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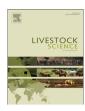
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Heat stress affects reproductive performance of high producing dairy cows bred in an area of southern Apennines

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ABSTRACT

A 5-year retrospective (from 2008 to 2012) survey was carried out by analyzing data of high producing dairy cows reared in farms located in an area of southern Apennines. The indicators of fertility obtained were related to either season variations or temperature-humidity index (THI). Conceptions were evaluated per month on an annual basis (NCY), i.e., a parameter obtained by subtracting gestation length to the calving date. A significant reduction of NCY was found during the summer months; furthermore, this parameter decreased along with THI increase. The number of heats detected varied similarly to NCY and represented the main cause of lower fertility consequent to heat stress (HS). The age at first calving was not significantly affected by either the season or the THI. The mean number of AI/pregnancy in relation to the calving date was significantly affected by the season but it was not related to THI. The number of days open was significantly larger in the animals calved from January to July than from August to December (163 ± 33 ys 123 ± 36 days; P < 0.001); this causes an annual economic loss of several thousand euro in each farm analyzed. In conclusion, HS causes severe economic loss in dairy farms located in southern Apennines that is mainly due to a lower number of heats detected as well as to a larger number of days open and semen doses used.

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1Introduction

Declining productive and reproductive performance of livestock in tropical and subtropical areas is a well-known phenomenon that limits high production dairy cattle breeding (Gauly et al., 2013; Kadzere et al., 2002). Global warming and the breeding of selected animals that are more and more sensitive to environmental effects have made this phenomenon, named heat stress (HS), particularly relevant even in temperate areas (Ferreira, 2013; Nardone et al., 2010). The origin of HS comes from the attempt to counteract hyperthermia induced by the high environmental temperature using mechanisms of thermal dispersion. These mechanisms are, however, also influenced

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by other input variables, such as relative humidity, ventilation, radiant energy and rainfall (Igono et al., 1992). Complex indexes, which combine some of the above climate parameters involved, have been proposed to monitor the effects of HS. The temperature-humidity index (THI), that combines the maximum temperatures with the minimum relative humidity, is the most used one (Ravagnolo and Misztal, 2000).

The infertility caused by the HS is due to both direct and indirect causes that affect reproductive performance. In particular, HS may act directly by reducing the quality of the gametes. In the male, there is an increase of oligoastheno-teratospermia (Meyerhoeffer et al., 1985). In the female, several alterations are described either in the processes of folliculogenesis, as prolongation of follicle dominance and co-dominance phenomena (Badinga et al., 1993; Wolfenson et al., 1995) with a significant reduction in follicular estradiol (Badinga et al., 1994), or luteogenesis,

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 as well as a delayed luteolysis (Wilson et al., 1998b) and a compromised luteal activity during the postpartum period
 (De Rensis et al., 2008). The follicle and oocyte maturations are compromised and the mature oocyte has a reduced
 competence for fertilization and embryonic development (Mihm et al., 1994; Rispoli et al., 2011; Roth, 2008).
 Lowering of the estrogen levels by the growing follicles translates into a lower intensity of estrous signs (Badinga

9 et al., 1994). This latter finding has been recently challenged by novel considerations on the role of GnRH in the
 11 control of estrus behavior in ruminants (Caraty et al.,

2002). The decrease of GnRH levels during stress (Smith and Dobson, 2002) suggested a possible involvement of GnRH in decreasing estrus behavior signs in cows during

15 the HS (van Eerdenburg, 2008b).

The reproductive failures attributable to HS involve 17 follicular growth and oocvte function even before the antral phase (42 days) or primary follicle (85 days) 19 (Lussier et al., 1987; Picton et al., 1998; Torres-Junior et al., 2008) and are prolonged up to two or three estrous 21 cycles from the end of the high environmental temperature (Roth et al., 2001). These effects are reduced with the 23 progress of the embryo-fetal development (Biggers et al., 1987). Indirect effects due to HS may consist in a reduction 25 of dry matter intake, which is responsible for deepening and extension of the negative energy balance postpartum 27 that results in a decreased fertility (Lucy et al., 1992). Further problems related to HS are attributable to an

ruriner problems related to HS are attributable to an
increase of some reproductive diseases, such as ovarian cysts (Lopez-Gatius, 2003). Dairy cows with high productive performance are more sensitive to environmental effects (Kadzere et al., 2002) and HS causes a decrease of
sexual cyclicity and increases the incidence of inactive ovaries (Lopez-Gatius, 2003).

35 Several techniques have been proposed in order to counteract the effects of HS on reproductive activity, as the 37 use of awnings, showers, fans (Hansen and Arechiga, 1999; Kendall et al., 2006; West, 2003); however, these strategies 39 can alleviate the productive inefficiency without solving reproductive failures. New strategies to combat infertility 41 are geared to accelerate the follicle renewal by hormonal treatments or ovum pick-up (Roth et al., 2001) as well as by 43 using reproductive procedures that do not require estrus detection, as fixed-time artificial insemination (Arechiga 45 et al., 1998) and embryo transfer (Al-Katanani et al., 2002).

The purpose of this study was to evaluate in an Apennine area of Southern Italy, namely the province of Potenza, the magnitude of the effects exerted by HS on reproductive performance in high producing dairy cows, the consequent economic loss and the compliance of the 51 THI for HS evaluations. The geographical area considered is characterized by a Mediterranean climate with mountain 53 winter temperatures, hot summer and large diurnal temperature variations. 55

2Materials and methods

2.1Animals

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Reproductive data from January 2008 to December 2012 were collected from a total number of 1743 Holstein Friesian (HF) cows bred in three dairy farms (named A, B and C) in the province of Potenza. Cows were barn-housed throughout the year and milked twice a day. Average milk production in 305 days was 9656 ± 188 kg and ranged among farms from 9534 (Farm C) to 9873 (Farm B) kg. The voluntary waiting period varied between 40 and 45 days. Clinical veterinary assistance was provided by private veterinarians employed by the Basilicata <u>Breeders'</u> Association (ARA). Data were collected on the dates of birth, calving, insemination and culling. The pregnancy length was postulated to the last 284 days on the basis of the information provided by the Italian Holstein Friesian Association (ANAFI) and updated to 2012.

2.2Estimation of reproductive parameters

Based on the above collected data, reproductive parameters were developed (Ferguson and Skidmore, 2013), as follows: number of conceptions evaluated per month on a per year basis (NCY). The conception date has been obtained by subtracting postulated pregnancy length (i.e., 284 days) from the calving date. The NCY was obtained by relating the number of conceptions on a month basis to the total conceptions per year. Number of heats detected per month on a per year basis (HDY) was calculated by relating the number of heats followed by an AI on a month basis to the total number of heats followed by AI detected each year. Conception Rate (CR) has been obtained by relating, on a month basis, the number of cows which conceived following an AI to the number of AI performed. Age at first calving (AFC), was the number of days from the birth to the first calving. Number of AI/pregnancy (NAIP) was the number of inseminations necessary to obtain a full term pregnancy. These data were related to the month of the previous calving. Number of days open (DO) was obtained by subtracting the postulated pregnancy length (i.e., 284 days) to the number of days between two consecutive parturitions (VanRaden et al., 2004). These data were related to the month of the first of the two parturitions.

2.3Climate data

Daily weather records from 2008 to 2012 were obtained from the three most nearby meteorological stations (less than 3 km) located at the same altitude of the examined dairy farms. These records were used to estimate means, variances, and covariances of monthly minimum and maximum temperatures, minimum and maximum relative humidity, and to calculate the temperature–humidity index (THI), using the standard THI equation in which the maximum temperature (*T*) was combined with the minimum humidity (*H*) (Ravagnolo and Misztal, 2000), as follows:

THI = (((9/5)T) + 32) - ((11/2) - (11/2)H)(((9/5)T) - 26))/100

THI classes were ranged according to arbitrary intervals in order to simplify further data analysis. A total of 9 THI classes were obtained, as follows: < 70, 70–79, 80–89, 90–99, 100–109, 110–119, 120–129, 130–139, and > 140.

Weather data were retrieved from the archives of the Agenzia Lucana di Sviluppo ed Innovazione in Agricoltura (ALSIA).

2.4Economic evaluation

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An economic assessment of the reproductive efficiency decline due to HS can be mainly evaluated considering the lowering of fertility caused by a reduction in the HDY and CR as well as the increase in the NAIP and the DO. The lower HDY and CR result in lengthening of DO, whose economic consequences have been recently evaluated in The Netherlands (van Eerdenburg, 2008a). The factors included in this estimation were (1) the loss of milk with respect to the EU milk quota system; (2) the loss of income due to less calves born per year; (3) problems around calving, like mastitis, negative energy balance and related problems (this aspect is positive in the case of short calving intervals but became negative in the case of long calving intervals). The economic losses were put in relation to the productive level of the dairy farms and evaluated in relation to the calving interval, considering 365 - d = day 0 for economic evaluation (van Eerdenburg, 2008a). A multiple regression linear analysis was performed by SigmaPlot (SigmaPlot for Windows, 11.0 release) on the values estimated by van Eerdenburg (R=0.974; P < 0.001) in order to obtain a model that could be adapted to our analysis. In particular

Economic loss = 42.9 - (0.460Y) + (0.245D)

where Y=average production of the dairy farm and D=calving interval-365 days.

For our economic evaluation, the cost of semen doses was not considered since it varied largely in relation to managerial choices.

2.5Statistical analysis

Statistical analysis was performed using Linear Regression and ANOVA (Systat 11.0 release) and including variables as year and farm in the model. Season variations were evaluated on a monthly basis. Before statistical processing, all parameters, expressed as annual incidence rate, were arcsine transformed before assessing the homogeneity of variance. Pairwise mean differences were evaluated by Fisher's least-significant-different (LSD) test. Data are presented as least squares means and standard deviations.

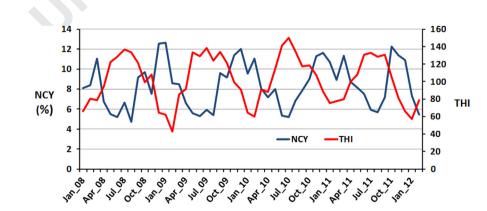
3Results

The analysis of climate data did not reveal significant differences among the evaluated years. However, a farm effect was found (P < 0.05). During the hottest months (i.e., June, July and August) Farms A and C showed, respectively, the lowest minimum temperature (Farm A=8.1 + 1.5 °C vs Farm $B = 11.6 + 2.0 \degree C$ and Farm $C = 11.0 + 1.9 \degree C$; P < 0.01) and the lowest maximum temperature (Farm $C = 33.3 + 2.1 \degree C$ vs Farm A=37.6 + 2.1 °C and Farm C=38.6 + 2.2 °C; P < 0.01). THI was significantly affected by the season, with a monthly variation ranging between 42 and 150, but it did not significantly vary among years and farms. High variability was also found in relation to the atmospheric temperature range (TR), *i.e.*, a parameter obtained by the numerical difference between the daily minimum and maximum values of temperature calculated on a monthly basis. In particular, this parameter significantly (P < 0.001) changed in relation to either the farm or the year and increased (P < 0.001) together with THI. On annual basis, TR showed a typical curvilinear pattern with the lowest value in January $(15.5 \pm 5.0 \degree C)$, a progressive increase peaking in August $(23.1 \pm 3.4 \degree C)$ and again a decline up to December (18.0 ± 4.2 °C).

The analysis of calving data distribution clearly showed a seasonal pattern which did not strictly fit with climatic variations (data not shown). When NCY was evaluated in relation to THI, the patterns of these two parameters showed a mirror trend (Fig. 1). NCY was significantly affected by either THI (P < 0.001) or month (P < 0.001). Comparing the THI classes to NCY, a significant (P < 0.01) decrease was observed along with the increase of THI (Fig. 2); this relationship was expressed by a straight linear regression with R = -0.437 (P < 0.001).

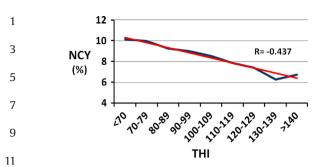
The incidence of HDY showed a pattern similar to NCY when related to THI. This trend was significantly affected by both THI (P < 0.04; R = -0.329) and months (P < 0.001). CR showed a pattern that was grossly opposite to THI and

Fig. 1. Temporal trends of the number of conceptions evaluated per month on a per year basis (NCY) and temperature-humidity index (THI) evaluated as the average of the three farms examined.



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Fig. 2. Relationship between the number of conceptions evaluated per month on a per year basis (NCY) and temperature-humidity index (THI) independently of farm and year effects. The straight line represents the linear trend regression.

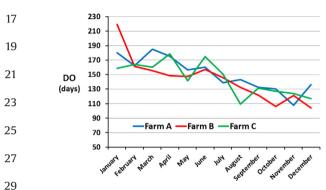


Fig. 3. Relationship between the number of days open evaluated in relation to the calving month (DO) and temperature-humidity index (THI) classes in the three farms evaluated independently on the year effect.

significantly affected by the year (P < 0.003). However, CR 35 was not significantly affected by either months or THI although CR significantly decreased in the period between 37 April and August with respect to the other months of the year (27.3% vs 35.4%; P < 0.01), with a sharp drop between 39 90 and 100 THI (R = -0.303).

AFC did not show statistically significant differences in relation to the year, month, THI and farms, ranging in relation to the latter parameter from 26 to 27 months.

43 Distribution of the NAIP was not significantly affected by THI but it was affected by either farm (P=0.003) or 45 month (P < 0.001). In particular, a lower number of AI was needed to obtain a pregnancy in the August–December 47 period with respect to January–July (2.37 ± 0.72 vs

3.06 ± 0.86; *P* < 0.001).
Evaluating the number of DO in relation to the month

of calving, a significant effect of the season (P < 0.001), but
 not of the THI, was found. In relation to the season, there
 was a significant reduction in the number of DO between
 August and December compared to January–July (123 ± 36

vs 163 ± 33 days; P < 0.001); the difference between these two periods ranged among farms from 36 to 45 days

(Fig. 3).
In Table 1, the total economic loss attributable exclusively to the greatest number of DO observed in animals
given birth in the first seven months of the year was evaluated and found to range among farms from 40.435 to

61 69.379 €/year.

Table 1

Estimation of economic loss produced by lengthening the calving interval, evaluated as number of days open, in the January–July (J–J) period compared to the August–December (A–D) period in the three dairy farms surveyed.

Farm	Days open		Cows calving on January–July	Economic loss cow/	Economic loss per
	J–J A (days) (A-D days)	period (n.)	day ^a (€)	year (€)
А	166 a 1	130 b	219	8.8	69.379
В	161 a 1	122 b	108	9.6	40.435
С	162 a 1	117 b	92	11.0	45.540

a,**b** (*P* < 0.01).

where Y=average production of the dairy farm and D=calving interval-365 days.

^a On the basis of the estimated values from van Eerdenburg (2008a, 2008b) and calculated by using an model obtained by multiple linear regression analysis as follows: economic loss = 42.9 - (0.460Y) + (0.245D).

4Discussion

The results of this study clearly demonstrated that the reproductive performance of dairy cows is greatly influenced by the season and that the THI allows only partly to highlight the relationship between HS and reproductive efficiency, as already suggested by other studies (Dikmen and Hansen, 2009). Similarly to Pregnancy Rate (Ferguson and Skidmore, 2013), NCY is affected by either HDY/heat detection rate or CR. Both these indicators of fertility are involved in determining the reproductive failures due to HS in cattle (Roth. 2008). Lower NCY during the hot season may be the result of either a decreased detection of estrus or a lower quality of gametes and embryos whose direct consequence is a lower CR. During the hottest period, the frequency of heats detected was significantly reduced. either when related to the season, expressed as months. or to THI. CR did not show statistically significant differences in relation to either the season or the THI. This may be due to the extension of the effects of HS even in months following the high environmental temperatures (Roth et al., 2001). In addition, the large atmospheric temperature range recorded in Apennine areas in the years analyzed may have weakened the negative effects of HS on the quality of gametes responsible for CR lowering, as recorded in other geographical areas (Roth, 2008). This information clearly indicates that the reduction in fertility observed during summer, as assessed by NCY, may be mainly due to a poor identification of animals in heat, as found in a study carried out in Argentina (Ferreira, 2013). This result shifts the focus to potential improvements in reproductive efficiency of the analyzed cows by using different strategies to correct the low HDY, as heatwatch, pedometer, or fixed-time artificial insemination.

AFC seemed not to be significantly affected by climate. This finding suggests a relative refractoriness of heifers to HS, as found in previous studies (West, 2003). Other studies (Wilson et al., 1998a) reported similar changes of ovarian function in heifers and lactating cows under HS-induced hyperthermia (Wilson et al., 1998b); however, heifers showed either a lower increase of the estradiol preceding estrus or did not show long estrous cycles when

compared to cows (Wilson et al., 1998a). Our data suggest that a good management of the distribution of calving dates of the heifers could be a reliable strategy to reduce the seasonal variability of milk production and/or to plane calving towards the period that provides the lowest number of DO.

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7 Another seasonal effect on the reproductive activity is the difference in DO in relation to the calving date; in fact, g a larger number of DO has been recorded in cows calved from January to July with respect to those calved in the August-December period. In this study, a possible expla-11 nation of this finding may be sought in the fact that the minimum mean number of DO, calculated on a monthly 13 basis, was 116 days: the cows calving after January might have overcome their negative energy balance postpartum 15 and rescued their reproductive activity after April, when 17 temperatures responsible for HS occur. Conversely, those cows calved after July overcame this situation by matching 19 their reproductive recovery with a more favorable climate. Since the animals calved in the period from January to July 21 were on average 42.9% of the annually calved animals, it follows that the HS affected the calving interval with an average annual increase of 17.1 days. A similar bimodal 23 distribution of DO has been found in a study aimed at 25 evaluating a seasonal pattern of DO in relation to states and regions within the United States (Oseni et al., 2003). 27 The discrepancy in the number of DO between the first and the second semester of the year was influenced by regional and seasonal variations; it increased in the South-29 east and partially in the Southwest and Midwest states 31 together with HS increase.

NAIP follows trends similar to those observed for DO as33expected due to the correlation between these two indicators (R=0.679; P < 0.001). However, the increase of NAIP35during the period from January to July provides further37that can be attributed to a CR decrease in addition to
reduced HDY.

39 The economic loss due to DO (or calving interval) increase represents an issue considered by different authors though 41 the estimates provided for these summary-indicators are quite discrepant (Gonzalez-Recio et al., 2004; Meadows et al., 43 2005). Methodological differences and economic context differences between areas and times are responsible for this, like frequently occurs in animal health economics (Seegers 45 et al., 1998). In addition, few papers focused primarily on the impact of reproductive indicators, as the CR (Boichard, 1990) 47 or the heat detection rate (de Vries and Conlin, 2003), and only some of these studies have considered the economic 49 aspects of seasonality on reproductive failures, as evaluated 51 under Finnish (Rajala-Schultz et al., 2000) or Florida (De Vries, 2004; Silva et al., 1992) conditions. To our knowledge, 53 this is the first study on the economic impact of heat stress on dairy cows in Italy. In addition, the peculiar location of the dairy farms examined, i.e., the southern Apennines, a moun-55 tainous area relatively far from the sea and characterized by large daily temperature ranges throughout the year, gave this 57 study a particular interest. Alongside the well-known production loss related with HS (West, 2003), the role of 59 reproductive inefficiency in production loss is less obvious 61 but equally significant. This latter damage extends several

weeks after temperatures reaching the values of thermal comfort. Even without considering the economic damages related to the cost of the semen, the economic loss is considerable and requires the referral of strategies to limit it.

In conclusion, heat stress caused many failures on reproductive activity in dairy cows reared in southern Apennines, <u>i.e.</u>, a lower number of heats detected and conceptions as well as a larger number of days open. Strategies should be ranged to counteract the effects of heat stress on reproduction in order to attenuate the relevant economic loss caused by high environmental temperatures.

Author disclosure statement

The authors declare that no conflicting financial interests exist.

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