rccp Revista Colombiana de Ciencias Pecuarias

Effects of heat stress on reproductive efficiency of high yielding Holstein cows in a hot-arid environment¤

Efecto del estrés calórico sobre la eficiencia reproductiva de vacas Holstein de alta producción de leche en un ambiente cálido y árido

Efeitos do estresse térmico sobre a eficiência reprodutiva de vacas leiteiras da raça Holandês de alto rendimento em um ambiente quente e árido

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(Received: May 4, 2012; accepted: October 31, 2012)

Summary

Background: high environmental temperature coupled with high humidity can result in reproductive failure in dairy cattle, with a drastic reduction in reproductive efficiency of dairy herds. **Objective**: to study the effect of high environmental temperature on reproduction performance of Holstein cows treated with recombinant bovine somatotropin (rbST) throughout lactation in an arid environment. **Methods**: reproductive variables (n=18,037 services) from a large dairy herd were evaluated with respect to the maximum temperature-humidity index (THI) prior to breeding, on the breeding day, and after breeding. The GENMOD procedure of SAS was used to assess the effect of THI and month of breeding on pregnancy by artificial insemination (P/AI). **Results**: increased THI from ≤ 70 to ≥ 95 units was associated with a decrease in P/AI from 47% to 26%. P/AI for cows inseminated on extremely hot days (THI= 85 to 90) preceded by cooler temperatures was six percent points higher than cows subjected to high temperatures before breeding. P/AI was higher ($p<0.05$) from January to March (39% to 41%) compared with the rest of the year (27% to 35%). The average number of inseminations per pregnancy was higher ($p<0.05$) from May to July (3.0 to 3.4) compared to other months (2.1 to 3.0). **Conclusions**: in this particular hot-arid environment (maximum temperature >38 ºC most of the year, and 230 mm mean annual rainfall), heat stress shortly before or after breeding severely compromises the breeding success of high yielding Holstein cows.

¤ To cite this article: Mellado M, Sepulveda A, Meza-Herrera C, Veliz FG, Arevalo JR, Mellado J, De Santiago A. Effects of heat stress on reproductive efficiency in high yielding Holstein cows in a hot arid environment. Rev Colomb Cienc Pecu 2013; 26:193-200.

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Key words: *days open, fertility, rbST, services per pregnancy.*

Resumen

Antecedentes: la asociación entre temperaturas ambientales elevadas y alta humedad conduce a fallas reproductivas, disminuyendo la fertilidad de hatos lecheros. **Objetivo**: determinar el efecto de temperaturas ambientales elevadas sobre el comportamiento reproductivo de vacas Holstein mantenidas en un ambiente árido y cálido y tratadas con hormona del crecimiento (rbST) durante toda la lactancia. **Métodos**: las variables reproductivas (n=18037 servicios) de una explotación comercial fueron evaluadas con respecto al máximo índice temperatura humedad (THI) antes, durante y después de la inseminación de las vacas. El procedimiento GENMOD de SAS se utilizó para determinar el efecto del THI y el mes de inseminación sobre las tasas de preñez (P/AI). **Resultados**: el incremento del THI de ≤ 70 a ≥ 95 unidades se asoció con una disminución en el P/AI de 47% a 26%. El P/AI para las vacas inseminadas en días con un ITH de 85 a 90, pero con temperaturas menos cálidas antes de la inseminación, fue de seis puntos porcentuales más altos (30% vs. 36%) que en las vacas expuestas a ITH más altos antes de la inseminación. El P/AI fue mayor (p<0,05) de enero a marzo (39% a 41%) comparado con el resto de los meses del año (27-35%). El número de servicios por preñez fue mayor (p<0,05) de mayo a julio (3,0 a 3,4) que en los otros meses del año (2,1 a 3,0). **Conclusiones**: en este ambiente extremadamente caliente (temperaturas máximas > 38 °C la mayor parte del año) y árido (promedio de precipitación anual de 230 mm), el estrés calórico poco antes o después de la inseminación disminuye drásticamente las probabilidades de establecer una gestación en vacas de alta producción de leche.

Palabras clave: *días abiertos, fertilidad, rbST, servicios por preñez.*

Resumo

Antecedentes: a associação entre alta temperatura e umidade no ambiente pode originar problemas reprodutivos, diminuindo o índice de fertilidade dos rebanhos leiteiros. **Objetivo**: avaliar o efeito da elevada temperatura ambiente sobre o desempenho reprodutivo de vacas da raça Holandês mantidas num ambiente quente e árido, as quais foram tratadas com somatotropina bovina recombinante (rBST) durante toda a lactação. **Métodos**: as variáveis reprodutivas (n = 18037 serviços) de um rebanho leiteiro foram avaliadas em relação ao máximo índice de temperatura-umidade (THI), antes, durante e depois da inseminação artificial das vacas. O procedimento GENMOD do SAS foi usado para avaliar o efeito do THI e do mês de reprodução sobre as taxas de gestação por inseminação artificial (P/AI). **Resultados**: o aumento do THI de ≤ 70 para ≥ 95 unidades foi associado com a diminuição no índice P/AI, de 47% a 26%. Em dias com THI de 85 - 90, mas com menor temperatura ambiental antes da inseminação, o índice P/AI foi 6% maior do que nas vacas expostas a superior THI. O índice P/AI foi maior (p<0,05) de janeiro a março (39% a 41%), em comparação aos outros meses do ano (27 a 35%). O número médio de inseminações por prenhez foi superior (p<0,05) de maio a julho (3,0 a 3,4) quando comparado aos outros meses do ano (2,1 a 3,0). **Conclusões**: em ambiente quente e seco (temperaturas máximas > 38°C a maior parte do ano, média de precipitação anual de 230 mm) o estresse térmico antes e depois da inseminação reduz drasticamente a possibilidade de estabelecer uma gestação em vacas de alta produção de leite.

Palavras chave: *dias abertos, fertilidade, rbST, serviços por prenhez.*

Introduction

The adverse effects of high environmental temperatures in temperate and tropical regions on the reproductive processes of dairy cattle have been well documented. They include an increased incidence of estrus-detection failures due to reduced estrus duration and intensity (Wolfenson *et al*., 1988; Younas *et al*., 1993), as well as reduced motor activity and other estrus signs (Hansen and Arechiga, 1999). Failures also include alterations of endocrine environment and follicular development patterns (Shehab-El-Deen *et al*., 2010) leading to low oocyte competence to develop into blastocysts (Ferreira *et al*., 2011), persistence of dominant follicles, and reduced quality of oocytes (Roth, 2008). High temperatures also negatively affect embryo survival rate prior to attachment (Ealy *et al*., 1993; García-Ispierto *et al*., 2006).

Most studies reporting negative effects of high environmental temperatures on dairy cow fertility were published more than 20 years ago when milk yield was much lower than it is today and before the commercial use of recombinant bovine somatotropin (rbST) in dairy cows. Genetic selection for milk production coupled with the use of rbST throughout lactation increases the metabolic heat production (Wheelock *et al*., 2010), which in turn increases susceptibility to high environmental temperatures. Thus, it is pertinent to revisit the impact of heat load on high yielding cows.

The objective of this study was to determine the relationship between environmental temperature and pregnancy by artificial insemination (P/AI) in a herd of high yielding Holstein cows. The hypothesis proposed that high temperature-humidity index (THI) on the day of breeding, 3 days prior to breeding, and 3 days following breeding are associated with reduced likelihood of pregnancy.

Materials and Methods

Herd, housing and feeding

The study was conducted in a large commercial dairy herd located in northeastern Mexico (26° 23' N, 104° 47' W; elevation 1140 m; mean annual temperature 27 ºC; mean annual rainfall 230 mm). This study followed institutional guidelines approved by the Animal Care and Use Committee of The Antonio Narro Agrarian Autonomous University. These guidelines are based on the norms set by the Canadian Council on Animal Care (1993).

The herd included approximately 5,000 lactating Holstein cows housed in open, dirt pens. All pens were equipped with electric fans installed on shade roofs. The animals were fed total mixed diets formulated to provide recommended total daily nutrients (1.62 Mcal/kg NEl, 18% crude protein) for 670 kg dairy cows producing > 33 kg milk/d (NRC, 2001). Cows were fed *ad libitum* four times per day at 06:00, 10:00, 12:00, and 16:00 h. Feed refusal constituted approximately 10% of the daily offer.

Throughout the study cows were milked three times per day (06:00, 14:00, and 21:00 h) in milking parlors. All cows were administered rbST (Lactotropin, 500 mg of zinc bovine somatotropin, Elanco Animal Health, Mexico) via subcutaneous injection every 14 d beginning at 60 days in milk (DIM) and continuing until 2 weeks before drying off. Lactation order in the study varied from 1 to 8, with cow body condition scores ranging from 2.75 to 3.5 (scale 1 to 5). The average milk yield for 305 days was approximately 13,200 kg throughout the duration of the study. The average number of inseminations was 3.7 (range = 1 to 14 inseminations). The average DIM was 234 (standard deviation = 89).

Reproductive management

Cows were routinely vaccinated against bovine viral diarrhea, infectious bovine rhinotracheitis, bovine respiratory syncytial virus, parainfluenza, and leptospirosis (with the five most common serovars, and all in the same vaccine; Bayovac Horizon 9®, Bayer of Mexico, Mexico City, Mexico). Inseminations began after 50 d of calving. The cows received typical reproductive management, which included estrus detection at 08:00 and 17:00 h, using the am/pm-breeding rule; well-trained herd personnel performed AI. Pregnancy status was assessed 45 to 50 days after AI by transrectal palpation, conducted by the herd veterinarian.

Breeding records representing 18,037 services were analyzed. Data were screened to include only cows that were lactating, had at least one service or a maximum of 8 services, had an interval to first service greater than 40 d and less than 160 d, with no previous abortion or hormonally induced lactation, and cows not culled <50 d after the last service. Lactation number and sire used for breeding were registered. P/AI was defined as the number of cows pregnant at 45 to 50 d after-service divided by the total number of cows bred.

Meteorological data

For the duration of the study, meteorological data were obtained from a station located 2 km away. Information, consisting of daily maximum temperatures and relative humidity, was used to calculate the temperature-humidity index (THI;

highest daily temperature in Celsius degrees; RH refers to maximum relative humidity) for each day using the following equation (Mader, 2003):

$$\text{THI} = (0.8 \times \text{temperature}) + [(\%\ \text{RH}/100) \times (\text{temperature} - 14.4)] + 46.4$$

Since nighttime conditions were warm during most of the year it is believed that an unimportant underestimation of the heat load existed. Likewise, given that wind speed was low at the study site and that this weather component in combination with other climatic variables has not been a good predictor of dairy cattle fertility (AL-Katanani *et al*., 1999), this variable was not included in the study.

Statistical analysis

The GENMOD procedure of SAS (SAS Institute, Cary, NC, USA) was used to assess the effect of THI (independent variable) on P/AI (dependent variable). The main effects in the models were THI at AI or 1 or 3 days before or after breeding; lactation number of cows and interval to service were included in the model as covariates. To test the effect of previous or subsequent THI with respect to THI on the day of service, THI classes were defined as 75 to 80, 80 to 85 and 85 to 90. The model used for P/AI was:

$$Y_{ijk} = \mu + b_1(X_k) + b_2(Z_k) + A_i + D_j + e_{ijk}$$

Where:

μ = overall mean;

$b_1(X_k)$ = covariate adjustment for lactation number;

$b_2(Z_k)$ = covariate adjustment for individual cow's previous interval to service;

A_i = temperature classes (75 to 80, 80 to 85 and 85 to 90 for maximum temperature on the day of breeding);

Dj = deviations of temperature 1 or 3 days before or 1 or 3 days after the day of breeding;

e_{ijk} = residual effect.

The GENMOD procedure of SAS was also used to assess the statistical significance of the effect of month of breeding on P/AI. Year and THI on the days of breeding were included in the analysis as covariates; therefore, pregnancy results are reported as covariate-adjusted means. Treatment means were separated using the probability of a statistical difference (PDIFF option of SAS). Statistical differences were considered significant at $p \leq 0.05$. The effect of month of breeding on the number of services per pregnancy was evaluated by the bivariate Wilcoxon rank sum test (proc npar1way; SAS) without adjustment for confounders. Days open for pregnant cows were analyzed by the LIFETEST procedure of SAS using both strata and time statements (SAS Institute Inc., Cary, NC, USA).

Exponential regression and Pearson correlation analyses were used to determine the relationship between THI and P/AI. For this particular analysis THI was divided into six classes, with the first class being <80; subsequent classes were set at each four units thereafter until the last class, which was >96.

Results

The nonlinear relationship between THI increase on the breeding day and P/AI is shown in figure 1. The P/AI ratio decreased sharply when THI exceeded 70 to 75 units. In contrast, P/AI decreased sharply when THI exceeded 70 to 75 units. Pregnancy rates declined to a lesser extend with THI >75 units. As THI increased >95 P/AI dropped from 32% to 26%. Eighty five percent of the total P/AI variation was accounted for by the THI on the breeding day.

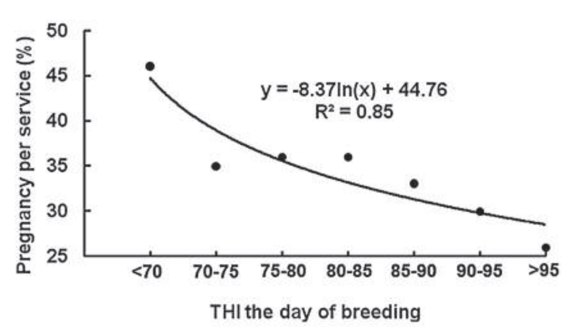

Figure 1. The relationship between temperature-humidity index (THI) and number of pregnancies per insemination based upon all services (n= 18,037 services, grouped according to THI classes).

When heat load was as its maximum on the breeding day (THI=85 to 90), low environmental temperature 1 or 3 d prior to breeding increased the likelihood of pregnancy ($p<0.05$; Table 1). Similarly, low THI 3 d after breeding improved P/AI ($p<0.05$). With less heat (THI=80 to 85) at breeding, low THI 3 d prior to breeding tended to increase P/AI ($p<0.07$). Likewise, low THI 1 or 3 d post-breeding tended to increase P/AI ($p<0.08$). With mild heat stress (THI=70 to 80), low THI 1 d post-breeding increased P/AI ($p<0.05$).

Table 1. Pregnancy changes per artificial insemination when the temperature-humidity index (THI) before or after breeding was cooler than on the breeding day (D_0).

THI D_0	D_{-1}		D_{-3}		D_{+1}		D_{+3}	
	n	%	n	%	n	%	n	%
85-90								
THI < D_0	968	36^{A}	972	36^{A}	1043	35^{A}	1026	36^{A}
THI > D_0	861	29^{B}	1389	30^{B}	913	32^{A}	1364	31^{B}
80-85								
THI < D_0	2428	36^{A}	942	37^{a}	1108	37^{a}	1108	38^{a}
THI > D_0	342	32^{A}	1328	34^{b}	992	33^{b}	1051	34^{b}
75-80								
THI < D_0	688	39^{A}	949	34^{A}	1059	39^{A}	603	37^{A}
THI > D_0	859	36^{A}	513	35^{A}	598	34^{B}	557	37^{A}

D_0= day of breeding, D_{-1}= one day before breeding, D_{-3}= three days before breeding, D_{+1}= one day after breeding, D_{+3}= three days after breeding. ^{A,B}Means within columns with different superscript differ ($p<0.05$). ^{a,b}Means within columns with different superscript tend to differ ($p=0.07$ to 0.08).

Influences of breeding month on fertility are presented in table 2. The P/AI decreased from April to December; the highest P/AI were observed in the winter months (approx. 40%). The time to first breeding was shorter among cows bred during fall, relative to those calving in other seasons. Likewise, cows bred in autumn showed lower days open than cows calving in other seasons. Services per pregnancy were higher among cows bred for the first time in spring and summer, relative to those bred in autumn and winter.

Discussion

THI effect on breeding

The overall P/AI (33%) was lower than that reported for artificially inseminated dairy cows in warm climates of the southeastern United States (Jordan, 2003; Huang *et al*., 2008), but higher than values for dairy operations in Georgia and Florida (De la Sota *et al*., 1998; De Vries and Risco, 2005). The low P/AI observed in the present study was apparently due to a drastic heat stress experienced by cows, associated with great metabolic heat load due to the high milk yield (Wheelock *et al*., 2010) derived from the rbST treatment and exacerbated by the environmental heat gain through solar radiation

Table 2. Reproductive variables of Holstein cows in a hot-arid environment, as a function of the breeding month. Values are means ± SD.

Month	n	Pregnancy per AI %	Calving to first breeding, days	Days open	Services per pregnancy
January	484	41^{a}	68 ± 36^{a}	122 ± 85^{bc}	26 ± 20^{de}
February	485	43^{a}	66 ± 23^{abc}	115 ± 76^{c}	26 ± 19^{de}
March	469	39^{a}	66 ± 29^{ab}	124 ± 86^{abc}	28 ± 23^{dc}
April	426	32^{bc}	67 ± 36^{ab}	135 ± 93^{a}	31 ± 24^{ab}
May	463	34^{b}	65 ± 30^{abc}	136 ± 86^{a}	34 ± 25^{a}
June	470	31^{bcd}	63 ± 33^{abcd}	123 ± 79^{abc}	30 ± 22^{bc}
July	528	27^{d}	63 ± 34^{abcd}	127 ± 73^{ab}	32 ± 21^{ab}
August	603	28^{d}	61 ± 38^{dc}	115 ± 66^{c}	30 ± 19^{bc}
September	797	28^{cd}	58 ± 31^{d}	104 ± 59^{d}	28 ± 18^{dc}
October	881	31^{bcd}	60 ± 36^{dc}	98 ± 58^{d}	24 ± 15^{ef}
November	661	35^{b}	62 ± 29^{bcd}	95 ± 59^{d}	21 ± 14^{f}
December	449	31^{bcd}	66 ± 42^{ab}	103 ± 70^{d}	23 ± 16^{f}

^{abcd} Within columns, means with different superscript differ ($p<0.01$)

input, elevated environmental temperature, mild air movement and poor night cooling.

The logarithmic association between THI and pregnancy rate found in the present study, showing that pregnancy rate diminishes with increasing environmental temperature, is congruous with patterns observed in dairy cattle maintained in hot weather, where pregnancy rates are drastically reduced when cows are exposed to high heat loads before, during, or after breeding (Garcia-Ispierto *et al*., 2007; Morton *et al*., 2007; Ben Salem and Bouraoui, 2009).

For the THI >80, the P/AI in the present study was sensitive to high THI prior or after breeding. Fertility has been found to be responsive to heat stress exerted 12 days before estrus, particularly on day 2 before breeding (AL-Katanani *et al*., 1999), the day after estrus (Cavestany *et al*., 1985), and days 1 to 7 d after breeding (Putney *et al*., 1989). The detrimental effect of elevated heat load before breeding is related with a low diameter of the dominant follicle and biochemical alterations of the follicular fluid (Leroy *et al*., 2004; Shehab-El-Deen *et al*., 2010), which result in inferior oocyte and granulosa cell quality (Ferreira *et al*., 2011; Payton *et al*., 2011). Hyperthermia of oocytes also hampers embryonic development, even in the absence of subsequent heat stress (Edwards *et al*., 2009), which compromises fertility (Lopez-Gatius, 2003). Heat stress before breeding causes delay in ovulation, and follicular persistency can lead to ovulation of a low quality, aged oocyte which is associated with low fertilization rate and embryonic mortality (Al-Katanani *et al*., 2001; Roth *et al*., 2001; Sartori *et al*., 2002).

On the other hand, hyperthermia after breeding compromises uterine environment with decreased blood flow to the uterus (Reynolds *et al*., 1985) and increased uterine temperature, which can lead to implantation failure and embryonic mortality (Hansen 2007; Morton *et al*., 2007). These effects are thought to be associated with the production of heat-shock proteins by the endometrium during heat stress (Jin-Xiang *et al*., 2009) and reduced interferon-tau production by the conceptus (Thatcher *et al*., 1994). Moreover, heat stress can affect endometrial prostaglandin secretion (Putney *et al*., 1989) causing abnormal luteal activity (Koivisto *et al*., 2008) leading to premature luteolysis and embryo loss (Ealy *et al*., 1993; Santolaria *et al*., 2010).

Month of breeding effect

The P/AI differed among months, peaking in winter months and sharply decreasing in spring and summer months (Table 2), which demonstrates a clear environmental impact on the reproductive performance of cows. Spring and summer are the warmest periods of the year in this area; thus, high environmental temperature during this period seems to explain this fertility depression. Reduction of fertility in cows during the warm months of the year has been reported in tropical and subtropical (AL-Katanani *et al*., 1999; Ben Salem and Bouraoui, 2009) as well as temperate (García-Ispierto *et al*., 2007; Nabenishi *et al*., 2011) regions of the world. This reduced summer fertility in dairy cows is due to poor estrus expression (Flores *et al*., 2004) as a result of reduced estradiol secretion from the dominant follicle developed in a low luteinizing hormone environment (De Rensis and Scaramuzzi, 2003). Heat stress has also been associated with impaired early embryo development (Ryan *et al*., 1993; Sartori *et al*., 2002) and increased embryo mortality in cattle (Wolfenson *et al*., 2000; Bényei *et al*., 2001; Hansen, 2007).

The P/AI in September and October did not differ from those values observed in summer, which suggests that the effects of heat stress had a carry-over effect into subsequent less warm months. Apparently, hyperthermia has a long-term effect on antral follicles that will develop into large dominant follicles some weeks later. Days open increased the most for cows bred during spring months, suggesting the greatest decrease in reproductive performance occurred during summer and fall. These results are in line with Cavestany *et al*. (1985). Likewise, services per pregnancy were greater from April to July, which agrees with Ray *et al*. (1992), who reported that cows calving in the spring or summer in Arizona required the highest number of services per conception.

These results reaffirm that high yielding dairy cows in a hot-arid environment at 26º N are extremely sensitive to heat load either few days before, during, or shortly after breeding. The sharp impact of heat load on P/AI is clearly manifested at THI>70. This study also shows there is a marked seasonal effect on P/AI in Holstein cows, indicating that efforts should be conveyed to minimize environmental stress of high yielding cows breeding during spring and summer in this hot arid environment.

Acknowledgments

This study was a part of the national research program, financially supported by the SAGARPA-CONACYT (Mexico); project: 2010-144591. The authors wish to thank Martha Alicia de la Rosa for her help ordering the data.

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