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A meta-analysis of heat stress in dairy cattle: The increase in temperature humidity index affects both milk yield and some physiological parameters

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Abstract: In this study, the relationships of temperature humidity index (THI) with milk yield and some physiological responses in dairy cattle were investigated. Our goal in the meta-analysis was to find the parameter(s) primarily affected under heat stress. A total of 16 studies with the temperature humidity index value higher than 72, which is an important factor in determining the effect of heat stress, were included in the meta-analysis. The variables of interest in the meta-analysis included: milk yield (kg/day), respiratory rate (breaths/min), rectal temperature (°C). In addition to the meta-analysis, principal component analysis (PCA) was also performed. In the meta-analysis, high variation or heterogeneity ($I^2 > 99\%$) was determined between the results of the studies. This may depend on many factors (climate, region, number of samples and management etc.). Heterogeneity is desirable in the meta-analysis, because it provides accurate and reliable interpretations of the variances of parameters. Due to high heterogeneity, the results of the studies were combined according to the mixed model. According to the mixed model and PCA results, a linear relationship was determined between the temperature humidity index and these physiological parameters. According to the meta-analysis, at THI > 72, the mean effect size of milk yield was 50%, and the effect sizes of respiratory rate and rectal temperature were approximately 65% and 38%. All three parameters have a significant effect under heat stress ($P < 0.000\,1$). As a result, there is a linear relationship between temperature humidity index, milk yield and physiological parameters. According to the other characteristics, the respiratory rate was determined as the primary response parameter in parallel with the increase in temperature humidity index.

Keywords: heat stress; dairy cow; metadata; correlation

High temperatures adversely affect farm animals as relative humidity values or solar radiation cause heat stress. When the ambient temperature exceeds the body temperature, it causes a failure in temperature loss due to the evaporation leading to a lack of thermoregulatory responses of the animals. In animals exposed to the heat stress, the regulation of body temperature becomes a priority. Therefore, while animals try to reach a balanced body temperature, some other physiological functions are impaired. The heat stress has negative effects on many characteristics such as growth, reproduction, animal welfare, physiological or metabolic properties (Romo-Barron et al. 2019). In general, an increase in respiratory rate and rectal temperature, sweating, decrease in activity, decrease in feed consumption, milk production and milk content can cause negative effects (Liu et al. 2019). The temperature humidity

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index (THI), which is calculated by using the ambient temperature and relative humidity and used to determine the heat stress, is widely used in dairy cattle (Mylostyvyi et al. 2020). According to the classification made by Armstrong (1994), it is stated that there is no heat stress when the temperature humidity index is < 72, mild stress between 72 and 79, moderate stress between 80 and 89, and extreme stress that can lead to death when it is ≥ 90. These studies indicated that different threshold values of THI are effective in the emergence of stress in dairy cattle. However, THI values greater than 72 are generally accepted as the beginning of heat stress (Liu et al. 2019; Pinto et al. 2020).

There are many studies examining the effects of heat stress on metabolic, physiological or behavioural characteristics in dairy cattle. However, since the conditions in which the studies were carried out and the methodologies used are different from each other, it is difficult to obtain general conclusions regarding the effect of heat stress on these traits. For this reason, there is a need for studies that examine independent studies conducted in different places and times in line with certain criteria. However, such studies are very scarce. In this direction, our aim was to examine the effects of heat stress on milk yield, rectal temperature and respiratory rate, which are frequently investigated, and their relationships with each other by meta-analysis.

MATERIAL AND METHODS

Metadata

The articles were obtained from searches in different electronic databases such as Scopus and Web of Sciences. The searches were done by using the keywords "heat stress", "milk yield" (MY), "physiological", "dairy cattle" and the combinations of these words. The selection process was constrained by the results of studies published between 2010 and 2020. A total of 67 articles were registered to select those suitable for meta-analysis.

Selection criteria

In the selection of studies to be included in the meta-analysis, a two-stage process was followed, as general and specific criteria. As a general criterion, (I) the publication of articles in the English language and in peer-reviewed journals; (II) the use of dairy cattle as experimental animals; (III) detailed description of the number, breed and experimental design of animals; and (IV) the studies in which the THI is higher than 72 and the relative humidity or ambient temperature is given for each experimental group were selected. As a special criterion, the papers involving MY (kg/day), rectal temperature (RT, °C) and respiratory rate (RR, breaths/min) measurements are included. In total, 16 out of 67 papers meeting the criteria were included in the meta-analysis.

In papers selected for the meta-analysis, an average of 31.36 kg/day for MY (range: 17.3 kg/day to 44.3 kg/day), an average of 39.21 °C for RT (range: 38.5 °C to 40.2 °C) and for RR, the mean was 70.55 breaths/min (range: 53.4 breaths/min to 85.8 breaths/min). According to the classification made by Armstrong (1994), 13 studies were in the mild stress group (72 ≤ THI ≤ 79), and three studies were in the moderate stress group (80 ≤ THI ≤ 89). The variation between the numbers of experimental animals was quite high (range: eight cows to 2 357 cows). Since the majority of the papers were conducted in the Holstein cows (except one), the breed comparison could not be made.

THI calculation and classification

THI is calculated with the following formula according to the National Research Council (1971):

$$THI = (1.8 \times AT + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times AT - 26.8)] \quad (1)$$

where:
AT – ambient temperature (°C);
RH – relative humidity (%).

According to the classification made by Armstrong (1994), the situation in which the animal does not have a thermal comfort zone or heat stress corresponds to values below THI < 72.

Statistical analysis

The database which includes information about THI values, MY, RR, RT, race, ambient tempera-

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ture, and the region where the study was conducted from 16 studies selected for the meta-analysis was generated in Microsoft Excel 2010 program. First, the principal component analysis (PCA) was performed to determine the global distributions of dairy cattle and the associations with THI, milk yield and some physiological responses. The Kaiser-Meyer-Olkin (KMO) measure was determined to detect the adequacy of the sample number for PCA. The KMO value was obtained as 0.794 and according to KMO > 0.5 criterion determined by Kaiser and Rice (1974), it was decided that the sample number was sufficient for PCA. In order to determine the suitability of THI, RR, RT and MY features for PCA, the diagonal values of the anti-image correlation matrix, which consists of Bartlett's test (Chi-square = 202.70; $P < 0.05$) and measures of sampling adequacy (MSA) scores, were examined and as a result, significant correlations were found between features (MSA > 0.50). The "HCPC" function of "FactoMineR" R package was used to compute PCA and AHC (Le et al. 2008). The "factoextra" R package was used. The PCA and AHC analyses were computed in the R environment (https://cran.r-project.org). In the meta-analysis, firstly, the heterogeneity test was performed to determine the variation between the studies. The Cochran-Q test (Cochran 1954) was used to determine heterogeneity between independent studies. The percentage of total variation (I^2) between the runs was obtained by the heterogeneity test. The I^2 statistic was calculated as follows:

$$I^2 = \frac{Q - (k - 1)}{Q} \tag{2}$$

where:

Q – heterogeneity statistic with a chi-square distribution;

k – number of runs.

Depending on the calculated size of the I^2 statistic, the more accurate interpretations of the inter-study variation will be possible. According to the study performed by Higgins et al. (2003) if $I^2 < 25\%$ low heterogeneity; $25\% < I^2 < 50\%$ moderate heterogeneity and $I^2 > 50\%$ is considered as high heterogeneity. Meta-analyses were performed in the Comprehensive Meta-Analysis v3 (Biostat, Inc., Englewood, NJ, USA).

RESULTS

Responses of physiological parameters and milk yield to the change of THI

PCA was performed to examine the relationships of THI, MY, RR, and RT. The equamax extraction method was performed to determine the number of factors explaining the total variation between variables in PCA and the first two dimensions with the highest rate of explaining the total change were determined (PC1 = 45.4% and PC2 = 29.9%) (Table 1).

In Table 1, the PC1 size showed a high correlation with RR and RT characteristics. At the same time, these two features are the two variables that contributed the most to the high explanation rate of PC1. This dimension is named for these two features. The PC2, on the other hand, has a high correlation with THI and MY features, and these two features contribute to PC2 explanation rate.

The scree plot was obtained from PCA and biplot graphics, in which the variables are shown in two-dimensional graphics (Figure 1). The first two components with an eigenvalue greater than 1 were determined in the scree plot graph and the biplot graph was designed according to these two components. In the biplot graphics, the PC1 and PC2 explained 75% of the total variability between the variables. The PC1 made the highest contribution to the explanation rate of variability. In Figure 1, depending on the increase in THI, the milk yield decreases while RR and RT increase. In particular, RR and RT increased linearly parallelly to each other depending on the value of THI.

Table 1. Correlations and contributions of variables to PC1 and PC2 axes

Variables	Variables' correlations		Variables' contributions (%)	
	PC1	PC2	PC1	PC2
THI	0.008	0.819	1.050	39.126
RT	0.935	0.042	40.810	4.171
RR	0.839	0.320	39.745	1.096
MY	0.485	–0.650	6.948	35.841

MY = milk yield; PC1 = first principal component of PCA; PC2 = second principal component of PCA; RR = respiratory rate; RT = rectal temperature; THI = temperature humidity index

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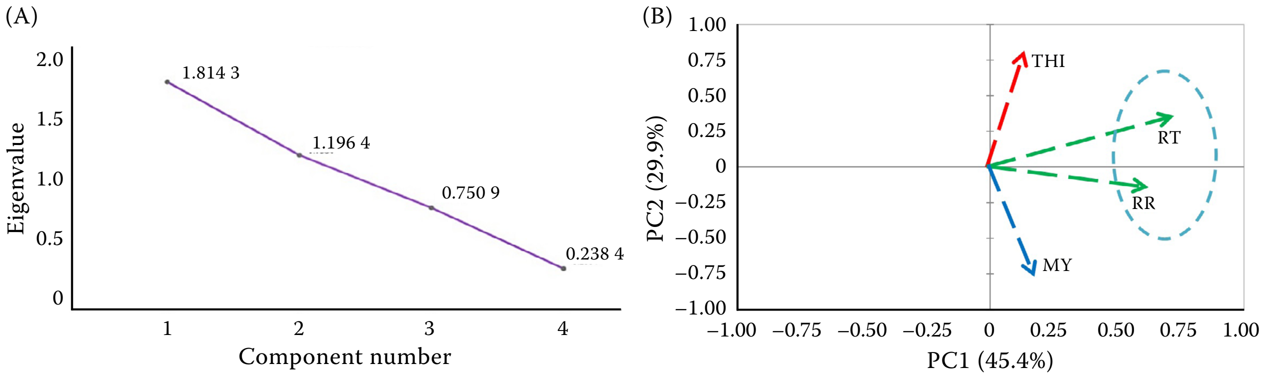

Figure 1. PCA plot evaluated variables of heat-stressed dairy cattle

(A) Scree plot of eigenvalues and cumulative variability; (B) correlations between temperature humidity index (THI) and milk yield (MY), respiratory rate (RR) and rectal temperature (RT)

PC1 = first principal component of PCA; PC2 = second principal component of PCA

Results of mixed meta-analysis

Details of the meta-analyses were presented based on the effects for mixed models. The heterogeneity of effect sizes was tested (ES) and quantified (I^2) and the obtained results are shown in Table 2. THI values ranged from 73 to 84 in 16 studies combined with meta-analysis. High heterogeneity was determined between studies combining milk yield, RT and RR results ($I^2 > 99$; $P < 0.001$).

The mixed meta-model was used as the combined model in order to reduce the heterogeneity and calculate the correct effect sizes. The heat stress had a moderate effect on both milk yield and physiological parameters, and these effects were statistically significant ($P < 0.000\,1$). The mean effect sizes on milk yield and respiratory rate were approximately 50% and 65%, and 38% at rectal temperature. Therefore, when THI > 72, respiratory rate and milk yield are more responsive than heat stress.

The forest plot is also given to show the summary of effect sizes for milk yield and physiological parameters (Figures 2–4).

The variance column in the graph shows the effect size of each study. Accordingly, the effect sizes of the studies vary between 0.735 and 226.000. Heat stress negatively affected milk yield in all studies ($P < 0.001$). However, since the variations in the effect sizes are very wide, the reductions in milk yield are quite different from each other. In the bar chart, it can be seen that studies with high variance and wider confidence intervals have lower values for the "weight" title, which varies between 4.9% and 6.7%, contribute less to the combination according to milk yield characteristics. Studies that contributed less to the combination had fewer animals and greater variation for milk yield than those that contributed more. Studies with higher contributions have low variance, and narrow confidence intervals. Unlike the fixed effects model, the forest plot assumes that each study has a different effect in the mixed model. Therefore, the weighted pooled estimations of milk yield differ from each other because the model assumes that the differences between studies are due to random effects.

Table 2. Effect of heat stress on milk yield and physiological parameters

Variables	Number of trials	ES	SE	95% CL		*P*	Heterogeneity	
							I^2	*P*
MY	16	50.289	3.038	44.335	56.243	0.000	99.93	< 0.001
RT	16	38.978	0.091	38.801	39.156	0.000	99.08	< 0.001
RR	16	65.152	3.652	61.994	76.310	0.000	99.72	< 0.001

ES = effect size; I^2 = heterogeneity as a percentage of total variability; MY= milk yield; PC1= first principal component of PCA; PC2= second principal component of PCA; RR= respiratory rate; RT= rectal temperature; SE = standard error; THI= temperature humidity index

Significance was accepted as $P < 0.001$

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Model	Study name	Statistics for each study				Weight (Random)		Residual (Random)			
		Variance	Lower limit	Upper limit	p-Value	Weight (Random)	Relative weight	Residual	Std Err	Std Residual	P-Val
	Ahmed et al. 2017	226.000	163.535	222.465	0.000	0.001	4.9	130.10	28.35	4.59	0.00
	Pinto et al. 2020	134.200	95.595	141.005	0.000	0.001	5.5	55.40	26.69	2.08	0.04
	Hall et al. 2018	105.542	70.865	111.135	0.000	0.001	5.7	28.10	26.14	1.08	0.28
	Perano et al. 2015	56.000	151.333	180.667	0.000	0.001	6.1	103.10	25.18	4.10	0.00
	Shapasand et al. 2010	56.000	81.333	110.667	0.000	0.001	6.1	33.10	25.18	1.31	0.19
	Yan et al. 2020	48.000	106.421	133.579	0.000	0.001	6.2	57.10	25.02	2.28	0.02
	Pan et al. 2014	30.000	64.265	85.735	0.000	0.002	6.4	12.10	24.66	0.49	0.62
	Gebremedhin et al. 2010	20.000	51.235	68.765	0.000	0.002	6.5	−2.90	24.45	−0.12	0.91
	Khan et al. 2018	14.764	4.029	19.091	0.003	0.002	6.5	−51.34	24.35	−2.11	0.03
	do Amaral et al. 2011	13.748	24.933	39.467	0.000	0.002	6.5	−30.70	24.32	−1.26	0.21
	Osei-Amponsah et al. 2020	12.740	15.934	29.926	0.000	0.002	6.5	−39.97	24.30	−1.64	0.10
	Kaufman et al. 2020	9.280	22.179	34.121	0.000	0.002	6.6	−34.75	24.23	−1.43	0.15
	Amamou et al. 2019	7.155	1.257	11.743	0.015	0.002	6.6	−56.40	24.19	−2.33	0.02
	Liang et al. 2013	5.645	12.773	22.087	0.000	0.002	6.6	−45.47	24.16	−1.88	0.06
	Dado-Senn et al. 2020	1.339	5.312	9.848	0.000	0.002	6.7	−55.32	24.07	−2.30	0.02
	de Andrade Ferrazza et al. 2017	0.735	19.219	22.581	0.000	0.002	6.7	−42.00	24.06	−1.75	0.08

Figure 2. Forest plot of the effect of heat stress on milk yield in dairy cattle

Model	Study name	Statistics for each study				Weight (Random)		Residual (Random)			
		Variance	Lower limit	Upper limit	p-Value	Weight (Random)	Relative weight	Residual	Std Err	Std Residual	P-Val
	Amamou et al. 2019	5.367	34.660	43.740	0.000	0.186	0.6	0.14	2.31	0.06	0.95
	de Andrade Ferrazza et al. 2017	4.265	34.452	42.548	0.000	0.234	0.8	−0.56	2.06	−0.27	0.78
	Liang et al. 2013	1.871	36.299	41.661	0.000	0.534	1.8	−0.08	1.36	−0.06	0.95
	Kaufman et al. 2020	1.224	36.462	40.798	0.000	0.817	2.8	−0.43	1.09	−0.40	0.69
	Osei-Amponsah et al. 2020	1.222	36.463	40.797	0.000	0.818	2.8	−0.43	1.09	−0.40	0.69
	Gebremedhin et al. 2010	1.100	37.644	41.756	0.000	0.909	3.1	0.64	1.03	0.62	0.54
	Yan et al. 2020	0.991	36.749	40.651	0.000	1.009	3.4	−0.36	0.98	−0.37	0.71
	Ahmed et al. 2017	0.693	37.869	41.131	0.000	1.443	4.9	0.44	0.81	0.54	0.59
	Pinto et al. 2020	0.660	37.408	40.592	0.000	1.515	5.1	−0.06	0.79	−0.08	0.94
	Pan et al. 2014	0.632	37.611	40.729	0.000	1.581	5.3	0.11	0.77	0.14	0.89
	Hall et al. 2018	0.548	36.549	39.451	0.000	1.826	6.1	−1.06	0.72	−1.48	0.14
	Khan et al. 2018	0.509	37.212	40.008	0.000	1.964	6.6	−0.45	0.69	−0.66	0.51
	do Amaral et al. 2011	0.367	37.803	40.177	0.000	2.728	9.2	−0.07	0.58	−0.13	0.90
	Shapasand et al. 2010	0.316	38.178	40.382	0.000	3.162	10.6	0.22	0.53	0.41	0.68
	Perano et al. 2015	0.200	38.623	40.377	0.000	5.000	16.8	0.44	0.41	1.07	0.28
	Dado-Senn et al. 2020	0.167	38.278	39.882	0.000	5.976	20.1	0.02	0.37	0.05	0.96

Figure 3. Forest plot of the effect of heat stress on rectal temperature in dairy cattle

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In the results of physiological parameters given in Figures 3 and 4, the rectal temperature (Z = 429.603; P < 0.001) and respiratory rate (Z =18.936; P < 0.001) of heat stress in the forest graph of the studies combined according to the mixed model were found to be significant. Since the rectal temperatures in the studies were close to each other, the effect sizes (variance) were obtained close to each other. While the first two studies contributed the least (0.6%, 0.8%), the last three studies contributed the most (10.6%, 16.8%, and 20.1%). Since the rectal temperatures in the studies included in the combination were close to each other, it is possible to say that the effect of heat stress on rectal temperature could not be clearly determined.

Contribution rates in respiratory rate are similar in all studies (Figure 4). Therefore, the combined results show that there is a tendency of an increase in respiratory rate due to heat stress. As a matter of fact, according to Table 2, since the respiratory rate has a larger effect size than the rectal temperature, it appears as the parameter that is primarily affected by heat stress.

Mild and moderate stress groups were created by using the THI values in the studies included in the meta-analysis, and the mean changes of MY, RR and RT according to these groups are examined in Figure 5. MY and RR are the features most affected by changes in THI. The RR is high in both mild and moderate stress situations. A significant increase was observed especially in the moderate stress group. RT was almost unchanged in both stress groups.

Model	Study name	Statistics for each study				Weight (Random)		Residual (Random)			
		Variance	Lower limit	Upper limit	p-Value	Weight (Random)	Relative weight	Residual	Std Err	Std Residual	P-Val
	Yan et al. 2020	73.361	39.013	72.587	0.000	0.003	5.2	−13.50	16.81	−0.80	0.42
	Amamou et al. 2019	67.976	44.641	76.959	0.000	0.003	5.3	−8.50	16.65	−0.51	0.61
	Osei-Amponsah et al. 2020	47.800	73.419	100.521	0.000	0.004	5.7	17.67	16.04	1.10	0.27
	Pan et al. 2014	36.176	57.311	80.889	0.000	0.004	5.9	−0.20	15.67	−0.01	0.99
	Kaufman et al. 2020	26.209	51.216	71.284	0.000	0.004	6.2	−8.05	15.35	−0.52	0.60
	Gebremedhin et al. 2010	24.400	81.318	100.682	0.000	0.004	6.2	21.70	15.29	1.42	0.16
	do Amaral et al. 2011	18.330	69.609	86.391	0.000	0.004	6.4	8.70	15.09	0.58	0.56
	Hall et al. 2018	16.432	21.605	37.695	0.000	0.004	6.4	−39.55	15.03	−2.63	0.01
	Khan et al. 2018	15.274	47.880	63.200	0.000	0.004	6.4	−13.76	14.99	−0.92	0.36
	de Andrade Ferrazza et al. 2017	14.255	46.400	61.200	0.000	0.004	6.5	−15.50	14.95	−1.04	0.30
	Liang et al. 2013	11.116	90.085	103.155	0.000	0.004	6.5	27.32	14.85	1.84	0.07
	Ahmed et al. 2017	11.085	42.974	86.026	0.000	0.004	6.5	10.20	14.85	0.69	0.49
	Dado-Senn et al. 2020	8.701	53.029	64.591	0.000	0.004	6.6	−10.49	14.77	−0.71	0.48
	Shapasand et al. 2010	7.779	81.493	92.427	0.000	0.004	6.6	17.66	14.74	1.20	0.23
	Perano et al. 2015	3.200	75.994	83.006	0.000	0.004	6.8	10.20	14.58	0.70	0.48
	Pinto et al. 2020	1.300	60.395	64.865	0.000	0.004	6.8	−6.67	14.51	−0.46	0.65

Figure 4. Forest plot of the effect of heat stress on respiratory rate in dairy cattle

DISCUSSION

The principal component analysis performed before the meta-analysis showed that for every unit increase in the temperature humidity index (THI) above 72, milk yield decreased, while respiratory rate and rectal temperature increased. It has been reported in many studies that the threshold value of THI is 72 and above for the negative effect of heat stress on milk yield and physiological parameters in Holstein cows (West 2003; Amamou et al. 2019).

Although high heterogeneity in classical studies is undesirable, it means that it is accurate and reliable regarding the variances of the parameters included in the study in the meta-analysis (Higgins et al. 2003). In the mixed meta-model, the most effective parameter was respiratory rate, followed

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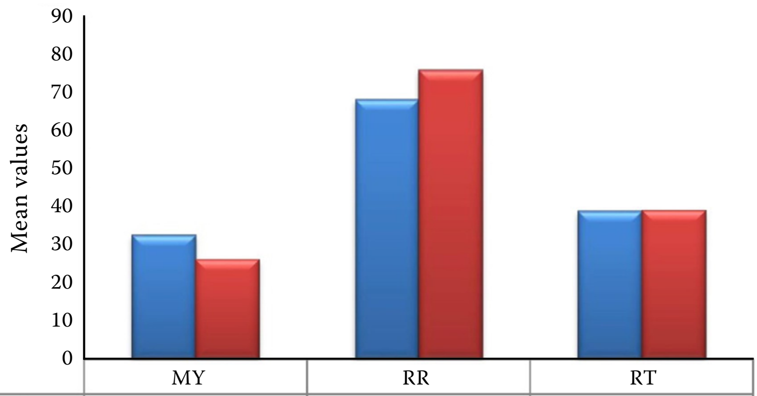

	MY	RR	RT
72 ≤ THI ≤ 79 (mild stress)	32.825	68.465	39.167
80 ≤ THI ≤ 89 (moderate stress)	26.433 3	76.276 3	39.391 8

Figure 5. Differences in mean milk yield (MY), respiratory rate (RR) and rectal temperature (RT) in studies combined for mild and moderate stress groups

by milk yield and rectal temperature. The mentioned parameters were in the class of parameters that are moderately (between 0.20 and 0.80) affected by heat stress (Cohen 1988). It was determined that the heat stress had a significant moderate effect on both milk yield and physiological parameters ($P < 0.000\,1$). However, considering the effect sizes of the parameters, it is possible to say that milk yield and respiratory rate are the characteristics most affected by heat stress. In line with the results obtained in our study, Najar et al. (2011) reported in a meta-analysis study that the respiratory rate and milk yield increased in parallel with the increase in THI.

Forest plots are a good tool for visual representations of effect size data to effectively present results combined in a meta-analysis (Lipsey and Wilson 2001). In this study, except for one study (4.9%), the variations of the other studies examined in terms of milk yield were close to each other, and they contributed to the combination at a similar rate (5.5–6.7%). It is possible to associate the differences between the contributions of the combined studies with random effects (Hedges and Olkin 1985). In the studies examined in the forest plot of milk yield, the effect of heat stress on milk yield was found to be significant at ≥ 76 values, which is the breakpoint for THI ($P < 0.000\,1$). Similarly, Amamou et al. (2019) stated that for the response to each unit change in the THI (breakpoint = 77), there was a significant decrease in milk yield. Ekine-Dzivenu et al. (2020) stated that daily milk yield was the highest in the THI range of 61–66 and the lowest in the THI range of 79–81. The threshold value of THI was reported as 82, with negative effects on milk yield reported by Dado-Senn et al. (2020).

The variability in contribution rates is high according to rectal temperatures (0.6–20.1%), both in milk yield and respiratory rate. Although rectal temperature has been accepted as a good indicator of thermoregulatory capacity (Godyn et al. 2019) in recent studies, it was not found as an effective parameter like milk yield and respiratory rate in our study. In this case it can be thought that the rectal temperature increases at the values when the heat stress is more severe. Likewise, Najar et al. (2011) reported that the change in rectal temperatures showed a significant increase in THI values of 90 and more.

Some studies have reported that there is a 3% (Dado-Senn et al. 2020) and 7% (Pinto et al. 2020) increase in rectal temperature in parallel with per unit increase in THI values of 70 and above. However, some investigators have reported minor changes in rectal temperatures (e.g. an increase from 38.5 °C to 40.4 °C) in the range of 55 ≤ TI ≤ 84 (Wheelock et al. 2010; Gantner et al. 2017).

In our study it was determined that respiratory rate was the most affected feature in both mild and moderate stress conditions. The respiratory rate in dairy cows is accepted as a reliable and early indicator of heat stress (Gaughan et al. 2000). However, when THI ≥ 72, there is an increase in the respiratory rate of dairy cows (Cook et al. 2007). In the study conducted by Pinto et al. (2020), the respiratory rate of dairy cattle increased on average by 2.9 per hour when THI = 70 while standing, accordingly THI = 65 and respiration increased by one unit in the lying position. Similarly, when THI ≥ 65, the

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animals' respiratory rate increased by an average of 2.5 breaths/min due to per unit increase in THI (Dado-Senn et al. 2020)

CONCLUSION

We investigated the effect of heat stress on milk yield and some physiological parameters in dairy cattle by meta-analysis. As the THI value increases, the respiratory rate increases and the milk yield decreases. This increase, especially the respiratory rate, gives a clear response. There is an increase in rectal temperatures, but not as remarkable as in the respiratory rate. In order to acquire more reliable and detailed information in future meta-analysis studies on heat stress, it may be suggested that new individual studies should be designed by considering different levels instead of THI threshold values.

Conflict of interest

The authors declare no conflict of interest.

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