

## Effect of heat stress on some reproductive traits in Holstein-Friesian cows under temperate continental climate

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### Abstract

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The aim of the present study was to determine the effect of heat stress (HS) on some reproductive traits in Holstein-Friesian cows in the central part of southern Bulgaria. The survey was conducted in a dairy cattle farm for the period 2015 - 2018 inclusive, on 155 lactations of cows on first to sixth parity. HS was determined by a modified Kelly and Bond (1971) equation using the maximum temperature (°C) and the average values of relative humidity (%) for each month of the study. Temperature and humidity data were taken from the nearest meteorological station in the area. It was found that Temperature-humidity index (THI) at first insemination had a significant ( $P < 0.05$ ) effect on the length of the Days open interval (DO) and on the number of inseminations for conception (NIC) ( $P < 0.01$ ). Depending on the different levels of THI at first insemination, the DO varied from 130.43 to 206.7 days, respectively for THI below 72 and over 90. The NIC varied from 2.43 to 4.24, respectively, at THI values within of 73-78 and over 90 reported during the month of the first insemination. The highest conception rate (CR) of inseminated cows and the highest conception rate at first insemination (CRFI) were found during the period of mild HS (THI 73-78) 41.7% and 32.5% respectively. With the onset of severe HS months (over 90), these trait values fall to 25% and 26.1%, respectively. In July and August the highest values of THI and the lowest CR 19.4% and CRFI 16.7% were reported. With an increase in THI an increase in the interval between inseminations (IBI) was observed from 35 days at THI below 72 to 45.6 days at THI over 90.

**Keywords:** Holstein-Friesian cows; heat stress; reproductive traits

### Introduction

Heat stress is the non-specific physiological response of the animals to the ambient temperature when they produce more heat than they can release (Yang, 2014). The decline in reproductive performance in dairy cows in subtropical and tropical climates under the influence of HS has been well studied. Given global warming and intense genetic progress for high productivity, the decline in reproductive traits is a limiting factor for dairy cattle farming in the coming years (Roth, 2017). According to the author HC in cows is no longer specific only to the hottest regions of the planet. In the literature begin to appear studies also on the negative effects of high temperatures on animals in regions of Europe with temperate continental and Mediterranean climate (Bernabucci et al., 2014).

Holstein-Frisian cows are the most widely used in the world milk production. This breed was created about 2000

years ago in the Netherlands (in the Friesland - North Holland region, where the climate is cool and humid (Wikipedia). In summer, this breed ability to relise body heat through the skin is limited by the relatively small body surface area against body weight, not well developed sweat glands and short, densely located hair coat (Yang et al. 2010). Due to this, as well as to the high milk performance, the breed is predisposed to HS. Holstein-Friesian cows are the main dairy breed distributed in Bulgaria, where the climate is temperate continental. It is important, from a scientific and practical point of view, to study how the levels of HS in different climatic zones of the world affect the physiological response of Holstein-Friesian cows, considered as the main dairy breed of the world.

The aim of the present study was to determine the effect of heat stress on some reproductive traits in Holstein-Friesian cows bred under conditions of temperate continental climate.

## Material and Methods

The study was conducted at a dairy cattle farm in Plovdiv district (Central South Bulgaria) with the data collected for the period from 2015 to 2018. The study included all Holstein-Friesian cows calved during this period. Cows were housed in semi-open free stall dairy barn. The average milk yield of the cows on the studied farm was 10 000 kg/lactation. One hundred and fifty five lactations of cows from first to sixth parity were studied. Depending on the number of parities, the cows were distributed in the following order: 48 cows on first, 59 cows on second, 18 on third, 17 on fourth, 9 on fifth and 4 cows on sixth parity. From the herd management software the calving date, the number of inseminations per conception and the dates of all inseminations were taken. From these data for each cow were calculated respectively: interval from calving to first insemination (CFI), DO and IBI.

Heat stress was determined by a modified Kelly and Bond (1971) equation:  $THI = t_{max} - (0.55 - 0.55 RH) (t_{max} - 58)$ , where:  $t_{max}$  - the maximum measured temperature (in °C) for the studied month, and RH are the average values for relative humidity (in %) for the studied month. The data on air temperature and humidity for all years of the study needed to calculate the THI were taken from the nearest meteorological station, which was at a distance of 30 km from the surveyed farm.

To obtain a better approximation, some factors were presented in classes as follows: for THI 4 classes were formed: no heat stress - THI up to 72; mild heat stress - THI from 73 to 78; moderate heat stress - THI from 79 to 89 and severe heat stress in THI over 90 (according to Armstrong (1994); for the season, respectively: 1 - spring (March, April and May); 2 - summer (June, July, August); 3 - autumn (September, October, November) and 4 - winter (December, January and February).

To assess the influence of THI on studied reproductive traits the following model was used:

$$Y_{ijkl} = \mu + THI_i + THI_j + THI_k + e_{ijkl}$$

Where:  $Y_{ijkl}$  - is the dependent variable (OPEN DAYS

number of inseminations for conception),

$\mu$  is mean effect,  $THI_i$  is the effect of THI (in classes) on calving,  $THI_j$  is the effect of THI (in classes) on first insemination,  $THI_k$  is the effect of THI (classes) on at conception and  $e_{ijkl}$  is the random residual effect.

To assess the influence of THI at insemination on the interval from calving to first insemination a one-way ANOVA was applied, using the following model:

$$Y_{ij} = \mu + THI_i + e_{ij}$$

Where:  $Y_{ij}$  is the dependent variable (interval from calving to first insemination),  $\mu$  is the mean effect,  $THI_i$  is the effect of THI (in classes) at insemination and  $e_{ij}$  is the random residual effect.

By analysis of variance (ANOVA) for the model were obtained by classes of the fixed factors the means of least squares (LSM).

For a basic statistical processing of the data a package MS Excel was used, and for obtaining the average values, errors, and analysis of variance, the corresponding modules of STATISTICA of StatSoft (Copyright 1990-1995 Microsoft Corp.)

## Results and Discussion

Table 1 presents the average values for the reproductive traits of the studied farm. The average number of inseminations for conception was  $2.89 \pm 0.15$ , the average duration of IBI was  $39.32 \pm 1.34$  days. The average duration of CFI was  $73.93 \pm 1.78$  days, and the average DO was  $149.89 \pm 7.08$  days. The reproductive traits values reported by us were similar to the reported by other authors (M'Hamdi et al., 2011; Zink et al., 2012). It is difficult to compare results from different populations and from different years due to different average productivity levels. It can be assumed that animals from earlier studies had lower milk yield, which could lead to better reproductive performance (Zink et al., 2012). Given that in studied by us farm the average milk yield was about 10 000 kg for 305 days in milk (DIM) we consider that presented reproductive traits values were very good.

**Table 1. Average values and variation of the reproductive traits in the studied farm**

Trait	Number	$\bar{x} \pm SE$	SD
Number of inseminations for conception	157	$2.89 \pm 0.15$	1.89
Interval from calving to first insemination (days)	157	$73.93 \pm 1.78$	22.36
Interval between inseminations (days)	278	$39.32 \pm 1.34$	22.37
Days open interval (days)	157	$149.89 \pm 7.08$	88.77

Table 2 presents the maximum values of THI by months for the years covered by the study. The presented data show that the months with high risk values of THI were from May to September, and the coldest - December, January and February. For all years from May to September inclusive, THI values representing a risk of HS were reported, as they reached TVI values above 80 and sometimes even above 90 according to the scale proposed by Arm-

strong (1994). This showed that in temperate climates, HS affected animals from the months of late spring to early autumn. Global warming is changing the classic even distribution of temperatures for the four seasons in temperate climates. Similar THI values for the temperate climate zone were also reported by Vitali et al. (2009) in northern Italy. In a study by Dimov (2017), similar maximum THI values were registered in cattle farms in Southern Bulgaria.

**Table 2. Maximum values of THI by months for the studied years**

Calendar months	2015	2016	2017	2018
January	64	64	64	64
February	64	74	70	64
March	64	74	73	70
April	76	84	78	82
May	82	85	82	76
June	88	91	95	80
July	90	89	91	91
August	89	90	91	87
September	91	86	87	88
October	74	81	77	77
November	73	77	70	74

Table 3 presents an analysis of variance for the influence of THI during calving, first insemination and conception on the duration of DO and NIC. It was found that THI at first insemination had a significant effect on the duration of the DO ( $P < 0.05$ ). The first insemination of cows usually takes place within 60-66 days after calving, but high THI values during this period usually reduce the

CR (Jordan, 2003). HS leads to a low CR (Hansen, 2019). According to the author, oocytes are damaged by HS in the earliest stages of folliculogenesis and become particularly sensitive. In the first seven days of its development, the embryo is particularly susceptible to damage under the influence of HS and is very likely not to be held up by the cow (Hansen, 2019).

**Table 3. Analysis of variance for the influence of THI during calving, first insemination and conception on the duration of DO and NIC**

Sources of variation	Degrees of freedom (n - 1)	Days open interval (days)		Number of inseminations for conception	
		MS	F P	MS	F P
Total for the model	3	10262.69	1.32 n.s.	6.36	1.85 n.s.
THI at calving	3	4796.00	0.62 n.s.	6.38	1.86 n.s.
THI at first insemination	3	25842.00	3.34 *	15.45	4.50 **
THI at conception	3	2538.00	0.32 n.s.	2.68	0.78 n.s.
Error	147				

Significance: \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; n.s. - no significance

Figure 1 shows the LS-means for the duration of the DO depending on the THI at first insemination. From the data in the figure it can be seen that the cows that were inseminated for the first time at THI up to 72, i.e. in conditions of temperature comfort according to Armstrong (1994) had the shortest DO - 130.43 days. As the values of THI increased, the duration of the DO increased. Under conditions of severe HS (THI over 90) at first insemination, the DO was 206.7 days. When cows were inseminated in such severe climatic conditions of HS, the CR was greatly reduced. According to Jordan (2003), the negative effects of HS on cows begin 42 days before and last 40 days after insemination. It is known HS cause some physiological changes in dairy cows such as rapid respiration rate, rapid heart rate,

and increased body temperature (Kadzere et al., 2002; Wheelock et al., 2010), but the most pronounced change is the reduced dry matter intake (West, 2003), which together with efforts for cooling leads to energy expenditure and metabolic changes (Bernabucci et al., 1999). The cows in which the first insemination was at the conditions of the most severe HS have calved at the beginning of the summer. In these animals during the first part of lactation when they were subjected to the strongest metabolic loading, as an addition and the HS effects were superimposed. Probably for this reason, these cows had the longest DO. These claims were also confirmed by Dash et al. (2016), who found that cows calved in the summer, had the longest DO.

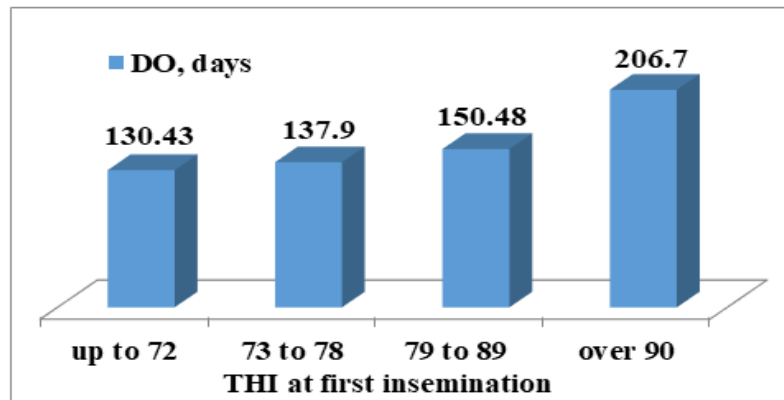


Fig. 1. LS-means for the duration of DO depending on the THI at first insemination

Fig. 2 shows the LS-means of NIC depending on THI at first insemination. The data showed that the NIC did not increase significantly at THI in the range of 72 to 90. There was even a slight decrease in the NIC when compar-

ing conditions without HS (THI below 72) - a value of 2.48 inseminations and in conditions of mild HS (THI from 73 to 78) - 2.43.

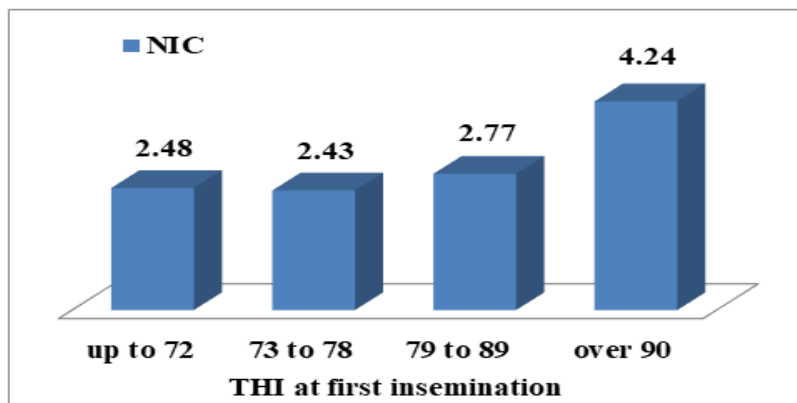


Fig. 2. LS-means of NIC depending on THI at first insemination

Our results did not corroborate the studies of (Morton et al., 2007; Schuller et al., 2014), according to which the CR decreases at THI above 72-73. Substantial impact on the cows NIC and CR have the measures taken against HS on farm. The presence of a cooling system, such as the one on the farm we studied, improves CR in cows under conditions of mild HS (Figure 3), which also affects the NIC. The positive effect of cooling on the CR was proven by Zolini et al. (2019). However, the increase of THI over 90 in the area of

the farm we studied caused a significant increase in the NIC to a value of 4.24 (Fig. 2). This also affects the CR, which drops to 25 % under conditions of severe HS. Similar results were reported by Voelz et al. (2016), who on the 36th day after insemination under HS conditions found 25-27% gestation. In the conditions of Israel Flamenbaum and Galon (2010) due to a high degree of HS found CR below 20%, and in case of poor cooling of cows only 3%.

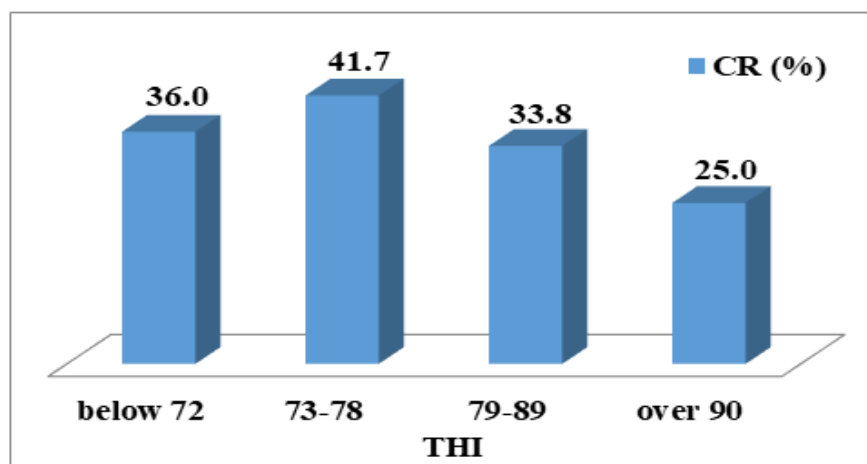


Fig. 3. Conception rate of cows depending on the THI class

The data presented in Figures 2, 3 and 4 show that in the cows on the farm we studied under conditions of mild heat stress (THI from 73 to 78) the CR was the highest. Ryan et al. (1992) found that at THI below 78.5 the cooling of cows had a positive effect on the reproductive performance of dairy cows, and at higher values of THI, the CR decreased and the NIC increased. In the studied farm, the cooling of the cows under mild heat stress may be the prob-

able reason for the good reproductive performance. Under conditions of moderate heat stress (THI from 79 to 89) there was a deterioration of reproductive indicators expressed by increased NIC, decreased CR and decreased CRFI (Fig. 2, 3 and 4). The occurrence of severe HS (THI over 90) led to a considerable increase in the NIC to 4.24; a decrease in the CR to 25% and a decrease in the CRFI to 26.1% (Fig. 2, 3 and 4).

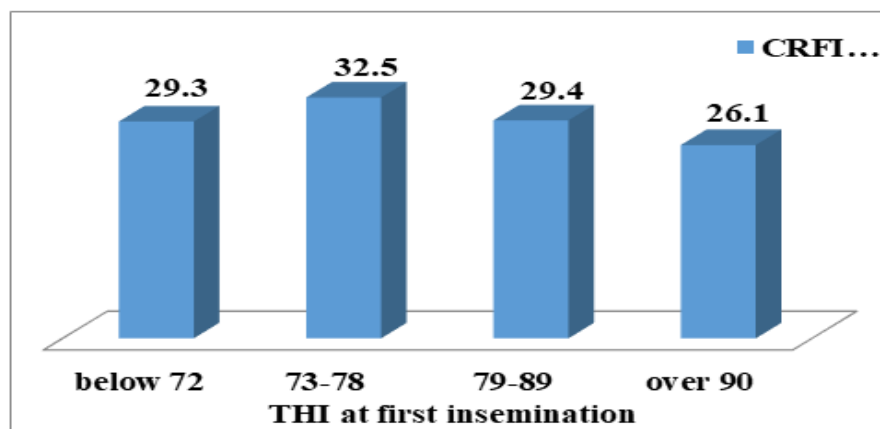


Fig. 4. Conception rate at first insemination depending on the THI class

According to McGowan et al. (1996) HS significantly reduces the CRFI and increases the NIC of cows at THI above 72. The presence of a cooling system according to the study of Ryan et al. (1992) improved reproductive performance, such as NIC and CR. Our study showed that under mild heat stress and cooling in temperate-continental climates the best reproductive performance in dairy cows were obtained, which was better than under conditions without heat stress. According to Wolfenson and Roth, (2019) the use of cooling of cows along with the hormones treatment such as GnRH and PGF<sub>2α</sub> significantly improves

reproductive performance. On the other hand, follicle development lasts 180 days (Lussier et al., 1987) and it is possible a negative effect of HS on folliculogenesis (Roth, 2017) to be observed. In another study, Jordan (2003) reported that the negative effects of heat stress on cow follicles begin 42 days before insemination. In other words, probably the good reproductive traits reported in the farm we studied under mild HS may be due to the fact that in previous periods (spring) the cows were in optimal conditions.

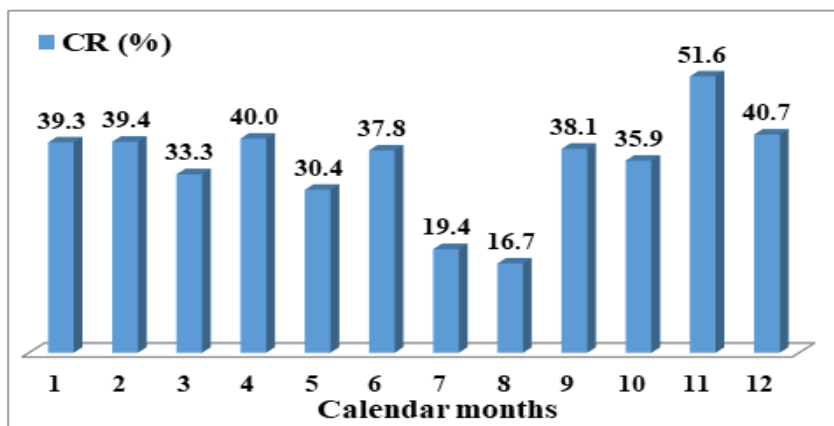


Fig. 5. Conception rate by calendar months

The data presented in Fig. 5 show the CR of cows from the total number inseminated by calendar months. From the data shown, it was clear that the CR of cows in June when the maximum values of THI varied throughout the years from 80 to 95 (Table 2) was close to the results achieved in January and February. In July and August, the CR dropped sharply to 19.4% and 16.7%, respectively. Our results confirm the studies of Ray et al. (1992) and Thompson et al. (1996) that reported a low CR in cows

inseminated during the summer months. With the onset of autumn (September, October) the CR of cows increased and reached 38.1% and 35.9% respectively. Our results did not support the claims of Roth et al. (1997) and Wolfenson et al. (1997) according to which in the autumn the CR is low due to the HS during the hot months.

Table 4 presents an analysis of variance for the effect of THI on the length of the IBI. It was found that THI had a significant effect ( $P < 0.05$ ) on this trait.

Table 4. Analysis of variance for the effect of THI on the length of the IBI

Sources of variation	Interval between inseminations (days)		
	Degrees of freedom (n -1)	MS	F P
Total for the model	3	1401.6	2.86 *
THI	3	1401.6	2.86 *
Error	274		

Significance: \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; n.s. - no significance

Fig. 6 shows the IBI in days depending on the THI class. It was reported that with increase of THI the IBI was increased. Increase between conditions without HS (THI below 72) and under a mild HS (THI from 73 to 78), the increase was a minimal - 1.6 days. With the occurrence of

moderate HS (THI from 79 to 89) this interval reached 40.8 days or 4.2 days more than the previous period under HS. When severe HS conditions occurred, the IBI increased to 45.6 days or 4.8 days longer than the period under moderate HS.

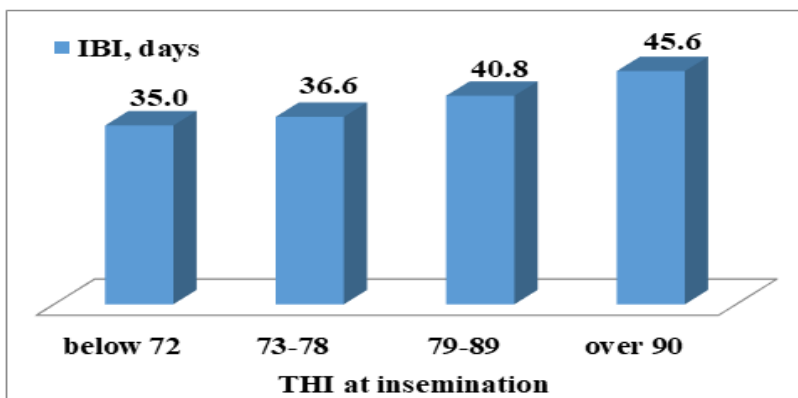


Fig. 6. IBI (days) depending on the THI class at insemination

The influence of HS on the IBI on the one hand may be due to the fact that the duration and intensity of estrus are affected under HS conditions (Gwazdauskas et al., 1981; Younas et al., 1993). During the hot months, motor activity and estrus decrease according to Nobel et al. (1997), and cases of anestrus and silent ovulation are increasing (Gwazdauskas et al., 1981). On the other hand, there are studies that prove an increase in cases of early embryonic mortality caused by HS (García-Ispuerto et al., 2006; Santolaria et al., 2010; El-Tarabany & El-Tarabany, 2015). We hypothesize that the increase in the IBI associated with an increase in THI, presented in Figure 6, was due to unregistered estrus as well as to early embryonic mortality, under conditions of moderate and severe heat stress.

## Conclusion

The heat stress in dairy cows in the region of Central Bulgaria was manifested from late spring (in May) to early autumn (in September). The highest THI values and respectively HS were reported in the summer (June, July and August). The high THI values at first insemination negatively affected the duration of DO and the IBI. The most substantial increase in these traits was found when the maximum THI values at first insemination were over 90 (under conditions of severe heat stress).

The highest CR as well as the highest CRFI was reported when cows were under conditions of mild heat stress (THI between 73 and 78). This was probably due to the favorable environmental conditions in the previous months, as well as to the cooling carried out on the farm. The accumulated negative effect of HS in May and June probably affected the development of follicles, due to which in July and August the lowest conception rates in cows were reported. The increase in THI led to an increase in the IBI.

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