Universidad Autónoma Agraria Antonio Narro

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Subdirección de Posgrado

COMPORTAMIENTO REPRODUCTIVO Y PRODUCCION DE LECHE DE VACAS CON LACTANCIAS PROLONGADAS (15 A 40 MESES).

Tesis

Que presenta JESSICA MARIA FLORES SALAS

como requisito parcial para obtener el grado de:

MAESTRO EN CIENCIAS EN PRODUCCION AGROPECUARIA

Torreón, Coahuila Julio 2015

UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO

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TESIS

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JESSICA MARÍA FLORES SALAS

Elaborada bajo la supervisión del comité particular de asesoría y aprobada como requisito parcial para optar al grado de

MAESTRO EN CIENCIAS EN PRODUCCIÓN AGROPECUARIA

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DEDICATORIA

A mis padres

Maria Salas Nevarez

Mario Flores Reyes

A mis hermanos

Claudia Angelica Flores Salas
Ulises Omar Flores Salas
Emara Monserrat Flores Salas
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Por darme tanto amor y apoyarme en cada paso de mi vida

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Quien me otorgó beca durante toda la estancia de la maestria.

INDICE GENERAL

Resumen

Factores de riesgo para sufrir lactancias > 15 meses en vacas Holstein altas productoras en un ambiente cálido.

Por:

Jessica María Flores Salas

MAESTRIA EN CIENCIAS DE PRODUCCION AGROPECUARIA

Estudio epidemiológico de los factores de riesgo para lactancias prolongadas involuntarias (15 a 40 meses) utilizando una regresión logística de múltiples variables se llevo a cabo en 3278 vacas lecheras Holstein de alto rendimiento en un rebaño administrado de forma intensiva, ordeñadas tres veces al día en el norte de México. Los objetivos fueron evaluar la asociación de la duración de la lactancia (15 a 40 meses) con la producción de leche y cuantificar el efecto de múltiples servicios (4 a ≥ 14) en la preñez por inseminación artificial. Además, se realizó un análisis de supervivencia, utilizando el modelo de Cox, para probar como la ocurrencia de la preñez afecta la duración de la lactancia. La Retención de placenta (Índice de riesgo (IR) = 1.3), metritis (IR = 1.8), cetosis (IR = 1.4), pico de producción (<50 *vs* >50 kg, IR = 1.4), índice de temperatura humedad a los 60 días postparto (<82 *vs* >82 unidades, IR = 1.4), cetosis (IR = 1.4) y producción de leche de 305 días (<11,000 *vs* >11,000 kg, IR = 1.6) aumentó significativamente el riesgo de lactaciones > 15 meses. Las vacas primíparas mostraron menos de la mitad el riesgo de lactancias prolongadas (450 a 1,349 días en leche, máxima producción de leche 37,852 kg; r= 0.71) y comparado con las pluriparas (450 a 1,221 días en leche, máxima producción de leche 38,021 kg; r= 0.75). La preñez por inseminación artificial (P/IA) en vacas con lactancia prolongada disminuyó linealmente a medida que el número de servicios aumentó (P/IA 50.5 % por cuatro servicios y 12.8 % por ≥ 14 servicios). Los datos mostraron que las vacas Holstein altas productoras de manera intensive y ordeñadas tres veces al día fueron capaces de mantener lactancias de hasta 40 meses con notable persistencia de leche hasta el momento del secado. Además, este estudio coincide con los hallazgos previos que indican que los trastornos reproductivos y metabólicos derivados de parto son factores de riesgo importantes para lactancias prolongadas, derivado de la relación de las enfermedades periparturientas con la reproducción deprimida en las vacas lecheras.

Palabras clave: Lactación prolongada, vacas Holstein.

Abstract

Risk factors for undergoing lactations >15 months in high producing Holstein cows in a hot environment

By:

Jessica Maria Flores Salas

MAESTRIA EN CIENCIAS DE PRODUCCION AGROPECUARIA

An epidemiological study of risk factors for involuntary extended lactations (15 to 40 months) using a multiple variable logistic regression was carried out on 3278 high-yielding dairy cows in an intensive well-managed Holstein herd, milked three times daily in northern Mexico. Additional objectives were to assess the association of lactation length (15 to 40 months) with milk yield and to assess the effect of multiple services (4 to ≥ 14) on pregnancy per artificial insemination. Also, a survival analysis was performed using the Cox proportional hazards model to test how the occurrence of pregnancy affect lactation length. Retained placenta (odds ratio (OR) = 1.3), metritis (OR = 1.8), ketosis (OR = 1.4), peak milk yield (<50 vs >50 kg, OR=1.4), temperature-humidity index at 60 days postpartum (<82 vs >82 units, OR = 1.4), ketosis (OR = 1.4) and 305-d milk yield (<11,000 vs >11,000 kg, OR = 1.6) significantly increased the risk for lactations >15 months. Primiparous cows had less than half the risk of extended lactations (OR = 0.3) compared to multiparous cows. Once a cow had conceived, her risk of having a prolonged lactation dropped sharply (P<0.01). A strong linear association was found between lactation length and total milk yield for primiparous (450 to 1349 days in milk, maximum milk yield 37,852 kg; r= 0.71) and pluriparous (450 to 1221 days in milk, maximum milk yield 38,021 kg; r= 0.75). Pregnancy per artificial insemination (P/AI) in cows with extended lactation dropped linearly as number of services increased (P/AI = 50.5% for 4 services and 12.8% for ≥ 14 services). The data showed that well-managed Holstein cows milked three times daily were capable of lactating up to 40 months with remarkable high persistency and with high milk yield at drying-off. Additionally, this study reinforces previous findings indicating that reproductive and metabolic disorders derived from calving are important risk factors for extended lactations, derived from the link of periparturient diseases with depressed reproduction in dairy cows.

Keys words: extended lactations, Holstein cows.

I. INTRODUCCIÓN

Las principales regiones productoras de leche en México se encuentran en un ambiente caluroso y árido en la parte noreste del país. Los largos periodos de altas temperaturas y la intensa radiación solar son una de las principales causas de la drástica reducción de la fertilidad de los grandes hatos de alto rendimiento que operan en esta área (Mellado *et al.*, 2011). El rendimiento reproductivo de las vacas lecheras en climas tropicales, subtropicales y áridos es influenciado por múltiples factores tales como: el medio ambiente, nivel socio-económico de los productores, disponibilidad de alimento, adaptabilidad y composición genética del ganado, sistema de manejo (intensivo o extensivo) y la disponibilidad de tecnologías de la reproducción (Thatcher *et al.*, 2010). En empresas lecheras intensivas una práctica común es fecundar a las vacas antes de los 120 días post-parto, con el objeto de tener intervalos entre partos entre 12 y 13 meses. Estas lactancias de alrededor de 10 meses son consideradas adecuadas para maximizar la rentabilidad de los establos lecheros (De Vries, 2006; Plaizier *et al.*, 1997). Estos intervalos entre partos son factibles de lograr en zonas templadas donde el estrés térmico es mínimo, pero preñar las vacas Holstein antes de los 4 meses postparto en zonas de intenso calor es muy difícil, ya que el estrés térmico crónico y agudo disminuye sustancialmente la fertilidad de las vacas lecheras (García-Ispierto *et al.*, 2007; Mellado *et al.*, 2013).

Además, en los sistemas de producción lechera que utilizan vacas que han sido seleccionadas por su alta producción de leche han sufrido bajas en la fertilidad (Walsh *et al.*, 2011). . Lo anterior porque la vaca entra en un balance energético negativo y reduce su consumo de alimento en su periodo posparto.

Debido a esto, una gran cantidad de vacas reducen su nivel reproductivo y no pueden quedar gestante en los primeros 120 días de lactación, lo que conduce que la vaca sea sometida a tratamientos hormonales para inducir la ovulación, y si no logra quedar gestante se incrementa la tasa de desecho de estas vacas por su infertilidad (García-Ispierto *et al.*, 2007; Wu *et al.*, 2012).

Dado que estos animales se ordeñan tres veces al día y en muchos establos se usa la hormona del crecimiento, la persistencia de estos animales permite tener lactancias de más de 15 meses, lo que permite preñar las vacas mas allá de los 200 días en leche. El efecto del número de lactancias o la edad de las vacas lecheras sobre la ocurrencia de desordenes reproductivos, así como la determinación de las estaciones de mayor incidencia de estos desordenes, han sido estudiados en países localizados en latitudes más al norte que México (Reyes y Mellado, 1994). Son muchos los factores que afectan la producción y composición de la leche y pueden dividirse en dos grandes grupos: fisiológicos y ambientales. Dentro de los factores fisiológicos, la capacidad genética de los animales afecta de manera notable la producción y composición de la leche de la vaca. Otros factores no hereditarios como edad del animal, número de lactancias, gestación y longitud del periodo seco, también inciden de forma importante en la variación de la cantidad y contenido de nutrientes de la leche (Mellado, 2010). El propósito de este estudio fue determinar los factores que afectan la producción de leche y duración de la lactancia de vacas infértiles/subfértiles con un alto potencial genético para la producción de leche en lactancias de más de 15 meses, en sistemas de producción intensivo en una zona de intenso calor.

1.1. Objetivos Específicos

1. Caracterizar la producción de leche en lactancias prolongadas (15 a 40 meses) con tres ordeñas por día tanto en vacas primerizas como en vacas multíparas y en vacas subfértiles (preñez después de 6 servicios) y estériles (no preñadas después de 10 servicios).
2. Determinar los factores asociados con la ocurrencia de las lactancias prolongadas (>15 meses).
3. Determinar las tasas de concepción en vacas subfértiles/infértiles con lactancias prolongadas e inseminadas más de 6 veces.
4. Determinar la relación entre los días abiertos y la producción de leche acumulada en vacas primerizas y multíparas.
5. Determinar la tasa de preñez de vacas con más de 5 servicios (5 a 15 servicios).

1.2. Hipótesis

1. Las vacas Holstein con una alta capacidad de producción de leche y con tres ordeñas por día pueden ordeñarse por más de 900 días, sin detrimento en la rentabilidad de la producción de leche.
2. Las vacas que se inseminan por primera vez durante los días más calurosos del año son más susceptibles a presentar lactancias prolongadas involuntarias en comparación con vacas que se inseminan en días de menor calor ambiental.
3. Las vacas con trastornos derivados del parto (retención de placenta y metritis son más susceptibles a presentar lactancia prolongadas involuntarias en comparación con vacas sin problemas asociados al parto.
4. Las vacas que presentan cetosis tienen mayor riesgo de presentar lactancias prolongadas en comparación con vacas sin este desorden metabólico.
5. En vacas con lactancias de más de 450 días existe una cercana relación entre la duración de la lactancia y la producción total de leche.
6. La tasa de concepción de las vacas Holstein en ambientes cálidos presentan una declinación marcada en sus tasas de concepción a medida que se acumulan los servicios infructíferos para la gestación.

II. REVISIÓN DE LITERATURA

La producción de leche y el desempeño reproductivo juegan un papel importante en la determinación de la rentabilidad de un hato lechero. En la mayoría de los establos modernos, la práctica general es inseminar a las vacas lo mas pronto posible después del parto, con el objetivo de establecer un intervalo entre partos de 12 a 13 meses, que se considera óptimo. Sin embargo, esta visión parece ya no ser sostenible debido al incremento notable de la producción de leche de las vacas en sistemas intensivos (la producción de leche en algunos hatos de los Estados Unidos presentan un promedio anual de 14,000 kg/vaca; (Kellogg *et al.*, 2001), al uso de la hormona del crecimiento a través de la lactancia, al incremento del número de ordeñas por día y a una mejor nutrición de las vacas (uso de dietas completas). Entonces, las actuales producciones de leche ya no se adecúan a la estrategia de las lactancias de 10 meses. Por otra parte, el consumo de alimento de las vacas lecheras no se ha incrementado al mismo ritmo que la producción de leche (Berry *et al.*, 2014), consecuentemente las vacas han incrementado la movilización grasa corporal y por lo tanto la eficiencia reproductiva ha declinado (Lucy, 2001). Por otra parte, tanto la preñez como el parto presentan costos ocultos para los productores, debido a los problemas que suelen presentar las vacas lecheras durante esta etapa. Por lo anterior, las lactancias prolongadas ofrecen una posibilidad de incrementar la rentabilidad de las empresas lecheras (Borman *et al.*, 2004). Otros estudios han demostrado una ventaja para un período más largo de días abiertos (DA) donde en hatos de alto rendimiento, la más alta productividad de las actuales y posteriores lactaciones se logró por las vacas primíparas que fueron inseminadas no antes de 70 días después del parto, y por las vacas multíparas en 41-90 días abiertos (Arbel *et al.*, 2001).

La forma de la curva de lactancia de las vacas lecheras está determinada por factores genéticos y ambientales. Un parámetro de la curva de lactancia que es de particular interés está en la persistencia que se puede describir como la capacidad de la vaca para mantener la producción después del rendimiento al pico de la lactancia. Las diferencias genéticas en la persistencia son de interés científico, debido a que reducen la precisión de las evaluaciones genéticas para producción de lactancia de 305 días. La persistencia también es un rasgo de interés económico directo, debido a su relación con la reproducción, la salud y la alimentación.

El rendimiento de leche a 305 días se usa como la medida estándar de productividad tanto para la administración y para la proyección futura de la granja. Sin embargo la mayoría de las lactancias rebasan los 305 días. Las vacas con alta persistencia tendrán rendimientos más altos de lactancia que aquellas con baja persistencia en duración de una lactancia mayor de 305 días (Dekkers *et al.*, 1998).

Un prolongado intervalo entre partos (IP) dá como resultado una mayor producción de leche en la siguiente lactancia. En los años 60 varios investigadores informaron que la producción anual de leche se maximiza con (IP) de 12 - 13 de meses. Sin embargo, es posible que éste ya no es hoy en día una duración óptima de la lactancia.

El efecto de intervalos entre partos de 12 y 15 meses sobre la producción y composición de la leche fue investigado por Rehn *et al.* (2000) en Suecia. En este estudio un grupo de vacas fue inseminado a los 50 días postparto y el otro hasta los 140 días posparto. Las vacas inseminadas tardíamente (lactancias de 15 meses produjeron 16% más leche que las vacas con intervalos entre partos de 12 meses. Tanto el contenido de grasa, proteína y lactosa de las vacas con lactancias de 15 meses fue ligeramente menor que las vacas con intervalos entre partos de 12 meses, sin embargo, el promedio de producción de leche por día tendió a ser menor en las vacas con intervalos entre partos de 15 meses (2–5%) debido a producciones de leche más bajas al final de la lactancia y periodos secos más prolongados.

Koc (2012) estudió en vacas Red Holstein en Turquía, los efectos del intervalo entre partos, mes del parto, año de parto y número de partos sobre la duración de la lactación, producción de leche por lactación, la producción de leche a 305 días y la producción diaria de leche. Los datos de intervalo entre partos se agruparon en tres clases: 12, 15 y 18 meses. En comparación con el intervalo entre partos de 12 meses, la producción de leche aumentó 18.9% y 30.9% y la producción a 305 días aumentó 8.8% y 8.1% en vacas con intervalos entre partos de 15 y 18 meses, respectivamente.

Sorensen *et al.* (2008) llevaron a cabo un estudio con vacas lecheras a las cuales sometieron a lactancias de 18 meses. Un grupo de vacas fue tratado con prácticas de manejo y nutrición estándar, mientras que un segundo grupo recibió suplemento alimenticio a partir de la semana 9 de lactancia y éste se prolongó hasta el final de la lactancia. A comenzar la lactancia la mitad de la ubre se ordeñó 2 veces por día y la otra mitad 3 veces por día. La producción de leche se incrementó 33% con 3 ordeñas comparada con 2 ordeñas y la suplementación nutricional se incrementó 11%, comparada con las vacas que no se suplementaron. Este estudio mostró que la persistencia de la lactancia es “plástica” y ésta puede ser incrementada con simples esquemas de manejo de las vacas.

En un estudio de campo de Arbel *et al.* (2001) se examinó el intervalo entre partos prolongado sobre la producción de leche y rentabilidad para un establo lechero. La primera inseminación se llevó a cabo a los 154 o a los 93 días posparto en vacas primíparas y a los 124 o 71 días postparto en vacas multíparas. El promedio de producción de leche en las vacas primerizas fue de 28.5 y 27.7 kg/día para vacas inseminadas a los 154 o 93 días. Para las vacas multíparas el promedio de producción de leche fue de 33.0 y 32.8 kg/día para ambos grupos de vacas. Tanto para las vacas primíparas como las multíparas la lactancia prolongada por mayores intervalos entre partos resultaron en mayor rentabilidad de la explotación lechera. Estos investigadores concluyeron que un retraso de 60 días con respecto al periodo voluntario de espera para la primera inseminación es ventajoso desde el punto de vista económico en vacas con un alto potencial para la producción de leche.

En un estudio de Grainger *et al.* (2009) midieron el efecto del tipo de dieta y nivel de consumo de energía sobre la producción de leche de vacas Holstein con lactancias prolongadas y mantenidas en praderas en Australia. Al completar 670 días de lactancia, la proporción de vacas produciendo leche fue similar entre vacas que recibían una dieta tradicional y vacas con dietas altas en energía. La producción de leche de las vacas testigo (300 días de lactancia) fue de 5087 kg, la de las vacas en praderas con lactancias de 670 días fue de 5963 kg, la de las vacas ordeñadas durante 670 días y que recibían una dieta alta en energía fue de 6672 kg y las que se ordeñaron por 670 días y que recibieron dietas completas fue de 8613 kg. Sin embargo, en aquellas vacas en pastoreo la lactancia prolongada no mejoró la producción de leche después de los 300 días de lactancia ajustado por la cantidad de sólidos en la leche y el porcentaje de vacas en ordeña al momento del secado. En contraste las vacas que recibieron la dieta completa tuvieron un menor contenido de sólidos en su leche a los 600 días de lactancia que las vacas mantenidas en pradera. Hubo sólo un 2.4% de decremento en sólidos de la leche en un periodo de 2 años de lactancia comparado con las vacas cuya lactancia duró 300 días.

En un estudio de Kolver *et al.* (2007) se exploró la posibilidad de extender la lactancia de vacas Holstein de Nueva Zelanda y de los Estados Unidos manejadas en pastoreo, con niveles de suplementación de 0, 3 o 6 kg de alimento por día. En un grupo, el 93% se estaban ordeñando a los 500 días de lactancia y 10% se ordeñaban a los 650 días de lactancia. La suplementación alimenticia no afectó la duración de la lactancia (605 ± 8.3 días; media ± EEM). Se observó una interacción entre genotipo y dieta para producción de leche, proteína y sólidos totales. Las diferencias entre genotipos fueron mayores con los niveles más altos de concentrado ofrecido. La producción anualizada de sólidos de la leche, definida como producción lograda en un intervalo entre partos de 2 años, fue 79% de la producción lograda por vacas con intervalos entre partos de 12 meses, en el caso de vacas neozelandesas y 94% para las vacas de origen norteamericano.

La frecuencia de lactancias prolongadas en vacas de la raza Blanco y Negro en Polonia fue determinada por Sawa y Bogucki (2009). La extensión de las lactancias fue en promedio de 84 días, tomando como base 305 días. Durante los días que excedieron los 305 días se produjeron 1064 kg de leche por vaca, lo cual representa un 17% más de leche por lactancia. Los factores que afectaron la ocurrencia de lactancias prolongadas fueron la edad de la vaca, el nivel de producción de leche en una lactancia estándar, el mes de parto y año de parto. La extensión de la lactancia más allá de los 305 días tuvo beneficios en la producción de leche, pero afectó negativamente a la eficiencia reproductiva. El número de inseminaciones por preñez se incrementó con la producción de leche. Para las vacas con producciones de 5000 kg en lactancias estándar los servicios se incrementaron de 1.48 a 3.33 con las lactancias prolongadas. Para vacas de mayor potencial lechero (7000 kg de leche por lactancia de 305 días) los servicios por preñez se incrementaron de 1.52 para vacas sin lactancias prolongadas a 4.73 en vacas con lactancias prolongadas.

Österman y Bertilsson (2003) examinaron el efecto de intervalos entre partos de 18 meses y dos frecuencias de ordeña (2 vs. 3 ordeñas diarias) sobre la producción y composición de la leche en vacas lecheras rojas y blanco de Suecia. En dos grupos de vacas se lograron intervalos entre partos de 12.2 y 17.5 meses. Las vacas con lactancias de 18 meses produjeron más leche que las vacas con lactancias de 12 meses, pero cuando las lactancias se ajustaron por intervalo entre parto no hubo diferencias entre grupos. Las vacas con lactancias de 18 meses con 3 ordeñas por día produjeron mayor cantidad de leche que las vacas con 18 meses de intervalo entre parto y dos ordeñas por día. Las vacas primíparas produjeron menos leche que las multíparas con intervalos entre partos de 12 meses; lo opuesto se observó con vacas multíparas.

Una forma de mejorar el uso de la capacidad de producción de las vacas lecheras podría ser una ampliación prevista de la lactancia. Vacas de ordeño tres veces al día (3 x) es una manera de aumentar la producción de leche y utilizar mejor la capacidad de las vacas. El efecto de la frecuencia de ordeño en la producción de leche ha sido estudiada por varios investigadores, informando un aumento del 20 % en la producción de leche durante los primeros 150 días de la lactancia de vacas ordeñadas (3 x) en comparación con las vacas son ordeñadas dos veces al día (2 x). Los mismos autores también informaron que el ordeño (3 x) no incrementó el pico de la curva, pero la producción se sostuvo a un nivel alto durante más tiempo (Österman y Bertilsson, 2003).

La raza Holstein tiene un potencial mayor en producción de leche que otras razas y se somete a mayores pérdidas de peso en lactación temprana cuando las reservas de tejido adiposo del cuerpo se movilizan para sostener estos rendimientos. Estos factores se han relacionado con menor concepción y las tasas de preñez. Bajo el sistema de parto estacionalmente concentrado se ve agravada por la falta de tiempo para recuperarse del balance negativo de energía del comienzo de la lactancia, porque el tiempo requerido de cría coincide con el pico de lactación (Auldist *et al.*, 2007).

El 65% de los incidentes de salud se producen en los primeros 45 días de lactancia, por lo tanto, al parir un menor número de veces está menos expuesta al riesgo. En su forma más simple, si los tres ciclos de lactancia de 12 meses típicos de toda la vida del ganado lechero en sistema de producción intensivo podrían ser reemplazadas por dos ciclos de la lactancia prolongados de duración de 18 meses, la exposición al riesgo se reduciría en un tercio. Es evidente que la lactancia prolongada beneficiará el bienestar de las vacas, aunque esto aún no ha sido probado rigurosamente (Sorensen *et al.*, 2008).

El factor natural que desencadena la actividad mamaria es el complejo preñez–parto. Debido a una selección genética en los bovinos productores de leche, la lactancia se prolonga más allá de la simple necesidad fisiológica de alimentar al becerro. Desde hace algunos años, el proceso de la galactopoyesis y lactogénesis se ha inducido con tratamientos hormonales, tanto en bovinos, como en ovinos y caprinos (Mellado *et al.*, 2011) .

2.1. Lactancias prolongadas en vacas lecheras

El objetivo primordial de los ganaderos en la mayoría de los países ha sido que cada vaca lechera de alto rendimiento produjera un becerro cada 12 meses, sin embargo, esto es difícil de conseguir y hay una discusión de cuál es el intervalo de parto ideal en la producción lechera. Extendiendo voluntariamente el primer servicio al parto y en consecuencia, el intervalo entre partos, puede beneficiarse la fertilidad de las vacas de alta producción, y tendrán así un periodo más largo para recuperarse después del parto (Refsdal, 2000). Esto reduce la necesidad de tratamientos hormonales debido al anestro pos-parto y reduce también el número de inseminaciones por vaca preñada (Larsson y Berglund, 2000).

2.2. Factores no infecciosos que afectan la reproducción de las vacas lecheras

La infertilidad está asociada a la creciente producción de leche en vacas altas productoras. La producción de leche y la eficiencia reproductiva no están bien correlacionadas genéticamente y es difícil determinar qué mecanismos están relacionados con el efecto que ejerce la producción de leche en la fertilidad. La reducción de la fertilidad ha sido asociada con el progreso genético para producir leche y también con las mejoras en la nutrición y las prácticas de manejo que han llevado a un continuo aumento en la producción láctea. La baja fertilidad de los establos modernos se ha atribuido a múltiples factores, alguno de ellos son: la frecuencia de ordeños (tres contra dos ordeños al día), inseminador, toro inseminador, edad, (número de lactancia), y el síndrome de la vaca repetidora (vaca sometida a más de 4 inseminaciones artificiales sin quedar preñada) son algunos de los factores que afectan la fertilidad de establos (López-Gatius, 2012).

2.3. Metabolismo y reproducción

El desarrollo del proceso de reproducción en la vaca es: el hipotálamo suministra hormona liberadora de gonadotropina (GnRH) que estimula la hormona luteinizante (LH) y a la hormona foliculoestimulante (FSH). Estas hormonas actúan en el ovario para iniciar y mantener las ondas de crecimiento folicular, lo cual se convierte en un folículo dominante que eventualmente es ovulado. Los folículos secretan estrógenos, los cuales tienen efectos tanto estimulantes como inhibitorios sobre la FSH y LH respectivamente. Finalmente, un folículo se rompe y emerge el ovocito, el cual se aloja en las trompas de Falopio. Los estrógenos circulantes proveen visibles signos de estro, que al ser detectados facilitan la oportuna inseminación del animal. El ovocito fecundado comienza a desarrollarse y un cuerpo lúteo inicia la secreción de progesterona. La disminución de los estrógenos circulantes y el aumento de progesterona dan como resultado una serie de adaptaciones en el útero que permiten el desarrollo y la implantación del embrión. Cada una de estas fases implica varios procesos intracelulares, entre ellos, señales endocrinas y neuronales, transcripción de genes, traducción, proteínas receptoras, segundos mensajeros, catálisis enzimática e interacciones químicas, y todos requieren energía. Cada uno de estos procesos tiene el componente genético de la heredabilidad variable y algunos de ellos pueden ser afectados por las características ambientales (características de los corrales, las interacciones sociales, temporada del año y clima), como calidad y cantidad de nutrientes. Por ejemplo, hay variaciones genéticas en la expresión de los receptores de la FSH y en la transcripción de genes inducidos de progesterona así como variaciones en los receptores y enzimas controlando el metabolismo de la grasa corporal, que dan lugar a diferencias en contenido de grasa corporal, señalización de IGF-I y producción de leptina. La prueba en el toro es suficiente para predecir que la reproducción es hereditaria y que la cría pueda producir grandes cantidades de leche en varias lactancias (McNamara y Shields, 2013).

2.4. Grasa corporal y reproducción

La grasa corporal y el buen estado nutricional son esenciales para una alta fertilidad. Al faltar o carecer de ciertos componentes en la alimentación, se retrasa la madurez sexual y se reduce la fertilidad en las hembras. Por lo tanto, cierta cantidad de tejido adiposo es necesaria para un exitoso ciclo reproductivo de la ovulación, la fertilización, la implantación y la preñez. El anestro posparto y la fertilidad varía entre especies y el tipo de función del animal y puede disminuir por el estado nutricional, mientras una cierta cantidad de grasa en el cuerpo puede relacionarse con una mayor fertilidad también puede ser demasiada perjudicial. El éxito en la producción láctea, fertilidad y preñez no solo dependen de la cantidad de grasa corporal, glucosa, vitaminas, minerales u otros nutrientes, existen hormonas como la leptina y la del factor de crecimiento insulínico tipo 1 (IGF-1) que son liberados por el tejido adiposo y tienen una función en los procesos reproductivos (McNamara y Shields, 2013). Es claro que el éxito reproductivo tiene una relación con un balance energético positivo (Wade y Schneider, 1992).

2.5. Glucosa y reproducción

Una de la principales causas de éxito reproductivo es el flujo de glucosa (Butler, 2003). Su papel se relaciona de manera integral con actividad endocrina, provocando respuestas específicas como la insulina e IGF-1. Hay relaciones bien establecidas entre las hormonas reproductoras tradicionales como los estrógenos, testosterona, progesterona, gonadotropinas, oxitocina y relaxina. Sin embargo, la insulina, somatotropina, factor de crecimiento insulínico tipo 1, hormona tiroidea, y corticosteroides están involucrados en los procesos reproductivos, ambos indirectamente mediante la regulación de la división celular y crecimiento de los tejidos y directamente a través de la regulación de entrada de glucosa en el ovario; crecimiento folicular; fetal, diferenciación de la glándula mamaria, crecimiento y metabolismo (Wathes *et al.*, 2007). Cada hormona tiene su papel en diversas funciones en la reproducción, la mayoría responde o es afectada por el flujo de glucosa en el cuerpo. La glucosa tiene un efecto directo en el hipotálamo, lo que causa la liberación de GnRH, lo que a su vez provoca la liberación de LH por la pituitaria. También la glucosa provoca aumento de insulina e IGF-1; que tiene efectos positivos en el crecimiento folicular. El flujo de glucosa está relacionado en el retorno al estro en cerdas y vacas lactando. Muchas vacas que producen grandes cantidades de leche pierden importantes cantidades de grasa corporal, sin embargo, muestran signos de estro, se preñan y paren. Esto indica que la variación genética y ambiental en todos los subsistemas es necesaria para el éxito reproductivo (McNamara y Shields, 2013).

2.6. Ácidos grasos y reproducción

Los ácidos grasos omega 3, omega 6 y sus metabolitos han sido identificados como positivos controladores en reproducción. Algunos investigadores han comunicado que estos ácidos grasos han mejorados la fertilidad en vacas lecheras lactantes(Santos *et al.*, 2008). El papel de los ácidos grasos es importante en el desarrollo y viabilidad de los ovocitos. Con esto se puede hacer una hipótesis cuantitativa en cuanto al porcentaje del éxito reproductivo y la calidad de los ovocitos (McNamara *et al.*, 2003).

2.7. Proteínas, aminoácidos y reproducción

Los aminoácidos también afectan la reproducción. Trabajos anteriores, sugieren que el exceso de aminoácidos causa aumentos de nitrógeno en orina y sangre por lo tanto concentraciones de urea o amoniaco en el útero que afectan la supervivencia del embrión (De Wit *et al.*, 2001; Sinclair *et al.*, 2014). Un reciente meta-análisis de varios estudios muestran que la mayoría de los animales alimentados con dietas altas en proteínas (de 19 a 21%) presentan una disminución de la preñez de aproximadamente el 10%. Además de la contribución del metabolismo de los aminoácidos, es probable una sutil interacción entre aminoácidos, transcripción de genes y regulación endocrina (Meier *et al.*, 2014). Los estudios genómicos han sugerido una conexión entre las variantes de los genes de miostatina, calpastatina y la fertilidad de la vaca. Estos estudios proporcionan pruebas de que existe un vínculo mecánico entre el metabolismo de proteínas, la expresión de los genes y la reproducción (McNamara y Shields, 2013). Así pues, se puede deducir que los aminoácidos son un elemento importante en la regulación del crecimiento embrionario (Mucci *et al.*, 2006).

2.8. Desordenes derivados del parto

La mayoría de los problemas de salud en la vaca lechera se producen durante el periparto, esto se relaciona al problema que tienen las vacas al adaptarse para la lactancia, lo cual resulta un desequilibrio fisiológico donde el regulador de mecanismos es insuficiente para que la vaca funcione de forma óptima y conduce a un alto riesgo de trastornos digestivos, metabólicos y problemas infecciosos (Ingvartsen, 2006). El aumento del número de lactancias es un factor pre-determinante para los desórdenes del periparto en vacas lecheras como retención de placenta, metritis, hipocalcemia, quistes ováricos y mastitis (Reyes y Mellado, 1994). El Intervalo parto-concepción, así como el número de servicios por concepción, aumenta en número de cojera en vacas (Sogstad *et al.*, 2006).

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IV. ANEXO ARTÍCULO

4.1 Risk factors for unplanned extended lactations (up to 46 months) In Holstein cows in a hot environment

Abstract

In temperate regions, 305-d lactation length with high-yielding dairy cows is considered optimum. Yet, this lactation length is unsustainable in zones with low reproductive efficiency due to high ambient temperature. Under these circumstances, extended lactations offer the possibility of improve reproductive performance, decrease culling rates, increase milk yield and reduce costs of production. A data set consisting of 3,278 lactations was used to characterize milk production of high-yielding Holstein cows milked three times per day and subjected to unplanned extended lactations (up to 1399 days) in a hot environment. Additional objectives were to identify risk factors for the occurrence of involuntary extended lactations, to assess the association between lactation length and milk yield and to determine the conception rates of cows with extended lactations and with multiple cumulative services. Thirty percent of lactations were between 450 and 1399 days. Full lactation yield of cows with lactations >900 days was over 30,000 kg. The epidemiological analysis of risk factors for involuntary extended lactations using a multiple variable logistic regression indicated that retained placenta (odds ratio (OR) = 1.3), metritis (OR = 1.8), ketosis (OR = 1.4), peak milk yield (<50 vs >50 kg, OR=1.4), temperature-humidity index at 60 days postpartum (<82 vs >82 units, OR = 1.4), ketosis (OR = 1.4) and 305-d milk yield (<11,000 vs >11,000 kg, OR = 1.6) significantly increased the risk for lactations >15 months. Primiparous cows had less than half the risk of extended lactations (OR = 0.3) compared to multiparous cows. Once a cow had conceived, her risk of having a prolonged lactation decreased sharply (P<0.01). A strong non-linear association was found between lactation length and total milk yield for primiparous (up to 1393 days in

milk, maximum milk yield 35,236 kg; r= 0.80) and pluriparous (up to 1399 days in milk, maximum milk yield 37,218 kg; r= 0.77). Conception rate in cows with extended lactation decreased linearly as number of services increased (conception rate = 50.5% for 4 services and 13% for ≥ 14 services). The data showed that well-managed Holstein cows milked three times daily were capable of lactating for over 40 months with remarkable high persistency and with high milk yield at drying-off. Additionally, this study showed that reproductive and metabolic disorders associated withcalving are important risk factors for involuntary extended lactations, derived from the link of periparturient diseases with depressed reproductive function in dairy cows.

Keywords: Conception rate, metritis, retained placenta, ketosis, heat stress

Implications

Extended lactations cycles (>900-days) are as productive and efficient as traditional 12-month intensive lactation cycles, both in primiparous and multiparous Holstein cows. Lactation persistency is plastic and can be sustained by simply using three milkings a day in high yielding animals. Dairy operations in hot environments and with substantial decrease in reproductive performance would benefits from this management system, because the likehood of stablishing and maintaining pregnancy would be increased, as the first insemination would occur after the stress of negative energy balance in early lactation, and cows would be recuperated from disorders associated with abnormal parturitions or periparturient reproductive diseases.

Introduction

In intensive dairy operations, a general practice is to get cows pregnant before 120 days in milk, with the aim of having a calving interval of 12 to 13 months, which is considered optimum in terms of profitability (Plazier *et al*., 1997; De Vries, 2006). This calving interval is very hard to attain with high milk-yielding Holstein cows in hot environments, because heat stress severely depress fertility (García-Ispierto *et al*., 2007). Pregnancy rates for Holstein cows in hot zones of northern Mexico is not greater than 33% (Mellado *et al*., 2012, 2014) and typically pregnancy rate decrease 10% in summer (Mellado *et al*. 2012). This low reproductive performance is due to a drastic heat stress experienced by cows for the most part of the year, associated with great metabolic heat load due to the high milk yield (Wheelock *et al*. 2010). Heat stress in cows in this environment is exacerbated by three times milking, intense solar radiation input, elevated ambient temperature, poor night cooling and mild air movement.

The low reproductive rate caused by the intense heat load leads to a high culling rates (García-Ispierto *et al*., 2007) and premature replacement of cows (Hadley *et al*., 2006). The cost associated with involuntary culling as a consequence of reproductive failure is an important component of the total cost of inadequate reproductive performance. Increased risk of culling imposes a hidden cost, which is not always obvious to the farmer. Involuntary cullings are associated with replacement costs, and hence include costs of rearing or acquiring a heifer (DeVries, 2006; Fetrow *et al*., 2006). If replacement heifer are not available at the time a group of cows are culled, capacity utilization is reduced as part of the facilities will be empty while the fixed costs remain unchanged. Further economic losses occur as milk yield of primiparous cows is lower than that of multiparous cows, and because there is a risk that the yield level of heifers sometimes is very low. Economic cost also arises as young cows culled due to reproductive failure do not reach their full production potential.

One alternative for dairy producers in zones on intense heat loads and with a low herd fertility and consequently with a low number of replacement heifers, is to extend lactations far beyond the traditional 10-month cycles. Extended lactations (up to 18 months) do not necessarily mean a lower productivity (Arbel *et al*., 2001; Osterman and Bertilsson, 2003). In fact, it has been shown that by combining longer calving intervals with increased milking frequency, the milk production per day from one calving to another might even be higher. The practice of prolonged lactations has also shown to be beneficial for fertility

(Larssonand Berglund, 2008) and animal welfare (Knight, 2001; Osterman and Redbo, 2001).

Additionally to the prolonged lactations due to failure of cows to become pregnant, a common practice of dairy producers in hot areas of northern Mexico is the continued use of artificial inseminations after five services. This practice is questionable, because cows requiring three or more consecutive services before conception, with no clinical signs of disease, are generally designated as repeat breeders (subfertil/unfertil; Katagiri*et al*., 2013; Pothmann *et al*., 2015). **Furthermore, numerous services** result in prolonged calving intervals, which is considered undesirable for dairy farmers because annual revenue per cow decreases, this is so because once cows reach the “breakeven point” they produce milk that equals the cost of production (van Arendonk and Liinamo, 2003).

This latter view has been recently challenged and some researchers consider extended lactation economically competitive, provided lactation persistency is maximized (Knight, 2005). Among the advantages of extended lactation would be a more even spread in labor requirements, input costs and income across the year. Additionally some cows regarded as infertile can get pregnant after the fifth service; thus, acquisition of replacement heifers may be reduced. Thus, extended lactations could be a suitable option for dairy enterprises in zones of intense heat where **high level** of **reproductive efficiency** is not possible to maintain.

The overall aim of this study was to quantify the milk production capacity of subfertil / infertil high milk-yielding cows undergoing extended lactations (15 to40 months) and to identify risk factors associated with the occurrence of extended lactations in Holstein cows. An additional objective was to determine the influence of number of cumulative previous services on conception rate of cows inseminated inadvanced lactation stage.

Material and methods

Study herd, housing and feeding

Data for this retrospective field study were from three neighboring large commercial dairy herds located in northeastern Mexico(26°N, elevation 1140 m, mean annual temperature 27ºC, mean annual rainfall 230 mm). This zone is characterized by high daytime temperatures in spring, summer and fall (around 40°C) and intense solar radiation associated with low relative humidity, while nights are warm.

All experimental procedures complied with The Guide for Care and Use of Agricultural Animals of the Agrarian Autonomous University Antonio Narro. The herds ranged from 2,900 to 3,400 lactating Holstein cows housed in open-lot, dirt floor pens with ample shade structures in each pen and with centralized feed alley. Pens in the dairy operations are equipped with electric fans installed on shade roofs. Most animals used in the study are born and raised at the herds studied. Cows are 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 >35 kg of milk/d (NRC, 2001). Cows are fed *ad libitum* for a daily **feed** refusal of approximately **10**% of that offered.

Cows are milked three times daily (04:00, 12:00, and 20:00 h) in milking parlors and are fed following each milking. Cows were not subjected to rbST administration. Lactation number of cows included in the study varied from one to eight; with body condition score of lactating cows ranging from 2.75 to 3.5 (scale 1 to 5). The actual 305-day rolling herd average for these dairy herds is about 10,900 kg and the herd replacement rate was around 27%.

Dara sets

Two data files were used. Data file 1 (n= 3,278 lactations) was from a single herd (2,900 lactating cows from 2012to 2014). This information was used to assess the risk factors involved in the occurrence of extended lactations and non-accumulative conception rates in Holstein cows. Data file 2 (n= 4,393 lactations from 2012to 2014) consisted of lactation ranging from 15 to 46months, from cows from three adjacent dairy farms with same management and facilities. This information was used to assess the association between lactation length and milk yield in cows with lactations >15 months, as well as the survival curves for cows pregnant and non-pregnant.

Reproductive management

All cows are routinely vaccinated against diseases that impair reproduction functions, such as bovine viral diarrhea, infectious bovine rhinotracheitis, bovine respiratory syncytial virus, para-influenza and leptospirosis (5-varieties; CattleMaster Gold FP5®, Zoetis, Mexico D.F., Mexico). The reproductive tract of each cow was routinely examined by rectal palpation around 30 d postpartum to check for normal ovarian structures and uterine involution. Additionally, a veterinary examined fresh cows to identify and treat cows with postpartum reproductive disorders, such as retained placenta (fetal membranes retained for more than 12 h), clinical metritis (enlarged uterus and a uterine discharge with fetid odour and red-brown fluid and pus), and endometritis (mucopurulent discharge after 30 days in milk), as well as ketosis (urine ketone evaluation dipstick (Ketostix; Bayer Corp. Diagnostics Division, Elkhart, IN)). The voluntary waiting period was 50 d postpartum regardless of peak milk production, after which cows were submitted for AI when detected in estrus. Detection of estrus was performed with the aid of pedometer technology integrated with the milking system, and artificial insemination (AI) was conducted based on pedometry-aided heat detection plus visual observation of estrous behavior, following the standard a.m./p.m. rule. Timed breeding protocols, e.g. Ovsynch and timed AI were performed in repeat breeder cows. Due to low conception rates in these dairy herds the majority of cows continued to be inseminated beyond 250 days in milk; the range for number of services was 1 to 24.

Commercial frozen-thawed semen from multiple sires from USA was used across all months of the year. Pregnancy was detected by palpation per rectum of the uterus and its contents about 45 days post AI. Conception rate was defined as the number of cows that conceive out of the ones that were detected in estrus at each estrous cycle.

Milk data collection

Milk yield per milking (three times per day) per cow was recorded daily electronically on each cow using the Alfa-Laval milking equipment installed in the barn and linked to a computer recording data for each cow separately. The milk meters were checked regularly and calibrated by the suppliers of the milking equipment. The data were used to calculate 305-d milk yield and total lactation yields for each cow. Cows with an average milk yield of 20 kg were dried off. All other cows that maintained production above this level for the entire lactation were dried off 60 days before their expected parturition date.

Edits required that 305-d milk yield range from 8,000 to 13,000 kg. Edits also were for age at calving (excluded if <20 or greater than 30 months), peak sample day milk (not greater than 75 kg). Lactations records were deleted if initiated by abortion or hormonal treatment. Records were also excluded if these were associated with mastitis at the beginning of lactation. A total of 228 cows (6.5%) did no metall inclusion criteria.

A second set of data were composed of 4,395 records of extended lactations (15 to 46 months) from cows calving from 2012 to 2013 from three large dairy herds (range 2100 to 3500 cows). These dairy herds were located in the same area described previously and followed the same milking, feeding, health and reproductive management than that described for the first herd. This information was used to assess the association between total milk yield and lactation length.

Climatic data

Climatic data was obtained from a meteorological station located 1 km away from the barn for the duration of the study. Information consisted of daily maximum temperatures and relative humidity. Air temperature was recorded with a mercury thermometer under full shade and at 1.4 m above the ground. This information was used to calculate the temperature–humidity index (THI) for each day, using the following equation (highest daily temperature in Celsius degrees; RH refers to maximum relative humidity):

THI = (0.8 × temperature) + (%RH/100) * (temperature − 14.4)] + 46.4.

Statistical evaluation

To analyze risk factors contributing to the occurrence of extended lactation (defined as a binary trait distinguishing between cows with more than 450 days in milk (1) and cows less than 450 days of lactation (0)), a multivariable logistic regression model of SAS(SAS Inst. Inc., Cary, NC, USA) was used, applying a backward step wise logistic model to eliminate all non-significant explanatory variables. Variables were continuously removed from the model by the Wald statistic criterion if the significance was greater than 0.20. To construct the final statistical model, a preliminary full model was conducted for the following potentially explanatory variables of interest: lactation number, year, temperature-humidity index at calving and at 60 days post-calving, seropositivity for brucellosis, 305-milk yield, milk production at peak yield, calving difficulty (dichotomized variable), season of calving, occurrence of ketosis, occurrence of retained placenta, occurrence of metritis. The full statistical model included main effects and all interactions. No significant interactions were found and the final model included only main effects. Per lactation, the time at risk started at the calving date, when the cow entered the milking group of cows and the time at risk ended when the cow was dried off or culled.

The GENMOD procedure of SAS was used to assess the statistical significance of number of previous cumulative services on conception rate. Treatment means were separated using the probability of a statistical difference (PDIFF option of SAS). Statistical differences were considered significant at $P<0.05$. The relationship between previous number of services and conception rate was examined by simple correlation and non-linear regression analyses.

A Kaplan-Meier survival analysis using the LIFETEST procedure in SAS was performed to illustrate the difference in lactation length between pregnant (fecundation at the final stage of lactation) and non-pregnant cows. The null hypothesis of no differences in the survivor functions of the strata (pregnant or not pregnant groups) was evaluated using the Wilcoxon tests.

A non-linear regression was used to describe the association between lactation length and total milk yield. Cow were classified by parity (first and later). Classes for THI at calving and 60 days post-calving were defined as less than 82 and >82 units. 305-d milk yield was categorized as <11,000 and >11,000 kg. Milk production at peak yield was classed as <50 and >50 kg. Months of calving were grouped into seasons, winter months being January–March; spring, April–June; summer, July–September; and fall, October–December. Classes for previous cumulative number of services at AI were defined as less than 4, 4-9 and >9.

Results

Milk yield

Out of a total 3,278 lactations analyzed from the first herd, 998 were over 15 months (30%; 95 % CI= 28-31%; range of milk yield 8,021 - 37,028 kg).Mean milk yield at peak was 48 kg at day 64 after the start of the lactation. Full lactation yield of cows with lactation length greater than 600 days was about twice as much as milk yield produced during the first 305 days in milk (Table 1). Lactation extension length averaged 395 days and milk produced during this period accounted for 52% of the total milk yield.

Table 1. Milk yield of high-yielding Holstein cows milked three times per day and undergoing unplanned lactations greater than 600 days in a hot environment. Values are means ± SD; n=256.

Traits	Primiparous	Multiparous	Combined
Lactation length cow >600 days in milk, d	702 ± 88	698 ± 115	700 ± 98
305-d milk yield, kg	9229 ± 1150	9892 ± 1403	9709 ± 1367
Milk yield between 305 - >600 d, kg	10935 ± 22371	11606 ± 3394	11179 ± 3091
Total milk yield in lactation >600 d, kg	20589 ± 2906	22548 ± 4017	21300 ± 3476
kg milk/day during 305 days in milk	30.2 ± 3.6	32.5 ± 4.7	31.8 ± 4.5
kg milk/day from 305 to >600 DIM	27.6 ± 3.5	29.8 ± 5.3	28.4 ± 4.4

Listed in Table 2 are factors which significantly affected the likelihood of cows undergoing lactations >15 months. Cows experiencing severe heat stress (THI >82) at 60 days post-partum were 1.4 times more likely ($P< 0.01$) to present extended lactations than cows with milder ambient temperature at around the time of first AI. Compared to cows without retained placenta, cows with retained placenta were 1.5 times more likely to present extended lactations ($P<0.05$).Cows experiencing metritis were 1.6 times more likely to present extended lactations than cows without this reproductive disorder. Cows experiencing a peak milk yield >50 kg were 1.4 times more likely to present extended lactation than cows producing <50 kg of milk at peak lactation. Odds of extended lactations were about 4 times lower in primiparous cows than older cows. The occurrence of ketosis was associated with increased risk of extended lactations ($P<0.05$). There was evidence that cows with 305-d lactations >11000 kg were 1.6 times more likely to experience extended lactations than cows with <11,000 kg milk yield.

Table 2. Odds ratios (OR) for the likelihood of extended lactation (>15 month)in high-yielding Holstein cows in a hot environment, as a function of climatic conditions, reproductive and metabolic disorders associated with parturition, number of lactations, and some lactation traits.

Variables	n	Cows with >450 days in milk (%)	Odds ratio (OR)*	95% C.I. OR	P
THI 60 d postpartum					0.004
>82	837	35.4	1.4	1.1 - 1.6	
<82	2441	27.9	1.0		
Retained placenta					0.001
Yes	557	32.7	1.5	1.2 – 1.8	
No	2721	29.2	1.0		
Metritis					0.0001
Yes	351	46.7	1.6	1.2 – 2.0	
No	2927	27.8	1.0		
Peak milk (kg)					0.0001
>50	899	34.7	1.4	1.3 – 1.6	
<50	2379	27.9	1.0		
Parity					
Primiparous	1362	22.0	0.3	0.3 – 0.4	0.0001
Multiparous	1916	40.8	1.0		
Ketosis					0.03
Yes	239	41.8	1.4	1.0 – 1.8	
No	3039	28.9	1.0		

305-d milk yield (kg)					0.0001
>11000	794	33.9	1.7	1.4 – 2.2	
<11000	2484	28.5	1.0		
Number of services					0.0001
>9	1032	49.9	4.3	3.5 – 5.3	
4-9	1193	27.6	1.2	1.0 – 1.4	
<4	1053	12.7	1.0		

*Odds ratios measure how much more or less likely the outcome is among variables with a given risk factor, compared with those without it, or reference category (odds ratio of 1.0).

As expected, there was a high correlation between days in milk and total milk yield for both primiparous and multiparous cows (Figure 1). Dispersion of data increased around700 days in milk and total milk yield showed a slight declined with lactations longer than 1000 days. The survival plot generated by the Kaplan-Meier survival analysis illustrated the longer lactations of non-pregnant cows compared with pregnant cows (Figure 2). The median lactation length was 354 and 411 d (P<0.01) for pregnant and non-pregnant cows, respectively.

Reproductive Performance

From the 3,278 cows enrolled in this analysis, 1180 were diagnosed pregnant (total services conception rate= 36%) by the end of lactations. The mean number (± SD) of services per pregnancy based only on data from pregnant cows was 4.7 ± 2.6. The non-linear regression analysis indicated that conception rates were negatively related with the previous cumulative number of services at AI. Conception rates in cows AI with 4 to 6 previous services were around 50%, whereas conception rates of cows with ≥ 14 services were only 13%.

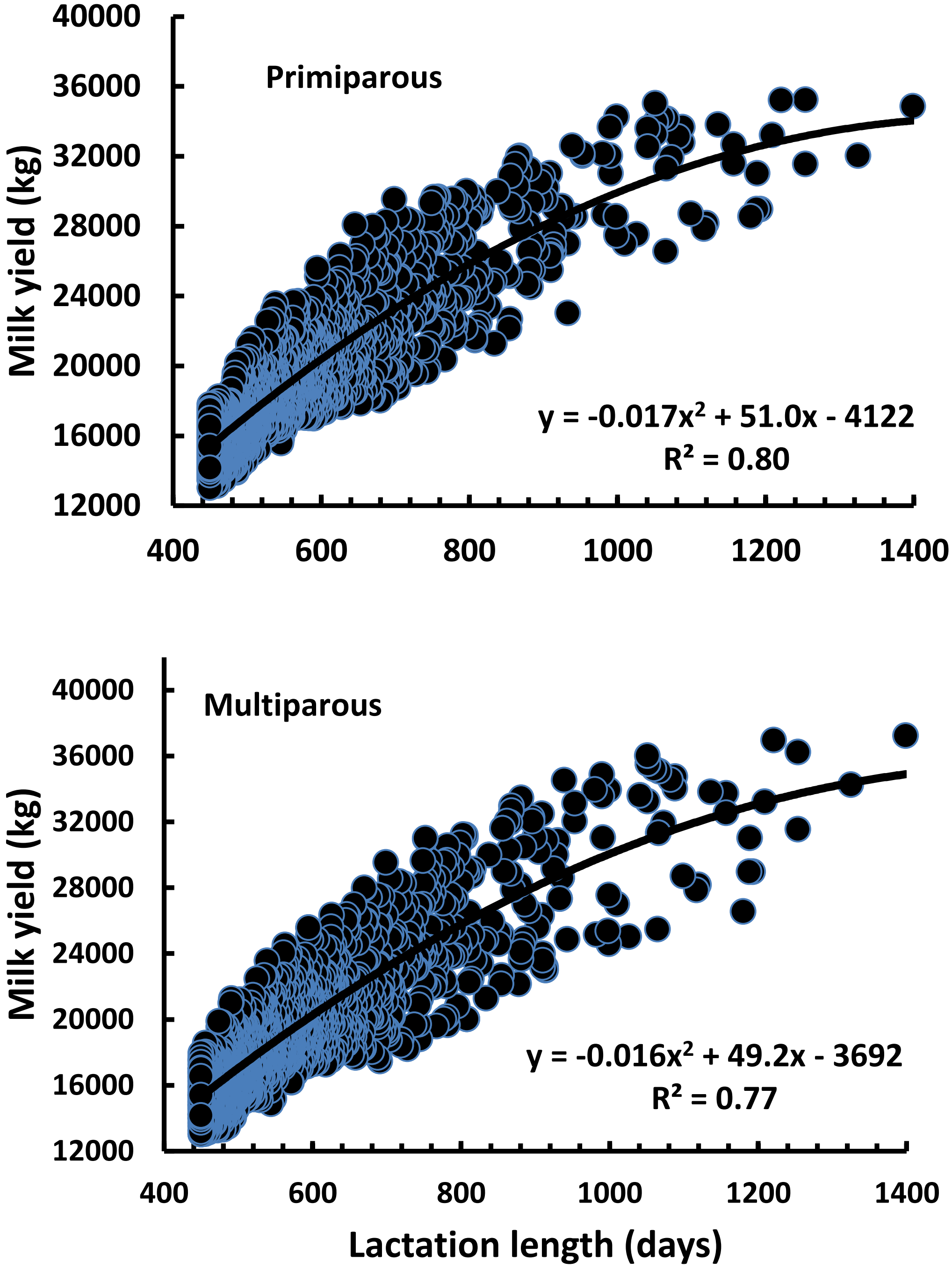

Figure 1. Association between lactation length and total milk yield of high-yielding Holstein cows with extended lactations in a hot environment.

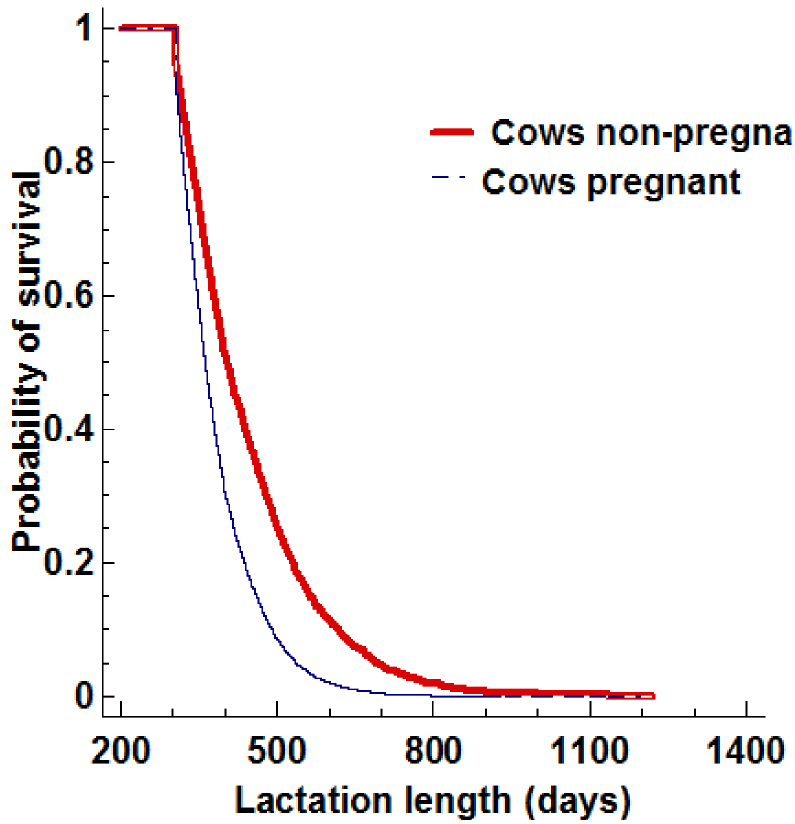

Figure 2. Lactation length in Holstein cows that were classified as pregnant or not pregnanat at any point in their lactation. Non-pregnant cows had increased (P< 0.001) median days of lactation compared with pregnant cows (411 and 354 d).

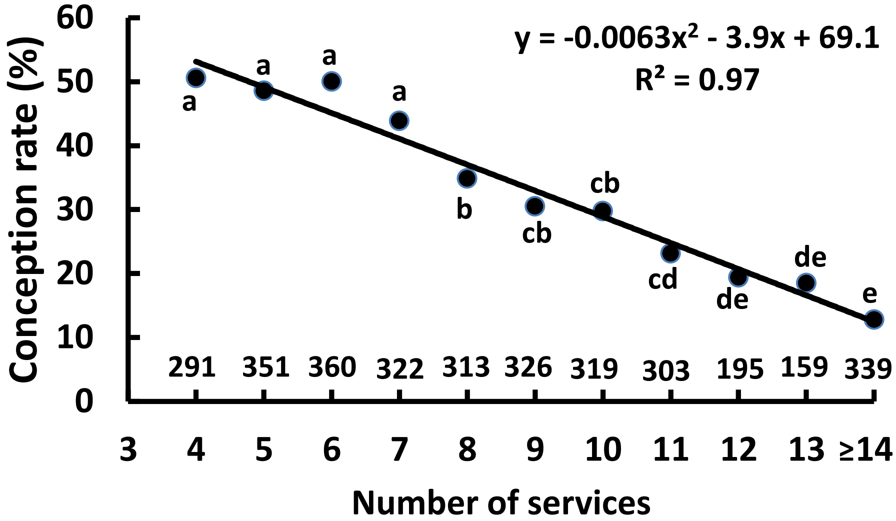

Figure 3. Non-cumulative conception rate as a function of number of services received previously, for high-yielding Holstein cows undergoing lactations >15 months in a hot environment

Discussion

Milk yield

During the past years, there has been increased interest in exploring the use of extended lactation as suitable option for some dairy enterprises, particularly in herbage-based systems (Borman *et al*., 2004; Williams *et al*., 2013). This interest, in part, is attributed to the fact that some producers are unable to maintain calving intervals around 365 days and must adapt their management systems to their new reality, as it is the case in the present study, where calving intervals are well over 13 months.

In the present study, cows with over 600 days in milk produced about twice as much milk as those cows with 305-d lactations, and milk yield of cows reaching 900 days in milk was over 30,000 kg. These data show that non-pregnant high-yielding Holstein cows managed intensively maintain a relatively stable level of milk production after peak yield. These results also reaffirm the view that lactation persistency is plastic and amenable to manipulate in order to maintain a high stable level of production after peak yield, thereby extending the lactation cycle (Sorensen and Knight, 2002). This is the first study to explore the capability of high-yielding Holstein cows to produce milk with lactations over 900 days in milk, therefore no comparisons can be made with other studies. However, these findings support previous reports of extended lactations in pasture-based systems (Auldist *et al*., 2007, Kolver *et al*., 2007) and back the view of Knight (2005), who holds that high-yielding dairycows maintain high milk production for much longer than the 305-day considered standard in intensive dairy operations. Therefore, there is no justification for the rule that calving interval should lastaround 365 days in high-yielding cows, because it truncates the productive life and reduces the life time yield of cows with high milk yield potential. The use of extended lactation in intensive farming systems in the region where this study took place is now considered commercially viable because the modern Holstein cow milked three times per day is capable of lactations that are twice or trice the traditional 300 days.

As anticipated, involuntary extended lactations were associated with high THI around the time offirst service. This extended lactation is mediated by the adverse effects of high environmental temperatures on the reproductive processes of dairy cattle. Heat stress salters endocrine environment and follicular development patterns (Shehab-El-Deen *et al*., 2010), reduce oocyte competence to develop into blastocysts (Ferreira *et al*., 2011), reduced quality of oocytes (Roth, 2001) and impaired early embryo development (García-Ispierto *et al*., 2006; Hansen, 2007), among other effects. Thus, heat stress is a major contributing factor to the occurrence of involuntary extended lactation in Holstein cows inseminated in periods of high ambient temperature, because of extended days open during the hot season.

Cows experiencing retained placenta and metritis were significantly more likely to experience involuntary extended lactations. The occurrence of both retained placenta (Han and Kim, 2005; López-Gatius *et al*., 2006) and metritis (Risco and Hernandez, 2003; Liu *et al*., 2011) in dairy cows reduces reproductive efficiency, which increases calving intervals, and as calving intervals increase, the average days in milk for the herd increases as well. With the traditional 12-13 months calving intervals in dairy cows in intensive production systems is been said that the longer the average days in milk is, the lower average daily milk production becomes. This was not the case in the present study. Thus, long calving intervals in Holstein cows managed intensively not necessarily negatively affect milk revenue through reducing lifetime milk production.

Clinical ketosis occurring during the negative energy balance period significantly increased the probability of cows experiencing involuntary extended lactations. This metabolic diseaseis characterized by marked changes in endocrine, metabolic and physiological statusis and it is associated with increased risk of periparturient disease such as metritis (Duffield *et al*., 2009; Suthar*et al*., 2013) displaced abomasum (Correa *et al*., 1993;Suthar*et al*., 2013) clinical endometritis (Shin et al., 2015) and ovarian cysts (Shin *et al*., 2015). These Calving-related disorders and diseases that affect the reproductive tract are major contributors to depression of fertility (Bisinotto *et al*., 2012, Shin *et al*., 2015). Thus, high-yielding Holstein cows undergoing a period of extensive tissue catabolism because of negative nutrient balance are prone to present ketosis, which in turn strongly decrease reproductive performance. Under these circumstances the intervals to uterine involution, first ovulation, first AI, and conception rate are prolonged (Aungier *et al*., 2014), which lead to the involuntary extended lactations.

Cows with peak milk yields greater than 50 kg milk per day were more likely to have involuntary extended lactations than cows with lower peak milk yields.

This response stems from the fact that cows with high peak milk yields experience a substantial increase in energy requirements to facilitate the dramatic increases in daily milk yield. This requirement is only partially met by increased feed intake and the remainder energy requirement for milk is derived from body reserves resulting in cows entering negative energy balance (Grummer, 2007). This negative energy balance creates higher risks of metabolic disorders and fertility failure (Roche et al., 2009; Vallimont *et al*., 2012), which in turn increases the chances of extended lactations. Previous studies have stablished that peak milk yield is closely linked to poorer fertility (López-Gatius *et al*., 2006; Wathes *et al*., 2007a**;** Albarrán-Portillo and Pollott, 2013).

Higher yielding cows were more likely to present unplanned extended lactations. Although some authors claim that milk yield and timely pregnancies of dairy cows do not necessarily play against each other (LeBlanc, 2010; Bello *et al*., 2012), an overall agreement exists that calving intervals have been unfavorably affected by the increase in the genetic potential of dairy cows for milk production (Lucy 2001; Norman et al. 2009; Albarrán-Portillo and Pollott, 2013). In fact, several authors have found a genetic link between calving interval and 305-d milk yield (Kadarmideen *et al*., 2003; Sewalem *et al*. (2010).Comparison of reproductive performance in a line of cows highly selected for milk production with a line reflecting the average genetic merit, showed that the selected cows commenced to cycle later and had their first heat later than animals of average genetic merit (Pollott and Coffey, 2008). Thus, it seems that reproductive parameters that reflect the reproductive status of dairy cows, such as days to first observed estrus and the start of luteal activity, are delayed in high-genetic-merit cows, which leads to a reduced fertility and consequently to longer lactations.

In the present study multiparous cows were more likely to present unplanned extended lactations than primiparous cows. This response seems to be due to the fact that primiparous cows tend to present higher fertility compared to multiparous cows (Mayne *et al*., 2002; López-Gatius *et al*., 2006). Primiparous cows are still growing and direct nutrients into growth as well as milk production, but by the second lactation, growth is almost complete (Coffey *et al*., 2006). The greater circulating concentrations of IGF-I in the primiparous cows (Wathes *et al*., 2007a) may direct partitioning of nutrients into body tissue rather than milk, so yield may not be such an significant factor in limiting fertility in the less mature dairy cows (Wathes *et al*., 2007b). With greater fertility, a great deal of primiparous cows did not have the chance to benon-pregnant after 200 days in milk, and consequently less young cows underwent prolonged lactations.

As it was expected, cows with more than four services had increased risk of presenting unplanned prolonged lactations. Given that the window of time within which dairy producers attempted to achieve pregnancies in their cows was extremely ample (over 300 days postpartum), the more services received the cows the greater the lactation length of cows.

Reproductive performance

The use of three time milking allowed milk producers to prolong the lactation of cows despite a non-pregnant reproductive status well beyond 8 months in milk. These cows then had additional opportunities to become pregnant over a longer period, thereby leading to an unusual great number of services and a very long lactations. This study provided substantial novel information on conception rates of cows with numerous services, a situation not practiced in intensive dairy herds in temperate regions.

Total service pregnancy rate at the end of lactations was 36%, which is similar to the 33% figure reported by Mellado *et al*. (2012) in commercial herds in this area. There was a significant repeat breeder problem in this dairy farm ascribed to hot climatefor the most part of year, which is in line with herds in this area (Mellado*et al*., 2012). Given the appropriate reproductive program utilized in these herds, it is believed that the reproductive difficulties were not management problem, but acow / herd problem associated with heat stress.

The probability of conception after few artificial inseminations declined at a decreasing rate as the number of cumulative services increased. A similar tendency has been reported in dairy cows in a hot environment (Badinga *et al*., 1985). The decrease in conception rates for subsequent inseminations can be explained by the fact that cows with greatest fertility become pregnant during the first inseminations, leaving a population of lesser fertility cows for subsequent inseminations. Also, the steady decline in conception rates as number of services accumulated suggests physiological alterations linked to individual animals as a possible cause of repeat breeding. However, given that when additional insemination were performed some cows got pregnant, suggests management and environmental influences as important factors behind the repeat breeding syndrome. Additionally, the fact that some cows with 10or greater consecutive services became pregnant, indicates that most of these repeat breeders were not sterile; rather, they suffered from lowered fertility.

These results indicate that, for dairy herds located in the area of the present study the optimal length of the voluntary waiting period is far beyond the traditional 50-60 days postpartum, because shorter days in milk at first AI is a risk factor for repeat breeding (Yusuf *et al*. 2010).Additionally, nutrient demands of high milk production negatively impact various physiological pathways which reduces the probability of establishment of pregnancy around peak of lactation. So, the initiation of breeding until cows are over 100 days in milk would seems reasonably in cows showing a high milk persistency, in combination with a high milk production levels and under intense heat stress. This scheme would drastically reduce semen and veterinarian costs.

Conclusions

These results indicate that productive extended lactations both in primiparous and multiparous high-yielding Holstein cows up to around 800 days are feasible on intensive systems with three milkings a day. These findings also highlight the complexity of the occurrence of involuntary extended lactations in high-yielding Holstein cows subjected to heat stress. Reproductive and metabolic disorders post-partum, high ambient temperature around the time of first services and high milk yield all raised the risk of involuntary extended lactation, which suggests that these are interrelated factors in their effect on reduced fertility and consequently on forced prolonged lactations. This study also found that an increase of at least 100 days in the usual voluntary waiting period after parturition before starting to begin rebreeding high producing cows has a productive and reproductive advantage, and should be considered by dairy producers with herds undergoing inefficient reproductive performance associated with decreased thermoregulatory competence due to hyperthermia.

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