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**EFFECTO DE LA CASTRACION SOBRE EL
COMPORTAMIENTO CONDUCTUAL Y CALIDAD DE LA
CANAL Y LA CARNE EN BOVINOS DE ENGORDA**

TESIS

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La presente tesis "EFECTO DE LA CASTRACIÓN SOBRE EL COMPORTAMIENTO CONDUCTUAL Y CALIDAD DE LA CANAL Y LA CARNE EN BOVINOS DE ENGORDA" realizada por el C. José Leonardo Bolado Sarabia, dirigido por la Ph.D. Cristina Pérez Linares, ha sido evaluada y aprobada por el Comité Particular abajo indicado, como requisito parcial para obtener el grado de:

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RESUMEN

Para evaluar el efecto de la inmunocastración sobre las características de la canal y carne se utilizaron machos Holstein de entre 7 y 8 meses de edad con un peso promedio de 232 ± 1.19 kg, se emplearon dos tratamientos, el placebo (machos enteros) contra inmunocastrados (Bopriva), posteriormente fueron sacrificados aproximadamente a los 239 días de engorda. Los resultados indicaron que mientras los niveles de testosterona se mantuvieron en 0.42 ng/ml para los machos enteros durante todo el estudio, para el día 181, se observaron diferencias ($P < 0.05$) en los animales inmunizados con valores de 0.21 ng/ml. Las canales de los animales tratados con Bopriva registraron pesos de canal caliente (CC) y canal fría (CF) mayores, también un mayor espesor de grasa dorsal, marmoleo y KPH ($P < 0.05$); sin embargo no se observaron diferencias en área del ojo de la costilla (AOC) ($P > 0.05$). No hubo diferencia significativa ($P > 0.05$) entre tratamientos para pH, L*, a*, b* C* y H*. Las canales de animales tratados con Bopriva fueron más pesadas, con mayor espesor de grasa dorsal y mejor marmoleo.

Palabras clave: inmunocastración, testosterona, canal, calidad de carne.

ABSTRACT

To evaluate the effect of immunocastration on carcass and meat characteristics, Holstein bulls aged between 7 and 8 months with a liveweight of 232 ± 1.19 kg were given two separate treatments, placebo (intact bulls) versus Bopriva, and then slaughtered after approximately 239 days of fattening. While the testosterone levels in intact bulls remained at 0.42 ng/ml throughout the study, by day 181, differences ($P < 0.05$) were observed in immunized bulls, with values of 0.21 ng/ml. The carcasses of animals treated with Bopriva recorded both a higher hot carcass weight (HCW) and a cold carcass weight (CCW), as well as higher dorsal fat density, marbling and KPH ($P < 0.05$); however, no differences ($P < 0.05$) were observed in the Longissimus lumborum area. No significant differences ($P > 0.05$) were recorded between the treatments for pH, L*, a*, b* C* and H*. The carcasses of the animals treated with Bopriva were heavier, with higher dorsal fat density and marbling score.

Key words

Immunocastration, testosterone, carcass, meat quality

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CAPITULO 1

INTRODUCCION

Las explotaciones pecuarias prefieren a los novillos sobre los animales enteros debido a que son menos agresivos y su actividad sexual es mínima (niveles más bajos de testosterona), además de que los toros son más susceptibles al estrés por lo tanto es más probable que presenten carne DFD (Seideman et al., 1982; Cohen et.al, 1990). No se le ha dado importancia al impacto de la castración y el estrés sobre los becerros al momento de intentar mejorar el manejo tradicional de los animales (Bretschneider, 2009). El crecimiento y maduración testicular son regulados por la hormona liberadora de gonadotropina (GNRH), la apropiada regulación de esta puede modular la concentración sanguínea de testosterona, manteniendo así un buen desarrollo durante el crecimiento mientras se reduce la masculinidad de la canal. La mayoría de los estudios recientes han registrado que las vacunas basadas en un agente anti-GNRH han sido eficaces para disminuir los niveles de testosterona y los comportamientos agresivos, pero este último solo se ha evaluado subjetivamente, por lo tanto no hay estudios donde se haya evaluado el comportamiento objetivamente (Jannet et al., 2012a).

Distintos estudios han registrado que no existen diferencias significativas sobre parámetros de calidad de la canal de interés comercial, por ejemplo Rodríguez et al. (2014) quienes no encontraron diferencias entre animales castrados a distintas edades respecto a la cobertura de grasa y área del ojo de la costilla y Giuliana et al. (2014) respecto al color de la carne, observaron que la condición sexual de los animales influía sobre los valores de pH, L*, a* y b*, siendo los valores de pH de los animales enteros mayores que los de los castrados ($P < 0.05$), los de L* menores en los enteros de igual manera la carne fue menos roja ($P < 0.05$) y menos amarillenta ($P < 0.05$) en los mismos.

Por lo anterior el objetivo de este trabajo es hacer una revisión de literatura acerca de la castración en el ganado bovino y su efecto sobre el comportamiento, bienestar animal y calidad de la carne y canal.

CAPITULO 2

REVISION DE LITERATURA

La castración en producción animal

La castración de los becerros destinados a la producción de carne es una práctica común en Norte América, el término hace referencia a la esterilización de los machos de cualquier especie, es decir la remoción *in situ* de los testículos destruyendo su función. Las implicaciones que esta práctica tiene sobre el bienestar resultan importantes desde el punto de vista del dolor agudo o crónico que genera en el animal independientemente del procedimiento (Rault et al., 2011).

Las explotaciones prefieren a los novillos sobre los toros debido a que son menos agresivos y su actividad sexual es mínima, además de que los toros son más susceptibles al estrés por lo tanto es más probable que presenten carne DFD (Seideman et al., 1982; Cohen et.al, 1990). A menudo se utiliza la castración como una herramienta de manejo que provee de ciertas ventajas, entre ellas una mejor calidad de la canal a través de la deposición de grasa, reducción de comportamiento agresivo y sexual lo que resulta en un manejo más sencillo, menor daño para la canal y mejor bienestar animal (Amatayakul-Chantler et al., 2012) y aunque los animales castrados crecen más lento y tienen una menor eficiencia alimenticia que sus similares enteros, su canal posee características deseadas por el consumidor (Adams, 2005).

En las explotaciones, la edad a la que se lleva a cabo la castración puede variar, con base a esto se escoge una técnica para realizarla y ya que dependiendo de esta la respuesta en términos hormonales (cortisol) así como conductual serán menos intensas, generalmente los becerros jóvenes (0-6

meses) son castrados mediante el uso de bandas de goma en el escroto, mientras que los mayores son castrados quirúrgicamente (Rault et al., 2011).

Formas de castración

Las opiniones respecto a la edad y al método de castración con relación a la eficacia y reducción del estrés y el dolor en los becerros son muy variables (Becker et al., 2012). Los métodos de castración se pueden dividir en

Mecánicas: Es el método más común y consiste en la remoción de los testículos y se puede llevar a cabo de dos formas: a) mediante la utilización de bandas de goma colocadas en la base del escroto (Bretschneider, 2009), esta forma es sencilla, barata y eficaz pero genera un dolor crónico en el animal y su tiempo de recuperación es más largo (Becker et al., 2012b).

Quirúrgica: Este proceso es el tradicional, pero resulta bastante estresante para el animal además de que está asociada a una reducción en la tasa de crecimiento del animal y a infecciones post operatorias (Price et al., 2003).

Químicos: Consiste en la inyección de agentes tóxicos o esclerosantes (lesionan el tejido causando inflamación y una eventual fibrosis) en el parénquima testicular causando daños irreversibles y pérdida de la función testicular (Mohammed y James, 2013).

Hormonales: La supresión inmunológica de las hormonas sexuales en los animales productores de carne reducen el comportamiento agresivo y mejora la calidad de la carne, las vacunas anti-GNRH estimulan la producción de anticuerpos neutralizando a la GNRH e inhibiendo la secreción de hormonas sexuales por lo que actualmente una opción es la inmunocastración, se ha observado que provoca una reducción de la conducta agresiva aumentando la economía a los productores de ganado mediante la reducción de la incidencia de lesiones en los animales y en sus cuidadores, la reducción de la carne con contusiones y la reducción de cortes de carne oscuros (Price et al., 2003).

Efectos de la castración

Incrementar la cantidad de becerros destetados al año, mediante correctas prácticas reproductivas y sanitarias, ha sido siempre la prioridad de cualquier explotación, sin embargo, no se le ha dado importancia al impacto de la castración y el estrés sobre los becerros al momento de intentar mejorar el manejo tradicional de los animales (Bretschneider, 2009). Los machos han sido castrados por su tendencia a acumular grasa, curiosamente esto va en contra del mercado actual que prefiere la carne magra; sin embargo, el contenido de grasa está estrechamente ligado a la calidad de la carne y se piensa que además la castración influye sobre la dureza, jugosidad y sabor de la carne de los bovinos (Rault et al., 2011).

La castración temprana reduce significativamente el dolor y la incomodidad para el animal, riesgo de sangrados e infecciones, tiempo de recuperación y pérdidas de peso. Existen mitos acerca de las propiedades anabólicas de los andrógenos por lo que muchos productores rehúsan castrar a los animales a una edad temprana (nacimiento), además considerando que las diferencias de producción debidas a la testosterona se manifiestan luego de la pubertad (10 meses promedio) y que los periodos de destete tradicionales son de 6 a 9 meses, se supone que los animales castrados tempranamente alcanzan pesos similares a los que se castran post-destete (Del Campo et al.,

2014). Por otro lado, se sostiene que si los animales son castrados tardíamente las pérdidas de peso se incrementan, lo que implica una desventaja con respecto a los castrados tempranamente, por lo tanto al momento de comenzar el periodo de engorda los animales castrados tempranamente llegan en una condición general superior a los castrados tardíos (Bretschneider, 2005). Sin embargo Prado et al. (2014) mencionan que la castración temprana o tardía no afecta los parámetros de calidad de la carne y la canal en becerros Holstein.

En un estudio utilizando 200 becerros Holstein para evaluar el efecto de la castración utilizando un Burdizzo (castración mecánica) sobre las características de la canal y calidad de la carne, para lo cual se dividieron en dos grupos; 1) castrados y 2) enteros, los resultados indicaron no diferencias en cuanto a valores de pH entre grupos, sin embargo los animales enteros registraron valores de L^* más bajos que los castrados ($P < 0.05$) y al igual que los valores de a^* y b^* ($P < 0.001$). Respecto a la dureza los animales castrados produjeron carne ligeramente más blanda que los enteros ($P < 0.001$). Frecuentemente se reporta que los animales enteros producen carne más oscura en comparación con los castrados. Y a pesar de que se han encontrado niveles de mioglobina similares independientemente de la condición sexual del animal, esta coloración oscura se asocia principalmente al comportamiento más agresivo de los animales enteros (Mach et al., 2009).

Se han realizado investigaciones para desarrollar una vacuna antagonista de las hormonas reproductivas que haría que los testículos se encogieran y disminuyeran su función debido a la falta de estimulación por parte de la hormona luteinizante, este proceso se conoce como inmunocastración, este inmuniza al animal frente a la GNRH, mediante la inyección de un análogo de esta hormona, el cual generara una respuesta inmune tal como si se tratase de una vacuna contra un virus o una bacteria (Rault et al., 2011). La inmunización contra la GNRH fue propuesta para una esterilización estratégica de animales machos, incluyendo toros como un método no quirúrgico más

amigable hacia el animal. Por lo tanto, la inmunocastración potencialmente puede hacer realidad las ganancias o ventajas que conlleva la engorda de animales enteros, incrementar la calidad de la carne y controlar comportamientos no deseados mediante una inmunización estratégica (Amatayakul-Chantler et al.,2012). Este procedimiento conlleva a una disminución de la secreción de hormona luteinizante (LH) y a la larga reduce el desarrollo testicular y los niveles sanguíneos de testosterona (Huxsoll et al., 1998).

La mayoría de los estudios recientes han registrado que las vacunas basadas en un agente anti-GNRH han sido eficaces para disminuir los niveles de testosterona y los comportamientos agresivos, pero este último solo se ha evaluado subjetivamente, por lo tanto no hay estudios donde se haya evaluado el comportamiento objetivamente (Jannet et al., 2012a).

Efecto de la inmunocastración en la producción animal

El crecimiento y maduración testicular son regulados por la hormona liberadora de gonadotropina (GNRH), la apropiada regulación de esta puede modular la concentración sanguínea de testosterona, manteniendo así un buen desarrollo durante el crecimiento mientras se reduce la masculinidad de la canal. Cook et al. (2000) evaluaron la inmunización contra la GNRH en becerros cruzados y su efecto sobre el desarrollo testicular y características de la canal, utilizando 58 animales divididos en dos grupos, en uno se aplicó la vacuna anti-GNRH y en el otro sin aplicación de vacuna (testigo). Se registró que el valor promedio de testosterona sanguínea disminuyó 75% debajo del límite de detección de la hormona en el grupo de animales inmunizados, la circunferencia escrotal también fue menor en este grupo que en el testigo, la dureza al corte no mostró significancia estadística ($P < 0.14$), sin embargo la concentración sanguínea de testosterona mostró una relación lineal positiva ($r^2 = 0.77$; $P < 0.01$) con la dureza de la carne, esto sugiere que entre más fuerte sea la respuesta inmune del animal frente a la vacuna anti-GNRH la carne será más blanda.

Los primeros registros sobre la inmunización de becerros contra la GNRH mostraron que si se alcanzaban los títulos de anticuerpos suficientes, el tamaño testicular, la producción de testosterona y el comportamiento agresivo podrían ser reducidos, por otra parte los animales inmunizados tienen aproximadamente 32% menos grasa y un área del ojo de la costilla 50% mayor a la altura de la décima costilla (Thompson, 2000).

Según Adams et al. (1993) la inmunización de becerros contra la GNRH a los 3.6 meses de edad mejoró la ganancia de peso, peso de la canal caliente y el área del ojo de la costilla con respecto a los novillos.

Lobley et al. (1992) reportaron que la inmunización contra GNRH de toros cruzados no afectó el consumo, sin embargo, alteró la proporción de tejido graso de la canal y el contenido de proteína.

La inmunocastración prepuberal reduce los niveles sanguíneos de testosterona por al menos cuatro meses, pero mantiene las tasas de crecimiento a niveles similares de animales enteros y superior a los castrados quirúrgicamente. Se utilizaron 90 becerros Holstein de entre ocho a diez semanas de edad para evaluar el efecto de una vacuna anti-GNRH sobre el comportamiento se utilizaron nueve grupos de animales a) castrados a los dos meses de edad; b) inmunizados a los 2, 2.5, 4 y 7.5 meses de edad; c) inmunizados a los 4, 4.5 y 7 meses de edad; d) 7.5 y 8 meses de edad y animales enteros, registrando el comportamiento de los animales (montas, signo de Flehmen, cornando el suelo, intentos de montas, manoteando el suelo, cabezazos, peleas, empujones, movimientos a lo largo de la cerca y daño de praderas). Se observó que los niveles sanguíneos de testosterona disminuyeron por debajo de 5 ng/ml en los animales inmunizados y mantuvieron un promedio menor a 5 ng/ml hasta 257 días después de que los animales fueran inmunizados, se redujeron el número de montas y comportamientos que provocan daño en las praderas además los comportamientos agonistas disminuyeron debido a que los animales tenían sus niveles de testosterona

bajos, reduciendo considerablemente la actividad del grupo (Jago y Matthews, 1997).

En un estudio conducido por Jannet et al. (2012a) donde se utilizaron 44 becerros Holstein negros y rojos (21) y pardo suizos (23) de entre seis y siete meses de edad, se conformaron dos grupos (inmunizados y testigo) donde el primero fue inmunizados contra la hormona GNRH. Se utilizó un sistema inalámbrico para medir la actividad física de los animales, registraron diferencia entre grupos en cuanto a la actividad física. Los animales inmunizados se mostraron más apáticos que aquellos en el grupo testigo luego de la vacunación durante un periodo de al menos 106 días. Los niveles de testosterona de los animales inmunizados se mantuvieron por debajo de los testigos (<0.01ng/ml vs 14 ng/ml), pero esto no afectó su ganancia diaria de peso, la mantuvieron al nivel de los animales testigos, atribuyéndose a una disminución en la actividad física.

Jannet et al. (2012b) llevaron a cabo un estudio en el cual utilizaron 12 becerros Holstein y los dividieron en dos grupos, uno inmunizado contra GNRH y un testigo, midieron niveles sanguíneos de testosterona, ellos observaron que los animales inmunizados, los cuales no vieron su ganancia diaria de peso comprometida y una disminución en los niveles sanguíneos de testosterona en el grupo inmunizado (testigos: >5ng/ml, inmunizados <5ng/ml).

En un estudio realizado por Amatayakul-Chantler et al. (2012) quienes utilizaron becerros *Bos indicus* x Pardo Suizo, los tratamientos fueron los siguientes: 1) Testigo, 2) implantes anabólicos, 3) Vacuna anti-GNRH y 4) implantes+ vacuna anti-GNRH. Los autores encontraron que los animales del tratamiento 3 mostraron una fuerte respuesta inmune contra la GNRH, esto lo asociaron a la supresión de los niveles sanguíneos de testosterona los cuales fueron menores a 5ng/ml, concluyendo que utilizando esta vacuna podían mejorar la calidad de la carne, debido a que la utilización de implantes ha mostrado una reducción en el marmoleo y en la terneza de la carne, por lo que

la utilización de este producto provee un método para mejorar la calidad de la carne cuando a los becerros se les están administrando implantes anabólicos.

Amatayakul-Chantler et al. (2013) utilizaron becerros Nelore (*Bos indicus*) para evaluar el efecto de un inmunocastrador sobre las características de la canal y calidad de la carne, en dos grupos, 1) castrados quirúrgicamente e 2) inmunocastrados; registraron que dos semanas después de castrados los animales del grupo 1 estos comenzaron a tener complicaciones como descargas purulentas, miasis, funiculitis del cordón espermático y hemorragias mientras que los inmunocastrados no presentaron complicación alguna. Con respecto a los niveles sanguíneos de testosterona fueron iguales entre grupos, además, no hubo diferencia entre grupos sobre las características de la canal y calidad de la carne, se observaron diferencias en el valor de b* (amarillo) en el grupo de inmunocastrados pero esta diferencia se considera irrelevante para aspectos generales de calidad de la carne.

Evaluación del estrés en los animales

En la industria de la carne el estrés provoca rendimientos inferiores a los esperados y bajas ventas del producto debido a una carne con coloración anormal, suave, seca o golpeada resultando de calidad inferior y en ocasiones con rechazo (Partida, 2007).

La observación del comportamiento animal y su productividad también se utilizan para determinar y medir el estrés y el temor. Los cambios en el comportamiento animal, como el cojeo, incremento en la vocalización, abortos espontáneos y patrones de sueño inusuales, son indicadores de estrés y temor, estos minimizan la ganancia de peso y el comportamiento normal. También se incrementan los desórdenes neuróticos, niveles de corticoides y la presión arterial además de suprimir el sistema inmune. Se dice que entre más joven el animal su sistema nervioso esta menos desarrollado, por lo tanto la respuesta de estrés genera menos impacto en el animal (Lanier, 2010).

Los cambios asociados al estrés (efectos negativos sobre la canal y la calidad de la carne) se relacionan con cambios en la concentración sanguínea de cortisol, sus niveles sanguíneos son buenos indicadores del estrés agudo experimentado por un animal e incrementa sustancialmente cuando el ganado se expone a situaciones adversas como el manejo, castración, reagrupamiento, transporte y tratamiento médico (Bøe y Færevik, 2003). También se puede medir a través de los niveles de glucosa, ácidos grasos volátiles (β -HBA) y hematocrito (VGA), así como con indicadores enzimáticos como la creatinfosfoquinasa (CK) y el cortisol. Estos parámetros se utilizan como indicadores de estrés, particularmente cuando se comparan valores previos y posteriores a un manejo que puede ocasionar estrés al animal (Amtann et al., 2006; Partida et al., 2007).

En un estudio donde se evaluó el efecto sobre los niveles de cortisol sanguíneos de distintos métodos de castración mediante bandas de goma, fueron utilizados 63 becerros de entre 4 y seis semanas de edad (Simmental, Holstein rojo y sus cruza), se dividieron en cinco grupos: 1) banda de goma tradicional, 2) banda de goma más burdizzo, 3) banda de goma en tejido testicular y remoción al noveno día, 4) triple banda de goma en la base escrotal y 5) control (castración simulada), referente a las concentraciones sanguíneas de cortisol no se registraron diferencias significativas entre grupos en ningún momento, sin embargo, la hubo al momento de hacer el manejo ($P < 0.001$). Las concentraciones fueron mayores de 15 a 20 minutos después de la castración, también se registraron diferencias entre grupos sobre la inflamación del tejido próximo (incremento en el diámetro escrotal) a la banda de goma ($P < 0.001$) en el grupo 4 (Becker et al., 2012), por lo tanto el dolor causado al aplicar este método incrementa los niveles de cortisol sanguíneos lo cual se traduce en niveles altos de estrés.

Si se mantiene un nivel bajo de secreción de testosterona se podría sostener la eficiencia de crecimiento y alimenticia sin que esto propicie la aparición de comportamientos sexuales y agonistas (Adams, 2005).

Se ha demostrado que la inmunocastración reduce los niveles sanguíneos de testosterona; en un estudio se utilizaron 12 becerros de entre 6 y 8 meses de edad, dos de estos se tomaron como animales testigo y el resto fue vacunado con un factor anti-GNRH, se observó que los niveles sanguíneos de testosterona disminuyeron a valores menores a 0.5 ng/ml después de la segunda aplicación de la vacuna y se mantuvieron en esos niveles alrededor de 10 semanas más, luego de esto los niveles de testosterona comenzaron a alcanzar sus niveles normales (>5ng/ml) (Theubet et al., 2010).

La marcada disminución en la secreción de testosterona es consecuencia de una inmunización efectiva que incrementa la deposición de grasa y disminuye el comportamiento agresivo, aunque la tasa de crecimiento se reduce en los animales inmunizados, el peso vivo final y el peso de la canal mejoran hasta equipararse a los animales que no han sido inmunizados debido a que se mantiene un nivel residual de secreción de testosterona (Adams, 2005).

Comportamiento animal

Cuando los animales están agrupados, interactúan y desarrollan relaciones con sus congéneres de naturaleza dominante-subordinado, lo que lleva al establecimiento de una jerarquía social, esto ocurre cuando dos animales de la misma especie se relacionan, entonces uno debe de aprender o intentar dominar al otro, mientras que el otro tratara de evitar cualquier confrontación, después de que los animales determinan su jerarquía la dominancia se establece, posteriormente las peleas tienden a disminuir mas no así comportamientos agonistas como persecuciones, empujones y cabezazos (Partida et al.,2007). En el ganado las relaciones de dominancia pueden causar estrés, contusiones y daños físicos (Fordyce et al., 2002).

Efecto de la castración sobre la calidad de la canal y carne

Distintos estudios han registrado que no existen diferencias significativas sobre parámetros de calidad de la canal de interés comercial como lo son el

área del ojo de la costilla y el espesor de la grasa de cobertura, Rodríguez et al. (2014) no encontraron diferencias entre animales castrados a distintas edades respecto a la cobertura de grasa y área del ojo de la costilla. Amatayakul-Chantler et al. (2013) no registraron diferencia entre animales castrados quirúrgicamente e inmunocastrados, en estudios similares conducidos por Cook et al. (2000); Ribeiro et al. (2004); Freitas et al. (2008); Mach et al. (2009); Giuliana et al (2014) y Rodríguez et al. (2014) se observan tendencias similares, por otro lado Amatayakul-Chantler et al. (2012) registraron que los animales que son inmunocastrados depositan mayor cantidad de grasa de cobertura mientras que el área del ojo de la costilla queda más pequeña en comparación con los animales enteros, Aïssat et al. (2002) realizaron un estudio similar y encontraron resultados similares además registraron que la deposición de grasa de riñon pelvis y corazón (KPH) no mostró diferencia entre animales inmunocastrados o enteros. Aricetti et al. (2008) condujeron un estudio donde compararon las características de la canal de animales castrados contra enteros observando que los animales enteros desarrollaron una cobertura de grasa menor a los castrados ($P < 0.02$), esto se lo atribuyeron a la presencia de hormonas masculinas que hacen que en lugar de acumular grasa desarrollen sus músculos, la coloración y el marmoleo también fueron mejores en los animales castrados ($P < 0.06$), la pobre coloración de la carne de los animales enteros se le atribuyó a la presencia de testosterona que hace que haya mayor cantidad de tejido conectivo en la carne y le da una tonalidad más oscura.

El pH de la carne es el parámetro más importante que influye sobre la calidad de la carne ya que afecta características importantes como el color, dureza, y capacidad de retención de agua (Andersen et al., 2004). La literatura reporta que los machos debido a su mayor agresividad, tienden a producir carne con valores de pH más altos que las hembras y se ha demostrado que las montas de un animal a otro para dominarlo son la actividad física que más causa depleción de glucógeno muscular por lo que afectara directamente al pH (Partida, 2007).

Se realizó un estudio para conocer el efecto de la castración y la edad sobre el desempeño, características de la canal y calidad de la carne en becerros Holstein; se utilizaron tres grupos de animales, dos castrados a distintas edades y un testigo, en el registraron que el peso de la canal caliente los animales enteros tuvieron un peso menor que los castrados ($P < 0.001$), el pH fue mayor en los animales enteros ($P < 0.001$), la dureza de la carne también fue mayor en este grupo ($P < 0.001$) la carne fue más oscura, menos roja y menos amarillenta que la de los animales castrados, como era de esperarse la castración mejoró la dureza de la carne y el pH (valores más bajos), además la carne de los castrados fue más luminosa, roja y amarillenta que la de los animales enteros ya que estos se estresan más fácilmente y se montan frecuentemente, siendo estos dos factores la posible razón por la cual la carne de los animales enteros tuvo un pH mayor y fue más oscura (Marti et al., 2013).

Micol et al. (2009) utilizaron 38 becerros Charolais para observar el efecto de la castración y la edad sobre la calidad de la carne, se dividieron en cuatro grupos y dos periodos, el primer grupo constaba de 9 animales castrados a los dos meses de edad y de otro de 10 animales castrados a los 10 meses, se utilizaron grupos similares para ambos periodos, no se registraron diferencias entre edades a la castración para valores de pH, a^* y b^* solo en el valor de L^* , donde los animales castrados a los 10 meses de edad mostraron una carne más oscura ($P < 0.05$).

Beceros de raza Nelore y Nelore x Aberdeen Angus fueron finalizados en corral de engorda durante 90 días, se utilizaron tres grupos por componente racial; 1) castrados quirúrgicamente, 2) inmunocastrados y 3) animales enteros, se registró que la condición sexual de los animales influyó sobre los valores de pH, L^* a^* y b^* , siendo los valores de pH de los animales enteros mayores que los de los castrados ($P < 0.05$), los valores de L^* fueron menores en los enteros ($P < 0.05$) esto se relacionó con los altos valores de pH de los animales enteros, de igual forma la carne fue menos roja ($P < 0.05$) y menos amarillenta ($P < 0.05$), pero no hubo diferencia entre grupos sobre la dureza de la carne (Giuliana et

al., 2014). En un estudio similar, se registró que los animales enteros mostraron tendencia a producir carne más oscura ($P = .10$) que los animales castrados quirúrgicamente y los inmunocastrados y esta tendencia se mostró también sobre la dureza de la carne, requiriendo un 16 % más de Nétwtones de fuerza ($P = .14$) para cortar la carne de animales enteros (Ribeiro et al., 2004) Por otra parte, Cook et al. (2000) registraron que se necesitaba un 16% menos de Nétwtones de fuerza para cortar la carne de animales inmunocastrados.

Costa et al. (2007) evaluaron el efecto de la castración sobre la calidad de la carne en 32 becerros Nelore-Simmental divididos en dos grupos; 1) castrados y 2) enteros, no existió diferencia entre grupos sobre los parámetros que determinan el color. El valor de L^* registró, una media de 31.4 valor que se puede considerar propio de una carne oscura, las medias de a^* y b^* (11.8 y 4.7 respectivamente) indicaron que el color rojo y amarillo de la carne fueron bajos, de igual forma el pH cuya media fue de 5.5 (normal) no mostró diferencia entre animales enteros y castrados.

Un estudio realizado por Destefanis et al. (2002) donde utilizaron 24 becerros Piemontes hipertrofiados para observar el efecto de la castración sobre la calidad de la carne en este tipo de ganado, se dividieron en tres grupos 1) castrados tempranamente (5 meses de edad), 2) castrados tardíos (13 meses de edad) y animales enteros, los dos primeros grupos fueron castrados quirúrgicamente. Generalmente la carne oscura de los machos se asocia a los altos valores de pH, como resultado de su temperamento más excitable y sus altas tasas de glucólisis *ante-mortem*, sin embargo, en este estudio no se observó significancia entre grupos en pH, dureza y color, esto se lo atribuyeron a que este tipo de ganado al igual que otras razas que desarrollan hipertrofia muscular son manejadas con sumo cuidado debido a su alto valor comercial.

Miguel et al. (2014) realizaron un estudio para demostrar que tanto la castración como la inmunocastración mejoran los atributos de color en la carne de animales de raza Nelore, para ello utilizaron 29 animales, de estos nueve se castraron quirúrgicamente, 10 fueron inmunocastrados y los 10 restantes fueron

animales enteros. Como resultado, observaron que no hubo diferencia entre grupos respecto a la dureza de la carne ($P > 0.05$) sin embargo, la medición de los atributos de color no mostró diferencias entre los castrados, pero si en comparación con los enteros (valores de L^* , a^* y b^* más bajos para los enteros; $P < 0.05$).

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CAPITULO 3



Effect of immunocastration with Bopriva on carcass characteristics and meat quality of feedlot Holstein bulls

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ABSTRACT

To evaluate the effect of immunocastration on carcass and meat characteristics, Holstein bulls aged between 7 and 8 months with a live weight of 232 ± 1.19 kg were given two separate treatments, placebo (intact bulls) versus Bopriva, and then slaughtered after approximately 239 days of fattening. While the testosterone levels in intact bulls remained at 0.42 ng/ml throughout the study, by day 181, differences ($P < 0.05$) were observed in immunized bulls, with values of 0.21 ng/ml. The carcasses of animals treated with Bopriva recorded both a higher hot carcass weight (HCW) and a cold carcass weight (CCW), as well as higher dorsal fat density, marbling and KPH ($P < 0.05$); however, no differences ($P > 0.05$) were observed in the *Longissimus lumborum* area. No significant differences ($P > 0.05$) were recorded between the treatments for pH, L*, a*, b* C* and H*. The carcasses of the animals treated with Bopriva were heavier, with higher dorsal fat density and marbling score.

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1. Introduction

Currently, producers of livestock for meat have considered the fattening of Holstein bull calves as an option, given that these animals offer certain advantages to the cattle farmer like the fact that they yield high quality carcasses, they can enter the feedlot with a weight of approximately 136 kg, and they can reach up to 590 kg after approximately one year of fattening (Duff & McMurphy, 2007).

However, if the calf bulls are not castrated, these animals can become dangerous (Duff & McMurphy, 2007), as a result of aggressive behavior both among themselves and toward their handlers, and they become difficult to handle. One method for reducing the occurrence of these events is through castration, this is accompanied with additional advantages, among which are enhanced carcass quality through improved body fat deposition, the reduction of aggressive and sexual behavior leading to easier handling, less carcass damage and improved animal welfare (Amatayakul-Chantler et al., 2012). However, this practice results stressful for the animal and is associated with reduced growth rates and post-operative infections (Price, Adams, Huxsoll, & Borgwardt, 2003). An alternative is immunocastration, which can bring the profits and advantages provided by the fattening of intact

animals, increase the quality of the meat, and control undesirable behavior through a strategic immunization (Amatayakul-Chantler et al., 2012). This procedure acts by the injection of a GnRH analog, which generates an immune response such as that stimulated by a vaccine against a virus or a bacteria (Rault, Lay, & Marchant-Forde, 2011), thus stimulating the production of antibodies that both neutralize the GnRH and inhibit the secretion of sexual hormones. In light of the above, the objective of this study was to evaluate the effect that immunocastration with Bopriva has on the carcass characteristics and meat quality in fattened Holstein bulls.

2. Material and methods

2.1. Geographical location

The study was carried out in the city of Mexicali, Baja California, which is found at 32° 32'00 N, 115° 12'41 W. The region is characterized by a dry desert climate with an average temperature of 34.7 °C (− 5 °C winter and 50 °C summer), with an annual rainfall of 37 mm, and a relative humidity above 50% (García, 1981). All animals were slaughtered at a Federal Inspection Type (TIF) slaughterhouse, following the methodology described in the Norma Oficial Mexicana NOM-033-ZOO-1995, "Humanitarian slaughtering of domestic and wild animals".

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2.2. Animals and design of the study

Holstein bulls from the same origin, aged between 7 and 8 months of age and with a live weight of 232 ± 1.19 kg were used. For the study two treatments were considered: four pens of 90 intact animals (placebo treatment) and four pens of 90 intact animals treated with Bopriva (Zoetis Laboratories Animal Health, México).

Twenty four hours after reception of the animals, the lots were structured, and the calves were vaccinated, dewormed, and an implant was placed (a trenbolone acetate, estradiol and tylosin product). Bopriva was administered subcutaneously on four occasions: (24 h after reception and on day 21, 101 and 181 of the experiment), while the calves in the placebo group were administered 1 ml of saline solution on the same days.

The live weight of each animal was recorded during each treatment. The animals were fed twice a day, following a program of 6 diets typical to the Northern region of the country, comprising wheat hay, sudangrass, tallow, DDG (Dried distillers grains), and a mineral premix. The animals were slaughtered once they had reached an average weight of 580 kg by day 239. The day of sacrifice the animals were herded by the cowboy on horseback for about 1 km to the slaughterhouse. The animals were kept in rest pens with access to water for approximately 6 h.

2.3. Serum testosterone levels

Ten animals per pen were randomly selected to measure serum testosterone levels. The animals selected for serum sampling were identified by an additional earing. Blood samples were taken every time that Bopriva was administered, and a sample was also taken during slaughter at the bleeding station on the production line at the slaughterhouse.

Approximately 5 ml of blood was extracted from the coccygeal vein. The samples were centrifuged at 3500 rpm to obtain serum, using a TRIAC centrifuge (Clay Adams, Model 0200, New Jersey, U.S.A.), and stored at -20 °C until the testosterone concentration was measured. The concentrations of serum testosterone were determined using the ENZO Testosterone Elisa Kit test (ADI-900-065), according to the manufacturer's instructions.

2.4. Carcass and meat quality

Carcasses from both treatments were chilled at 2 °C for 24 h and ribbed between the 12th and 13th ribs to collect additional carcass data. A total of 226 carcasses from the immunocastrated group and 208 carcasses from the placebo group were available by the slaughterhouse to be considered for the study of all the variables. The measurements of HCW and CCW, dorsal fat density, KPH (kidney, pelvic and heart fat), marbling, ribeye area, pH and color of each carcass were taken. Dorsal fat was measured in mm using a metric ruler, according by Hale, Goodson, and Savell (2013). The ribeye area was evaluated using a plastic grid method suggested by Iowa State University. The estimated quantity of kidney, pelvic and heart fat (% KPH) which was subjectively evaluated and expressed as a percentage of hot carcass weight (HCW), and the marbling score (scale of slight; small; modest; moderate; slight abundant; moderately abundant), were both evaluated following the methodology described by AMSA (2001). The pH was determined using a potentiometer (HANNAH INSTRUMENTS Inc. pH 101), the color values (L^* , a^* , b^* , C^* , H^*) were measured on the surface of the cut from the *Longissimus lumborum* muscle between the twelfth and thirteenth intercostal space using a MINOLTA CM-2002 spectrophotometer (Minolta camera, Co., Ltd., Japan) with a specular component included (SCI), a D_{65} illuminant, and a 10° observer, where L^* is the index of luminosity, a^* is the red color intensity and b^* is the yellow color intensity. Forty eight hours postmortem, a total of 80 meat samples of approximately 500 g were obtained from *Longissimus lumborum* muscle, 10 random samples per pen. The samples were vacuum packed, refrigerated and sent to the Meat Quality Laboratory for products of

animal origin at the Instituto de Investigaciones en Ciencias Veterinarias at the Universidad Autónoma de Baja California, and subjected to shear force (SF) analysis using Warner-Bratzler blades. This test was conducted on pre-cooked 1 cm² meat samples obtained perpendicular to the muscle fibers using a meat core sampler, with measurements made using a texturometer (Lloyd Instruments, England). All measurements were carried out in triplicate.

3. Statistical analysis

The effects of the treatments on pH, color, and SF traits in the meat were analysed using the GLM procedure (SAS Inst. Inc., Cary, NC). The linear statistical model for the trial was as follows: $Y_{ij} = \mu + \tau_i + \xi_{ij}$ $i = 1, 2; j = 1, 2, \dots, r$ where Y_{ij} is the response variable, μ is the true mean effect, τ_i is the fixed treatment effect (Bopriva vs placebo) and ξ_{ij} is the random residual error iid $N(0, \sigma_e^2)$. When the treatments represented a significant ($P \leq 0.05$) source of variation, differences between means for treatment were compared using Tukey's procedure.

Testosterone levels of each animal, which was recorded at days 1, 21, 101, 181, and at the day of slaughter, was analysed with a linear mixed model for repeated measures using the MIXED procedure (SAS Inst. Inc., Cary, NC) using REPEATED statement. The linear statistical model was $Y_{ijk} = \mu + \tau_i + A_{k(i)} + D_j + (\tau D)_{ij} + \xi_{ijk}$ $i = 1, 2; j = 1, 2, \dots, 5, k = 1, 2, \dots, r$ where Y_{ijk} is the response variable, μ is the true mean effect, τ_i is the fixed treatment effect (Bopriva vs placebo), $A_{k(i)}$ is the animal within treatment effect assumed iid $N(0, \sigma_a^2)$, D_j is the fixed days effect, $(\tau D)_{ij}$ is the treatment \times days interaction effect, and ξ_{ijk} is the random residual error effect iid $N(0, \sigma_e^2)$. The analysis of repeated measures data requires appropriate accounting for correlations between the observations made on the same animal and heterogeneous variances among records over time, therefore unstructured (UN), compound symmetric (CS), and autoregressive of order 1 [AR(1)] covariance structures were tried and evaluated by using Akaike's information criterion (AIC) and Schwarz's Bayesian information criterion (BIC). The covariance structure with values of the criteria closest to zero was the most desirable. Thus, UN structure was chosen. When the treatment \times days interaction represented a significant ($P \leq 0.05$) source of variation, differences between least square means for treatments by each day were compared using Tukey-Kramer's procedure (Steel & Torrie, 1985).

4. Results and discussion

4.1. Serum testosterone levels

The average testosterone levels on the day 1 were similar ($P > 0.05$) between immunocastrated group (0.26 ± 0.61 ng/ml) and placebo group of bulls (0.28 ± 0.60 ng/ml) (Fig. 1), at day 21 of fattening by

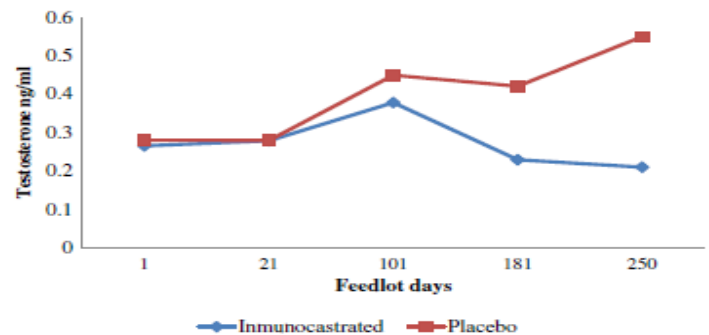


Fig. 1. Concentrations of serum testosterone by treatment.

both treatments there were no differences ($P > 0.05$) (0.27 ± 0.05 and 0.27 ± 0.06 ng/ml) respectively.

The testosterone levels increased gradually until day 101 without significant differences ($P > 0.05$) in both immunocastrated (0.38 ± 0.05 ng/ml) and placebo bulls (0.44 ± 0.05 ng/ml). From day 181, the testosterone levels in the intact bulls remained stable (0.42 ± 0.06 ng/ml) until the end of the study, while they gradually decreased in the group of immunized bulls, falling to 0.22 ± 0.05 ng/ml by day 181, with significant differences between both treatments recorded from this point onwards ($P < 0.05$). At the end of feedlot days, when animals were slaughtered, testosterone levels were 0.21 ± 0.06 ng/ml in immunocastrated bulls and 0.54 ± 0.06 ng/ml in placebo bulls. These results are consistent with those obtained by Theubet, Thun, Hilbe, and Janett (2010), who recorded values lower than 0.5 ng/ml after the second application of the vaccine in immunocastrated bulls.

Jannet et al. (2012a) observed a similar decrease in testosterone (<0.01 ng/ml immunocastrated bulls vs 14 ng/ml control) in Holstein bull calves, while Jannet et al. (2012b) recorded levels of >5 ng/ml in intact and <5 ng/ml in immunocastrated bulls.

Amatayakul-Chantler et al. (2012) reported that immunocastrated animals showed a strong immune response against GNRH, which they associated with the suppression of testosterone levels in the blood, that were lower than 5 ng/ml.

According to Adams (2005), if a residual level of testosterone secretion remains, growth and feed efficiency can be maintained without the appearance of sexual behavior and agonists. The average daily weight gain in this study was similar between treatments (Bopriva: 1.508 kg vs 1.503 kg control), analogous to results obtained by Jannet et al. (2012a), whom observed that immunocastrated animals besides lowering testosterone levels they maintained a daily weight gain similar to control animals. Nevertheless, in this study feed conversion in animals treated with Bopriva was more efficient (8.32:1) compared to the control group (9.46:1). By the end of production period, the immunocastrated animals reached a final average weight of 602.25 kg compared to 589.5 kg in placebo treatment. This effect may be attributed to the decrease in testosterone levels that as a consequence, will ensure that levels of physical activity decrease, and therefore the animals use the energy normally spend socializing, exercise and fighting in their growth (Jannet et al., 2012a).

4.2. Carcass and meat quality

The carcasses of animals treated with Bopriva recorded a higher average HCW and CCW, higher dorsal fat density and KPH percentage ($P < 0.05$). However, no difference was recorded for the ribeye area ($P > 0.05$) (Table 1). These findings oppose to a previous study which used *Bos indicus* animals and crossbreeds and did not observe significant differences ($P > 0.05$) in HCW and CCW between immunocastrated and intact animals (Cook, Popp, Kastelic, Robbins, & Harland, 2000; Giuliani et al., 2014).

Table 1
Mean values \pm S.E. for the variables in the treatment of Holstein bulls.

Variable	Treatment	
	Immunocastrated	Placebo
HCW (kg)	360 \pm 3.24 ^a	349 \pm 3.46 ^b
CCW (kg)	357.68 \pm 3.13 ^a	347.84 \pm 2.50 ^b
Dorsal fat (mm)	6.77 \pm 0.18 ^a	4.48 \pm 0.19 ^b
KPH (%)	1.97 \pm 0.05 ^a	1.68 \pm 0.05 ^b
<i>Longissimus lumborum</i> area (cm ²)	32.16 \pm 0.24 ^a	32.67 \pm 0.25 ^a

Mean \pm SE; different letters on the same row indicate difference ($P < 0.05$); n = 434 carcasses evaluated. HCW: hot carcass weight; CCW: cold carcass weight; KPH: kidney, pelvic and heart fat.

The results obtained for dorsal fat in this study coincided with the differences recorded for immunocastrated and intact bulls by D'Occhio, Aspden, and Trigg (2001) (5 mm vs 1.5 mm), Amatayakul-Chantler et al. (2012) (5 mm vs 4 mm), Andreo et al. (2013) (4.2 mm vs 3.3 mm), and Andreo et al. (2016) (4.22 vs 3.34) ($P < 0.05$). The scores for the marbling classification per treatment is showed in Table 2. The Bopriva group showed more carcasses with better marbling scores (small, modest, moderate, abundant) than the placebo group ($P < 0.05$). Similar differences were reported by Ribeiro et al. (2004), Amatayakul-Chantler et al. (2012), and Andreo et al. (2013), all of whom observed a higher grade of marbling for immunocastrated animals compared to the placebo group. Furthermore, in a study by De Freitas et al. (2015) conducted in immunocastrated and intact bulls, it was found that testosterone levels decreased in immunocastrated animals, but marbling score was higher compared to intact animals.

The differences observed in HCW and CCW between treatments in this study could be attributed to the amount of fat deposits in the carcass. In Mexico, KPH percentage is included in the HCW and CCW values. Coutinho, Peres, and Justo (2006) suggest that these differences in fat density and marbling could be attributed to the fact that fat takes longer to be deposited in intact bulls than in immunocastrated animals, due to the anabolic effect of testosterone, suggesting that testosterone has a lipogenic inhibitory effect on the enzymatic activity of the adipose tissue, which increase the basal levels of lipolytic activity (Andreo et al., 2016; De Freitas et al., 2015).

While the value for the *Longissimus lumborum* area for both treatments was lower than that recorded by Lehmkuhler and Ramos (2008) (79.8 cm²) and Bjorklund, Heins, Dicostanzo, and Chester-Jones (2014) (76.0 cm²), other studies found a smaller *Longissimus lumborum* area of 29.2 cm² (García, Walter, Reed, Rogers, & Lawrence, 2016) and 29.4 cm² (Velásquez, Noguera, & Posada, 2013).

No significant differences ($P > 0.05$) between treatments were found for the physicochemical variables of the meat, although the color values L*, a*, b*, C* and H* were typical of DFD (dark, firm and dry meat), the pH was that of a normal meat (Wulf, Emmett, Leheska, & Moeller, 2002); therefore, for both treatments the meat can be classified as a dark meat (Table 3). This can be attributed to a high level of antemortem stress which leads to a depletion of glycogen in the muscle and therefore affects the meat color and pH (Viljoen, De Kock, & Webb, 2002). According to Grandin (2000) the transport for short distances did not interfere on the ultimate pH of the carcasses. However, handling conditions, novelty of environment and fatigue, contribute to stress reactions, according to Miranda de la Lama, Villaroel, and María (2014). The pH values in both treatments correspond to that of a normal pH in the meat, so that the stress experienced prior to slaughter must have been for a prolonged period. Nevertheless similar studies have recorded differences ($P < 0.05$) in pH and color between intact and immunocastrated animals, with high pH values in intact animals, as well as a less red meat ($P < 0.05$) and less yellow meat ($P < 0.05$) but there was no difference recorded for SF between groups ($P > 0.05$).

Table 2
Classification of marbling in the carcasses according to treatment.

Classification	Treatment	
	Immunocastrated n = 226	Placebo n = 208
Traces	10	41
Slight	137	153
Small	75	14
Modest	2	0
Moderately abundant	2	0

Values in the table correspond to the number of times that any of the categories were presented.

Table 3
Physicochemical variables of the meat.

Variable	Treatments	
	Immunocastrated	Placebo
pH	5.64 ± 0.08 ^a	5.62 ± 0.01 ^a
L*	27.18 ± 0.307 ^a	26.30 ± 0.325 ^a
a*	21.38 ± 0.298 ^a	20.32 ± 0.316 ^a
b*	17.81 ± 0.274 ^a	17.71 ± 0.290 ^a
C*	27.91 ± 0.380 ^a	25.88 ± 0.402 ^a
H*	41.13 ± 0.354 ^a	41.13 ± 0.375 ^a
SF (N)	54.48 ± 1.96 ^a	54.48 ± 1.96 ^a

Mean ± SE; different letters on the same line indicate difference ($P < 0.05$). SF: shear force

(Giuliana et al., 2014). Ribeiro et al. (2004) observed that intact animals tend to produce a darker meat ($P = 0.10$) than immunocastrated animals, and also the tenderness of meat was different, moreover meat from intact animals required 16% more shear force ($P = 0.14$) to be cut. In a study conducted by Andreo et al. (2013), significant differences were found for variables a*, b* and C* ($P < 0.05$), but not for L* and H*, between treatments. The variables a* and b* were higher in immunocastrated and this was attributed to the great amount of fat in the meat. Although the animals in both groups were exposed to stress agents prior to slaughter, in this study the SF values between treatments were similar ($P > 0.05$). Ferreira et al. (2006) reported that neither transportation time nor rest period have any effect on the SF values, where they reported values of 48.1 N in DFD meat, and similar values were found in this study, and in accordance with the criteria established by Boleman et al. (1997), correspond to meat of intermediate tenderness (tender: 22.26–35.10 N; intermediate: 40.01–52.95 N; tough: 57.85–70.60 N). The same was observed by Amatayakul-Chantler et al. (2013) who found no differences ($P > 0.05$) on SF values between surgically castrated animals and immunocastrated animals. Moreover, Giuliana et al. (2014) did not report differences ($P > 0.05$) on SF values due to sexual conditions (surgically: 56.60 ± 0.36 N; immunocastrated: 53.37 ± 0.35 N and intact bulls: 48.85 ± 0.35 N). However, in this study, the results observed on SF values could be attributed to the age of the animals at the slaughter time that was at 15 to 17 months of age, while Amatayakul-Chantler et al. (2012) reported differences ($P = 0.107$) between immunocastrated (75.31 N) and intact animals (80.41 N) but that were slaughtered at 20 months of age, and similar to Andreo et al. (2013) who also found differences ($P < 0.02$) between intact animals (77.96 N) and immunocastrated (62.95 N) slaughter at 24 months of age.

5. Conclusions

Immunocastration of Holstein bulls with Bopriva represents an attractive alternative for today's livestock industry, since considering animal welfare is less traumatic than traditional castration, and decreased the serum testosterone levels, making the animal handling easier.

The carcasses of animals treated with Bopriva were heavier, with a higher dorsal fat density and greater marbling score, all these being features which confers a higher market value than carcasses from intact Holstein bulls.

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Effect of Immunocastration on Behavior and Blood Parameters (Cortisol and Testosterone) on Beef Holstein Steers

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Abstract

To evaluate the effect that immunocastration has on behavior, testosterone and cortisol levels of feedlot Holstein bull calves, 720 intact animal between 7 and 8 months of age and weight of 232 ± 1.19 kg were divided in two groups and given separate treatments: immunocastration using the bopriva vaccine and a placebo. The bulls were slaughtered at day 239. The vaccine was applied on days 1, 21, 101, and 181 and on that same days testosterone levels were measure; while cortisol, glucose and creatin kinase measurements were done on day 181 and during exanguination. Sexual, aggressive and social behaviors were evaluated and found that intact bulls showed a higher average of head butts, mounting, threats, Flehmen sign and sniffing ($P < 0.05$), while no difference were found for vocalizations, lowering of the head and immunocastrator has

shown to be effective to reduce testosterone, sexual and aggressive behaviors on Holstein bulls.

Keywords: immunocastration, Holstein bulls, testosterone

Resumen

Para evaluar el efecto de la castración sobre el comportamiento conductual, niveles de testosterona y cortisol en machos Holstein en engorda, se utilizaron 720 machos enteros entre 7 y 8 meses de edad con un peso de 232 ± 1.19 kg divididos en dos grupos para separar tratamientos: inmunocastrados vacunados con Bopriva y el grupo placebo. Los animales fueron sacrificados a los 239 días de engorda. La vacuna fue aplicada en los días 1, 21, 101 y 181 días de engorda y en los mismos días fue medido los niveles de testosterona, mientras que las mediciones de cortisol, glucosa y creatin kinasa fueron hechas en el día 181 y durante el desangrado postmortem. Se evaluó el comportamiento sexual, agresivo y social y se observó que los machos enteros mostraron mas altos valores promedios en montas, topetazos, amenazas, signos de Flehmen y olfateos ($P < 0.05$), mientras que no hubo diferencias ($P > 0.05$) reportadas en vocalizaciones, cabeza baja y acicalamiento. Los niveles de testosterona en machos enteros permaneció en 0.47 ng/mL durante el estudio, pero apartir del día 181 de engorda, se presentaron diferencias ($P < 0.05$) observadas con respecto a los machos inmunocastrados con valores de 0.22 ng/mL. Al sacrificio los niveles de testosterona fueron de 0.21 ± 0.06 ng/mL en los machos inmunocastrados y de 0.54 ± 0.06 ng/mL en el grupo placebo. El uso de un inmunocastrador mostró ser más efectivo para reducir los niveles de testosterona así como el comportamiento sexual y agresivo en machos Holstein.

Palabras clave: Inmunocastración, machos Holstein, testosterona

Introduction

Castration of calves for meat production is a common practice in North America, the term refers to the sterilization of males of any species, that involves the *in situ* removal of the testicles suppressing their function. However, this practice affects the animal welfare due to acute or chronic pain caused regardless of the

At present times beef production using Holstein calves results attractive to meat producers in northern Mexico because male Holstein calves are not useful in milk production and are sold at a lower price than beef calves. However, if this bulls are not castrated (Duff and McMurphy 2007) their aggressive behavior among them and against their handlers makes them hard to handle and also more susceptible to stress.

To reduce both the aggressive and sexual behaviors immunocastration is considered an alternative to traditional castration as it decreases blood testosterone levels and as a result handling is easier and less stressful to the animals and therefore carcasses obtained from this animals show less lesions (Amatayakul-Chantler *et al* 2012). Immunocastration consist of the injection of an analog of GnRH which generates an immune response similar to that of a vaccine against a virus or bacteria (Rault *et al* 2011) stimulating the production of antibodies that would neutralize GnRH to inhibit the secretion of sexual hormones. Also, immunocastration requires less labor and equipment and represents less danger for the animals and humans (Price *et al* 2003).

The objective of this work is to determine if immunocastration has an effect on animal behavior, testosterone, and cortisol levels of feedlots Holstein steers.

Material and methods

Geographical location

The study was carried out in the city of Mexicali, Mexico, which is located at 32° 32'00 N, 115° 12'41W. The region is characterized by a dry desert climate with an average temperature of 34.7 °C (-5 °C winter and 50 °C summer), with an annual rainfall of 37mm, and a relative humidity above 50% (García 2004).

Animals and design of the study

Holstein bulls from the same origin, between 7 and 8 months of age and with an average live weight of 232 ± 1.19 kg were used. For the study two treatments were applied: four pens of 90 intact animals (placebo group) and four pens of 90 intact animals treated with Bopriva (immunocastrated group) (Zoetis Laboratories Animal Health, México). Twenty four hours after the animals arrived to the feedlot, lots were formed, and the calves were vaccinated,

dewormed, and an implant was placed (trenbolone acetate, estradiol and tylosin). Bopriva was administered subcutaneously to the vaccinated group, on four occasions: (24 h after reception and on days 21, 101 and 181 of the experiment), while the calves in the placebo group received 1 ml of saline solution on the same days. The live weight of individual animals was recorded during each treatment. The animals were fed twice a day, following a program of 6 diets typical to the Northern region of the country, comprising of wheat hay, sudangrass, tallow, dried distillers grains (DDG), and a mineral premix. The animals were slaughtered once they had reached an average weight of 580 kg by day 239. Prior to slaughter the cattle was kept in rest pens with access to water for approximately 6 hours. On the day of slaughter the animals were herded by a handler on horseback for about 1 km to the slaughterhouse.

Behavior assessment

Animal behavior was assessed in each of the lots, one hour a day, for two weeks, alternating one hour in the morning and one hour in the afternoon. The evaluation was performed in three stages: the first stage started on day 21 and concluded on day 101; the second stage was between days 102 and 181; the final stage comprised the last 7 days of the fattening period. The following variables were evaluated by recording the number of events: sexual behavior (mounting and Flehmen sign), aggressive behavior (head butting and threatening), and social behavior (sniffing, grooming, lowered head, and vocalization).

Serum testosterone levels

Ten animals per pen were randomly selected to measure serum testosterone levels. Animals selected for serum sampling were identified by an additional ear tag. Blood samples were taken every time that Bopriva was administered, and a sample was also taken during slaughter at the bleeding station on the production line at the slaughterhouse. Approximately 5 ml of blood was extracted from the coccygeal vein. The samples were centrifuged at 3500 rpm to obtain the serum, using a TRIAC centrifuge (Clay Adams, Model 0200, New Jersey, U.S.A.), and stored at -20°C until testosterone concentration was measured. Concentrations

of serum testosterone were determined using the ENZO Testosterone Elisa Kit test (ADI-900-065), according to manufacturer's instructions.

Physiological indicators of stress

To measure stress parameters two additional blood samples were taken at day 181 of the experiment during the last vaccination with Bopriva, one with anticoagulant EDTA to measure hematocrit, glucose and creatine kinase (CK), and the second sample was collected with sodium heparin to measure seric cortisol. Also, additional blood samples were taken during slaughter of the animals to repeat these measurements. All samples were refrigerated at 4°C and sent for analysis to the Laboratory of Clinical Pathology of the Institute of Veterinary Science Research at the Autonomous University of Baja California. To determine glucose and CK an enzymatic analysis was performed with automated chemistry equipment (SPIN 200, Spin React, Spain). Briefly, samples were tempered for two minutes and latter centrifuged at 3400 rpm for 10 minutes on a clinical centrifuge (VE-400, VELAB) to separate the serum which was placed in 1.5 ml Eppendorf microtubes. Glucose and CK was determined by the GOD-POD method where glucose oxidase (GOD) catalyzes glucose oxidation to gluconic acid. The hydrogen peroxide (H₂O₂) produced is detected by an oxygen, phenol, 4 aminofenazone (4-AF) chromogenic acceptor, under the presence of peroxidase (POD). The intensity of the color developed was proportional to glucose concentration present in the sample. The creatin kinase reactive (CK-NAC), catalyzes the reversible transfer of a phosphate group from phosphocreatine to Adenosin diphosphate (ADP). This reaction is coupled with other reactions catalyzed by hexokinase and glucose-6-phosphate dehydrogenase. The speed of NADPH formation, photometrically determined, was proportional to the catalytic concentration of CK in the sample. To determine seric cortisol was performed with Cortisol Test System (Accu-Bind ELISA microwells, Monobind, Inc., Lakeforest, CA, USA).

Statistical analysis

To compare testosterone levels between treatments (Bopriva and placebo) and its performance over time (day 1, 21, 101, 181 and slaughter), a time-repeated

measures model was used that included the common effect plus the fixed effects of treatment, time factor, treatment x time interaction and the random effect of experimental error. To study the treatment x time interaction, a covariance structure was modeled between the records, evaluating three possible covariance structures: unstructured, compound symmetry and first order self-regression, using the Akaike and Schwartz criteria, the best one being the one that presented lowest values for these two indicators. In order to analyze the behavioral variables (mounts, head butts, threats, Flehmen, sniffing, grooming, low head and vocalizations) by treatment effect, records were taken in three post-vaccination periods (days 101, 181 and 7 days before the end of the finishing Period), and 47 visits were made in total, in addition to the ante-mortem rest time in the resting pens. The analysis was performed with the average value divided by total visits. To test the differences between treatments in their behavioral form in the periods was analyzed using a linear model that included the fixed effects of treatment and the interaction treatment x period as a covariable plus the random effect of the error. The comparison between slopes was performed with the Student's T statistic and the comparison between means of treatment was performed using means adjusted by the covariable in the model, the significance was declared for P values $<.05$. The analysis was performed using the SAS 9.4 statistical package with the Proc GLM procedure (general linear model), the comparison of inclines through the estimate statement and for the least squares means the ls means statement

Results

The mean values of the behavior variables evaluated per treatment are presented in Table 1. immunocastrated males showed an evident and effective reduction of sexual and aggressive behaviors.

The average testosterone levels on day 1 were similar ($P>0.05$) between the immunocastrated group (0.26 ± 0.61 ng/ml) and placebo group (0.28 ± 0.60 ng/mL) (Fig. 1). Likewise, at day 21 there were no differences between treatments ($P>0.05$) (0.27 ± 0.05 and 0.27 ± 0.06 ng/mL) respectively. Testosterone levels increased gradually until day 101 without significant

differences ($P>0.05$) in both immunocastrated (0.38 ± 0.05 ng/mL) and placebo bulls (0.44 ± 0.05 ng/mL). From day 181, testosterone levels of placebo bulls remained stable (0.42 ± 0.06 ng/ml) until the end of the study, while levels gradually decreased in the immunized group of bulls, falling to 0.22 ± 0.05 ng/mL by day 181, with significant differences between both treatments recorded from this point forward ($P<0.05$). At the end of fattening period, when animals were slaughtered, testosterone levels were 0.21 ± 0.06 ng/mL in immunocastrated and 0.54 ± 0.06 ng/mL in the placebo group.

Table 2 showed the mean values of the physiological indicators of stress. Cortisol values only showed differences ($P <0.05$) per treatment, with the highest values observed in the immunocastrated bulls at exsanguination. For Glucose values differed ($P <0.05$) over time. For CK differences were observed only among periods, with the highest values for both treatments at exsanguination. Differences in these physiological parameters were expected in both groups, as those observed for testosterone levels and behavior variables. However the absence of differences might be due to a deficient animal handling previous to slaughter.

Discussions

The testosterone results observed in the present study are in agreement with Jago *et al* (1997) who observed an increase in testosterone levels accompanied by an increase in the frequency of agonist behaviors in uncastrated animals such as mounting, fights among others. Other studies have reported a decrease in the frequency of sexual behaviors, mounting, threats, and head butts in immunocastrated males when compared with uncastrated males (Huxsoll *et al* 1998, Marti *et al* 2015, Freitas *et al* 2015). These differences may be due to the effect that immunocastration has on serum testosterone levels through the effect of the synthetic GNRH contained in the vaccine (Bopriva™) which stimulates the production of antibodies by the animal immune system. The antibodies block the animal's GNRH receptors located in the hypophysis and therefore inhibit the release of the hormones FSH and LH that are responsible for stimulating the production of testosterone in the gonads, and as a result testosterone levels in

bloodstream will be reduced (Herbert and Trig 2005). Freitas *et al* (2015) showed that there is a correlation among testosterone levels and frequency of mounts and agonistic behavior, and they believe that testosterone levels stimulate a homosexual behavior. Therefore, when testosterone levels are reduced, also physical activity will be decreased and so sexual and aggressive behavior occurrence will be less.

These results are consistent with those obtained by Theubet *et al* (2010), who recorded values below 0.5 ng/mL after the second application of the vaccine on immunocastrated bulls. Jannet *et al* (2012a) observed a similar decrease in testosterone (<0.01 ng/mL immunocastrated bulls vs 14 ng/mL control bulls) in Holstein bull calves, while Jannet *et al* (2012b) recorded levels of >5 ng/mL in uncastrated and <5 ng/mL in immunocastrated bulls. Amatayakul-Chantler *et al* (2012) reported that immunocastrated animals showed a strong immune response against GNRH, which was associated with suppression of testosterone levels in blood below 5 ng/mL.

According to Palme *et al* (2000), cortisol levels aid as a reference parameter to evaluate stress in animals and may be increased when animals face a hostile situation (transportation, handling, different social environment) (Van de Water *et al* 2003; Bretschneider *et al* 2005; Amtmann *et al* 2006). The present study showed that during pre-slaughter management immunocastrated bulls exhibited a more docile behavior that made them easily distracted and thus making their mobilization within the chute slower which led to the indiscriminate use of the electric cattle prods when animals were herded. This may explain the high levels of cortisol in immunocastrated animals at exsanguination. When animals undergo a stressful event their blood levels of catecholamines are increased affecting the metabolism of carbohydrates (glycogenolysis and gluconeogenesis), so that there is an increase in the blood levels of glucose and lactate (Bond *et al* 2004). However, Shaw and Tume (1992) state that blood glucose levels alone is not a reliable indicator of stress, and cortisol levels have to be considered altogether. Moreover, Mórmede *et al* (2007) sustain that the response to stress vary among species and is related to cortisol basal levels:

cattle have low basal levels of cortisol (often lower than 0.54 ng/dL) and cortisol levels in response to stress can increase from 2.17 ng/dL to 7.24 ng/dL. According to the categories of cortisol levels classified by Grandin (1997), cortisol levels found in this study correspond to a basal level for Holsteins during handling; however, according to the authors cortisol levels among studies should not be compared because of their variability. Also, Mórmede *et al* (2007) state that serum glucocorticoid levels are affected by several factors such as time of day, season, and environmental factors; also, that cortisol levels differ according to the level of stress experienced by the animals during the experiment, and either are not consistent among studies where similar treatments were used because every individual respond different to a stressful stimulus in addition to environmental factors.

Regarding the levels of CK recorded, these were higher at exsanguination time than at day 181 when the last vaccine was applied. This might be due to stress inflicted during mobilization of the animals from the feedlots to the slaughter house and also the pre-slaughtering handling. According to Earley and Murray (2010), CK is an enzyme that is released from the sarcoplasm of the muscle cells into the bloodstream due to the increased cell membrane permeability in response to muscle damage and stress (Kannan *et al* 2000), and therefore high levels of this enzyme are a good indicator of muscle activity or damage, and similar to glucose, the increase in CK levels is attributed to the stress caused to cattle by a deficient handling prior to slaughter. The use of an immunocastrator has shown to be effective to reduce the serum levels of testosterone and the sexual and aggressive behaviors, and therefore can help to reduce the levels of physiological stress indicators such as cortisol, glucose and CK. However, all these the advantages may not be exploited due to a deficient animal management practices at the slaughter house; therefore, in order to ensure the successful performance of the vaccine it is important to modify and improve the animal management practices prior to slaughter.

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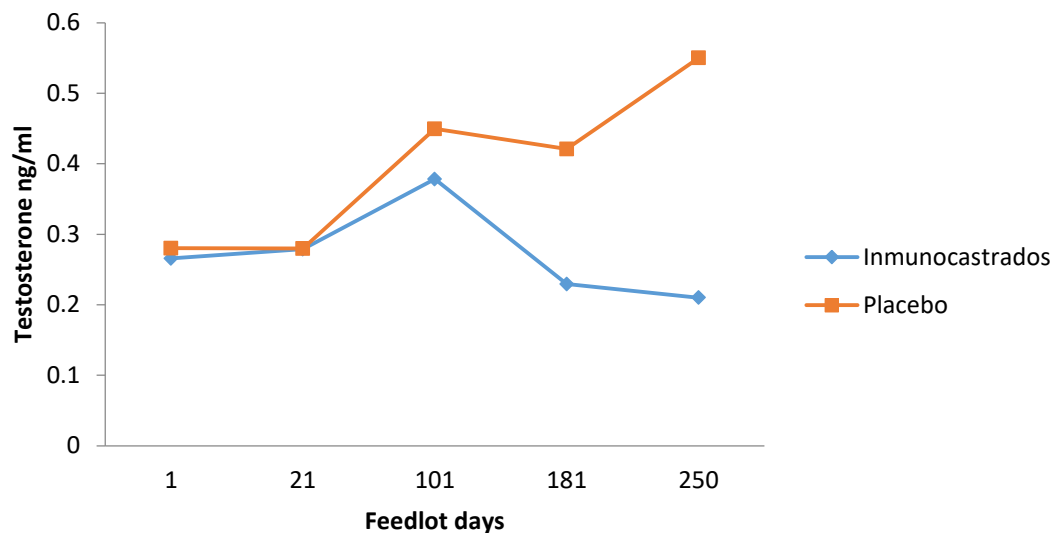


Figure 1. Concentrations of serum testosterone by treatment.

Table 1. Mean \pm S.E. of behavior variables during the fattening period.

Variable	Treatment		Pr > t
	Bopriva	Control	
Mounting	8.60 \pm 1.78 ^b	16.32 \pm 1.78 ^a	0.0068
Head butting	4.45 \pm 0.79 ^b	6.97 \pm 0.79 ^a	0.0365
Threatening	10.23 \pm 0.61 ^b	15.22 \pm 0.61 ^a	0.0001
Flehmen signals	7.90 \pm 0.90 ^b	13.22 \pm 0.90 ^a	0.0006
Sniffing	11.82 \pm 1.03 ^b	19.85 \pm 1.03 ^a	0.0001
Grooming	12.15 \pm 1.00 ^a	11.43 \pm 1.00 ^a	NS
Lowered head	4.33 \pm 0.35 ^a	5.11 \pm 0.35 ^a	NS
Vocalitations	2.20 \pm 0.60 ^a	2.41 \pm 0.60 ^a	NS

Different literals in the same row indicate statistical difference ($P < 0.05$).

Table 2. Mean \pm S.E. of physiological indicators of stress

Variable	Treatments			
	Bopriva		Control	
	Día 181	Bled	Día 181	Bled
Cortisol (ng/dL)	3.84 \pm 0.28 ^b	5.06 \pm 0.36 ^a	3.79 \pm 0.32 ^b	3.78 \pm 0.38 ^b
Glucose (mmol/L)	4.54 \pm 0.10 ^{a,b}	4.80 \pm 0.11 ^{a,b}	4.86 \pm 0.10 ^a	4.55 \pm 0.11 ^b
CK (U/L)	307.97 \pm 48.11 ^b	567.12 \pm 52.89 ^a	195.32 \pm 46.54 ^b	590.29 \pm 52.89 ^a

Different literals in the same row indicate statistical difference ($P < 0.05$). CK: Creatinkinase.

CAPITULO 4

CONCLUSIONES GENERALES

Es importante tener conocimiento sobre los metodos de castración existentes, ya sean tradicionales o nuevos como la inmunocastración y sobre todo sobre las repercusiones que tiene cada procedimiento en el animal y el efecto que puede tener sobre el product final como lo es la carne; esto resulta útil desde el punto de vista economico y ético ya que se debe buscar brindar y mantener un buen nivel de bienestar animal desde que el animal inicia su period de engorda hasta el sacrificio y si es posible mejorar el bienestar animal y hacer el manejo de los animals más facil mediante el uso de una vacuna esto se verá reflejado sobre la calidad de la canal y de la carne y por consiguiente obtener un beneficio económico mayor.