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### **Sensitivity of Dairy Buffalo to Temperature-Humidity Index: Preliminary Study**

#### **Fiorella SARUBBI1\* , Gennaro PICCIRILLO<sup>2</sup> , Giuseppe AURIEMMA<sup>3</sup> , Giuseppe GRAZIOLI<sup>4</sup> , Raffaele PAPPALARDO<sup>5</sup>**

1-5 Institute for the Animal Production System in the Mediterranean Environment, National Research Council, 80055 Portici, NA, ITALY

<sup>1</sup>https://orcid.org/0000-0002-9241-5718, <sup>4</sup>https://orcid.org/0009-0005-3683-0609, <sup>2</sup>https://orcid.org/0009-0002-8197-9929, <sup>3</sup>https://orcid.org/0009-0000-0981-5108 <sup>5</sup>https://orcid.org/0009-0004-6726-936X

\* Corresponding author: fiorella.sarubbi@cnr.it



#### **INTRODUCTION**

The buffalo species is known for its remarkable ability to adapt to various environments. In subtropical regions, buffaloes are commonly raised due to their tolerance for high temperatures and humidity. Their high resistance to parasites and ability to efficiently utilize food in time of scarcity have made it possible to establish economies in swampy areas (Bengis et al., 2023)

Currently, the primary focus of buffalo breeding and husbandry is milk production for dairy processing. Buffalo farmers' foresight in investing in agriculture was the reason for this change, who continued to invest in the potential of these animals despite concerns from analysis, that the reclamation of the swampy territories: would lead to a decline in buffalo populations.

Instead, the number of buffaloes in Italy has increased significantly, with the country now leading Europe with 400 thousand heard.

The thermal environment plays a crucial role in the performance of farm animals, as temperatures outside the thermo-neutral range can cause stress. Dairy cattle are particularly sensitive to temperature fluctuations (Wathes et al*.*, 1983), with ideal performance occurring between 5-15 °C (Hahn, 1999), while buffaloes thrive between 5 and 25 °C (Dash et al., 2015)

Thermal stress can have negative impacts on feed intake, growth efficiency, and overall animal production and reproduction (Ravagnolo and Misztel*,* 2002; West et al*.,* 2003; Gantner et al*.*, 2015), especially in temperature climates.

Studies on dairy cattle, in Africa (Du Preez et al., 1990), Australia (Davison et al., 1996), and the Indian subcontinent (Zewdu et al., 2014) have highlighted the detrimental effects of heat stress on livestock productivity.

According to the Intergovernmental Panel on Climate Change (IPCC), the average temperature of the Earth will gradually increase by 1.4°C during this century, reaching 5.8°C above its historical average. The increased frequency of heat waves will have serious effects especially on dairy cow milk production, thus leading to crucial changes in animal farming for species with hereditary compensatory and adaptive mechanisms to overcome the heat stress (Lacetera, 2019). The expansion of breeding the most thermo-tolerant animal species is foreseeable to sustain animal production. Buffaloes, having originated and being mainly distributed in tropical and subtropic environments, present anatomy and physiology mechanisms, such as sparse hairs and substantial melanin pigment in the epidermis, that are able to counter hot and humid climate conditions rather than the cooler conditions of internal areas (Serrapica et al., 2022). Buffalo leather's sweat glands are 6 times less dense than cowhide, resulting in less efficient heat dissipation through sweating (Marai and Haeeb, 2010). Petrocchi Jasinski et al. (2023) reported that for buffalo, a value of THI <72 is considered optimal, THI between 72 and 79 is considered mild stress, THI from 80 to 89 is considered moderate stress, and THI ≥90 is considered severe stress. In Mediterranean Italian buffalo exposed to hot conditions, a lower milk yield was registered (De Rosa et al., 2009). THI variances have been employed to calculate the effects of climate change on livestock management strategies to overcome heat stress exposure (Morgado et al*.,* 2023). Yadav et al*.* (2022), studying the effect of THI on milk yield and on some physiological parameters of lactating Murrah buffalo cows during the summer season, observed a reduced milk yield when passing from 80 to 85 THI, as well as an increase in respiration rate and an alteration of leukocyte populations, and of urea nitrogen in the blood. Costa et al*.* (2020), investigating the effect of THI on milk composition and

coagulation traits of Italian Mediterranean buffalo throughout a whole year, found a negative relationship between THI and fat, casein, and lactose content in milk together with a positive correlation between THI and milk urea nitrogen. According to Matera et al*.* (2022), Italian Mediterranean buffalo cows need an optimal THI range of between 59 and 63 to sustain milk yield, quality, and udder health. Furthermore, Vale's study (2007) recommended that THI above 75 has detrimental effects on the reproductive performance of buffaloes.

In Italy, buffalo farming, particularly for "Mozzarella" production, has expanded beyond region like "Lazio" and "Campania" to other part of Italy, including the north.

The economic advantage of buffalo milk, with higher yields and commercial value compared to cow milk, has contributed to its increased popularity.

The Italian Mediterranean Buffalo population has grown steadily, 12,000 registered animals in 1947 to approximately 413 thousand today. Italy now accounts for 95% of the European buffalo population, with the ratio of buffalo milk to cow milk increasing continuously in the country. This study will test how the effects of THI on milk yield and reproductive performance vary with regional differences.

# **MATERIALS and METHODS**

The meteorological records obtained from a weather archive, while data related to production and reproductive aspects were sourced from the Italian Breeders' Association (AIA) archive. The dataset consists of approximately 34,000 registrations collected between January and December 2022. The selection of 2022 was based on its status as one of the warmest years in recent history. Weather-related information includes environmental temperature, humidity, wind speed, and dew point. Production aspects included kilograms of milk produced per lactation, fat and protein percentages, and lactation duration. Reproductive data includes the conception interval.

Approval for animal welfare was not required for this study since the data originated from pre-existing databases.

The data was divided into northern Italy (Veneto, Piedmont, Lombardy, Friuli and Emilia-Romagna), central (Lazio, Marche and Tuscany) and southern Italy (Abruzzo, Basilicata, Calabria, Campania, Molise, Puglia and Sicily).

For this study, production parameters such as total milk production in the total lactation (total MY, kg/lactation), protein percentage (PP), fat percentage (FP), lactation length (days) and conception interval were considered. To account for the varying sizes of the individual parameters, the data were normalized by converting them into log10.

Temperature humidity index (THI) has been widely used as an indicator of thermal stress (Ravagnolo et al., 2002; Gantner et al., 2015; Vitali et al., 2009).

Heat stress is caused by a combination of temperature, relative humidity, solar radiation, air movement, and precipitation. Many studies on heat stress in livestock focus on the two main environmental stressors: ambiental temperature and relative humidity.

Based on the availability of maximum and minimum temperature and relative humidity (Ravagnolo et al*.,* 2002; St-Pierre et al., 2003), this study calculated THI equation:

THI=  $(9/5 ET + 32) - (11/2 - 11/2 RH) * (9/5 ET + 26)$ 

where ET=environmental temperature and RH=relative humidity. ET is expressed in degrees Celsius and RH is expressed as percentage.

Water vapor concentration of the air is important since it can drastically reduce the ability of the animal to use evaporative heat loss through the skin and lungs.

Data are presented as the mean ± SD. One-way ANOVA was used to assess the effect of THI level on the quality characteristics of milk and certain reproductive aspects. Tukey's test was used as a post hoc test to identify the mean values that differed significantly from each other (SPSS® v27, 2017). Values of P<0.01 and P<0.05 were considered to indicate statistically significant differences.

# **RESULTS and DISCUSSION**

Descriptive statistics for each trait are shown in Table 1. When Table 1 is analyzed, the differences in weather conditions between the regions examined are revealed. Class 1 had a maximum temperature of 10.34-19.82 °C, class 2 had a temperature of 13.22-22.15 °C, and class 3 had a temperature of 13.01-21.94 °C. It is also apparent that thermal excursions are present in the central areas of Italy as reported by Wathes et al. (1983). In all the cases examined, the southern regions consistently show higher values. The daily relative humidity reached a maximum of 70% in all 3 classes. When analyzing the data on milk production, there was no discernible difference between northern and southern Italy, while the central region exhibited lower milk production.

Similar trends can be observed in both qualitative and productive aspects. For milk production and characteristics similar results were found by Albenzio et al. (2024). The data regarding differences in lactation length is interesting, as it appears that even if statistical significance is not reached, the length of lactation is lower than in central Italy.

Table 1. Descriptive Statistics for milk yield total (MYtot), fat percentage (FP), protein percentage (PP), environmental temperature (ET), dew point, relative humidity (RH), lactation length, conception interval, and THI.

Parameters	<b>Class</b>	Units	<b>Means</b>	<b>SD</b>	<b>SEM</b>
Environmental	$\mathbf{1}$	$\rm ^{\circ}C$	15.32 <sup>b</sup>	8.56	0.078
Temperature	2	$\prime\prime$	17.69a	7.76	0.103
	3	$\prime\prime$	17.47a	7.31	0.058
Dew point	$\mathbf{1}$	$\rm ^{\circ}C$	9.45c	6.45	0.058
	$\overline{2}$	$\prime\prime$	10.53 <sup>b</sup>	5.66	0.075
	3	$\prime\prime$	11.77a	6.25	0.050
<b>Relative Humidity</b>	$\mathbf{1}$	$\%$	62.20c	20.29	0.187
	2	$\prime\prime$	63.99b	14.34	0.191
	3	$\prime\prime$	68.99 <sup>a</sup>	12.34	0.098
Milk Yield Total	$\mathbf{1}$	$\mathrm{Kg}$	249	413.15	3.74
	$\overline{2}$	$\prime\prime$	2272	282.93	3.76
	3	$\prime\prime$	2381	334.30	2.66
Fat	$\mathbf{1}$	$\frac{0}{0}$	7.85	0.32	0.002
	$\overline{2}$	$\mu$	7.37	0.56	0.007
	3	$\boldsymbol{\mathcal{U}}$	7.75	0.52	0.004
Protein	$\mathbf{1}$	$\%$	4.62	0.11	0.01
	$\overline{2}$	$\%$	4.63	0.16	0.02
	3	$\%$	4.72	0.50	0.03
Lactation Length	$\mathbf{1}$	Day	254.65	10.09	0.097
	$\overline{2}$	$\prime\prime$	239.10	23.23	0.308
	3	$\boldsymbol{\mathcal{U}}$	248.89	10.33	0.084
Conception Interval	$\mathbf{1}$	Day	156a	65	0.59
	$\overline{2}$	$\prime\prime$	117 <sup>b</sup>	48	0.64
	3	$\prime\prime$	158 <sup>a</sup>	110	0.88
THI	$\mathbf{1}$		64.24	5.74	0.040
	$\overline{2}$		65.41	2.80	0.037
	3		64.56	3.89	0.031

Class: 1=North, 2=Central, 3=Southern. a, b and c: p<0.05

The conception interval also shows this trend. This has resulted in statistical significance. The coefficient correlation ( $r=0.019$ ;  $p<0.01$ ) between the class conception rate appears to be highly significant, suggesting a close relationship between environmental conditions and their effects on relevant aspects.

Table 2 displays the correlation between THI and various aspects of milk quantity, quality, and reproduction.

	Milk	Milk	Milk	Conception	Lactation
	kg	fat	protein	interval	length
THI	$0.442**$	$-0.784**$	$-0.468**$	$0.496**$	$0.288**$

Table 2. Correlation between THI and milk production and reproduction characteristics

#### $*$  $P<0.01$

Table 2 shows a close association between the THI and all the parameters considered, indicating a negative effect on the quality of milk. The raw data in Table 1 reflects these results as well. Bouraoui et al. (2002) found similar results in a study on dairy cows, quantifying a 21% reduction in milk production related to the TH index. They attributed the decrease in feed intake in animals to temperature and humidity. The same condition may also be linked to the relationship between the TH index and reproductive parameters. An increase in temperature and humidity can lead to difficulty breathing and, increased stress on animals. This condition is even more pronounced in buffalo for the physiological reasons described in the introduction. Muller et al. (1994) also observed similar results.

Figure 1 displays a clear and effective quantitative relationship between THI and the quantity of milk produced. All subgroups of THI are represented in the data, covering all production levels. The figure clearly illustrates that when THI value range between 65 and 70, there is a higher expression of productive potential. Conversely, when the value drops below 60, lower potential is evident. These results emphasize the significant impact on the index on the productivity of the herd. THI and milk quality are both subject to the same criteria, as shown in Figure 2.





and milk yield

Figure 1. Subgroup effect between THI Figure 2. Subgroup effect between THI and milk quality

Upon examination of the figure, when compared to the previous one it is evident that the influence of THI on the quantity of milk produced is not significant, especially on production aspects, such as the protein content of milk, despite significant correlation coefficients. There is a particularly low effect: around the value of 55.

The relationship between THI and the conception interval is illustrated in Figure 3.





and Conception interval

Figure 3. Subgroup effect between THI Figure 4. Subgroup effect between THI and Lactation length

In this case, it is evident that at THI between 60 and 70, there is a longer period between delivery and subsequent conception. Hahn et al. (1999) also found similar results in a study on the effect of temperature and humidity on pregnancy rate. The breeding of milk buffalo has become increasingly industrialized, requiring significant attention to fertility, which play a crucial role in determining economic yield. Having data that enables the farm to objectively evaluate fertility trends is essential. In 2023, Nishisozu *et al*. investigated the impact of the temperature-humidity index on the conception rate of heifers and Holstein cows. The conception rate at THI values between 61-65 and 71-75 was higher than at THI > 75, while the other THI values had no effect. They report, in conclusion, that THI values greater than 75 negatively affect the rate of conception.

As depicted in Figure 4, which correlates the length of lactation with to the TH index, this influence is further.

It appears that the TH index is most susceptible when it has lower values for both productive and reproductive aspects, as suggested by the results. This data is supported by the fact that buffalo, which are subtropical animals, use energy reserves to maintain thermal homeostasis when subjected to thermal stress, influencing both

production and reproductive capacity. Nasr et al. (2010) and Yehia et al. (2020) have studied these results. Significant changes in fat and protein content in milk composition have been observed in relation to THI, especially for THI values above 62. Thermal stress in cattle has been associated with a decrease in fat and protein percentages, unlike studies conducted in humans (Arieli et al., 2004; Rejeb et al., 2012; Bernabucci et al., 2015; Nasr et al., 2017).

# **CONCLUSION**

The results of this preliminary study have shown the potential impact of the TH index on both productivity and reproduction. Based on our study, we can suggest an ideal THI range for this species in the specific environmental conditions of the study (between 60 and 70). Farmers should prioritize managing the farm to create environmental conditions that will support rather than hinder the essential productive and reproductive aspects crucial for farm profitability. Different farmers should conduct more comprehensive studies to further define the effects of THI limit values on the well-being and production of buffalo in intensive farming conditions.

# **Conflict of Interest Statement**

The authors have declared that there are no competing interests.

# **Authors' Contribution**

FS contributed to the project idea, design and execution of the study. GP, RP, GA, GG conducted the laboratory analyses. FS and GP supervised the experiment and wrote the manuscript.

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