Centro de Investigación en Alimentación y Desarrollo, A.C.

USO COMBINADO DEL CLORHIDRATO DE ZILPATEROL E IMPLANTE ESTEROIDAL SOBRE LA MODULACIÓN DEL CRECIMIENTO EN CORDEROS DE PELO EN FINALIZACIÓN

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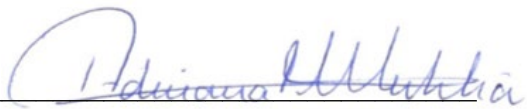

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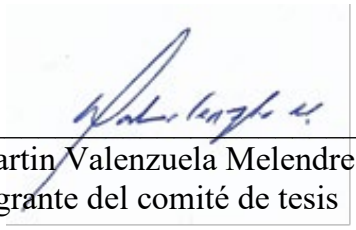

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Al Laboratorio de Ciencia y Tecnología de la Carne y su equipo de trabajo.

DEDICATORIA

A la vida…

CONTENIDO

RESUMEN

En bovinos de engorda, la administración combinada de clorhidrato de zilpaterol (CZ) con implantes esteroidales (IE) ha sido una estrategia efectiva para incrementar la producción de carne, ya que interactúan en forma aditiva para mejorar la ganancia de peso y de canal, sin embargo, en corderos aún no se ha estudiado su efecto combinado. El objetivo del estudio fue evaluar los efectos de CZ e IE sobre el comportamiento productivo, características de la canal, rendimiento de cortes primarios, morfología del músculo *Longissimus thoracis* (LT) y las características físico-químicas de la carne en corderos de pelo. Se confinaron individualmente 40 corderos Dorper × Pelibuey para asignarlos en un arreglo factorial 2^2 a las siguientes combinaciones de tratamientos: 1) sin CZ ni IE (testigo), 2) sin CZ y con IE (IES), 3) con CZ y sin IE (CZS), 4) con CZ e IE (CZ+IE). La interacción CZ×IE no afectó el comportamiento productivo, características de la canal ni el rendimiento de cortes. Sin embargo, el CZ aumentó la tasa de crecimiento, la eficiencia alimenticia, peso y rendimiento de canal, sin afectar la deposición de grasa interna ni externa. Mientras que el IE no afectó estas variables. Comparado con cualquier otro tratamiento, la combinación CZ+IE incrementó el área del LT, el tamaño de las fibras musculares tipo IIb y el contenido de colágeno soluble, mientras que el CZS aumentó el porcentaje de fibras tipo IIb. El CZS y el IES redujeron tamaño de las fibras tipo I y aumentaron el tamaño de las fibras tipo IIa comparado con el testigo y el tratamiento CZ+IE. Los promotores de crecimiento no afectaron los contenidos de colágeno total, proteína, grasa y humedad en la carne. El CZS incrementó el pH después de 24 h, 72 h y 14 d *postmortem* comparado con los tratamientos testigo, IES y CZ+IE. En general, ni la interacción CZ×IE, ni el IE afectaron el color, la capacidad de retención de agua, el esfuerzo al corte y el perfil de ácidos grasos. Sin embargo, el CZ administrado de manera individual redujo los valores de color e incrementó el esfuerzo al corte en carne no madurada y madurada, y tendió afectar el perfil de ácidos grasos. Se concluye que, en corderos enteros, el CZ y el IE interactúan sinérgicamente para promover hipertrofia muscular en el músculo LT; sin embargo, esto no se refleja en mejoras en comportamiento productivo y características de la canal. Además, el IE actuó como un antagonista de los efectos negativos que genera el CZ en la calidad de la carne.

Palabras claves: Agonistas adrenérgicos, estradiol, fibras musculares, ovinos de pelo.

ABSTRACT

In feedlot cattle, the combined administration of zilpaterol hydrochloride (ZH) with steroid implants (SI) has been an effective strategy to increase meat production, since they interact in an additive way to improve live weight gain and carcass weight. However, in lambs this combined effect of ZH and SI has been not studied. The aim of the study was to evaluate the effects of ZH and SI on productive performance, carcass characteristics, wholesale cut yields, *Longissimus thoracis* (LT) muscle morphology, and physicochemical characteristics of meat in hair male lambs. Forty Dorper × Pelibuey entire male lambs were individually confined and assigned in a 2^2 factorial arrangement to the following treatment combinations: 1) Without ZH and SI (control), 2) Without ZH and with SI (SIA), 3) With ZH and without SI (ZHA), and 4) With ZH and SI (ZH+SI). The ZH×SI interaction did not affect productive performance, carcass traits, and wholesale cut yields. Supplemental ZH increased growth rate, feed efficiency, carcass weight and dressing, but internal and external fat depositions were unaffected, while SI did not affect these variables. Compared to any treatment combination, ZH+SI increased LT area, type IIb muscle fiber size, and soluble collagen content, while ZHA increased the percentage of type IIb fiber. Both ZHA and SIA decreased type I fiber size and increased type IIa fiber size compared to control and ZH+SI. The growth promoters did not affect the contents of total collagen, protein, fat, and moisture in meat. ZHA increased *postmortem* pH at 24 h, 72 h, and 14 d compared to control, SIA, and ZH+SI. Overall, neither the ZH x SI interaction nor the IE affected color parameters, water-holding capacity, shear force, and fatty acid profile. However, ZH decreased color values and increased shear force both in unaged and aged meat, but had little effect on fatty acid profile. It was concluded that in entire male lambs, ZH and SI interacted synergistically to promote muscle hypertrophy in LT, however, this was not reflected in improvements in feedlot performance and carcass characteristics. In addition, SI acted as an antagonist of the negative effects that develops ZH in lamb meat.

Keywords: Adrenergic agonists, estradiol, hair sheep, muscle fiber.

1. SINOPSIS

1.1 Justificación

En la última década, el inventario y la productividad en los rebaños de ovinos mexicanos ha crecido significativamente, pero sólo satisface el 70 % de la demanda de carne ovina nacional, por lo que se ha tenido que importar de otros países como Australia, Nueva Zelanda y Estados Unidos (SIAP, 2015). Esto ha traído como consecuencia que la industria de la carne de ovino en el mundo demande estrategias para mejorar el comportamiento productivo y características de canal de los corderos de engorda, ya que la producción de carne de esta especie es insuficiente para satisfacer su demanda en el mercado (Montossi *et al.*, 2013). Debido a lo anterior, el uso de promotores de crecimiento en la engorda de corderos es una estrategia productiva cada vez más empleada por los productores, ya que permite mejorar el crecimiento, la eficiencia alimenticia y las características de la canal.

El clorhidrato de zilpaterol (CZ) y los implantes esteroidales (IE) que contienen acetato de trembolona (ATB) y estradiol $17\beta_2$ (E_2), son dos promotores de crecimiento que se han utilizado con cierto éxito en la engorda de ovinos (Galbraith *et al.*, 1997; McClure *et al.*, 2000; Ortíz *et al.*, 2013; Avendaño-Reyes *et al.*, 2018; Rojo-Rubio *et al.*, 2018; Cayetano-De-Jesús *et al.*, 2020). La suplementación de CZ ha mostrado ser una estrategia más efectiva para mejorar el crecimiento y las características de la canal (Avendaño-Reyes *et al.*, 2018; Cayetano-De-Jesús *et al.*, 2020), sin embargo, esto ha causado efectos contraproducentes para la calidad de la carne (carne oscura, firme y seca, DFD, por sus siglas en inglés; Dávila-Ramírez *et al.*, 2013; 2017). Por su parte, los efectos del IE sobre el comportamiento productivo y las características de la canal han sido controversiales, pues mientras algunos autores reportan mejoras en estas variables (Galbraith *et al.*, 1997; Ortíz *et al.*, 2013), otros estudios no han reportado efecto alguno de los mismos (McClure *et al.*, 2000). A pesar de que no se encontraron antecedentes y evidencias en la literatura sobre los efectos del IE en la calidad de la carne de los ovinos, se ha reportado que en la carne de bovinos de engorda el IE provoca cambios negativos (Parr *et al.*, 2011). El perfil de ácidos grasos también se ha visto parcialmente modificado por la suplementación con CZ en la carne de ovinos (Dávila-Ramírez *et*

al., 2017; 2018). Cabe mencionar que aún, cuando el CZ modifica el perfil de ácidos grasos de la carne ovina, estos cambios en las concentraciones de ácidos grasos al parecer no ponen en riesgo la salud del consumidor (Dávila-Ramírez *et al.*, 2017).

Tanto el CZ como el IE funcionan como promotores de crecimiento porque mejoran la deposición de masa muscular a través de aumentar la síntesis de proteína, reducir la proteólisis y evitar la lipogénesis (Mersmann, 1998; Beermann, 2002; Smith y Johnson, 2020). Se ha reportado que, a nivel de músculo estriado, estos promotores también cambian el metabolismo celular y, consecuentemente, la remodelación de las fibras musculares en cuanto a su proporción y tamaño, específicamente favoreciendo un recambio para incrementar el porcentaje y el tamaño de fibras IIb, las cuales son las más grandes y tienen un metabolismo glucolítico rápido por ser ricas en glucógeno (Parr *et al.*, 2016; Ebarb *et al.*, 2016). Resulta importante señalar que, a pesar de generar efectos biológicos similares, el CZ y el IE presentan diferentes mecanismos de acción. El CZ es un agonista adrenérgico β_2 (AA-β_2) que se administra en la dieta durante los últimos 30 días antes del sacrificio cuando la deposición de grasa corporal es mayor; esto se debe a que funciona redistribuyendo sustratos energéticos para la formación de músculo esquelético (Mersmann, 1998; Beermann, 2002). En cambio, los IE son hormonas anabólicas (ATB y E_2) que pueden ser aplicadas durante toda la etapa de engorda (Johnson *et al.*, 1996, Pampusch *et al.*, 2014), las cuales, a través del factor de crecimiento similar a la insulina I (IGF-I), estimulan la proliferación y unión de las células satélite a las fibras musculares para aumentar la disponibilidad de ADN y la síntesis de proteínas muscular (Dayton y White, 2014). Además, este IE puede bloquear la producción de adrenocorticotropina para reducir la proteólisis (Sillence, 2004) y aumentar la disponibilidad de receptores adrenérgicos β (RA-β) (Harris *et al.*, 2020).

Cabe mencionar que la industria de ganado de engorda administra en forma combinada el CZ y los IE para potencializar sus efectos como promotores de crecimiento. Esto partiendo del principio de que actúan por diferentes mecanismos de acción, además del hecho de que el IE aumenta la disponibilidad de RA-β. En este sentido, varios estudios han demostrado que el ganado de carne tiene mejor ganancia de peso, eficiencia alimenticia, peso y rendimiento de canal, y en general deposición de masa muscular por un efecto aditivo entre CZ e IE (González *et al.*, 2007; Kellermeier *et al.*, 2009; Baxa *et al.*, 2010; Bryant *et al.*, 2010; Parr *et al.*, 2011). Aunque esto

también se ha asociado con una menor calidad en la carne (Kellermeier *et al.*, 2009). Ebarb *et al.* (2017) y Kellermier *et al.* (2009) señalan que el CZ e IE interactúan incrementando el porcentaje de fibras tipo IIb y el tamaño de todas las fibras musculares, lo cual se refleja en una mayor deposición de proteína en canal.

Hasta la fecha no existen investigaciones que hayan examinado los efectos combinados de CZ e IE sobre el comportamiento productivo, las características de la canal, el rendimiento de cortes primarios, la calidad de la carne y la fisiología del músculo, así como el mecanismo por el cual podrían interactuar (sinérgico o aditivo) en la engorda de ovinos de ningún genotipo. Como previamente se mencionó, estos promotores de crecimiento también se usan en la engorda de corderos con buenos resultados sobre la ganancia de peso vivo y la deposición de masa muscular en la canal, sin embargo, su uso es menor en la industria ovina debido a que está menos desarrollada en el país. No obstante, los productores demandan estrategias efectivas que ayuden a mejorar la deposición de músculo en la canal y, consecuentemente, el crecimiento de los corderos de engorda, al mismo tiempo que reduzcan los costos de alimentación y el periodo de engorda. Por lo anterior, se requiere dilucidar si los corderos implantados con IE y luego alimentados con CZ modulan su crecimiento y producen hipertrofia muscular, asimismo se hace necesario evaluar el impacto que la combinación de ambos promotores tiene en las características físico-químicas de la carne.

1.2 Antecedentes

1.2.1 Producción de Carne Ovina

A nivel mundial, la producción anual de carne de ovino es de 14 millones de toneladas, esto constituye alrededor del 3 % de la producción mundial de la carne (FAO, 2016). El comercio entre países productores de carne de ovina representa entre el 7 y 9 % de la producción total, aunque la mayor parte se consume en el país donde se produce (FAO, 2016). China (20.1 %) seguido de Australia (5.6 %) y Nueva Zelanda (3.8 %) son los principales productores de carne ovina (FAO,

2018); sin embargo, mientras China es el principal importador de esta carne, Australia y Nueva Zelanda exportan grandes volúmenes a diferentes partes del mundo incluyendo México, la Unión Europea, Asia del Norte, Oriente Medio y América del Norte (Morris, 2009; FAO, 2018). En general, las estadísticas recientes señalan que el inventario mundial de esta especie incrementó 9 % entre 2010 y 2016, y en 2017 se cuantificó una población de alrededor de 1,202.4 millones de cabezas (FAOSTAT, 2018). A pesar de esto, la demanda de carne a nivel mundial sigue siendo sin cubrirse, y países como China necesitan importar carne de ovino y de cualquier otra especie debido a que su población sigue creciendo y los espacios para producir sus alimentos son escasos (Mao *et al.*, 2016).

En México, según datos del Servicio de Información Agroalimentaria y Pesquera (SIAP), el inventario ovino al 2018 era de 8,902,451 cabezas, las cuales están distribuidas a través del país de la siguiente manera: 55 % en el centro, 24 % en el sur y 23 % en el norte (SIAP, 2018). El inventario nacional de ovinos, al igual que la producción de carne de esta especie, han estado creciendo paulatinamente a partir del año 2000, reportándose un desarrollo en esta industria de alrededor del 30 % en los últimos 10 años (SIAP, 2015). Así, en el 2017, México producía 61,600 toneladas de carne ovina (SIAP, 2018), y sólo cubría entre 70 y 80 % de la demanda nacional, teniendo que importar carne de países como Australia, Nueva Zelanda, Uruguay y Estados Unidos (Bobadilla *et al.*, 2017). Este escenario de la industria ovina mexicana muestra que la producción de cordero para abasto es un nicho de oportunidad dentro del sector agropecuario. De modo que ha provocado un aumento en el interés de producir esta especie para satisfacer la demanda nacional y de ser posible exportar a países como Corea del sur y China, donde México ya lo está haciendo con carne de res (Arteaga, 2012).

La producción de ovinos es una actividad que se desarrolla en todos los estados del país bajo diferentes sistemas de producción, dependiendo de las condiciones climáticas y la disponibilidad de los recursos forrajeros. Sin embargo, la producción de carne ovina se ha convertido en los últimos años en una actividad que está en desarrollo y transitando de una producción de traspatio a una de tipo comercial (Cárdenas-Villegas y Cortez-Romero, 2012). Como consecuencia, los ovinocultores están interesados en el desarrollo e implementación de tecnologías que permitan aumentar la eficiencia de los rebaños. Específicamente, se busca aumentar la ganancia diaria de

peso, la eficiencia alimenticia y el rendimiento en canal, ya que la mayoría de la carne ovina se consume en el país en el platillo típico conocido como barbacoa, donde la calidad de la carne tiene poca importancia (Camacho-Ronquillo *et al.*, 2018). No obstante, en los últimos años ha surgido el interés por producir cortes finos para restaurantes gourmet, siendo el corte más valioso el rack.

El uso de promotores de crecimiento en corderos de engorda ha sido una tecnología que ha demostrado ser efectiva para mejorar crecimiento y peso de la canal. Cabe mencionar que muchos de estos promotores de crecimiento han sido desarrollados para bovinos y se han ido adaptando para la engorda de ovinos. Si bien, algunos de estos promotores han mostrado ser buenos promoviendo el crecimiento en los ovinos, se han observado ciertas discrepancias en sus modos de acción y farmacocinética comparados con los bovinos. Los AA-β_2 y los IE que contienen ATB con E_2 son dos promotores de crecimiento ampliamente usados en la engorda de bovinos, porque son los que mejores resultados han dado para acelerar la producción de carne de esta especie (Parr *et al.*, 2016; Smith y Johnson, 2020). Ambos también se han probado para mejorar la producción de carne en ovinos, sin embargo, sus efectos benéficos no han sido muy consistentes como en bovinos (McClure *et al.*, 2000; Ortíz *et al.*, 2013; Avendaño-Reyes *et al.*, 2016; 2018). A continuación, se hace una descripción más amplia del uso de estos dos promotores de crecimiento en la engorda de ovinos.

1.2.2 Clorhidrato de Zilpaterol en Ovinos

El CZ es un AA-β_2 que se ofrece en la dieta durante el periodo de finalización y tiene un periodo de retiro antes del sacrificio de tres días. Este agonista, junto con el clorhidrato de ractopamina, son los únicos autorizados en la alimentación del ganado en México y otros países del continente Americano y Africano, ya que han mostrado ser menos agresivos en los animales y tienen baja residualidad en la carne (Shelver y Smith, 2006). El CZ, a diferencia del clembuterol que es el AA-β_2 más potente y está prohibido por daños a la salud humana, es 12 veces menos potente debido a que en su estructura química solo tiene un cloro (clembuterol = dos cloros) (Verhoeckx *et al.* 2005); sin embargo cuenta con un anillo aromático, un grupo hidroxilo unido al carbono β, un nitrógeno

alifático y una cadena R (Smith, 1998). En consecuencia, la estructura química del CZ es diferente a la de otros AA-β_2 y esto ha llevado a que su afinidad con el receptor RA-β_2 sea alta pero relativamente menor comparado con clembuterol (Johnson *et al.*, 2014), mientras que con RA-β_1 ha mostrado ser muy débil (Verhoeckx *et al.*, 2005). La baja afinidad con RA-β_1 explica porque el CZ es recomendado para su uso en la alimentación de rumiantes, ya que estos tienen abundante cantidad de RA-β_2 en músculo esquelético (99 %) y tejido adiposo (90 %; Mersmann, 1998).

En general, la respuesta fisiológica de los AA-β inicia con su unión al RA-β, formando un complejo agonista-receptor que activa la proteína G (Bermann, 2002); específicamente, la subunidad α de la proteína G estimula a la enzima adenil-ciclasa (AC), dando como resultado un incremento en los niveles de adenosin monofosfato cíclico (AMPc), mensajero químico intracelular relacionado con la regulación de la proteína cinasa A (PKA) (Mersmann, 1998; Johnson, 2006). La PKA es encargada de modular la actividad de varias proteínas y hormonas, y en consecuencia es la responsable directa de ajustar el metabolismo celular para desencadenar los efectos biológicos que se atribuyen a los AA-β. Algunas de estas son enzimas que pueden activarse o inactivarse, por ejemplo, la desactivación de la enzima acetil-CoA relacionada con la biosíntesis de ácidos grasos y la activación de la lipasa, enzima limitante para lipólisis (Mersmann, 1998); de este modo, el AA-β reduce la lipogénesis y ejerce efectos lipolíticos en tejido adiposo, al mismo tiempo que promueve la síntesis de proteína (Johnson *et al.*, 2014). La PKA también regula la activación génica que fosforila los factores de transcripción como la proteína de unión al elemento de respuesta (siglas en ingles CREB) al AMPc, el cual se une al ADN (Mersmann, 1998). Esta unión del CREB al ADN estimula la actividad de transcripción que ayuda a regular los diferentes genes que responden a los RA-β en las células de los mamíferos (Johnson, 2004).

No obstante, se han sugerido modos de acción indirectos de los AA-β, como el aumento del flujo sanguíneo hacia la periferia del cuerpo, lo que podría causar mayor aporte de nutrientes y movilización de ácidos grasos del tejido adiposo, lo cual también favorece una mayor disponibilidad de sustrato energético para la síntesis proteica en el músculo (Mersmann, 2002). Otros mecanismos indirectos que estimulan el crecimiento muscular son las variaciones endocrinas y metabólicas (Beermann, 2002). Animales alimentados con AA-β han mostrado modificar la secreción de una serie de hormonas que incluyen insulina, somatotropina, hormona de crecimiento

y factor de liberación de somatotropina, las cuales actúan como mediadores de los efectos de los agonistas (Bermann, 2002).

En el 2009, Park *et al.* reportaron que la fosforilación y la activación de la proteína cinasa activada por AMP (AMPK por sus siglas en inglés), es un complejo enzimático que se activa con el aumento de relación AMP-ATP. La AMPK es considerada un detector de energía celular que ayuda al balance en el metabolismo energético de la célula y se correlaciona positivamente con el aumento en los niveles de ARNm de las cadenas pesadas de miosina-IIX (MHC IIX por sus siglas en inglés). Esto se evidenció cuando la inyección de un activador AMPK (AICAR) al animal aumentó la fosforilación de AMPK y los niveles de ARNm de las cadenas pesadas de miosina, mejorando sinérgicamente la MHC IIX sin afectar los niveles de ARNm de MHC I o IIA.
Debido a que la fosforilación de AMPK puede regularse mediante AMPc (Park *et al.*, 2009), estos datos ayudaron a apoyar la teoría de que el mecanismo celular de la hipertrofia muscular postnatal puede verse influenciado por los AA-β (Mersmann, 1998).

En estudios donde administraron diferentes AA-β (ractopamina, clembuterol, L644-969 y CZ), han encontrado cambios significativos en los niveles de ARNm de α-actina y miosina en el músculo de diferentes especies (Helferich *et al.*, 1990; Smith *et al.*, 1995; Johnson *et al.*, 2014). Estos resultados fueron inferidos debido al incremento en la tasa de transcripción de estos mensajeros en el músculo, así como por el aumento en la estabilidad del ARN total que a su vez incrementó la tasa de síntesis de ARNm en las fibras musculares (Helferich *et al.*, 1990; Smith *et al.*, 1995). En general, la hipertrofia de las fibras musculares es producto de cambios en la frecuencia del tipo de fibra muscular, tasas diferenciales de ARN y ADN muscular, y acreción de proteínas (Smith *et al.*, 1989; Beermann, 2002). Varios estudios en rumiantes han reportado que los AA-β_2 pueden aumentar el área de la sección transversal (AST) de todas las fibras musculares, asimismo que las fibras tipo II son las más sensibles al AA-β_2 (Johnson *et al.*, 2014).

Cabe mencionar que el uso de CZ en la engorda de ovinos se ha estudiado ampliamente en la última década, siendo los resultados variables pero la mayoría han coincidido en señalar una mejora en el comportamiento productivo y las características de la canal, con efectos controversiales a nivel de rendimiento de cortes primarios. Así, diversos estudios conducidos en ovinos de pelo reportan

mejoras en ganancia diaria de peso (GDP), eficiencia alimenticia (EA), peso de la canal (PCC) y rendimiento de la canal (RC) tanto en corderos y corderas con la suplementación de CZ durante las últimas cuatro o cinco semanas antes de sacrificio (Estrada-Angulo *et al.*, 2008; Macías-Cruz *et al.*, 2010; Avendaño-Reyes *et al.*, 2011; 2018; López-Carlos *et al.*, 2012; Dávila-Ramírez *et al.*, 2015; Rojo-Rubio *et al.*, 2018). Por su parte, López-Carlos *et al.*, (2010) y Dávila-Ramírez *et al.*, (2014), todos los estudios sólo reportaron mejoras en las características de la canal, pero no en el comportamiento productivo. Otros estudios no han encontrado beneficio alguno por administrar el CZ en corderos de engorda bajo sistema de alimentación en pastoreo (Salinas-Chavira *et al.*, 2006; Macías-Cruz *et al.*, 2016). Estas discrepancias en los resultados posiblemente se deban a variaciones genéticas (Nourozi *et al.*, 2008) o factores ambientales (Macías Cruz *et al.*, 2013), considerando que el mecanismo de acción *in vivo* del AA-β_2 es sensible a otros eventos secundarios que se alteran a nivel fisiológico y endocrino en tejidos abundantes en RA-β (Mersmann, 1998).

El mecanismo por el cual el CZ mejora el crecimiento, la eficiencia alimenticia, el PCC y el RC ha sido asociado con una movilización de nutrientes a partir de tejido graso hacia la formación de masa muscular (Ortíz-Rodea *et al.*, 2016). Esta redistribución de nutrientes se da porque el CZ promueve la lipólisis y la síntesis de proteína muscular al mismo tiempo que reduce la lipogénesis y la proteólisis (Avendaño-Reyes *et al.*, 2011). Sin embargo, recientemente se demostró que en ovinos no siempre el sustrato de nutrientes para formar músculo se obtiene del tejido graso, sino que también los despojos de la canal pueden aportar estos nutrientes (Avendaño-Reyes *et al.*, 2018; Rivera-Villegas *et al.*, 2019). Al parecer, la marca del producto es determinante en definir que tejido liberará nutrientes para formación de músculo. El CZ de la marca Zilmax® favorece la redistribución de nutrientes del tejido adiposo, pero el CZ de la marca genérica Grofactor® moviliza nutrientes de piel, cabeza y patas (Rivera-Villegas *et al.*, 2019). Se conoce que la forma en como está fijada la molécula de CZ al vehículo es diferente entre marcas y posiblemente esta sea la razón de los cambios que se observan en cuanto a su funcionalidad dentro del cuerpo (Avendaño-Reyes *et al.*, 2016).

En el caso de calidad de la carne ovina, la suplementación con CZ causa una tendencia a producir carne DFD por mantener alto el pH final *postportem*. Así, algunos estudios en ovinos (López-Carlos *et al.*, 2012; Dávila-Ramírez *et al.*, 2013; 2017; Cayetano-De-Jesus *et al.*, 2020) indican que

la suplementación con CZ durante 30 días antes del sacrificio produce una carne con pH final alto, decolorada (bajo valores para L*, a*, b*, C* y H*) y con mayor dureza del músculo *Longissimus dorsi* (LD). Por su parte, López-Carlos *et al.* (2012) informaron que no hubo diferencias en pérdida por goteo, pérdida de peso por cocción, composición proximal y pH a las 24 h *postmortem* por la suplementación de CZ en la etapa de finalización de corderos engorda. En 2015, Vahedi *et al.* reportaron que en ovinos finalizados con CZ, no hubo impacto negativo en la coloración de la carne, pero sí en el esfuerzo al corte. Cabe mencionar que si bien, algunos estudios reportan efectos negativos en calidad de la carne debido al uso de CZ, estos cambios no se han percibido totalmente por los panelistas en la evaluación sensorial. Dávila-Ramírez *et al.* (2013) reportaron que los panelistas detectaron en la carne una ligera disminución en su color y terneza, pero no en la apariencia general, el sabor, el olor y la jugosidad. Más recientemente, los mismos autores señalaron que los panelistas no detectaron cambios en el color y terneza de la carne, y contrario a lo esperado, ellos detectaron una mejora en la apariencia general de la carne (Dávila-Ramírez *et al.*, 2017). Esto sugiere la necesidad de hacer más estudios donde se evalué el efecto del CZ sobre las características organolépticas de la carne ovina.

Por otra parte, dado el modo de acción del CZ, se espera que existan cambios en la oxidación de la carne y el perfil de ácidos grasos. Contrario a lo esperado, este AA-β_2 parece generar ligeras modificaciones en las concentraciones de algunos ácidos grasos, específicamente reduce las concentraciones de ácidos grasos poliinsaturado n-3 (Dávila-Ramírez *et al.*, 2017). Sin embargo, los mismos autores reportaron posteriormente que no se encontraron cambios en el perfil de ácidos grasos por efecto del CZ en ovinos, lo cual lo atribuyeron al efecto del esfuerzo al corte (EC) (Dávila-Ramírez *et al.*, 2018). La información sobre este tema es limitada por lo que en el futuro es recomendable hacer más investigación al respecto.

Por otra parte, la suplementación con AA-β_2 favorece el metabolismo glucolítico muscular en ovinos y bovinos, por lo que estos agonistas remodelan el músculo para aumentar la proporción y el tamaño de las fibras de contracción rápidas (Strydom *et al.*, 2009; Hemmings *et al.* 2015). En el 2015, Hemmings *et al.* adicionaron en la dieta un AA-β_2 en ovinos durante 60 y 120 días y reportaron que este indujo la expresión del ARNm de MHC IIB en LD, una isoforma que normalmente no se expresa en el LD de ovinos. La expresión de las isoformas MHC más rápidas

(IIX y IIB) se asoció con una disminución en la actividad de la enzima isocitrato deshidrogenasa, pero ningún cambio en la actividad de lactato deshidrogenasa, indicando una capacidad reducida para el metabolismo oxidativo. Adicionalmente, se han reportado cambios en la expresión de factores reguladores metabólicos que podrían inducir ajustes en el metabolismo muscular/tipo de fibra; es decir, disminuyeron el ARNm del coactivador β-1 de PPAR-γ y aumentaron el ARNm de la proteína 140, factores que interactúan con los receptores adrenérgicos. Esto sugiere que los dos factores funcionan a través de diferentes mecanismos, induciendo cambios en la masa muscular y transiciones de fibras oxidativas a fibras glucolíticas (Taylor, 2004).

1.2.3 Implantes Esteroidales en Ovinos

Los esteroides son un grupo de hormonas que secretan tanto machos como hembras de manera natural, ya que cumplen funciones relacionadas con la reproducción, el desarrollo y el crecimiento corporal de los animales (Squires, 2010). Dados los efectos anabólicos que han demostrado tener dichas hormonas, las empresas farmacéuticas comenzaron a producirlas sintéticamente y a fijarlas en pellets o gomas elaboradas de silicón y/o plástico, los cuales ahora se conocen comúnmente como IE (Reinhardt, 2007). Esta tecnología es ampliamente usada como un promotor de crecimiento en la engorda de ganado de carne, a tal grado que en la actualidad resulta difícil encontrar becerros no implantados en los corrales de engorda de finalización (Dikeman, 2007). Las hormonas esteroidales más utilizadas para hacer estos IE son: andrógenos, estrógenos y progesterona (Herago y Agonafir, 2017). Diversas sustancias que contienen estos implantes están aprobadas para su uso en forma individual o en combinación, sin embargo, el implante que combina ATB con E_2 es el más utilizado (Reinhardt, 2007). El IE de ATB+E_2 se emplea en diferentes etapas productivas del animal y bajo distintas estrategias de implantación; éste se coloca en el tercio medio de la parte posterior de la oreja del animal, de donde se liberan lentamente las hormonas para ejercer sus efectos biológicos durante un periodo de 60 a 120 días si es bien colocado (Dikeman, 2007). Los IE tienen diferente potencia de acción de acuerdo a la concentración de las hormonas, siendo los implantes más potentes aquellos que se aplican en fase de finalización en corral de engorda (Smith y Johnson, 2020).

Los IE incrementan el peso maduro y promueven hipertrofia muscular en los animales de engorda estimulando una mayor síntesis de proteína y reduciendo la proteólisis y lipogénesis (Pressantino *et al.*, 2012; Parr *et al.*, 2014). Cabe mencionar que el mecanismo de acción a nivel celular de los IE es muy complejo y hasta la fecha no está dilucidado completamente. No obstante, recientemente Smith y Johnson (2020) hicieron una revisión donde describen dichos mecanismos tanto a nivel genómico y no genómico. La principal diferencia entre los efectos genómicos y no genómicos está relacionada con la regulación de los procesos transcripcionales. A nivel genómico, los IE promueven un proceso de transcripción que se regula a través de receptores citoplasmático/nuclear, es decir, después de la unión del ligando a un receptor hormonal ubicado en el citosol, se transloca al núcleo de la célula donde se inicia la actividad transcripcional nuclear, que es mucho más lenta de desarrollar en comparación de la no genómica. Mientras que, a nivel no genómico, la señalización es provocada por receptores nucleares translocados a la membrana y funcionan por medio de sistemas de mensajeros secundarios capaces de alterar las respuestas fisiológicas. Por lo tanto, estas interacciones en las que participan dichas regiones del receptor son importantes para la transmisión de señales rápidas (Simoncini *et al.* 2004).

En términos generales, estas hormonas activas se unen a las proteínas albumina y globulina para ser transportadas a través del torrente sanguíneo hasta los músculos donde se encuentran las células que tienen receptores específicos para ajustarse y desencadenar su acción genómica y no genómica (Smith y Johnson, 2020). Así, en el músculo esquelético se observa un incremento en la expresión del gen IGF-I y en la síntesis de la proteína IGF-I producidos localmente en el músculo esquelético (Pampusch *et al.*, 2008). En 2007 Wu *et al.* señalan que los andrógenos directamente estimulan la transcripción de los genes IGF-I en la región específica, mientras que el estradiol promueve la producción de IGF-I por estimular el receptor de estrógenos 1 acoplado a proteína G (GPER-1 por sus siglas en inglés; Dayton y White, 2014). Esta producción local de IGF-I en el músculo promueve la proliferación y diferenciación de células satelitales entre la lámina basal y el sarcolema de las fibras musculares, las cuales al final se unen a las fibras para donar su núcleo y de esta manera incrementar la disponibilidad de ADN para la síntesis de proteína y reducir la proteólisis (Johnson y Beckett, 2014; Smith y Johnson, 2020). La proliferación de células satelitales también juega un rol importante en la síntesis de colágeno, el cual es esencial durante remodelación de las fibras musculares a medida que se va dando la hipertrofia de las mismas debido al IE (Fry *et al.*, 2017).

Adicionalmente, los IE pueden mejorar la ganancia de masa muscular por un efecto indirecto regulado endócrinamente a través del sistema hormona de crecimiento (GH por sus siglas en inglés; Johnson y Beckett, 2014). Los estrógenos anabólicos estimulan a nivel de hipotálamo la producción del factor liberador de la GH, la cual a su vez promueve la síntesis y liberación de la GH a nivel de hipófisis anterior. Esta hormona entra a la circulación sanguínea para promover el crecimiento directo de los tejidos, al mismo tiempo que acelera el metabolismo estimulando la actividad de glándula tiroidea (Smith y Johnson, 2020). También la GH estimula la liberación de IGF-I a nivel hepático, hormona que está asociada con el crecimiento de los diferentes tejidos corporales. Dado que el IGF-I hepático puede fácilmente unirse a insulina para regular el metabolismo de energía, los IE se encargan de aumentar los niveles circulantes de la isoforma IGFBP-3, la cual ayuda al transporte sanguíneo de este IGF-I hasta el lugar de su acción directa como promotor de crecimiento (Johnson *et al.*, 1996). Cabe mencionar que la disponibilidad del IGF-I hepático se ha relacionado más con el crecimiento de los huesos (Smith y Johnson, 2020).

Resulta importante señalar que hay pocos estudios donde se ha evaluado el efecto del IE en la producción de carne ovina. La mayoría de los estudios existentes se han centrado en determinar sus efectos en comportamiento productivo y características de la canal, sin embargo, no hay información en relación a los efectos en calidad de la carne, perfil de ácidos grasos y morfología del músculo. Adicionalmente, en años recientes se ha estudiado muy poco sobre el efecto del IE que contiene ATB y E_2 en ovinos, por lo que los estudios que a continuación se describirán en su mayoría son de la década de los 80´s y 90´s. Esto sugiere la necesidad de desarrollar más investigación en relación al uso de esta tecnología para hacer más eficiente la engorda de corderos.

En corderos enteros Katahdin × Pelibuey se encontró que el IE mejoró el comportamiento productivo, mientras que la administración de un implante no esteroidal, no generó cambio alguno en el crecimiento y la eficiencia alimenticia (Ortiz *et al.*, 2013). Por su parte, Coelho *et al.* (1981) observaron un incremento del 26.6 % en GDP, 20 % en EA y 6.5 % en PCC debido al uso de implantes que contenía ATB+E_2. Esto se encuentra en concordancia con estudios donde se usaron corderos castrados, la implantación con ATB+E2 mejoró la GDP (Grandadam *et al.*, 1975; Johnson *et al.*, 1998; McClure *et al.*, 2000). Sin embargo, estos efectos benéficos de los IE sobre el crecimiento no se observaron en corderas (McClure *et al.*, 2000).

Respecto a las características de la canal, Grandadam *et al.* (1975) reportaron valores similares en PCC y RC en corderos implantados con ATB+E_2 respecto a los corderos no implantados. Contrariamente, McClure *et al.* (2000) observaron una reducción en el peso de la canal fría sin cambio alguno en área del músculo *Longissimus* o en la deposición de grasa interna por efecto del IE. Cabe mencionar que los efectos de los IE en las características de la canal han sido muy consistentes en ganado de carne, ya que la mayoría de estudios reportan un aumento en el PCC, RC y área del músculo *Longissimus dorsi* (Neill *et al.*, 2009; Baxa *et al.*, 2010).

Como previamente se indicó, a la fecha no se cuenta con registros de estudios sobre los efectos del IE en la calidad de la carne y morfología del músculo en ovinos, mientras que los efectos en bovinos son algo contradictorios. Algunos estudios han reportado un aumento en la dureza de la carne por efecto del IE (Platter *et al.*, 2003; Garmyn *et al.*, 2011), mientras que otros no encontraron cambios en esta variable de calidad (Crouse *et al.*, 1987; Boucque *et al.*, 1988). Por su parte, Neill *et al.* (2009) no encontraron cambios en el color, la textura, la firmeza y el pH final de carne magra en vacas de desecho implantadas con ATB+E_2. Por otra parte, en el músculo *Semitendinosus* de ganado de carne, Girard *et al.* (2012) encontraron que el IE no afectó la fracción miofibrilar, pero el esfuerzo al corte aumentó en 1.04 kg debido a un alto contenido de tejido conectivo y bajo porcentaje de colágeno soluble. Por su parte, Kellermeier *et al.* (2009) midieron la cantidad de colágeno total en filetes de novillos implantados y no implantados. A diferencia del estudio previo, la carne de novillo implantado tuvo 34 % menos colágeno total y un aumento en el esfuerzo al corte con respecto a la carne del testigo.

Es importante destacar que el contenido de colágeno no es el único factor que puede modificar el esfuerzo al corte, este también puede verse afectado por el tamaño de la fibra muscular. Es bien sabido que el uso de promotores de crecimiento puede aumentar el tamaño de la fibra, especialmente las de tipo glucolíticas, lo que aumenta el rendimiento muscular (Parr *et al.*, 2016). Kellermeier *et al.* (2009) reportaron un incremento en el diámetro de las fibras musculares en novillos implantados con (ATB+E2) en comparación con novillos no implantados. Posteriormente, en dos estudios Ebarb *et al.* (2016; 2017) también encontraron un aumento significativo en el área de sección transversal (AST) de todos los tipos de fibras debido a la colocación de IE en novillos; adicionalmente, ellos reportaron una asociación entre el aumento en el AST de las fibras con un

mayor esfuerzo al corte. Existen variaciones acerca del impacto de los implantes sobre la calidad de la carne, es posible que estas se deban a diferencias multifactoriales en las que se conducen los diferentes estudios. En el 2009, Boles *et al.* señalaron que las variaciones entre razas, sexo y estrategia de implantación contribuyen en gran medida a explicar las variaciones que hay entre los estudios sobre el efecto del IE en la calidad de la carne. En general, los estudios establecen que el IE, al igual que otros promotores de crecimiento, produce una mejora en la deposición de masa muscular, pero con efectos negativos en la calidad de la carne de bovinos.

1.2.4 Uso Combinado de Clorhidrato de Zilpaterol e Implante Esteroidal

El uso combinado de CZ e IE en ovinos de engorda aún no se ha estudiado, en cambio, en bovinos es una estrategia ampliamente investigada y usada de forma práctica en los corrales de engorda. A pesar del costo elevado que implica el uso de esta estrategia, se ha documentado que el efecto benéfico de estos dos promotores de crecimiento se potencializa cuando los becerros se implantan durante todo el periodo de engorda y luego en el último mes antes del sacrificio se suplementan con CZ (Kellermeier *et al.* 2009; Baxa *et al.*, 2010; Parr *et al.*, 2011; 2014; Ebarb *et al.*, 2016). Según Ebarb *et al.* (2016), la aplicación combinada de ambos promotores de crecimiento favorece un mayor tamaño de todos los tipos de fibras musculares y sin modificar la deposición de colágeno, lo cual se traduce en una mayor hipertrofia muscular comparado a cuando se ofrecen individualmente.

Cabe señalar que todos los estudios conducidos en bovinos han encontrado mejoras en GDP, EA, PCC, RC y área del músculo *Longissimus toracis* (LT) por la aplicación individual de CZ o IE, y la interacción entre ellos no ha sido significativa para ninguna de estas variables. No obstante, numéricamente, los becerros tratados con CZ+IE han mostrado mejor ganancia de peso vivo y de canal, así como área del músculo LT con una disminución en la deposición de grasa en canal. Basado en esto, los autores de esos estudios han señalado que la aplicación combinada de CZ e IE causa un efecto aditivo de tipo positivo sobre el comportamiento productivo y características de la canal. En el 2014 Parr *et al.* y recientemente Harris *et al.* (2020) encontraron que la expresión del

ARNm de RA-β_2, así como la cantidad de estos receptores, aumentaron por efecto del IE en becerros. Dichos autores mencionaron que probablemente, el IE potencializa el efecto de la suplementación de CZ en ganado de engorda por aumentar la disponibilidad de RA- β_2, lo cual explica el efecto aditivo causado por administrar ambos promotores de crecimiento en forma combinada.

Finalmente, se debe tener en cuenta que la aplicación de CZ+IE promueve una mayor hipertrofia muscular, lo cual produce un aumento en la dureza de la carne (Kellermeier *et al.*, 2009; Ebarb *et al.*, 2016). Al parecer, este aumento en la dureza de la carne no está asociado con cambios en su bioquímica, ya que el pH ha mostrado no ser afectado cuando se administran en forma conjunta estos dos promotores de crecimiento (Ebarb *et al.*, 2017). El aumento en el tamaño de las fibras musculares es en gran medida responsable de la dureza de la carne (Ebarb *et al.*, 2016). Cabe mencionar que también hay estudios reportando que la combinación de CZ+IE en vaquillas no afectó los valores de esfuerzo al corte (Kerth *et al.*, 2003; Boles *et al.*, 2009). A nivel de perfil de ácidos grasos, Webb *et al.* (1995) reportaron cambios en las concentraciones de ácido palmítico, ácido palmitoleico y ácido oleico por efecto del CZ+IE.

1.3 Hipótesis

La combinación de CZ e IE interaccionan para potencializar sus efectos sobre el comportamiento productivo y características de la canal, sin afectar la calidad de la carne, en ovinos de pelo en finalización, lo cual se alcanza por alteraciones en la morfología del músculo.

1.4. Objetivo General

Evaluar los efectos de la suplementación de clorhidrato de zilpaterol en combinación con un implante de acetato de trembolona con estradiol sobre la modulación del crecimiento en ovinos de

pelo en etapa de finalización.

1.5. Objetivos Específicos

1. Determinar el efecto de la suplementación de CZ sobre el comportamiento productivo, las características de la canal y el rendimiento de cortes primarios en corderos de pelo implantados o no con hormonas esteroidales.

2. Analizar el efecto de la suplementación de CZ sobre la calidad de la carne madurada del músculo *Longissimus thoracis* en corderos de pelo implantados o no con hormonas esteroidales.

3. Analizar el efecto de la suplementación de CZ sobre las propiedades fisicoquímicas y el perfil de ácidos grasos de la carne del músculo *Longissimus thoracis* en corderos de pelo implantados o no con hormonas esteroidales.

4. Determinar el efecto de la suplementación de CZ sobre las características histoquímicas de las fibras musculares del *Longissimus thoracis* en corderos de pelo implantados o no con hormonas esteroidales.

1.6. Sección Integradora

En este trabajo de investigación se plantearon cuatro objetivos específicos de inicio, mismos que se cumplieron en su totalidad, obteniéndose como productos dos artículos, el primero de ellos ya publicado en la revista Meat Science (2019, 158: 107890, https://doi.org/10.1016/j.meatsci.2019.107890), y el segundo ha sido sometido a la misma revista (ID: MEATSCI-D-20-00193). La revista Meat Science se encuentra indizada en el Journal Citation

Reports con un factor de impacto de alrededor de 3.6 (Q1 del área), considerada como una revista con altos estándares de calidad.

El primer artículo publicado lleva por título "*Growth, carcass characteristics, cut yields and meat quality of lambs finished with zilpaterol hydrochloride and steroid implant*". Su contenido corresponde con la primera etapa experimental de este estudio, en donde se evaluaron los efectos del uso combinado de los dos promotores de crecimiento (CZ y IE) sobre el comportamiento productivo, las características de la canal, rendimiento de cortes primarios y calidad de la carne madura en ovinos de pelo. Para el cumplimiento del objetivo, 40 corderos enteros Dorper × Pelibuey se confinaron individualmente en corraletas y se asignaron en un arreglo factorial 2^2 a las siguientes combinaciones de tratamientos: 1) sin CZ e IE (testigo), 2) sin CZ y con IE (IES), 3) con CZ y sin IE (CZS), 4) con CZ e IE (CZ+IE). Los resultados mostraron que el CZ y el IE no interactuaron para modificar el comportamiento productivo, las características de la canal y el rendimiento de cortes primarios. Por su parte, en los animales administrados con sólo el CZ mejoró tanto el comportamiento productivo, como el peso y el rendimiento de la canal sin modificar la deposición de grasa interna y externa. Por otro lado, los efectos del IE fueron mínimos, sólo observándose una mejora en el rendimiento de la canal y del corte del pescuezo. Los resultados también mostraron que el CZ y el IE administrados en conjunto actuaron sinérgicamente para mejorar el área del *Longissimus thoracis*, sin embargo, el IE tuvo un efecto antagonista sobre el CZ para disminuir el pH de la carne a un rango normal, en consecuencia, la calidad de la carne madurada durante 14 días no se vio afectada por la administración combinada de ambos promotores.

El segundo artículo producto de esta investigación que ha sido sometido a evaluación lleva por título "*Muscle fiber morphometry and physicochemical characteristics of the Longissimus thoracis muscle of hair male lambs fed zilpaterol hydrochloride and implanted with steroids*". El manuscrito contiene los resultados obtenidos después de evaluar los efectos del uso combinado de los dos promotores de crecimiento (CZ y IE) sobre la morfometría miofibrilar, el área de la sección transversal, el contenido de colágeno, la composición proximal, la oxidación lipídica y de mioglobina, y el perfil de ácidos grasos del músculo *Longissimus thoracis*, el cual se obtuvo de los corderos usados en la primera parte del experimento. El CZ y el IE interactuaron sinérgicamente

para aumentar el área del músculo, así como el AST de las fibras tipo IIb y el porcentaje de colágeno soluble. Sin embargo, estos promotores de crecimiento también actuaron sinérgicamente para reducir la oxidación de mioglobina y el porcentaje de colágeno insoluble. El IE fue un antagonista del efecto del CZ para reducir el pH final a un rango normal, mientras que el CZ actuó en forma antagónica sobre el IE para reducir el contenido de cenizas en la carne. El contenido de proteína, grasa, humedad y colágeno total en la carne, así como oxidación lipídica, el esfuerzo al corte, los parámetros de color y la capacidad de retención de agua en la carne no fueron afectados por la interacción CZ × IE. Sin embargo, la suplementación de CZ afectó negativamente la calidad de la carne, ya que tendió a promover la presencia de carne DFD. En general, el IE no modificó la calidad de la carne ovina. El perfil de ácidos grasos fue parcialmente modificado por CZ, pero no por el IE.

2. GROWTH, CARCASS CHARACTERISTICS, CUT YIELDS AND MEAT QUALITY OF LAMBS FINISHED WITH ZILPATEROL HYDROCHLORIDE AND STEROID IMPLANT

M. Angeles López-Baca, Marco Contreras, Humberto González-Ríos, Ulises Macías-Cruz, Noemí Torrentera, Martín Valenzuela-Melendres, Adriana Muhlia-Almazán, Sergio Soto-Navarro, Leonel Avendaño-Reyes

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Growth, carcass characteristics, cut yields and meat quality of lambs finished with zilpaterol hydrochloride and steroid implant

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ABSTRACT

Forty hairbreed male lambs were used to evaluate the effects of zilpaterol hydrochloride (ZH, 0 and 0.15 mg/kg BW) and steroid implant (SI, without and with 52.5 mg trenbolone acetate and 7.5 mg 17β-estradiol) on feedlot performance, carcass characteristics, non-carcass components, wholesale cut yield, and meat quality. Supplemental ZH increased growth rate, feed efficiency, carcass weight, and dressing percentage, with no effect on wholesale cut yields. Feeding ZH increased muscle pH at 24 h. Supplemental ZH increased meat shear force, but decreased lightness, redness, and yellowness after frozen storage followed by a 14-day aging period. The SI administration increased dressing percentage and neck yield, but decreased testicle weight and meat redness, without affecting other variables. The LT area was greater with ZH + SI administration than with individual application of ZH or SI. Compared to individual administration, simultaneous application of ZH and SI did not result in improved growth performance, carcass traits and wholesale cut yields in hairbreed male lambs.

1. Introduction

The sheep meat industry in the world demands strategies to improve feedlot performance, carcass weight and dressing percentage of fattening lambs, since the meat production from this specie is insufficient to meet its demand in the market (Montossi et al., 2013). Zilpaterol hydrochloride (ZH) and steroid implants (SI) containing trenbolone acetate (TBA) and 17β-estradiol (E_2) are two growth promotants authorized for cattle fattening in several countries on the American continent, including Mexico (FAO, 2016). For feedlot lambs, both growth promotants have shown effectiveness for increased growth rate, feed efficiency, carcass weight, dressing percentage and *Longissimus* muscle area (Avendaño-Reyes et al., 2018; McClure, Solomont, & Loerch, 2000; Rojo-Rubio et al., 2018); however, effects on wholesale cut yields and meat quality due to supplemental ZH have been inconsistent (Dávila-Ramírez et al., 2013; Dávila-Ramírez et al., 2017; Macías-Cruz et al., 2016), while effects on those variables due to TBA + E_2 implant have not been studied. Notably, the improvement in feedlot performance and carcass characteristics of sheep has been more evident using ZH supplementation than the SI administration.

Some studies in beef cattle have reported that steers implanted with TBA + E_2 have better growth performance and carcass characteristics without negative effects on meat quality when receiving a diet supplemented with ZH during the finishing phase (Garmyn et al., 2011; Kellermeier et al., 2009; Neill et al., 2009). Those studies indicated that simultaneous administration of ZH and SI in beef cattle produced an additive effect to increase muscle tissue deposition, since each promotant may stimulate a greater muscle protein synthesis using different action mechanisms. The TBA and E_2 released by the implant bind to steroid receptors in the cytoplasm to stimulate the synthesis of insulin-like growth factor-I (IGF−I) and growth hormone; these hormones stimulate the proliferation and binding of satellite cells which are responsible for increasing protein synthesis in muscle fibers (Dayton & White, 2014). Also, application of SI has been positively associated with β-adrenergic receptor (β-AR) mRNA expression (Parr et al., 2014), so that implanting could be beneficial to increase the amount β-AR, and consequently, effectiveness of ZH as a growth promotant. For its part, β_2-adrenergic agonist (β_2-AA) ZH binds to β_2-AR located in the cellular membrane to promote hypertrophy of muscle fibers though increasing protein synthesis (myosin and actin) and decreasing proteolysis. In

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addition, this β_2-AA binds to β receptors from the fatty tissue to stimulate lipolysis and to decrease lipogenesis, provoking a redistribution of the energetic substrate toward the formation of muscle tissue (Mersmann, 1998; Mills, 2002). Other studies also have reported mobilization of nutrients from non-carcass tissue for myogenesis (Avendaño-Reyes et al., 2018; Rivera-Villegas et al., 2019). Thus, the combined administration of these two growth promotants is more beneficial than the sole administration of each promotant.

It is worth mentioning that the combined effect of ZH and SI has not been studied in fattening lambs. Recently, Webb, Allen, and Morris (2018) evaluated the simultaneous administration of ZH with a zeranol implant in feedlot lambs, and they found an additive effect by applying both promotants to improve live weight gain and cold carcass weight. Other variables such as dressing percentage and shear force were greater for ZH + implant lambs than for lambs solely implanted, but not compared to ZH-fed lambs. Given this background, the hypothesis tested in the current study was that growth promotants ZH and SI are more effective in combined application than applied individually to improve growth performance, carcass characteristics and wholesale cut yields, with some negative effects on meat quality (e.g. color traits and tenderness) in feedlot lambs. Therefore, the objective of this study was to evaluate the effects of zilpaterol hydrochloride and steroidal implants on feedlot performance, carcass characteristics, wholesale cut yields and meat quality of hairbreed male lambs finished in confinement.

2. Materials and methods

2.1. Study site

The feedlot performance period and carcass evaluation was conducted at the Instituto de Ciencias Agrícolas, Universidad Autónoma de Baja California (UABC), located in the Mexicali Valley, Baja California, México. Meat quality evaluation was developed at the Meat Science and Technology Laboratory of the Centro de Investigación en Alimentación y Desarrollo A.C. (CIAD A.C.), located in Hermosillo, Sonora, México. All procedures of management and slaughter of animals were conducted according to approved Mexican Official Standards for production, care, use of laboratory animals and their slaughter (NOM-062-ZOO-1999, NOM-051-ZOO-1995 and NOM-033-ZOO-1995). Additionally, the Research Ethics Committee of the CIAD A.C., approved and supervised all experimental procedures (ID: CE/021/2018). It should be noted that in Mexico, the promotants ZH and SI are not authorized to be used in sheep, by which the aforementioned Ethics Committee approved the use of both promotants only for experimental purposes. In addition, no product derived from lambs treated with these promotants was used for human consumption.

2.2. Animals, management and treatments

Forty Dorper × Pelibuey crossbred intact male lambs averaging 34.98 ± 0.24 kg and 4.5 mo old were used to carry out this study. All lambs were adapted to individual pens and basal diet (Table 1) for 15 d before beginning the experimental feeding phase. All pens were housed in a shaded area with galvanized sheet metal (2.5-m height). The dimensions of the pens were 1.0 m wide × 1.5 m long, and each one was equipped with two buckets placed in the front, one for feeding and the second for drinking. In addition, they were treated against parasites with 7 mg of ivermectin (Ivermectin; Sanfer Laboratory, Mexico City, Mexico), and injected with 5000 IU of vitamin A, 75,000 IU of vitamin D3 and 50 mg of vitamin E1 (Vigantol; Bayer, Mexico City, Mexico). The basal diet was formulated according to nutritional requirements for feedlot finishing lambs (11.7 MJ metabolizable energy [ME]/kg dry matter [DM] and 16% crude protein [CP]; NRC, 2007).

Lambs were weighed individually at the beginning of the experimental period and arranged into blocks of four animals with similar body weight (blocking factor = initial BW), resulting in 10 blocks. Lambs from each block were randomly assigned to four treatment combinations obtained from a 2 × 2 factorial arrangement, where the study factors were ZH supplementation (with and without) and SI application (with and without). Thus, the treatment combinations were: 1) without ZH and SI; 2) without ZH and with SI; 3) with ZH and without SI; and 4) with ZH and SI. Lambs treated with SI were implanted subcutaneously at the middle third into the back of the ear with three pellets of the implant Revalor® (52.5 mg TBA and 7.5 mg E_2; Intervet Laboratory, Mexico) during the complete feeding period (63 d). The dose of steroids administrated across the SI was approximated according to previous studies in which the following doses were implanted in lambs weighing between 24 and 40 kg: 40 to 60 mg TBA and 5.6 to 15 mg E_2 (Kongsuwan, Knox, Allingham, Pearson, & Dalrymple, 2012; McClure et al., 2000; Ortiz et al., 2013). For ZH-fed lambs, a β_2-AA (Grofactor®, Virbac Laboratory, Mexico) was offered daily at a dose of 0.15 mg/kg of BW on each animal. Supplementation of ZH lasted 30 d and was provided between days 31 and 60 of the feeding period, followed by a 3-d withdrawal period before slaughter. The ZH doses were adjusted on days 31, 38, 46, and 53 of the feeding period. While ZH dosage at the beginning of its administration was calculated based on BW recorded before the morning feeding, ZH dosages in the other days were adjusted by estimating the BW from an average daily gain (ADG) of 250 g, which has been reported in previous studies for feedlot hairbreed male lambs (Avendaño-Reyes et al., 2018; Dávila-Ramírez et al., 2014). Notably, estimated and observed ADG were similar, so dosage adjustments were appropriate. The beta-agonist Grofactor® was offered to the lambs mixed with wheat meal (6 mg of ZH into 30 g of wheat meal). Thus, each lamb supplemented with ZH received the amount of mixture that corresponded to its daily dosage of ZH before the morning feeding. At the same time, lambs without ZH supplementation received an amount of wheat meal alone based on a hypothetical estimate of the amount of ZH that they should consume daily.

Throughout the experimental feeding period, the basal diet was offered twice daily at 0700 and 1700 h in similar proportions (50:50). The daily amount of feed offered was adjusted to have a feed refusal rate of around 5%. Fresh drinking water was always available during the experimental period. The health status of lambs was also

Table 1
Ingredients and chemical composition of the experimental diet.

Ingredients[a] (%)	
Wheat straw	16.0
Alfalfa hay	14.0
Wheat milled	54.0
Soybean meal	12.0
Soybean oil	2.5
White salt	0.5
Calcium phosphorus	0.7
Limestone	0.3
Chemical composition[b] (%)	
Dry matter	90.8
Organic matter	92.6
Ash	7.4
Crude protein	15.0
Ether extract	7.6
Neutral detergent fiber	44.1
Acid detergent fiber	14.7
Hemicellulose	29.4
Cellulose	10.2
Lignin	4.5
Energies[b] (MJ/kg)	
Metabolizable energy	11.7
Net energy for maintenance	7.9
Net energy for gain	5.4

[a] As fed basis.
[b] Dry matter basis.

Table 2
Effect of zilpaterol hydrochloride (ZH) and steroid implant (SI) on productive performance of hairbreed lambs finished in feedlot.

Items	ZH (mg/kg BW)[a]			SI[b]			P-Value		
	0	0.15	SEM	Without	With	SEM	ZH	SI	ZH × SI
Body weight (kg)									
d 1	35.01	34.95	0.24	34.99	34.97	0.24	0.84	0.95	0.87
d 30	42.97	42.10	0.45	42.46	42.59	0.46	0.18	0.84	0.54
d 60	49.95	50.03	0.57	49.94	50.05	0.57	0.92	0.90	0.97
Total weight gain (kg)									
d 1 a 30	7.95	7.16	0.51	7.47	7.64	0.51	0.28	0.82	0.61
d 31 a 60	6.99	7.84	0.35	7.48	7.34	0.35	0.05	0.78	0.27
d 1 a 60	14.94	15.13	0.62	14.95	15.11	0.61	0.82	0.85	0.96
Average daily gain (g/d)									
d 1 a 30	265	239	17.2	249	255	16.5	0.28	0.82	0.61
d 31 a 60	232	261	10.4	249	245	11.7	0.05	0.78	0.28
d 1 a 60	249	252	10.1	249	252	10.3	0.82	0.85	0.96
Feed intake (kg)									
d 1 a 30	1.63	1.62	0.05	1.63	1.62	0.05	0.86	0.90	0.49
d 31 a 60	1.66	1.58	0.06	1.63	1.61	0.06	0.38	0.79	0.79
d 1 a 60	1.64	1.61	0.05	1.63	1.62	0.05	0.68	0.98	0.75
Feed efficiency (g/kg)									
d 1 a 30	166	150	9.4	159	157	9.4	0.25	0.88	0.34
d 31 a 60	142	168	6.3	155	154	6.9	< 0.01	0.96	0.27
d 1 a 60	154	160	4.6	157	156	4.6	0.35	0.99	0.81

[a] Lambs fed 0 or 0.15 mg of ZH/kg BW per day from day 31 to 60 of the feeding period.
[b] Lambs without and with steroidal implant administered in the ear (52.5 mg of trenbolone acetate +7.5 mg of 17β-estradiol) during the 63-d feeding period.

yields (Table 4). In the case of non-carcass components, while the weights of skin, head, and feet expressed as a percentage of empty BW were lower ($P \leq .02$) by ZH effect (Table 5), other components were unaltered ($P \geq .24$; i.e. blood, testicle, heart, kidney, liver, spleen, lungs, stomach and intestine). On the other hand, the SI administration did not affect ($P \geq .78$) growth performance (overall or by periods; Table 2). For carcass characteristic (Table 3) and wholesale cut yields (Table 4), only dressing percentage increased ($P = .01$) and neck yield decreased ($P = .05$) by SI administration. In non-carcass components (Table 5), the SI administration caused lower ($P < .01$) testicular weight (as % empty BW), without affecting ($P \geq .11$) other components.

3.2. Area and meat quality of the LT

The ZH × SI interaction affected ($P \leq .05$) LT area (Fig. 1), as well as ultimate pH and WBSF (Fig. 2). The LT area was greater ($P < .01$) with ZH + SI administration compared to controls or the individual application of ZH or SI. The LT area did not differ ($P > .05$) among controls and lambs treated only with ZH or SI. Additionally, ultimate pH and WBSF were greater ($P < .01$) in lambs treated only with ZH than in lambs any other treatment combination. The control lambs and lambs treated with ZH + SI or only with SI had similar ($P > .05$) ultimate pH and WBSF.

In relation to other quality traits, ZH supplementation did not affect *postmortem* pH at 45 min ($P = .25$) but whether at 24 h, being greater ($P = .02$) due to ZH (Table 6). With exception of H*, which only tended to decrease ($P = .07$), the colors of the meat (L*, a*, b* and C*) decreased ($P \leq .04$) by ZH effect. Supplemental ZH unaffected ($P = .71$) cooking loss and tended to increase ($P = .08$) WHC. On the other hand, SI caused lower ($P = .05$) a* values, without affecting ($P \geq .11$) any other color traits, *postmortem* pH (45 min and 24 h), cooking loss or

Table 3
Effect of zilpaterol hydrochloride (ZH) and steroid implant (SI) on carcass characteristics and fat deposition of hairbreed lambs finished in feedlot.

Items	ZH (mg/kg BW)[d]			SI[e]			P-value		
	0	0.15	SEM	Without	With	SEM	ZH	SI	ZH × SI
HCW[a] (kg)	23.96	25.17	0.29	24.26	24.87	0.28	< 0.01	0.15	0.77
CCW[b] (kg)	23.38	24.61	0.29	23.72	24.27	0.29	< 0.01	0.19	0.72
Dressing (%)	54.71	57.34	0.41	55.20	56.86	0.43	< 0.01	0.01	0.91
Conformation (units)	7.35	7.54	0.12	7.42	7.47	0.12	0.27	0.80	0.80
Body fat									
Fat thickness (cm)	3.15	2.72	0.23	2.85	3.03	0.23	0.20	0.58	0.81
KPH fat[c] (%)	2.27	2.01	0.14	2.15	2.13	0.14	0.21	0.93	0.52
Omental fat (%)	3.00	2.73	0.17	2.99	2.74	0.17	0.26	0.32	0.41
Mesenteric fat (%)	1.95	1.72	0.10	1.91	1.76	0.10	0.12	0.34	0.52
Morphometric measures (cm)									
Carcass length	63.25	63.86	0.48	63.82	63.29	0.48	0.37	0.43	0.81
Neck perimeter	37.22	37.14	0.62	36.75	37.61	0.62	0.92	0.33	0.31
Thorax depth	15.56	16.08	0.20	15.76	15.88	0.21	0.09	0.69	0.31
Leg length	28.26	29.04	0.27	28.63	28.67	0.28	0.06	0.93	0.79
Leg perimeter	44.98	47.00	0.55	46.09	45.88	0.57	0.02	0.79	0.24

[a] HCW = Hot carcass weight.
[b] CCW = Cold carcass weight.
[c] KPH fat = kidney, pelvic and heart fat.
[d] Lambs fed 0 or 0.15 mg of ZH/kg BW per day from day 31 to 60 of the feeding period.
[e] Lambs without and with steroidal implant administered in the ear (52.5 mg of trenbolone acetate +7.5 mg of 17β-estradiol) during the 63-d feeding period.

continuously monitored by direct observation. Three feed samples were collected every time the basal diet was mixed. All samples were dried in a forced-air oven at 60 °C for 24 h, milled across a 2-mm screen and mixed to obtain two subsamples which were used to determine the following: DM, organic matter, ash, CP, ether extract, neutral and acid detergent fiber, cellulose and lignin (Chemists & Horwitz, 1990; Van Soest, Robertson, & Lewis, 1991). Metabolizable energy and net energy for maintenance and gain were calculated using formulas proposed by NRC (1985).

2.3. Feedlot performance

Individual BW was recorded at days 1, 31, and 61 of the experimental fattening period, just before offering food in the morning. All BW were multiplied by 0.96 to eliminate the weight due to gastrointestinal content (Cannas, Tedeschi, Fox, Pell, & Van Soest, 2004). The daily amount of food offered and refused was also recorded before the morning feeding. From this data collected, total weight gain (TWG), ADG, feed intake and feed efficiency were estimated for the following periods: days 1 to 30, 31 to 60 and 1 to 60 (overall).

2.4. Carcass characteristics and non-carcass components

All lambs were slaughtered after the 3-d withdrawal period of the ZH dietary supplementation. First, lambs were fasted from feed and water for 12 h before recording slaughter BW and then were slaughtered by exsanguination. Next, lambs were skinned and eviscerated in order to register hot carcass weight (HCW) and weights of the following non-carcass components: blood, skin, head, feet, heart, liver, lungs, kidney, full and empty gastrointestinal tract, empty stomach complex, empty intestine, spleen, kidney, pelvic, and heart (KPH) fat, and omental and mesenteric fats. Carcasses were then chilled at 4 °C for 24 h to register cold carcass weight (CCW), conformation using an 8-point scale [1 = bad and 8 = excellent; (Smith & Griffin, 2001)], and the morphometric measurements carcass length, neck perimeter, thorax depth, and leg length and perimeter (Parés-Casanova, 2013). Carcasses were split along the mid-line and then the left half of each carcass was ribbed between the 12th and 13th rib to measure *Longissimus thoracis* (LT) muscle area using a dot square grid of 64 mm^2, and also fat thickness with a vernier calliper.

With exception of the weight of KPH fat (expressed as % of HCW), weights of all non-carcass components were expressed as percentages of the empty BW (EBW). The HCW was also expressed as a percentage of the EBW to obtain dressing percentage. The EBW was obtained by difference between slaughter BW and gastrointestinal content weight; this last weight was calculated by difference between weights of full and empty gastrointestinal tract.

2.5. Wholesale cut yields

The left half carcass was weighed into wholesale cuts according to the methodology described by Avendaño-Reyes et al. (2011). Initially, the half carcass was weighted and sectioned into the following cuts: forequarter, hindquarter, neck, shoulder, ribs, plain loin, loin, leg, breast, and flank. The wholesale cut yields were obtained by expressing their weights as a percentage of the weight of the half carcass.

2.6. Meat quality evaluation

Meat quality was evaluated on the LT muscle and included pH at 45 min and 24 h *postmortem*, which were measured directly in carcasses (12th and 13th rib) using a pH meter (Hanna Instruments, model HI 98140, Woonsocket, RI, USA) equipped with a standardized penetration electrode. Additionally, after 24 h of maintaining the carcasses in cooling room at 4 °C, LT muscle was dissected from the loin wholesale cut (portion of the loin located between the 4th and 12th ribs), vacuum packaged and stored at −20 °C during two weeks. Then, LT meat was thawed for 48 h (24 h at −10 °C and the remaining time at 0 °C), and finally aged at 4 °C for 14 d to evaluate: ultimate pH, color variables, cooking loss, water-holding capacity (WHC), and *Warner-Bratzler* shear force (WBSF). Quality variables were measured repeatedly in each sample to record an average value for each.

The ultimate pH was determined in a mixture obtained by homogenizing 5 g of muscle sample with 25 mL of distilled water, using a pH meter (Model HI-2210, Hanna Instruments Digital, Woonsocket, RI) equipped with a glass electrode. The potentiometer was previously calibrated using two buffers (pH at 4.0 and 7.0) and each measurement was repeated three times.

Color measurements were performed directly on the meat surface after 30 min blooming using a Minolta CR-400 colorimeter (Konica Minolta Sensing, Inc., Osaka, Japan) equipped with D65 illuminator, 10° observer and a 8 mm diameter aperture. Lightness (L*), redness (a*) and yellowness (b*) were recorded according to the Hunter-Lab method. Color saturation index (Chroma, C*) was calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$ and the color Hue angle (H*) as $H^* = (\tan^{-1}(b^*/a^*)$ (Priolo, Waghorn, Lanza, Biondi, & Pennisi, 2000). A total of eight measurements per sample were taken at different locations.

The WHC was determined using a previously described methodology (Sutton, Ellis, Lan, McKeith, & Wilson, 1997). A 3-g meat sample was placed in a micron nylon mesh to be suspended in a centrifuge tube and then centrifuged at 2000 × *g* for 5 min at 5 °C in the order to record again the sample weight. The difference of sample weights before and after centrifugation was expressed as a percentage of the 3 g sample to obtain WHC. Each sample was evaluated three times. For WBSF and cooking loss, steaks were cooked until reaching an internal temperature of 71 °C using an electric skillet (Cook Master Oster, model 3222–3, Mississauga, Ontario, Canada). After cooking, steaks were cooled to room temperature (25–30 °C) and chilled at 4 °C for 24 h to evaluate WBSF using a TA. XT *plus* Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). From each steak, seven prismatic pieces of 1.27 cm diameter were cut in longitudinal orientation to the muscle fibers to record the WBSF value in Newtons. In addition, steaks were weighed before and after cooking to determine cooking loss as follows: [(initial weight - weight after cooking and draining)/ (initial weight)] × 100.

2.7. Statistical analysis

Data were analyzed as a 2 × 2 factorial in a randomized complete block design using PROC MIXED from SAS (SAS Inst. Inc., Cary, NC). Animal was the experimental unit for all analyses. The model included fixed effects of ZH supplementation, SI application and the interaction between them. The random terms were block and experimental error. Means were compared with a Tukey test at $P \leq .05$, considering trend when $0.05 \leq P \leq .10$ and non-significant effect to $P > .10$.

3. Results

3.1. Feedlot, carcass and non-carcass traits, and wholesale cuts

No ZH × SI interaction was detected for growth performance ($P \geq .27$), carcass characteristics ($P \geq .24$), non-carcass components ($P \geq .18$) or wholesale cut yields ($P \geq .52$). Supplemental ZH affected only feedlot traits between the day 31 and 60 of the feeding period, being TWG, ADG and feed efficiency greater ($P \leq .05$) due to ZH (Table 2). Feed intake was unaffected ($P \geq .38$) by ZH supplementation. In addition, feeding ZH promoted greater ($P \leq .02$) HCW, CCW, dressing percentage and leg perimeter (Table 3). Thorax depth ($P = .09$) and leg length ($P = .06$) tended to increase with ZH supplementation. Conformation, body fat deposition and the remaining carcass morphometric measures were unaltered ($P \geq .12$) by ZH supplementation. In addition, ZH supplementation did not affect ($P \geq .13$) wholesale cut

Table 4
Effect of zilpaterol hydrochloride (ZH) and steroid implant (SI) on wholesale cut yields of hairbreed lambs finished in feedlot.

Items[a]	ZH (mg/kg BW)[b]			SI[c]			*P*-Value		
	0	0.15	SEM	Without	With	SEM	ZH	SI	ZH × SI
Forequarter (%)	53.62	53.57	0.38	53.18	54.01	0.38	0.91	0.13	0.62
Hindquarter (%)	46.37	46.43	0.38	46.82	45.99	0.38	0.91	0.13	0.62
Neck (%)	4.37	4.23	0.21	4.60	4.00	0.21	0.63	0.05	0.98
Ribs (%)	9.55	9.82	0.19	9.46	9.90	0.20	0.35	0.13	0.88
Loin (%)	12.02	11.78	0.20	11.81	11.99	0.20	0.41	0.53	0.52
Plain loin (%)	9.23	9.59	0.16	9.35	9.47	0.16	0.13	0.60	0.86
Breast and flank (%)	7.44	7.34	0.21	7.60	7.17	0.21	0.74	0.17	0.92
Shoulder (%)	30.47	29.93	0.42	29.77	30.63	0.43	0.38	0.16	0.75
Leg (%)	26.91	27.31	0.41	27.40	26.82	0.41	0.50	0.32	0.84

[a] Weights of wholesale cuts expressed as a percentage of the half carcass weight.
[b] Lambs fed 0 or 0.15 mg of ZH/kg BW per day from day 31 to 60 of the feeding period.
[c] Lambs without and with steroidal implant administered in the ear (52.5 mg of trenbolone acetate +7.5 mg of 17β-estradiol) during the 63-d feeding period.

Table 5
Effect of zilpaterol hydrochloride (ZH) and steroid implant (SI) on non-carcass components of hairbreed lambs finished in feedlot.

Items[a]	ZH (mg/kg BW)[c]			SI[d]			*P*-value		
	0	0.15	SEM	Without	With	SEM	ZH	SI	ZH × SI
Blood (%)	4.59	4.43	0.09	4.62	4.40	0.09	0.24	0.11	0.60
Skin (%)	11.48	10.00	0.23	10.89	10.59	0.23	< 0.01	0.38	0.08
Head (%)	5.43	5.12	0.09	5.31	5.24	0.09	0.02	0.57	0.12
Feet (%)	2.41	2.19	0.03	2.34	2.27	0.04	< 0.01	0.20	0.96
Testicles (%)	1.20	1.24	0.08	1.51	0.93	0.08	0.71	< 0.01	0.30
Heart (%)	0.42	0.42	0.007	0.43	0.42	0.07	0.93	0.15	0.65
Kidney (%)	0.32	0.31	0.01	0.31	0.31	0.01	0.72	0.78	0.17
Liver (%)	2.06	2.02	0.06	2.05	2.04	0.06	0.66	0.92	0.23
Spleen (%)	0.36	0.31	0.06	0.31	0.35	0.06	0.58	0.59	0.53
Lungs (%)	1.37	1.44	0.05	1.42	1.39	0.05	0.35	0.69	0.79
Stomach[b] (%)	2.80	2.79	0.06	2.87	2.72	0.06	0.93	0.11	0.76
Intestines (%)	2.93	2.55	0.24	2.92	2.56	0.24	0.26	0.30	0.65

[a] Weights of each non-carcass component expressed as a percentage of the slaughter BW.
[b] Stomach complex composed of rumen, omasum, abomasum and reticulum.
[c] Lambs fed 0 or 0.15 mg of ZH/kg BW per day from day 31 to 60 of the feeding period.
[d] Lambs without and with steroidal implant administered in the ear (52.5 mg of trenbolone acetate +7.5 mg of 17β-estradiol) during the 63-d feeding period.

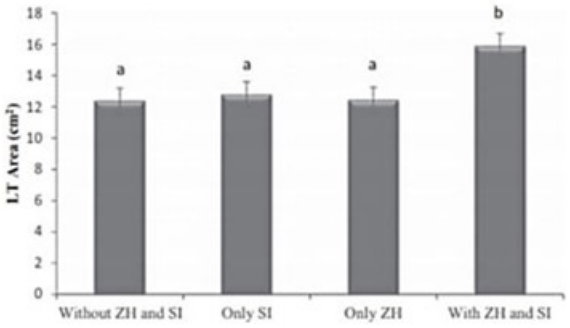

Fig. 1. Effect of zilpaterol hydrochloride (ZH) and steroid implant (SI) on *Longissimus toracis* (LT) muscle area of hairbreed lambs finished in feedlot. Different letters indicate significant differences at $P < .05$. The ZH × SI interaction was significant ($P = .05$).

WHC.

4. Discussion

Dietary supplementation of ZH during the finishing phase improves feedlot performance of beef cattle by increasing growth rate and feed efficiency (Avendaño-Reyes et al., 2016; Meraz-Murillo et al., 2017), and in fact, some studies suggest that the ZH effect as growth promotant is still greater in cattle already implanted with TBA + E_2 (Mills, 2002; Parr et al., 2014). Based on the aforementioned, the current study hypothesized that feedlot performance and carcass characteristics of economic importance could be further improved with some affectations on the meat quality of lambs if ZH and SI are administrated simultaneously instead of individually. However, results partially supported this hypothesis given that there was no interaction between both growth promotants to affect feedlot performance, carcass characteristics and wholesale cut yield of lambs; despite this, muscle deposition (greater LT area) was increased for lambs treated with ZH + SI compared with the other treatment combinations. Also, the implantation with TBA + E_2 increased tenderness of the meat in ZH-fed lambs. In addition, this is the first study evaluating a possible synergistic or additive effect on growth and carcass traits by administering at the same time supplemental ZH and TBA + E_2 implant using fattening lambs. Thus, our results suggest that there is not a synergistic or additive effect by the simultaneous application of these promotants to improve growth rate, feed efficiency, carcass characteristics or wholesale cut yields in entire male sheep. This was evidenced by the lack of interaction effects, as well as, we did not observe numerical differences among lambs of SI, ZH and ZH + SI as reported previous studies in beef (Baxa et al., 2010; Parr et al., 2011). However, results in LT muscle indicate that SI and ZH acted synergistically to improve the growth of this muscle without affecting tenderness in meat. This could have beneficial applications for a market of mutton cuts.

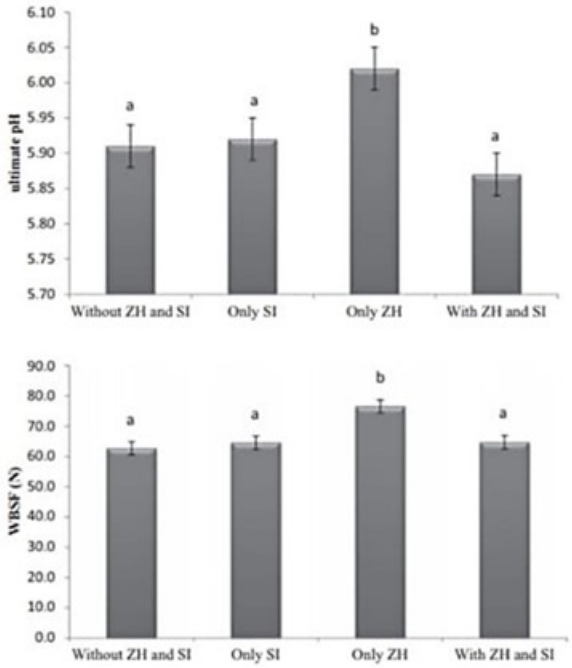

Fig. 2. Effect of zilpaterol hydrochloride (ZH) and steroid implant (SI) on ultimate pH and *Warner-Bratzler* shear force (WBSF) in *Longissimus toracis* (LT) muscle of hairbreed lambs finished in feedlot. LT muscle was freezing/thawing and then aged by 14 d before the evaluation of meat quality. Different letters indicate significant differences at $P < .05$. The ZH × SI interaction was significant ($P = .05$).

4.1. Feedlot, carcass and non-carcass traits, and wholesale cuts

Growth performance, HCW, and CCW of hairbreed lambs were improved by ZH supplementation. Similarly, most studies have reported greater ADG, feed efficiency and carcass weight in fattening lambs fed ZH during the last 4 to 5 weeks before slaughter (Avendaño-Reyes et al., 2018; López-Carlos et al., 2011; Macías-Cruz, Álvarez-Valenzuela, Soto-Navarro, Águila-Tepato, & Avendaño-Reyes, 2013; Rojo-Rubio et al., 2018). Although, other studies in male sheep have only reported an improvement in carcass weight and dressing percentage without any other effect on feedlot performance (Dávila-Ramírez et al., 2014; López-Carlos et al., 2010). These discrepancies in results are possibly due to genetic variations (Nourozi, Abazari, Raisianzadeh, Mohammadi, & ZareShahne, 2008) or environmental factors (Macías-Cruz et al., 2013), considering that the in vivo mechanism of action of the β_2-AA is very sensitive to other secondary events that alter at the physiological and endocrine level in those tissues with a large number of β-adrenergic receptors (Mersmann, 1998).

Interestingly, feeding ZH did not modify body fat deposition (i.e. KPH, omental, mesenteric and thickness), but decreased the weights (% empty BW) of some non-carcass components such as skin, head and feet. These findings are in agreement with reports in fattening lambs when ZH of brand Grofactor® (Avendaño-Reyes et al., 2018) was used as in the current study. However, these results partially differed with respect to studies that used ZH of the brand Zilmax® (Dávila-Ramírez et al., 2014; Rojo-Rubio et al., 2018), where both fat deposition and some weights of non-carcass components were decreased by use of the β_2-AA. This suggests that the action mechanism of the ZH molecule to promote muscle hypertrophy varies with the origin of the commercial product, confirming those findings as indicated in other reports about this topic (Avendaño-Reyes et al., 2018; Rivera-Villegas et al., 2019). Therefore, in the case of this study where ZH Grofactor® was used, improvements in ADG, feed efficiency, carcass weight and dressing yield due to ZH were attributed to a greater muscle mass deposition (Avendaño-Reyes et al., 2011; Mersmann, 1998). Unlike the ZH Zilmax®, this positive effect of ZH Grofactor® on muscle formation was not primarily due to the redistribution of the energetic substrate from fat tissue toward muscle to increase the protein synthesis, but possibly to a mobilization of nutrients from some non-carcass components (i.e. skin, head and feet); Avendaño-Reyes et al. (2018) also suggested this point. Given that feed intake did not change by ZH effect, other action mechanisms by which β_2-AA could have promoted muscle hypertrophy in lambs were: 1) low muscle proteolysis (Mersmann, 1998), 2) greater nitrogen retention (Brake, Titgemeyer, & Jones, 2011) and 3) better dietary energy partitioning (Macías-Cruz, Álvarez-Valenzuela, et al., 2013).

In agreement with previous studies (Avendaño-Reyes et al., 2018;

Table 6
Effect of zilpaterol hydrochloride (ZH) and steroid implant (SI) on area, *postmortem* pH, and meat quality of the *Longissimus thoracis* (LT) muscle of hairbreed lambs finished in feedlot.

	ZH (mg/kg BW)[d]			SI[e]			*P*-values		
	0	0.15	SEM	Without	With	SEM	ZH	SI	ZH × SI
LT Area (cm^2)	12.60	14.17	0.57	12.42	14.35	0.57	0.05	0.03	0.05
pH postmortem									
45 min	6.75	6.71	0.02	6.73	6.72	0.22	0.25	0.78	0.90
24 h	5.81	5.92	0.03	5.84	5.89	0.03	0.02	0.27	0.46
Meat quality after aging[a]									
Ultimate pH	5.92	5.94	0.02	5.99	5.90	0.02	0.38	0.04	0.02
Lightness (L*)	40.74	38.74	0.64	39.74	39.74	0.66	0.04	0.99	0.16
Redness (a*)	17.33	15.11	0.31	16.68	15.76	0.31	< 0.01	0.05	0.45
Yellowness (b*)	8.55	6.91	0.30	7.81	7.66	0.30	< 0.01	0.73	0.67
Hue angle (H*)	26.11	24.34	0.67	24.79	25.67	0.69	0.07	0.37	0.94
Chroma (C*)	19.35	16.64	0.38	18.45	17.55	0.39	< 0.01	0.11	0.47
Cooking loss (%)	27.20	26.74	0.86	27.03	26.92	0.86	0.71	0.93	0.43
WHC[b] (%)	86.75	87.52	0.30	87.47	86.81	0.31	0.08	0.14	0.18
WBSF[c] (N)	63.64	70.60	2.15	69.62	64.62	2.15	0.03	0.12	0.03

[a] LT muscle was vacuum packed, frozen/thawed and then aged during 14 day before evaluation of meat quality.
[b] WHC = Water-holding capacity.
[c] WBSF = Warner-Bratzler shear force.
[d] Lambs fed 0 or 0.15 mg of ZH/kg BW per day from day 31 to 60 of the feeding period.
[e] Lambs without and with steroidal implant administered in the ear (52.5 mg of trenbolone acetate + 7.5 mg of 17β-estradiol) during the 63-d feeding period.

Macías-Cruz et al., 2016; Rojo-Rubio et al., 2018), supplemental ZH did not modify the yield of wholesale cuts of our hair male lambs. However, there are some studies in sheep reporting variations in cut yields due to ZH, but the effects are not consistent. For instance, a greater leg yield in ZH-fed rams was reported by Rivera-Villegas et al. (2019) and Dávila-Ramírez et al. (2014), but in the first study lower loin yield was also found. Remarkably, supplemental ZH was expected to improve the yield of some cuts such as loin and leg because they have large quantity of type-II muscle fibers, and ZH stimulates the development of these fibers (Walker et al., 2010). Perhaps, the development of the muscular fibers achieved by effect of ZH was not enough to reflect a greater yield in the wholesale cuts of lambs. This hypothesis could be the object of study of future research.

On the other hand, overall results evidenced that SI containing 52.5 mg TBA plus 7.5 mg E_2 did not act as a growth promotant for fattening lambs in the finishing phase, even when previous studies reported otherwise (Johnson, White, Hathaway, Christians, & Dayton, 1998; McClure et al., 2000; Ortiz et al., 2013). The SI administration in the last 63 d before slaughter did not improve growth rate, feed efficiency, carcass weight or wholesale cut yield. Only a better dressing percentage was observed for the SI-treated lambs. Similar results were reported for Greyface breed lambs after being implanted with 57.5–70 mg of TBA and 8.1–12 mg of E_2 during 63 d (Yasin & Galbraith, 1981). Even though, a lower body fat deposition due to SI was also found in that study. For their part, another study did not find any benefit in growth or carcass traits implanting 60 mg of TBA and 12 mg of E_2 to Targhee × Hampshire ewe lambs, but an enhancement in weight gain and lean accretion was reported by effect of this implant in wethers (McClure et al., 2000). There is no clear explanation regarding the lack of effects of SI on growth and carcass traits in our study; however, most studies have reported positive effects from the SI application in sheep when wethers were used. Steroid implant seemed to work differently in wethers and ram lambs due to variations in the growth metabolism and activation of androgen receptors. Thus, SI is better as growth promotant in wether because TBA and E2 released by implant compensate the lack of natural testosterone synthesis in castrated males (Johnson et al., 1998; Lee, Henricks, Skelley, & Grimes, 1990). Testosterone is a hormone that stimulates somatotropin/IGF-I axis, which is important to increase muscle protein accretion and weight gain in fattening ruminants (Dayton & White, 2014). Contrarily, in entire male, the steroids administered through implants seem to enter into a competition with natural testosterone causing a decrease in circulating testosterone levels and amount of androgen receptors. Notably, circulating concentrations of IGF-I and growth hormone are maintained despite the decline in testosterone levels in these males, by which they follow normally growth (Lee et al., 1990). Therefore, gender seems to play an important role in the functioning of SI as a growth promotant for sheep.

While it is true that SI application did not improve growth and carcass weight in lambs, we observed an increase in muscle development caused by administration of SI (greater LT area), which was sufficient to increase carcass yield but not carcass or live weight gain. So, greater dressing percentage in SI-treated lambs was due to that TBA + E_2 implant stimulated protein synthesis and, consequently, muscle hypertrophy (Dayton & White, 2014; Preston, 1999). The SI increases the production of IGF-I and IGF-I mRNA in muscle fibers to favor the proliferation of satellite cells, which divide and fuse with fibers to donate their nuclei (Dayton & White, 2014; Parr et al., 2014). Thus, the major presence of nuclei in muscle fibers promotes greater protein synthesis in SI-treated lambs. As expected, given that several studies in different species (i.e. sheep, cattle and human) agree with this finding (Deaver, Glass, Grieger, & Reeves, 1988; Greyling, Kotze, Taylor, & Hagendijk, 1993; Passantino, 2012), the implantation of lambs with TBA + E_2 decreased the testicular development. This is because both steroidal hormones caused alterations in the male reproductive axis (hypothalamic-pituitary-gonadal), which led to an inhibitory effect on the LH and FSH secretion, hormones required to promote the testicular synthesis and to release the hormone testosterone (Deaver et al., 1988). Testosterone is the hormone responsible for regulating all testicular reproductive processes, and therefore, the growth and development of the same (Passantino, 2012).

4.2. Area and meat quality of the LT

Results of the LT area suggest that both growth promotants had a synergistic effect to improve muscle hypertrophy in the LT, which does not agree with those reports for beef cattle where the simultaneous application of ZH and SI increased LT area due to an additive effect (Garmyn et al., 2011; Neill et al., 2009; Parr et al., 2014). Therefore, this confirms that ZH + SI treated lambs compared with control lambs or treated with implant or ZH alone had a better protein synthesis in skeletal muscle; perhaps this was due to a redistribution of nutrients toward muscle tissue and to an increase in the availability of nuclei in muscle cells, at the same time that decreased the proteolysis process (Dayton & White, 2014; Mersmann, 1998). Additionally, Parr et al. (2014) demonstrated that ZH could potentiate their effects as growth promotant in implanted steers because implanting with TBA + E_2 can improve the activation of β-AR, specifically the β_1-AR and β_2-AR mRNA expression. Thus, the increase of protein synthesis through the mechanisms previously indicated could lead to a greater LT fiber diameter (Kellermeier et al., 2009), muscle hypertrophy and LT area. More studies in sheep are required to test this hypothesis.

The combined treatment ZH + SI did not improve loin weight in lambs even when LT muscle area increased with this treatment. Similar findings have been reported by other studies where supplemental ZH was added in the basal diet of feedlot lambs (Avendaño-Reyes et al., 2018; Rojo-Rubio et al., 2018). In all studies, including this, loin weight was adjusted as a percentage of the weight of half of the carcass, which could explain the inconsistency observed in the ZH + SI treatment (greater LT area with no effect on loin yield).

Surprisingly, the better accretion of muscle tissue observed in ZH + SI treated lambs did not lead to a decrease in the meat tenderness, because WBSF of the LT was similar among the treatments control, ZH + SI and SI alone. This result does not match with those reported in beef cattle, where WBSF of the *Longissimus* muscle was not affected by the ZH × SI interaction, but its effects separately increased WBSF at 7, 14 and 21 d *postmortem* (Garmyn et al., 2011; Kellermeier et al., 2009). Perhaps, discrepancies in WBSF results between our study and those previously reported may be due to the specie (sheep vs. bovine), and because we freeze the meat before the aging period. In fact, WBSF was greater in ZH alone than ZH + SI, which suggests that TBA + E_2 implant helped to decrease the toughness of the meat after the aging period in ZH-fed lambs. Additionally, this finding indicates that, in male lambs, ZH increased meat toughness by a direct effect at biochemical level and not by stimulating a greater protein synthesis and fiber diameter in muscle. More studies in sheep are required to confirm these results on WBSF. We strongly believe that the differences in WBSF among treatment combinations could be attributed to variations recorded in the ultimate pH (Korn et al., 2013). Wu, Farouk, Clerens, and Rosenvold (2014) indicate that ultimate pH plays a critical role in the degradation of myofribrillar proteins during the aging period, since levels of insoluble collagen and activity of the calpain-calpastatin proteolytic enzyme system are mainly altered (Dikeman, 2007; Korn et al., 2013). In sheep meat, Watanabe, Daly, and Devine (1996) reported a curvilinear association between ultimate pH and WBSF values, with a toughness peak at pH around 6.0 and improvements in tenderness at pH below and above 6.0. The authors explain the that the activity of the calpastatin enzyme and the myofribillar fragmentation index increase stopping the proteolysis of structural myofibrillar protein at intermediate pH (6.0), while an increase in the calpain activity is observed at pH > 6.0 y < 6.0. The calpain is the main enzyme responsible of aged meat tenderization. As LT meat from ZH and ZH + SI lambs had

pH = 6.0 and 5.8, respectively, our results show that SI acted to improve LT tenderness of ZH-fed lambs by reducing ultimate pH.

Moreover, without effect of the interaction, feeding ZH caused a greater *postmortem* pH at 24 h in the LT, coinciding with previous reports of sheep (Dávila-Ramírez et al., 2013; Dávila-Ramírez et al., 2017). However, there are some studies reporting lack of ZH effect on pH at 24 h *postmortem* in *Longissimus* muscle (López-Carlos et al., 2012; Rojo-Rubio et al., 2018). Increased muscle pH due to feeding ZH could be explained by a drop in muscle glycogen levels at slaughter, and this has been reported previously for cimaterol-fed lambs (Lee & Kim, 1994). The β_2-AA change the muscle metabolism from an aerobic type to an anaerobic to increase protein accretion; however, the anaerobic metabolism promotes a fast utilization of glycogen before slaughter, which causes a greater muscle pH at 24 h *postmortem* (Dunshea, D'Souza, Pethick, Harper, & Warner, 2005). The pH at 24 h *postmortem* for the LT of ZH-fed lambs was 5.9, which is slightly above the reference range (Sañudo, Santolaria, María, Osorio, & Sierra, 1996). A *postmortem* pH at 24 h above the reference range is related with dark, firm and dry meat (Adzitey & Nurul, 2011). So, ZH supplementation negatively impacted meat quality of hairbreed male lambs by an inadequate decline in meat pH during the first 24 h *postmortem*.

In congruence with the previously mentioned, ZH-fed lambs had a meat of lower quality than lambs fed without ZH, given that supplemental ZH decreased all color traits (a*, b*, H* and L*), as well as a trend to increase WHC. Similar results of feeding ZH on color of lamb meat were found by Dávila-Ramírez et al. (2017) and Dávila-Ramírez et al. (2013). These findings were attributed to the *postmortem* pH at 24 h, which is a relevant indicator of the physicochemical characteristics and the quality that meat exhibits during the aging time. A DFD meat, as observed in the current study, has few protein denaturation and high WHC within and among muscle cells, which provokes a packaging and storage of meat with few extracellular spaces (Warriss, 2000). The lack of intramuscular spaces limits the refractive index of light and the entrance of sufficient amount of oxygen to muscular tissue after unpacking, influencing the oxymyoglobin formation and the intensity of meat coloration (Dávila-Ramírez et al., 2013).

Finally, the TBA + E_2 implantation had a little effect on meat quality of fattening lambs, being a relevant aspect the decrease of redness. Probably, the hypertrophy of LT fibers caused a dilution in the muscle myoglobin content, which reflected a slight lower tonality of the meat redness color in SI-treated lambs (Garmyn & Miller, 2014).

5. Conclusions

Supplemental ZH and TBA + E_2 implant administered simultaneously in intact male lambs did not cause a better growth rate, feed efficiency, carcass characteristic and wholesale cut yields (absence of synergistic or additive effects). Supplementation of ZH alone to male lambs was effective to improve feedlot performance, carcass weight and dressing yield, without affecting wholesale cut yields and decreasing meat quality (harder and slightly dark meat). Contrarily, the sole application of TBA + E_2 implant during 63 d before slaughter was an ineffective strategy to improve growth performance and carcass characteristics of feedlot finishing male lambs. Finally, implanting TBA + E_2 to lambs fed ZH had a positive effect on muscle growth and meat tenderness in the LT muscle; and this finding can be indicative of a local synergistic effect between these growth promotants.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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References

Adzitey, F., & Nurul, H. (2011). Pale soft exudative (PSE) and dark firm dry (DFD) meats: Causes and measures to reduce these incidences-A mini review. *International Food Research Journal, 18*, 11–12.

Avendaño-Reyes, L., Macías-Cruz, U., Álvarez-Valenzuela, F., Águila-Tepato, E., Torrentera-Olivera, N., & Soto-Navarro, S. (2011). Effects of zilpaterol hydrochloride on growth performance, carcass characteristics, and wholesale cut yield of hair-breed ewe lambs consuming feedlot diets under moderate environmental conditions. *Journal of Animal Science, 89*, 4188–4194.

Avendaño-Reyes, L., Meraz-Murillo, F., Pérez-Linares, C., Figueroa-Saavedra, F., Correa, A., Álvarez-Valenzuela, F., ... Macías-Cruz, U. (2016). Evaluation of the efficacy of Grofactor, a beta-adrenergic agonist based on zilpaterol hydrochloride, using feedlot finishing bulls. *Journal of Animal Science, 94*, 2954–2961.

Avendaño-Reyes, L., Torrentera, N., Correa-Calderón, A., López-Rincón, G., Soto-Navarro, S., Rojo-Rubio, R., ... Macías-Cruz, U. (2018). Daily optimal level of a generic beta-agonist based on zilpaterol hydrochloride for feedlot hair lambs. *Small Ruminant Research, 165*, 48–53.

Baxa, T. J., Hutcheson, J. P., Miller, M. F., Brooks, J. C., Nichols, W. T., Streeter, M. N., ... Johnson, B. J. (2010). Additive effects of a steroidal implant and zilpaterol hydrochloride on feedlot performance, carcass characteristics, and skeletal muscle messeger ribonucleic acid abundace in finishing steers. *Journal of Animal Science, 88*, 330–337.

Brake, D., Titgemeyer, E., & Jones, M. (2011). Effect of nitrogen supplementation and zilpaterol-HCl on urea kinetics in steers consuming corn-based diets. *Journal of Animal Physiology and Animal Nutrition, 95*, 409–416.

Cannas, A., Tedeschi, L. O., Fox, D. G., Pell, A. N., & Van Soest, P. J. (2004). A mechanistic model for predicting the nutrient requirements and feed biological values for sheep. *Journal of Animal Science, 82*, 149–169.

Chemists, A. A., & Horwitz, W. (1990). *Official methods of analysis* (15th ed.). *Vol. I.* Arlington, VA: AOAC.

Dávila-Ramírez, J., Avendaño-Reyes, L., Macías-Cruz, U., Peña-Ramos, E., Islava-Lagarda, T., Zamorano-García, L., ... González-Ríos, H. (2017). Fatty acid composition and physicochemical and sensory characteristics of meat from ewe lambs supplemented with zilpaterol hydrochloride and soybean oil. *Animal Production Science, 57*, 767–777.

Dávila-Ramírez, J., Avendaño-Reyes, L., Macías-Cruz, U., Torrentera-Olivera, N. G., Zamorano-García, L., Peña-Ramos, A., & González-Ríos, H. (2013). Effects of zilpaterol hydrochloride and soybean oil supplementation on physicochemical and sensory characteristics of meat from hair lambs. *Small Ruminant Research, 114*, 253–257.

Dávila-Ramírez, J., Macías-Cruz, U., Torrentera-Olivera, N., González-Ríos, H., Soto-Navarro, S., Rojo-Rubio, R., & Avendaño-Reyes, L. (2014). Effects of zilpaterol hydrochloride and soybean oil supplementation on feedlot performance and carcass characteristics of hair-breed ram lambs under heat stress conditions. *Journal of Animal Science, 92*, 1184–1192.

Dayton, W., & White, M. (2014). Meat science and muscle biology symposium—Role of satellite cells in anabolic steroid-induced muscle growth in feedlot steers. *Journal of Animal Science, 92*, 30–38.

Deaver, D., Glass, J., Grieger, D., & Reeves, J. (1988). Effects of estradiol on secretion of LH, hypothalamic function and testicular development in bull calves. *Domestic Animal Endocrinology, 5*, 307–316.

Dikeman, M. (2007). Effects of metabolic modifiers on carcass traits and meat quality. *Meat Science, 77*, 121–135.

Dunshea, F. R., D'Souza, D. N., Pethick, D. W., Harper, G. S., & Warner, R. D. (2005). Effects of dietary factors and other metabolic modifiers on quality and nutritional value of meat. *Meat Science, 71*, 8–38.

FAO (2016). *The state of food insecurity in the world 2015. Meeting the 2015 international hunger targets: Taking stock of uneven progress. Joint FAO/who food standards Programme. 30th session, Astana, Kazakhstan.* Rome: Food and Agriculture Organization Publications.

Garmyn, A., Knobel, S., Spivey, K., Hightower, L., Brooks, J., Johnson, B., ... Yates, D. (2011). Warner-Bratzler and slice shear force measurements of 3 beef muscles in response to various aging periods after trenbolone acetate and estradiol implants and zilpaterol hydrochloride supplementation of finishing beef steers. *Journal of Animal Science, 89*, 3783–3791.

Garmyn, A., & Miller, M. (2014). Meat science and muscle biology symposium—Implant and beta agonist impacts on beef palatability. *Journal of Animal Science, 92*, 10–20.

Greyling, J., Kotze, W., Taylor, G., & Hagendijk, W. (1993). Effect of an anabolic steroid on body measurements in ram lambs. *Small Ruminant Research, 11*, 351–357.

Johnson, B., White, M., Hathaway, M., Christians, C., & Dayton, W. (1998). Effect of a combined trenbolone acetate and estradiol implant on steady-state IGF-I mRNA concentrations in the liver of wethers and the longissimus muscle of steers. *Journal of Animal Science, 76*, 491–497.

Kellermeier, J., Tittor, A., Brooks, J., Galyean, M., Yates, D., Hutcheson, J., ... Miller, M. (2009). Effects of zilpaterol hydrochloride with or without an estrogen-trenbolone acetate terminal implant on carcass traits, retail cutout, tenderness, and muscle fiber

diameter in finishing steers. *Journal of Animal Science, 87*, 3702–3711.
Kongsuwan, K., Knox, M. R., Allingham, P. G., Pearson, R., & Dalrymple, B. P. (2012). The effect of combination treatment with trembolone acetate and estradiol-17β on skeletal muscle expression and plasma concentrations of oxytocins in sheep. *Domestic Animal Endocrinology, 43*, 67–73.
Korn, K., Lemenager, R., Claeys, M., Waddell, J., Engstrom, M., & Schoonmaker, J. (2013). Supplemental vitamin D3 and zilpaterol hydrochloride. II. Effect on calcium concentration, muscle fiber type, and calpain gene expression of feedlot steers. *Journal of Animal Science, 91*, 3332–3340.
Lee, C. Y., Henricks, D. M., Skelley, G. C., & Grimes, L. W. (1990). Growth and hormonal response of intact and castrate male cattle to trenbolone acetate and estradiol. *Journal of Animal Science, 68*, 2682–2689.
Lee, Y. B., & Kim, Y. S. (1994). Muscle characteristics and meat tenderness of cimaterol-fed lambs. *Journal of Food Science, 59*, 33–37.
López-Carlos, M., Ramírez, R., Aguilera, S., Rodríguez, H., Aréchiga, C., & Méndez, L. (2012). Effect of the administration program of two beta-adrenergic agonists on growth performance, carcass, and meat characteristics of feedlot ram lambs. *Journal of Animal Science, 90*, 1521–1531.
López-Carlos, M., Ramírez, R., Aguilera-Soto, J., Aréchiga, C., Méndez-Llorente, F., Rodríguez, H., & Silva, J. (2010). Effect of ractopamine hydrochloride and zilpaterol hydrochloride on growth, diet digestibility, intake and carcass characteristics of feedlot lambs. *Livestock Science, 131*, 23–30.
López-Carlos, M., Ramírez, R., Aguilera-Soto, J., Plascencia, A., Rodríguez, H., Aréchiga, C., ... Gutierrez-Bañuelos, H. (2011). Effect of two beta adrenergic agonists and feeding duration on feedlot performance and carcass characteristics of finishing lambs. *Livestock Science, 138*, 251–258.
Macías-Cruz, U., Álvarez-Valenzuela, F., Soto-Navarro, S., Águila-Tepato, E., & Avendaño-Reyes, L. (2013). Effect of zilpaterol hydrochloride on feedlot performance, nutrient intake, and digestibility in hair-breed sheep. *Journal of Animal Science, 91*, 1844–1849.
Macías-Cruz, U., Avendano Reyes, L., Vicente Perez, R., Alvarez Valenzuela, F. D., Correa Calderon, A., Gonzalez Rios, H., ... Mellado, M. (2016). Growth and carcass characteristics of lambs finished with zilpaterol hydrochloride in grazing alfalfa. *Revista Mexicana de Ciencias Pecuarias, 7*, 243–252.
Macías-Cruz, U., Avendaño-Reyes, L., Álvarez-Valenzuela, F. D., Torrentera-Olivera, N. G., Meza-Herrera, C., Mellado-Bosque, M., & Correa-Calderón, A. (2013). Crecimiento y características de canal en corderas tratadas con clothidrato de zilpaterol durante primavera y verano. *Revista Mexicana de Ciencias Pecuarias, 4*, 1–12.
McClure, K., Solomont, M., & Loerch, S. (2000). Body weight and tissue gain in lambs fed an all-concentrate diet and implanted with trenbolone acetate or grazed on alfalfa. *Journal of Animal Science, 78*, 1117–1124.
Meraz-Murillo, F., Avendaño-Reyes, L., Pérez-Linares, C., Figueroa-Saavedra, F., Torres-Rodríguez, V., Guerra-Liera, J., ... Macías-Cruz, U. (2017). Feedlot performance, carcass characteristics and meat quality of zebu heifers supplemented with two β-adrenergic agonists. *Animal Production Science, 57*, 2125–2132.
Mersmann, H. J. (1998). Overview of the effects of β-adrenergic receptor agonists on animal growth including mechanisms of action. *Journal of Animal Science, 76*, 160–172.
Mills, S. (2002). Biological basis of the ractopamine response. *Journal of Animal Science, 80*, 28–32.
Montossi, F., Font-i-Furnols, M., del Campo, M., San Julián, R., Brito, G., & Sañudo, C. (2013). Sustainable sheep production and consumer preference trends: Compatibilies, contradictions, and unresolved dilemmas. *Meat Science, 95*, 772–789.
Neill, S., Unruh, J., Marston, T., Jaeger, J., Hunt, M., & Higgins, J. (2009). Effects of implanting and feeding zilpaterol hydrochloride on performance, carcass characteristics, and subprimal beef yields of fed cows. *Journal of Animal Science, 87*, 704–710.
Nourozi, M., Abazari, M., Raisianzadeh, M., Mohammadi, M., & ZareShahne, A. (2008). Effect of terbutaline and metaproterenol (two beta-adrenergic agonists) on performance and carcass composition of culled Moghani ewes. *Small Ruminant Research, 74*, 72–77.
NRC (1985). *Nutrient requeriments of sheep*. Washington, D.C., USA: National Academy Press.
NRC (2007). *Nutrient requirements of small ruminants: Sheep, goat, cervids, and new world camelids*. Washington, D.C., USA: National Academy Press.
Ortiz, B., Camacho, A., Villalba, N. E., Flores, L. R., Romo, J. A., Aguirre, J., ... Barajas, R. (2013). Efecto de la potencia de los implantes con zeranol o trenbolona + estradiol en la respuesta productiva de ovinos de pelo en engorda intensiva en clima caluroso. *Zootecnia Tropical, 31*, 71–77.
Parés-Casanova, P. M. (2013). Morphometric dimensions allow differentiation of lamb carcasses for some breeds. *Egyptian Journal of Sheep and Goat Sciences, 8*(1), 167–170.
Parr, S. L., Brown, T., Ribeiro, F., Chung, K. Y., Hutcheson, J. P., Blackwell, B., ... Johnson, B. J. (2014). Biological responses of beef steers to steroidal implants and zilpaterol hydrochloride. *Journal of Animal Science, 92*, 3348–3363.
Parr, S. L., Chung, K. Y., Galyean, M. L., Hutcheson, J. P., DiLorenzo, N. D., Hales, K. E., ... Johson, B. J. (2011). Performance of finishing beef steers in response to anabolic implant and zilpaterol hydrochloride supplementation. *Journal of Animal Science, 89*, 560–570.
Passantino, A. (2012). Steroid hormones in food producing animals: Regulatory situation in Europe. *Steroid hormones in food producing animals: A bird's-eye view of veterinary medicine*. InTech.
Preston, R. (1999). Hormone containing growth promoting implants in farmed livestock. *Advanced Drug Delivery Reviews, 38*, 123–138.
Priolo, A., Waghorn, G., Lanza, M., Biondi, L., & Pennisi, P. (2000). Polyethylene glycol as a means for reducing the impact of condensed tannins in carob pulp: Effects on lamb growth performance and meat quality. *Journal of Animal Science, 78*, 810–816.
Rivera-Villegas, A., Estrada-Angulo, A., Castro-Pérez, B., Urías-Estrada, J., Ríos-Rincón, F., Rodríguez-Cordero, D., ... Zinn, R. (2019). Comparative evaluation of supplemental zilpaterol hydrochloride sources on growth performance, dietary energetics and carcass characteristics of finishing lambs. *Asian-Australasian Journal of Animal Sciences, 32*, 209–216.
Rojo-Rubio, R., Avendaño-Reyes, L., Albarrán, B., Vázquez, J. F., Soto-Navarro, S. A., Guerra, J. E., & Macías-Cruz, U. (2018). Zilpaterol hydrochloride improves growth performance and carcass traits without affecting wholesale cut yields of hair sheep finished in feedlot. *Journal of Applied Animal Research, 46*, 375–379.
Sañudo, C., Santolaria, M., María, G., Osorio, M., & Sierra, I. (1996). Influence of carcass weight on instrumental and sensory lamb meat quality in intensive production systems. *Meat Science, 42*, 195–202.
Smith, G. C., & Griffin, D. B. (2001). *Meat evaluation handbook*. American Meat Science Association.
Sutton, D., Ellis, M., Lan, Y., McKeith, F., & Wilson, E. (1997). Influence of slaughter weight and stress gene genotype on the water-holding capacity and protein gel characteristics of three porcine muscles. *Meat Science, 46*, 173–180.
Van Soest, P. V., Robertson, J., & Lewis, B. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science, 74*, 3583–3597.
Walker, D., Titgemeyer, E., Baxa, T., Chung, K., Johnson, D., Laudert, S., & Johnson, B. (2010). Effects of ractopamine and sex on serum metabolites and skeletal muscle gene expression in finishing steers and heifers. *Journal of Animal Science, 88*, 1349–1357.
Warriss, P. (2000). *The effects of live animal handling on carcass and meat quality*. Cambridge, USA: Cabi, North American Office131–155.
Watanabe, A., Daly, C. C., & Devine, C. E. (1996). The effects of the ultimate pH of meat on terdernedd changes during ageing. *Meat Science, 42*, 67–78.
Webb, E. C., Allen, J., & Morris, S. (2018). Effects of non-steroidal growth implant and dietary zilpaterol hydrochloride on growth and carcass characteristics of feedlot lambs. *South African Journal of Animal Science, 48*, 601–608.
Wu, G., Farouk, M. M., Clerens, S., & Rosenvold, K. (2014). Effect of beef ultimate pH and large structural protein chenges with aging on meat tenderness. *Meat Science, 98*, 637–645.
Yasin, A., & Galbraith, H. (1981). A note on the response of wether lambs to treatment with trenbolone acetate combined with oestradiol-17β or zeranol. *Animal Science, 32*, 337–340.

3. MUSCLE FIBER MORPHOMETRY AND PHYSICOCHEMICAL CHARACTERISTICS OF THE LONGISSIMUS THORACIS MUSCLE OF HAIR MALE LAMBS FED ZILPATEROL HYDROCHLORIDE AND IMPLANTED WITH STEROIDS

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Abstract

Muscle fibers morphometry and physicochemical characteristics were evaluated in LT muscles obtained from entire male lambs treated with zilpaterol hydrochloride (ZH, 0 and 0.15 mg/kg body weight) and/or steroidal implant (SI, with and without trenbolone acetate/estradiol). ZH and SI acted synergistically to increase LT area, type-IIb fiber cross-sectional area and soluble collagen content, as well as to decrease metmyoglobin and insoluble collagen content. Ash content and ultimate pH showed to decrease by an antagonist effect between ZH and SI. Content of total collagen, protein, fat, moisture, oxidized lipids and water-holding capacity were unaffected by ZH and SI. Supplemental ZH, but not SI, decreased all color parameters and tended to increase shear force. Fatty acid profile was slightly modified by ZH only. Overall, the SI implantation to male lambs followed by a ZH supplementation promoted greater LT hypertrophy, without affecting protein and fat content, quality and fatty acid profile in their meat.

Keywords: Growth promotants, hair breed sheep, myofibrillar histochemical, collagen, fatty acid profile, DFD meat.

1. Introduction

The β_2-adrenergic agonists (β_2-AA) are synthetic growth promotants working as redistribution agents of body (i.e. fat and visceral tissue) and dietary nutrients to promote skeletal muscle hypertrophy, and improve carcass and live weight gain in fattening animals (Johnson, Smith, & Chung, 2014), including sheep (Cayetano-De-Jesus *et al.*, 2020; Avendaño-Reyes *et al.*, 2018; Hemmings, Daniel, Buttery, Parr, & Brameld, 2015). Zilpaterol hydrochloride (ZH) is a β_2-AA supplied in the finishing diet of fattening lambs to stimulate protein synthesis, lipolysis, myofibrillar glycolytic metabolism (type II muscle fiber), and glucose oxidation and hypertrophy

in muscle (Barnes *et al.*, 2019; Parr *et al.*, 2016; Johnson *et al.*, 2014). This in turn improves feedlot performance, carcass weight and *Longissimus thoracis* (LT) cross-sectional area (CSA; Cayetano-De-Jesus *et al.*, 2020; Avendaño-Reyes *et al.*, 2018) while decreasing internal body fat deposition (Dávila-Ramírez *et al.*, 2018; 2017) and concentration of n-3 polyunsaturated fatty acids (PUFA; Dávila-Ramírez *et al.*, 2017). However, the meat quality also decreases by supplementing ZH to feedlot lambs, since typically there is a tendency to produce dry, firm and dark (DFD) meat (López-Baca *et al.*, 2019; Dávila-Ramírez *et al.*, 2017). The low meat quality is because ZH supplementation maintains the high ultimate pH (> 5.8) by promoting muscle glycogen depletion before slaughter, and consequently, a low lactic acid synthesis and poor muscle acidification (Lee & Kim, 1994). In meat, this causes alterations in the calpain-calpastatin system, increased myoglobin oxidation (Metmyoglobin [MetMb]), discoloration, and increased *Warner-Bratzler* shear force (WBSF) and water holding capacity (WHC; Wu *et al.*, 2020; Ponnampalam *et al.*, 2017; Suman & Joseph, 2013; Strydom, Hope-Jones, Frylinck, & Webb, 2011).

Recently, a study demonstrated that the muscle hypertrophy effect due to supplemental ZH in male lambs may be potentiated by implanting them with trenbolone acetate (TBA) and 17β-estradiol (E_2) before and during the ZH supplementation period, without affecting meat quality (López-Baca *et al.*, 2019). The authors found that both growth promotants interacted synergistically to increase LT muscle area, but the steroidal implant (SI) acted as ZH antagonist to decrease ultimate pH into a normal range, avoiding changes in color parameters, WBSF and WHC. The mechanisms that led the implant to modulate the effects of supplemental ZH on muscle growth and meat quality in lambs are unknown. So, further studies of the combined effect of ZH and SI on muscle histochemical and meat physicochemical characteristics are required to understand their joint mode of action.

Overall, SI increases circulating concentrations of growth hormone and IGF-I, and IGF-I in turn stimulates satellite cell proliferation and differentiation into skeletal muscle to promote greater protein synthesis and low proteolysis (Smith & Johnson, 2020; Parr *et al.*, 2014). High activity of satellite cells in muscle motivates the collagen synthesis as part of the myofribrillar growth and remodeling (Fry, Kirby, Kosmac, McCarthy, & Peterson, 2017; Fry *et al.*, 2014), and then collagen content could partially explain changes in the meat tenderness of sheep (Starkey, Geesink, Oddy, & Hopkins, 2015; Purslow, 2005). The SI is also known to increase both expression and number of β_2-adrenergic receptors (β_2-AR), and this is likely how implant enhances the hypertrophic effects of ZH (Harris *et al.*, 2020; Parr *et al.*, 2014). In feedlot heifers, ZH+SI treatment increased significantly CSA without altering proportion of all muscle fiber types; however, this was positively associated with WBSF but not with ultimate pH (Ebarb *et al.*, 2016; Kellermeier *et al.*, 2009). Therefore, the objective was to evaluate the effects of ZH supplementation and implantation of steroidal hormones in feedlot male lambs on area, myofibrillar morphometry, collagen content, proximate composition, lipid and myoglobin oxidation, fatty acid profile and meat quality of the LT muscle.

2. Materials and methods

The Research Ethics Committee of the Centro de Investigación en Alimentación y Desarrollo A.C. (CIAD A.C.), in Hermosillo campus, Sonora, Mexico, evaluated and approved all methodologies used (ID: CE/021/2018). Growth promotants were authorized only for experimental use.

2.1. Animals and experimental design

This study was carried out with samples of the LT muscles obtained from experimental sheep used in the article published by López-Baca *et al.* (2019). In consequence, animal characteristics, management procedures, experimental design, and treatments were similar. In brief, 40 Dorper ×

Pelibuey crossbred intact male lambs (initial body weight [BW] = 35 ± 0.2 kg and age= 4.5 months) were housed in individual pens to develop a 63-d feedlot trail. Lambs were assigned under a 2^2 factorial arrangement in a randomized complete block design to four treatment combinations (n= 10) of growth promotants: 1) without SI and ZH, 2) with SI but without ZH, 3) without SI but with ZH and 4) with SI and ZH. The applied SI had into three pellets 52.5 mg TBA and 7.5 mg E_2 (Revalor®, Intervet Laboratory, Mexico), and was administered at the middle third of the ear from the first day of test until the end (d 63). The ZH was offered orally at a dose of 0.15 mg/kg of BW/d (Grofactor®, Virbac Laboratory, Mexico) before the morning feeding between days 31 and 60 of trial, with a 3-d withdrawal period before slaughtering by exsanguination of animals. Lambs were fed a basal total mixed diet that meet NRC requirements for finishing phase (NRC, 2007; metabolizable energy= 11.7 MJ/kg dry matter and crude protein= 16 %). The diet was formulated with 54 % wheat milled, 12 % soybean meal, 2.5 % soybean oil, 14 % alfalfa hay, 16 % wheat straw and 1.5 % mineral. The availability of feed and water was ad libitum during entire experimental period.

2.2. Collection of samples

After slaughter, muscle samples of LT (approximately 5 g from 10^{th} intercostal space of right carcass) were taken approximately 5 min *postmortem* to be rapidly collocated by duplicate in vials, frozen in isopentane and submerged in liquid nitrogen. These samples were stored at - 80 °C ultra-cold freezer until analysis of muscle fiber histology. Note that the carcasses of lambs of the blocks 2, 4, 6, 7 and 9 were selected to collect tissue samples (n= 5/treatment combination). Then, 40 carcasses were cooled at 4 °C for 24 h to dissect the LT muscle from loin portion located between the 4^{th} and 12^{th} rib. Muscles were individually vacuum packaged and stored at - 20 °C, and then transported along with tissue samples to the Meat Science and Technology Laboratory of the CIAD

A.C., for analysis. All measurements in meat were conducted immediately after the LT muscle was thawed.

2.3. LT area and fiber histochemical

The LT muscle area was measured before its vacuum packaged with a dot square grid of 64 mm^2. For histochemical, muscle samples were changed of freezing temperature from - 80 to - 20 °C during the 24 h prior to sectioning them with a Leica CM-1100 cryostat (Leica Microsystems, Nussloch, Germany). Four cryosections (10 μm thick) of every sample were cut, and then fixed by pairs on Superfrost Microscope slides (n= 2 slides /sample; Fisher Scientific, Waltham, MA). Slides were subjected to the staining procedure for nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) activity (Ogata, 1958). Slides were observed across an inverted phase contrast microscope (Eclipse TS100-F, Nikon, Melville, USA), equipped with a digital camera (Model SCA-EA05, Swiftcam, HK) and connected to a computer. In each slide, the two cryosections were reviewed and one of them (better tissue fixation and staining) was selected to take three random images with the 40X objective. Images were processed in the Image J version 1.45s software to measure histomorphometric variables of approximately 700 fibers per animal. Cross-sectional area and perimeter of muscle fibers were measured. Fibers were also classified as type I (intense dark color), IIa (moderate dark color) and IIb (weak dark color) according to their coloration and following the methodology described by de Freitas, Freitas, Lopes, Pai-Silva, & Piçarro, 2009). Thus, fiber type composition was calculated by expressing the number of each fiber type as a percentage of the total number of fibers analyzed.

2.4. Meat quality

The physical evaluation of the meat was conducted following the same methodology described by López-Baca *et al.* (2019), including measured variables (i.e. ultimate pH, color, cooking loss, WBSF, and WHC), meat handling procedures, measuring equipment and repetitions per sample. In brief, LT samples were thawed during 48 h at - 10 °C the first 24 h, and at 0 °C the last 24 h, before meat evaluation. Ultimate pH was measured on a homogeneous mixture of meat (5 g) with distilled water (25 mL) using a pH meter for liquids (HI-2210, Hanna Instruments, RI). For color, the meat was bloomed for 30 min and then a colorimeter (CR-400, Konica Minolta Sensing, Japan) was put on its surface to measure lightness (L*), redness (a*) and yellowness (b*), using the Hunter-Lab procedure. These color data were used to calculate chroma (C*) and hue angle (H*) (Priolo *et al.*, 2000). For cooking loss, one steak per animal was weighed before and after cooking up to 71 °C of internal temperature, and the difference of weights was expressed as a percentage of the steak weight before cooking. Same cooked steak was chilled (4 °C for 24 h) and next cut as prismatic pieces of 1.27 cm of diameter to measure WBSF with a texture analyzer (TA. XT *plus* Texture Analyzer, Texture Technologies Corp, NY, USA). The WHC was measured according to the procedure of Sutton, Ellis, Lan, McKeith, & Wilson (1997).

2.5. Proximate analysis and collagen content

The proximate evaluation of the meat considered dry matter, moisture (950.46), protein (Kjeldahl method; 920.123), lipid (920.39) and ash (920.153), and these analyzes were developed with standardized procedures (AOAC, 2000). For its part, the total collagen content was determined using the standardized procedure 990.26 (AOAC, 2000), while soluble collagen content was determined with the procedure described by Starkey, Geesink, Oddy, & Hopkins (2015), which is the AOAC procedure 990.26 (AOAC, 2000) with some modifications. Total and soluble collagen content was registered as µg OH-pro/g of muscle. Insoluble collagen content was calculated by

difference between total and soluble collagen. Both soluble and insoluble collagen contents were expressed as a percentage of the total collagen content.

2.6. Lipid oxidation and metmyoglobin

Lipid oxidation was assessed using the technique of thiobarbituric acid reactive substances (TBARS; Witte, Krause, & Bailey, 1970), which determines lipid oxidation by measuring the malondialdehyde concentration (MDA). In brief, 10 g of meat and 20 mL trichloroacetic acid (TCA) were homogenized with Ultra-Turrax mixer (T25 digital, IKA®, Staufen, DEU) at 1100 rpm for one minute within 25-mL centrifuge tube placed on ice, which then was centrifuged at 2300 × *g* for 30 min at 5 °C, and finally filtered with Whatman no. 42 filter paper. A mixture of 2.0 mL of filtrate and 2.0 mL of TBARS were incubated in water bath for 20 min at 97 °C and, after cooling at room temperature, the absorbance of the mixture was measured with a 531 nm wavelength using a spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Penang, MY). From this absorbance, MDA milligrams per kilogram of meat were calculated.

The metmyoglobin (MetMb) technique was performed according to the methodology of Stewart, Hutchins, Zipser, & Watts (1965). Briefly, 5 g of meat and 20 mL phosphate buffer (40 mM and pH = 6.8) were homogenized with Ultra-Turrax mixer (T25 digital, IKA®, Staufen, DEU) at 1300 rpm for 30 s, and then centrifuged for 28000 × *g* for 30 min at 4 °C. After centrifugation, the mixture was filtered using Whatman no.1 filter paper and a sample of the filtering was used to measure absorbance with 700, 572 y 525 nm wavelength using a spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Penang, MY). MetMb percentage was calculated from absorbance.

2.7. Fatty acid profile

Initially, the lipid fraction from meat was separated using the procedure described by Bligh & Dyer (1959), where a meat sample is treated with a mixture of chloroform and methanol (v/v, 2:1). Then, the lipid fraction was methylated to obtain fatty acid methyl esters (FAME) according to the method of Park & Goins (1994). Measurements of FAME were developed by gas chromatography (6890 series, Hewlett Packard, California, USA), which had an Omegawax® 250 capillary GC column (30 m × 0.25 mm × 0.25 μm) and a flame ionization detector (FID). Injector and FID temperatures were set at 260 °C and helium was used as a carrier gas at a constant flow of 0.8 mL/min. The column temperature was programmed to increase 5 °C/min, starting at 150 °C and ending at 220 °C. Individual fatty acids were identified by comparing its retention time with an external standard (FAME mix C4-C24 component, Supelco, Missouri, USA). The quantification was performed using tridecanoic acid (C13:00; Sigma-Aldrich, Missouri, USA) as an internal standard. Fatty acid concentrations in meat were reported in mg/100 mg FAME detected. In addition, fatty acid concentrations classified as saturated (SFA), monounsaturated (MUFA), n-3, n-6 and polyunsaturated (PUFA) were calculated. Rations of PUFA:SFA, MUFA:SFA and n-6:n-3 were also calculated, likewise the atherogenic index (AI= C12:0+C14:0+C16:0/MUFA+PUFA).

2.8. Statistical analysis

Statistical analysis was performed using different procedures of the SAS program (SAS Inst. Inc., Cary NC). Initially, all data were subjected to normality analysis with the PROC UNIVARIATE, and then they were analyzed for analysis of variance under a 2^2 factorial arrangement in a randomized complete block design with the PROC MIXED, where main factors were ZH supplementation and SI implantation, and blocking factor was initial BW. A Tukey test was used to compare means declaring differences at $P \leq .05$ and trend at $.05 < P \leq .10$.

3. Results

3.1. LT area, fiber morphometry, collagen and proximate analysis

In LT area and fiber morphometry, the ZH × SI interaction affected ($P \leq .02$) LT area, type IIb fiber percentage, and CSA and perimeter of all fiber types (Table 1). The LT area was greater ($P < .01$) with ZH+SI administration than with any other treatment combination, with no differences ($P \geq .20$) among groups treated as control, ZH alone and SI alone. Percentages of type I and type IIa fibers did not change ($P \geq .18$) with the individual or combined application of ZH and SI, but percentage of type IIb fibers was greater ($P = .02$) with supplemental ZH alone than with any other treatment combination. Perimeter and CSA for type I fibers were smaller ($P \leq .01$) applying ZH or SI individually compared to control, but increased to a similar level ($P = .14$) as control when growth promotants were administered in combination (ZH+SI). Results of CSA and perimeter for type IIa fibers due to ZH or SI were completely opposite to those observed for type I fibers, while control and ZH+SI had similar ($P = 0.47$) size of type IIa fibers. Relative to type IIb fibers, CSA and perimeter were smaller ($P < .01$) with SI application only and similar ($P = .35$) with supplemental ZH alone compared to control, but ZH+SI administration caused the largest ($P < .01$) size of this type of fibers.

The ZH × SI interaction also affected ($P = .01$) soluble and insoluble collagen percentage, while total collagen content was not affected ($P \geq .29$) by neither the interaction nor the main factors (Table 1). Soluble collagen percentage in control was greater ($P < .01$) than in feeding ZH alone, but lower ($P < .01$) than with SI application only, although ZH+SI administration caused the highest ($P < .01$) soluble collagen percentages. For insoluble collagen percentage, results were completely opposite to those obtained for soluble collagen percentage.

The percentages of protein, fat and moisture in the LT muscle (Table 1) were not modified by the main factors or their interaction ($P \geq .13$). However, the ZH × SI interaction tended to affect ash

percentage (P = .07). Ash percentage was lower (P < .01) by supplementing ZH both with and without SI compared to control and SI application only. Overall, the highest (P < .01) ash percentage was observed with the SI placement only.

3.2. Physicochemical characteristics and fatty acid profile

There were no effects (P = .80) of the ZH × SI interaction, nor by the individual administration of ZH or SI ($P \geq .71$), on lipid oxidation (MDA; Table 2). MetMb percentage tended (P = .06) to change due to the ZH × SI interaction, being lower ($P \leq .03$) in control than in any other treatment combination (Fig. 1). In addition, MetMb percentage was similar ($P \geq .39$) with separated application of ZH and SI, but greater (P = .02) in ZH application alone than in ZH+SI administration. The ZH × SI interaction modified (P = .03) ultimate pH without affecting ($P \geq .30$) any other physicochemical traits (Table 2). Ultimate pH changed (P < .01) only by administering ZH alone, being higher than in any other treatment combination (Fig. 2). Based on main factors, ZH supplementation decreased ($P \leq .04$) color parameters (L*, a*, b*, H* and C*) and tended (P = .08) to increase WBSF, without affecting ($P \geq .44$) cooking loss and WHC. No physicochemical parameter was affected ($P \geq .13$) by SI placement.

Neither ZH × SI interaction ($P \geq .11$) nor SI alone ($P \geq .18$) affected the fatty acid profile (Table 3). Supplementation with ZH alone caused a trend ($.07 \leq P \leq .09$) to decrease concentrations of C10:0, C18:2CIS, C20:3n-6 and total PUFA, as well as PUFA:SFA ratio. There were no effects ($P \geq .12$) of ZH supplementation on individual concentration of any other fatty acid, or total concentrations of SFA, MUFA, n-3 and n-6. The MUFA:SFA and n6:n3 ratios, and the AI were also unaffected ($P \geq .55$) by administrating ZH alone.

4. Discussion

4.1. LT area, fiber morphometry, collagen and proximate analysis

Both supplemental ZH and SI containing TBA+E_2 cause increased protein accretion and low proteolysis, which in turn leads to a myofibrillar remodeling and skeletal muscle hypertrophy (Ebarb *et al.*, 2016). In hair breed sheep, there are no studies describing the changes in the muscle fibers due to ZH supplementation and/or SI application, notwithstanding that both growth promotants have evidenced to increase LT area, suggesting muscle hypertrophy (Avendaño-Reyes *et al.*, 2018; Galbraith, Singh, & Scaife, 1997). Note that this beneficial effect of ZH and SI on LT area is not consistent in all studies, as there are works reporting no effect for sheep (Macías Cruz *et al.*, 2016; McClure, Solomont, & Loerch, 2000). The latter agrees with our results, since individual administration of ZH or SI did not caused changes in LT area. Despite this, both growth promotants acted synergistically to increase LT area.

According to our results of fiber histochemical and collagen content, both fiber morphometry and collagen solubility changed depending on the growth promotant administered, which explains findings observed for LT area. Apparently, SI-treated lambs maintained a similar LT area compared to control and ZH lambs, because they had a greater soluble collagen synthesis but not due to beneficial changes in size and proportion of the different muscle fiber types. In fact, SI application decreased the type I and IIb muscle fiber CSA by approximately 5%, and this could have caused a smaller LT area if the soluble collagen content was low. Soluble collagen is a less compacted protein than insoluble collagen, so it can increase muscle volume (Purslow, 2005). For its part, ZH-fed lambs did not alter its LT area as β_2-AA did not modify the size of type IIb muscle fibers and increased in 3.5 % the size of type IIa fibers. In the lamb LT muscle predominant glycolytic fibers, mostly type IIb fibers that are larger than any other muscle fiber type (Starkey, Geesink, Oddy, & Hopkins, 2015); so, variations in size and amount of type IIb fibers can be

reflected more evidently in the skeletal muscle development of this specie (Parr *et al.*, 2016). Remarkably, lambs treated simultaneously with both growth promotants were the only ones that increased its LT area, which was associated with a rise in the size of type IIb muscle fibers, as well as to a greater secretion of soluble collagen.

The cattle implantation with TBA and E_2 increases local production of IGF-I in skeletal muscle, which in turn triggers both proliferation and differentiation of satellite cells into muscle fibers to increase DNA availability and protein synthesis (Smith & Johnson, 2020). In addition, this implant has shown to increase the β_2-AR quantity in β_2-AA fed cattle (Harris *et al.*, 2020; Parr *et al.*, 2014). Based in the aforementioned, we suggested in a previous study (López-Baca *et al.*, 2019) that combined treatment ZH+SI increased LT area because these growth promotants worked in synergy to promote a greater muscle hypertrophy, particularly SI increased the availability of β_2-AR and this potentiated the ZH effects for protein synthesis and greater size in muscle fibers. In the current study, β-AR population and expression were not measured, however, the increase in type IIb fiber CSA and perimeter with ZH+SI application compared to control or individual application of ZH or SI could support this hypothesis. It is well documented that feeding ZH favors a predominant muscle glycolytic metabolism, so this agonist remodels the muscle to increase proportion and size of fast fibers (type II; Hemmings, Daniel, Buttery, Parr, & Brameld, 2015), manly fast glycolytic fibers (type IIb) as they are highly receptive to anabolic stimuli of ZH (Parr *et al.*, 2016). Note that the high amount of soluble collagen observed in ZH+SI treatment also contributed to improve LT area, and its high availability is a strong evidence that there was increased activity of satellite cells by offering together ZH and SI. Satellite cells encourage the appearance of myogenic progenitor cells (MPC) within the myofibrillary extracellular matrix, which is composed primarily of collagen secreted by fibrogenic cells (Fry *et al.*, 2014). The MPC are responsible to regulate the secretory activity of fibrogenic cells through secreting exosomes containing miR-206, which represses

Rrbp1, a master regulator of collagen biosynthesis (Fry *et al.*, 2017). Overall, our results suggest that, in hair male lambs, ZH and SI interacted synergistically to improve size of type IIb muscle fibers, soluble collagen synthesis and, consequently, LT area (skeletal muscle hypertrophy). However, this was not reflected in changes for protein, fat and moisture content in the LT muscle of lambs.

We do not have a clear explanation for proximate analysis results; however, we believe that the hypertrophy promoted by the synergistic effect of ZH and SI on LT was not enough to make a difference in its protein, fat and moisture content. However, as in this study, ZH and SI in cull cows acted synergistically to increase LT area without affecting protein, fat and moisture content of this muscle (Neill *et al.*, 2009). Interestingly, there was an antagonist effect between ZH and SI on LT ash content, since the ash content increased with SI alone and decreased with the combination (ZH+SI). Coelho, Galbraith, & Topps (1981) also reported greater ash content in lamb carcass by implanting TBA+E_2, and attributed this effect to an increase in calcium, phosphorus, and magnesium content. Whereas the LT ash content did not change in cull cows implanted and fed ZH (Neill *et al.*, 2009). Recently, a study reported that ash content in beef meat has a high positive correlation with potassium content, but low relationship with calcium content (Patel, Bergamaschi, Magro, Petrini, & Bittante, 2019). Potassium is the mineral most abundant in meat, and both potassium and calcium are essential elements for the formation of muscle mass (NRC, 2007). In steers, ZH has shown to decrease parathyroid hormone synthesis (Strydom *et al.*, 2011) and to regulate calcium influx into LT muscle cells, which together with the high intake of intracellular calcium causes a decrease in muscle calcium content (Korn *et al.*, 2013). Some β_2-AA different to ZH have also been associated with a decrease in plasma potassium concentration (Aliaga, Arizon, Bermúdez, Castán, & Santandreu, 2020). Therefore, it was believed that the antagonist effect of

ZH on SI for LT ash content is the result of a muscle hypokalemia and hypocalcemia caused by the β_2-AA.

4.2. Physicochemical characteristics and fatty acid profile

This is the first study evaluating the effects of ZH and SI on lipid and myoglobin oxidation in sheep. In cattle, available information in this regard is also scarce. Here, no growth promotants altered LT lipid oxidation in hair male lamb, and this could be because the fatty acid profile experienced minimal changes due to supplemental ZH and no changes due to the implant. It is widely documented that meat oxidation is high correlated (positive) with PUFA content (Domínguez *et al.*, 2019). Feeding steers with ZH has also showed no modification of fat oxidation (Hansen, Frylinck, & Strydom, 2012).

The ZH × SI interaction suggests a synergistic effect between promotants to decrease MetMb percentage in LT muscle. This could have been caused by the change in myofibrillar metabolism from oxidative to predominantly glycolytic (Suman & Joseph, 2013), which was evident as LT muscle from ZH+SI had type IIb fibers with greater CSA and perimeter than LT muscle from control or individual administration of SI or ZH. The ype IIb muscle fibers are characterized by having high glycogen content, low blood supply and low myoglobin, and in consequence, they present the highest glycolytic-anaerobic metabolic capacity. In contrast, type I muscle fibers, and partially type IIa fibers, exhibit a higher oxidative-aerobic metabolism as they have great amount myoglobin, mitochondrial activity and oxygen (Parr *et al.*, 2016); however, mitochondria compete for oxygen with oxymyoglobin, causing a deoxygenation of myoglobin and an increase in MetMb levels (Wu *et al.*, 2020).

It is important pointing out that all changes observed in muscle fiber development (metabolism), collagen and MetMb content, and ultimate pH by the ZH × SI interaction were not reflected on

meat quality. The fact that ZH fed lambs were previously implanted with TBA+E2 led to a normal ultimate pH (5.4 to 5.8; Ponnampalam *et al.*, 2017) in LT meat, while lambs fed only ZH recorded an ultimate pH slightly above the normal range (5.87). Currently, it is known that feeding β_2-AA in sheep increases muscle glycogen depletion before slaughter (Lee & Kim, 1994), and this could reduce both lactic acid production and *postmortem* muscle acidification, favoring the presence of a high ultimate pH in the meat (pH > 5.8; Ponnampalam *et al.*, 2017). Therefore, our results suggest that SI application before and during the ZH supplementation to male lambs could regulate muscle glycogen depletion before slaughter. Therefore, this could have practical implications, given that ZH is associated with poor quality meat production.

Regardless of the SI application which did not affected the meat quality, LT meat from ZH fed lambs had decreased color values (L*, a*, b*, H* and C*) and a tendency to be tough, i.e. meat with tendencies to be DFD. These results are explained by the high ultimate pH observed in ZH meat. Ponnampalam *et al.* (2017) mentioned that an ultimate pH > 5.8 is associated with alterations in color (high myoglobin oxidation), WHC and WBSF (high calpastatin and low calpain), leading to the production of DFD meat. In this study, insoluble collagen content increased by ZH effect, which could also be associated with an increase in meat toughness (Purslow, 2005). Similar findings for WBSF were reported in hair ewe and male lambs by including ZH in finishing diet (Cayetano-De-Jesus *et al.*, 2020; Dávila-Ramírez *et al.*, 2017). Note that feeding ZH did not affect WHC, whereas previous studies found increased WHC due to ZH supplementation in hair lambs (Cayetano-De-Jesus *et al.*, 2020; López-Baca *et al.*, 2019). This discrepancy in WHC could be due to a higher ultimate pH observed in those published studies (> 5.9). As meat pH approaches 5.5, the myofibrillar proteins are closer to their isoelectric points and the net charges will equalize, reducing the meat capacity to hold water (Ponnampalam *et al.*, 2017).

Supplemental ZH alone, but not SI alone, marginally modified the concentration of some fatty acids in meat; in particular, ZH tends to decrease contents of C10:0, C18:2CIS, C20:3n-6 and total PUFA. Similarly, Dávila-Ramírez *et al.* (2017) found in hair ewe lambs that ZH slightly decreased concentrations of some PUFA in LT muscle, but these same authors did not report changes due to ZH using heat-stressed male lambs (Dávila-Ramírez *et al.*, 2018). Here, both ZH and SI did not alter body fat deposition and lipid oxidation, and in consequence, the fatty acid profile had minimal changes. It is worth mentioning that meat is classified as healthy for human consumption if PUFA:SFA ratio is within 0.45-0.65, n6:n3 ratio has a 5:1maximum value (Razminowicz, Kreuzer, & Scheeder, 2006) and atherogenicity index is < 1.0 (Pilarczyk & Wojcik, 2015). Our meat meets these parameters and, therefore, both ZH and SI are two growth promotants that can be used to produce healthy lamb meat based on its lipid profile.

5. Conclusions

In conclusion, the combined administration of TBA+E_2 implants with supplemental ZH in hair male lambs was more effective in triggering a hypertrophy into LT muscle than individual administration of ZH or SI. This was associated to a synergic effect between growth promotants, which helped to increase type IIb muscle fiber size (CSA and perimeter) and soluble collagen content. Despite this, muscle protein, fat and moisture content did not change by applying any growth promotant. Meat quality and fatty acid profile were affected only by ZH supplementation, causing a tendency to produce DFD meat with minimal changes in the concentration of any fatty acids. Finally, regardless of the application of growth promoters, the lamb meat had a fatty acid profile that could be considered healthy for consumers.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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References

Aliaga, C. A., Arizon, L. F. de, Bermúdez, R. M., Castán, J. A. B., & Santandreu, A. V. (2020). Severe hypokalemia secondary to abuse of β-adrenergic agonists in a pediatric patient: Case report. *Brazilian Journal of Nephrology*, *42*(2), 250–253. https://doi.org/10.1590/2175-8239-jbn-2019-0020

AOAC. (2000). *Official methods of analysis of A.O.A.C. international; agriculture, chemical, contaminant, drugs* (17a ed.). Maryland, USA.

Avendaño-Reyes, L., Torrentera-Olivera, N. G., Correa-Calderón, A., López-Rincón, G., Soto-Navarro, S. A., Rojo-Rubio, R., … Macías-Cruz, U. (2018). Daily optimal level of a generic beta-agonist based on zilpaterol hydrochloride for feedlot hair lambs. *Small Ruminant Research*, *165*, 48–53. https://doi.org/10.1016/j.smallrumres.2018.06.014

Barnes, T. L., Cadaret, C. N., Beede, K. A., Schmidt, T. B., Petersen, J. L., & Yates, D. T. (2019). Hypertrophic muscle growth and metabolic efficiency were impaired by chronic heat stress, improved by zilpaterol supplementation, and not affected by ractopamine supplementation in feedlot lambs1. *Journal of Animal Science*, *97*(10), 4101–4113. https://doi.org/10.1093/jas/skz271

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911–917. https://doi.org/10.1139/o59-099

Cayetano-De-Jesus, J. A., Rojo-Rubio, R., Grajales-Lagunes, A., Avendaño-Reyes, L., Macias-Cruz, U., Gonzalez-del-Prado, V., … Lee-Rangel, H. A. (2020). Effect of zilpaterol hydrochloride on performance and meat quality in finishing lambs. *Agriculture*, *10*(6), 241. https://doi.org/10.3390/agriculture10060241

Coelho, J. F. S., Galbraith, H., & Topps, J. H. (1981). The effect of a combination of trenbolone acetate and oestradiol-17β on growth performance and blood, carcass and body characteristics of wether lambs. *Animal Science*, *32*(3), 261–266. https://doi.org/10.1017/S000335610002715X

Dávila-Ramírez, J. L., Avendaño-Reyes, L., Macías-Cruz, U., Peña-Ramos, E. A., Islava-Lagarda, T. Y., Zamorano-García, L., … González-Ríos, H. (2017). Fatty acid composition and physicochemical and sensory characteristics of meat from ewe lambs supplemented with zilpaterol hydrochloride and soybean oil. *Animal Production Science*, *57*(4), 767. https://doi.org/10.1071/AN15311

Dávila-Ramírez, J. L., Avendaño-Reyes, L., Peña-Ramos, E. A., Islava-Lagarda, T. Y., Macías-Cruz, U., Torrentera-Olivera, N. G., … González-Ríos, H. (2018). Impact of zilpaterol hydrochloride and soybean-oil supplementation on intramuscular fat, fatty acid profile and

cholesterol concentration in the longissimus muscle of male hair lamb under moderate heat-stress conditions. *Animal Production Science*, *58*(10), 1932. https://doi.org/10.1071/AN16747

de Freitas, C. E. A., Freitas, S. de B. Z., Lopes, F. da S., Pai-Silva, M. D., & Piçarro, I. da C. (2009). Skeletal muscles with antagonistic muscular actions: Morphological, contractile and metabolic characteristics. *International Journal of Morphology*, *27*(4), 1173–1178. https://doi.org/10.4067/S0717-95022009000400034

Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants*, *8*(10), 429. https://doi.org/10.3390/antiox8100429

Ebarb, S. M., Drouillard, J. S., Maddock-Carlin, K. R., Phelps, K. J., Vaughn, M. A., Burnett, D. D., … Gonzalez, J. M. (2016). Effect of growth-promoting technologies on Longissimus lumborum muscle fiber morphometrics, collagen solubility, and cooked meat tenderness12. *Journal of Animal Science*, *94*(2), 869–881. https://doi.org/10.2527/jas.2015-9888

Fry, C. S., Kirby, T. J., Kosmac, K., McCarthy, J. J., & Peterson, C. A. (2017). Myogenic progenitor cells control extracellular matrix production by fibroblasts during skeletal muscle hypertrophy. *Cell Stem Cell*, *20*(1), 56–69. https://doi.org/10.1016/j.stem.2016.09.010

Fry, C. S., Lee, J. D., Jackson, J. R., Kirby, T. J., Stasko, S. A., Liu, H., … Peterson, C. A. (2014). Regulation of the muscle fiber micro environment by activated satellite cells during hypertrophy. *The FASEB Journal*, *28*(4), 1654–1665. https://doi.org/10.1096/fj.13-239426

Galbraith, H., Singh, S. B., & Scaife, J. R. (1997). Response of castrated male sheep to oestrogenic and androgenic compounds implanted alone or in combination. *Animal Science*, *64*(2), 261–269. https://doi.org/10.1017/S1357729800015824

Hansen, S., Frylinck, L., & Strydom, P. E. (2012). The effect of vitamin D3 supplementation on texture and oxidative stability of beef loins from steers treated with zilpaterol hydrochloride.

Meat Science, *90*(1), 145–151. https://doi.org/10.1016/j.meatsci.2011.06.014

Harris, T. L., Smith, Z. K., Ribeiro, F. R. B., Jennings, M. A., Vogel, G. J., & Johnson, B. J. (2020). Ractopamine hydrochloride and estradiol + trenbolone acetate implants alter myogenic mRNA, β-adrenergic receptors, and blood metabolites. *Open Journal of Animal Sciences*, *10*(03), 447–467. https://doi.org/10.4236/ojas.2020.103028

Hemmings, K. M., Daniel, Z. C. T. R., Buttery, P. J., Parr, T., & Brameld, J. M. (2015). Differential effects of short-term β agonist and growth hormone treatments on expression of myosin heavy chain IIB and associated metabolic genes in sheep muscle. *Animal*, *9*(2), 285–294. https://doi.org/10.1017/S175173111400233X

Johnson, B. J., Smith, S. B., & Chung, K. Y. (2014). Historical overview of the effect of β-adrenergic agonists on beef cattle production. *Asian-Australasian Journal of Animal Sciences*, *27*(5), 757–766. https://doi.org/10.5713/ajas.2012.12524

Kellermeier, J. D., Tittor, A. W., Brooks, J. C., Galyean, M. L., Yates, D. A., Hutcheson, J. P., … Miller, M. F. (2009). Effects of zilpaterol hydrochloride with or without an estrogen-trenbolone acetate terminal implant on carcass traits, retail cutout, tenderness, and muscle fiber diameter in finishing steers1. *Journal of Animal Science*, *87*(11), 3702–3711. https://doi.org/10.2527/jas.2009-1823

Korn, K. T., Lemenager, R. P., Claeys, M. C., Waddell, J. N., Engstrom, M., & Schoonmaker, J. P. (2013). Supplemental vitamin D3 and zilpaterol hydrochloride. II. Effect on calcium concentration, muscle fiber type, and calpain gene expression of feedlot steers1. *Journal of Animal Science*, *91*(7), 3332–3340. https://doi.org/10.2527/jas.2012-5962

Lee, Y. B., & Kim, Y. S. (1994). Muscle characteristics and meat tenderness of cimaterol-fed lambs. *Journal of Food Science*, *59*(1), 33–37. https://doi.org/10.1111/j.1365-2621.1994.tb06891.x

López-Baca, M. Á., Contreras, M., González-Ríos, H., Macías-Cruz, U., Torrentera, N., Valenzuela-Melendres, M., … Avendaño-Reyes, L. (2019). Growth, carcass characteristics, cut yields and meat quality of lambs finished with zilpaterol hydrochloride and steroid implant. *Meat Science*, *158*, 107890. https://doi.org/10.1016/j.meatsci.2019.107890

Macías Cruz, U., Avendaño Reyes, L., Vicente Pérez, R., Álvarez Valenzuela, F. D., Correa Calderón, A., González Ríos, H., … Mellado, M. (2016). Crecimiento y características de la canal de corderos finalizados con clorhidrato de zilpaterol en pastoreo de alfalfa. *Revista Mexicana de Ciencias Pecuarias*, *7*(2), 243. https://doi.org/10.22319/rmcp.v7i2.4177

McClure, K. E., Solomont, M. B., & Loerch, S. C. (2000). Body weight and tissue gain in lambs fed an all-concentrate diet and implanted with trenbolone acetate or grazed on alfalfa. *Journal of Animal Science*, *78*(5), 1117. https://doi.org/10.2527/2000.7851117x

Neill, S., Unruh, J. A., Marston, T. T., Jaeger, J. R., Hunt, M. C., & Higgins, J. J. (2009). Effects of implanting and feeding zilpaterol hydrochloride on performance, carcass characteristics, and subprimal beef yields of fed cows12. *Journal of Animal Science*, *87*(2), 704–710. https://doi.org/10.2527/jas.2008-1254

NRC. (2007). *Nutrient requirements of small ruminants: Sheep, goat, cervids, and new world camelids*. *National Research Council*. Washington, D.C., USA: National Academies Press. https://doi.org/10.17226/11654

Ogata, T. (1958). A histochemical study of the red and white muscle fibers Part III . Activity of the diphosphopyridine nucleotide diaphorase and triphosphopyridine nucleotide diaphorase in muscle fiber. *Acta Medica Okayama*, *12*(3), 233–240. https://doi.org/10.18926/AMO/31361

Park, P. ., & Goins, R. E. (1994). In Situ preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. *Journal of Food Science*, *59*(6), 1262–1266. https://doi.org/10.1111/j.1365-2621.1994.tb14691.x

Parr, S. L., Brown, T. R., Ribeiro, F. R. B., Chung, K. Y., Hutcheson, J. P., Blackwell, B. R., … Johnson, B. J. (2014). Biological responses of beef steers to steroidal implants and zilpaterol hydrochloride1. *Journal of Animal Science*, *92*(8), 3348–3363. https://doi.org/10.2527/jas.2013-7221

Parr, T., Mareko, M. H. D., Ryan, K. J. P., Hemmings, K. M., Brown, D. M., & Brameld, J. M. (2016). The impact of growth promoters on muscle growth and the potential consequences for meat quality. *Meat Science*, *120*, 93–99. https://doi.org/10.1016/j.meatsci.2016.04.022

Patel, N., Bergamaschi, M., Magro, L., Petrini, A., & Bittante, G. (2019). Relationships of a detailed mineral profile of meat with animal performance and beef quality. *Animals*, *9*(12), 1073. https://doi.org/10.3390/ani9121073

Pilarczyk, R., & Wojcik, J. (2015). Fatty acids profile and health lipid indices in the Longissimus lumborum muscle of different beef cattle breeds reared under intensive production systems. *Acta Sci Pol Zootechnica*, *14*(1), 109–126.

Ponnampalam, E. N., Hopkins, D. L., Bruce, H., Li, D., Baldi, G., & Bekhit, A. E. (2017). Causes and contributing factors to "dark cutting" meat: Current trends and future directions: A review. *Comprehensive Reviews in Food Science and Food Safety*, *16*(3), 400–430. https://doi.org/10.1111/1541-4337.12258

Purslow, P. P. (2005). Intramuscular connective tissue and its role in meat quality. *Meat Science*, *70*(3), 435–447. https://doi.org/10.1016/j.meatsci.2004.06.028

Razminowicz, R. H., Kreuzer, M., & Scheeder, M. R. L. (2006). Quality of retail beef from two grass-based production systems in comparison with conventional beef. *Meat Science*, *73*(2), 351–361. https://doi.org/10.1016/j.meatsci.2005.12.013

Smith, Z. K., & Johnson, B. J. (2020). Mechanisms of steroidal implants to improve beef cattle growth: a review. *Journal of Applied Animal Research*, *48*(1), 133–141.

https://doi.org/10.1080/09712119.2020.1751642

Starkey, C. P., Geesink, G. H., Oddy, V. H., & Hopkins, D. L. (2015). Explaining the variation in lamb longissimus shear force across and within ageing periods using protein degradation, sarcomere length and collagen characteristics. *Meat Science*, *105*, 32–37. https://doi.org/10.1016/j.meatsci.2015.02.011

Stewart, M. R., Hutchins, B. K., Zipser, M. W., & Watts, B. M. (1965). Enzymatic reduction of metmyoglobin by ground beef. *Journal of Food Science*, *30*(3), 487–491. https://doi.org/10.1111/j.1365-2621.1965.tb01790.x

Strydom, P. E., Hope-Jones, M., Frylinck, L., & Webb, E. C. (2011). The effects of a beta-agonist treatment, Vitamin D3 supplementation and electrical stimulation on meat quality of feedlot steers. *Meat Science*, *89*(4), 462–468. https://doi.org/10.1016/j.meatsci.2011.05.012

Suman, S. P., & Joseph, P. (2013). Myoglobin chemistry and meat color. *Annual Review of Food Science and Technology*, *4*(1), 79–99. https://doi.org/10.1146/annurev-food-030212-182623

Sutton, D. S., Ellis, M., Lan, Y., McKeith, F. K., & Wilson, E. R. (1997). Influence of slaughter weight and stress gene genotype on the water-holding capacity and protein gel characteristics of three porcine muscles. *Meat Science*, *46*(2), 173–180. https://doi.org/10.1016/S0309-1740(97)00006-5

Witte, V. C., Krause, G. F., & Bailey, M. E. (1970). A new extraction method for determing 2-Thiobarbituric acid values of pork and beef during storage. *Journal of Food Science*, *35*(5), 582–585. https://doi.org/10.1111/j.1365-2621.1970.tb04815.x

Wu, S., Luo, X., Yang, X., Hopkins, D. L., Mao, Y., & Zhang, Y. (2020). Understanding the development of color and color stability of dark cutting beef based on mitochondrial proteomics. *Meat Science*, *163*, 108046. https://doi.org/10.1016/j.meatsci.2020.108046

Table 1

Area, fiber morphometry, collagen content and proximate analysis of the *Longissimus thoracis* muscle obtained from male lambs fed zilpaterol hydrochloride (ZH) and implanted with trenbolone acetate and 17β-estradiol (SI).

Items	No ZH		ZH			*P*-Value		
	No SI	SI	No SI	SI	SEM	ZH [a]	SI [b]	ZH×SI
LT area (cm^2)	12.40a	12.84a	12.44a	15.89b	0.38	0.05	0.03	0.01
Proportion of fibers (%)								
Type I	23.35	20.84	17.01	22.39	2.8	0.41	0.62	0.18
Type IIa	33.00	33.14	30.59	33.24	1.57	0.47	0.39	0.44
Type IIb	43.64a	46.01a	52.42b	44.37a	1.90	0.08	0.16	0.02
Cross-sectional area (μm^2)								
Type I	2155a	2046b	1856c	2101ab	26.91	<0.01	0.01	<0.01
Type IIa	2389a	2458b	2473b	2362a	20.76	0.77	0.31	<0.01
Type IIb	3065a	2918b	3081a	3312c	22.75	<0.01	0.06	<0.01
Perimeter (μm)								
Type I	179a	173b	169c	178a	1.15	<0.01	0.30	<0.01
Type IIa	189a	189a	192b	188a	0.82	0.69	<0.01	<0.01
Type IIb	215a	207b	217a	223d	0.83	<0.01	0.05	<0.01
Collagen content								
Total (OH-μg/g)	0.643	0.639	0.640	0.641	0.002	0.83	0.41	0.29
Soluble (%)	17.22a	19.11b	16.44c	20.73d	0.27	0.16	<0.01	<0.01
Insoluble (%)	82.78a	80.89b	83.57c	79.28d	0.29	0.17	<0.01	<0.01
Proximate analysis (%)								
Moisture	71.02	71.03	70.40	71.00	0.50	0.50	0.55	0.56
Lipid	2.75	2.47	2.20	2.13	0.29	0.13	0.53	0.70
Protein	25.22	25.28	25.84	25.35	0.45	0.44	0.63	0.53
Ash	5.32a	6.13b	4.77c	4.84c	0.20	<0.01	0.03	0.07

[a] Lambs fed 0 or 0.15 mg of ZH/kg body weight/day from day 31 to 60 of the feeding period.
[b] Lambs without or with steroidal implant administered in the ear (52.5 mg of trenbolone acetate + 7.5 mg of 17β-estradiol) during the 63-d feeding period.

Table 2

Lipid oxidation, metmyoglobin concentration and meat quality of the *Longissimus thoracis* muscle obtained from lambs fed zilpaterol hydrochloride (ZH) and implanted with trenbolone acetate and 17β-estradiol (SI).

Items [a]	ZH [b]			SI [c]			*P*-Value		
	0	0.15	SEM	Without	With	SEM	ZH	SI	ZH×SI
Malondialdehyde (mg/kg)	0.55	0.55	0.06	0.56	0.53	0.06	0.98	0.71	0.80
Metmyoglobin (%)	29.17	26.99	1.18	28.36	27.80	1.22	0.22	0.74	0.06
Ultimate pH	5.72	5.82	0.03	5.79	5.72	0.03	0.06	0.04	0.03
Color									
Lightness (L*)	38.99	36.53	0.57	37.83	37.69	0.57	<0.01	0.86	0.71
Redness (a*)	16.21	13.12	0.38	14.84	14.49	0.38	<0.01	0.52	0.61
Yellowness (b*)	7.39	5.41	0.31	6.61	6.19	0.30	<0.01	0.35	0.98
Hue angle (H*)	24.31	22.32	0.64	23.84	22.78	0.64	0.04	0.26	0.67
Chroma (C*)	17.83	14.21	0.46	16.26	15.78	0.46	<0.01	0.47	0.69
Cooking loss (%)	23.27	23.22	0.59	23.92	22.58	0.59	0.95	0.13	0.76
WHC (%)	84.34	84.83	0.44	84.66	84.51	0.44	0.44	0.81	0.72
WBSF (N)	68.45	74.62	2.35	72.76	70.41	2.35	0.08	0.50	0.30

[a] WHC = Water-holding capacity; WBSF = *Warner-Bratzler* shear force.

[b] Lambs fed 0 or 0.15 mg of ZH/kg body weight/day from day 31 to 60 of the feeding period.

[c] Lambs without or with steroidal implant administered in the ear (52.5 mg of trenbolone acetate + 7.5 mg of 17β-estradiol) during the 63-d feeding period.

Table 3

Fatty acid profile of the *Longissimus thoracis* muscle obtained from lambs fed zilpaterol hydrochloride (ZH) and implanted with trenbolone acetate and 17β-estradiol (SI).

Items [a]	ZH [b]	SI [c]	*P*-Value

	0	0.15	SEM	Without	With	SEM	ZH	SI	ZH×SI
Saturated fatty acids (SFA)									
C10:0	0.12	0.10	0.005	0.11	0.11	0.004	0.07	0.96	0.64
C12:0	0.15	0.09	0.03	0.10	0.13	0.03	0.18	0.52	0.42
C14:0	1.98	1.97	0.10	2.07	1.88	0.10	0.94	0.21	0.32
C15:0	0.30	0.27	0.03	0.26	0.31	0.03	0.50	0.31	0.31
C16:0	25.07	24.88	0.43	25.05	24.90	0.43	0.76	0.81	0.29
C17:0	0.99	1.02	0.05	0.95	1.05	0.05	0.76	0.18	0.94
C18:0	15.37	16.14	0.34	15.68	15.83	0.34	0.12	0.76	0.73
C24:0	0.55	0.50	0.05	0.49	0.56	0.05	0.44	0.37	0.47
Monounsaturated fatty acids (MUFA)									
C15:1	1.58	1.52	0.12	1.50	1.60	0.12	0.74	0.54	0.26
C16:1	1.56	1.53	0.06	1.60	1.49	0.06	0.70	0.23	0.54
C17:1	0.59	0.60	0.03	0.58	0.60	0.03	0.90	0.66	0.73
C18:1CIS	39.79	40.88	0.66	40.84	39.83	0.66	0.26	0.29	0.52
Polyunsaturated fatty acids (PUFA)									
C18:2TRANS	1.49	1.30	0.10	1.40	1.38	0.10	0.21	0.90	0.63
C18:2CIS	7.17	6.10	0.43	6.26	7.02	0.43	0.09	0.22	0.23
C20:2	0.36	0.32	0.02	0.34	0.34	0.02	0.26	0.97	0.11
C18:3n-3	0.59	0.56	0.03	0.56	0.59	0.03	0.46	0.55	0.44
C20:3n-6	0.25	0.20	0.02	0.21	0.23	0.02	0.08	0.48	0.99
C20:4n-6	2.21	2.02	0.17	1.98	2.25	0.17	0.45	0.28	0.12
ΣSFA	44.55	44.98	0.51	44.74	44.79	0.51	0.56	0.94	0.48
ΣMUFA	43.53	44.53	0.62	44.52	43.53	0.62	0.26	0.27	0.59
ΣPUFA	12.07	10.50	0.63	10.76	11.82	0.63	0.09	0.24	0.19
PUFA:SFA	0.27	0.23	0.01	0.24	0.26	0.01	0.09	0.29	0.18
MUFA:SFA	0.98	0.99	0.02	0.99	0.97	0.02	0.65	0.42	0.94
n3	0.59	0.56	0.03	0.56	0.59	0.03	0.46	0.55	0.44
n6	2.46	2.22	0.19	2.19	2.48	0.19	0.26	0.36	0.15
n6:n3	4.16	3.96	0.32	3.91	4.20	0.32	0.84	0.90	0.56
AI [d]	0.57	0.56	0.01	0.57	0.56	0.01	0.55	0.82	0.25

[a] Milligrams of fatty acid /100 mg fatty acid methyl esters detected.

[b] Lambs fed 0 or 0.15 mg of ZH/kg body weight/day from day 31 to 60 of the feeding period.

[c] Lambs without or with steroidal implant administered in the ear (52.5 mg of trenbolone acetate + 7.5 mg of 17β-estradiol) during the 63-d feeding period.

[d] AI= Atherogenic index ([C12:0+C14:0+C16:0]/[MUFA+PUFA).

Caption of Figures

Fig. 1. Metmyoglobin concentration and ultimate pH in the *Longissimus thoracis* muscle obtained from lambs fed zilpaterol hydrochloride and implanted with trenbolone acetate and 17β-estradiol (SI). Different letters indicate significant differences at $P < 0.05$.

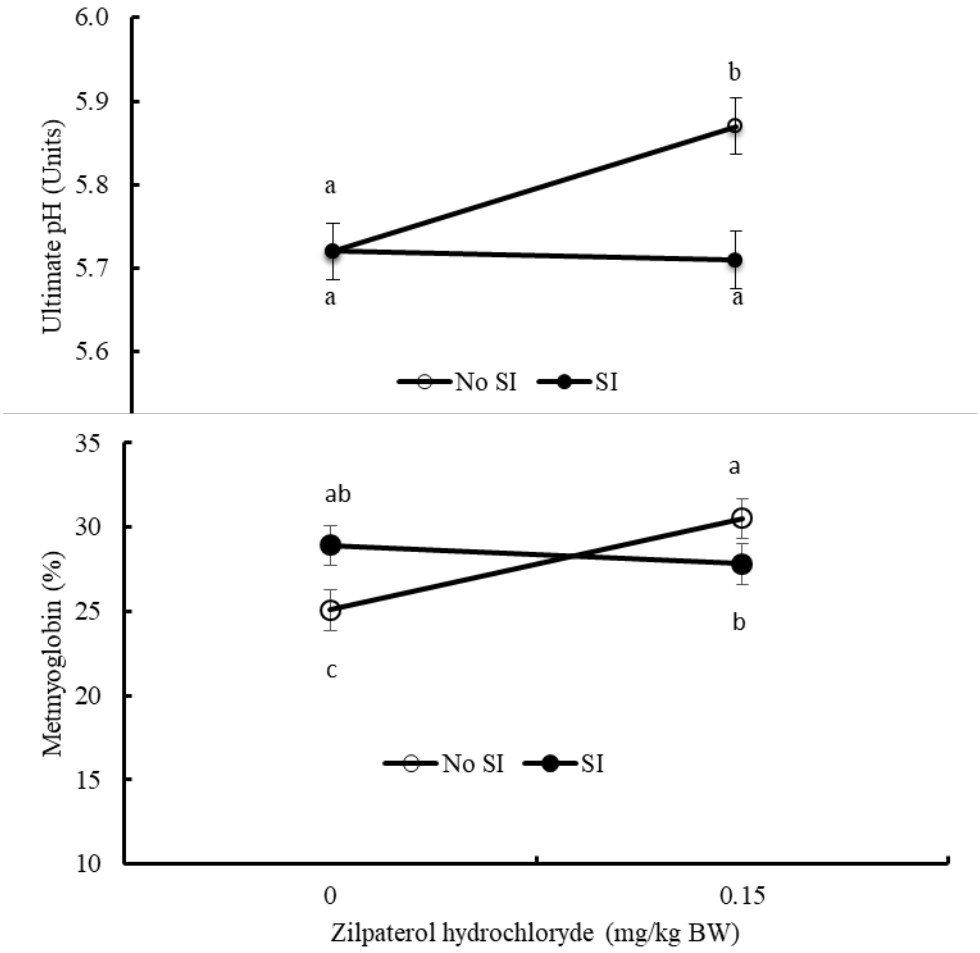
6.0
5.9
5.8
5.7
5.6
Ultimate pH (Units)
a
b
a
a
No SI
SI
35
30
25
20
15
10
Metmyoglobin (%)
ab
a
c
b
No SI
SI
0
0.15
Zilpaterol hydrochloryde (mg/kg BW)

Fig. 1

4. RESULTADOS Y DISCUSIÓN

Los resultados del presente estudio mostraron que la interacción entre el implante esteroidal (IE) y la suplementación con clorhidrato de zilpaterol (CZ) no afectó ($P > 0.05$) ni el comportamiento productivo, ni las características de la canal, ni el rendimiento de cortes primarios en los corderos de pelo finalizados en corral de engorda. Mientras que la aplicación individual del IE tuvo efectos mínimos en estas variables, mostrando mayor rendimiento en la canal y de pescuezo. Por su parte, la suplementación con CZ en forma individual mostró un efecto marcado en el comportamiento en corral y en las características de la canal, pero no el rendimiento de los cortes primarios. Específicamente, el CZ incrementó ($P \leq 0.05$) la ganancia de peso y la eficiencia alimenticia durante los 30 días que se ofreció; pero su efecto no se reflejó ($P \geq 0.35$) en un mejor comportamiento productivo durante el periodo completo de la prueba (63 d). En las características de la canal, la suplementación con CZ incrementó ($P < 0.01$) el peso de canal caliente y frío, así como el rendimiento en canal ($P < 0.01$) y el tamaño de la pierna ($P = 0.02$), pero sin afectar ($P \geq 0.12$) la deposición de grasa corporal (espesor de grasa dorsal, grasa KPH, y grasa omental y mesentérica).

La interacción no significativa entre ambos promotores de crecimiento es un resultado inesperado de acuerdo a la hipótesis planteada, que sugiere que la aplicación combinada de CZ y IE en corderos de pelo en finalización no promueve efectos sinérgicos o aditivos para mejorar el comportamiento productivo y las características de la canal. Debido a que la suplementación de CZ incrementó la ganancia de peso vivo y de canal, pero no así el IE, la hipertrofia generada por administrar ambos promotores en forma conjunta no fue suficiente para reflejarse en mayor ganancia de peso, eficiencia alimenticia, y peso y rendimiento de canal. Estos resultados de la interacción de ambos promotores son congruentes con los reportados en ganado de carne cuando se implantó y suplementó con CZ (Baxa *et al.*, 2010; Kellermeier *et al.*, 2009; Neill *et al.*, 2009; Parr *et al.*, 2011). A pesar de no haber efecto de la interacción CZ × IE en esos estudios de ganado, los autores concluyeron que ambos promotores actuaron de manera aditiva para mejorar el comportamiento productivo y las características de la canal de importancia económica porque numéricamente tuvieron mejor ganancia de peso, peso de canal y rendimiento de canal con la administración conjunta de los promotores que con la administración individual.

El efecto benéfico individual por la suplementación de CZ sobre el crecimiento (últimos 30 días), el peso y rendimiento de la canal y el tamaño de la pierna es consistente con lo reportado por otros estudios en corderos de similar sexo, manejo y genotipo (Macías-Cruz *et al.*, 2013; Avendaño-Reyes *et al.*, 2018; Rojo-Rubio *et al.*, 2018; Cayetano-De-Jesus *et al.*, 2020). Debido a que el CZ no modificó la deposición de grasa corporal y sí redujo el porcentaje de peso de algunos despojos (cabeza, piel y patas), se puede sugerir que el CZ si mejoró el comportamiento productivo y algunos rasgos de canal asociados con la deposición de masa muscular, puesto que promovió una mayor síntesis de proteína, redujo la proteólisis y movilizó nutrientes de algunos tejidos de despojo para formar músculo. Estos resultados junto con los reportados previamente por Avendaño-Reyes *et al.* (2018) y Rivera-Villegas *et al.* (2018) rompen el paradigma de que CZ aumenta la masa muscular porque redistribuye sustrato energético de tejido lipídico para la formación de músculo. Los estudios donde usaron CZ de patente Zilmax® en la engorda de corderos reportan mayor acción lipolítica en tejido graso y síntesis de proteína muscular (Lopez-Carlos *et al.*, 2011; 2012), mientras que aquellos en donde se usó el CZ genérico Grofactor®, incluyendo este estudio, no encontraron acción de lipólisis, pero sí incremento en síntesis de proteína muscular (Avendaño-Reyes *et al.*, 2018; Rivera-Villegas *et al.*, 2018). Esto se puede explicar porque aunque las moléculas de CZ son similares entre marcas, al parecer el vehículo está modificando la biodisponibilidad y el mecanismo de acción en los ovinos.

Por otra parte, los efectos de aplicar solamente IE sobre el comportamiento productivo, características de la canal y rendimiento de cortes primarios fueron mínimos en los corderos de pelo, incrementando en 1.6 % el rendimiento en canal y reduciendo en 0.6 % el rendimiento de pescuezo. Sin embargo, la mayoría de los estudios publicados han reportado beneficios en crecimiento, eficiencia alimenticia y ganancia de peso de canal por implantar a corderos (Johnson *et al.*, 1998; McClure *et al.*, 2000; Ortiz *et al.*, 2013). La falta de coincidencia entre los resultados previos y los del presente estudio puede deberse dos factores: 1) la diferencias en el sexo de los corderos; 2) las diferencias entre corderos enteros (este estudio) y corderos castrados (investigaciones previas); pues se ha documentado que el IE promueve un mejor crecimiento en corderos castrados porque las hormonas esteroidales que contiene el implante compensan la ausencia de la síntesis natural de testosterona a nivel de testículos (Johnson *et al.*, 1998).

Los resultados de este estudio muestran que la interacción CZ × IE modificó el área, la morfometría de fibras y el contenido de colágeno soluble en *Longissimus thoracis*. La aplicación combinada de CZ e IE hizo más grande ($P < 0.01$) el área del LT, así como el área de la sección transversal (AST) y el perímetro de fibras muscular tipo IIb comparado con la aplicación individual de cada promotor de crecimiento, o bien la falta de aplicación de estos. Contrariamente, la suplementación sola de CZ incrementó ($P < 0.01$) el porcentaje de fibras tipo IIb comparado con cualquier otra combinación de tratamientos. Los porcentajes de fibras tipo I y IIa no variaron ($P \geq 0.18$) con la administración junta o separada de los promotores de crecimiento. Sin embargo, el AST y el perímetro disminuyeron ($P < 0.01$) en fibras tipo I y aumentaron ($P \leq 0.01$) en fibras tipo IIa por aplicar CZ o IE, con ningún cambio ($P = 0.25$) por su aplicación combinada. El contenido de colágeno total no cambió ($P \geq 0.29$) por aplicar estos promotores de crecimiento, aunque los porcentajes de colágeno soluble e insoluble fueron diferentes ($P < 0.01$) entre las cuatro combinaciones de tratamiento. El porcentaje de colágeno soluble fue más alto ($P < 0.01$) con CZ+IE y más bajo sin la aplicación de los promotores de crecimiento; los resultados de porcentaje de colágeno insoluble fueron contrarios a los de porcentaje de colágeno soluble.

Los resultados del área del LT demostraron que el CZ y el IE actuaron sinérgicamente para promover una hipertrofia en este músculo. Probablemente, esta hipertrofia se dio porque ambos promotores de crecimiento también actuaron sinérgicamente para aumentar el tamaño de las fibras musculares tipo IIb y la deposición de colágeno soluble. En congruencia con esto, Parr *et al.* (2014) señalaron que las fibras glucolíticas rápidas (tipo IIb) son más receptivas al estímulo de los promotores de crecimiento, mientras que las fibras oxidativas lentas (tipo I) son relativamente menos sensibles al estímulo anabólicos. Se debe tomar en cuenta que las fibras tipo IIb son de mayor tamaño que las fibras tipo I y IIa, en consecuencia, cualquier cambio en el tamaño de las fibras glucolíticas rápidas puede reflejarse en el tamaño del músculo (Picard and Gagaoua, 2020), tal como se observó en este estudio. Por su parte, el colágeno soluble está menos compactado que el colágeno insoluble y, como se encuentra en el perimisio del músculo, una acumulación de este tipo de colágeno puede también incrementar el volumen muscular (Purslow, 2005).

En términos generales, el mayor tamaño de las fibras tipo IIb puede deberse a que el IE potencializó el efecto de CZ aumentando el número de RA-β_2 (Parr *et al.*, 2014; Harris *et al.*, 2020), mientras

que la mayor acumulación de colágeno soluble puede ser el resultado de una alta proliferación y diferenciación de células satelitales en el LT debido a que CZ ayudó al IE a estimularlas (Smith y Johnson, 2020). Las células satelitales son responsables de regular la síntesis de colágeno durante el crecimiento del músculo, ya que éste juega un rol clave en la remodelación y acomodo miofibrilar (Fry *et al.*, 2017).

Los resultados del análisis proximal del músculo LT mostraron que el porcentaje de proteína, grasa y humedad no fueron afectados ($P \geq 0.13$) por la interacción CZ × IE o la aplicación individual de CZ o IE en los corderos. Esto sugiere que el efecto sinérgico de CZ e IE causó una hipertrofia que no provocó una gran acumulación de proteína muscular como para reflejarse en un mayor porcentaje de proteína en la carne. Adicionalmente, el resultado de porcentaje de grasa muscular coincide con los resultados de grasa KPH, espesor de grasa dorsal y grasa omental y mesentérica. Al parecer, ni CZ ni IE ejercieron efecto sobre la actividad lipolítica, aun cuando estudios previos si lo han reportado (Lopez-Carlos *et al.*, 2011; 2012; Dávila-Ramírez *et al.*, 2014). Estos resultados de composición proximal coinciden con lo reportado en vacas de desecho implantadas con hormonas esteroidales y alimentadas con CZ (Neill *et al.*, 2009).

En ovinos de pelo suplementados con CZ también se ha reportado una hipertrofia en el LT sin que esto se refleje en cambios en el porcentaje de proteína o humedad (López-Carlos *et al.*, 2012). Por otra parte, la interacción CZ × IE tendió ($P = 0.07$) a afectar el porcentaje de cenizas en el músculo LT, mientras que el CZ de manera individual disminuyó ($P \leq 0.01$) el contenido de cenizas en el músculo LT de corderos implantados por un efecto antagonista. Se ha documentado que los AA-β_2 causan hipocalcemia e hipocalemia en los animales (Korn *et al.*, 2013) y humanos (Aliaga *et al.*, 2020) que los consumen. Tanto el potasio como el calcio son minerales que se encuentran en altas cantidades en el músculo, así que una reducción de ellos podría explicar el resultado de cenizas. Sin embargo, se requiere en el futuro realizar algunos estudios del efecto de CZ e IE sobre el perfil de minerales del músculo LT para comprobar esta hipótesis.

En los resultados de calidad de la carne, el pH no fue afectado 24 h *postmortem* ($P = 0.46$) por la interacción CZ × IE; sin embargo, la suplementación individual de CZ si aumentó el pH ($P = 0.02$), pero el IE no lo afectó ($P = 0.27$). La interacción CZ × IE afectó ($P \leq 0.03$) el pH y el porcentaje

de metamioglobina (MetMb), tanto en la determinación puntual (72 h *postmortem*) como después de 14 días de maduración. La aplicación individual de CZ incrementó ($P < 0.01$) el pH puntual y el pH de carne madura comparado con los pH observados en carne de las otras combinaciones de tratamiento, entre los cuales no hubo diferencias ($P \geq 0.20$) para ninguno de los pH. El porcentaje de MetMb fue más bajo ($P \leq 0.01$) sin la aplicación de los promotores de crecimiento o cuando se aplicaron de forma combinada en comparación a cuando se administraron individualmente CZ o IE, entre los cuales no hubo diferencia ($P = 0.39$). En la evaluación puntual, la interacción CZ × IE y la aplicación del IE (como factor principal) no modificaron ($P \geq 0.13$) los parámetros de color, la pérdida por cocción, la capacidad de retención de agua, ni el esfuerzo de corte; sin embargo, la suplementación con CZ redujo ($P \leq 0.04$) los valores promedio de todos los parámetros de color y tendió ($P = 0.08$) a incrementar el esfuerzo de corte. En la evaluación de carne madurada por 14 d, la interacción de CZ × IE afectó ($P = 0.03$) solamente el esfuerzo al corte, siendo mayor con la suplementación individual de CZ comparado con cualquier otra combinación de tratamiento, entre los cuales no hubo diferencias ($P \geq 0.16$). Basado en factores principales, mientras que el IE solamente redujo ($P = 0.05$) el valor promedio del color rojizo (a*), la suplementación de CZ nuevamente redujo ($P \leq 0.04$) todos los valores promedios de los parámetros de color y tendió ($P = 0.08$) a incrementar la capacidad de retención de agua.

Los resultados en términos generales mostraron que la suplementación de CZ, pero no la aplicación de IE, en corderos de pelo es un factor que afecta negativamente la calidad de la carne del músculo LT, tanto en las primeras horas *postmortem* como después de un periodo de maduración. La carne de corderos alimentados con CZ presentó una decoloración y baja luminosidad combinada con un aumento en su dureza y capacidad de retención de agua. Todos estos cambios en la calidad debido a la suplementación con CZ son característicos de una producción de carne clasificada como oscura, firme y seca (DFD siglas en inglés), la cual no es muy aceptada por el consumidor por su baja terneza y apariencia oscura (Ponnampalam *et al.*, 2017; Wu *et al.*, 2020). Estudios previos en corderos (Dávila-Ramírez *et al.*, 2013; 2017) y ganado de carne (Avendaño-Reyes *et al.*, 2016; Meraz-Murillo *et al.*, 2017) también han reportado este efecto negativo en la carne por alimentarlos con CZ antes del sacrificio.

De acuerdo a estudios previos, el principal responsable de la presencia de carne DFD es el hecho

de que el pH final se mantiene alto (>5.8) por efecto del CZ (Dávila-Ramírez *et al.*, 2014; Ponnampalam *et al.*, 2017). De acuerdo con lo anterior, en este estudio se mostró que el pH de la carne del LT a las 24 h, 72 h y 14 d *postmortem* se mantuvo por arriba de 5.8 y significativamente más alto debido al CZ. Lo anterior permite concluir que la carne de corderos tratados con CZ indujo la condición DFD debido a que el pH *postmortem* se mantuvo alto, tal como se ha indicado en estudios previos (Dávila-Ramírez *et al.*, 2013, 2014).

De acuerdo a reportes previos, el pH alto de la carne generado por la suplementación de CZ se debe a que este AA-β_2 causa un aumento en el consumo del glucógeno muscular antes del sacrificio, lo cual limita la producción de ácido láctico y la acidificación muscular *postmortem* (Lee y Kim, 1994; Cayetano-De-Jesus *et al.*, 2020). A su vez, la presencia de pH final alto en carne provoca que disminuya la degradación de proteína muscular durante la maduración e incremente la dureza de la carne, ya que reduce la actividad de la enzima calpaína, al mismo tiempo que favorece la actividad de las enzimas calpastatinas (Simmons *et al.*, 1997). Adicionalmente, este pH alto también provoca una mayor respiración mitocondrial a nivel de fibras musculares, por lo cual las cadenas de transporte de electrones compiten por el oxígeno con la oximioglobina, causando una desoxigenación y aumento en la producción de MetMb (Suman y Joseph, 2013; Ponnampalam *et al.*, 2017) como se observó en este estudio. Así, todo este mecanismo desencadenado por el cambio en el pH puede llevar a la carne DFD en los corderos alimentados con CZ.

Cabe mencionar que el IE tuvo un efecto antagonista sobre la acción del CZ en el pH, reduciéndolo a un rango normal como cuando no se administró ninguno de los dos promotores de crecimiento. Este efecto resultó favorable para evitar que la suplementación del CZ en los corderos promoviera la presencia de carne DFD. De hecho, el IE y el CZ actuaron sinérgicamente para reducir el porcentaje de MetMb, y esto explica porque cuando se administró el CZ a corderos implantados no causó una decoloración en la carne. También, como previamente se describió, estos promotores de crecimiento tuvieron un efecto sinérgico para aumentar el contenido de colágeno soluble, lo cual también resultó positivo para mejorar la terneza de la carne cuando CZ es ofrecido a los corderos. Estos hallazgos sugieren que los efectos negativos que causa el CZ en la calidad de la carne pueden ser eliminados si los corderos se implantan con hormonas acetato de trembolona y estradiol antes y durante el periodo de suplementación del AA-β_2. Este es el primer estudio que demuestra este efecto benéfico por combinar IE y CZ en corderos.

La oxidación lipídica y el perfil de ácidos grasos no fueron afectados por la interacción CZ × IE o el efecto principal de IE; por su parte, la suplementación de CZ solamente tendió ($0.08 \leq P \leq 0.09$) a disminuir las concentraciones de C10:0, C18:2CIS, C20:3n-6 y total de ácidos poli-insaturados (PUFA siglas en inglés). Los efectos de CZ e IE sobre el perfil de ácidos grasos han sido poco estudiados. En coincidencia con nuestros resultados, Dávila-Ramírez *et al.* (2017) encontraron una ligera disminución en las concentraciones de PUFA por efecto del CZ en corderas de pelo, aunque en otros estudios donde expusieron a los corderos a estrés calórico no reportaron efecto alguno asociado con el CZ (Dávila-Ramírez *et al.*, 2018). En este estudio, los escasos efectos observados debido a CZ y nulos efectos debido a IE podrían deberse a que ninguno de los promotores de crecimiento alteró la oxidación de lípidos y la deposición de grasa.

5. CONCLUSIONES GENERALES

Los promotores de crecimiento CZ e IE actuaron sinérgicamente para incrementar el tamaño de fibras musculares tipo IIb y el contenido de colágeno soluble, lo cual favoreció una mayor hipertrofia muscular en corderos de pelo. Sin embargo, esto no se reflejó en mayor comportamiento productivo, características de la canal ni rendimiento de cortes primarios. No obstante, la colocación del IE a los corderos alimentados con CZ funcionó como modulador de los efectos del CZ a nivel del músculo LT, ya que potencializó el efecto hipertrófico de CZ; además, en el manejo *postmortem* de la carne favoreció que el pH de la carne no madurada y madura se redujera a un nivel considerado como rango normal (5.4 a 5.8). La presencia de un pH normal en la carne de corderos suplementados con CZ redujo la oxidación de mioglobina (MetMb) y evitó la presencia de cambios en su color y terneza. Las concentraciones de los ácidos grasos y las relaciones entre ellos no cambiaron por la aplicación combinada de CZ e IE, y en términos generales, el perfil de ácidos grasos que presentó la carne se consideró saludable para el humano.

En general, el IE en los corderos no funcionó por si sólo como promotor de crecimiento, tampoco alteró la calidad de la carne ni el perfil de ácidos grasos. Contrariamente, el AA-β_2 CZ mostró ser un potente promotor de crecimiento en corderos de pelo de finalización, ya que mejoró el comportamiento productivo durante el periodo que se ofreció, así como las características de la canal, el porcentaje de fibras IIb y el tamaño de fibras IIa. Sin embargo, el CZ también provocó que el pH *postmortem* de la carne se mantuviera alto, aumentando la oxidación de mioglobina, la decoloración y la dureza de la carne. Cabe mencionar que el CZ usado fue genérico, y éste no actúo como un redistribuidor de sustratos energéticos obtenidos a partir de tejido graso para promover la formación de masa muscular, sino que removió nutrientes a partir de tejidos de despojo, y muy probablemente también de la dieta, para incrementar la masa muscular.

6. RECOMENDACIONES

Los principales hallazgos obtenidos sugieren que la combinación de CZ × IE en corderos enteros de pelo actúan de manera sinérgica para mejorar la calidad de la carne por medio de alteraciones en la deposición de colágeno soluble y la morfología del músculo. Sin embargo, es necesario realizar estudios que puedan integrar el conocimiento necesario para dilucidar el mecanismo de acción que explique estos resultados. En este sentido, en un estudio posterior se requiere llevar a cabo un análisis de expresión de genes (MHC, RA-β_2 e IGF-I) para confirmar su relación con los cambios en la morfología del músculo; asimismo, otro donde se establezca la relación de la activación y proliferación de las células satélites con la deposición de colágeno en el músculo.

7. BIBLIOGRAFÍA

Aliaga, C.A., Arizon, L.F. de, Bermúdez, R.M., Castán, J.A.B., Santandreu, A.V., 2020. Severe hypokalemia secondary to abuse of β-adrenergic agonists in a pediatric patient: Case report. Brazilian Journal of Nephrology 42, 250–253. https://doi.org/10.1590/2175-8239-jbn-2019-0020

Arteaga J. C. 2012. Mensaje institucional en el acto Inaugural del VII. Foro Ovino del Estado de México. INIFAP. ICAMEX.

Avendaño-Reyes, L., Macías-Cruz, U., Álvarez-Valenzuela, F. D., Águila-Tepato, E., Torrentera-Olivera, N.G, Soto-Navarro, S.A., 2011. Effects of zilpaterol hydrochloride on growth performance, carcass characteristics, and wholesale cut yield of hair-breed ewe lambs consuming feedlot diets under moderate environmental conditions. Journal of Animal Science, 89, 4188–4194. htts:// doi:10.2527/jas.2011-3904

Avendaño-Reyes, L., Meraz-Murillo, F.J., Pérez-Linares, C., Figueroa-Saavedra, F., Correa, A., Álvarez-Valenzuela, F.D., Guerra-Liera, J.E., López-Rincón, G., Macías-Cruz, U., 2016. Evaluation of the efficacy of Grofactor, a beta-adrenergic agonist based on zilpaterol hydrochloride, using feedlot finishing bulls. Journal of Animal Science 94, 2954–2961. https://doi.org/10.2527/jas.2015-9878

Avendaño-Reyes, L., Torrentera-Olivera, N.G., Correa-Calderón, A., López-Rincón, G., Soto-Navarro, S.A., Rojo-Rubio, R., Guerra-Liera, J.E., Macías-Cruz, U., 2018. Daily optimal level of a generic beta-agonist based on zilpaterol hydrochloride for feedlot hair lambs. Small Ruminant Research 165, 48–53. https://doi.org/10.1016/j.smallrumres.2018.06.014

Baxa, T.J., Hutcheson, J.P., Miller, M.F., Brooks, J.C., Nichols, W.T., Streeter, M.N., Yates, D.A., Johnson, B.J., 2010. Additive effects of a steroidal implant and zilpaterol hydrochloride on feedlot performance, carcass characteristics, and skeletal muscle messenger ribonucleic acid abundance in finishing steers. Journal of Animal Science 88, 330–337. https://doi.org/10.2527/jas.2009-1797

Beermann, D.H., 2002. Beta-adrenergic receptor agonist modulation of skeletal muscle growth. Journal of Animal Science. 80, E18–E23. https://doi.org/10.2527/animalsci2002.0021881200800ES10004x

Bobadilla, S.E.E., Flores, P.J.P., Perea, P.M. 2017. Comercio exterior del sector ovino mexicano antes y después del tratado de libre comercio con América del norte. Economía y Sociedad, 21, 35-49. https://www.redalyc.org/jatsRepo/510/51054506003/html/index.html

Boles, J.A., Boss, D.L., Neary, K.I., Davis, K.C., Tess, M.W., 2009. Growth implants reduced tenderness of steaks from steers and heifers with different genetic potentials for growth and marbling. Journal of Animal Science. 87, 269-274. https://doi.org/10.2527/jas.2008-1256

Boucque, C.V., Fiems, L.O., Casteels, M., Cottyn, B.G., Buysse., F.X., 1988. Effect of anabolic steroids, alone or in combination with antibiotics, on bull performance, carcass traits, and meat quality characteristics. Archiv für Tierernaehrung. Animal Nutrition. 38, 317-326.

https://doi:10.1080/17450398809428298

Bryant, T.C., Engle, T.E., Galyean, M.L., Wagner, J.J., Tatum, J.D., Anthony, R.V., Laudert. S.B., 2010. Effects of ractopamine and trenbolone acetate implants with or without estradiol on growth performance carcass characteristics, adipogenic enzyme activity, and blood metabolites in feedlot steers and heifers. Journal of Animal Science. 88, 4102-4119. https://doi:10.2527/jas.2010-2901

Camacho-Ronquillo, J.C., Hernández-Hernández, J.E. Villareal Espino-Barros, O.A., Franco Guerra, F.J., Camacho-Becerra, C.A. 2018. Análisis económico de la engorda de ovinos en una granja integral en el Estado de Puebla, México. Revista Mexicana de Agronegocio 42, 1-10. https://doi:10.22004/ag.econ.275173

Cárdenas-Villegas, S., Cortez-Romero, C. 2012. Aplicación de biotecnología reproductiva para el mejoramiento genético de rebaños de ovinos. Agroproductividad 5:25-33.

Cayetano-De-Jesus, J.A., Rojo-Rubio, R., Grajales-Lagunes, A., Avendaño-Reyes, L., Macias-Cruz, U., Gonzalez-del-Prado, V., Olmedo-Juárez, A., Chay-Canul, A., Roque-Jiménez, J.A., Lee-Rangel, H.A., 2020. Effect of zilpaterol hydrochloride on performance and meat quality in finishing lambs. Agriculture 10, 241. https://doi.org/10.3390/agriculture10060241

Coelho, J.F.S., Galbraith, H., Topps. J.H., 1981. The effect of a combination of trenbolone acetate and oestradiol-17β on growth performance and blood, carcass and body characteristics of wether lambs. Animal Production. 32, 261-266. https://doi: 10.1017/S000335610002715X

Crouse, J. D., Schanbacher, B.D., Cross, H.R., Seideman, S.C., Smith, S.B., 1987. Growth and carcass traits of heifers as affected by hormonal treatment. Journal of Animal Science. 64, 1434-1440. https://doi:10.2134/jas1987.6451434x

Dávila-Ramírez, J.L., Avendaño-Reyes, L., Macías-Cruz, U., Peña-Ramos, E.A., Islava-Lagarda, T.Y., Zamorano-García, L., Valenzuela-Melendres, M., Camou, J.P., González-Ríos, H., 2017. Fatty acid composition and physicochemical and sensory characteristics of meat from ewe lambs supplemented with zilpaterol hydrochloride and soybean oil. Animal Production Science. 57, 767. https://doi.org/10.1071/AN15311

Dávila-Ramírez, J.L., Avendaño-Reyes, L., Macías-Cruz, U., Torrentera-Olivera, N.G., Zamorano-García, L., Peña-Ramos, A., González-Ríos, H., 2013. Effects of zilpaterol hydrochloride and soybean oil supplementation on physicochemical and sensory characteristics of meat from hair lambs. Small Ruminant Research. 114, 253–257. https://doi.org/10.1016/j.smallrumres.2013.07.009

Dávila-Ramírez, J.L., Avendaño-Reyes, L., Peña-Ramos, E.A., Islava-Lagarda, T.Y., Macías-Cruz, U., Torrentera-Olivera, N.G., Rojo-Rubio, R., González-Ríos, H., 2018. Impact of zilpaterol hydrochloride and soybean-oil supplementation on intramuscular fat, fatty acid profile and cholesterol concentration in the longissimus muscle of male hair lamb under moderate heat-stress conditions. Animal Production Science 58, 1932. https://doi.org/10.1071/AN16747

Dávila-Ramírez, J.L., Macías-Cruz, U., Torrentera-Olivera, N.G., González-Ríos, H., Soto-Navarro, S.A., Rojo-Rubio, R., Avendaño-Reyes, L., 2014. Effects of zilpaterol hydrochloride and soybean oil supplementation on feedlot performance and carcass

characteristics of hair-breed ram lambs under heat stress conditions. Journal of Animal Science. 92, 1184–1192. https://doi.org/10.2527/jas.2012-6214

Dávila-Ramírez, J.L., Macías-Cruz, U., Torrentera-Olivera, N.G., González-Ríos, H., Peña-Ramos, E.A., Soto-Navarro, S.A., Avendaño-Reyes, L., 2015. Feedlot performance and carcass traits of hairbreed ewe lambs in response to zilpaterol hydrochloride and soybean oil supplementation. Journal of Animal Science 93, 3189-3196. https://doi.org/10.2527/jas.2014-8723

Dayton, W.R., White, M.E., 2014. Meat science and muscle biology symposium—Role of satellite cells in anabolic steroid-induced muscle growth in feedlot steers. Journal of Animal Science. 92, 30–38. https://doi.org/10.2527/jas.2013-7077

Dikeman, M.E., (2007). Effects of metabolic modifiers on carcass traits and meat quality. Meat Science. 77, 121–135. https://doi.org/10.1016/j.meatsci.2007.04.011

Ebarb, S. M., Drouillard, J. S., Maddock-Carlin, K. R., Phelps, K. J., Vaughn, M. A., Burnett, D.D., Van Bibber-Krueger, C.L., Paulk, C.B, Grieger, D.M., Gonzalez, J.M., 2016. Effect of growth-promoting technologies on Longissimus lumborum muscle fiber morphometrics, collagen solubility, and cooked meat tenderness. Journal of Animal Science, (94) 2, 869–881. https://doi.org/10.2527/jas.2015-9888

Ebarb, S, M., Phelps, K. J., Drouillard, J. S., Maddock-Carlin, K. R., Vaughn, M. A. , Burnett, D. D., Noel, J. A., Van Bibber- Krueger, C. L., Paulk, C. B., Grieger, D. M., Gonzalez, J. M. 2017. Effects of anabolic implants and ractopamine-HCl on muscle fiber morphometrics, collagen solubility, and tenderness of beef longissimus lumborum steaks. Journal of Animal Science, 95, (3), 1219–1231. https://doi.org/10.2527/jas.2016.1263

Estrada-Angulo, A., Barreras-Serrano, A., Contreras, G., Obregon, J.F., Robles-Estrada, J.C., Plascencia, A., Zinn, R.A., 2008. Influence of zilpaterol clorhydrate supplementation on growth performance and carcass characteristics of feedlot lambs. Small Ruminant Research. 80, 107–110. https://doi.org/10.1016/j.smallrumres.2008.09.006

FAO (Food and Agricultural Organisation), 2016. Food Outlook. June 2016. Biannual Report on global food markets June 2016. Available from: http://www.fao.org/3/a-I5703E.pdf

FAO. 2018. Perspectivas alimentarias. Resúmenes de mercado. Organización de las Naciones Unidas para la Alimentación y la Agricultura. Disponible en: http://www.fao.org/3/CA0910ES/ca0910es.pdf. (Accesado: 13 de enero de 2019).

FAOSTAT. 2018. Data of live animals. Food and Agriculture Ornagization of the United Nations. Disponible en: http://www.fao.org/faostat/en/#data/QA. (Accesado: 20 de marzo de 2020).

Fry, C.S., Kirby, T.J., Kosmac, K., McCarthy, J.J., Peterson, C.A., 2017. Myogenic progenitor cells control extracellular matrix production by fibroblasts during skeletal muscle hypertrophy. Cell Stem Cell 20, 56–69. https://doi.org/10.1016/j.stem.2016.09.010

Galbraith, H., Singh, S.B., Scaife, J.R., 1997. Response of castrated male sheep to oestrogenic and androgenic compounds implanted alone or in combination. Animal Science, 64, 261–269. https://doi.org/10.1017/S1357729800015824

Garmyn, A., Knobel, S., Spivey, K., Hightower, L., Brooks, J., Johnson, B., ... Yates, D., 2011. Warner-Bratzler and slice shear force measurements of 3 beef muscles in response to

various aging periods after trenbolone acetate and estradiol implants and zilpaterol hydrochloride supplementation of finishing beef steers. Journal of Animal Science, 89, 3783–3791. https://doi.org/10.2527/jas.2011-4134

Girard, I., Bruce, H.L., Basarab, J.A., Larsen, I.L., Aalhus, J.L., 2012. Contribution of myofibrillar and connective tissue components to the Warner-Bratzler shear force of cooked beef. Meat Science. 92, 775-782. https://doi:10.1016/j.meatsci.2012.06.037

Gonzalez, J.M., Carter, J.N., Johnson, D.D., Ouellette, S.E., Johnson, S.E., 2007. Effect of ractopamine-hydrochloride and trenbolone acetate on longissimus muscle fiber area, diameter, and satellite cell numbers in cull beef cows. Journal of Animal Science. 85, 1893-1901. https://doi:10.2527/jas.2006-624

Grandadam, J.A., Scheid, J.P., Jobard, A., Dreux, H., Boisson, J.M., 1975. Results Obtained with Trenbolone Acetate® in Conjunction with Estradiol 17β in Veal Calves, Feedlot Bulls, Lambs and Pigs. Journal of Animal Science. 41, 969-977. https://doi.org/10.2527/jas1975.413969x

Harris, T.L., Smith, Z.K., Ribeiro, F.R.B., Jennings, M.A., Vogel, G.J., Johnson, B.J., 2020. Ractopamine hydrochloride and estradiol + trenbolone acetate implants alter myogenic mRNA, β-adrenergic receptors, and blood metabolites. Open Journal of Animal Sciences 10, 447–467. https://doi.org/10.4236/ojas.2020.103028

Helferich, W.G., Jump, D.B., Anderson, D.B., Skjaerlum, D.M, Merkel, R.A., Bergen, W.G., 1990. Skeletal muscle α-actin is increased pretranslational in pigs fed the phenotholamine ractopamine. Endocrinology. 126, 3096-3100. https://doi.org/10.1210/endo-126-6-3096

Hemmings, K.M., Daniel, Z.C.T.R, Buttery, P.J., Parr, T.., Brameld, J.M., 2015. Differential effects of short-term β agonist and growth hormone treatments on expression of myosin heavy chain IIB and associated metabolic genes in sheep muscle. Animal, 9(2), 285–294. https://doi.org/10.1017/S175173111400233X

Herago, T., Agonafir, A., 2017. Growth promoters in cattle. Advances in Biological Research. 1, 24-34. https:10.5829/idosi.abr.2017.24.34

Johnson, B.J., Anderson, P.T., Meiske, J.C., Dayton, W.R., 1996. Effect of a combined trenbolone acetate and estradiol implant on feedlot performance, carcass characteristics, and carcass composition of feedlot steers. Journal of Animal Science. 74, 363-371. https://doi.org/10.2527/1996.742363x

Johnson, B.J., 2004. β-adrenergic agonists: Efficacy and potential mode of action in cattle. Proc. Plains Nutrition Council. AREC: 04-14.

Johnson, M., 2006. Molecular mechanisms of β 2-adrenergic receptor function, response, and regulation. Journal of Allergy and Clinical Immunology. 117, 18-24. https://doi:10.1016/j.jaci.2005.11.012

Johnson, B.J., Smith, S.B., Chung, K.Y., 2014. Historical overview of the effect of β-adrenergic agonists on beef cattle production. Asian-Australasian Journal of Animal Sciences. 27, 757-766. https://doi:10.5713/ajas.2012.12524

Johnson BJ, Beckett J. 2014. Application of growth enhancing compounds in modern beef production executive summary. American Meat Association Reference Paper For 2014:1–

15. Disponible en: https://meatscience.org/publications-resources/white-papers/docs/default source/publications-resources/white-papers/application-of-growth-enhancing-compounds-in-modern-beef-production-2015-final

Kellermeier, J.D., Tittor, A.W., Brooks, J.C., Galyean, M.L., Yates, D.A., Hutcheson, J.P., Nichols, W.T., Streeter, M.N., Johnson, B.J., Miller, M.F., 2009. Effects of zilpaterol hydrochloride with or without an estrogen-trenbolone acetate terminal implant on carcass traits, retail cutout, tenderness, and muscle fiber diameter in finishing steers. Journal of Animal Science 87, 3702–3711. https://doi.org/10.2527/jas.2009-1823

Kerth, C. R., Montgomery, J.L., Morrow, K.J., Galyean, M.L., Miller, M.F., 2003. Protein turnover and sensory traits of Longissimus muscle from implanted and nonimplanted heifers. Journal of Animal Science. 81, 1728-1735. https://doi:/2003.8171728x

Korn, K.T., Lemenager, R.P., Claeys, M.C., Waddell, J.N., Engstrom, M., Schoonmaker, J.P., 2013. Supplemental vitamin D3 and zilpaterol hydrochloride. II. Effect on calcium concentration, muscle fiber type, and calpain gene expression of feedlot steers1. Journal of Animal Science 91, 3332–3340. https://doi.org/10.2527/jas.2012-5962

Lee, Y.B., Kim, Y.S., 1994. Muscle characteristics and meat tenderness of cimaterol-fed lambs. Journal of Food Science 59, 33–37. https://doi.org/10.1111/j.1365-2621.1994.tb06891.x

López-Carlos, M.A., Ramírez, R.G., Aguilera-Soto, J.I., Aréchiga, C.F., Méndez-Llorente, F., Rodríguez, H., Silva, J.M., 2010. Effect of ractopamine hydrochloride and zilpaterol hydrochloride on growth, diet digestibility, intake and carcass characteristics of feedlot lambs. Livestock Science 131, 23–30. https://doi.org/10.1016/j.livsci.2010.02.018

López-Carlos, M.A., Ramírez, R.G., Aguilera-Soto, J.I., Rodríguez, H., Aréchiga, C.F., Méndez-Llorente, F., Chavez, J.J., Medina, C.A., Silva, J.M., 2012. Effect of the administration program of 2 β-adrenergic agonists on growth performance and carcass and meat characteristics of feedlot ram lambs. Journal of Animal Science 90, 1521–1531. https://doi.org/10.2527/jas.2010-3513

Macías-Cruz, U., Avendaño-Reyes, L., Vicente-Perez, R, Alvarez-Valenzuela, F.D., Correa-Calderon, A., Gonzalez-Rios, H., Soto-Navarro, S.A., Mellado, M., 2016. Crecimiento y características de la canal de corderos finalizados con clorhidrato de zilpaterol en pastoreo de alfalfa. Revista Mexicana de Ciencias Pecuarias (7) 2, 243-252.

Macías-Cruz, U., Alvarez-Valenzuela, F.D., Torrentera-Olivera N.G., Velázquez-Morales, J.V., Correa-Calderon, A., Robinson, P.H., Avendaño-Reyes, L., 2010. Effect of zilpaterol hydrochloride on feedlot performance and carcass characteristics of ewe lambs during heat-stress conditions. Animal Production Science 50, 983-989. https://doi.org/10.1071/AN10094

Macías-Cruz, U., Álvarez-Valenzuela, F.D., Soto-Navarro, S.A., Águila-Tepato, E., Avendaño-Reyes, L., 2013. Effect of zilpaterol hydrochloride on feedlot performance, nutrient intake, and digestibility in hair-breed sheep. Journal of Animal Science 91, 1844–1849. https://doi.org/10.2527/jas.2011-4911

Mao, Y., Hopskins, D., Zhang, Y., Luo, X., 2016. Consumption patterns and consumer attitudes to beef and sheep meat in China. American Journal of Food and Nutrition 4, 30-39 https://doi.org/10.12691/ajfn-4-2-1

McClure, K.E., Solomont, M.B., Loerch, S.C., 2000. Body weight and tissue gain in lambs fed an all-concentrate diet and implanted with trenbolone acetate or grazed on alfalfa. Journal of Animal Science 78, 1117. https://doi.org/10.2527/2000.7851117x

Meraz-Murillo, F.J., Avendaño-Reyes, L., Pérez-Linares, C., Figueroa-Saavedra, F., Torres-Rodríguez, V., Guerra-Liera, J.E., Mellado, M., Macías-Cruz, U., 2017. Feedlot performance, carcass characteristics and meat quality of Zebu heifers supplemented with two β-adrenergic agonists. Animal Production Science 57, 2125. https://doi.org/10.1071/AN15369

Mersmann, H. J. 1998. Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action. Journal of Animal Science 76, 160-172. https://doi.org/10.2527/1998.761160x

Mersmann, H. J. 2002. Beta-adrenergic receptor modulation of adipocyte metabolism and growth. Journal of Animal Science (80) 1, E24-E29. https://doi.org/10.2527/animalsci2002.0021881200800ES10005x

Montossi, F., Font-i-Furnols, M., del Campo, M., San Julián, R., Brito, G., Sañudo, C., 2013. Sustainable sheep production and consumer preference trends: Compatibilies, contradictions, and unresolved dilemmas. Meat Science 95, 772–789. https://doi.org/10.1016/j.meatsci.2013.04.048

Morris, S.T., 2009. Economics of sheep production systems. Small Ruminant Research. 86, 59-62. https://doi.org/10.1016/j.smallrumres.2009.09.019

Neill, S., Unruh, J.A., Marston, T.T., Jaeger, J.R., Hunt, M.C., Higgins, J.J., 2009. Effects of implanting and feeding zilpaterol hydrochloride on performance, carcass characteristics, and subprimal beef yields of fed cows12. Journal of Animal Science 87, 704–710. https://doi.org/10.2527/jas.2008-1254

Nourozi, M., Abazari, M., Raisianzadeh, M., Mohammadi, M., ZareShahne, A., 2008. Effect of terbutaline and metaproterenol (two beta-adrenergic agonists) on performance and carcass composition of culled Moghani ewes. Small Ruminant Research 74, 72-77. https://doi.org/10.1016/j.smallrumres.2007.03.009

Ortiz, B., Camacho, A., Villalba, N.E., Flores, L.R., Romo, J.A., Aguirre, J., García, D.E., Barajas, R., 2013. Efecto de la potencia de los implantes con zeranol o trembolona + Estradiol en la respuesta productiva de ovinos de pelo en engorda intensiva en clima caluroso. Zootecnia Tropical (31) 1, 74–79.

Ortíz Rodea, A., Barbosa Amezcua, M., Partida de la Peña, J.A., Ronquillo, M.G., 2016. Effect of zilpaterol hydrochloride on animal performance and carcass charatceristics in sheep. A meta-analysis. Journal of Applied Animal Research. 44, 104-112. https://doi.org/10.1080/09712119.2015.1013966

Pampusch, M.S., White, M.E., Hathaway, M.R., Baxa, T.J., Chung, K.Y., Parr, S.L., Johnson, B.J., Weber, W.J., Dayton. W.R., 2008. Effects of implants of trenbolone acetate, estradiol, or both, on muscle insulin-like growth factor-I, insulin-like growth factor-I receptor, estrogen receptor-α, and androgen receptor messenger ribonucleic acid levels in feedlot steers. Journal Animal Science. 86, 3418–3423. https://doi.org/10.2527/jas.2008-1085

Parr, S.L., Brown, T.R., Ribeiro, F.R.B., Chung, K.Y., Hutcheson, J.P., Blackwell, B.R., Smith,

P.N., Johnson, B.J., 2014. Biological responses of beef steers to steroidal implants and zilpaterol hydrochloride1. Journal of Animal Science 92, 3348–3363. https://doi.org/10.2527/jas.2013-7221

Parr, S.L., Chung, K.Y., Galyean, M.L., Hutcheson, J.P., di Lorenzo, N., Hales, K.E., May, M.L., Quinn, M.J., Smith, D.R., Johnson, B.J., 2011. Performance of finishing beef steers in response to anabolic implant and zilpaterol hydrochloride supplementation. Journal of Animal Science 89, 560–570. https://doi.org/10.2527/jas.2010-3101

Parr, T., Molebeledi-Mareko, H.D., Kevin-Ryan, J.P., Krystal-Hemmings, M., David-Brown, M., John-Bramelda, M., 2016. The impact of growth promoters on muscle growth and the potential consequences for meat quality. Meat Science 120, 93-99. http://doi.org/10.1016/j.meatsci.2016.04.022

Park, S.K., Sheffler, T.L., Spurlock, M.E., Grant, A.L., Gerrard, D.E., 2009. Chronic activation of 5′-AMP-activated protein kinase changes myosin heavy chain expression in growing pigs. Journal of Animal Science (87) 10, 3124-3133. https://doi.org/10.2527/jas.2009-1989

Picard, B., Gagaoua, M., 2020. Muscle fiber properties in cattle and their relationships with meat qualities: An overview. Journal of Agricultural and Food Chemistry 68, 6021–6039. https://doi.org/10.1021/acs.jafc.0c02086

Platter, W.J, Tatum, J.D., Belk, K.E., Scanga, J.A., Smith, G.C., 2003. Effects of repetitive use of hormonal implants on beef carcass quality, tenderness, and consumer ratings of beef palatability. Journal of Animal Science 81, 984-996. https://doi.org/10.2527/2003.814984x

Ponnampalam, E.N., Hopkins, D.L., Bruce, H., Li, D., Baldi, G., Bekhit, A.E., 2017. Causes and contributing factors to "dark cutting" meat: Current trends and future directions: A review. Comprehensive Reviews in Food Science and Food Safety 16, 400–430. https://doi.org/10.1111/1541-4337.12258

Purslow, P.P., 2005. Intramuscular connective tissue and its role in meat quality. Meat Science 70, 435–447. https://doi.org/10.1016/j.meatsci.2004.06.028

Reinhardt, C., 2007. Growth-promotant implants: Managing the tools. Veterinary Clinics of North America: Food Animal Practice. 23, 309-319. https://doi.org/10.1016/j.cvfa.2007.03.004

Rivera-Villegas, A., Estrada-Angulo, A., Castro-Pérez, B.I., Urías-Estrada, J.D., Ríos-Rincón, F.G., Rodríguez-Cordero, D., Barreras, A., Plascencia, A., González-Vizcarra, V.M., Sosa-Gordillo, J.F., Zinn, R.A., 2019. Comparative evaluation of supplemental zilpaterol hydrochloride sources on growth performance , dietary energetics and carcass characteristics of finishing lambs. Asian-Australasian Journal of Animal Sciences 32, 209–216. https://doi.org/https://doi.org/10.5713/ajas.18.0152

Rojo-Rubio, R., Avendaño-Reyes, L., Albarrán, B., Vázquez, J.F., Soto-Navarro, S.A., Guerra, J.E., Macías-Cruz, U., 2018. Zilpaterol hydrochloride improves growth performance and carcass traits without affecting wholesale cut yields of hair sheep finished in feedlot. Journal of Applied Animal Research 46, 375–379. https://doi.org/10.1080/09712119.2017.1307756

Salinas-Chavira, J., Domínguez-Muñoz, M., Díaz-Martínez, R., Cruz-Bautista, P., Montaño-Gómez, M. F., Arzola-Alvarez, C., 2006. Effect of duration of zilpaterol hydrochloride treatment on carcass characteristics and weight gain in grazing Pelibuey lambs. Journal of

Applied Animal Research, 29(1), 25-28. https://doi.org/10.1080/09712119.2006.9706564

Shelver, W.L., and Smith, D.J. 2006. Tissue residues and urinary excretion of zilpaterol in sheep treated for 10 days with dietary zilpaterol. Journal of Agricultural and Food Chemistry, 54, 4155-4161. https://doi.org/10.1021/jf060552m

SIAP. 2018b. Producción de carne ovina. Servicio de Información Agroalimentaria y Pesquera. Disponible en: http://infosiap.siap.gob.mx/repoAvance_siap_gb/pec CompaEspProd.jsp. (Accesado: 20 de diciembre de 2018).

SIAP. 2015. Resumen de la producción nacional y comercio de carne de ovino. http://infosiap.siap.gob.mx/gobmx/datosAbiertos_p.php

Sillence, M.N., 2004. Technologies for the control of fat and lean deposition in livestock. The Veterinary Journal 167, 242-257. https://doi.org/10.1016/j.tvjl.2003.10.020

Squires E.J. 2010. Applied Animal Endocrinology, CABI 2nd ed. Cambridge, UK. 281. www.cabi-publishing.org

Simoncini T, Mannella P, Fornari L, Caruso A, Varone G, Genazzani AR. 2004. Genomic and non-genomic effects of estrogens on endothelial cells. Steroids. 69(8-9):537–542. https://doi:10.1016/j.steroids.2004.05.009

Simmons, N.J., Young, O.A., Dobbie, P.M., Singh, K., Thompson, B.C., Speck, P.A., 1997. Post-mortem calpain-system kinetics in lamb: Effects of clenbuterol and preslaughter exercise. Meat Science 47, 135–146. https://doi.org/10.1016/S0309-1740(97)00048-X

Smith, Z.K., Johnson, B.J., 2020. Mechanisms of steroidal implants to improve beef cattle growth: a review. Journal of Applied Animal Research 48, 133–141. https://doi.org/10.1080/09712119.2020.1751642

Smith, D.J., 1998. The pharmacokinetics, metabolism, and tissue residues of beta-adrenergic agonists in livestock. Journal of Animal Science 76, 173-194. https://doi.org/10.2527/1998.761173x

Smith, S.B., Garcia, D.K., Anderson, D.B., 1989. Elevation of a specific mRNA in longissimus muscle of steers fed ractopamine. Journal of Animal Science 67, 3495-3502. https://doi.org/10.2527/jas1989.67123495x

Smith, S.B., Davis, S.K., Wilson, J.J., Stone, R.T., Wu, F.Y., Garcia, D.K., Lunt, D.K., Schiavetta, A.M., 1995. Bovine fast-twitch myosin light chain 1: cloning and mRNA amount in muscle of cattle treated with clenbuterol. American Journal of Physiology-Endocrinology and Metabolism 268, E858-E865. https://doi.org/10.1152/ajpendo.1995.268.5.E858

Strydom, P.E., Frylinck, L., Montgomery, J.L., Smith, M.F., 2009. The comparison of three β-agonists for growth performance, carcass characteristics and meat quality of feedlot cattle. Meat Science. 81, 557–564. https://doi.org/10.1016/j.meatsci.2008.10.011

Suman, S.P., Joseph, P., 2013. Myoglobin chemistry and meat color. Annual Review of Food Science and Technology 4, 79–99. https://doi.org/10.1146/annurev-food-030212-182623

Taylor, S.S., Yang, J., Wu, J., Haste, N.M., Anand, G., 2004. PKA: a portrait of protein kinase dynamics. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics 1697, 259-269. . https://doi.org/10.1016/j.bbapap.2003.11.029

Vahedi, V., Towhidi, A., Hedayat-Evrigh, N., Vaseghi-Dodaran, H., Motlagh, M.K., Ponnampalam, E.N., 2015. The effects of supplementation methods and length of feeding of zilpaterol hydrochloride on meat characteristics of fattening lambs. Small Ruminant Research, 131, 107-112. https://doi.org/10.1016/j.smallrumres.2015.08.018

Verhoeckx, K.C.M., Doornbos, R.P., van der Greef, J., Witkamp, R.F., Rodenberg, R.J.T., 2005. Inhibitory effects of the β_2-adrenergic receptor agonist zilpaterol on the LPS-induced production of TNF-α *in vitro* and *in vivo*. J. Veterinary Pharmacology and Therapeitics. 28, 531-537. https://doi.org/10.1111/j.1365-2885.2005.00691.x

Webb, E.C., Casey, N.H. 1995. Fatty acids in carcass fat of steers treated with a β-adrenergic agonist individually or in combination with trenbolone acetate+ oestradiol-17β. Meat Science, 41(1), 69-76. https://doi.org/10.1016/0309-1740(94)00049-D

Wu Y, Zhao W, Zhao J, Pan J, Wu Q, Zhang Y, Bauman WA, Cardozo CP. 2007. Identification of androgen response elements in the insulin-like growth factor I Upstream promoter. Endocrinology 6, 2984–2993. https: doi:10.1210/en.2006-1653

Wu, S., Luo, X., Yang, X., Hopkins, D.L., Mao, Y., Zhang, Y., 2020. Understanding the development of color and color stability of dark cutting beef based on mitochondrial proteomics. Meat Science 163, 108046. https://doi.org/10.1016/j.meatsci.2020.108046