable logistic regression models of maximum hours per day of breeding ≥ THI of 73 associated with conception rate in lactating dairy cows (n = 7,252 inseminations).

Maximal h/d	B slope	Odds Ratio	95% Confidence interval	n	*P*	Conception rate (%)
0	0.25	1.28	1.12-1.47	6018	<0.01	31.90
1	-0.25	0.78	0.68-0.89	1234	<0.01	26.74
2	-0.25	0.78	0.68-0.90	1144	<0.01	26.75
3	-0.25	0.78	0.68-0.90	1070	<0.01	26.82
4	-0.25	0.78	0.68-0.91	995	<0.01	26.83
5	-0.26	0.77	0.66-0.90	940	<0.01	26.49
6	-0.29	0.75	0.64-0.88	896	<0.01	26.00
7	-0.27	0.76	0.65-0.91	773	<0.01	26.39
8	-0.26	0.77	0.65-0.92	684	<0.01	26.61
9	-0.32	0.73	0.60-0.88	601	<0.01	25.46
10	-0.34	0.67	0.55-0.82	556	<0.01	23.52
11	-0.53	0.59	0.46-0.74	445	<0.01	21.57
12	-0.53	0.59	0.46-0.76	392	<0.01	21.68
13	-0.54	0.58	0.45-0.75	374	<0.01	21.39
14	-0.63	0.53	0.40-0.70	330	<0.01	20.00
15	-0.68	0.5	0.37-0.68	293	<0.01	19.11
16	-0.78	0.46	0.33-0.63	271	<0.01	17.71
17	-0.89	0.41	0.29-0.58	235	<0.01	16.17
18	-0.89	0.41	0.28-0.60	204	<0.01	16.18
19	-0.86	0.42	0.29-0.62	188	<0.01	16.49
20	-0.64	0.53	0.35-0.80	146	<0.01	19.86
21	-0.65	0.52	0.34-0.79	143	<0.01	19.58
22	-0.62	0.54	0.35-0.82	139	<0.01	20.14
23	-0.61	0.54	0.35-0.83	133	<0.01	20.30
24	-0.65	0.52	0.32-0.85	102	<0.01	19.61

Table 3. Results of univariable logistic regression models of heat load indices associated with conception rate in lactating dairy cows (n = 7,252 inseminations).

Heat load index	Odds ratio	95% Confidence interval	n	*P*
THImean			7252	< 0.01
< 73		Referent	6813	
≥ 73	0.61	0.49-0.77	439	< 0.01
THImax			7252	< 0.01
< 73		Referent	6018	
≥ 73	0.78	0.68-0.89	1234	< 0.01
THIhour			7252	< 0.01
< 9hr/d		Referent	6651	
≥ 9hr/d	0.74	0.61-0.90	601	< 0.01

3.6 Discussion

The present study examined the effect of heat stress on CR of lactating dairy cows in the moderate climate of the temperate latitude. In this study, CR was strongly dependent on mean daily THI at the day of breeding ($r = -0.49$). Conception rate decreased continuously, beginning at the lowest THI threshold of 41 with each unit increase in mean THI at the day of breeding. These findings indicate that heat stress in lactating dairy cows already occurs at low THI in the moderate climates as evidenced by decreasing CR.

Temperature-humidity-index of 73 was chosen as threshold based on the observed relationship between the mean THI at the day of breeding and the resulting CR which is similar to previous research describing thresholds of 70 [38] and 72 [23], respectively. The negative effect of climate on CR was apparent from THI threshold 41, but the fitted line was approximately linear above THI ≥ 73 at the day of breeding. Over this range the fitted effect of each incremental THI unit was constant and additive, as reported previously [23]. The B slope of the logistic regression model started decreasing clearly from THI threshold 73. The increase in CR at the THI threshold of 77, however, might be related to low number of breedings (n = 58) compared to the other THI thresholds. It has been reported that CR was negatively influenced by average daily THI ≥ 70 in Georgia and Florida [38] and ≥ 75 in Spain [4]. Our results demonstrate that the THI threshold for decreasing CR in the temperate latitudes is comparable to the THI threshold from tropical or subtropical areas.

In the past, studies investigating the influence of heat stress on CR of dairy cows used different THI-formulas to estimate THI thresholds. Temperature-humidity-indices differ in their ability to detect heat stress and different indices are used in different climate areas [39]. One common THI formula to estimate THI should be used as standard, to compare heat stress in dairy cows between different climate conditions and to compare effectiveness of heat abatement strategies from all over the world. Our findings indicate that the THI-formula described by Kendall et al. (2009; $THI = (1.8 \times AT + 32) - ((0.55 - 0.0055 \times RH) \times (1.8 \times AT - 26))$) can be used both in tropical or subtropical areas [4,38] and in the moderate climate to estimate heat stress in dairy cows.

The relationship between CR and the number of hours with THI ≥ 73 at the day of breeding showed that 1 h of exposure to THI ≥ 73 is already sufficient to decrease CR by about 5 percentage points. The fitted line was approximately linear above 9 h with a mean THI ≥ 73 at the day of breeding. Over this range, the fitted effect of each incremental h per day with a mean THI ≥ 73 was constant and additive until 19 h per day [23]. Furthermore the B slope of the logistic regression model started decreasing from 9 h with a mean THI ≥ 73. With a threshold of 20 h with a mean THI ≥ 73 CR decreased about 3 percentage points and remained at this level until 24 h with a mean THI ≥ 73 at the day of breeding. Unlike most of the studies researching heat stress in cattle, our data were collected from moderate climates in the temperate latitudes in Central Europe. Compared to tropical or subtropical areas such geographical regions experience discriminative seasons with high variation of climate conditions between winter and summer months. Interestingly, cows which were acclimated to cold climate conditions showed higher reactions (heat production, respiration rate) to heat stress than cows which were acclimated to warmer climate conditions [40]. In the present study we observed considerable fluctuation in AT and THI in relative short periods which may be related to decreasing CR up to 19 h per day with a mean THI ≥ 73. We assume that on d with more than 20 h with a mean THI ≥ 73 only minor fluctuations in THI occur. This observation demonstrates that dairy cows in the moderate climates can be more severely affected by heat stress which is often fluctuating, than cows in tropical or subtropical climates which are exposed to a constant climate. Also, it highlights the importance of using heat abatement strategies in moderate climates, avoiding fluctuations in heat stress in cows that are less acclimated. Further research is warranted to quantify the effects of such climate fluctuations in the subtropical and the moderate climates.

To assess the effects of heat stress on CR in lactating dairy cows, maximum THI [37], mean THI [36] and total h above a certain THI threshold [23] have been used as heat load indices. Morton [23] compared daily maximum THI with total h above the THI threshold of 72 from a data set generated in Australia from 25,505 breedings and suggested total h above the THI threshold of 72 as the most useful heat load index. Both heat load indices were strongly correlated (r = 0.92). The authors concluded that further work is required in other regions where they might not be so closely correlated [23]. In our study mean daily THI ≥ 73, maximum daily THI ≥ 73 and hours above a threshold of 9 h per day at the day of breeding were compared but coefficients of correlation (r = 0.56, 0.66, 0.84) were lower. In contrast to Morton [23], the climate data in our study was obtained in the moderate climates of the temperate latitudes. In the moderate climates high daily fluctuations of climate conditions are observed during day and night, which results in lower correlations of head load indices. Furthermore, the climate conditions were measured onsite. The climate conditions inside the barn are dependent on different factors (e.g. stocking rate, cleaning processes) which may lead to short time fluctuations also affecting correlations. Based on the odds ratios, the strongest decrease in CR was observed for mean daily THI ≥ 73 at the day of breeding. These results indicate that the mean THI is the most sensitive heat load index relating to CR in lactating dairy cows in the moderate climates. Furthermore, our results confirm previous findings that effects of THI on CR are more likely to be mediated through prolonged periods of time by a mean THI than by a high daily maximum THI [23].

When the average THI in the period from d 42 before to d 31 after the day of breeding was ≥ 73, CR decreased significantly for all 7 analyzed periods of heat stress. Our findings indicate that CR of lactating dairy cows is negatively affected by heat stress both before and after the day of service. These results are in line with previous research that identified negative effects of heat stress on CR for the whole interval from d 42 before to d 40 after breeding [25]. In our study, the period from d 21 to d 1 before service was most sensitive to heat stress. When the mean THI was ≥ 73 in this period, CR decreased to 12%. It is well documented, that heat stress beginning on d 1 of the estrus cycle leads to a reduced number of follicles, a reduced concentration of estradiol in follicles [32,33] and to a reduced growth [41] and incomplete dominance of the dominant follicle [32]. Incomplete dominance can result in ovulation of aged oocytes which may be responsible for significant reduced pregnancy rates [42]. The period from d 1 to d 21 after the day of breeding also appears particularly important. When the mean THI was ≥ 73 in this period, CR decreased to 16%. Indeed, the decrease in CR was higher in the periods of 3 weeks before and after the day of breeding compared to the periods 2 d before and 3 d after the day of breeding. These findings suggest that when heat stress is prevalent over prolonged periods the CR is more negatively affected than when heat stress occurs only few days around breeding [23]. Also, it was observed herein that the effects of THI on CR are more likely to be mediated through prolonged periods of time above the THI threshold than by a high daily maximum THI, as showed previously [23].

In previous studies the peri-implantation period was identified as a period of significant decrease in CR due to increased fetal loss [36] because the embryo and the endometrial stroma undergo dramatic morphological changes [43]. Changes in the uterine environment and the endometrial and luteal prostaglandin secretion due to heat stress inhibit the embryonic development and increase early embryonic loss [13,18]. Although previous studies demonstrated that older embryos appear to be more tolerant to heat stress than the 2-cell embryos soon after conception [44], in our study the period 21 d after the day of breeding was more sensitive to heat stress than the period 3 d after the day of breeding. The maximum THI during the study period was 79.9 and did not reach heat stress level of 82.3 and 85 that have been described for tropical or subtropical areas [39,45]. Our results suggest that heat stress in the moderate climates influences embryo mortality particularly over prolonged periods of heat stress rather than on single days of heat stress, although there is a strong negative impact of heat stress on CR in the period 3 d after the day of breeding. We observed that even after the period of embryo initial development, the heat stress remains deleterious for the CR. In our study the climate conditions were measured onsite while in previous studies climate data were obtained from meteorological stations in the vicinity of the study site. Inside the barn microclimates are generated through different factors (e.g. cleaning processes, building insulation) [24] which might cause moderate heat stress over prolonged periods.

Further research is necessary to investigate the effect of short and long term fluctuations of heat stress on the conception rate of dairy cattle. Furthermore, THI thresholds for different effects of heat stress on the reproductive performance of dairy cattle should be compared between tropical or subtropical climates and the moderate climates.

3.7 Conclusions

Conception rate of lactating dairy cows in the moderate climate was highly affected by heat stress. The THI of 73 was the most likely threshold for the influence of heat stress on CR based on the observed relationship between the mean THI at the day of breeding and the resulting CR. Negative effects of heat stress, however, were apparent already at lower levels of THI and 1 h of mean THI ≥ 73 decreased CR significantly. This indicates that dairy cows in the moderate climates might be more affected by heat stress than cows acclimatized to tropical or subtropical climates which are exposed to a constant heat stress. The CR of lactating dairy cows was negatively affected by heat stress both before and after the day of breeding. The period from d 21 to d 1 before the day of breeding was most sensitive to heat stress. Thus, heat abatement strategies should maintain low and constant THI to avoid fluctuations in heat stress over a period of at least 3 weeks before to 3 weeks after the day of service.

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4 ADDITIONAL UNPUBLISHED WORK

Evaluation of natural and artificial breeding programs affecting conception rates of dairy cows under short and long term heat stress

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4.1 Abstract

The objectives of this retrospective study were to examine the effect of heat stress on natural service and artificial insemination (AI) breeding programs. We investigated the influence of short and long term heat stress on conception rate (CR) of dairy cows under breeding at natural estrus and breeding at timed AI after 2 different Ovsynch programs. In addition, relationship among heat stress, parity and type of semen were determined. Cows experiencing short (mean temperature-humidity index (THI) ≥ 73 at the day of breeding) and long (mean THI ≥ 73 in the period 3 weeks before breeding) term heat stress were 37% and 65% less likely to get pregnant compared to cows not experiencing heat stress. Cows treated with an Ovsynch or modified Ovsynch program were 20% and 36% less likely to get pregnant, respectively, than cows inseminated at natural estrus. Cows inseminated with frozen-thawed or fresh extended sperm were 32% and 31% less likely to get pregnant, respectively, than cows bred by natural service. Multiparous cows in the third lactation or higher were 27% less likely to get pregnant than primiparous cows. The present study indicate, that climate conditions should be considered to optimize breeding programs and resulting CR. Our findings suggest that synchronization programs decrease CR in addition to heat stress and can therefore not generally be recommended to compensate low summer fertility during periods of heat stress in the moderate climate. Particularly under periods of long term heat stress, breeding at natural estrus instead of Ovsynch program can be an approach to avoid a major decrease in resulting CR.

4.2 Key words

heat stress, conception rate, artificial insemination, ovsynch, natural service, dairy cow

4.3 Introduction

Reproductive efficiency is one of the key components of a profitable dairy system (Rabiee et al., 2005) and heat stress drastically reduces reproductive performance in lactating dairy cows (Wolfenson et al., 2000). Heat stress can have major effects on fertility and embryonic survival in lactating dairy cows (Hansen and Arechiga, 1999). These include compromised endometrial function and secretory activity, smaller follicular size and suppressed dominance of the large follicle (Wolfenson et al., 2000). Disturbances in hormonal balance include decreased serum estradiol concentration (Wilson et al., 1998a, Wilson et al., 1998b), decreased plasma concentration of LH and decreased progesterone secretion (Wolfenson et al., 2000). Furthermore, oocyte quality, embryo development (Wolfenson et al., 2000, De Rensis and Scaramuzzi, 2003) and embryo mortality (Wolfenson et al., 2000) are negatively affected by heat stress. These processes lead to a decrease in conception rate (CR) during the hot season ranging between 20 and 30% compared to the winter season (De Rensis et al., 2002). Even in the moderate climates of the temperate latitude CR declined from 31% during cold climate conditions to less than 13% during periods of heat stress (Schüller et al., 2014). Due to these effects heat stress causes important economic losses to the dairy industry during summer months (Collier et al., 2006).

As a result of impaired reproductive performance and less efficient detection of estrus during heat stress, timed artificial insemination (AI) breeding programs have become popular because of their predictability and the likelihood of producing a greater proportion of total pregnancies in the herd (Stevenson, 2005). The primary benefits of timed AI breeding programs include decreased days to first insemination, day-to-day convenience in managing the breeding herd and efficient use of labor for detection of estrus and AI (Stevenson, 2005). The Ovsynch program is probably the most popular breeding program employed (Stevenson, 2005).

The use of timed AI breeding programs have been considered as a profitable alternative for managing dairy herds when estrus detection rates are abysmal during summer months (Jobst et al., 2000), however, this effect differed between reports (De Rensis et al., 2002, Stevenson, 2005). Studies comparing breeding programs with timed AI after synchronization of estrus or AI at natural estrus indicate mixed results on CR (Chebel et al., 2004). In a meta-analysis (Rabiee et al., 2005) based on 71 Ovsynch treatment and control comparisons it was shown, that there is little or no significant improvement in pregnancy rates using the Ovsynch program over other breeding programs. Furthermore, the authors pointed out that the costs of labor and hormone administration should be considered when selecting an Ovsynch program for routine use. Irrespective of this observations, heat stress has led to increased use of timed AI during summer months to improve reproductive performance (Collier et al., 2006) because management limitations induced by heat stress on estrus detection are eliminated (De la Sota et al., 1998, DeJarnette et al., 2001). However, there is a dearth of information on comparisons of timed AI programs with conventional farm programs of reproductive management during heat stress (Burke et al., 1996) and a lack of controlled field data evaluating reproductive performance of herds that implement such breeding programs (Jobst et al., 2000). Furthermore, reproductive efficiency in dairy cows is decreasing worldwide. Therefore, methods for timed insemination of dairy cattle still need to be optimized (Lucy, 2001).

The objectives of the study were to examine the effect of heat stress on natural and AI breeding programs. Specifically we investigated the relationship between different breeding programs and resulting CR and determined the influence of short and long term exposure to heat stress on the CR of lactating dairy cows in different breeding programs. In addition, the relationship among heat stress, lactation number and type of sperm is discussed.

4.4 Materials and methods

4.4.1 Design of the barn

The retrospective study was conducted on a commercial dairy farm in Sachsen-Anhalt, Germany from May 2010 to October 2012. The herd consisted of 1,150 Holstein dairy cows with an average milk production of 10,345 kg (4.0% fat, 3.3% protein). The barn was positioned in a NE-SW orientation (51° 77´ N, 12° 91´ E) with open ventilation and a mechanical fan-system. Sixty fans were installed above the stalls and controlled manually by the farm manager. All cows were housed in a free-stall facility with slatted floors and beds equipped with rubber mats. Group composition was dynamic with cows entering and leaving the experiment depending on their calving dates. Cows were fed a TMR. The rations were formulated to meet or exceed the requirements of the NRC (2001). Lactating cows were milked 3 times a day at starting at 6.00, 14.00 and 22.00.

4.4.2 Reproductive management

The voluntary waiting period was set at d 49 postpartum. Between d 35 and 49 postpartum cows received an initial injection of 25 mg PGF2α (Dinoprost, Dinolytic, Zoetis Deutschland GmbH, Berlin, Germany) and a second injection of 25 mg PGF2α 2 weeks later to regress the corpora lutea. Cows that showed estrus after the second injection of PGF2α were artificially inseminated. Pedometers (Milkline, Gariga di Podenzano, Italy) and visual observation were used to detect estrus. Cows that did not show estrus after the second injection of PGF2α within 3 d were treated with an Ovsynch program starting 12 d later. On this day, treated cows received an initial injection of 100 µg of GnRH (Gonadorelin, Gonavet Veyx, Veyx-Pharma GmbH; Schwarzenborn, Germany). Seven d later at the same time, cows received 25 mg PGF2α to regress the corpora lutea and 48 h later, a second injection of 100 µg of GnRH to induce ovulation of the dominant follicle. The AI was performed 16 to 17 h after the second injection of GnRH. Cows that showed estrus after the first injection of GnRH of the Ovsynch program were inseminated prematurely. Cows with a corpus luteum at the time of rectal examination (i.e. after the second injection of PGF2α) were treated with a modified Ovsynch program. Cows received 25 mg PGF2α to regress the corpora lutea and 48 h later an injection of 100 µg of GnRH to induce ovulation of the dominant follicle. The AI was performed 16 to 17 h after the injection of GnRH.

Inseminations at detected estrus were performed by 7 technicians within 12 h after detection of estrus or at designated time for the timed insemination with frozen-thawed or fresh extended sperm from bulls (n = 219) used randomly across treatment. Cows after caesarean section, with adhesions of the uterus and repeat breeder cows in third or higher lactation received natural service by a bull. Pregnancy diagnoses were performed 35 to 42 d after the day of breeding with transrectal ultrasonography by the herd veterinarian. Cows with unclear pregnancy diagnosis were reexamined with transrectal ultrasonography 1 week later. Cows that were not pregnant were reassigned to an Ovsynch program (e.g. standard or modified) based on the presence of a corpus luteum as described above. Cows that were observed in estrus > 10 d after AI were assumed nonpregnant and re-inseminated.

4.4.3 Data collection

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 3 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 and relative humidity data were used to calculate the temperature-humidity index (THI) according to the equation reported by Kendall et al. (2009): THI = (1.8 x AT + 32) - ((0.55 - 0.0055 x RH) x (1.8 x AT - 26)).

Cow ID, parity, service dates, breeding program, type of sperm, number of services and results of pregnancy diagnosis were obtained from computerized herd records managed using Herde (Version 5.5, Software Projektierungs- und Handels GmbH, Aschare, Germany).

4.4.4 Statistical analyses

Data from the onsite climate logger and from Herde were exported into Excel spreadsheets (Office 2010, Microsoft Deutschland GmbH, Munich, Germany) and statistical analysis was performed using SPSS for Windows (Version 19.0, SPSS Inc., IBM, Ehningen, Germany). Daily mean ambient temperature, relative humidity and resulting THI were calculated from hourly measures. Months with minimal 1 day with a mean THI ≥ 73 were determined as hot months. This threshold for the influence of heat stress at the day of breeding on CR had been determined in a previous study based on the same dataset (Schüller et al., 2014). Short term heat stress was defined as a mean THI ≥ 73 at the day of breeding because heat stress around the day of breeding was consistently associated with reduced CR (García-Ispierto et al., 2007, Morton et al., 2007). Long term heat stress was defined as a mean THI ≥

73 for the period from d 21 to d 1 before day of breeding because heat stress beginning on d 1 of the previous estrus cycle leads to a reduced number of follicles, a reduced concentration of estradiol in follicles and to an earlier emergence of the dominant follicle (Wolfenson et al., 1995, Wolfenson et al., 1997). In a study that compared the impact of heat stress on CR between 7 different periods around the day of breeding, heat stress in the period 3 wk before breeding had the greatest negative influence on CR (Schüller et al., 2014). Descriptive statistics of continuous variables were presented by median and range. For categorical data, frequencies of occurrence were presented. Conception rate was defined as the number of pregnant cows divided by the number of total services multiplied by 100 (Schefers et al., 2010).

In analysis 1, the association between short term heat stress (THI < 73, THI ≥ 73), parity (1., 2., ≥ 3. lactation), breeding program (natural estrus, Ovsynch, modified Ovsynch), type of sperm (AI with frozen-thawed sperm, AI with fresh extended sperm, natural service), number of services (1, 2, 3, ≥ 4 services) and CR was determined in a multivariable logistic regression model. Furthermore, the interactions between short term heat stress and each variable was determined. The model was constructed in a backward stepwise manner with variables being continuously removed from the model by the Wald statistic criterion if the significance was greater than 0.20. Odds ratios, including 95% CI were reported for all one way interactions and significance was set at P < 0.05. The multivariable logistic regression model was calculated as follows:

$$\ln\left(\frac{1}{1-\mathrm{P}}\right) = \propto + \beta_1\,\chi_1 + \beta_2\,\chi_2 + \cdots + \beta_{15}\,\chi_{15}$$

where P is the probability of conception, α is constant, χ_1 is primiparous (0, no | 1, yes), χ_2 is 1. lactation (0 | 1), χ_3 is ≥ 3. lactation (0 | 1), χ_4 is natural estrus (0 | 1), χ_5 is Ovsynch (0 | 1), χ_6 is modified Ovsynch (0 | 1), χ_7 is covered by the bull (0 | 1), χ_8 is AI with frozen-thawed sperm (0 | 1), χ_9 is AI with fresh extended sperm (0 | 1), χ_{10} is 1 service (0 | 1), χ_{11} is 2 services (0 | 1), χ_{12} is 3 services (0 | 1), χ_{13} is ≥ 4 services (0 | 1), χ_{14} is short term heat stress (0, THI < 73 | 1, THI ≥ 73), and χ_{15} is long term heat stress (0, THI < 73 | 1, THI ≥ 73).

In analysis 2, the association between long term heat stress (THI < 73, THI ≥ 73), parity, breeding program, type of sperm, number of services and CR was determined in a multivariable logistic regression model. Furthermore, the interactions between long term heat stress and each variable was determined. The model was constructed in a backward stepwise manner with variables being continuously removed from the model by the Wald statistic criterion if the significance was greater than 0.20. Odds ratios, including 95% CI were reported for all interactions and significance was set at P < 0.05.

For all variables, one class of each variable was considered as the reference and an odds ratio significantly higher (or lower) than 1 for any other class of this variable was indicative of an increased (or reduced) risk for conception when compared to the class used as reference.

4.5 Results

The dataset contained 7,252 breeding records from 1,707 lactating dairy cows on a single dairy farm. The overall CR obtained was 31.0%. Synchronization by an Ovsynch program was conducted in 19.1% of the breedings, 8% were synchronized by a modified Ovsynch program and 72.9% of the inseminations were conducted on a natural estrus. Inseminations with frozen-thawed and fresh extended sperm were conducted in 76.2% and 13.7%, of the cows, respectively and 10.1% of the cows were bred by natural service. The population of cows enrolled in the study consisted of 32.9% primiparous cows, 31.0% cows in the second lactation, and 36.1% cows in the third or higher lactation. Frequency distribution of number of inseminations needed to establish a pregnancy was 36.5% (1 insemination), 24.8% (2 inseminations), 16.1% (3 inseminations) and 22.7% (≥ 4 inseminations).

Descriptive statistics for the climate variables are depicted in Table 1. The THI showed seasonal fluctuations with lowest monthly means during cold climate conditions (October to February) and highest monthly means during hot climate conditions (May to August, Figure 1). Number of days with an average THI ≥ 73 was 57 (6.2%) of the 913 experimental days.

4.5.1 Analysis 1

In the multivariable logistic regression model the variables short term heat stress, breeding program, type of sperm and parity remained significant (Table 2). No interactions between short term heat stress and the variables breeding program, type of sperm and parity remained significant. Number of services had no significant association to CR ($P = 0.60$) and therefore was not included in the multivariable models. Cows experiencing short term heat stress were 37% less likely to get pregnant than those not exposed to short term heat stress.

4.5.2 Analysis 2

In the multivariable logistic regression model the variables long term heat stress, breeding program, type of sperm and parity remained significant (Table 3). No interactions between long term heat stress and the variables breeding program, type of sperm and parity remained significant. Number of services had no significant association to CR ($P = 0.60$) and therefore was not included in the multivariable models. Cows experiencing long term heat stress were 65% less likely to get pregnant than those experiencing no long term heat stress.

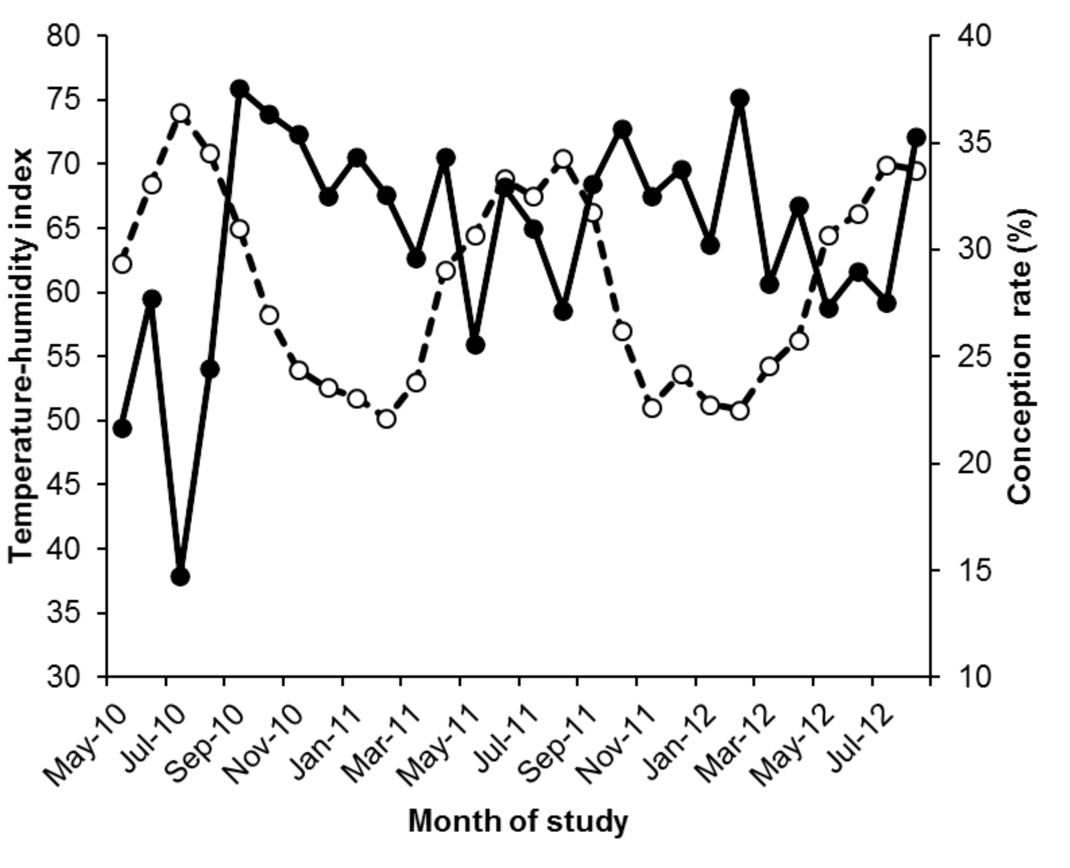

Figure 1. Monthly means of temperature-humidity index (- - -) and conception rate (―) during 913 experimental days.

Table 1. Mean, maximum and minimum ambient temperature, relative humidity and temperature-humidity index in the barn from April 2010 to November 2012 (n = 913 experimental days).

Climate variable	Mean ± SD	Maximum	Minimum
Ambient temperature (°C)	16.0 ± 5.5	29.8	2.3
Relative humidity (%)	76.0 ± 8.2	95.8	48.9
Temperature-humidity index	60.4 ± 8.5	79.9	40.8

Table 2. Results of multivariable logistic regression model of factors associated with conception rate in dairy cows under short term heat stress (average THI ≥ 73 at the day of breeding).

Variable	Odds ratio	Confidence interval (95%)	n	*P*
Breeding program			7252	< 0.05
Natural estrus	Referent		5293	
Ovsynch	0.80	0.69 - 0.92	1372	< 0.05
Modified Ovsynch	0.64	0.51 - 0.79	587	< 0.05
Type of sperm			7252	< 0.05
Bull	Referent		729	
AI frozen-thawed	0.68	0.58 - 0.80	5529	< 0.05
AI fresh extended	0.69	0.55 - 0.86	994	< 0.05
Parity			7252	< 0.05
1.	Referent		2387	
2.	0.84	0.74 - 0.95	2252	< 0.05
≥ 3.	0.73	0.65 - 0.83	2613	< 0.05
Short term heat stress			7252	< 0.05
THI < 73	Referent		6813	
THI ≥ 73	0.63	0.50- 0.79	439	< 0.05

Table 3. Results of multivariable logistic regression model of factors associated with conception rate in dairy cows under long term heat stress (average THI ≥ 73 in the period 3 weeks before breeding).

Variable	Odds ratio	Confidence interval (95%)	n	*P*
Breeding program			7252	< 0.05
Natural estrus	Referent		5293	
Ovsynch	0.80	0.70 - 0.92	1372	< 0.05
Modified Ovsynch	0.63	0.51 - 0.78	587	< 0.05
Type of sperm			7252	< 0.05
Bull	Referent		729	
AI frozen-thawed	0.68	0.58 - 0.79	5529	< 0.05
AI fresh extended	0.68	0.54 - 0.85	994	< 0.05
Parity			7252	< 0.05
1.	Referent		2387	
2.	0.84	0.74 - 0.95	2252	< 0.05
≥ 3.	0.73	0.64 - 0.83	2613	< 0.05
Long term heat stress			7252	< 0.05
THI < 73	Referent		6936	
THI ≥ 73	0.35	0.23- 0.53	179	< 0.05

4.6 Discussion

The present study examined the effects of short and long term heat stress on CR of dairy cows considering natural and artificial breeding programs. In this study, cows experiencing short and long term heat stress were 37% and 65% less likely to get pregnant, respectively, than cows not exposed to heat stress (Tables 2 and 3). These findings indicate, that the negative impact on CR is higher for long term heat stress than for short term heat stress. These results are in agreement with previous studies demonstrating that a reduction in CR under heat stress occurs due to the combined effects of environmental heat over prolonged periods rather than on single days around the day of breeding (Wolfenson et al., 2000, Morton et al., 2007, Schüller et al., 2014). Although these results indicate that the negative impact of heat stress on CR is higher for long term heat stress than for short term heat stress, it is obvious that even heat stress only on the day of breeding has a considerable negative influence on CR. To consider these observations a distinction was made between short and long term heat stress in the recent study.

Timed AI breeding programs are a proactive response to optimize reproductive performance of dairy herds during periods of heat stress. The primary benefits of timed AI breeding programs include decreased days to first AI, day-to-day convenience in managing the breeding herd and efficient use of labor for AI (LeBlanc et al., 1998, Tenhagen et al., 2004b, Stevenson, 2005). Further benefit occurs because the need of estrus detection is eliminated (Hansen and Arechiga, 1999, Bucher et al., 2009) which compensates a decrease in estrus detection rate under heat stress (Orihuela, 2000, Peralta et al., 2005). Synchronization programs that allow for timed AI usually increase pregnancy rates in herds with low rates of detected estrus, because all eligible cows are inseminated but CR are either similar to or lower than those of cows inseminated based upon natural estrus (Cerri et al., 2004). In the Ovsynch program all cows are inseminated which drives the AI submission rate to 100% and the CR becomes the limiting factor (Cartmill et al., 2001). In our study, cows bred by timed AI after Ovsynch program were 20% less likely to get pregnant than cows inseminated at natural estrus (Table 2). These results are consistent with previous work in which the CR after Ovsynch program was similar (Pursley et al., 1997, Chebel et al., 2004) or lower (Jobst et al., 2000) than CR after natural estrus. A meta-analysis demonstrated, that the conception and pregnancy rates obtained with the Ovsynch program and breeding at natural estrus did not differ (Rabiee et al., 2005). It remains speculative if our lower results of CR obtained with the Ovsynch program were caused by a less competent corpus luteum and consequently an increased prevalence of short estrous cycles (Macmillan et al., 2003). Furthermore, cows subjected to timed AI programs experience lower CR because follicles of differing maturity can be induced to ovulate, resulting in a less competent corpus luteum and consequently a slower rise in plasma progesterone and lower midluteal concentrations (Macmillan et al., 2003, Wiltbank et al., 2011).

Cows bred by timed AI after Ovsynch program were more likely to get pregnant than cows bred by timed AI after modified Ovsynch program (Table 2 and 3). Our results suggest that the Ovsynch program is more suitable for synchronization of dairy cows than the modified Ovsynch program. Previous studies demonstrated, that a PGF2α injection in early or mid diestrus leads to an incomplete luteolysis because the early corpus luteum is nonresponsive to a single injection of PGF2α (Moreira et al., 2000, Colazo et al., 2013). The corpus luteum in these cows failed to respond to PGF2α and, as a consequence, ovulation is not synchronized with timed AI after modified Ovsynch program (Moreira et al., 2000). In our study, only cows with a corpus luteum, diagnosed by rectal examination, were treated with a modified Ovsynch program. Rectal diagnosis of corpora lutea is error prone (Kelton et al., 1991) and cows in early or mid diestrus cannot be differentiated from cows in late diestrus. These findings suggest that the modified Ovsynch program is confounded by the diagnostic errors and susceptible for incomplete synchronization of ovulation and therefore should not be used.

It has been argued that estrus detection in dairy herds can be eliminated by using synchronization (Rabiee et al., 2005, Bucher et al., 2009). Using an automated activity monitoring system for estrus detection in combination with visual observation lead to estrus detection rates of 56.3% in the first 21 d after the voluntary waiting period (Michaelis et al., 2014). Furthermore, CR between cows inseminated with an automated activity monitoring system or timed AI did not differ (conception risk of 31% and 30% respectively) and the time to pregnancy was shorter with automated activity monitoring system compared to timed AI (Neves et al., 2012). While manufacturer of automated activity monitoring systems claim high estrus detection rates even under effects that influence the cow movements because of individual threshold monitoring (Milkline, 2014) science-based information is not available. Further research is necessary to evaluate such estrus detection systems under heat stress conditions as an alternative to synchronization programs because hormone use is a critical issue (Refsdal, 2000). Consumers have a growing interest in animal health and animal welfare issues as well as ethical concerns regarding the use of hormones and antibiotics (Refsdal, 2000). Endogenous hormones and hormones induced by synchronization programs in dairy livestock can enter the environment by excretions from the farm animals (Tomlinson et al., 1985, Johnson et al., 2006) and lead to ecological and health problems in humans and animals (Vos et al., 2000, Lange et al., 2002). Therefore, a hormone use with low efficacy (i.e. under periods of heat stress) should be avoided.

In the present study, cows inseminated with frozen-thawed or fresh extended sperm were 32% and 31% less likely to get pregnant, respectively, than cows bred by natural service

(Table 2 and 3). These effects are consistent with a previous study that demonstrated an increase in cumulative pregnancy rate by 9.4 percentage points (Lima et al., 2009) compared to timed AI, respectively. There is evidence, that the use of natural service increases herd reproductive performance because bulls are better at detecting estrus than humans and errors in estrous detection are avoided (Vishwanath, 2003, Lima et al., 2009). Although, in our study, the group of cows that were bred by natural service consisted of cows after caesarean section, with adhesions of the uterus and repeat breeder cows in third or higher lactation, CR was higher than for cows that received AI. These findings are in contrast to Overton and Sischo (2005) who found that cows with difficulties to get pregnant that were bred by natural service had lower reproductive performance than cows without difficulties to get pregnant that were bred by AI. Further research is needed to identify the most effective breeding program for cows where a low reproductive performance can be expected. In our study, natural service at natural estrus was detected as the most effective breeding program. We emphasize that this benefit should be weighed against considerable risks associated with natural service such as occupational safety, omission of gain in genetic merit, decreased predictability of time of birth, birth weight and infertility in the dominant bull (Vishwanath, 2003, Lima et al., 2010).

In our study, cows inseminated with frozen-thawed sperm had almost the same likelihood to get pregnant as cows inseminated with fresh extended sperm (Table 2 and 3). This finding is in line with previous studies describing comparable CR for frozen-thawed and fresh extended sperm considering current sperm processing techniques (Buckley et al., 2004, Bucher et al., 2009).

In our study, the variable number of services had no significant association with CR which is in contrast to recent publications that report an inverse relationship for number of inseminations to conception rate (González-Recio et al., 2005, Weller et al., 2006). We determined a significant association ($r = 0.33$, $P < 0.01$) between the number of services and type of breeding. With increasing number of insemination (1, 2, 3 or ≥ 4 inseminations) proportion of cows bred by timed AI after Ovsynch program decreased from 42.1% to 1.4% whereas the proportion of cows bred by natural service increased from 56.6% to 92.8%. We suspect the negative impact of increasing number of services on CR was offset by the inverse use of breeding programs with increasing number of services.

We observed, that multiparous cows in the second and third lactation were 16% and 27% less likely to get pregnant, respectively, than primiparous cows (Table 2 and 3). This observation agrees with previous reports (Chebel et al., 2004, Tenhagen et al., 2004a, Astiz and Fargas, 2013). A possible reason for better fertility in primiparous cows is the reduced risk of metabolic disorders in early lactation impairing reproductive efficiency (Tenhagen et al., 2004a).

Interestingly, in our study, there were no significant interactions between short or long term heat stress and the variables parity, breeding program, and type of sperm. These findings indicate, that the relationships between CR and the tested variables did not differ between periods with and without heat stress. However, overall likelihood of CR is cumulatively calculated out of the likelihoods of CR of each variable calculated in the logistic regression model. Therefore, the negative impact of short and long term heat stress on CR is added to the likelihood of each tested variable. Hence, multiparous cows in the third or higher lactation that were bred by timed AI after modified Ovsynch program under long term heat stress are expected to have lowest CR. These findings support our hypothesis that climate conditions should be considered to optimize breeding programs and resulting CR on dairy farms. It is noteworthy, that synchronization programs decreased CR in addition to heat stress and can therefore not generally be recommended to compensate low summer fertility during periods of heat stress in the moderate climate as proposed (Wolfenson et al., 2000, De Rensis et al., 2002). In a study of Cartmill et al. (2001) CR and rates of embryo survival decreased as THI increased particularly after an Ovsynch program. Although Ovsynch improved initial pregnancy rates during periods of heat stress, greater embryonic death after d 27 to d 30 negated most of that advantage compared with cows inseminated after detection of natural estrus (Cartmill et al., 2001). Particularly, under periods of long term heat stress, breeding at natural estrus instead of breeding by timed AI after Ovsynch program could be an approach to avoid a major decrease in resulting CR.

In the dairy industry, AI upon natural estrus is still the most prevalent method to breed cows for first and subsequent services (Overton and Sischo, 2005). Nevertheless, in our study cows that received AI under short and long term heat stress were 32% less likely to get pregnant than cows bred by natural service. Our findings are in line with previous studies, that reported lower pregnancy rates for AI breeding herds (8.1%) than for natural service herds (9.8%) and combinated breeding herds (9.3%) during periods of heat stress (de Vries et al., 2005). Our findings indicate that especially for problem and repeat breeder cows and for cows exposed to long term heat stress, higher CR can be expected with breeding at natural service.

A most recent study conducted in Spain and including 7,805 cows supports our findings, that heat stress significantly reduces CR for both, primiparous and multiparous cows (Astiz and Fargas, 2013). Therefore, we propose to re-consider breeding programs based on timed AI especially for multiparous cows in the third or higher lactation. With our data set submission rates could not be determined and survival curves comparing different breeding programs under periods of heat stress could not be evaluated. Therefore further research is warranted to customize breeding programs to certain climate conditions considering pregnancy and economic outcomes and to determine if suspending breeding for certain periods of heat stress and subpopulations of cows might be advantageous.

4.7 Conclusions

Our data indicate that even under short and long term heat stress, breeding at natural estrus was the most effective breeding program, compared to an Ovsynch or modified Ovsynch program. Our findings provide evidence that breeding by timed AI after Ovsynch program decrease CR in addition to heat stress. Therefore it can not generally be recommended to compensate low summer fertility during periods of heat stress in the moderate climate. Particularly, under periods of long term heat stress, breeding at natural estrus instead of Ovsynch program can be an approach to avoid a major decrease in resulting CR.

4.8 Acknowledgments

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5 DISCUSSION

Heat stress in dairy cows is an issue of increasing importance because the tolerance of heat stress decreases with increasing milk yield (Kadzere et al., 2002, West, 2003) and a further increase in milk yield can be expected (Hansen, 2000, Van Arendonk and Liinamo, 2003). Compared to the tropical or subtropical areas, not much is known about the incidence of heat stress in the moderate climate and the impact on reproductive performance of dairy cows. Therefore, the overall objectives of this work were to determine the incidence of heat stress in dairy livestock in the moderate climate and to examine the impact of heat stress on the reproductive performance of dairy cows.

The first study of my thesis was designed to investigate, if the climate data obtained from a meteorological station located in the vicinity of the experimental barn can be used to consider heat stress inside dairy livestock. The results of this study demonstrate that inside a dairy barn microclimates are generated that differ significantly from the climate conditions at the meteorological station. Especially the holding area and the adjacent high-yielding pen were detected as locations with the highest heat stress incidence inside the barn compared to the close-up pen and the fresh cow pen. Ambient temperature and THI in the high-yielding pen and the holding area were significantly higher compared to the ambient temperature and the THI in the close-up pen and the fresh cow pen ($n = 270$, $P < 0.01$). Furthermore, number of days with a mean THI ≥ 72 were 44, 36, 26, and 23 for the high-yielding pen, the holding area, the close-up pen and the fresh cow pen, respectively ($n = 270$; $P = 0.06$).These result are in line with a previous postulation that particularly the holding pen is a location of intensive heat stress (Collier et al., 2006). Microclimates inside a dairy farm are generated due to different functions of pens, construction characteristics, and environmental conditions. Especially the holding area is a location where stocking density reaches high level during milking which might increase the risk of suboptimal climate conditions (Cooper et al., 1998, Wagner-Storch and Palmer, 2002). Furthermore, the holding area is a location of intensive cleaning processes, especially during milking, which might increase relative humidity and resulting THI. In the current studies cows were milked 3 times per day and thus exposed to the climate conditions in the holding area for approximately 30 minutes prior to each milking. As demonstrated in the second study, already 1 hour of heat stress (THI ≥ 73) at the day of breeding decreased conception rate about 16% ($n = 1234$, $P < 0.01$). These results support the conclusion that short time exposure to heat stress conditions inside the holding area can lead to a decrease in the conception rate of dairy cows. These findings underline the need of an effective climate management in the holding area and all locations inside the barn in which cows are exposed to short time heat stress. Due to the central location inside the barn and the absence of natural ventilation, an effective climate management in the holding area is often challenging.

Therefore, further studies should compare the efficiency of different ventilation systems to reduce heat stress in the holding area.

The objective of the first study was to compare the climate conditions in 7 experimental barns on 6 dairy farms to the climate recorded at the closest corresponding meteorological station. The climate conditions in all barns differed significantly from the climate recorded at the corresponding meteorological stations. Ambient temperature and THI were significantly higher in all barns compared to the corresponding meteorological stations ($P < 0.01$). Furthermore, the number of days with a mean THI ≥ 72 was higher in all experimental barns and significantly higher in 5 experimental barns compared to the corresponding meteorological stations (P < 0.01). These results clearly demonstrated, that heat stress is underestimated in its magnitude and duration when climate data is obtained from a meteorological station in the vicinity of the study site. To assess heat stress in dairy livestock appropriately further studies should obtain climate data directly inside the dairy barn.

The objectives of the second study were 1) to investigate the impact of heat stress on the conception rate of dairy cows, 2) to determine a threshold for the impact of heat stress on the conception rate of dairy cows and 3) to detect the period around the day of breeding in which the conception rate of dairy cows is most sensitive to heat stress. The results of the second study illustrate that heat stress has a major impact on the conception rate of dairy cows in the moderate climate. The THI of 73 was determined as the threshold for the influence of heat stress on the conception rate, which is similar to thresholds described for tropical or subtropical areas (García-Ispierto et al., 2007, Morton et al., 2007). Nevertheless, negative effects of heat stress were apparent already at lower levels of THI. Conception rates decreased continuously with increasing THI beginning at a THI-threshold of 56 at the day of breeding. The THI ≥ 56 was reached on 62.7% (n = 2752) of all inseminations during the study period and cows exposed to a THI ≥ 56 at the day of breeding were 14% less likely to get pregnant than cows exposed to a THI < 56. Therefore, in 62.7% of all inseminations in the study period cows were at least 14% less likely to get pregnant due to heat stress. To my knowledge, this is the first study that describes an impact of heat stress on the reproductive performance of dairy cows at such a low THI-threshold. According to the equation of Kendall et al. (2009), a THI of 56 is reached by a relative humidity of 76% and an ambient temperature of 13 °C (e.g.) which is below the mean climate conditions during the study period (16.0 ± 0.5 °C and 76.0 ± 8.2%). The thermoneutral zone of dairy cows is described to range between 5°C and 25°C ambient temperature (National Research Council, 1981, Wheelock et al., 2010) and is defined as the zone of minimal heat production at normal rectal temperature (Kadzere et al., 2002). The results of this work provide evidence that heat stress already decreases reproductive performance of dairy cows below the upper limit of the thermoneutral zone. High yielding dairy cows are more affected by heat stress than low yielding dairy cows because increased milk production is related to elevated metabolic heat production (Kadzere et al., 2002). As milk yield is expected to increase further (Van Arendonk and Liinamo, 2003), the negative impact of heat stress will become more important. Therefore, milk yield and physiological constitution of dairy cows should be considered to assess heat stress, and further studies should identify upper limits of the thermoneutral zone for modern high yielding dairy cows.

The conception rate decreased continuously with the increasing number of hours with a mean THI ≥ 73 at the day of breeding. At a threshold of 20 hours with a mean THI ≥ 73 at the day of breeding the conception rate increased about 3% points and remained at this level. Cows exposed to a high but constant level of heat stress showed lower decrease in conception rate than cows exposed to a lower but fluctuating level of heat stress. The objective of this work was to investigate the influence of heat stress on dairy cows in the moderate climate of the temperate latitude. In contrast to the climate conditions from studies conducted in the tropical or subtropical areas, the climate conditions in the moderate climate are characterized by distinctive seasons with high climate variations between summer and winter months. In a study that was conducted in a climate chamber it was demonstrated that cows that were acclimatized to cold climate conditions showed higher physiological response (respiration rate, heat production) to heat stress than cows that were acclimatized to hot climate conditions (Robinson et al., 1986). The results of that study are in line with our observations that cows exposed to a high but constant level of heat stress showed lower decrease in conception rate than cows exposed to a lower but fluctuating level of heat stress. Thus, heat abatement strategies should maintain low and constant THI to avoid a major decrease in conception rate during the summer. Further research is necessary to compare the effects of short and long term fluctuations of heat stress on the reproductive performance of dairy cows.

The objectives of the third study were 1) to examine the effects of heat stress on the conception rate under natural and AI breeding programs and 2) to determine the effects of short and long term heat stress on the conception rate of dairy cows under different breeding programs. To avoid a major decrease in reproductive performance in periods of heat stress the use of artificial insemination programs enjoy great popularity due to decreased days to first artificial insemination and the efficient use of labor for day-to-day convenience in managing the herd (Tenhagen et al., 2004, Stevenson, 2005). Furthermore, the need of estrus detection is eliminated (Hansen and Arechiga, 1999, Bucher et al., 2009); and estrus detection is often challenging under periods of heat stress due to shorter estrus duration (Orihuela, 2000) and decreased mounting activity (Pennington et al., 1985). Because all eligible cows are inseminated, the use of artificial insemination programs lead to an estrus detection rate of 100%, and the conception rate becomes the limiting factor (Cartmill et al., 2001). Therefore, the use of artificial insemination programs lead to an increase in the pregnancy rate, especially in herds with a low estrus detection rate (Cerri et al., 2004). In this work the impact of heat stress on the conception rate of dairy cows was investigated. However, the heat detection rate was not provided in the current data set, therefore, the pregnancy rate could not be determined. A meta-analysis demonstrated that the conception rate and the pregnancy rate obtained with the Ovsynch program and breeding at natural estrus did not differ (Rabiee et al., 2005). However, the impact of heat stress on the pregnancy rate of dairy cows under different breeding programs in the moderate climate remains unclear and should be investigated in further studies.

This study provided evidence that breeding after Ovsynch program cannot be recommended in general to compensate poor reproductive performance during periods of heat stress. Especially under short and long term heat stress, breeding at natural estrus was the most effective breeding program and can be an approach to avoid a major decrease in the conception rate in the summer months. Nevertheless, estrus detection remains a critical issue under periods of heat stress. A recent study demonstrated that visual observation in combination with an automated activity monitoring system can lead to estrus detection rates of 56.3% in the 21 days after the voluntary waiting period (Michaelis et al., 2014). Furthermore, the time to pregnancy was shorter with an automated activity monitoring system compared to timed artificial insemination, and the conception rate between cows inseminated with timed artificial insemination and at detected estrus with an automated activity monitoring system did not differ (Neves et al., 2012). Manufacturers of automated activity monitoring systems claim high estrus detection rates even under effects that influence the cows movement due to individual threshold monitoring. Therefore, the use of automated activity monitoring systems could be a solution to avoid a major decrease of the reproductive performance in periods of heat stress. Nevertheless, science-based information is not available, and further studies should evaluate automated activity monitoring systems in periods of heat stress.

6 SUMMARY

The overall objective of this study was to determine the incidence of heat stress in dairy livestock in the moderate climate and to examine the impact of heat stress on the reproductive performance of dairy cows.

The first study investigated if the climate data obtained from a meteorological station located in the vicinity of the experimental barn can be used to consider heat stress inside dairy livestock. Climate conditions in all barns differed significantly from the climate recorded at the corresponding meteorological stations. The ambient temperature and THI were significantly higher in all barns compared to the corresponding meteorological stations. Furthermore, the number of days with heat stress (THI ≥ 72) was higher in all experimental barns. The results of this study indicated that inside dairy barns microclimates are generated that differ significantly from the climate conditions at the meteorological station. Therefore, heat stress is underestimated in its magnitude and duration when climate data is obtained from a meteorological station in the vicinity of the study site. Especially the holding pen is a location of intensive heat stress that requires effective heat abatement strategies.

The first study provided evidence that heat stress occurs in dairy livestock in the moderate climate, however, the effect of heat stress on the reproductive performance remained unclear. Therefore, a second study was conducted 1) to investigate the impact of heat stress on the conception rate of dairy cows, 2) to determine a threshold for the impact of heat stress on the conception rate of dairy cows and 3) to detect the period around the day of breeding in which the conception rate of dairy cows is most sensitive to heat stress. The results of this study demonstrated that heat stress has a major impact on the conception rate of dairy cows in the moderate climate. The THI of 73 was determined as threshold for the influence of heat stress on conception rate in dairy cows. Negative effects of heat stress were apparent already at lower levels of THI, and 1 h of mean THI ≥ 73 at the day of breeding decreased the conception rate significantly. In the current study, cows exposed to a high but constant level of heat stress showed lower decrease in conception rate than cows exposed to a lower but fluctuating level of heat stress. Thus, heat abatement strategies should maintain low and constant THI to avoid a major decrease in the conception rate during the summer. The conception rate of lactating dairy cows was negatively affected by heat stress both before and after the day of breeding. The period from d 21 to d 1 before the day of breeding was most sensitive to heat stress. Thus, heat abatement strategies should maintain low and constant THI to avoid fluctuations in heat stress over a period of at least 3 weeks before to 3 weeks after the day of service.

To avoid a depressed reproductive performance during summer months, the use of timed artificial insemination programs has become popular because of their predictability and the elimination of estrus detection. Therefore, the objectives of the third study were 1) to examine the effects of heat stress on the conception rate of dairy cows under natural and artificial insemination breeding programs and 2) to determine the effects of short and long term heat stress on the conception rate under different breeding programs. The results of this study indicate that even under short and long term heat stress, breeding at natural estrus was the most effective breeding program compared to an Ovsynch or modified Ovsynch program. These findings provide evidence that breeding by timed artificial insemination after Ovsynch programs lead to a decrease in the conception rate in addition to heat stress. Breeding by timed artificial insemination after Ovsynch programs cannot be recommended in general to compensate poor reproductive performance during periods of heat stress. Especially under short and long term heat stress, breeding at natural estrus can be an approach to avoid a major decrease in the conception rate in the summer months.

Overall, the 3 studies clearly demonstrated that heat stress in dairy cows plays a great role in the moderate climates of the temperate latitude. The location of climate data collection in dairy livestock is an important factor to determine heat stress appropriately, and therefore, heat stress is underestimated in its duration and magnitude. Heat stress significantly depresses the reproductive performance of dairy cows in the moderate climate, and already low duration and magnitude of heat stress can significantly decrease the conception rate of dairy cows. The use of timed AI programs is not a general solution to compensate low summer fertility. Therefore, further research is necessary to investigate in approaches to avoid a major decrease in conception rates during periods of heat stress in the moderate climate.

7 ZUSAMMENFASSUNG

Einfluss von Hitzestress auf die Fruchtbarkeit von Milchkühen im gemäßigten Klima der mittleren Breiten

Ziel dieser Arbeit war es, das Vorkommen von Hitzestress in Milchviehställen der gemäßigten Klimazone in seiner Dauer und Intensität zu identifizieren und den Einfluss auf die Fruchtbarkeit von Milchkühen zu bestimmen.

In der ersten Studie wurde untersucht, ob von den Klimadaten der nächstgelegenen Wetterstation auf das Vorkommen von Hitzestress innerhalb eines Milchviehstalles geschlossen werden kann. Die Klimadaten aus allen Ställen unterschieden sich signifikant von den Klimadaten der jeweils nächstgelegenen Wetterstation. Die Luft-Temperatur und der THI waren in allen Ställen signifikant höher als an der jeweils nächstgelegenen Wetterstation. Auch die Anzahl an Tagen mit Hitzestress (THI ≥ 72) war in allen Ställen signifikant höher als an der jeweils nächstgelegenen Wetterstation. Die Ergebnisse der ersten Studie zeigen deutlich, dass sich innerhalb eines Stalles Mikroklimata ausbilden, welche sich signifikant von den Klimabedingungen an der nächstgelegenen Wetterstation unterscheiden. Hitzestress innerhalb eines Milchviehstalles wird daher in seiner Dauer und Intensität unterschätzt, wenn Klimadaten ausschließlich an einer Wetterstation erhoben werden. Innerhalb des Stalles ist besonders der Vorwartebereich für das Auftreten von Hitzestress gefährdet, weswegen hier ein effektives Klima-Management gefordert ist.

Die Ergebnisse der ersten Studie zeigten deutlich, dass Hitzestress auch in Milchviehställen der gemäßigten Klimazone von Bedeutung ist, wobei der Einfluss auf die Fruchtbarkeit von Milchkühen bisher nicht bekannt war. Das Ziel der zweiten Studie war es daher, 1) den Einfluss von Hitzestress auf die Konzeptionsrate von Milchkühen im gemäßigten Klima zu untersuchen, 2) einen Grenzwert für diesen Einfluss fest zu legen und 3) den Zeitraum um den Tag der Besamung zu bestimmen an dem Hitzestress den größten Einfluss auf die Konzeptionsrate hat. Insgesamt machen die Ergebnisse dieser Studie deutlich, dass Hitzestress die Konzeptionsrate von Milchkühen in der gemäßigten Klimazone drastisch beeinflussen kann. Der THI von 73 wurde als Grenzwert für den Einfluss von Hitzestress auf die Konzeptionsrate von Milchkühen berechnet. Allerdings kann ein negativer Einfluss von Hitzestress auf die Konzeptionsrate von Milchkühen bereits ab einem THI von 56 beobachtet werden und schon eine Stunde Hitzestress am Tag der Besamung kann die Konzeptionsrate signifikant senken. In der vorliegenden Studie zeigten Kühe, die einem hohen aber konstanten Hitzestress-Level ausgesetzt waren, einen geringeren Abfall der Konzeptionsrate als Kühe, die einem niedrigen und schwankenden THI ausgesetzt waren. Die Konzeptionsrate von

Milchkühen wurde sowohl in dem Zeitraum vor, wie auch in dem Zeitraum nach der Besamung negativ beeinflusst. Der Zeitraum von Tag 21 bis Tag 1 vor der Besamung war am sensibelsten für den Einfluss von Hitzestress. Strategien im Klima-Management sollten daher einen niedrigen und konstanten THI im Stall über den gesamten Zeitraum von 3 Wochen vor bis 3 Wochen nach der Besamung gewährleisten, um einen starken Abfall der Konzeptionsrate in den Sommermonaten zu vermeiden.

Um einen Abfall der Fruchtbarkeit in Hitzestressperioden zu vermeiden, werden terminierte Besamungsprogramme aufgrund ihrer Vorhersagbarkeit und des Wegfalls der Brunstbeobachtung vermehrt eingesetzt. Das Ziel der dritten Studie war es daher 1) den Einfluss von Hitzestress auf die Konzeptionsrate von Milchkühen unter Einsatz natürlicher und terminierter Besamungsprogramme zu bestimmen, sowie 2) den Einfluss von Kurzzeit- und Langzeit-Hitzestress auf die Konzeptionsrate unter verschiedenen Besamungsprogrammen zu untersuchen. Die Ergebnisse dieser Studie zeigen, dass die Besamung zur natürlichen Brunst im Vergleich zur terminierten Besamung nach Einsatz von Ovsynch-Programmen das effektivste Besamungsprogramm unter Kurzzeit- sowie unter Langzeit-Hitzestress ist. Die vorliegenden Ergebnisse verdeutlichen, dass der Einsatz von terminierter Besamung nach Ovsynch-Programmen zu einem zusätzlichen Abfall der Konzeptionsrate unter Hitzestress führt. Die terminierte Besamung nach Einsatz von Ovsynch-Programmen stellt daher keinen generellen Lösungsansatz dar, um eine verminderte Fruchtbarkeit unter Hitzestress zu kompensieren. Die Besamung zur natürlichen Brunst kann allerdings eine Möglichkeit darstellen um einen Abfall der Fruchtbarkeit unter Kurzzeit- sowie unter Langzeit-Hitzestress zu begrenzen.

Die vorliegenden Studien verdeutlichen, dass Hitzestress bei Milchkühen im gemäßigten Klima der mittleren Breiten eine große Rolle spielt. Die Erhebung der Klimadaten in Milchviehställen ist ein entscheidender Faktor um Hitzestress angemessen beurteilen zu können, weswegen Hitzestress bei Milchkühen in seiner Dauer und Intensität bisher deutlich unterschätzt wurde. Hitzestress im gemäßigten Klima wirkt sich negativ auf die Fruchtbarkeit von Milchkühen aus und schon geringe Intensitäten und kurze Phasen von Hitzestress führen zu einem signifikanten Abfall der Konzeptionsrate. Der Einsatz von terminierten Besamungsprogrammen stellt dabei keinen generellen Lösungsansatz dar, um dem Abfall der Konzeptionsrate unter Hitzestress entgegen zu wirken. Die Erforschung weiterer Lösungsansätze ist notwendig, um den Abfall der Konzeptionsrate bei Milchkühen unter Hitzestress zu reduzieren.

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9 PUBLICATIONS

Research articles

Schüller, L. K.; O. Burfeind and W. Heuwieser. (2013):
Short communication: Comparison of ambient temperature, relative humidity, and temperature-humidity index between on-farm measurements and official meteorological data. Journal of Dairy Sciece. 96:7731-738.

Schüller, L. K.; O. Burfeind and W. Heuwieser. (2014):
Impact of heat stress on conception rate of dairy cows in the moderate climate considering different temperature–humidity index thresholds, periods relative to breeding, and heat load indices. Theriogenology. 81:1050-1057.

Oral presentations at conferences

Schüller, L. K.; O. Burfeind and W. Heuwieser. (2014):
Einfluss von Hitzestress auf die Fruchtbarkeit von Milchkühen. 10. Berlin-Brandenburgischer Rindertag. Berlin, Germany. 02.-04.10.2014. In: 10. Berlin-Brandenburgischer Rindertag, p. 124 – 127.

Schüller, L. K.; O. Burfeind and W. Heuwieser. (2014):
Comparison of climate conditions between on farm measurement and official meteorological data. 19. Congreso Internacional ANEMBE de Medicina Bovina, 9. ECBHM Symposium. Oviedo, Spain. 25.-27.06.2014. In: Presentations, oral presentations and poster book - ANEMBE, p. 249.

Schüller, L. K.; O. Burfeind and W. Heuwieser. (2014):
Measurement of heat stress and effects on conception rate in lactating dairy cows. 8th PhD-Symposium - bringing future scientists together & DRS presentation seminar. Berlin, Germany. 15.07.2013. In: Programm und Abstracts - Freie Universität Berlin, Fachbereich Veterinärmedizin, p. 16.

W. Heuwieser, L. K. Schüller. (2013):
Hitzestress in Deutschland? Auswirkungen auf Fruchtbarkeit, Milchleistung und Behandlungsentscheidungen. bpt Kongress 2013. Mannheim, Germany. 26.-29.09.2013. In: bpt-Kongress 2013: Vortragsband – bpt-Akademie GmbH (Hrsg.), p. 154–159.

Poster presentations at conferences

Schüller, L. K.; O. Burfeind and W. Heuwieser. (2014):
Impact of heat stress on conception rate of dairy cows in the moderate climate. 28. World Buiatrics Congress. Cairns, Australia. 27.07-01.08.2014. In: Proceedings of the 28. World Buiatrics Congress, Cairns 2014, p. 242.

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11 DECLARATION OF INDEPENDENCE

Hiermit erkläre ich, dass ich, Laura-Kim Schüller alle Studien selbständig durchgeführt und die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

Tabelle 1. Eigener Anteil[1] an den Forschungsprojekten der vorliegenden Dissertation

	Studie 1[a]	Studie 2[b]	Studie 3[c]
Studienplanung	+++	+++	+++
Datenerhebung	+++	+++	+++
Datenanalyse	+++	+++	+++
Verfassen des Manuskripts	+++	+++	+++
Editieren des Manuskripts	++	++	++

[1]Legende: +++: > 70 %
++: 50-70 %
+: < 50 %

[a] *Short communication:* Comparison of ambient temperature, relative humidity, and temperature-humidity index between on-farm measurements and official meteorological data

[b] Impact of heat stress on conception rate of dairy cows in the moderate climate considering different temperature–humidity index thresholds, periods relative to breeding, and heat load indices

[c] Evaluation of natural and artificial breeding programs affecting conception rates of dairy cows under short and long term heat stress

Berlin, den 09.09.2014

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