UNIVERSIDAD AUTONOMA DE BAJA CALIFORNIA
Instituto de Ciencias Agrícolas

MANIPULACIÓN DEL CRECIMIENTO Y CALIDAD DE LA CARNE DE OVINOS MEDIANTE EL USO DE CLORHIDRATO DE ZILPATEROL Y ACEITE RICO EN ÁCIDOS GRASOS INSATURADOS

T E S I S
QUE COMO REQUISITO PARCIAL PARA OBTENER EL GRADO DE:

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Los miembros del comité designado para la revisión de la tesis de José Luis Dávila Ramírez, la han encontrado satisfactoria y recomiendan que sea aceptada como requisito parcial para obtener el grado de Doctor en Ciencias Agropecuarias.

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RESUMEN

La producción intensiva de ganado de carne ha cambiado dramáticamente en los últimos años. El conocimiento en aspectos de nutrición ha conducido a desarrollar nuevas tecnologías para mejorar la eficiencia alimenticia, composición de la canal y calidad de la carne. El uso de agonistas adrenérgicos-β (AA-β) favorece el crecimiento del músculo esquelético, disminuye el contenido de grasa y aumenta la hipertrofia muscular; sin embargo, afecta la calidad de la carne. Por lo tanto, se deben buscar estrategias para contrarrestar este problema. Recientemente, se ha utilizado la adición de grasas poliinsaturadas en la dieta de animales rumiantes; sin embargo, los resultados no han sido totalmente concluyentes. Por lo tanto, se cree que la suplementación en forma conjunta de Clorhidrato de Zilpaterol (CZ) y aceite rico en ácidos grasos poliinsaturados puede tener un efecto favorable en ovinos de pelo. Por lo tanto, el objetivo del trabajo fue evaluar la suplementación de CZ y aceite de soya (AS) en ovinos de pelo (40 ovinos en verano/ 32 ovinos en invierno) y sus efectos en comportamiento productivo, características de la canal, calidad de la carne (parámetros fisicoquímicos) y cambios en el perfil de ácidos grasos del músculo *longissimus dorsi* provocados por el efecto lipolítico y lipogénico que tiene el CZ. En verano, la suplementación conjunta de CZ + AS no provoco ($P>0.05$) cambios en ninguna de las variables analizadas. El uso de CZ provocó un aumento ($P<0,05$) en el peso de la canal, área del ojo de músculo *longissimus dorsi* (AOC) y desarrollo muscular de piernas. Mientras que provocó una disminución en los parámetros de color, grasa intramuscular y ácidos grasos monoinsaturados ($P<0.05$). Por su parte, la suplementación de AS no provoca cambios en comportamiento productivo ni características de la canal. Sin embargo, aumentó los valores de a*, croma, capacidad de retención de agua y contenido de colesterol ($P<0.05$). Por otra parte, en invierno, la suplementación conjunta de CZ + AS no provocó

(P>0.05) cambios en ninguna de las variables analizadas. El uso de CZ en invierno aumentó (P<0.05) el peso de la canal, perímetro de pierna y AOC, disminuyendo los parámetros de color y contenido de ácidos grasos totales omega-3. Por su parte, la suplementación de AS no provoca cambios (P>0.05) en comportamiento productivo, características de la canal, calidad de la carne ni contenido de ácidos grasos poliinsaturados. De manera general, se observa que la suplementación simultanea de CZ y AS durante la época de verano e invierno, no provoca ningún efecto en comportamiento productivo, características de la canal y calidad de la carne. Por su parte, el suplementar CZ y AS en forma individual provoca cambios modestos en algunas de las variables evaluadas. Por lo tanto, es importante evaluar nuevas estrategias de alimentación en corderos de pelo para lograr cambios sustanciales.

Palabras clave: Aceite de soya, Agonistas adrenérgicos-β, Características de la canal, Comportamiento productivo, Ovinos de pelo.

ABSTRACT

Intensive beef production has changed dramatically in recent years. Knowledge in the area of nutrition has led to develop new technologies to improve feed efficiency, carcass composition and meat quality. Use of β-adrenergic agonists (β-AA), promotes growth of skeletal muscle, fat content decreases and increases muscle hypertrophy; however, affects the quality of the meat. Therefore, look for strategies to counteract this problem. Recently, we have used the addition of polyunsaturated fat in the diet of ruminant animals; however, the results have not been conclusive. Therefore, it is believed that supplementation of jointly zilpaterol hydrochloride (ZH) and oil rich in polyunsaturated fatty acids may have a favorable effect on hair lambs. Therefore, the aim of the study was to evaluate supplementation of ZH and soybean oil (SO) in hair lambs (40 lambs in summer / 32 lambs in winter) and its effects on feedlot performance, carcass characteristics, quality meat (physicochemical parameters) and changes in the fatty acid profile in muscle *longissimus dorsi* caused by lipolytic and lipogenic effect of the ZH. In summer, the joint supplementation of ZH + SO did not cause (P> 0.05) changes in any of the variables analyzed. Using of ZH caused an increase (P <0.05) in carcass weight, LM area, and leg muscle development. While caused a decrease in the parameters of color, intramuscular fat and monounsaturated fatty acids (P <0.05). Meanwhile, SO supplementation does not cause changes in feedlot performance and carcass characteristics. However, increased values of a *, chroma, water holding capacity and cholesterol content (P <0.05). Moreover, in winter, joint supplementation did not cause ZH + SO (P> 0.05) changes in any of the variables analyzed. The use of ZH in winter increased (P <0.05) carcass weight, leg perimeter, and LM area, decreasing color parameters and content of total omega-3. Meanwhile, SO supplementation does not cause changes (P> 0.05) on feedlot performance, carcass characteristics, meat quality or content of

polyunsaturated fatty acids. In general, the supplement simultaneously of ZH and SO during the summer and winter, have no effect on feedlot performance, carcass characteristics and meat quality. While individual ZH and SO supplementation causes modest changes in some of the variables evaluated. Therefore, it is important to evaluate new strategies for feeding lambs hair to make substantial changes.

Keywords: β-adrenergic agonists, Carcass traits, Feedlot performance, Hair lambs, Soybean oil

CAPÍTULO I

Introducción General

1. INTRODUCCIÓN

Los sistemas de producción intensivos de carne han cambiado en los últimos años. El uso de técnicas modernas ha permitido mejorar el comportamiento productivo, características de la canal y calidad de carne en algunas especies, lo cual ha generado ganancias económicas importantes. El uso de agonistas adrenérgicos-β (AA-β) es considerado como una de las técnicas más potentes en aumentar el desarrollo de masa muscular (Dikeman, 2007; Chung y Johnson, 2008). Hoy en día, el clorhidrato de zilpaterol (CZ) es el β-AA más utilizado en dietas de finalización, al cual se le han atribuido efectos potentes en aumentar el crecimiento del músculo esquelético (Delmore et al., 2010), disminución del marmoleo graso y aumentar la hipertrofia muscular (Kellermeier et al., 2009). El uso de CZ ha sido usado en ovinos (Aguilera- Soto et al., 2008; Avendaño-Reyes et al., 2011), en donde se han observados resultados positivos.

La investigación en torno al uso de CZ se ha centrado principalmente en el efecto que ha ejercido la molécula en el comportamiento productivo, calidad y composición de la canal, asimismo, el aspecto de terneza en la carne. Sin embargo, poco se ha investigado sobre su efecto en características físicas y sensoriales involucradas en calidad de la carne, así como su efecto en el perfil de ácidos grasos de la carne. En el caso específico de la especie ovina, los resultados obtenidos hoy en día no son concluyentes (Macías-Cruz et al 2010; Avendaño-Reyes et al 2011). Existe un gran número de factores que pueden estar involucrados en la manera de responder al CZ. En este sentido, factores dependientes del animal como raza, edad y sexo, externos al animal como el manejo (alimentación y medio ambiente), así como otros procesos *postmortem* (método de sacrificio, refrigeración y maduración de la carne) pueden estar relacionados con los efectos del CZ (Thompson, 2002). Poletto et al. (2009) reportan que la utilización de AA-β ocasiona una activación de receptores adrenérgicos en células grasas musculares, lo cual origina un aumento en la lipólisis, con lo cual disminuye el contenido de grasa en el músculo.

Por otra parte, con la finalidad de concentrar el contenido energético en el alimento de animales rumiantes, se ha utilizado la adición de grasas en dietas de

finalización; sin embargo, los efectos de estos ingredientes en comportamiento productivo, características de la canal, calidad de la carne y perfil de ácidos grasos no han sido concluyentes. En el caso especifico del contenido graso de la carne; hoy en día, ha tomado gran importancia la repercusión que pueda tener el consumo de alimentos ricos en ácidos grasos saturados debido a que están relacionados con problemas de obesidad y problemas cardiovasculares (Webb y O'Neill, 2008). Para contrarrestar este grave problema, el mercado exige productos cárnicos magros y con un alto contenido de proteína. Por lo cual, para atender la demanda de mercado, las estrategias en producción animal deben estar orientadas a generar carne con esta característica. Actualmente, una estrategia empleada para obtener carne con una mejor composición saludable de lípidos, es la adición de grasas y aceites en la dieta de animales rumiantes. Hasta la fecha, la grasa que ha sido utilizada en dietas de finalización han sido principalmente saturadas, mientras que el uso de aceites con un alto contenido de ácidos grasos poliinsaturados es limitado, debido a que pueden causar un impacto en la flora ruminal al momento de su consumo (Manson et al., 2009). La composición del perfil de ácidos grasos en la carne de animales rumiantes está determinada por el metabolismo de lípidos constituyentes de la dieta. En el rumen, el metabolismo de los lípidos se caracteriza por una intensa lipólisis y una biohidrogenación de los ácidos grasos por acción de microorganismos ruminales (Harfoot y Hazlewood, 1997).

En la actualidad, la industria ganadera ha evaluado el efecto de suplementar AA-β para incrementar el comportamiento productivo y características de la canal; además, de la suplementación de aceite rico en ácidos grasos insaturados para modificar el perfil de lípidos en la carne. Sin embargo, los resultados no han sido concluyentes. Hoy en día, no se ha evaluado de manera conjunta el efecto que pueda tener la suplementación de una molecula con potencial lipolítico (CZ) y la adición de aceite rico en ácidos grasos poliinsaturados en la dieta de corderos de pelo (machos-hembras) confinados bajo condiciones ambientales de estrés (calor-frío) y el efecto que puedan tener estos dos

suplementos en el comportamiento productivo, características de la canal, calidad de la carne y perfil de ácidos grasos.

Por lo tanto, el objetivo general del trabajo fue evaluar el efecto de la suplementación de clorhidrato de zilpaterol y aceite de soya en comportamiento productivo, características de la canal y calidad de la carne en diferentes épocas del año en corderos de pelo confinados de manera intensiva en el noroeste de México. Los objetivos particulares del estudio fueron: 1) Evaluar el comportamiento productivo, características de la canal y rendimiento de cortes primarios de corderos de pelo confinados en condiciones de estrés por calor y frio. 2) Evaluar las características químicas, fisicas y sensoriales del músculo *Longissimus thoracis*. 3) Evaluar el porcentaje de grasa intramuscular, perfil de ácidos grasos y contenido de colesterol en el músculo *Longissimus thoracis*.

CAPÍTULO II

Revisión de Bibliografía

2. REVISIÓN DE LITERATURA

2.1. Producción Ovina en México

Actualmente, el inventario nacional de ganado ovino es cercano a 7 millones de cabezas distribuidas geográficamente en la mayoría de los Estados de la República Mexicana (SAGARPA, 2006). Sin embargo, a nivel nacional, el Estado de México e Hidalgo son los que poseen la mayor producción. De manera general, la actividad ovina está orientada a la producción de carne, en la cual se obtienen altos precios ya sea en pie y canal en comparación con otras especies pecuarias. En México, las principales razas existentes son de lana (Suffolk, Hampshire, Rambouillet, Dorset, Charoláis, Romanov), sin embargo, 25% del inventario total de ganado ovino es de cobertura de pelo (Pelibuey, Blackbelly, Dorper, Katahdin), siendo las razas terminales productoras de carne: Kathadin, Dorper, Suffolk y Charoláis (SAGARPA, 2006).

En México, ante la construcción de rastros tipo inspección federal (TIF) en diversas zonas, se abre la extraordinaria oportunidad de ofrecer productos cárnicos de calidad, lo cual permitirá una retroalimentación para garantizar inocuidad y calidad. Hoy en día, la ovinocultura nacional no satisface la cada vez más grande demanda de carne de esta especie, situación que es muy alentadora para los próximos años. Por lo tanto, es indispensable fortalecer investigaciones hacia este sector y definir claramente los objetivos de producción, ser competitivos en calidad y precio a nivel internacional, y con ello, crear un negocio rentable.

2.2. Promotores del Crecimiento

Ante el incremento del consumo de proteína de origen animal, se ha tenido que hacer uso de sistemas de producción más intensivos, en los cuales se han utilizado promotores del crecimiento para hacer más eficiente el comportamiento productivo y reducir los costos de producción. Un promotor de crecimiento se define como una sustancia natural o sintética que al ser proporcionada al animal a

través de la dieta u otra vía, produce una respuesta mayor en el crecimiento animal al normalmente existente (Duckett et al., 1996). Se clasifican en dos grandes grupos en base a su modo de acción y naturaleza: 1) Aditivos alimenticios, actúan modulando la fermentación ruminal produciendo cambios en poblaciones de microorganismos presentes en el tracto digestivo y mejorando la eficiencia animal (ionóforos, probióticos, prebióticos, ácidos orgánicos y enzimas); y 2) Compuestos hormonales, actúan como agentes anabolizantes del metabolismo animal. Dentro de este grupo se encuentran los implantes hormonales que pueden ser del tipo androgénico, estrogénico o combinado. Así como los compuestos agonistas β-adrenérgicos (Sumano et al., 2002).

En el caso específico de los agonistas β-adrenérgicos, son usados generalmente en la etapa de finalización para promover el crecimiento de varias especies. Estos compuestos favorecen la síntesis de proteína, incrementan la ganancia diaria de peso y mejora la conversión alimenticia (Ekpe et al., 2000). En este sentido, el clorhidrato de zilpaterol es el agonista-β de mayor uso en los corrales de finalización, el cual es empleado los últimos 30 días de finalización, mejorando el crecimiento y deposición de músculo en machos y hembras (Montgomery et al., 2009).

2.3. Agonistas Adrenérgicos-β (AA-β)

Los agonistas-β son potentes fármacos broncodilatadores, anabólicos y agentes lipolíticos en muchas especies (Scott et al., 1991; Sillence et al., 2000). Están catalogados como agentes involucrados en la repartición de nutrientes, ya que fomentan la producción de proteína y reducen la cantidad de grasa (Mersmann, 1998). Actualmente, los β-agonistas comerciales existentes son moléculas análogas a hormonas endógenas catecolaminas (epinefrina y norepinefrina), y son reconocidas por receptores β-adrenérgicos (β-AR) en la membrana celular.

Los compuestos AA-β, dentro de su estructura química, presentan una cadena lateral que puede ser levógira o dextrógira (Morgan, 1990). Las moléculas

existentes en el mercado presentan ambos estereoisómeros; por tanto, pueden tener actividad variable dependiendo de su posición quiral. Morgan (1990) indica que algunos compuestos como el clembuterol, clorhidrato de ractopamina, clorhidrato de zilpaterol y terbutalina, tienen la parte levógira como activa. Por su parte, Sumano et al. (2002) indican que existen compuestos que de manera experimental funcionan con la parte dextrógira. Un aspecto importante en este tipo de compuestos son las sustituciones del anillo aromático para obtener una actividad biológica definida. Algunos compuestos, como el isoproterenol y dobutamina, se transforman e inactivan rápidamente por enzimas tisulares catecol-O-metil-transferasas (COMT), las cuales metilan los hidroxilos en el anillo aromático (Peters, 1989). En contraparte, la ractopamina, salmeterol y salbutamol no son sustratos para la enzima COMT, y sólo son biotransformados por glucuronidación hepática (Courtheyn et al., 1996). Por su parte, la presencia del cloro en la molécula de clembuterol lo hace más liposoluble al compararlo con sus análogos y, por ende, tiende a difundirse más profundamente en los tejidos y la grasa animal (Martin et al., 1992; Waldeck y Widmark, 1995).

A pesar de existir una respuesta favorable en comportamiento productivo en animales suplementados, el uso de AA-β exhiben importantes diferencias en la manera de responder debido a las características de los grupos sustituyentes. El uso de AA-β provoca un aumento en la hipertrofia del músculo esquelético, lo cual es resultado de cambios en síntesis de proteína y tasas de degradación; mientras que en tejido adiposo estos compuestos promueven la lipólisis (Beermann, 2002; Birkelo, 2003; Verhoeckx et al., 2005). En el caso específico del clorhidrato de zilpaterol, su uso durante 30 días en la etapa de finalización mejora la ganancia diaria de peso, eficiencia alimenticia, rendimiento de la canal y área del músculo *longissimus dorsi* en ganado bovino y ovino (Vasconcelos et al., 2008; Avendaño-Reyes et al., 2011;). Los efectos biológicos del uso de clorhidrato de zilpaterol (CZ) son por el resultado de la unión de esta molécula con un receptor β-adrenérgico (β-AR), el cual se ubica en la superficie celular de los tejidos, incluyendo músculo esquelético y tejido adiposo (Mersmann, 1998).

Existen tres subtipos de β-AR (β1, β2 y β3), los cuales se encuentran en la mayoría de las células de mamíferos; sin embargo, en músculo esquelético y tejido adiposo, el más abundante es el β2-AR (Sillence y Matthews, 1994). Sin embargo, Verhoeckx et al. (2005) indican que el CZ se puede unir también al β1-AR, pero exhibe mayor afinidad por el β2-AR.

2.4. Mecanismo de Acción de AA-β

La molécula AA-β, al ser administrada oralmente al ganado, atraviesa intacta el rumen y es absorbida en el tracto digestivo posterior, en donde se incorpora a la circulación sanguínea hasta llegar al tejido blanco. Posteriormente, estas moléculas orgánicas se unen a receptores AA-β, dando lugar al complejo agonista-receptor, el cual es reconocido por las proteínas Gs, las cuales se activan (Smith y Smith, 1995). La subunidad β de la proteína G, activa a la enzima adenilato-ciclasa produciendo el monofosfato de adenosina cíclico (AMPc), el cual es una de las principales moléculas de señalización intracelular. La molécula de AMPc produce sus efectos al unirse a la subunidad reguladora de la proteína quinasa A, con lo cual libera la subunidad catalítica que fosforila algunas proteínas intracelulares relacionadas con permitir la entrada de Ca^{+2} a la célula y fosforila otras proteínas que van a regular la síntesis de proteínas, claves para el funcionamiento celular (Ruffulo, 1991; Sillence et al., 2000).

Aunque los receptores AA-β están presentes en la mayoría de las células de animales mamíferos, su distribución de subtipos (β1, β2, β3) y su proporción varía entre tejidos de una misma especie (Mersmann, 1998). En este sentido, el subtipo β1 se encuentran en mayor proporción en el corazón (estimula contracción) y en músculo liso intestinal (induce relajación) (Peters, 1989). Los β2 se localizan en los bronquios y músculo uterino, induciendo su relajación. Sin embargo, la magnitud del efecto de cada agonista empleado dependerá de su actividad intrínseca en el receptor y distribución en los tejidos blanco (Smith, 1998). Mersmann (1998) reporta que diversos ensayos muestran que uniones competitivas entre los diferentes receptores β-AA indican que existe una alta

proporción de subtipos β2 en músculo esquelético y tejido adiposo. Mientras que Van Liefde et al. (1994) indican que, aproximadamente, 75% de los receptores existentes son del subtipo β2, mientras que 25% son del subtipo β1 en el tejido adiposo; además, sugieren que no existe evidencia de la presencia del subtipo β3 en animales productores de carne.

La unión de AA-β con los receptores antes mencionados causa un incremento en la cantidad de ácido ribonucleico de transferencia (RNAt) de varias proteínas del músculo esquelético. En este sentido, la administración de AA-β incrementa la cantidad de RNA mensajero para la proteína miosina de cadena ligera, RNAm de β-actina y también del inhibidor de la proteasa calpaína-calpastatina (Yang y McElligott, 1989). La modificación de estas proteínas se debe a que el uso de AA-β incrementa el flujo sanguíneo en ciertas regiones corporales, con lo cual acelera el proceso de hipertrofia en el músculo esquelético al transportar mayores cantidades de sustratos y fuentes de energía para la síntesis de estas proteínas. Por su parte, la unión de los AA-β con los receptores afines también se ha observado que provocan una disminución en la cantidad de grasa de la canal, lo cual es atribuido a una degradación de triacilgliceroles en adipocitos y a una inhibición de síntesis de ácidos grasos y triacilglicerol (Vanbelle y Teller, 1991). MacNeel y Mersmann (1995) indican que después del suministro de AA-β, se observa un aumento en la actividad lipolítica y una disminución en la actividad lipogénica en tejido adiposo. De esta forma, Mersmann (1998) indica que después de la administración de AA-β se observa una elevación en la concentración plasmática de ácidos grasos no esterificados, lo cual confirma una actividad lipolítica que ocurre en los adipocitos.

2.5. Dosis y Toxicidad de los AA-β

Los productos AA-β autorizados no son tóxicos en las dosis recomendadas porque no producen mutagenicidad, genotoxicidad, no tienen potencial carcinogénico y no afectan el estatus reproductivo (Intervet, 2006). En el

caso específico del CZ y del clorhidrato de ractopamina, la dosis recomendada por el fabricante son 6 y 30 ppm/animal/d, respectivamente. Pruebas metabólicas indican que niveles plasmáticos de estos compuestos alcanzan sus niveles máximos entre 10 y 30 d después de haberlo consumido, tanto en bovinos como en ovinos; sin embargo, después de 24 h, de ser suspendido el suministro de AAβ, ocurre una eliminación progresiva en varios tejidos (músculo, riñón, hígado y grasa). No obstante, en el caso particular del músculo, la cantidad de residuo es eliminada de manera más rápida, ya que 80% del compuesto ya no es encontrado después de 24 h. En contraparte, los tejidos en donde ocurre más lenta eliminación, son en hígado y riñones, ya que hasta después de 72 h de ser retirado el suministro del producto, se elimina por completo. Por lo tanto, las empresas fabricantes de estos productos (MSD y Elanco), recomiendan retirar la administración del producto entre 3 y 5 d antes de enviar a los animales a sacrificio.

Sin embargo, a pesar de las normativas vigentes, existen productores que utilizan ilegalmente algún AA-β como el clembuterol, además de no seguir las recomendaciones de retiro adecuado. En México se han presentado brotes de intoxicaciones en personas que consumieron vísceras contaminadas con este AA-β. A raíz de los brotes antes citados, autoridades mexicanas (SAGARPA), a partir del 2002, formalizaron públicamente una norma emergente (NOM-EM-015-ZOO-2002) que establece las especificaciones técnicas para el control y uso de AA-β en animales suplementados con estos compuestos. Dichas normas incluyen un programa de verificación y certificación de explotaciones que utilizan estos productos como fuente promotora del crecimiento animal, estableciendo el uso de clorhidrato de zilpaterol y de ractopamina en bovinos y cerdos, respectivamente. Por otro lado, en Europa solo han autorizado el uso de estos productos como broncodilatadores en algunas especies, pero no como agentes repartidores de nutrientes y su posterior efecto como promotores del crecimiento, con lo cual evitan el riesgo de intoxicación en la población (Kuiper et al., 1998). Es importante hacer mención que el problema en salud pública se debe a una cuestión de

concentraciones de clembuterol en los alimentos consumidos, y no a una toxicidad genómica acumulable por el uso de este tipo de compuesots (Kuiper et al., 1998).

La ingesta diária admisible (IDA) es un concepto útil para evaluar cuestiones de toxicidad. La IDA sirve para calcular la concentración de resíduos en tejidos en un medicamento y sus metabolitos, tomando como factor de alimento diário ingerido un total de 300 g de carne, 300 g de grasa, 300 g de vísceras, dos huevos y un litro de leche (Elliot et al., 1995; De Wasch et al., 1998; Cerni et al., 1998). Por ejemplo, el valor de ingesta diaria admisible (IDA) del clembuterol es 0.04 μg/kg/d (Elliot et al., 1995), lo cual equivale a 2.4 μg/d para una persona de 60 kg. Para determinar el valor de IDA para clembuterol se determinó el nivel de no efecto del compuesto en 2.4 mg / día (Boenisch y Quirke, 1992). Esto indica que si se toma como referencia una ingesta diaria de 2 kg de productos cárnicos y grasa, el valor máximo en concentración de residuos será equivalente a 125 ng/kg de alimento (125 ppb), lo que sugiere que cada kg de producto de origen animal puede contener 125 ng de clembuterol activo, y con estas dosis no se presentarían reacciones adversas en el organismo.

Por su parte, en el uso de clorhidrato de ractopamina, la IDA es 230 veces mayor que en la molécula de clembuterol, calculado a partir de su administración oral y con una biodisponibilidad del 100%. Esto indica que se pueden administrar 57.5 ng de ractopamina para obtener el nivel de no efecto similar al del clembuterol. Sin embargo, considerando una biodisponibilidad del 60%, se requiere más ractopamina para lograr un nivel de no efecto similar al presentado en el clembuterol (Dalidowicz et al., 1992; Smith, 1998). Mientras que con el uso de clorhidrato de zilpaterol, para obtener un efecto ligeramente cardioestimulador y broncodilatador, se requerirán 40 mg de zilpaterol en contraste a 20 μg de clembuterol, el cual ocasionaría el mismo efecto broncodilatador y cardioestimulador (Mitchell y Glora, 1998).

2.6. Uso de AA-β en ganado ovino de carne

En ovinos productores de carne se han evaluado algunos AA-β de forma experimental; sin embargo, a pesar de que son varios los trabajos en donde evalúan el efecto anabólico en comportamiento productivo y características de la canal (Estrada- Angulo et al., 2008; Macías- Cruz et al., 2010; Avendaño Reyes et al., 2011: Lopéz-Carlo et al. 2010, 2011) su uso a nivel comercial es muy restringido. Algunos reportes indican que el uso de AA-β en ovinos provoca una disminución en la deposición de grasa y es observado un incremento en la acreción muscular (Koohmaraie et al., 1991; Avendaño-Reyes et al 2011). Ante estos cambios favorables, la composición química de la canal se ve mejorada y el peso de los cortes primarios comerciales (Baker et al., 1984). Rikhardsson et al. (1991) reportaron que en corderos Suffolk que fueron suplementados con Cimaterol observaron un incremento en el peso vivo final (15.3%), conversión alimenticia y rendimiento en canal, y una disminución del 42% en la grasa dorsal. Por su parte, el agonista L644-969 también ha sido utilizado de manera experimental por Kretchmar et al. (1990), generando una ganancia de peso mayor al 8.3%, con una mejor conformación de la canal, reducción del 30% en grasa dorsal y un incremento del 27.6% en el área del ojo de costilla (AOC). Sin embargo, la calidad de la carne es afectada en el parámetro esfuerzo al corte, ya que se aumenta de manera importante, lo que se debe a que se presenta una disminución (14.4%) en la actividad de enzimas calpainas y un incremento (59%) en la actividad de calpastatinas (inhibidor de las calpainas) (Wheeler y Koohmaraie.1992).

En la actualidad, un AA-β ampliamente utilizado es el clorhidrato de zilpaterol. Macías-Cruz et al. (2010) indican que la suplementación de CZ en corderas confinadas bajo estrés por calor (34.1 °C y 85% humedad) no generó cambios en el comportamiento productivo, longitud de la canal y porcentaje de grasa dorsal. Sin embargo, mejoró el peso de la canal caliente y fría e incrementó la conformación y el AOC. En este sentido, algunos estudios han reportado

mejoras en peso corporal, GDP, y en la eficiencia alimenticia (Avendaño-Reyes et al., 2011; López-Carlos et al., 2011, 2012), una mejoría parcial (Estrada-Angulo et al., 2008; Mondragón et al., 2010), o ningún efecto (Macías- Cruz et al., 2010) por el uso de CZ en el comportamiento productivo de corderas después de 30 a 34 días de ser alimentadas con dietas de finalización conteniendo este β-AA. En cuanto a las características de la canal, se observa que la adición de CZ en dietas de finalización de corderos Dorper × Pelibuey mejora el peso de la canal caliente, peso de la canal fría, porcentaje en el rendimiento de la canal y en el AOC de LM (López-Carlos et al., 2010, 2012; Macías-Cruz et al, 2010a.; Mondragón et al., 2010; Avendaño-Reyes et al., 2011), resultados que son atribuidos al efecto anabólico que el CZ ejerce sobre el músculo, incluyendo la hipertrofia de la fibra muscular, al cambio en la frecuencia del tipo de fibra en el músculo, a la tasa diferencial de ARN y ADN en el músculo, y a la acumulación de proteínas. En cuanto al efecto del CZ en el contenido graso, Estrada-Angulo et al. (2008) y Ríos-Rincón et al. (2010) reportaron una menor grasa KPH en canales de corderos machos cruzados Pelibuey × Katahdin suplementados con este compuesto, en comparación con las canales de corderos sin suplementar. Sin embargo, otros estudios indican que no hay efecto del uso de CZ sobre la deposición de grasa corporal en corderas de pelo (Macías-Cruz et al., 2010; Avendaño-Reyes et al., 2011). Por lo tanto, la variación en el grado de respuesta después de la suplementación de β-agonistas puede ser por efecto de la edad, especie, sexo, dieta, genotipos, entre otros factores (Mersmann, 1998). Por lo tanto, resulta de interés investigar y evaluar el impacto de estos productos en ganado ovino de pelo bajo diferentes esquemas de alimentación, épocas del año, raza etc, y aprovechar el incremento en el rendimiento en canal, y por el efecto lipolítico que se les ha atribuido a estos compuestos lograr modificar algunas características de la carne y principalmente el perfil de ácidos grasos de la misma.

2.7. Ácidos grasos dietarios en ovinos

Los rumiantes en general, y los ovinos en particular, tienen una alta proporción de ácidos grasos monoinsaturados en sus fracciones de lípidos (Boles et al., 2005). La carne de cordero tiene aproximadamente 22% de ácido palmítico, 18% de ácido esteárico y 32% de ácido oleico (Enser et al., 1996). Sin embargo, en animales rumiantes, el ácido esteárico se puede desaturar por la acción de la enzima estearoil-CoA desaturasa (SCD) y, de esta manera, convertirse en ácido oleico. Ademas, en rumiantes, hormonas como insulina puede ejercer cambios en la expresión del gen de la enzima SCD, lo cual sugiere que en ganado ovino es posible desarrollar estrategias para manipular la dieta y producir con ello tejidos con un perfil menor de ácidos grasos saturados (Daniel et al., 2004).

En ovinos, se ha evaluado la suplementación de β-AA, tales como cimaterol, clembuterol, zilpaterol y ractopamina, encontrando que estas moléculas reducen el espesor de grasa subcutánea y contenido de grasa en el músculo Longissimus de corderos (Hamby et al, 1986; López-Carlos et al., 2011) en comparción a corderos no suplementados. Tansey et al. (2004) reportan que en el adipocito, el uso de compuestos β-agonistas aumenta el catabolismo de los lípidos debido a que hay una activación de la hormona sensible a la lipasa (HSL), la cual estimula el proceso de lipólisis a través de una estimulación de la vía dependiente de cAMP, resultando en una aceleración de la hidrólisis de triglicéridos convirtiendo ácidos grasos no esterificados (NEFA) y glicerol (Beermann, 2002). Valenzuela-Grijalva et al. (2012) indican que el uso de zeranol en corderos cruzados Dorper x Black Belly provoca un incremento en la cantidad de ácidos grasos monoinsaturados en la carne. Por lo tanto, se cree que el uso de sustancias con potencial anabólico y que tienen un efecto en la lipolisis, generaran cambios moderados en el perfil lipídico muscular, ya que la composición de lípidos en el tejido de animales rumiantes en gran medida estará determinado por el metabolismo que ocurre en el rumen de los lípidos dietarios, debido a que se caracteriza por existir una intensa lipólisis, biohidrogenación de ácidos grasos y síntesis de novo por los mismos microorganismos (Harfoot y Hazlewood, 1997).

En el caso específico del efecto del consumo de alimento, reportes indican que dietas que contienen altas concentraciones de pastos aumentan el contenido de CLA cis-9, trans-11 en animales rumiantes (Dhiman et al., 1999). Sin embargo, en dietas formuladas a base de concentrados se observa un aumento en el contenido de ácido oleico en la carne de animales rumiantes (Rowe et al., 1999). En animales rumiantes, se han realizado varias modificaciones en la dieta con la finalidad de aumentar la cantidad de ácidos grasos insaturados y principalmente los niveles de CLA en la carne (Park et al., 1999; Madron et al., 2002).

En este sentido, Kott et al. (2003) reportan que alimentar ovinos con semillas de girasol provoco un incremento en el contenido de ácidos grasos insaturados y en la concentración de CLA cis-9, trans-11 en el tejido muscular. Por su parte, Bolte et al. (2002) reportaron un aumento en los niveles de ácidos grasos poliinsaturados y CLA en la carne de corderos alimentados con dietas conteniendo semillas de cártamo. Las semillas de girasol y cártamo contienen un alto contenido de CLA, lo cual indica que un aumento en los niveles de precursores de CLA en la dieta puede resultar en un incremento en el contenido de CLA en la carne. Por su parte, Daniel et al. (2004) reportan un aumento en el contenido de ácido oleico en la carne, la cual es causada por un aumento de este ácido graso en la dieta. Byers y Schelling (1993) indican que la modificación ruminal de los ácidos grasos poliinsaturados provenientes de la dieta, es parcial debido a la hidrólisis limitada de estas grasas a nivel ruminal. Por su parte, la adición de 4% de aceite hidrogenado de palma y girasol, provoca una disminución en el consumo de materia seca y en la composición de ácidos grasos C16:0, C18:1 cis-11 y C18:3, aunado a que se observa un incremento en el nivel de C18:1 trans, C18:2 y C18:3 (Manson et al., 2009). Jerónimo et al. (2009) reportan que la utilización de una mezcla de aceites ricos en ácidos grasos poliinsaturdos (girasol-linaza) es buena estrategia para la obtención de carne de cordero enriquecida con CLA y ácidos grasos poliinsaturados omega-3. Por lo tanto, el lograr una modificación en el perfil de ácidos grasos insaturados, especialmente CLA, tiene beneficios potenciales para la salud (Park et al., 1999).

2.8. Ácidos grasos en carne de ovinos

En la actualidad, la cantidad elevada de ácidos grasos saturados que trae la carne de animales rumiantes, en comparación con el contenido graso de otras fuentes proteicas, se ha convertido en un tema de preocupación debido a que se relaciona íntimamente con la etiología de obesidad, hipertensión y enfermedades coronarias en humanos. Sin embargo, el consumir ciertos tipos de ácidos grasos es importante para el organismo. En este sentido, Belury (2002) indica que el CLA tiene beneficios importantes en la salud humana a través de ejercer acción anticancerígena, antioxidante y contra el padecimiento de diabetes. Por esto, lograr alterar la composición de ácidos grasos insaturados en la carne de animales rumiantes puede originar un valor importante en la industria de producción de ganado ovino.

Como se mencionó anteriormente, el proceso de biohidrogenación genera una disminución en el efecto de suplementar ácidos grasos poliinsaturados. Sin embargo, ácidos grasos trans, como el CLA, se originan como resultado de una biohidrogenación incompleta del ácido linoleico y del ácido α-linolénico por efecto de los microorganismos ruminales involucrados en dicho proceso. Algunos reportes indican que la adición de ácidos grasos insaturados en las dietas de corderos puede provocar un aumento en el contenido de CLA y ácidos grasos poliinsaturados en músculo (Knott et al., 2003; Boles et al., 2005). Por otra parte, Bessa et al. (2005) indican que la suplementación de aceite rico en ácidos grasos insaturado y con un contenido alto de concentrado en las dietas resulta en un aumento en el ácido linoleico. Jerónimo et al. (2009) reportan que la utilización de aceites ricos en ácidos grasos insaturados (girasol y linaza) en dieta de corderos, es útil para obtener carne enriquecida con ácido linoleico conjugado (CLA) y ácidos grasos poliinsaturados omega-3. Por lo tanto, es de interés buscar y comprobar estrategias y aditivos que mejoren la calidad de la carne y modifiquen el perfil de ácidos grasos en corderos estabulados en épocas de verano e invierno, ya que existen reportes indicando que condiciones climáticas adversas de mucho calor o frio modifican el consumo de alimento (Bernabucci et al. 2009), aunado a

modificar la calidad de la carne, ya que aspectos como pH, capacidad de retención de agua, textura, color y principalmente deposición de tejido graso pueden varian dependiendo de la época de sacrificio (Miranda de la Lama et al., 2009).

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CAPÍTULO III

Comportamiento productivo y calidad de la canal (Época de Verano)

Efecto de la suplementación de clorhidrato de zilpaterol y aceite de soya en comportamiento productivo y características de la canal de corderos de raza de pelo bajo condiciones de estrés por calor

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Effects of zilpaterol hydrochloride and soybean oil supplementation on feedlot performance and carcass characteristics of hair-breed ram lambs under heat stress conditions

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3.1. ABSTRACT

Forty Dorper × Pelibuey ram lambs initially weighing 31.7 ± 2.30 kg were stratified by BW and randomly assigned to treatments under a completely randomized design with a 2 × 2 factorial arrangement of treatments to evaluate effects of zilpaterol hydrochloride (ZH; 0 or 10 mg/lamb daily) and soybean oil (SBO; 0 or 6%) on feedlot performance, carcass characteristics, and wholesale cut yield of ram lambs under heat stress conditions. After a 34-d feeding period, all lambs were harvested. Climatic conditions were of moderate heat stress (average temperature 35.7°C) for lambs during the study. Interactions ZH × SBO were not observed ($P \geq 0.11$) for any of the variables evaluated. During the first 17 d of experiment, ZH increased ($P \leq 0.05$) BW, ADG, and G:F without affecting feed intake ($P = 0.40$), but from d 18 to 34 and the entire 34-d feeding period, feedlot performance was not affected ($P = 0.18$) by ZH.Also, ZH decreased KPH, dressing percent, LM area, LM pH at 24 h postmortem, and leg perimeter ($P \leq 0.04$). Renal fat ($P = 0.03$) decreased with ZH while other noncarcass components were not affected ($P \geq 0.06$) by ZH supplementation. Leg yield ($P = 0.01$) and plain loin ($P =$

0.04) decreased with ZH and yields of other wholesale cuts were not affected ($P \geq 0.10$) by ZH. Feedlot performance ($P \geq 0.20$) and wholesale cut yield ($P \geq 0.21$) were not affected by SBO. Additionally, dressing percentage decreased ($P < 0.01$) with SBO while Other carcass characteristics ($P \geq 0.12$) were not affected by SBO. In conclusion, inclusion of both ZH and SBO in feedlot finishing diets did not improve feedlot performance, carcass characteristics, or wholesale cut yield of hair-breed ram lambs under moderate heat stress. Feedlot performance responded only to ZH and only during the first 17 d of the feeding period. In addition, some carcass characteristics of economic importance, such as dressing, LM area, and leg yield, were improved by ZH.
Key words: β-adrenergic agonist, feedlot sheep, growth rate, high temperatures, wholesale cut.

3.2. RESUMEN

Cuarenta corderos machos de la cruza Dorper × Pelibuey con peso inicial de 31.7 ± 2.30 kg se estratificaron por peso corporal y se asignaron aleatoriamente a cada tratamiento usando un arreglo factorial 2 × 2 en un diseño completamente al azar, para evaluar el efecto del clorhidrato de zilpaterol (CZ; 0 ó 10 mg/cordero/d) y aceite de soya (AS; 0 ó 6%) en el comportamiento productivo, características de la canal y rendimiento de cortes primarios de corderos confinados bajo condiciones de estrés por calor. Después de un período de suplementación de 34 d, los corderos fueron sacrificados. Las condiciones climáticas durante el periodo experimental fueron catalogadas como estrés calórico moderado (temperatura media de 35.7 °C). No se observó efecto significativo de la interacción CZ x AS ($P \geq 0.11$) en ninguna de las variables evaluadas. En los primeros 17 d del estudio, el uso de CZ incrementó ($P \leq 0.05$) el peso corporal, GDP y eficiencia alimenticia (G: F), sin afectar el consumo de alimento ($P = 0.40$); sin embargo, en el periodo comprendido del día 18 al 34 y en todo el período de alimentación (0-34 d), el comportamiento productivo no fue afectado ($P = 0.18$) por la suplementación de CZ. El uso de CZ disminuyó la grasa KPH, rendimiento de la canal, AOC de LM, pH a las 24 h *postmortem*, y el

perímetro de la pierna (P ≤ 0.04). En los componentes de la no-canal, el contenido de grasa renal (P=0.03) disminuyó con la suplementación de CZ, mientras que los demás componentes no fueron afectados (P≥ 0.06). En cortes primarios, se observó un mayor rendimiento de pierna (P=0.01) y menor de lomo liso (P=0.04) por la suplementación de CZ, mientras que los demás cortes no fueron afectados (P≥0.10) por el CZ. Por otra parte, la suplementación de AS no generó cambios en el comportamiento productivo (P≥0.20) ni en rendimiento de cortes primarios (P ≥ 0.21). El rendimiento de la canal disminuyó (P<0.01) por la suplementación de AS, mientras que las otras características de la canal (P≥0.12) no fueron afectadas. En conclusión, la adición de CZ y AS en dietas de finalización no mejora el comportamiento productivo, características de la canal ni rendimiento de cortes primarios de corderos de raza de pelo confinados bajo condiciones de estrés por calor moderado. El comportamiento productivo respondió solamente al uso de CZ y sólo durante los primeros 17 d del período de suplementación. Sin embargo, algunas características de la canal de importancia económica, como el rendimiento de la canal, AOC del músculo Longissimus y el rendimiento de la pierna, mejoraron por la suplementación de CZ.

Palabras clave: Agonista β-Adrenergico; Altas temperaturas; Comportamiento productivo; Cortes primarios; Velocidad de crecimiento.

3.3. INTRODUCTION

Heat stress conditions result in reduced feed intake by finishing lambs (Bernabucci et al., 2009), so factors such as ADG, G:F, carcass yield, and meat quality may decreased in heat-stressed animals (Marai et al., 2007).Use of β-adrenergic agonists (β-AA), such as zilpaterol hydrochloride (ZH), may be a feeding management strategy to improve ADG and carcass characteristics in lambs, even when feed intake is reduced because of heat stress. In steers, Baxa et al. (2010) reported that ZH resulted in mobilization of tissue from noncarcass (i.e., visceral organs) components and directed these nutrients to carcass tissues during periods of ZH supplementation, which translates to lower energy requirements for

growth and, consequently, lower feed intake (Reeds and Mersmann, 1991). However, studies to evaluate effects of ZH on growth and carcass traits of ram lambs under heat stress conditions are lacking. Nonetheless, Macías-Cruz et al. (2010a) found that ZH improved carcass dressing and LM area, without effects on feedlot performance using ewe lambs.

High energy dense ingredients such as vegetable oils might improve growth and carcass traits of feedlot sheep under heat stress conditions. Adding fats to diets of animals under heat stress reduces rumen heat production and improves fat deposition (West, 1999). Vegetable oils at 3 to 5% do not negatively affect rumen fermentation (Boles et al., 2005). However, oil supplementation has not been evaluated in sheep experiencing heat stress. The combination of ZH with vegetable oil supplementation in finishing diets could increase caloric intake and therefore improve performance and carcass characteristics. Therefore, the objective of this study was to evaluate effects of ZH and soybean oil (SBO) on feedlot performance, carcass characteristics, and wholesale cut yield of hair-breed ram lambs under heat stress conditions.

3.4. MATERIALS AND METHODS

All procedures involving ram lambs were conducted within the guidelines of approved local official techniques for animal care in México (NOM-051-ZOO-1995: humanitarian care of animals during mobilization; NOM- 033-ZOO-1995: slaughter of domestic and wild animals).

3.4.1. *Study Site*

The experiment was conducted during the summer season at the Sheep Experimental Unit of the Instituto de Ciencias Agrícolas (ICA), at the Universidad Autónoma of Baja California (UABC), in Mexicali Valley, located in northwestern México (114.6° N, 32.8° W). Climatic conditions in the Mexicali Valley are similar to those of the Sonoran desert, with extreme temperatures during summer (above 40°C) and winter (below 10°C) and average annual precipitation of 85 mm (García, 1985). Environmental temperatures (T) and relative humidity (RH) during the

experimental period were collected from the UABC Climatic Experimental Station, which is located 20 km from the study site. These climatic data were used to estimate the temperature-humidity index (THI) following the formula proposed by Hahn (1999) as THI = (0.81 × T) + RH (T – 14.4) + 46.4.

3.4.2. *Animal, Housing, and Treatments*

Forty Dorper × Pelibuey crossbred ram lambs (31.7 ± 2.30 kg; 4 mo of age) were individually housed in pens equipped with shade, feed troughs, and an automatic waterer. Ram lambs were adapted to pens and a basal diet, which was the same as control diet (Table 1), during a 20-d period immediately before initiating the experiment. Animals received an injection of vitamins A, D, and E (Vigantol; Bayer, México City, México; 1 mL/animal) and were treated for internal and external parasites (Invermectin; Sanfer Laboratory, Mexico City, Mexico; 0.5 mL/animal). One week before initiation of the experimental phase, lambs were individually weighed, stratified by BW, and randomly assigned to treatments within BW groups under a completely randomized design. Treatments were arranged in a 2 × 2 factorial. Factors were ZH (0 or 10 mg/ lamb daily; Zilmax; Intervet, México City, Mexico) and SBO (0 or 6% SBO/kg DM; Chef's Pride; Ventura Foods LLC, Brea, CA). Lambs fed SBO were adapted to the vegetable oil during the week before beginning the experiment while lambs fed ZH were not adapted to the β-AA. To ensure a total intake of the β-AA, ZH (133.33 g) was mixed with 19.1 kg of wheat meal, and 30 g/lamb daily of mixture was offered to lambs before the morning feeding. At the same time, groups treated without ZH were fed only with 30 g/lamb daily of wheat meal. Table 1 shows ingredients and chemical composition of experimental diets. The health status of lambs was monitored daily. The ZH was withdrawn on d 32 of the feedlot phase (i.e., 48 h before slaughter).

3.4.3. *Feedlot Performance*

The feedlot performance phase lasted 34 d. During that experimental period, diets were offered twice daily (0700 and 1700 h). The amount of feed offered and refused was weighed and recorded daily to determine feed intake. Also, feed

offered to each animal was adjusted to minimize refusals (<5.0%). Two samples per week of feed offered were collected, dried in forced-air oven at 60°C for 24 h, and stored to determine chemical composition. Dried samples were ground in a Wiley mill (2-mm screen; Wiley mill model 4; Thomas Scientific, Swedesboro, NY) and then analyzed for DM, ash, ether extract, and CP (method number 930.15, 942.05, 945.16, and 984.13, respectively; AOAC, 1990). Concentration of NDF (Van Soest et al., 1991, as modified by Ankom Technology) and ADF (Goering and Van Soest, 1970, as modified by Ankom Technology) were determined using an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). Gross energy was determined with an adiabatic bomb calorimeter (Parr 1281 Automatic Energy Analyzer; Parr Instrument Co., Moline, IL). Organic matter content was estimated as 100 – ash content. Also, ME was calculated by multiplying DE × 0.82. All lambs were individually weighed at the beginning, middle, and end of the experimental period before the morning feeding. From data collected, ADG, total BW gain (TWG), G:F, and daily feed intake were calculated. Lambs were fasted 24 h before recording the final BW. All feedlot performance variables were calculated for the following periods: d 1 to 17, d 18 to 34, and d 1 to 34.

3.4.4. *Carcass and Noncarcass Data*

After recording final BW, all lambs were harvested in the Meat Laboratory of the ICA UABC. At harvesting, blood was collected in plastic bags and weighed. Skin and head were removed from the carcass and weighed. In addition, the peritoneum, rumen, intestine, liver, lungs, heart, and renal fat were also removed and weighed. Carcasses were individually weighed to record HCW and were then chilled for 24 at 4°C to obtain cold carcass weight (CCW), carcass length, thorax depth, leg length and perimeter, and conformation based on methodology reported by Smith et al. (2001; numerical scale from 1 = bad [thinly muscled throughout] to 10 excellent [thickly muscled throughout]). Longissimus muscle pH was measured 45 min and 24 h postmortem using a portable pH meter (model HI 98140; Hanna Instruments, Woonsocket, RI) with a puncture electrode. Backfat thickness and LM were measured at the 12th rib. Finally, cooling loss (difference between HCW and

CCW) and dressing (expressing HCW as percentage of the final BW) were calculated. Also, KPH was expressed as a percentage of HCW whereas noncarcass components (head, blood, skin, heart, lungs, liver, kidney, peritoneum, renal fat, rumen, and intestine) were expressed as a percentage of final live BW.

3.4.5. *Wholesale Cut*

Carcasses were split in half, and right sides were used to obtain wholesale cuts following the methodology described by Avendaño-Reyes et al. (2011). The forequarter was divided into neck, ribs, loin, and shoulder whereas the hindquarter was divided into leg, plane loin, and sirloin. Each wholesale cut was expressed as a percentage of HCW.

3.4.6. *Statistical Analysis*

All data collected was analyzed with analysis of variance using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Data were analyzed as a 2 × 2 factorial arrangement under a completely randomized design, considering the fixed effects of factors ZH (0 or 10 mg/ lamb daily), SBO (0 or 6% SBO/kg DM), and ZH × SBO interaction with no random effects. Means were separated using pairwise comparisons (PDIFF STDERR statement). Significance was declared at $P \leq 0.05$ and tendency when $0.05 < P \leq 0.10$.

3.5 RESULTS

3.5.2. *Climatic Conditions*

Temperature, relative humidity, and THI during the experimental period were 35.72°C (28.79 to 42.42°C), 32.63% (15.45 to 54.12%), and 83.23 units (74.14 to 92.97 units), respectively (Table 2). During the first 17 d of the performance study, the environmental temperatura was warmer (37.57 vs. 33.88°C) and humidity was lower (29.03 vs. 36.24%) compared with the second 17 d. Consequently, the THI average was greater by almost 5 units for the first 17 d of the study.

3.5.2. *Feedlot Performance*

No ZH × SBO interactions were detected ($P \geq 0.11$) for feedlot performance, carcass characteristics, noncarcass components, or wholesale cut yield. Therefore, only main effects are discussed.

3.5.3. *Zilpaterol Hydrochloride*

Final BW, total gain, ADG, DMI, and G:F were not affected ($P \geq 0.18$) by ZH after a 34-d feeding period (Table 3). Body weight, total gain, ADG, and G:F were greater ($P \leq 0.05$) and DMI was not affected ($P = 0.40$) by ZH from d 1 to 17 of the feeding period. During d 18 to 34 of the feeding period no effects ($P \geq 0.72$) of ZH were observed for total gain, ADG, DMI, and G:F. Effects of ZH on carcass characteristics of ram lambs are presented in Table 4. Percentage of KPH fat decreased ($P = 0.01$) and carcass dressing percentage, LM area, and leg perimeter increased ($P \leq 0.04$) with ZH supplementation. Hot carcass weight ($P = 0.07$) and CCW ($P = 0.08$) tended to be greater in lambs receiving ZH. Additionally, LM pH at 24 h postmortem was greater ($P < 0.01$) for ZH-fed lambs. Others carcass characteristics (cooling loss, conformation, fat thickness, carcass and leg length, thorax depth, and pH of the LM at 45 min postmortem) were not affected ($P \geq 0.11$) by ZH treatment. In the case of noncarcass components, renal fat percentage decreased ($P = 0.03$) and liver percentage tended to decrease ($P = 0.06$) in ZH-fed lambs (Table 5). However, percentages of head, blood, skin, heart, lungs, kidney, peritoneum, rumen, and intestine were not affected ($P \geq 0.12$) by ZH. Effects of ZH on whole sale cuts yield are presented on Table 6. Leg yield increased ($P = 0.01$), plain loin yield decreased ($P = 0.04$), and shoulder yield tended to decrease ($P = 0.10$) for ZH-fed lambs. The yield of the Other wholesale cuts (forequarter, neck, ribs, loin, hindquarter, and sirloin) was not affected by ZH ($P \geq 0.17$).

3.5.4. *Soybean Oil*

Inclusion of SBO in finishing diets did not affect ($P \geq 0.20$) feedlot performance of lambs during d 1 to 17, d 18 to 34, and d 1 to 34 (Table 3).

Dressing percentage was the only carcass characteristic affected by SBO (Table 4) and it was decreased ($P < 0.01$) by SBO. In noncarcass components (Table 5), inclusion of SBO decreased lung and liver percentages ($P \leq 0.01$) and increased ($P = 0.02$) peritoneum percentage. The percentages of the remaining noncarcass components (head, blood, skin, heart, kidney, renal fat, rumen, and intestine) were not affected by SBO ($P \geq 0.20$). Moreover, wholesale cut yields were not affected ($P \geq 0.21$) by SBO feeding (Table 6).

3.6. DISCUSSION

3.6.1. *Climatic Conditions*

High environmental temperatures have been negatively related with growth traits and DMI in feedlot sheep (Marai et al., 2007; Macías-Cruz et al., 2010b). Animals respond to heat stress by making physiological, metabolic, and hormonal adjustments induced by thermoregulatory mechanisms to reduce body heat load (Bernabucci et al., 2009). Activation of these thermoregulatory mechanisms generally results in decreased feed intake and increased water intake and as a result decreased ADG and G:F (Marai et al., 2007). During the current study, environmental temperatures (approximately 36°C) were elevated with low levels of relative humidity (approximately 33%), which led to conditions categorized as moderate heat stress (THI of about 83 units; Marai et al., 2007). During the first 17 d of the feeding period, heat stress was considered severe as the average temperature and THI were 38°C and 86 units, respectively. Therefore, the climatic conditions prevalent during the overall course of the present study were significant enough to negatively impact feedlot performance of hair-breed ram lambs.

3.6.2. *Zilpaterol Hydrochloride*

Zilpaterol hydrochloride has been demonstrated to improve ADG and G:F during the finishing phase of feedlot cattle (Avendaño-Reyes et al., 2006; Montgomery et al., 2009). However, the effect of ZH on sheep has not been consistent. Some studies have reported improvements in BW, ADG, and G:F

(Avendaño-Reyes et al., 2011; López-Carlos et al., 2011, 2012), partial improvement (Estrada-Angulo et al., 2008; Mondragón et al., 2010), or no effects (Macías-Cruz et al., 2010a) of ZH on growth performance of sheep after 30 to 34 d of feeding with feedlot diets containing this β-AA. In general, in the present study, there was no major effect of ZH on any feedlot trait after a 34-d feeding period. This lack of ZH effect may be due to the high environmental temperatures recorded during the study. Evaluating the effect of season on productive performance of sheep fed ZH under a desert climate conditions, Macías-Cruz et al. (2012) found that ZH action depended on season, with improved performance in Winter compared with summer. Heat stress may alter effects of ZH by influencing receptor binding, signal transduction systems, or ZH transport to sites of action or by limiting the number of β2-receptors in target tissues (Mersmann, 1998; Ekpe et al., 2000). Zilpaterol hydrochloride improved BW, ADG, TWG, and G:F during the first 17 d, but these growth traits were not affected by ZH during the last 17 d of feeding. These results may be attributed to β2-receptors in muscle and adipose tissue induced by high environmental temperaturas (Ekpe et al., 2000) and an increase in the desensitization of β-adrenergic receptors over time from chronic exposure to β-AA (Beermann, 2002). In general, feed intake was not affected by ZH, which agrees with findings in previous studies under thermoneutral (López-Carlos et al., 2010; Avendaño-Reyes et al., 2011) and heat stress (Macías-Cruz et al., 2010a) conditions.

Regardless of the environmental temperature, several studies indicate that addition of ZH in finishing diets ofmDorper × Pelibuey lambs improves HCW, CCW, dressing percentage, and LM area (López-Carlos et al., 2010, 2012; Macías-Cruz et al., 2010a; Mondragón et al., 2010; Avendaño-Reyes et al., 2011), and those results have been attributed to the anabolic effect that ZH exerts on muscle including muscle fiber hypertrophy, change in muscle fiber type frequency, differential rate of muscle RNA and DNA, and protein accretion. Similar results of carcass characteristics were found in the present study although the effect of ZH on HCW and CCW was not as clear because only trends to increase these variables were observed. Additionally, carcasses from ZH-fed lambs had less

percentage of KPH fat and similar depth of fat thickness than those lambs fed without ZH. Estrada-Angulo et al. (2008) and Ríos-Rincón et al. (2010) reported lower KPH fat in carcasses from Pelibuey × Katahdin intact male lambs supplemented with ZH compared with carcasses from lambs fed without β-AA and/or with ractopamine hydrochloride. López-Carlos et al. (2010) also found that both ZH and ractopamine hydrochloride decreased fat thickness. Other studies did not report effects of ZH on body fat deposition (Macías-Cruz et al., 2010a; Avendaño-Reyes et al., 2011). Variation in the degree of response from supplementation of β agonists among different studies can be attributed to age, species, sex, diet, and genotypes, among other factors (Mersmann, 1998). The behavior of meat pH from slaughter until 24 h postmortem directly impacts meat quality. A fast fall in meat pH (pH < 6.0) during the first 45 min postmortem is associated with a low capacity for water retention and tenderness while a pH greater than 6.0 after 24 h postmortem is associated with dark, firm, and dry meat (Martínez-Cerezo et al., 2005). In the present study, the average pH of the LM after 45 min postmortem was of 6.81 ± 0.02 without effect of ZH, but pH at 24 h postmortem was greater (6.19 vs. 5.97 ± 0.07) in the LM obtained from lambs fed ZH. These results suggest that this β-AA negatively affected meat quality of hair-breed ram lambs due to alterations in the normal decrease in the pH during the first 24 h after slaughter. Similar effects of ZH on muscle pH have been previously reported for beef cattle (Avendaño-Reyes et al., 2006; Strydom et al., 2009; Hope-Jones et al., 2010). However, effects of ZH on muscle pH are limited and contradictory in sheep (Mondragón et al., 2010; López-Carlos et al., 2012). Effects of ZH on noncarcass components are consistent with other results previously published for hair-breed ewe lambs under heat stress conditions (Macías-Cruz et al., 2010a) and thermoneutral conditions (Avendaño- Reyes et al., 2011). Similarly, Holland et al. (2010) reported no effect of ZH on noncarcass components of beef steers. Reeds and Mersmann (1991) found that the β-AA receptors are mainly distributed in skeletal muscle while in smooth muscle the presence of these receptors is small, which explains why weights of rumen, omasum, abomasum, small intestine, skin, head, heart, kidney, and lungs expressed as a percentage of

final BW were not affected by ZH supplementation. The decrease in renal fat and the tendency to decrease liver weights as a percentage of final BW were the only effects of ZH on noncarcass components. With respect to renal fat, it is well documented that β-AA promote lipolysis in adipose tissue (Beermann, 2002; Birkelo, 2003; Verhoeckx et al., 2005). Liver and gut account for a disproportionate amount of whole-body energy consumption (52%) compared with relative mass (<10% of body mass; Burrin et al., 1989). The liver of ruminants is very active metabolically (Huntington and Tyrrell, 1985). Therefore, lower liver mass contributes to a decrease maintenance energy and spare energy for BW gain. Montgomery et al. (2009) hypothesized that differences in HCW and dressing percentage observed in cattle fed ZH could be due to a shift in mass from noncarcass to carcass tissue, and more substrate repartitioning in carcass vs noncarcass tissue. Even though ZH did not affect liver mass of finishing steers (Holland et al., 2010) or hair-breed ewe lambs (Avendaño-Reyes et al., 2011), decreases in liver mass have been observed as an effect of different β-AA in different species. In agreement with the present study, liver mass decreased in ram lambs fed ZH (López-Carlos et al., 2012). Lamb liver mass decreased due to the effect of cimaterol and L-644,969 (Kim et al., 1987). Liver mass of finishing Friesian steers linearly decreased with increasing L-644,969 in the diet (Moloney et al., 1990). Salbutamol decreased liver mass of swine (Hansen et al., 1994), and clenbuterol decreased liver mass in mice (Sharma et al., 1997). The effect of ZH on yield of whole cuts was evident because an increase in leg yield and a decrease in plane loin yield were detected. One possible explanation is that heat-stressed sheep redirect a great amount of blood to the legs to dissipate the body heat load, leaving other parts of the body (i.e., plane loin) with low amount of blood (Marai et al., 2007). Thus, activation of β2-receptors by ZH is greater with high availability of blood and, consequently, muscle development in that body region. Another possible explanation might be the availability of type II muscle fibers, given that Walker et al. (2010) reported that type II muscle fibers had a greater response to β-AA stimulation. In agreement withthese results, Macías-Cruz et al. (2010a) also reported an increase in leg yield by supplementation of ZH in heat-stressed ewe

lambs. However, in that study ZH decreased yield of forequarter, neck, and shoulder. This discrepancy between studies may be due to the intensity of heat stress prevailing during the 34-d feeding period.

3.6.3. *Soybean Oil*

This is the first study to evaluate the effect of a vegetable oil in finishing diets for heat-stressed lambs on performance and carcass characteristics. Diets containing greater oil levels are known to have a lower caloric increment compared to carbohydrates, reducing the amount of heat generated during feed digestion (Li and Sauer, 1994). The lower metabolic heat production leads to a lower energy expenditure for the homeothermy maintenance, making energy available for tissue growth (Najafi et al., 2012). However, inclusion of 6.0% SBO in the diet of finishing ram lambs did not affect feedlot performance after a 34-d feeding period and had only minimal effect on carcass characteristics (lower dressing in SBO lambs). Similarly, in thermoneutral conditions, Beaulieu et al. (2002) reported that addition of 5.0% of SBO did not improve DMI, growth traits, or carcass components in finishing Angus-Wagyu heifers even though a trend to decrease dressing percentage by effect of SBO supplementation was detected. Additionally, in Merino ram lambs (Manso et al., 2009), Hampshire × Dorset lambs (Radunz et al., 2009), and Mahabadi goat kids (Najafi et al., 2012), there was no effect of soybean, sunflower, or palm oils on productive performance and carcass characteristics (i.e., HCW, CCW, dressing, fat thickness, LM area, carcass length, and ultimate pH from LM). Regardless of environmental temperatures, findings of the current study and others indicate that animal performance and carcass characteristics may not be influenced by supplementation of vegetable oils when feed intake is similar from diets formulated isoenergetic with or without some type of oil. Results of the effect of vegetable oils on noncarcass components are limited in the literature. In the present study, SBO decreased lung and liver percentages and increased the peritoneum percentage. A clear explanation for these results is not available because other studies using finishing lambs (Soares et al., 2012) and pigs (Wang et al., 2011) reported no effect of SBO on noncarcass components. This

discrepancy between our study and previously published results may be due to species, amount of supplemented oil, fiber level in diet, climatic conditions, live weight, and age. Results of the effects of SBO on wholesale cut yields agree with those reported previously for sheep (Manso et al., 2009; Soares et al., 2012) and goats (Najafi et al., 2012) because SBO supplementation did not improve cut yields. The lack of effect of diet on wholesale cuts is consistent with the absence of differences in growth performance and carcass traits, and changes in these parameters are often related to changes in growth rates, age, genotype, and maturity (Manso et al., 2009). Therefore, under heat stress conditions, SBO supplementation at 6.0% in finishing diets did not affect cut yield of hairbreed finishing lambs.

In conclusion, ZH reduced KPH and increased dressing percentage and LM area without affecting feedlot performance of hair-breed ram lambs under heat stress conditions. Also, ZH improved muscle development of legs because its perimeter and yield in cut were greater in ZH lambs than in lambs fed without ZH. Additionally, supplementation of SBO had no effects on feedlot performance, carcass characteristics, or wholesale cut yields of finishing hair-breed ram lambs under heat stress.

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Table 1. Composition of experimental diets fed to hairbreed rams

Item	Treatments			
	Control	ZH[1]	SBO[2]	SBO+ZH
Ingredient, % of DM				
Wheat grain	68.0	68.0	50.5	50.5
Alfalfa hay	12.1	12.1	10.0	10.0
Wheat straw	3.0	3.0	12.0	12.0
Soybean meal	10.5	10.5	15.0	15.0
Cane molasses	5.0	5.0	5.0	5.0
White salt	0.5	0.5	0.5	0.5
Calcium phosphate	0.7	0.7	0.8	0.8
Limestone	0.2	0.2	0.2	0.2
Soybean oil	---	---	6.0	6.0
Zilpaterol hydrochloride	---	10.0	---	10.0
Chemical composition, DM basis				
DM, %	93.4	93.4	94.2	94.2
OM, %	86.9	86.9	87.2	87.2
CP, %	19.4	19.4	19.7	19.7
Ether extract, %	1.3	1.3	4.2	4.2
NDF, %	17.7	17.7	17.9	17.9
ADF, %	8.1	8.1	10.1	10.1
Ash, %	6.5	6.5	7.1	7.1
DE,[3] Mcal/kg	3.57	3.57	3.56	3.56
ME,[3] Mcal/kg	2.92	2.92	2.92	2.92
NE_m,[3] Mcal/kg	1.97	1.97	1.96	1.96
NE_g,[3] Mcal/kg	1.32	1.32	1.32	1.32

[1] ZH= Zilpaterol hydrochloride
[2] SBO= Soybean oil
[3] Calculated based on tabular energy values for individual feeding ingredients (NRC, 2007).

Table 2. Climatic conditions[1] during the 34-d feeding period

	Days of the feeding period		
Item	0 al 17	18 al 34	0 al 34
Temperatures, °C			
Maximum	44.60	40.24	42.42
Minimum	30.58	27.00	28.79
Average	37.57	33.88	35.72
Relative humidity, %			
Maximum	48.56	59.68	54.12
Minimum	12.88	18.02	15.45
Average	29.03	36.24	32.63
THI,[2] units			
Maximum	96.24	89.69	92.97
Minimum	76.30	71.95	74.14
Average	85.75	80.71	83.23

[1]Data taken from Universidad Autónoma of Baja California Climatic Experimental Station, Mexicali, BC, México.
[2] THI = temperatura-humidity index.

Table 3. Productive performance of hair-breed ram lambs supplemented with zilpaterol hydrochloride (ZH) and soybean oil (SBO) under heat stress conditions

Item	ZH, [1] mg/día			SBO, [2] %			*P-value*[3]		
	0	10	SEM	0	6	SEM	ZH	SBO	ZH x SBO
Replicates	20	20	-	20	20	-	-	-	-
Initial BW, kg	30.32	30.32	0.11	30.07	30.57	0.78	0.952	0.007	0.952
d 0 to 34									
BW at 34 d, kg	37.70	38.40	0.55	37.80	38.10	0.57	0.35	0.85	0.59
Gain, kg	7.34	8.13	0.52	7.92	7.54	0.54	0.29	0.59	0.65
ADG, kg/d	0.23	0.25	0.016	0.25	0.24	0.017	0.29	0.56	0.70
DMI,kg/d	1.03	1.03	0.02	1.03	1.04	0.02	0.91	0.76	0.25
G:F	0.22	0.24	0.013	0.24	0.22	0.013	0.18	0.42	0.98
d 1 to 17									
BW at 34 d, kg	34.4	35.4	0.34	34.6	35.2	0.39	0.05	0.20	0.51
Gain, kg	4.07	5.04	0.31	4.49	4.62	0.31	0.04	0.76	0.45
ADG, kg/d	0.25	0.32	0.019	0.28	0.29	0.019	0.03	0.75	0.45
DMI,kg/d	1.00	1.04	0.03	1.02	1.03	0.03	0.40	0.46	0.46
G:F	0.25	0.30	0.015	0.28	0.28	0.015	0.02	0.96	0.46
d 18 to 34									
Gain, kg	3.18	3.29	0.38	3.56	2.91	0.40	0.83	0.24	0.50
ADG, kg/d	0.20	0.21	0.024	0.22	0.18	0.025	0.85	0.24	0.49
DMI,kg/d	1.06	1.06	0.03	1.08	1.04	0.03	0.93	0.35	0.18
G:F	0.18	0.19	0.021	0.21	0.17	0.022	0.72	0.23	0.79

[1]ZH supplementation: lambs receiving 29.5 g/d of wheat grain containing 0 mg of ZH (0) and lambs receiving wheat grain (29.5 g/d) containing 10 mg of ZH (10).
[2] SBO supplementation: lambs receiving a diet without SBO (0) and lambs receiving a diet containing 6% of supplemental SBO (6).
[3]Probability values associated with ZH supplementation, SBO supplementation, and ZH × SBO interactions.

Table 4. Carcass characteristics of hair-breed ram lambs supplemented with zilpaterol hydrochloride (ZH) and soybean oil (SBO) under heat stress conditions

Item	ZH, mg[1]			SBO, %[2]			P-value[3]		
	0	10	SEM	0	6	SEM	ZH	SBO	ZH × SBO
Replicates	20	20	-	20	20	-	-	-	-
HCW, kg	17.4	18.3	0.34	18.0	17.8	0.34	0.07	0.62	0.55
Cold carcass weight, kg	17.1	17.9	0.33	17.6	17.4	0.33	0.08	0.72	0.52
Cooling loss, %	2.13	2.26	0.18	2.36	2.02	0.18	0.62	0.18	0.55
Conformation[4]	8.06	8.40	0.18	8.39	8.08	0.19	0.21	0.26	0.81
KPH fat, %	3.85	2.84	0.27	3.19	3.50	0.27	0.01	0.41	0.43
Fat thickness, cm	1.35	1.18	0.11	1.15	1.37	0.12	0.29	0.17	0.12
LM área, cm^2	17.5	19.1	0.50	18.9	17.7	0.50	0.03	0.12	0.73
Dressing, %	47.7	49.2	0.39	49.2	47.7	0.39	<0.01	<0.01	0.65
pH *postmortem* of LM									
45 min	6.80	6.82	0.02	6.80	6.81	0.02	0.44	0.69	0.79
24 h	5.97	6.19	0.07	6.03	6.13	0.07	0.01	0.24	0.43
Carcass length, cm	62.1	61.0	0.53	61.1	62.0	0.53	0.11	0.26	0.97
Thorax depth, cm	16.6	16.3	0.32	16.5	16.4	0.32	0.61	0.85	0.68
Leg length, cm	31.8	31.5	0.52	32.1	31.3	0.53	0.67	0.27	0.84
Leg perimeter, cm	41.8	43.5	0.63	43.1	42.2	0.63	0.04	0.29	0.41

[1]ZH supplementation: lambs receiving 29.5 g/d of wheat grain containing 0 mg of ZH (0) and lambs receiving wheat grain (29.5 g/d) containing 10 mg of ZH (10).
[2]SBO supplementation: lambs receiving a diet without SBO (0) and lambs receiving a diet containing 6% of supplemental SBO (6).
[3]Probability values associated with ZH supplementation, SBO supplementation, and ZH × SBO interactions.
[4]Ranked from 1 (bad) to 10 (excellent).

Table 5. Noncarcass components of hair-breed ram lambs supplemented with zilpaterol hydrochloride (ZH) and soybean oil (SBO) under heat stress conditions

Item, %[1]	ZH, mg/d[2]			SBO, %[3]			P-value[4]		
	0	10	SEM	0	6	SEM	ZH	SBO	ZH × SBO
Replicates	20	20	-	20	20	-	-	-	-
Head	5.61	5.70	0.11	5.65	5.66	0.12	0.53	0.90	0.14
Blood	4.10	3.93	0.09	4.00	4.03	0.08	0.17	0.78	0.56
Skin	7.40	7.06	0.20	7.35	7.12	0.20	0.23	0.41	0.56
Heart	0.42	0.40	0.42	0.41	0.41	<0.01	0.18	0.81	0.86
Lungs	1.38	1.55	0.08	1.62	1.31	0.06	0.12	0.01	0.43
Liver	1.78	1.67	0.04	1.81	1.65	0.04	0.06	0.01	0.61
Kidney	0.30	0.28	0.01	0.30	0.28	<0.01	0.16	0.25	0.11
Peritoneum	3.74	3.22	0.25	3.06	3.90	0.21	0.13	0.02	0.87
Renal fat	1.83	1.40	0.14	1.57	1.67	0.11	0.03	0.58	0.54
Rumen	2.89	2.95	0.16	2.78	3.06	0.14	0.77	0.20	0.80
Intestine	2.69	2.68	0.11	2.68	2.70	0.11	0.94	0.90	0.51

[1] Weight of each noncarcass component is expressed as a percentage of final BW.
[2] ZH supplementation: lambs receiving 29.5 g/d of wheat grain containing 0 mg of ZH (0) and lambs receiving wheat grain (29.5 g/d) containing 10 mg of ZH (10).
[3] SBO supplementation: lambs receiving a diet without SBO (0) and lambs receiving a diet containing 6% of supplemental SBO (6).
[4] Probability values associated with ZH supplementation, SBO supplementation, and ZH × SBO interactions.

Table 6. Wholesale cut yields of hair-breed ram lambs supplemented with zilpaterol hydrochloride (ZH) and soybean oil (SBO) under heat stress conditions

	ZH, mg/día[2]			SBO, %[3]			P-value[4]		
Item[1], %	0	10	SEM	0	6	SEM	ZH	SBO	ZH × SBO
Forequarter	56.77	56.16	0.28	56.53	56.41	0.28	0.167	0.784	0.658
Neck	5.75	5.88	0.26	6.04	5.59	0.26	0.726	0.213	0.660
Ribs	22.70	23.00	0.50	22.68	23.01	0.50	0.651	0.628	0.211
Loin	10.07	9.84	0.22	10.02	9.89	0.22	0.445	0.678	0.102
Shoulder	18.25	17.44	0.38	17.78	17.91	0.38	0.118	0.807	0.981
Hindquarter	43.23	43.84	0.28	43.47	43.59	0.28	0.167	0.783	0.657
Legs	23.73	25.15	0.33	24.44	24.43	0.33	0.010	0.987	0.165
Plain loin	7.36	6.60	0.23	6.83	7.13	0.23	0.041	0.402	0.357
Sirloin	12.14	12.20	0.17	12.20	12.03	0.17	0.858	0.524	0.437

[1] Weight of each wholesale cut is expressed as a percentage of HCW.
[2] ZH supplementation: lambs receiving 29.5 g/d of wheat grain containing 0 mg of ZH (0) and lambs receiving wheat grain (29.5 g/d) containing 10 mg of ZH (10).
[3] SBO supplementation: lambs receiving a diet without SBO (0) and lambs receiving a diet containing 6% of supplemental SBO (6).
[4] Probability values associated with ZH supplementation, SBO supplementation, and ZH × SBO interactions.

CAPÍTULO IV

Calidad Fisicoquímica y Sensorial (Época de Verano)

Efecto de la suplementación de clorhidrato de zilpaterol y aceite de soya en las características fisicoquímicas y sensoriales de la carne de corderos de pelo

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Effects of zilpaterol hydrochloride and soybean oil supplementation on physicochemical and sensory characteristics of meat from hair lambs

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4.1. Abstract

Forty Dorper × Pelibuey male lambs were used to obtain the Longissimus dorsi muscle to evaluate the effect of zilpaterol hydrochloride (ZH) and soybean oil (SO) on chemical (mois-ture and intramuscular fat), physicochemical quality (pH, water holding capacity [WHC], Warner Bratzler shear force [WBSF], color) and sensory attributes of meat. Lambs were indi-vidually housed in pens and assigned randomly to 1 of 4 treatments (n = 10): (1) control (no ZH or SO); (2) supplemented with ZH (10 mg/animal daily); (3) addition of 6% SO, and (4) supplemented with ZH (10 mg/animal daily) + addition of 6% SO. Feeding ZH decreased color parameters and intramuscular fat ($P < 0.05$), and increased pH and WBSF. Panelists observed a decreased in overall color, harder meat and higher content of connective tis-sue ($P < 0.05$) by ZH supplementation. Feeding SO increased ($P < 0.05$) a* value and WHC, without modifying the sensory characteristics.

Keywords: β-Adrenergic agonist, Hair lambs, Meat quality, Soybean oil, Zilpaterol

4.2. Resumen

Se usaron 40 corderos cruzados Dorper × Pelibuey para obtener el músculo *Longissimus dorsi* y evaluar el efecto del clorhidrato de zilpaterol (CZ) y aceite de soya (AS) en parámetros de calidad química (humedad y grasa intramuscular), calidad fisicoquímica (pH, capacidad de retención de agua [CRA], textura por esfuerzo al corte Warner Bratzler [WBSF] y color) y atributos sensoriales de la carne de corderos. Los corderos se alojaron en corraletas individuales y posteriormente fueron asignados al azar a 1 de 4 tratamientos (n=10): tratamiento 1 (testigo) (sin suplemento de CZ y AS); tratamiento 2: suplementación de CZ (10 mg/animal/d); tratamiento 3: adición de 6% de AS en la dieta de finalización, tratamiento 4: suplementación de CZ (10 mg/animal/d) + adición de 6% de AS en la dieta de finalización. La suplementación de CZ disminuyó los parámetros de color y grasa intramuscular ($P<0.05$), mientras que se observó un aumento en los valores de pH y WBSF. En parámetros sensoriales, los panelistas observaron una disminución en el color total, dureza de la carne y un elevado tejido conectivo ($P<0.05$) por la suplementación de CZ. Por otra parte, la suplementación de AS aumentó ($P<0.05$) el valor de a* y de CRA, sin modificar las características sensoriales.

Palabras clave: Aceite de soya; Agonista β-Adrenergico; Calidad de la carne; Cordero de pelo; Zilpaterol.

4.3. Introduction

β- Adrenergic agonists (β-AA) are classified as chemical compounds that stimulate β-adrenergic receptors of the autonomic nervous system, used as growth promoters in different domestic species in order to increase growth and feed efficiency, and improve product quality meat (Johnson and Chung, 2007). The β-AA improve weight gain and carcass characteristics of cattle and pigs

(Montgomery et al., 2009). Currently, the agonists zilpaterol hydrochloride (ZH) and ractopamine hydrochloride (RH) are approved in Mexico and United States for use in pigs and beef cattle feeding. Results of using ZH in lambs are inconclusive. In cattle, ZH increases hardness of the meat, but did not cause color changes (Avendano-Reyeset al., 2006). However, there is not information reported of other physicochemical factors involved in meat quality, which can be modified by the addition of β-AA. Large number of factors may be involved in maintaining quality. Some depend on the animal, such as breed, age, sex, handling operation (feeding-environment), and others relatedto the postmortem process, such as the method of slaughter, refrigeration and meat aging (Thompson, 2002). Today, with health problems affecting the population (obesity, cardiovascular diseases), the interest of consumer about the benefits of meat rich in essential fatty acid has been increased, and therefore the dietary supplementation of polyunsaturated fats in ruminants should be considered. Currently, saturated fat is mainly used in ruminants, while the use of oils with high content of unsaturated fatty acids is restricted, due to the fact that can impact on the flora and affect rumen digestion and feed intake (Manso et al., 2009). However, there are few reports in which the effect of oil supplementation in ruminants on physicochemical characteristics of meat quality has been evaluated. Reports indicate that an increase in unsaturated fatty acids content in the diet leads to an increased lipid oxidation in the meat and a decrease in color stability (Yang et al., 2002), but others reports indicate no changes in meat color of lambs supplemented with oils rich in polyunsaturated FA (Moloney et al., 2012). However, the combined dietary supplementation of β-AA and oils rich in polyunsaturated fatty acids and their effect on meat quality of lambs have not been studied yet. Therefore, the objective of this study was to evaluate the effect of ZH and SO on physicochemical and sensory characteristics of meat from male hair lamb sunder intensive feeding of northwestern Mexico.

4.4. Materials and methods

All procedures involving male lambs were conducted within the guidelines of approved local official technique of animal care in México (NOM-015-ZOO, 2002:

Humanitarian care of animals during mobilization; NOM-033-ZOO, 1995: Slaughter of domestic and wild animals).

4.4.1. Animal, housing, and treatments

The experiment was conducted during summer season at the Sheep Experimental Unit of the Instituto de Ciencias Agrícolas, at the Universidad Autónoma de Baja California (ICA-UABC), in Mexicali Valley, located in northwestern México (latitude 114.6° and longitude 32.8°). Climatic conditions in the Mexicali Valley are similar to those of the Sonoran desert, with extreme temperatures throughout summer (above 40°C) and winter (below 10°C). During the feeding study there was a mean temperature and mean relative humidity of 35°C and 50.4%, respectively. Forty Dorper × Pelibuey crossbred ram lambs (31.70 ± 2.30 kg, 4 months of age) were individually housed in pens equipped with shade, feed troughs and automatic waterer. Prior to experimental period, rams were adapted to basal diet and pens during 20-d. Also, animals were treated against internal and external parasites (Ivermectin, Sanfer Laboratory, Mexico City, Mexico; 0.5 mL/animal), and received an injection ofvitamins A, D, and E (Vigantol; Bayer, México City, México; 1 mL/animal). Lambs were individually weighed at the beginning of the feeding phase. Animals were randomly assigned to 1 of the following 4 treatments(n = 10): (1) control (without ZH or SO); (2) supplemented with ZH (10 mg/animal daily; ZilmaxTM, Intervet, México City, México); (3) addition of 6% SO (Chef's PrideTM; Ventura Foods LLC; Brea, CA) and (4) supplemented with 10 mg ZH + addition of 6% SO. Treatments were arranged in a 2 × 2 factorial. Factors were ZH (0 or 10 mg) and SO (0 or 6%). In order to ensure a total intake of the β-AA, ZH (133.3 g) was mixed with 19.1 kg of wheat meal, and daily 30 g of mixture/lamb was offered before the morning feeding. At the same time, groups treated without ZH were daily fed with only 30 g of wheat meal/lamb. ZH was withdrawn on the 32 day of feedlot phase (i.e. 48 h before slaughter). Therefore, the feedlot performance phase lasted 34 d. Ration for all experimental groups contained 17.4% CP and 2.7 Mcal/kgME. The ration for groups without SO,

contained wheat meal (68%), alfalfahay (12.1%), wheat straw (3%), soybean meal (10.5%), cane molasses (5%), limestone (0.20%), calcium phosphorus (0.8%), and common salt (0.5%). For experimental groups supplemented with SO, 6% of soybean oil was added, and percentages of wheat meal, alfalfa hay, wheat straw and soybean meal were changed to 50.5%, 10%, 12.0%, and 15%, respectively. Mixed ration was provided twice daily in a similar proportion. The amount of feed offered and refused was weighed and recorded daily to determine feed intake. Also, feed offered to each animal was adjusted to minimize refusals (<5.0%). The final body weight for each animal was registered too.

4.4.2. Slaughter and obtaining *Longissimus dorsi* muscle

At the end of the 34-d feeding period, lambs were slaughtered in the Meat Laboratory of the ICA-UABC following the conventional procedures. Feed and water were withdrawn 24 h before slaughter. At 24 h postmortem, m. *Longissimus dorsi* (LD) was removed from the left side of each carcass, vacuum packed and freeze at −20°C. Frozen samples were transported to the Research Laboratory of Meat Products located in CIAD AC (Centro de Investigación en Alimentación y Desarrollo AC) at Hermosillo, Sonora, Mexico, for further analysis.

4.4.3. Chemical and physicochemical analysis

Upon arrival at the meat laboratory, samples were kept frozen at −20°C. Before analyses, samples were thawed for 24 h at 4◦C and then sectioned to carry out chemical, physicochemical and sensory determinations. Sectioning started from the distal end (12th rib interface) and cranially toward the chuck end of the rib. The 1st steak (1.5 cm) was identified and used for proximate analysis, the following 2 pairs (2.54 cm each) for Warner Bratzler shear force tests and sensory analysis, respectively; a slice of 1 cm was used for color and pH. Samples to be evaluated for chemical composition were trimmed of surrounding fat and epimysium and ground to be analyzed in triplicate for moisture and intramuscular fat content following the AOAC official methods (AOAC, 1990). Results were expressed as a percentage of fresh muscle weight. Determination of pH was performed at 4°C

using a portable digital pH meter (Hanna, Model HI 98140, Woonsocket, RI, USA). Color measurements of LD muscle surfaces after 30 min blooming were carried out using a Minolta CR-400 colorimeter (Konica Minolta Sensing, Inc., Japan) with D65 illuminant and 10° in the observer. Color parameters L* (lightness), a* (redness), and b* (yellowness) were evaluated (Cassens et al., 1995). Hue angle (Hue) was calculated by the formula: Hue = tan−1 (b*/a*), and Chroma, using the formula: Chroma = $(a^* + b^*)^{½}$). Color determinations were made at 5 different locations from surface of the samples cold (4-6°C). Analysis of water holding capacity (WHC) was carried out following the procedure described by Sutton et al. (1997), which is based on centrifugation (3600 rpm × 5 min) of the meat sample. The WHC percentage was calculated based on the difference in weight of the sample before and after centrifugation. For the evaluation of Warner Bratzler shear force (WBSF) a Texture Analyzer texturometer T.A.X.T. Plus was used. To measure WBSF on LD muscle, sections of 2.5 cm thick were obtained, and cooked in an electric skillet (Cook Master Oster, model 3222-3, Mississauga, Ontario, Canada) until reaching a final internal temperature of 71°C. Once cooked, the samples were cooled (25-30°C) and chilled at 4°C for 24 h. Subsequently, the meat was cut into pieces of 1.27 cm of diameter in longitudinal direction of muscle fibers, and WBSF was determined with Warner Bratzler attachment cutter on 8 specimens by sample. The value WBSF was expressed in kg. To measure cooking loss, samples were weighed in the raw state and immediately after reaching the final cooking temperature.

4.4.4. Sensorial analysis

Sensory traits were evaluated by a trained 8-member panel (ISO8586-1, 1993). Meat samples were prepared according to AMSA (1995) guidelines. On the day before sensory evaluation, steaks were removed from the freezer and thawed out at 4°C for 24 h. Steaks were cooked following the same procedure previously described for WBSF determination. Each steak was cut into 1.27 cm × 1.27 cm thickness of cooked samples.The sensory panel evaluated cooked samples for “odor intensity”, “flavor intensity”, “fat mouth feeling”, “tenderness”, “juiciness” and

“amount of connective tissue” using a unstructured 10.0-cm line scale. This line scale was anchored on the left (0 cm) with a descriptive term representing the lowest degree of odor intensity, flavor intensity, fat mouth feeling, tenderness, juiciness, and amount of connective tissue. The right end (10.0 cm) of the scale was a descriptive term representing the highest sensorial degree for each sensory trait. Cooked samples were evaluated under soft red light while raw samples were evaluated under white light. Two visual traits, overall color and overall appearance, were evaluated on raw samples using the same type of scale.

4.4.5. Statistical analysis

Data were analyzed as a completely randomized design with a 2 × 2 factorial arrangement, using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC, USA). The model included ZH, SO, and ZH × SO interaction as fixed effects, and slaughter weight of animals as a covariate. Lamb was the experimental unit. For sensorial data, the model included also the random effect of panelist. Means comparison was performed by LSMEANS procedure. Significances were estimated at a 0.05 probability level in error type I.

4.5. Results and discussion

4.5.1. Moisture and intramuscular fat content

Moisture and intramuscular fat content are listed inTable 1. Moisture content of LD muscle was not affected (P >0.05) by ZH and SO factors, and was in the rangeof 74-75 g/100. On the other hand, ZH supplementation decreased 30.38% intramuscular fat content compared to the control group (P <0.01). This reduction is due to the anabolic and lipolytic effects of the β-AA. In the adipocyte, the β-AA increase the catabolism of lipids through the activation of hormone sensitive lipase through PKA, which degrades triglycerides into glycerol and fatty acids (Mersmann, 2002). Previous reports of the chemical analysis of muscle from lambs supplemented with β-AA are inconsistent. In this sense, Koohmaraie et al. (1996) reported no effects in any chemical value of meat, while Boler et al. (2009) reported increases in moisture and reductions in percentages of fat.

4.5.2. Physicochemical analysis

Physicochemical parameters (Table 1) were not affected by ZH×SO interaction (P >0.05). However, ZH supplementation affected (P <0.001) the color parameters. Coordinates L*, a*, b* and Chroma decreased 8.60%, 25.12%, 26.44% and 25.36%, respectively, in the meat of animals supplemented with ZH. Also, ZH increased 3.68% the pH (P <0.01), respect to not supplemented with ZH. Meat shear force increased 48.78% (P < 0.001) in the ZH group respect to control group (10.98 vs 7.38 kg). Cooking loss decreases 10.01%, but it was not significant (P >0.05). Hue values were not altered (26.35° and 26.12° for control and ZH supplemented, respectively). On the other hand, SO supplementation increase 15.61% a* value (P <0.001), Chroma 15.56% (P <0.0001), and WHC 4.15% (P <0.01), respect to animals not supplemented with SO. Meanwhile, the Other characteristics were not affected (P >0.05) by use of SO. Dietary supplementation of ZH to hair lambs affected physicochemical parameters of meat. In the current study, pH values found in LD muscle of different groups analyzed, were higher than those reported previously for sheep meat (Sañudo et al., 1996), as these authors report a normal range of 5.5-5.8 for this variable. It is important to mention that is essential to maintain the final pH value of meat within the normal range, so that other quality characteristics are not affected. Reports indicate that increasing the speed and pH drop in the meat may increase the hardness and the quantity of juice released, and therefore affects the qualityof the meat (Beriain and Lizaso, 1997). The information on the effect of β-AA on the color of lamb meat is limited. In this regard, Shackelford et al. (1992) found no change in the color of meat from lambs supplemented with cimaterol. Research indicates that the use of anabolic implants increases the appearance of darkcutters, which is observed in lower L* values (Dikeman, 2007), and this L* reduction is consistent with those found in current study. On the other hand, reports indicate that lambs implanted with zeranol did not cause changes in L*value of LD muscle (Reiling and Johnson, 2003). Although the focus of most meat color research emphasizes on a*or redness, some research has stated that b* or yellowness plays an important role in meat color (Insausti et al., 1999). It could be assumed that the small changes in

b*are just as important as substantial variations in L* and a* when are related to the discoloration of beef and changes in percentages of pigments when they are determined by the reflectance spectra (Insausti et al., 1999). A trend to pale meat has been reported in studies using β-AA, probably due to reduced heme pigmentation and to a larger proportion of fast twitch glycolytic fibers (Mersmann, 2002). However, several studies agree that supplementation of ZH could increase the L* values of LM, indicating that a brighter red lean color could be observed (Avendano-Reyes et al., 2006). Carr et al. (2005) reported a reduction in muscle color (a*, b*) of pigs fed RH, which could be due to a dilution muscle oxymyoglobin content caused by muscle fiber hypertrophy. Therefore, one possible explanation for the reduction in a* value observed in LM muscles in this study could be due to a decrease in the amount ofoxymyoglobin in muscle lambs fed ZH. Previous investigations of ZH supplementation on the color stability and shelflife of whole muscle cuts from beef cattle vary widely, with results indicating both advantages and disadvantages from ZH supplementation (Rogers et al., 2010). Respect to the increased in WBSF observed from dietary supplementation of ZH to hair lambs of present study, this effect has been a general result in studies using feedlot cattle supplemented with β-AA. Studies conducted by Strydom and Nel (1999) with ZH on cattle have shown an increased in beef LM WBSF and a decreased in sensory tenderness scores. Avendaño-Reyes et al. (2006) reported an increase of 14.09 % in WBSF in meat from cattle supplemented with ZH, which was attributable to low enzyme activity that occurs during freezing, with certain proteolytic activity and, therefore, meat tenderness is reduced (Chacón, 2004). In contrast to these results, O'Neill (2001) reported that there were no differences in tenderness between meat from steers fed ZH and controls. Dietary supplementation of SO to hair lambs affected physicochemical parameters of meat, causing an increasein the values of a*, Chroma and WHC. Moreover, some studies indicate that administration of fat affected the ruminal flora, digestion of food and consumption (Manso et al., 2009). Meanwhile, Yang et al. (2002) indicate that alterations in fatty acid composition alter the color stability. Poulson et al. (2004) found that calves fed a diet supplemented with synthetic CLA isomers had lower stability in red meat due to a

higher susceptibility to oxidation. However, the present results do not agree with those reported by Poulson et al. (2004), since the values of a* from LD muscle in this experiment were higher because of SO supplementation, without affecting WBSF.

4.5.3. Sensory analysis

Sensory analysis (Table 2) was not affected by interaction ZH×SO ($P > 0.05$). However, ZH supplementation in fresh meat decrease 6.39% ($P < 0.05$) the overall color of the LD muscle, but no differences were detected in appearance ($P > 0.05$). In cooked product, the panelists did not detect differences in odor, flavor, juiciness and fat mouth feeling ($P > 0.05$). However, in assessments related to the hardness of the meat, ZH supplementation decreased 19.74 % meat tenderness ($P < 0.01$) and increased connective tissue in 57.25% ($P < 0.01$), respect to control group. On the other hand, SO supplementation did not modify ($P > 0.05$) the sensory attributes. Schroeder (2004) reported an increase in WBSF of meat from steers supplemented with RH, but the sensory panel did not detect differences in juiciness or flavor. Studies indicate that sensory analysis realized by trained panelists, described more desirable score meat color with ZH (Hilton et al., 2009). Luno et al. (1999) reported the heifers supplemented with clembuterol, dramatically decreased sensory panel scores for both tenderness and juiciness. In the present experiment, the LD muscle sensory variables of tenderness, connective tissue and overall color decreased, however, the variables juiciness, flavor intensity and odor, showed no differences by the panel. This effect can be attributed to the fact that maybe there were no changes in LD muscle marbling by the use of zilpaterol, as thereare reports that when the trained panel detects changes in juiciness, flavor and odor is attributed to the decrease in marbling by using zilpaterol (Montgomery et al., 2009). In relation to SO supplementation, in previous studies conducted in lambs, supplementation with oils rich in polyunsaturated fatty acids did not show any differences in sensory properties of meat (Santos-Silva et al., 2004; Najafi et al., 2012). On the other hand, Madron et al. (2002) observed a higher juiciness on

meat from steers supplemented with oily seeds (extruded soybean). Beriain and Lizaso (1997) mentioned that small amounts of fat in the muscle are necessary to lubricate the fibers and promote juiciness and flavor of the cooked product. Productive strategies used to alter the fatty acid composition of beef could also alter the type of volatiles produced and therefore the aroma and flavor (Elmore et al., 2004). Wood et al. (2003) indicate that meat flavor intensity is higher when animals are supplemented with a diet rich inunsaturated fatty acids, especially linolenic acid. In present study have not been observed significant differences in parameters odor intensity, fat mouth feeling by supplementation of SO. Contrary to the provisions of this work, some authors note that the fatty acid composition of the diet can alter the type of volatiles that occur in meat and thus alter its flavor (Elmore et al., 2004).

4.6. Conclusions

Feeding ZH to Dorper × Pelibuey lambs decreased a*, b*, L* values, as well as chroma and intramuscular fat. Adding ZH lowered overall color and tenderness. Feeding SO increased the value of a*, Chroma and WHC, with no alteration of sensory variables of the *Longissimus dorsi* muscle.

4.7. References

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Table 1. Composition of experimental diets fed to hairbreed rams

Item	Treatments			
	Control	ZH[1]	SBO[2]	SBO+ZH
Ingredient, % of DM				
Wheat grain	68.0	68.0	50.5	50.5
Alfalfa hay	12.1	12.1	10.0	10.0
Wheat straw	3.0	3.0	12.0	12.0
Soybean meal	10.5	10.5	15.0	15.0
Cane molasses	5.0	5.0	5.0	5.0
White salt	0.5	0.5	0.5	0.5
Calcium phosphate	0.7	0.7	0.8	0.8
Limestone	0.2	0.2	0.2	0.2
Soybean oil	---	---	6.0	6.0
Zilpaterol hydrochloride	---	10.0	---	10.0
Chemical composition, DM basis				
DM, %	93.4	93.4	94.2	94.2
OM, %	86.9	86.9	87.2	87.2
CP, %	19.4	19.4	19.7	19.7
Ether extract, %	1.3	1.3	4.2	4.2
NDF, %	17.7	17.7	17.9	17.9
ADF, %	8.1	8.1	10.1	10.1
Ash, %	6.5	6.5	7.1	7.1
DE,[3] Mcal/kg	3.57	3.57	3.56	3.56
ME,[3] Mcal/kg	2.92	2.92	2.92	2.92
NE_m,[3] Mcal/kg	1.97	1.97	1.96	1.96
NE_g,[3] Mcal/kg	1.32	1.32	1.32	1.32

[1] ZH= Zilpaterol hydrochloride
[2] SBO= Soybean oil
[3] Calculated based on tabular energy values for individual feeding ingredients (NRC, 2007).

Table 2. Least square means for chemical (moisture and intramuscular fat) and physicochemical parameters of meat quality of hair sheep supplemented zilpaterol hydrochloride and soybean oil.

	ZH,[a] mg/día		SBO,[b] gr/100 gr DM			P-value[c]		
Item	0	10	0	6	SEM[d]	ZH	SBO	ZH x SBO
Replicates	20	20	20	20	—	—	—	
Moisture	74.56	74.45	74.82	74.19	0.53	0.81	0.16	0.46
Intramuscular fat	2.37	1.65	1.82	2.21	0.22	0.007	0.16	0.60
Cooking loss, %	21.27	19.14	20.82	19.59	1.08	0.06	0.27	0.43
Lightness (L*)	40.81	37.30	37.94	40.18	1.13	0.004	0.06	0.72
Redness (a*)	18.63	13.95	15.11	17.47	0.78	0.0001	0.005	0.47
Yellowness (b*)	9.34	6.87	7.57	8.70	0.60	0.0003	0.06	0.24
pH	5.97	6.19	6.03	6.13	0.07	0.006	0.24	0.43
WHC[e]	81.91	82.67	80.62	83.97	1.19	0.53	0.009	0.71
WBSF [f], kg	7.38	10.98	8.80	9.56	0.83	0.0001	0.37	0.94
Hue angle [g]	26.35	26.12	26.18	26.29	0.92	0.80	0.91	0.17
Chroma [h]	20.86	15.57	16.90	19.53	0.94	0.010	0.001	0.37

[a] ZH, zilpaterol hydrochloride

[b] SO, soybean oil.

[c] Probability values associated with ZH supplementation, SBO supplementation, and ZH × SBO interactions.

[d] SEM, standard error of mean.

[e] WHC, water holding capacity.

[f] WBSF, Warner Bratzler shear force.

[g] Hue = tan−1(b*/a*) × 57.29.

[h] Chroma = (a* + b*) ½

Table 3. Least square means for sensory characteristics of meat quality of hair sheep supplemented with zilpaterol hydrochloride and soybean oil.

	ZH,[a] mg/día		SBO,[b] gr/100 gr DM			P-value[c]		
Variable	0	10	0	6	SEM[d]	ZH	SBO	ZH x SBO
Replicates	20	20	20	20	—	—	—	
Overall color	7.51	7.03	7.77	7.34	0.20	0.04	0.19	0.90
Overall appearance	7.03	6.83	7.37	6.81	0.20	0.08	0.46	0.40
Odor	6.71	7.48	7.13	6.81	0.26	0.43	0.66	0.06
Flavor	7.13	7.22	7.31	6.63	0.27	0.31	0.48	0.18
Fat mouthfeeling	4.78	4.94	4.85	4.62	0.19	0.87	0.56	0.38
Tenderness	7.65	6.14	7.30	6.21	0.45	0.01	0.77	0.66
Juiciness	6.26	6.46	5.68	5.99	0.32	0.45	0.13	0.85
Connective tissue	1.31	2.06	1.59	2.65	0.34	0.02	0.25	0.67

It was used an unstructured 10.0-cm line scale. Line scale was anchored on the left (0 cm) with a descriptive term representing the lowest degree of odor intensity, flavor intensity, fat mouthfeeling, tenderness, juiciness, and amount of connective tissue. On the right end (10.0 cm) of the scale was a descriptive term representing the highest sensorial degree for each sensory trait.

[a] ZH, zilpaterol hydrochloride

[b] SO, soybean oil.

[c] Probability values associated with ZH supplementation, SBO supplementation, and ZH × SBO interactions.

[d] SEM, standard error of mean.

CAPÍTULO V

Perfil de ácidos grasos (Época de Verano)

Efecto de la suplementación de clorhidrato de zilpaterol y aceite de soya en la grasa intramuscular, perfil de ácidos grasos y contenido de colesterol de corderos de pelo bajo condiciones de estrés por calor

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Effects of zilpaterol hydrochloride and soybean oil supplementation on intramuscular fat, fatty acid profile and cholesterol content of hair lambs under heat stress conditions

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5.1. ABSTRACT

Forty Dorper x Pelibuey crossbred male lambs (31.70 ± 2.30 kg, 4 mo of age) were used to evaluate the effect of zilpaterol hydrochloride (ZH; 0 or 10 mg/lamb daily) and soybean oil (SBO, 0 or 6%) supplementation on intramuscular fat, fatty acid profile and cholesterol content of meat. The study was conducted during summer in a geographical area having desert conditions, therefore heat stress environmental conditions reached near 50°C at noon (average ambient temperature 35°C). One week before initiation of the experimental phase, lambs

were randomly assigned to treatments under a completely randomized design with a 2 x 2 factorial arrangement (n=10 animals by treatment). Animals fed with SBO were adapted to the vegetable oil during the week before beginning the experiment. Lambs were fed twice daily in a 50:50 amount proportion, respectively. The experimental period was 34 days. The ZH was withdrawn on day 32 of the feedlot phase (48 h before slaughter). Subsequently, animals were slaughtered and 24 h *postmortem*, *m. Longissimus thoracis* (LT) was removed of the left carcass, stored at -20 °C under vacuum and transported for further analysis. Before analyses, samples were thawed for 24 h at 4 °C and then sectioned to carry out intramuscular fat, fatty acid profile and cholesterol determinations. Interactions ZH x SBO were not significant (P>0.05) for any of the variables evaluated. The ZH supplementation decreased 30.38% intramuscular fat content compared to zero ZH group (P<0.01). However, ZH supplementation no affected any of the individual fatty acids (P>0.05). Meanwhile, the inclusion of ZH decreased amount MUFA (3.51%) and MUFA/SFA ratio (4.88%) (P≤0.05). On the other hand, supplementation of SBO decreased CFA (5.63%) and fatty acid C18:1 9c (7.70%) and C20:4n-6 (30.19%) (P<0.05), while TFA (141.63%) and cholesterol (35%) content was increased (P<0.05). However, Σ SFA, MUFA, PUFA, n−6, n−3; and MUFA/SFA, PUFA/SFA, and n−6/ n−3 ratios were not affected (P>0.05) by SBO. The results indicated that principal changes at lipid composition of hair lamb meat under heat stress conditions were caused by SBO supplementation, however, the changes are modest and would have minimal impact on the human health.

Abbreviations: β-AA, β-adrenergic agonist; β-AR, β-adrenergic receptors; CFA, *cis* fatty acids; DM, dry matter; FAME, fatty acid methyl ester; FID, flame ionization detector; GC, gas chromatography; HDL, high density lipoprotein; HSL, hormone sensitive lipase; KCl, potassium chloride; KOH, potassium hydroxide; LT, *Longissimus thoracis;* MUFA, monounsaturated fatty acids; MUFA/SFA, Monounsaturated fatty acids / saturated fatty acids; ME, metabolizable energy; n3, omega-3 fatty acids; n6, omega-6 fatty acids; n6/n3, omega-6 fatty acids/omega-3 fatty acids; NEFAs, non esterified fatty acids; PC, crude protein;

PUFA, poliinsaturated fatty acids; PUFA/SFA, poliinsaturated fatty acids / saturated fatty acids; RH, ractopamine hydrochloride; SFA, saturated fatty acids; SBO, soybean oil; TFA, *trans* fatty acids; ZH, zilpaterol hydrochloride.

Keywords: Cholesterol; fatty acid profile; hair lamb; intramuscular fat; soybean oil; zilpaterol.

5.2. RESUMEN

Se utilizaron 40 corderos cruzados Dorper x Pelibuey (31.70 ± 2.30 kg, de 4 meses de edad) para evaluar el efecto de la suplementación de clorhidrato de zilpaterol (CZ; 0 ó 10 mg/cordero al día) y aceite de soya (AS, 0 ó 6%) en el porcentaje de grasa intramuscular, perfil de ácidos grasos y contenido de colesterol en carne de cordero. El estudio se realizó en la época de verano en una zona desértica, por lo que las condiciones ambientales se clasificaron como estrés por calor, ya que la temperatura ambiental alcanzó 50°C a las 15:00 h (temperatura media de 35°C). Una semana antes de iniciar la fase experimental, los corderos se asignaron al azar cada tratamiento bajo un diseño completamente al azar con arreglo factorial 2 x 2 (n= 10 animales por tratamiento). Los animales que se suplementaron con AS fueron adaptados al consumo de aceite vegetal por una semana antes de iniciar el experimento. Los corderos se alimentaron dos veces al día (06:00 y 18:00 h). El periodo experimental duró 34 días. La suplementación de CZ se retiró el día 32 de la engorda (48 h antes del sacrificio). Posteriormente, los animales se sacrificaron y 24 hr *postmortem* se retiró de la canal izquierda el músculo *Longissimus thoracis,* el cual se almacenó a -20°C bajo vacío y después se transportó para su análisis. Antesdel análisis, las muestras se descongelaron por 24 h a 4°C y posteriormente seccionadas para realizar las determinaciones de grasa intramuscular, perfil de ácidos grasos y contenido de colesterol. La interacción CZ x AS no fue significativa (P>0.05) en ninguna de las variables evaluadas. La suplementación de CZ disminuyó en 30.38% el contenido de grasa intramuscular al compararlo con los animales sin suplementar (P<0.01). Sin embargo, a pesar de modificar el contenido de grasa intramuscular, la suplementación de CZ no afectó la concentración de ninguno de los ácidos grasos

individuales evaluados (P>0.05). Además, la adición de CZ disminuyó la cantidad de ácidos grasos monoinsaturados (MUFA) (3.51%) y la relación ácidos grasos monoinsaturados/ácidos grasos saturados (MUFA/SFA) (4.88%) (P<0.05). Por otro lado, la suplementación de AS redujo los ácidos grasos que están en posición *cis* (CFA) (5.63%) y del ácido graso C18: 1 9c (7.70%) y C20: 4n-6 (30.19%) (P<0.05), mientras que el contenido de ácidos grasos en posición *trans* (TFA) (141.63%) y colesterol (35.0%) fueron aumentados (P<0.05). Sin embargo, la sumatoria de ácidos grasos saturados, monoinsaturados (MUFA), poliinsaturados (PUFA), omega 6 (n-6), omega 3 (n-3), y las relaciones MUFA/SFA, PUFA/SFA y n-6/n-3 no fueron afectadas (P>0.05) por la adición de AS. De manera general, los resultados indican que los principales cambios en la composición de lípidos en la carne de cordero de pelo bajo condiciones de estrés por calor fueron a causa de la suplementación de AS. Sin embargo, los cambios presentados son modestos y tendrían un impacto mínimo sobre la salud humana.

Abreviaciones: Aceite de soya (AS); Ácidos grasos *cis* (CFA); Ácidos grasos monoinsaturados (MUFA); Ácidos grasos monoinsaturados/ácidos grasos saturados (MUFA/SFA); Ácidos grasos no esterificados (NEFA); Ácidos grasos omega-3 (n-3); Ácidos grasos omega-6 (n-6); Ácidos grasos omega-6/ácidos grasos omega-3 (n-6/n-3); Ácidos grasos poiinsaturados/ácidos grasos saturados (PUFA/SFA); Ácidos grasos poliinsaturados (PUFA); Ácidos grasos saturados (SFA); Ácidos grasos *trans* (TFA); Agonista β-adrenérgico (β-AA); Clorhidrato de ractopamina (CR); Clorhidrato de zilpaterol (CZ); Cloruro de potasio (KCl); Cromatografía de gases (CG); Detector de ionización de flama (FID); Energía metabolizable (EM); Éster metílico de ácidos grasos (FAME); Hidróxido de potasio (KOH); Hormona sensible a la lipasa sensible a hormonas (HSL); Lipoproteína de alta densidad (HDL); *Longissimus thoracis* (LT); Materia seca (MS); Proteína cruda (PC); Receptor β-adrenérgico (β-AR).

Palabras clave: Aceite de soya; Colesterol; Cordero de pelo; Grasa intramuscular; Perfil de ácidos grasos; Zilpaterol.

5.3. Introduction

Today, our society suffers from obesity problems, caused in part by a high consumption of animal fat, saturated fat to a greater extent (Moreno and Rodríguez, 2007). Fatty acid composition of red meats has received much attention as a consequence of its negative perceived implications in human health (Webb and O'Neill, 2008). Therefore nowadays, meat market demands leaner products but also with a high content of protein. As a result of this trend, animal production practices have been oriented to produce meat with these features. One strategy would be to use of growth promoters, which have led to the production of leaner meat with the additional benefits of a more efficient and reduced cost animal production. Zilpaterol hydrochloride (ZH) is an orally active β-adrenergic agonist (β-AA) approved for use in cattle feedlots in the United States (Delmore et al., 2010). Zilpaterol hydrochloride elicits a response throughout binding to β-adrenergic receptors (β-AR), which are membrane-bound located on most mammalian cells (Mills and Mersmann, 1995). The activation of protein kinase A, as elicited by β-AA, is responsible for changes in protein synthesis and degradation, particularly in skeletal muscle (Mersmann, 1998), and ZH putatively works to increase muscle growth via binding to the β-AR. Activation of β-AR in muscle and fat cells results in increased lipolysis, decreased lipogenesis, increased protein accretion, or a combination of these effects in several animal species (Poletto et al., 2009).

Health-conscious consumers are not only demanding leaner meat, but also with a higher content of essential polyunsaturated fatty acids. A strategie to achive this new goal is to supplement polyunsaturated fats in ruminants' feed. Currently fats supplemented in ruminant ration are mainly saturated, while the use of oils with a high content of polyunsaturated fatty acids is restricted, as they can cause a great impact on the flora ruminal digestion (Manson et al., 2009). Therefore, changing the composition of fatty acids in ruminants' meat is a great challenge for producers.

Today, adding β-AA to improve the feedlot performance, and supplementation of unsaturated fatty acids to modify the lipid profile of meat has

been studied. Additionaly, it has been reported that heat stress conditions results in reduced feed intake by finishing lambs (Bernabucci et al., 2009). However, so far no studies there which have been jointly prove the use of β-AA and oils rich in polyunsaturated fatty acids to assess it the lipolytic and lipogenic effect in hair lambs under heat stress conditions. Therefore, the aim of this study was to evaluate the effect of ZH and soybean oil (SBO) dietary supplementation on intramuscular fat, fatty acid profile and cholesterol content of the *Longissimus thoracis* muscle male hair lambs under heat stress environmental conditions.

5.4. Materials and Methods

All procedures involving animal handling were conducted within the approved Mexican official guidelines for animal care (NOM-051-ZOO-1995: Humanitarian care of animals during mobilization; NOM-033-ZOO-1995: Slaughter of domestic and wild animals).

5.4.1. Animal, housing, and treatments

Forty Dorper x Pelibuey crossbred male lambs (31.70 ± 2.30 kg, 4 mo of age) were used in a 34-day feeding study. The experiment was conducted at the Sheep Experimental Unit of the Instituto de Ciencias Agrícolas, at the Universidad Autónoma de Baja California (ICA-UABC), in the Mexicali Valley, north-western México (latitude 114.6º and longitude 32.8º). This geographical area has desert conditions with extreme temperatures during summer (above 40 °C) and winter (below 10 °C). The experiment was conducted during summer, therefore heat stress environmental conditions reached near 50°C at noon (average ambient temperature 35°C), relative humidity average of 32.63% and temperature-humidity index of 83.2 units. One week before initiation of the experimental phase lambs were randomly assigned to treatments arranged in a 2 x 2 factorial arrangement of treatments (n=10 animals by treatment). Factors were ZH (0 or 10 mg/lamb daily; zilmax; Intervet, México, City) and SBO (0 or 6% SBO/kg DM, Chef's Pride, Venture Foods LLC, Brea CA). All animals were treated for internal and external

parasites (Invermectin, Sanfer Laboratory, Mexico City, Mexico; 0.5 mL / animal). Lambs were individually housed in pens equipped with shade, feed throughs and waterers. Animals fed with SBO were adapted to the vegetable oil during the week before beginning the experiment, while lambs supplemented with ZH were not adapted to the β-AA. In order to ensure a total intake of ZH in the treated groups, it was mixed into 29.5 g of wheat grain and offered before providing the corresponding diet during the morning feeding. Animals groups without ZH were fed only 29.5 g of wheat grain. The experimental diets were formulated containing 190 g/kg of CP and 12.13 MJ/ ME of kg DM. Table 1 shows ingredients and chemical composition of experimental diets, and Table 2 shows their fatty acid profile. Lambs were fed twice daily in a 50:50 amount proportion, respectively. The health status of lambs was monitored daily. The ZH was withdrawn on day 32 of the feedlot phase (i.e. 48 h before slaughter, based on the technical note of zilmax; Intervet, México, City). The final BW mean of lambs ranged between 37.7-38.4 kg for all treatments.

5.4.2. Slaughter and obtaining Longissimus thoracis muscle

At the end of the 34-d feeding period, lambs were slaughtered in the Meat Laboratory of the ICA-UABC following the conventional procedures (NOM-033-ZOO-1995). Feed and water were withdrawn 24 h before slaughter. The hot carcass weigth ranged between 17 kg and 18.3 kg for all treatments. At 24 h *postmortem*, *m. Longissimus thoracis* (LT) was removed (4^{th} to 12^{th} intercostal space) of the left carcass, stored at -20 °C under vacuum and transported for further analysis at the Meat Science and Technology Lab in CIAD AC (Centro de Investigación en Alimentación y Desarrollo AC) at Hermosillo, Sonora, Mexico. Upon arrival at the Meat lab, samples were kept frozen at 20◦C. Before analyses, samples were thawed for 24 h at 4◦C and then sectioned to carry out intramuscular fat, fatty acid profile and cholesterol determinations.

5.4.3. Intramuscular fat content

The intramuscular fat content was obtained following method 991.36 of AOAC (AOAC, 2000).

5.4.4. Fatty acid profile evaluation

The lipid fraction of each sample was recovered using an adaptation of the method described by Bligh and Dyer (1959). Approximately 20 g (rounded to the nearest 1 mg) of meat samples were added to a beaker with 40 mL of a 2:1 (v/v) chloroform: methanol solution. The mixture was then filtered through a sheet of Whatman No. 541 paper, and the filtrate was recovered in a 100 mL graduated cylinder. The extraction procedure was repeated, and the two filtrates were combined. Next, 20 mL of 0.37% KCl was added to the pooled filtrates. After 8 h, the top (aqueous) layer was decanted, and the remaining fraction, solid fraction was transferred to a volumetric flask. Chloroform: methanol solution was added to the organic layer to reach a final volume of 100 mL.

Fatty acid methylation was performed according to the method of Park and Goins (1994). First, 5–10 mL of the lipid extraction was dried at 40 ◦C in a rotary evaporator under vacuum. The lipid residue was washed in a glass vial with 10 mL heptane, and 0.5 mL of 2 mol L^{-1} methanolic KOH solution was added. The vial was gently mixed and allowed to stand for approximately 4 min. Only the upper phase was removed for fatty acid methyl ester (FAME) determination. The fatty acid proportions of phospholipids and triglycerides were not measured as separate fractions.

The FAME fatty acid composition was analyzed by gas chromatography (GC) using a Hewlett Packard (Palo Alto, CA, USA) 6890 series machine with a flame ionization detector Agilent (FID) and a 6890 auto-sampler. A Supelco (Bellefonte, PA, USA) SP2560 (0.25 mm × 100 m, 0.20 µm film width) melted-capillary, silicon-based column was used. The oven temperature was programmed from an initial temperature of 150 °C (20 min) to a final temperature of 220 °C at the rate of 5 °C min^{-1}. Injector temperature was set at 250 °C, and FID temperature was adjusted to 300 °C. The chromatograms were recorded and downloaded using ChemStation software. Tridecanoic acid (13:0 Sigma-Aldrich, St Louis, MO, USA)

was used as the internal standard. The identification of fatty acids was performed according to their retention times and the elution patterns. Fatty acid data were reported as g/100 g of FAME. Total amounts of SFA, MUFA, PUFA, *trans* fatty acids (TFA) and *cis* fatty acids (CFA) were calculated, as well as the PUFA/SFA and the n-6/n-3 ratios.

5.4.5. Cholesterol content evaluation

A technique originally reported by Thompson and Merola (1993) was used to extract and quantify cholesterol. First, 5–8 mL of the lipid extract was vaporized in a water bath at a temperature below 40 °C. The residue was weighed until it reached 100±0.05 mg fat. Then, 200 µL of a 1mg mL^{-1} coprostane internal standard (Sigma- Aldrich) in ethanol was added to the residue, followed by 8 mL of 3% ethanol pyrogagol and 0.5 mL KOH (1.5 g mL^{-1}). The samples were then placed in a water bath at 80 °C for 8 min. After cooling to room temperature, 12 ml of water and 20 ml of cyclohexane were added to the samples. Then, 17 mL of supernatant was removed and dissolved in 500 mL of a derivatizing agent (bis-trimethylsilyltrifluoride acetamide, Sigma-Aldrich), and 100 µL aliquots were removed. To obtain a diluted sample (with a 2.5 dilution factor) for further analysis, 150 µL cyclohexane was added.

Cholesterol content was quantified by GC using a Hewlett Packard 6890 series chromatographer with an FID and a 6890 autosampler. A cyano-propyl-phenyl-methyl-polysiloxane capillary silicon-based column was used (Hewlett Packard 19091; 0.20 mm×25 m, 0.33 µm film). The oven was set at 260 °C and the temperature of the injection port and detector were maintained at 330 °C. The chromatograms were recorded using ChemStation software. A standard curve was constructed to quantify the concentration of cholesterol using a 1 mg mL^{-1} stock solution of cholesterol in ethanol (Sigma-Aldrich); 0.2, 0.4, 0.6, 0.8, 1.0 or 1.2 mL was added to tubes that were placed in a water bath at a temperature between 35 and 40 °C. Then, 0.5 mL of derivatizing agent and 150 µL cyclohexane were added to each sample. The samples were then injected into the chromatograph.

Chromatograms for each dilution were obtained, and cholesterol content was reported as cholesterol mg g^{-1} fresh tissue.

5.4.6 Statistical analysis

Data of intramuscular fat, fatty acid profile and cholesterol were analyzed using a completely randomized design in a 2 x 2 factorial arrangement, using the PROC MIXED procedure of SAS (SAS, 2004) according to the following statistical model: $Y = \mu + Z_i + S_j + (ZS)_{ij} + E_{ijk}$, where μ= the overall mean; Z_i = the fixed effect of zilpaterol hydrochloride supplementation (0 or 10); S_j = the fixed effect of soybean oil supplementation (0 or 6); ZS_{ij} = the interaction of zilpaterol x soybean oil; and E_{ijk} = the residual error. Significances were estimated at a 0.05 probability level for error type I. Due the interaction was not significant for any response variable was removed from the original model. Comparison of means was performed by the LSMEANS procedure.

5.5. Results

Interactions ZH x SBO were not significant (P>0.05) for intramuscular fat content, fatty acid prolife, fatty acid ratios and cholesterol content of meat. Therefore, only main effects are presented and discussed.

5.5.1. Intramuscular fat content

Intramuscular fat content (Table 3) decreased 30.38% by ZH supplementation compared to the control group (P<0.05). On the other hand, SBO supplementation no affected intramuscular fat content (P>0.05).

5.5.2. Intramuscular fatty acid profile and cholesterol content

ZH supplementation to the lambs no affected any of the individual fatty acids from the intramuscular fat (P>0.05). On the other hand, SBO supplementation changed the content of various individual fatty acids (Table 3). The content of

C18:1 n-9 t, C18:2 n-6t was increased, while the content of C18:1 9c and C20:4 n-6 was reduced by addition of SBO ($P<0.05$).

Cholesterol content only was affected by SBO supplementation. Cholesterol was increased 35% by addition of SBO to the diet of lambs.

Respect to partial sums of fatty acid and nutritional values (Table 4), the sums of monounsaturated fatty acids (Σ MUFA) decreased 3.51% ($P<0.05$) and MUFA/SFA ratio 4.88% ($P<0.05$) by ZH supplementation. However, Σ SFA, PUFA, TFA, CFA, n–6, n–3, and PUFA/SFA, and n–6/n–3 ratios were not affected by ZH ($P>0.05$).

Respect to SBO effects, TFA was twice higher ($P<0.001$), while CFA decreased 5.63% ($P<0.001$) in lambs supplemented with SBO. However, Σ SFA, MUFA, PUFA, n–6, n–3; and MUFA/SFA, PUFA/SFA, and n–6/ n–3 ratios were not affected ($P>0.05$) by SBO.

5.6. Discussion

5.6.1. Intramuscular fat content

The present study confirms the ability of ZH to reduce the percentage of intramuscular fat content in lamb meat. Supplementation of other β-AA, such as cimaterol and clenbuterol, have also been reported reduced subcutaneous fat thickness in lamb and heifers (Hamby et al., 1986; Miller et al., 1988). López-Carlos et al. (2011) reported that fat content of LM was reduced in lambs fed with ractopamine hydrochloride (RH) or ZH in comparison with control lambs. Tansey et al. (2004) indicated that in the adipocyte, the β-AA increase the catabolism of lipids by activation of a hormone sensitive lipase (HSL) that stimulates lipolysis through a cAMP-dependent process, resulting in accelerated triglyceride hydrolysis into non esterified fatty acids (NEFAs) and glycerol.

Diets containing high oil levels are known to have a lower caloric increment compared to carbohydrates, reducing the amount of heat generated during feed digestion (Li and Sauer, 1994). The lowest metabolic heat production leads to a lower energy expenditure for the homoeothermic maintenance, making energy

available for tissue growth (Najafi et al., 2012). In the present study, inclusion of SBO in the finishing diet of hair lambs did not affect intramuscular fat content after a 34-d feeding period. Radunz et al. (2009) also reported that the intramuscular fat content in meat was not affected by supplementation of SBO and linseed oil in Hampshire x Dorset lambs.

5.6.2. Fatty acid profile and cholesterol content

To our knowledge this is the first study that evaluates the effect of ZH, in finishing diets lambs, on the fatty acid prolife and cholesterol content of meat. Therefore, results are discussed and compared to previous studies performed in lamb raised under normal weather conditions and/or other ruminants.

Cholesterol content in the present study was not affected by ZH supplementation. These results contrast with those reported by Valenzuela-Grijalva et al. (2012) which indicated that zeranol implantation decreased the cholesterol content of hair lambs meat. The different response caused on the cholesterol content by the use of different animal growth promoters (implants and β-AA) in lambs finishing diets, could be explained by other factors such as sexual and climate, which need to be evaluated in future studies. No effect of ZH supplementation was detected on the amount of total saturated fatty acids in meat from heat-stressed lambs. This result was different to those reported by Ibrahim et al. (2006) and Dixon (1983) in steers with zeranol and estradiol benzoate implants, respectively, who observed an increase in the relative proportion of SFA. Contrary to the increasing effect of ZH over the amount of MUFA in meat from crossbred Dorper x Black belly lambs reported earlier by Valenzuela-Grijalva et al. (2012), in the present study ZH decreased the total MUFA content of heat-stressed lamb meat. This contradicting result may be explained due to the differences between the weather conditions in which animals were raised in each study. In the study of Valenzuela-Grijalva et al. (2012), no heat-stress conditions were prevalent during their experiment in comparison to the high temperature prevalence in the present experiment. Heat stress may alter the effects of ZH by influencing receptor binding

and signal transduction systems, or ZH transport to the sites of action, or limit the number of β2-receptors in target tissues (Mersmann, 1998; Ekpe et al., 2000).

Additionally, ZH supplementation has been reported to increase TFA content of beef (Fritsche et al., 2001), and n-6 and n-3 fatty acids in steers and non-heat stressed lamb meat (Ibrahim et al., 2006; Valenzuela- Grijalva et al., 2012). However in the current study, no effect was found in the content of these types of fatty acids.

With the purpose to guide consumers for a healthy fat intake, Health Institutions recommend that dietary PUFA/SFA ratio should be between 0.45 and 0.65 and n-6/n-3 ratio should not exceed 4.0 (Department of Health, 1994). Low values of PUFA/SFA ratio in the diet may increase the risk of cardiovascular disease. PUFA/SFA ratio values for heat-stressed lamb meat found in this study were below the healthy range proposed by the Department of Health (Department of Health, 1994). However, Hoffman et al. (2003) suggested that 0.12 can be considered a more accurate minimum healthy value for this ratio. Although ZH supplementation had no effect on omega fatty acids ratio (n-6/n-3), values were considerably above the recommended value of 4.0. These high values of the n-6/n-3 ratio were attributed to the low content of n-3 fatty acids detected in the intramuscular fat. On the other hand, in the current study the inclusion of SBO not increase the n6 / n3 ratio in the meat, possibly heat stress presented in the evaluation period causes changes in the metabolism of dietary lipids in the rumen of lambs (West, 1999; Wang et al., 2010).

In this sense, lipid composition of ruminant tissues is also determined by the metabolism of dietary lipids in the rumen, which is characterized by intense lipolysis, fatty acid (FA) biohydrogenation and novo lipid synthesis by microorganisms (Harfoot and Hazlewood, 1997). In the current study, SBO dietary supplementation decreased the proportion of C18:1 9c, while C18:1n-9t and C18:2n-6t was increased. Concentrations of C18:1n-9 and C18:2n-6t obtained in the present study in LT from SBO supplemented lambs, in general were in concordance with those reported by Bessa et al. (2005) in LT of Merino Branco

lambs fed with high concentrate diets (90% of dietary DM) containing 10% soybean oil.

Beaulieu et al. (2002) reported that increased dietary intake of C18:2n-6 did not affect the concentrations of this in loin muscle of steers fed a soybean oil supplement. On the other hand, others studies have reported unsaturated oil supplementation in beef (Gillis et al., 2004) and lamb (Bessa et al., 2005) fed high-concentrate diets resulted in greater proportions of C18:2 n-6.

Regarding cholesterol, there are no reports that evaluate the impact of soybean oil supplementation on the lipid in muscle tissue. The significant increase of cholesterol content of meat found in this study due to SBO supplementation is in concordance with the increase of serum cholesterol in heifers supplemented with coconut oil and lambs supplemented with palm oil, respectively (Bindel et al., 2000; Solomon et al., 1992). In this sense, Choi et al. (2006), observed a simultaneous increase in content of total cholesterol and HDL cholesterol in serum of lambs supplemented with 5% soybean oil. Nestel et al. (1978) proposed that an increase in dietary fat stimulated intestinal cholesterol synthesis increased absorption and transport of fat in ruminants.

In the current study, feeding SBO had no effect over total SFA content of meat. However, Lough et al. (1992) reported a reduction of saturated fatty acids (primarily due to decreased C16:0) in subcutaneous adipose and longissimus muscle in rams supplemented with canola seed. Nonetheless, Boles et al. (2005) reported that total SFA proportion in muscle and adipose tissue was not different in lambs with safflower oil supplementation in finishing diets.

Consumption of the n-3 fatty acids, particularly EPA and DHA, is important to human health (Ponnampalam et al., 2001). The recommended level of consumption for EPA and DHA for adults ranges from 0.3 to 0.65 g/d (Kris-Etherton et al., 2000). Based on the recommended levels, the low values of EPA and DHA detected in present study does not appear to be biologically important. Low values found in the current study were probably due to the high consumption of concentrate in the diets supplied. Ruminants fed with high concentrate diets and low percentage of forage, has been reported to have low levels of EPA and DHA

fatty acids in meat. Green pastures are rich souces of α-linolenic acid (18:3n-3, ALA), which may be converted to long chain omega-3 fatty acids, such as EPA and DHA, in the body tissues (Simopoulos, 1999). Recently, Ponnampalam et al. (2014a and 2014b) reported that the level of long chain polyunsaturated omega-3 fatty acid (EPA + DHA) decreased when grain was supplemented to lambs during periods of low availability of good quality pasture; and established that when different factors such as gender, sire breed, dam breed, rearing type, site, and environmental conditions were considered, nutrition was the main factor of variation of the intramuscular content of EPA and DHA fatty acids.

5.7. Conclusion

Results showed that feeding ZH to Dorper x Pelibuey cross breed lambs under heat stress decreased the content of intramuscular fat of the LT muscle. However, this reduction did not affect the fatty acid profile. The inclusion of ZH decreased amount MUFA and MUFA/SFA ratio. Supplementation with SBO increased the cholesterol content, with modest changes in fatty acid composition in meat, which would have minimal impact on the human health. New strategies should be tested to increase content of healthy fatty acids in lamb meat. The efficacy of these new strategies must be confirmed on a wide variation of environmental conditions, animal management, and other finishing systems.

5.8. References

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Table 1. Ingredients and proximate analysis and energy estimates analysis of the experimental diets provided to hair lambs**.**

Item	Control	SBO
Ingredient, % of DM		
Wheat grain	68.0	50.5
Alfalfa hay	12.1	10.0
Wheat straw	3.0	12.0
Soybean meal	10.5	15.0
Cane molasses	5.0	5.0
White salt	0.5	0.5
Calcium phosphorus	0.7	0.8
Limestone	0.2	0.2
Soybean oil	---	6.0
Chemical composition, g/kg DM basis		
DM	934	942
OM	869	872
CP	194	197
EE	13	42
NDF	177	179
ADF	81	101
Ash	65	71
DE, MJ/kg	14.93	14.89
ME, MJ/kg	12.21	12.21
NE_m, MJ/kg	8.24	8.20
NE_g, MJ/kg	5.52	5.52

SBO, soybean oil; DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergente fibre; ADF, acid detergent fibre; DE, digestible energy; ME, metabolizable energy; NE_m, net energy maintenance; NE_g, net energy gain.

Table 2. Fatty acid composition (g/100 g FAME) of the experimental diets and oils provided to hair lambs.

Item	Diets		Oilse[a]
	without SBO	With SBO	
Fatty acid			
C14:0	0.80	0.60	0.08
C16:0	19.99	15.33	10.81
C18:0	6.66	6.64	3.07
C18:1, cis 9	26.81	25.34	23.69
C18:2, n-6	41.65	46.44	53.33
C18:3, n-3	4.09	5.65	6.65
SFA [b]	27.45	22.57	15.00
MUFA [c]	26.81	25.34	24.75
PUFA [d]	45.74	52.09	60.25

SBO, soybean oil.
[a] Chef's Pride[R] ,Ventura Foods LLC, Brea, CA, USA.
[b] SFA, total saturated fatty acids.
[c] MUFA, Total monounsaturated fatty acids.
[d] PUFA, Total poliinsaturated fatty acids.

Table 3. Intramuscular fat (g/100 g tissue), fatty acid profile (g/ 100 g FAME) and cholesterol content (mg/100 g tissue) of *longissimus thoracis* muscle from male lambs supplemented with zilpaterol hydrochloride and soybean oil.

	ZH[a], mg/d			SBO[b], g/100 g			P-value[c]	
	0	10	SEM[d]	0	6	SEM	ZH	SBO
Replicates	20	20		20	20			
Intramuscular fat	2.37	1.65	0.22	1.82	2.21	0.22	0.007	0.15
Fatty acid								
C14:0	2.10	2.10	0.14	2.04	2.16	0.14	0.98	0.39
C15:1	1.18	1.52	0.20	1.51	1.19	0.20	0.10	0.12
C16:0	23.86	24.19	0.50	24.09	23.96	0.50	0.50	0.80
C16:1	2.17	2.11	0.16	2.15	2.13	0.16	0.73	0.88
C17:0	1.44	1.21	0.18	1.36	1.30	0.18	0.21	0.76
C18:0	11.78	12.38	0.55	11.61	12.54	0.55	0.27	0.10
C18:1, n-9t	3.97	3.30	0.52	2.11	5.16	0.52	0.21	0.001
C18:1, 9c	40.74	39.71	0.91	41.84	38.62	0.93	0.27	0.001
C18:2, n-6t	0.15	0.12	0.03	0.10	0.18	0.03	0.39	0.01
C18:2, n-6c	6.39	7.02	0.53	6.46	6.96	0.54	0.24	0.37
C:18:3, n-6	0.59	0.61	0.05	0.06	0.59	0.05	0.72	0.93
C18:3, n-3	0.08	0.07	0.01	0.09	0.07	0.01	0.66	0.09
C20:3, n-3	0.21	0.23	0.03	0.26	0.17	0.03	0.65	0.01
C20:3, n- 6	2.14	2.47	0.27	2.41	2.19	0.28	0.23	0.44
C20:4, n-6	0.39	0.51	0.07	0.53	0.37	0.07	0.11	0.04
C24:1	0.002	0.02	0.02	0.003	0.02	0.02	0.35	0.43
C22:6, n-3	0.14	0.25	0.11	0.14	0.26	0.11	0.34	0.30
Cholesterol	63.0	55.2	5.5	50.4	68.4	5.0	0.17	0.003

[a] Zilpaterol hydrochloride supplementation, lambs receiving 29.5 g/d of wheat grain containing 0 mg of ZH (0) and lambs receiving wheat grain (29.5 g/d) containing 10 mg of ZH (10).

[b] Soybean oil supplementation, lambs receiving a diet without SBO (0) and lambs receiving a diet containing 6 g/100 g DM of SBO (6).

[c] Probability values associated with ZH supplementation or SBO supplementation effects.

[d] SEM, standard error of mean.

Table 4. Partial sums of fatty acid and nutritional value of intramuscular fat from *longissimus thoracis* muscle of hair lambs supplemented with zilpaterol hydrochloride and soybean oil.

Item	ZH,[a] mg/d		SEM[d]	SBO,[b] g/100 gr MS		SEM	P-value[c]	
	0	10		0	6		ZH	SBO
Replicates	20	20	—	20	20	—	—	—
Partial sums								
Σ SFA[e]	40.36	40.93	0.58	40.42	40.86	0.58	0.33	0.46
Σ MUFA[f]	49.52	47.78	0.82	48.98	48.32	0.83	0.04	0.43
Σ PUFA[g]	10.11	11.29	0.81	10.59	10.81	0.82	0.15	0.79
Σ TFA[h]	4.12	3.43	0.53	2.21	5.34	0.53	0.20	<0.0001
Σ CFA[i]	47.14	46.74	0.66	48.30	45.58	0.67	0.55	0.0003
Σ n-6[j]	9.66	10.73	0.77	10.10	10.30	0.78	0.18	0.80
Σ n-3[k]	0.43	0.55	0.12	0.49	0.50	0.12	0.32	0.91
Ratios								
Σ MUFA/ ΣSFA[l]	1.23	1.17	0.03	1.22	1.18	0.03	0.05	0.33
Σ PUFA/ ΣSFA[m]	0.25	0.28	0.02	0.26	0.26	0.02	0.26	0.90
n-6/n-3	22.94	20.27	2.33	21.92	21.29	2.35	0.26	0.79

[a] Zilpaterol hydrochloride supplementation, lambs receiving 29.5 g/d of wheat grain containing 0 mg of ZH (0) and lambs receiving wheat grain (29.5 g/d) containing 10 mg of ZH (10).
[b] Soybean oil supplementation, lambs receiving a diet without SBO (0) and lambs receiving a diet containing 6% of supplemental SBO (6).
[c] Probability values associated with ZH supplementation, SBO supplementation, and ZH × SBO interactions.
[d] SEM, standard error of mean
[e] SFA, Total saturated fatty acids.
[f] MUFA, Total monounsaturated fatty acids.
[g] PUFA, Total poliinsatured fatty acids.
[h] TFA, Total trans fatty acids.
[i] CFA, Total cis fatty acids.
[j] Total omega-6 fatty acids.
[k] Total omega-3 fatty acids.
[l] Monounsaturated fatty acids / saturated fatty acids.
[m] Poliinsaturated fatty acids / saturated fatty acids.

CAPÍTULO VI

Comportamiento productivo y calidad de la canal (Época de Invierno)

Comportamiento productivo y características de la canal de corderas hembras en repuesta a la suplementación de clorhidrato de zilpaterol y aceite de soya

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Running head: Use of growth promoters in ewe lambs feeding

Feedlot performance and carcass characteristics of hairbreed ewe lambs in response to zilpaterol hydrochloride and soybean oil supplementation

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6.1. ABSTRACT

The objective of the present study was evaluate the impact of zilpaterol hydrochloride (ZH; 0 or 10 mg /lamb daily) and soybean oil (SBO; 0 or 6%) on feedlot performance, carcass characteristics and wholesale cut yield of thirty-two Dorper x Pelibuey ewe lambs initially weighing 30.55 ± 2. 57 kg, which were stratified by BW and randomly assigned to treatments under a completely randomized design with a 2 × 2 factorial arrangement. After a 32-d feeding period, all ewes were harvested. Interactions ZH × SBO were not observed (P ≥ 0.08) for

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any of the variables evaluated. In the first 16 d of experiment, feedlot performance was not affected (P ≥ 0.33) by ZH supplementation; but from d 17 to 32, ZH increased (P ≤ 0.05) total gain (kg), ADG, and G:F without affecting DMI (P ≥ 0.58). Also, ZH increased HCW, cold carcass weight, dressing, LM area, and leg perimeter (P ≤ 0.05). Lungs decreased (P = 0.03) with ZH, while other noncarcass components were not affected (P ≥ 0.12) by ZH supplementation. Yield of wholesale cuts were not affected (P ≥ 0.06) by ZH. Feedlot performance (P ≥ 0.34) and wholesale cut yield were not affected (P ≥ 0.08) by SBO. Additionally, weight of peritoneum and liver decreased (P = 0.05) with SBO, while other carcass characteristics (P ≥ 0.07) and noncarcass components (P ≥ 0.08) were not affected by SBO. In conclusion, supplementation of ZH in feedlot finishing diets improved feedlot performance only during the last 16 d of the feeding period, while some carcass traits of economic importance, such as dressing percent, LM area, and leg perimeter were also improved by ZH. On the other hand, feedlot performance, carcass characteristics, and yield of wholesale cuts were not affected by SBO supplementation.

Key words: β-adrenergic agonist, hairsheep ewes, soybean meal, carcass traits.

6.2 RESUMEN

El objetivo del presente estudio fue evaluar el impacto de la suplementación de clorhidrato de zilpaterol (CZ; 0 ó 10 mg/cordera al día) y aceite de soya (AS; 0 ó 6%) en el comportamiento productivo, características de la canal y rendimiento de cortes primarios, para lo cual se utilizaron 32 corderas hembras cruzadas con peso inicial de 30.55 ± 2 57 kg, las cuales fueron estratificadas por peso vivo inicial y asignadas aleatoriamente a los tratamientos bajo un arreglo factorial 2 × 2 en un diseño completamente al azar. Después de un período de alimentación de 32 d, todas las corderas fueron sacrificadas. No existió efecto de la interacción CZ × AS (P ≥0.08) en ninguna de las variables evaluadas. Por otra parte, en los primeros 16 días de experimentación se observa que el comportamiento productivo no se afectó (P ≥0.33) por la suplementación de CZ; sin embargo, del periodo

comprendido del día 17 al día 32, la suplementación de CZ incrementó (P≤ 0.05) la ganancia total (kg), la GDP y la eficiencia alimenticia (G: F), sin afectar el consumo de alimento (DMI) (P ≥0.58). También, la suplementación de CZ aumentó el peso de la canal caliente y canal fría, rendimiento de la canal, área de LM y perímetro de pierna (P≤ 0.05). La suplementación de CZ redujo el porcentaje de peso de los pulmones (P=0.03), mientras que los otros componentes de la no-canal no fueron afectados (P≥ 0.12) por el uso de CZ. El rendimiento de cortes primarios no se vio afectados (P≥0.06) por el uso de CZ. Por otra parte, el comportamiento productivo (P≥0.34) y el rendimiento de cortes primarios no fueron afectados (P ≥0.08) por la suplementación de AS. Además, la suplementación de AS provocó disminución del peso del peritoneo y del hígado (P=0.05), mientras que las características de la canal (P≥ 0.07) y los otros componentes de la no-canal (P ≥ 0.08) no fueron afectados por la adición de AS. En conclusión, la suplementación de CZ en dietas de finalización bajo un esquema de producción intensiva mejora el rendimiento del comportamiento productivo solamente durante los últimos 16 días del período de alimentación, mientras que algunas características de la canal, las cuales son de importancia económica como el rendimiento de la canal, área de LM, y perímetro de la pierna también se mejoran por el uso de CZ. Por otro lado, el comportamiento productivo, características de la canal y rendimiento de cortes primarios no fueron afectados por la adición de AS en dietas de finalización.

Palabras clave: aceite de soya, agonistas β-adrenérgicos, características de la canal, corderas de pelo.

6.3. INTRODUCTION

Beef production has changed in recent years. Modern techniques have been used to improve feedlot performance and carcass characteristics in some species. In this sense, interest in improving these aspects has been focused on the use of β-adrenergic agonists (β-AA). In ruminant animals, the use of β-AA causes a

substantial increase in skeletal muscle mass (Chung and Johnson, 2008). Recently, an β-AA which has been used extensively in finishing diets is zilpaterol hydrochloride (ZH), which has been attributed a potent effects to increase skeletal muscle growth (Delmore et al., 2010), with a decrease in marbling score to increase muscle hypertrophy (Kellermeier et al., 2009). In lambs, the use of ZH has focused on male lambs (Dávila-Ramírez et al., 2014; López-Carlos et al., 2011), but only few have reported data from ewe lambs (Macías-Cruz et al., 2010; Avendaño-Reyes et al., 2011). The natural differences between genders can cause differences in ZH response. In this sense, ewe lambs have reduced growth rate and carcass yield compared with male lambs (Gonçalves, 2009). Likewise, reduced in feedlot performance and some carcass characteristics (Tejeda et al., 2008), and greater fat deposition in carcasses (Lind et al., 2011) have been observed for ewe lambs compared to male lambs. Using cattle, Montgomery et al. (2009) supplemented ZH to steers and heifers and reported an increase of 36 and 18% in ADG, 28 and 21% in G:F, 11.6 and 6.7 kg in final BW, 16.4 and 12.1 in HCW, 8.23 and 6.37 cm2 in LM area, respectively. They also found a decrease of 6.2 and 2% in DMI for heifers and steers respectively, and no effect on KPH and 12th rib fat. The use of high energy dense ingredients such as vegetable oils might improve growth and carcass traits of feedlot ewe lambs. Soybean oil (SBO) is an energy dense ingredient; however, its addition to the diet exceeding 9% may negatively affect rumen fermentation or nutrient digestibility (Kucuk et al., 2004; Radunz et al., 2009). We hypothesized that the combination of ZH and SBO supplementation in sheep finishing diets could be additive and thus improve feedlot performance and carcass characteristics of ewe lambs. For these reasons, this study focuses on evaluating the effect of ZH and SBO on feedlot performance, carcass characteristics, and wholesale cut yield of hairbreed ewe lambs.

6.4. MATERIALS AND METHODS

All procedures involving ewe lambs were made following the guidelines of approved local official techniques of animal care in México (NOM-015-ZOO-2002:

Humanitarian care of animals during mobilization of animals; NOM-033-ZOO-1995: slaughter of domestic and wild animals).

6.4.1. Study Site

The experiment was conducted during winter season at the Sheep Experimental Unit of the Instituto de Ciencias Agrícolas (ICA), at the Universidad Autónoma of Baja California (UABC), in Mexicali Valley, located in northwestern México (114.6º N and 32.8º W). Climatic conditions in the Mexicali Valley are similar to those of the Sonoran desert, with extreme temperatures during summer (above 40 °C) and winter (below 10 °C), and average annual precipitation of 85 mm (García, 1985). During the study, ambient temperatures and relative humidity varied from 12-18 °C and 25 to 55%, respectively.

6.4.2. Animal, Housing, and Treatments

Thirty-two Dorper × Pelibuey crossbred ewe lambs (30.55 ± 2.57 kg, 6 mo of age) were individually housed in pens equipped with shade, feed troughs and waterer. All ewe lambs were adapted to pens and to a basal diet during a 30-d period immediately before initiating the experiment. Animals received an injection of vitamins A, D, and E (Vigantol; Bayer, México City, México; 1 mL/animal), and were treated for internal and external parasites (Invermectin, Sanfer Laboratory, México City, México; 0.5 mL/animal). One week before initiation of the experimental phase, ewe lambs were individually weighed, stratified by BW, and randomly assigned to treatments within BW groups under a completely randomized design. Treatments were arranged in a 2 × 2 factorial. Factors were ZH (0 or 10 mg/lamb daily; ZilmaxTM, MSD, México City, México) and SBO (0 or 6% SBO/kg DM; Chef´s PrideTM; Ventura Foods LLC; Brea, CA). Lambs fed SBO were adapted to the vegetable oil during the week before beginning the experiment

while lambs fed ZH were not adapted to the β-AA. To ensure a total intake of the β-AA, ZH (133.33 g) was mixed with 19.1 kg of wheat meal, and 30 g/ lamb daily of mixture was offered to lambs before the morning feeding. At the same time, groups treated without ZH were fed only with 30 g/lamb daily of wheat meal. Table 1 shows ingredients and chemical composition of experimental diets. The health status of lambs was monitored daily. The ZH was withdrawn on d 32 of the feedlot phase (i.e. 48 h before slaughter).

6.4.3. Feedlot Performance

The feedlot performance phase lasted 34 d. During that experimental period, diets were offered twice daily (0700 and 1700 h). The amount of feed offered and refused was weighed and recorded daily to determine DMI. Also, feed offered to each animal was adjusted to minimize refusals (< 5.0 %). Two samples per week of feed offered were collected, dried in forced-air oven at 60oC for 24 h, and stored to determine chemical composition. Dried samples were ground in a Wiley mill (2-mm screen; Wiley mill model 4, Thomas Scientific, Swedesboro, NY) and then analyzed for DM, ash, ether extract, and CP, (Method No. 930.15, 942.05, 945.16, and 984.13, respectively; AOAC, 1990). Concentration of NDF (Robertson and Van Soest, 1991, as modified by Ankom Technology) were determined using an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). Gross energy was determined with an adiabatic bomb calorimeter (Parr 1281 Automatic Energy Analyzer, Parr Instrument Co., Moline, IL). Organic matter content was estimated as 100 – ash content. Also, metabolizable energy was calculated by multiplying DE × 0.82. All lambs were individually weighed at the beginning, middle, and end of the experimental period before the morning feeding. From data collected, ADG, total BW gain (TWG), G:F, and DMI were calculated. Lambs were fasted 24 h before recording the final BW. All feedlot performance variables were calculated for the following periods: d 1 to 16, d 17 to 32, and d 1 to 32.

6.4.4. Carcass and Non-Carcass Data

After recording final BW, all ewe lambs were slaughtered in the Meat Laboratory of the ICA-UABC. At harvesting, blood was collected in plastic bags and weighed. Skin and head were removed from the carcass and weighted. In addition, the peritoneum, rumen, intestine, liver, lungs, heart, and renal fat were also removed and weighed. Carcasses were individually weighed to record HCW, and were then chilled for 24 h at 4 °C to obtain cold carcass weight (CCW), carcass length, thorax depth, leg length and perimeter, and conformation based on methodology reported by Smith et al. (2001; numerical scale from 1 = bad [thinly muscled throughout] to 10 excellent [thickly muscled throughout]). Longissimus muscle pH was measured 45 min and 24 h postmortem using a portable pH meter (model HI 98140; Hanna Instruments, Woonsocket, RI, USA) with a puncture electrode. Backfat thickness and LM were measured at the 12th rib. Finally, cooling loss (difference between HCW and CCW) and dressing percent (expressing HCW as percentage of the final BW) were calculated. Also, KPH was expressed as a percentage of HCW, whereas noncarcass components (head, blood, skin, heart, lungs, liver, kidney, peritoneum, renal fat, rumen and intestine) were expressed as a percentage of final live BW.

6.4.5. Wholesale Cut

Carcasses were split in half, and right sides were used to obtain wholesale cuts following the methodology described by Avendaño-Reyes et al. (2011). The forequarter was divided into neck, ribs, loin, and shoulder, whereas the hindquarter was divided into leg, plane loin and sirloin. Each wholesale cut was expressed as a percentage of HCW.

6.4.6. Statistical Analysis

All data collected were analyzed with ANOVA using the MIXED procedure of SAS (SAS Inst., Cary, NC). Data were analyzed as a 2 x 2 factorial arrangement under a completely randomized design, considering the fixed effects of factors ZH (0 or 10 mg/lamb daily), SBO (0 or 6% SBO/kg DM), and ZH × SBO interaction with no random effects. Significance was declared at $P \leq 0.05$ and tendency when $0.05 < P < 0.10$.

6.5. RESULTS

6.5.1. Feedlot Performance

Interaction between ZH and SBO was not significant ($P \geq 0.08$) for feedlot performance, carcass characteristics, noncarcass components, or wholesale cut yield. Therefore, only main effects are discussed.

6.5.2. Zilpaterol Hydrochloride

Final BW, total gain, ADG, DMI, and G:F were not affected ($P \geq 0.26$) by ZH after a 32 d feeding period or during d 0 to 16 ($P \geq 0.46$) of the feeding period (Table 2). However, total gain, ADG, and G:F were greater ($P \leq 0.03$), and DMI was not affected ($P = 0.58$) by ZH treatment during d 17 to 32 of the feeding period. Effects of ZH on carcass characteristics of ewe lambs are presented in Table 3. Hot and cold carcass weights, dressing percent, LM area, and leg perimeter were increased ($P \leq 0.01$) by ZH. Percentage of KPH fat tended to decrease ($P = 0.07$) and conformation tended to increase ($P = 0.07$) in lambs supplemented with ZH. Other carcass characteristics (cooling loss, fat thickness,

carcass and leg length, thorax depth, and pH of LM at 45 min and 24 h postmortem) were not affected (P ≥ 0. 89) by ZH treatment. Effects of ZH on noncarcass components are presented on Table 4. Lung percentage decreased (P = 0.05) as a result of ZH. However, percentage of head, skin, heart, liver, kidney, peritoneum, renal fat, rumen, blood, and intestine were not affected (P ≥ 0. 12) by ZH. Effects of ZH on yield of whole sale cuts are presented on Table 5. Percentage of forequarter yield tended to decrease (P = 0.06), and percentage of hindquarter yield tended to increase (P = 0.06) for ZH-fed lambs. However, the yield of the other wholesale cuts (neck, ribs, loin, shoulder, legs, plain loin, and sirloin) did not differe in ZH-fed and control lambs (P ≥ 0.17).

6.5.3. Soybean Oil

Inclusion of SBO in finishing diets did not affect (P ≥ 0.34) feedlot performance of ewe lambs during d 1 to 16, 17 to 32, and 0 to 32 (Table 2). The pH of the LM at 24 h postmortem increased (P = 0. 05) by SBO feeding compared to controls (Table 3). Cooling loss (P = 0.08) tended to be greater in ewe lambs receiving SBO. Other carcass characteristics were not affected (P ≥ 0. 15) by inclusion of SBO. In noncarcass components (Table 4), inclusion of SBO decreased liver and peritoneum percentages (P ≤ 0.05) compared to data obtained for control lambs. Also, skin (P = 0.09) and kidney (P = 0.08) tended to decrease with inclusion of SBO. The percentages of the remaining noncarcass components (head, blood, heart, lungs, renal fat, rumen, and intestine) did not differ (P ≥ 0. 27) between control and SBO-fed lambs. In yield of wholesale cuts, only loin percentage tended (P = 0. 08) to decrease with supplementation of SBO. The remaining percentages of wholesale cuts (forequarter, neck, ribs, shoulder, hindquarter, legs, plain loin, and sirloin) were not affected (P ≥ 0. 16) by SBO feeding (Table 5).

6.6. DISCUSSION

6.6.1. Zilpaterol Hydrochloride

In finishing diets of steers and heifers, the use of ZH on feed has been demonstrated to increase ADG, LM area, and dressing percent. It has also improved feed efficiency and carcass yield grade (Montgomery et al., 2009). The use of ZH in lambs has not induced a constant in steers and heifers response. In this sense, Aguilera-Soto et al. (2008) reported an increase in ADG and improvement in G:F using ZH in lambs, while other researchrs reported not effects in feedlot performance (López-Carlos et al., 2010; Avendaño-Reyes et al., 2011). Feeding ZH at 10 mg/animal daily during the last 30 d of feeding had no influence on performance in ewe and male lambs (Macías-Cruz et al 2010; Dávila-Ramírez et al., 2014). The null response of ZH in these experiments probably due was performed during heat-stress conditions.

Some studies in lambs have documented a decrease in ADG of 38.2% (López-Carlos et al., 2011; 0 vs. 10 d ZH groups) and increase of 25.6% (Avendaño-Reyes et al., 2011; 0 vs. 30 d ZH group) during the experimental phase, while in G:F decreased and increased 25.44% and 24.1%, respectively. López-Carlos et al. (2011) detected a 13.4% decrease in DMI, while not difference is reported by Avendaño-Reyes et al. (2011). Moreover, reports indicate an increase of 25.8% in male lambs (Aguilera-Soto et al., 2008), and 25.6% in ewe lambs (Avendaño-Reyes et al., 2011); however, greater gains have been observed in males (319 vs 304 g/d), respectively. In this sense, the use of ZH responde in male and ewe, however, is more modest in ewe lambs. Gonçalves, (2009) indicates that there physiologicas differences in male and female lambs that can cause a variable response in performance, independent of use of growth-enhancing agents. Despite physiologicas differences between the sexes, so that the use of ZH it is correct and acted as a promoter of growth, the rate of absorption from the digestive tract plays a very important role (Murdoch et al., 2005). However, rate excretion and endogenous transformation can affect β-adrenergic

agonists effect (Smith, 1998). In the particular case of ZH, the responses decreases when is preslaughter withdrawal period is prolonged, which may be related to variation in feedlot performance in varias especies. However, the use of to β-AA causes increase in muscle tissue (Johnson, 2004).

In the particular case of ZH, studies in cultured bovine satellite cells, indicate that ZH causes a decrease of the expression of β-adrenergic receptor mRNA, which may indicate that administration of ZH for a long period may result in desensitized in tissues due to an absence of receptors for these molecules (Sissom et al., 2007). Meanwhile, Winterholler et al. (2007) report that the amount of β1-AR mRNA decreased by increasing day of supplementation of ractopamine hydrochloride (RH). Regarding the β2-AR, Sillence and Matthews (1994), reported that bovine skeletal muscle cells are the most abundant receptors. Verhoeckx et al. (2005) indicate that the β1-AR and β2-AR can bind to ZH, however, the β2-AR have more affinity. Vasconcelos et al. (2008) reported that supplementation of ZH for more than 20 d on feedlot cattle results in low feedlot performance and en carcass traits, however, this response is not due to a downregulation of the β-AR. Therefore, a desensitization or internalization of the β-AR may be the cause of performance attenuation which is observed when using ZH for a long period of evaluation (Benovic, 2002). Moreover, reports indicate that prolonged β-AA exposure not maintain increases in protein synthesis, and due a no activation of satellite cells DNA by the β-AA (Johnson and Chung, 2007).

Supplementation of β-AA usually causes an increase of carcass weight and dressing percent. This is due to the β-AA stimulate muscle hypertrophy, causing in proteins, changes in the synthesis (increase) and degradation (decrease) (Moody et al., 2000; Dikeman, 2007). Another important effect that has been observed to be supplemented ZH in cattle is a reduction in fat thickness (Vasconcelos et al., 2008; Montgomery et al., 2009). The changes observed by supplementation of β-AA may be due to that these compounds modify redirection of nutrients, which cause muscle protein synthesis increase (Mersmann, 1998), and promote lipolysis in adipose tissue (Verhoeckx et al., 2005). In the present study, ZH increased

HCW and LM area. These findings agree with previous studies by Avendaño-Reyes et al. (2011) and Macías-Cruz et al. (2010) in which the supplementation of ZH causes carcass weights increased in lambs. However, Dávila-Ramírez et al. (2014) evaluated the supplementation of ZH in lambs to near the end of the feeding period, were not reporting increase in carcass weight. Lopez-Carlos et al. (2012) in male lambs documented an increase of 9.2% in HCW and 17.4% in LM area by ZH (6 mg/kg of DM during 30 d). In ewes lambs, Avendaño-Reyes et al. (2011) reported an increase of 15.1% in HCW and 34.5% in LM area (10 mg/animal daily). These reports indicate that independently of sex, the supplementation of ZH has a clear impacts carcass tissue gain. However, despite this clear impact in lambs are less consistent. In this sense, the supplementation for 56 d of 4.5 or 6.7 mg / kg of DM of ZH in Pelibuey lambs not unaffected cold carcass weight, dressing percent, LM area, and fat thickness (Félix et al., 2005). Meanwhile, in Rambouillet lambs supplemented with ZH, Aguilera- Soto et al. (2008) report increases for cooling loss.

Regarding the hot carcass weight, Avendaño-Reyes et al. (2011) and Macías-Cruz et al. (2010) reported increase from 3.7 to 4.5%. In the present study, the HCW increase 2.9% by supplementation of ZH. The increase in HCW was greater than the presented in final BW when ZH was supplemented. Several factors may be involved in this behavior. In this sense, reports indicate that the differences observed in dressing percent and HWC by supplementation ZH, could be due to a change in mass from noncarcass to carcass tissue, or by a decrease in gull fill caused by a decrease in DMI (Montgomery et al., 2009). Studies indicate that in every cell type the β-AA are present, and upon the occurrence of this process, a large number of metabolic and physiological functions are controlled exceptionally (Mersmann, 2002). Regarding the weight of visceral organs, previous reports in ewe lambs agreed with those reported by Avendaño-Reyes et al. (2011), those indicate that the β-AA supplementation was not altered or was reduced the weight of visceral.

In the present study, wholesale cut yields of hindquarter and forequarter did not responde to ZH. Results that do not match those reported by Smith et al. (1995), as indicating that β-AA supplementation generates a large response in type II muscle fibers, and the muscles that contain a high amount of these fibers have a development due to this response. Muscle development by ZH supplementation is probably because this compound increases in larger muscle fibers due to the transcription of various MHC (myosin heavy chain) mRNA (Smith et al., 1995). In this sense, the use of ZH in feedlot steers increased weight (expressed as a percentage of carcass weight) of single subprimals, while others subprimals decreased by ZH administration (Plascencia et al., 1999). Today, they have reported inconsistencies in the procedures the ZH after administration, however, tissue specific and endocrine changes, may be related to modifications in fat and muscle metabolism (Gilson et al., 1996). Although, both alpha and beta-adrenergic receptors are present in ovine adipose tissue (Watt et al., 1991), variations in the sensitivity and responsiveness of individual adipose depots to cathecholamines stimulation have been demonstrated in lambs. Because of these inconsistencies in animals after ZH supplementation, further research should be conducted to try to understand because these modifications occur.

6.6.2. Soybean Oil

In rumiants, the fat supplementation had variable effects on nutrients digestibility, feedlot performance, and body composition. This variability in response may be due to the level of fat in the diet and used mainly the source of fat, which can directly affect the ruminal microbes (Abou Ward et al., 2008). In this sense, Khorasani et al. (1992) indicate that dietary inclusion of fats reduced energy available to bacteria, therefore fat digestión in the rumen is limited, resulting in a decrease in microbial protein synthesis and production of volatile fatty acids (Khorasani et al., 1992). Other reports indicate that supplementation of safflower sedes causes no changes in DMI, however, indicate a slight increase in feedlot performance when diets se suplementaron up to 6% dietary oil (Kott et al., 2003). However, Bolte et al. (2002) and Boles et al. (2005) evaluated the addition oil diets

in finishing lambs, not reporting effects in feedlot performance. Lipid rich in n-3 PUFA supplementation could influence feed intake, but data have been controversial (Ponnampalam et al., 2001). In carcass quality lambs, the effect by the addition of unsaturated oil in finishing diets (high content of grain), our results agree with those reported by Bolte et al. (2002) and Bessa et al. (2005), which found no effect in subjective measurements (marbling and conformation score) and intramuscular fat content, respectively.

Specifically, the addition of SBO in this study had no effect on carcass characteristics. In agreement with Boles et al. (2005) indicate that the addition of oil in finishing diets (high- concentrate) of lambs , there is no effect on HWC , fat thickness , and LM area. Moreover, the addition of SBO in diets of lambs not causes changes in backfat thickness or in KPH. In this sense, reports indicate no changes in the fat thickness in lambs by supplementation of fish oil (Ponnampalam et al., 2001). Meanwhile, Wistuba et al. (2006) suggest that fish oil supplementation in ruminants cause decreased subcutaneous fat. In this study, fat depots decreased by supplementation of SBO, KPH fat decreased 7.18%, fat thickness 4.88%, and peritoneum 21.35% with respect to non-supplemented lambs. Dávila-Ramírez et al. (2014) in a similar experiment with lambs of the same age but opposite sex, reported an increase in fat deposits of 9.72% in KPH fat, 19.13% in fat thickness, 27.45% in peritoneum, and renal fat in 6.37% by effect of SBO supplementation. This performance was not expected because females are more efficient in fat metabolism due to early growth, lower adult weight, and greater fat accumulation resulting from the action of their sex hormones. The reason for these differences in lipid deposition and metabolism by supplementation of SBO are not yet fully understood completely.

In conclusion, ZH showed an impact on carcass traits reducing KPH and increasing HCW, dressing percent, conformation, leg perimeter, and LM area without affecting feedlot performance of hair-breed ewe lambs. Also, the wholesale cuts forequarter and hindquarter tended to be greater in ZH -fed lambs than in lambs fed no ZH. Additionally, supplementation of SBO tended to decrease the

weight of some noncarcass components and had no effect on feedlot performance, carcass characteristics, or wholesale cut yields of finishing hair-breed ewe lambs. However, the combination of ZH and SBO supplementation in finishing hair-breed ewe lams was not additive.

6.7. LITERATURE CITED

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Table 1. Ingredients and chemical analysis of the experimental diets provided to ewe lambs

Item	Treatments			
	Control	ZH	SO	SO+ZH
Ingredient, % of DM				
Wheat grain	68.0	68.0	50.5	50.5
Alfalfa hay	12.1	12.1	10.0	10.0
Wheat straw	3.0	3.0	12.0	12.0
Soybean meal	10.5	10.5	15.0	15.0
Cane molasses	5.0	5.0	5.0	5.0
White salt	0.5	0.5	0.5	0.5
Calcium phosphorus	0.7	0.7	0.8	0.8
Limestone	0.2	0.2	0.2	0.2
Soybean oil	---	---	6.0	6.0
Zilpaterol hydrochloride	---	10.0	---	10.0
Chemical composition, DM basis				
DM, %	93.4	93.4	94.2	94.2
OM, %	86.9	86.9	87.2	87.2
CP, %	19.4	19.4	19.7	19.7
EE, %	1.3	1.3	4.2	4.2
NDF, %	17.7	17.7	17.9	17.9
ADF, %	8.1	8.1	10.1	10.1
Ash, %	6.5	6.5	7.1	7.1
DE, Mcal/kg[1]	3.57	3.57	3.56	3.56
ME, Mcal/kg[1]	2.92	2.92	2.92	2.92
NE_m, Mcal/kg[1]	1.97	1.97	1.96	1.96
NE_g, Mcal/kg[1]	1.32	1.32	1.32	1.32

Control = ewe lambs fed without Zilpaterol Hydrochloride (ZH) or Soybean oil (SO); ZH = ewe lambs fed 10 mg ZH/animal/d; SO = ewe lambs fed 6% SO; and SO+ZH = ewe lambs fed 10 mg/animal/d plus 6% SO. [1]Calculated based on tabular energy values for individual feeding ingredients (NRC, 2007).

Table 2. Productive performance of ewe lambs supplemented with zilpaterol hydrochloride (ZH) and soybean oil (SO).

Item	ZH, mg/d[1]			SBO, %[2]			P-value[3]		
	0	10	SEM	0	6	SEM	ZH	SBO	ZH × SBO
Initial BW, kg	30.51	30.59	0.23	30.51	30.59	0.23	0.74	0.74	0.67
d 0 to 32									
BW at 32 d, kg	35.58	36.23	0.56	35.77	36.04	0.56	0.26	0.63	0.19
Gain, kg	5.07	5.64	0.52	5.26	5.46	0.52	0.29	0.71	0.11
ADG, kg/d	0.17	0.19	0.02	0.17	0.18	0.02	0.29	0.71	0.12
Feed Intake, kg/d	1.12	1.14	0.04	1.12	1.14	0.04	0.75	0.61	0.89
G:F	0.15	0.16	0.013	0.16	0.16	0.013	0.42	0.97	0.08
d 1 to 16									
BW at 16 d, kg	32.52	32.77	0.34	32.59	32.70	0.34	0.46	0.76	0.68
Gain, kg	2.01	2.13	0.41	2.06	2.07	0.41	0.77	0.98	0.59
ADG, kg/d	0.14	0.15	0.029	0.148	0.148	0.029	0.77	0.99	0.59
Feed intake, kg/d	1.063	1.064	0.05	1.065	1.062	0.05	0.99	0.97	0.81
G:F	0.14	0.14	0.026	0.14	0.14	0.026	0.92	0.99	0.56
d 17 to 32									
Gain, kg	3.06	4.04	0.37	3.50	3.60	0.37	0.02	0.79	0.15
ADG, kg/d	0.19	0.25	0.02	0.22	0.22	0.02	0.02	0.79	0.15
Feed Intake, kg/d	1.18	1.20	0.05	1.17	1.21	0.05	0.58	0.34	0.63
G:F	0.16	0.21	0.019	0.19	0.19	0.019	0.03	0.89	0.23

[1]ZH supplementation = ewe lambs receiving 37.31 g/d of wheat grain containing 0 mg of ZH (0) and ewe lambs receiving wheat grain (37.31 g/d) containing 10 mg of ZH (10).
[2]SO supplementation = ewe lambs receiving a diet without SO (0) and ewe lambs receiving a diet containing 6% of supplemental SO (6).
[3]Probability values associated with ZH supplementation (ZH), SO Supplementation (SO), and ZH × SO interactions (ZH × SO).

Table 3. Carcass characteristics of ewe lambs supplemented with zilpaterol hydrochloride (ZH) and soybean oil (SO) under heat stress conditions.

Item	ZH, mg[1]			SBO, %[2]			P-value[3]		
	0	10	SEM	0	6	SEM	ZH	SBO	ZH × SBO
HCW, kg	16.64	17.69	0.38	16.89	17.44	0.38	0.01	0.16	0.87
Cold carcass weight, kg	16.09	17.09	0.35	16.36	16.81	0.35	0.01	0.22	0.94
Dressing, %	48.51	50.48	0.55	49.38	49.62	0.55	0.02	0.76	0.37
Cooling loss, %	3.35	3.43	0.25	3.16	3.62	0.25	0.74	0.08	0.48
Conformation[4]	7.53	8.06	0.27	7.59	8.00	0.27	0.07	0.15	0.43
KPH fat, %	4.26	3.26	0.53	3.90	3.62	0.53	0.07	0.61	0.86
Fat thickness, cm	1.23	1.17	0.16	1.23	1.17	0.16	0.72	0.70	0.54
LM area, cm^2	13.22	15.56	0.66	14.23	14.55	0.66	<0.01	0.63	0.81
pH *postmortem* of LM									
45 min	5.44	5.47	0.07	5.50	5.41	0.07	0.66	0.19	0.11
24 h	5.27	5.28	0.04	5.32	5.22	0.04	0.89	0.05	0.89
Carcass length, cm	61.18	61.70	0.42	61.67	61.21	0.42	0.39	0.44	0.73
Thorax depth, cm	15.72	15.14	0.41	15.59	15.27	0.41	0.17	0.45	0.82
Leg length, cm	33.75	33.84	0.66	33.75	33.84	0.66	0.89	0.89	0.38
Leg perimeter, cm	41.17	42.92	0.71	41.72	42.38	0.71	0.02	0.37	0.57

[1]ZH supplementation = ewe lambs receiving 37.31 g/d of wheat grain containing 0 mg of ZH (0) and ewe lambs receiving wheat grain (37.31 g/d) containing 10 mg of ZH (10).
[2]SO supplementation = ewe lambs receiving a diet without SO (0) and ewe lambs receiving a diet containing 6% of supplemental SO (6).
[3]Probability values associated with ZH supplementation (ZH), SO Supplementation (SO), and ZH × SO interactions (ZH × SO).
[4]Ranked from 1 (bad), 10 (excellent).

Table 4. Non-carcass components of ewe lambs supplemented with zilpaterol hydrochloride (ZH) and soybean oil (SO) under heat stress conditions.

Item, %[1]	ZH, mg/d[2]			SBO, %[3]			P-value[4]		
	0	10	SEM	0	6	SEM	ZH	SBO	ZH × SBO
Head	5.17	5.11	0.14	5.18	5.11	0.14	0.66	0.62	0.79
Blood	4.11	3.90	0.13	4.01	4.00	0.13	0.12	0.90	0.67
Skin	8.98	8.52	0.31	9.03	8.47	0.31	0.16	0.09	0.17
Heart	0.44	0.42	0.02	0.43	0.43	0.02	0.31	0.88	0.93
Lungs	1.49	1.24	0.12	1.30	1.43	0.12	0.05	0.27	0.24
Liver	2.08	2.06	0.09	2.21	1.93	0.09	0.76	<0.01	0.62
Kidney	0.30	0.29	0.01	0.31	0.28	0.01	0.24	0.08	0.71
Peritoneum	5.47	4.83	0.60	5.76	4.53	0.60	0.30	0.05	0.49
Renal fat	1.02	0.85	0.17	0.98	0.98	0.17	0.34	0.63	0.70
Rumen	2.86	2.81	0.13	2.88	2.80	0.13	0.66	0.54	0.58
Intestine	2.72	2.86	0.18	2.76	2.83	0.18	0.46	0.68	0.29

[1] Weight of each non-carcass component is expressed as a percentage of final BW.
[2] ZH supplementation = ewe lambs receiving 37.31 g/d of wheat grain containing 0 mg of ZH (0) and ewe lambs receiving wheat grain (37.31 g/d) containing 10 mg of ZH (10).
[3] SO supplementation =ewe lambs receiving a diet without SO (0) and ewe lambs receiving a diet containing 6% of supplemental SO (6).
[4] Probability values associated with ZH supplementation (ZH), SO Supplementation (SO), and ZH × SO interactions (ZH × SO).

Table 5. Wholesale cut yields of ewe lambs supplemented with zilpaterol hydrochloride (ZH) and soybean oil (SO) under heat stress conditions.

	ZH, mg/día[2]			SBO, %[3]			P-value[4]		
Item[1], %	0	10	SEM	0	6	SEM	ZH	SBO	ZH × SBO
Forequarter	58.75	57.64	0.57	58.21	58.17	0.57	0.06	0.94	0.54
Neck	3.58	3.38	0.34	3.69	3.27	0.34	0.55	0.24	0.29
Ribs	25.89	25.07	0.58	25.35	25.60	0.58	0.17	0.67	0.43
Loin	12.22	12.39	0.26	12.54	12.06	0.26	0.53	0.08	0.14
Shoulder	17.06	16.80	0.44	16.62	17.23	0.44	0.55	0.18	0.88
Hindquarter	41.25	42.36	0.57	41.78	41.82	0.57	0.06	0.94	0.54
Legs	22.43	23.15	0.50	22.53	23.04	0.50	0.17	0.33	0.82
Plain loin	7.14	7.47	0.23	7.47	7.13	0.23	0.18	0.16	0.13
Sirloin	11.75	11.68	0.17	11.77	11.65	0.17	0.64	0.47	0.56

[1]Weight of each wholesale cut is expressed as a percentage of HCW.
[2]ZH supplementation =ewe lambs receiving 37.31 g/d of wheat grain containing 0 mg of ZH (0) and ewe lambs receiving wheat grain (37.31 g/d) containing 10 mg of ZH (10).
[3]SO supplementation = ewe lambs receiving a diet without SO (0) and ewe lambs receiving a diet containing 6% of supplemental SO (6).
[4]Probability values associated with ZH supplementation (ZH), SO Supplementation (SO), and ZH × SO interactions (ZH × SO).

CAPÍTULO VII

Calidad Fisicoquímica, Sensorial y Perfil de Ácidos Grasos (Época de Invierno)

Composición de ácidos grasos, características fisicoquímicas y sensoriales de carne de corderas suplementadas con clorhidrato de zilpaterol y aceite de soya

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Fatty acid composition and physicochemical and sensory characteristics of meat from ewe lambs supplemented with zilpaterol hydrochloride and soybean oil

Running title: zilpaterol hydrochloride and soybean oil effects on meat quality of ewe hair lambs

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7.1 Abstract

The effect of supplementation of zilpaterol hydrochloride (ZH; 0 or 10 mg/lamb daily) and soybean oil (SBO; 0 or 6%) on chemical, physicochemical, sensory quality, and fatty acid composition of the *longissimus thoracis* muscle of ewe lambs was studied using a randomized complete design with a 2 × 2 factorial arrangement. After a 32-d feeding period, all ewes were slaughtered. Interactions ZH × SBO were not observed ($P > 0.05$) for any of the variables evaluated. Feeding ZH decreased color parameters ($P < 0.05$), while others characteristics physicochemical were not affected ($P > 0.05$). Panelists only observed an increase in appearance ($P < 0.001$) by ZH supplementation. Aditionally, ZH decreased ($P < 0.05$) the amount of C20:5n3, C22:6n-3 and total omega-3 fatty acids, compared to zero ZH group. Physicochemical and sensory characteristics, and fatty acid composition of meat were not modificated by SBO ($P > 0.05$). Supplementation of ZH to ewe hair lambs decreased color parameters of meat, while feeding SBO caused no changes in the meat lamb quality. Regarding fatty acid composition, the results indicated that the supplementation of ZH and SBO cause minimal changes in meat of ewe lambs during winter season.

Keywords: Ewe lambs; Fatty acid profile; Meat quality; Soybean oil; Zilpaterol

7.2. Resumen

Los efectos de la suplementación de clorhidrato de zilpaterol (CZ; 0 ó 10 mg/cordera/día) y el aceite de soya (AS; 0 ó 6%) en la calidad química, fisicoquímica, sensorial y composición de ácidos grasos del músculo *Longissimus thoracis* de 32 corderas de pelo cruzadas Dorper x Pelibuey fueron analizados bajo un arreglo factorial 2×2 en un diseño completamente al azar. Después de un período de alimentación de 32 d, las corderas se sacrificaron. No se observó efecto de la interacción CZ×AS ($P>0.05$) en ninguna de las variables evaluadas. La suplementación de CZ disminuyó los parámetros de color L*, a*, b* y Croma ($P<0.05$), mientras que las otras características fisicoquímicas no fueron afectados ($P>0.05$). En lo que respecta a la evaluación sensorial, los panelistas sólo

observaron un aumento (P<0.001) en la apariencia en la carne con la suplementación de CZ. Adicionalmente, el uso de CZ disminuyó (P<0.05) la cantidad de los ácido grasos C20: 5n3, C22:6n-3 y ácidos grasos totales omega-3, en comparación al grupo no suplementado con CZ. Por otra parte, la suplementación de AS no modificó las características fisicoquímicas y sensoriales, ni la composición de ácidos grasos de la carne (P>0.05). La suplementación de CZ en corderas de pelo provocó una disminución en los parámetros de color en la carne, mientras que la suplementación de AS no causó cambios en la calidad de la carne en las corderas. En cuanto a la composición de ácidos grasos, los resultados indican que la suplementación de CZ y AS causan cambios mínimos en el perfil de ácidos grasos de la carne de corderas durante la temporada invernal.

Palabras clave: aceite de soya; agonista β-adrenérgico; calidad de la carne; corderas; perfil de ácidos grasos; zilpaterol.

7.3. INTRODUCTION

A great number of factors are involved in lamb carcass and meat quality. Intrinsic factor and productive management directly influence carcass and meat quality; therefore, it is always necessary to search for new strategies that enhance meat quality. Modern cattle production uses a great variety of products and additives such as Beta-adrenergic agonists (β-AA), to improve feedlot performance, quality and chemical composition of meat. The β-AA's are considered one of the most potent growth promoters that increases carcass weigth and lean tissue development, improves feed efficiency, and reduces fat deposition (Dikeman 2007). Today, zilpaterol hydrochloride (ZH) is the most used β-AA. Recent research has focused on the effect of ZH on growth, carcass composition, and meat tenderness, although little has been done to investigate its effect on physical-chemical and sensory characteristics of meat. With respect to these two last characteristics, Avendaño-Reyes et al. (2006) indicated that Warner bratzler shear force (WBSF) was increased, meanwhile meat color was not altered by ZH

supplementation (60 mg/d) to steers. In a different study, Dávila-Ramírez et al. (2013) reported that in male lambs ZH decreased color parameters of Longissimus thoracis (LT) muscle (L*, a*, b* and Chroma by 8.60%, 25.12%, 26.44% and 25.36%, respectively), increased pH and WBSF, when compared to control samples. In adittion, panelists observed a decreased in overall color, harder meat and higher content of connective tissue by ZH supplementation. However, the impact of ZH on fatty acid composition of meat has not been investigated. Reports indicate that β-AA can causes an increase in lipolysis, and a decrease in lipogenesis in several animal species (Poletto et al. 2009); therefore, it is possible that ZH usage modify the fatty acid profile in meats. Fatty acid composition of red meats has received much attention as a consecuence of its perceived negative implications in human health due to its high levels of saturated fat (40 to 50%) and low levels of polyunsaturated fat (Vanderveen 1996; Webb and Oneill 2008). However, modifying the composition of fatty acids in ruminants' meat is a great challenge for meat industry. A strategy that has been implemented to modify the fatty acid content of meat is by dietary supplementation of polyunsaturated fats in ruminants (Manson et al. 2009) A study performed in Targhee × Rambouillet wethers, Boles et al. (2005) found that safflower oil supplementation did not cause changes in total SFA in muscle, while Bolte et al. (2002) observed a decrease in total SFA in adipose tissue. In this regard, lipid composition of ruminant tissue is also determined by the metabolism of dietary lipids in the rumen, which is characterized by intense lipolysis and fatty acid (FA) biohydrogenation by microorganisms (Harfoot and Hazlewood 1997). Regarding oils supplementation and its relationship to the meat quality, recently Dávila-Ramírez et al. (2013) report that dietary supplementation of soybean oil (SBO) to hair lambs modified physicochemical parameters of meat, causing an increase in a* values, Chroma and water holding capacity (WHC). Nonetheless, studies indicated no changes in meat color of lambs supplemented with oils rich in polyunsaturated fatty acids (Bessa et al. 2008). Nowadays, there are few reports in which the effect of oil supplementation in ruminants on meat quality has been evaluated. Therefore, it is important to evaluate the combined dietary supplementation of β-AA and oils rich in

polyunsaturated fatty acids and their effect on quality and lipid composition of ewe lamb meat. The objective of this study was to evaluate the effect of ZH and SBO on physicochemical and, sensory characteristics, as well as the fatty acid profile of meat from ewe hair lambs under intensive feeding.

7.4. MATERIALS AND METHODS

All procedures involving ewe lambs were conducted within the guidelines of approved local official techniques for animal care in México (NOM-015-ZOO-2002; NOM-033-ZOO-1995).

7.4.1. Study Site

The experiment was conducted during winter season at the Sheep Experimental Unit of the Instituto de Ciencias Agrícolas, at the Universidad Autónoma of Baja California (ICA-UABC), in Mexicali Valley, located in northwestern México (latitude 114.6º and longitude 32.8º). Climatic conditions in the Mexicali Valley are similar to those of the Sonoran desert, with extreme temperatures during summer (above 40^{o}C) and winter (below 10 ^{o}C), and an average annual precipitation of 85 mm.

7.4.2. Animal, Housing, and Treatments

Thirty-two Dorper x Pelibuey crossbred ewe lambs (30.55 ± 2.57 kg, 6 mo of age) were individually housed in pens equipped with shade, feed troughs and waterer. Ewe lambs were adapted to pens and a basal diet during a 30-d period immediately before initiating the experiment. Animals received an injection of vitamins A, D, and E (Vigantol; Bayer, México City, México; 1 mL/animal), and were treated for internal and external parasites (Invermectin, Sanfer Laboratory, Mexico City, Mexico; 0.5 mL/animal). One week before initiation of the experimental phase, ewe lambs were individually weighed (BW) and completely

randomized to one of four treatments (n=8). The treatments were arranged in a 2 × 2 factorial. Factors were ZH (0 or 10 mg Zilmax^{TM} /lamb daily; Intervet, México City, Mexico) and SBO (0 or 6% SBO/kg DM; Chef´s Pride^{TM}; Ventura Foods LLC; Brea, CA, USA).

Ewe lambs fed SBO were adapted to the vegetable oil during the week before beginning the experiment, while lambs fed ZH were not adapted to the β-AA. In order to ensure the total intake of β-AA, ZH (10 mg/d/animal) was mixed with 37.31 g of wheat meal offered to ewe lambs before the morning feeding. At the same time, groups treated without ZH were fed only with 37.31 g/ewe lamb daily of wheat meal. The experimental diets were formulated containing 190 g/kg of CP and 12.13 MJ/ ME of kg DM. Table 1 shows ingredients, chemical composition and fatty acid profile of experimental diets. Ewe lambs were fed twice daily in a 50:50 amount proportion, respectively. The health status of ewe lambs was monitored daily. The ZH was withdrawn on day 30 of the feedlot phase (i.e. 48 h before slaughter, based on the technical note of zilmax; Intervet, México, City). The final BW mean of ewe lambs ranged between 35.5-36.2 kg for all treatments.

7.4.3. Slaughter and *Longissimus thoracis* muscle disection

At the end of the 34-d feeding period, ewe lambs were slaughtered in the Meat Laboratory of the ICA-UABC following the conventional procedures (AOAC 2000). Feed and water were withdrawn 24 h before slaughter. The hot carcass weigth ranged between 16.6 kg and 17.7 kg for all treatments. At 24 h *postmortem*, *m. Longissimus thoracis* (LT) was removed (4th until 12th intercostal space) from both sides of the carcass, vacuum packed and freeze at -20 °C. Frozen samples were transported, for further analysis, to the Meat Science and Technology Lab in CIAD AC (Centro de Investigación en Alimentación y Desarrollo AC) located at Hermosillo, Sonora, Mexico.

7.4.4. Sample sectioning

Upon arrival at the Meat lab, samples were kept frozen at -20 °C. Before analyses, samples were thawed for 24 h at 4 °C and then sectioned to carry out chemical, physicochemical, sensory determinations, and fatty acid composition. Sectioning started from the distal end (12th rib interface) and cranially toward the chuck end of the rib. The 1st pairs steak (1.5 cm each) was used for moisture, intramuscular fat content and fatty acid composition, the following 2 pairs (2.54 cm each) for Warner Bratzler shear force test and sensory analysis, respectively; a slice of 1 cm was used for color and pH.

7.4.5. Determination of moisture and intramuscular fat content

Before determination of moisture and intramuscular fat content, meat samples' surrounding fat and epimysium were trimmed off and then grounded to be analyzed in triplicate following the AOAC oficial methods (AOAC 2000). Results were expressed as a percentage of fresh muscle weight.

7.4.6. pH determinations

Determination of pH was performed at 4 °C using a portable digital pH meter (Hanna, Model HI 98140, Woonsocket, RI, USA).

7.4.7. Meat color

Color measurements of LT muscle surfaces after 30 min blooming were carried out using a Minolta CR-400 colorimeter (Konica Minolta Sensing, Inc., Japan) with D65 illuminant and 10° in the observer. Color parameters L* (lightness), a* (redness), and b* (yellowness) were evaluated (Cassens *et al.* 1995). Hue angle (Hue) was calculated by the formula: Hue = $\tan^{-1}$ (b*/a*), and Chroma, using the formula: Chroma = (a* + b*)½). Color determinations were made at 5 different locations at the surface of the cold samples (4–6 °C).

7.4.8. Water holding capacity (WHC)

Analysis of water holding capacity (WHC) was carried out following the procedure described by Sutton *et al.* (1997) which is based on the ability of meat samples to hold water after centrifugation (3600 rpm × 5 min). The WHC percentage was calculated based on the difference in weight of the sample before and after centrifugation.

7.4.9. Warner Bratzler shear force (WBSF)

For the evaluation of Warner Bratzler shear force (WBSF) a Texture Analyzer texturometer T.A.X.T. Plus was used. To measure WBSF on LT muscle, sections of 2.5 cm thick were obtained, and cooked in an electric skillet (Cook Master Oster, model 3222-3, Mississauga, Ontario, Canada) until reaching a final internal temperature of 71 °C. After cooking, samples were cooled (25-30 °C) and chilled at 4 °C for 24 h. Subsequently, meat was cut into pieces of 1.27 cm of diameter in longitudinal direction of muscle fibers, and WBSF was determined with Warner Bratzler attachment cutter on 8 specimens by sample. The value WBSF was expressed in kg. To measure cooking loss, samples were weighed in the raw state and immediately after reaching the final cooking temperature.

7.4.10. Sensorial analysis

Sensory traits were evaluated by a trained 8-member panel (ISO 8586-1 1993). Meat samples were prepared according to AMSA guidelines (AMSA 1995). On the day before sensory evaluation, steaks were removed from the freezer and thawed out at 4 °C for 24 h. Steaks were cooked following the same procedure previously described for WBSF determination. Each cooked steak was cut into 1.27 cm × 1.27 cm thickness. Sensory panel evaluated cooked samples, under soft red light, for “odor intensity”, “flavor intensity”, “fat mouthfeeling”, “tenderness”, “juiciness” and “amount of connective tissue” using a unstructured 10.0-cm line

scale. This line scale was anchored on the left (0 cm) with a descriptive term representing the lowest degree of odor intensity, flavor intensity, fat mouthfeeling, tenderness, juiciness, and amount of connective tissue. The right end (10.0 cm) of the scale was a descriptive term representing the highest sensorial degree for each sensory trait. Two visual traits, overall color and overall appearance were evaluated on raw samples, under white light, using the same type of scale.

7.4.11. Fatty acid profile evaluation

The lipid fraction was extracted using the method described by Bligh and Dyer (1959). Methylation of fatty acid was done according to the method reported by Park and Goins (1994). Analysis of methyl-fatty acids was performed with a gas chromatograph (Hewlett Packard model 6890, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and a 6890 auto-sampler. A Supelco (Bellefonte, PA, USA) SP2560 (0.25 mm × 100 m, 0.20 µm film width) melted-capillary, silicon-based column was used. The oven temperature was programmed from an initial temperature of 150 °C (20 min) to a final temperature of 220 °C at the rate of 5 °C min^{-1}. Injector temperature was set at 250 °C, and FID temperature was adjusted to 300 °C. Tridecanoic acid (13:0 Sigma-Aldrich, St Louis, MO, USA) was used as internal standard. The identification of fatty acids was performed according to their retention times and the elution patterns. Fatty acid proportions of phospholipids and triglycerides were not measured as separate fractions. Fatty acid data were reported as percentage of total fatty acid methyl esters detected. Total amounts of SFA, MUFA, PUFA, *trans* fatty acids (TFA) and *cis* fatty acids (CFA) were calculated, as well as the PUFA/SFA and the n-6/n-3 ratios.

7.4.12. Statistical analysis

Data of physical-chemical and, sensory characteristics, and fatty acid composition were analyzed using a completely randomized design in a 2 x 2 factorial arrangement, using the PROC MIXED procedure of SAS (SAS 2004). The model included ZH, SBO, and ZH × SBO interaction as fixed effects, and slaughter

weight of animals as a covariate. Lamb was the experimental unit. For sensorial data, the model included also the random effect of panelist. Means comparison was performed by LSMEANS procedure. Significances were estimated at a 0.05 probability level in error type I.

7.5. RESULTS AND DISCUSSION

Interactions of evaluated factors were not significant ($P > 0.05$) for any of the response variables analyzed. Therefore, only main effects are discussed.

7.5.1. Moisture and intramuscular fat content

Effects of ZH and SBO on moisture and intramuscular fat content in meat of ewe lambs are presented in Table 2. The content of moisture and intramuscular fat was not affected ($P > 0.05$) by ZH or SBO supplementation. Currently, there is more investigations which have evaluated ZH effect on meat quality and there are few studies in lambs, and on this last, effects are inconsistent. Currently, reports examining the effects of β-AA have primarily focused on the meat quality attributes of *Longissimus dorsi* (LD) muscle in beef cattle. However, our results indicate that ZH not changes the percentage of intramuscular fat and moisture. In this sense, Mondragon *et al.* (2010) reported that use of ZH (5.3 and 15.9 mg ZH/ kg DM) in male Rambouillet lambs decreased moisture and intramuscular fat in LD muscle. However, Brand *et al.* (2013) did not observed any effect of ZH treatment on moisture and intramuscular fat percentages in meat of merino lambs. Contrary to our study, in a similar research with male lambs (Dorper x Pelibuey), ZH supplementation causes a reduction of 30% intramuscular fat content of LT, with respect to animals non supplemented (1.65% vs 2.37%, respectively) (Dávila-Ramírez *et al.* 2013). This behavior is attributed to lipolytic effect of use of β-AA (Mersmann 1998). In present experiment, the use of ewe lambs, possibly caused that ZH supplementation had no effect in intramuscular fat. In this sense, reports indicate that ewe lambs deposit more fat compared to ram lambs at same age

(Kirton *et al.* 1995), indicating that is strongest physiological process of fat accumulation in females, with respect to that attributed to β-AA lipolytic effect.

7.5.2. Physicochemical evaluation

The effects of ZH or SBO on the physicochemical characteristics of ewe lamb meat are presented in Table 2. Color parameters L*, a*, b* and Chroma decreased ($P < 0.05$) 5.80%, 15.94%, 24.73% and 17.25%, respectively, in meat from animals supplemented with ZH. Other physicochemical characteristics such as pH, HUE, cooking loss, WHC, and WBSF were not affected by ZH treatment ($P > 0.05$). On the other hand, SBO supplementation did not affect ($P > 0.05$) any of the meat physicochemical characteristics evaluated. In accordance with the above, Lopéz-Carlos *et al.* (2012) reported no differences in ultimate pH values (5.74-5.77) of the LD muscle from lambs with different doses and exposure time of ZH. However, recently other study reported that ZH supplementation in male lambs increased pH in LM (5.97 vs 6.19) with respect to non-supplemented animals (Dávila-Ramírez *et al.* 2013). It is important to recall that final pH of meat has to be maintained within its normal range to avoid detrimental effects on quality characteristics. In the present study, cooking loss was not modified by ZH and SBO. In agreement with the current results, studies indicated no variation in cooking loss in LT from lambs supplemented with ZH (6 mg/kg, 30 d) (Lopéz-Carlos *et al.* 2012), and Dávila-Ramírez *et al.* (2013) (10 mg/kg, 30 d) in hair lambs. However, Geesink *et al.* (1993) reported that supplementation of β-AA increase the cooking loss, and this may be due to underlying muscle properties, such as larger muscle cells with weaker supporting structure, or increased water/protein ratio.

Meat color is the most important factor to select fresh meat at the time of purchase. Color stability is mainly a function of myoglobin reducing activity and overall oxygen consumption rate of a muscle. Although numerous studies have evaluated the impact of ZH on carcass quality, limited data exist on the effect of β-AA on the color stability of meat from ewe lambs. In the present study, almost all

color parameters decreased by ZH. A similar effect was recently reported in ZH-supplemented male lambs (Dávila-Ramírez *et al.* 2013). The decrease in the color parameters was technologically insignificant, since it did not cause the presence of dark cuts, plus the observed pH values were within the normal range.

The low color values found in the present study with the use of ZH probably due to reduced heme pigmentation and to a larger proportion of fast twitch glycolytic fibers (Mersmann 2002), or dilution muscle myoglobin content caused by muscle fiber hypertrophy (Carr *et al.* 2005). Regarding to the SBO supplementation, in the present study no significant variations in ewe meat color were found. In accordance with present study, Bessa *et al.* (2008) indicate that meat color stability was not affected by supplementing of soybean oil to lambs. On the other hand, other study reported that 6% SBO supplementation in male lambs caused an increase of a* value and Chroma of LT muscle (Dávila-Ramírez *et al.* 2013). The discrepancy with respect to present study, can be explained by the animal sex condition and the year season different of the tests. In this sense, Walker *et al.* (2010) reported that there are differences in metabolites and gene expression between animals with sex different, which may be influencing these results.

In our study, WBSF was not affected by ZH supplementation. No studies have investigated ZH supplementation in ewe lambs and its effect on WBSF. The use of β-AA increase performance and lean gain efficiency, however, some studies indicate negative impact on meat quality, especially an increase in the hardness of the meat in several species (Dikeman 2007; Dávila-Ramírez *et al.* 2013). However, in steers fed with ZH reports that do not exist differences in tenderness with respect to non-supplemented animals (O'Neill 2001). In the case of lambs, recently study indicant that 30 day supplementation of ZH, in male lambs under heat stress conditions, caused an increase of 48.78% (10.98 kg vs 7.38 kg) with respect to non-supplemented animals (Dávila-Ramírez *et al.* 2013). These discrepancies in the results of these studies and ours can be attributed to the sexual condition of animals as it can influence the effects of ZH on meat tenderness. In other species,

Claus *et al.* (2010) report that ZH supplementation cause value higher in heifer LL muscles when compared with steer LL muscles. Meanwhile, Choat *et al.* (2006) indicant that steers typically produce more tender meat than heifers. However, the impact of ZH on meat tenderness in ewe lambs needs to be documented. Regarding oil supplementation, in the current study no differences in WBSF value were found. In this sense, Gibb *et al*. (2004) did not detected differences in meat hardness between cattle supplemented with sunflower seed rich in oleic and linoleic acids. Meanwhile, Simitzis *et al.* (2005) found that oregano oil supplementation did not influence meat shear force values of lambs.

7.5.3. Sensory evaluation

Effects of ZH and SBO on sensory characteristics of ewe lamb meat are presented on Table 3. Only the attribute of appearance was significantly higher ($P < 0.05$) for meat samples from animals supplemented with ZH in comparison with meat from control animals. Similar results were reported by McKeith *et al.* (1988) where no differences in juiciness, tenderness, flavor intensity or off-flavor intensity were observed among treatments when RH (Ractopamine Hydrochloride) was fed to pigs at levels of 0, 5, or 10 ppm. Crome *et al.* (1996) suggested that feeding RH at the 10 ppm level will not affect fresh loin quality and palatability in meat of pork. On the other hand, Hilton *et al.* (2009) reported decreased sensory scores for juiciness, tenderness, and beef flavor of the LT muscle, but felt those differences may be attributed to the decreased marbling of ZH carcasses.

Likewise, Leheska *et al.* (2009) also reported a decrease in sensory scores of tenderness, juiciness, and flavor of cattle fed ZH, attributing these differences to protein structure or function modifications caused by ZH. In general, analysis of the results presented in this and of other studies indicate that effects of β-AA on meat quality parameters have been confusing due to factors such as differences in expression of β-adrenergic receptors in cells, tissues, dose of β-AA and effectiveness, sex, breed, age of the animals, and hormones that may influence metabolic pathways associated to β-AA effects (Mersmann 1995). More research

is needed to clarify the role of β-adrenergic agonists on sensory evaluation of meat ewe lambs.

Addition of SBO to ewe lamb diet (Table 3) had not effect in any of sensory attributes of fresh and cooked ewe meat ($P > 0.05$). Mir *et al.* (2003) supports that inclusion of oils in the diet of beef had no effect on meat tenderness or palatability. Albeit *et al*. (2004) observed higher off-flavor values in diets of beef with high content of unsaturated fatty acids, inclusive an increase of rancid flavor intensity. Some authors noted that fatty acid composition of the diet can alter the type of volatiles that occur in meat and thus alter its flavor (Mc Niven *et al.* 2004; Elmore *et al.* 2004). However, others authors have reported that supplemented with oils rich in polyunsaturated fatty acids had no effects in the sensory properties of male lamb meat (Dávila-Ramírez *et al.* 2013) and male Mahabadi goat meat (Najafi *et al.* 2012).

7.5.4. Intramuscular fatty acid profile

Effects of ZH and SBO on the fatty acid profile of meat from ewe lambs are presented in Table 4. ZH supplementation to ewe lambs only decreased ($P < 0.05$) the amount of C20:5n3 (36.56%) and C22:6, n-3 (61.54 %) compared to zero ZH group; whereas the content of all other individual fatty acids was not affected ($P > 0.05$) by the ZH addition.

Respect to partial sums of fatty acid and nutritional values (Table 5), the sums of omega-3 fatty acids (Σ n-3) decreased ($P < 0.05$) 20.99% by ZH addition. However, Σ SFA, MUFA, PUFA, TFA, CFA, n-3, and MUFA/SFA, PUFA/SFA, and n−6/n−3 ratios were not affected by ZH ($P > 0.05$). No previous studies were found regarding the effect of ZH supplementation in lamb diets, on the fatty acid profile of its meat. Therefore, results are discussed and compared to previous studies performed in lamb supplemented with other anabolic agents and other ruminant supplemented with ZH. As same as in the present study, ZH supplementation by 20 d also had no effect on the amount of saturated and polyunsaturated fatty acid in meat of Hanwoo steer (Choi *et al.* 2013). However, Ibrahim *et al.* (2006)

observed an increase in the relative proportion of SFA using Zeranol in steers. Regarding the effect of ZH supplementation in the content of TFA, n-3 and n-6 fatty acids, Fritsche *et al.* (2001) reported that ZH supplementation in steers increased the amount of these groups of fatty acids. The different response caused in different fatty acids content by the use of ZH in various species, could be explained by factors such as sexual and climatic conditions, which need to be evaluated in future studies.

Concepts of fatty acid ratios (PUFA/SFA and n-6/n-3) are very important health issue for human. In this regard, in current experiment the values of PUFA/SFA ratios were between 0.20-0.23 and for n-6/n-3 were 10.48-11.29. Health institutions have recommended that dietary PUFA/SFA ratio should be between 0.45-0.65 and that n-6/n-3 ratio should not exceed 4.0 (Department of Health 1994). Additionally, ZH supplementation causes an average 37% decrease of EPA and DHA content in ewe lamb's meat. Ponnampalam *et al.* (2001) reported that consume of these fatty acids is important to human health, with recommended values for adults of 0.3 to 0.65 g/d. However, because in general ruminant meat is not a main source of EPA and DHA in the human diet, the reduction detected in the current study by ZH supplementation, may not be biologically important. Nevertheless, potential health effects of ZH supplementation on all fatty acid concentrations requires further investigation.

On the other hand, SBO supplementation as source of PUFA in ewe lamb finishing diets had no effect ($P > 0.05$) on the content of any individual fatty acid of the muscle (Table 4), whence either ($P > 0.05$) on the partial sums of fatty acid and nutritional values (Table 5). Previous studies performed to evaluate the effect of oil supplementation on high concentrate diets, have showed inconsistent responses on variation of individual fatty acid concentration. The percentage of oleic fatty acid observed in this study is much higher than reported by Santos *et al.* (2007) in lambs supplemented with soybean oil (8%), and those reported by Randunz *et al.* (2009) in lambs supplemented with soybean oil (1.78%) and linseed (0.89%). The highest content of oleic acid in the muscle could be related to highest enzyme

activity (Dervish *et al.* 2010). In this sense, Dinh *et al.* (2010) indicate that high presence of oleic acid is therefore of Δ9 desaturase, since this enzyme may convert a high percentage of estearic to oleic acid in the enterocyte (Byers and Schelling 1993).

In agreement with the present study, Boles *et al.* (2005) demonstrated that total SFA proportion in muscle was not different in lambs finishing diets supplemented with safflower oil. Meanwhile, the means for PUFA of animals supplemented with SBO were lower than those reported by Randunz *et al.* (2009) in ewe Hampshire x Dorset supplemented with soybean and linseed oils (8.38 vs 14.64 ΣPUFA).

This behavior possibly is due to the lower amount of intramuscular fat found in our study in comparison to intramuscular fat found by Randunz *et al.* (2009) (3.66 vs 4.77 g/100 g) whose intramuscular fat content possibly is related to the final BW of sacrifice of ewe. In this sense, reports indicate that the metabolism of dietary lipids in the rumen determines lipid composition of ruminant muscle (Harfoot and Hazlewood 1997). Therefore, the synthesis of fat is the major factor affecting the fatty acid composition in the muscle of several species (Wood *et al.* 2008). However, the amount of fat synthesized dependent of rate of growth and maturing of animal because they are controlled by many physiological aspects such the hormones biosynthesis, genetic and environmental factor, and diet (Santos-Silva *et al.* 2004).

7.6. CONCLUSIONS

According to the results of the present study, feeding ZH to Dorper x Pelibuey crossbred ewe lambs during winter season in the Mexicali Valley caused a decrease in color parameters (L*, a*, b* and Chroma) and total omega-3 fatty acids. Meanwhile, feeding SBO did not affect physical-chemical and sensory parameters of the *Longissimus thoracis* muscle. Besides, SBO supplementation was not able to increase the PUFA content of ewe lamb meat or any other health or nutritional important fatty acid ratio. Therefore, new strategies in finishing lambs

should be tested, that may help to increase some of the nutritional important fatty acids in ewe lamb's meat and also that are useful to be applied in a wide variety of environmental conditions and finishing systems.

7.7. REFERENCES

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Table 1. Ingredients and proximate analysis and energy estimates analysis of the experimental diets provided to ewe lambs**.**

Item	Control	SBO	Oil[a]
Ingredient, % of DM			
Wheat grain	68.0	50.5	---
Alfalfa hay	12.1	10.0	---
Wheat straw	3.0	12.0	---
Soybean meal	10.5	15.0	---
Cane molasses	5.0	5.0	---
White salt	0.5	0.5	---
Calcium phosphorus	0.7	0.8	---
Limestone	0.2	0.2	---
Soybean oil	---	6.0	---
DM	934	942	---
OM	869	872	---
CP	194	197	---
EE	13	42	---
NDF	177	179	---
ADF	81	101	---
Ash	65	71	---
DE, MJ/kg	3.57	3.56	---
ME, MJ/kg	2.92	2.92	---
NE_m, MJ/kg	1.97	1.96	---
NE_g, MJ/kg	1.32	1.32	---
Fatty acid (mg^{-1}/100 mg total FA)			
SFA [b]	27.45	22.57	15.00
C14:0	0.80	0.60	0.08
C16:0	19.99	15.33	10.81
C18:0	6.66	6.64	3.07
MUFA [c]	26.81	25.34	24.75
C18:1, cis 9	26.81	25.34	23.69
PUFA [d]	45.74	52.09	60.25
C18:2, n-6	41.65	46.44	53.33
C18:3, n-3	4.09	5.65	6.65

SBO, soybean oil; DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergente fibre; ADF, acid detergent fibre; DE, digestible energy; ME, metabolizable energy; NE_m, net energy maintenance; NE_g, net energy gain.
[a] Chef's Pride[R] ,Ventura Foods LLC, Brea, CA, USA.
[b] Total saturated fatty acids.
[c] Total monounsaturated fatty acids.
[d] Total poliinsaturated fatty acids

Table 2. Least square means for chemical (moisture and intramuscular fat) and physicochemical parameters of meat quality of ewe lambs supplemented zilpaterol hydrochloride and soybean oil.

	ZH,[a] mg/d			SBO,[b] gr/100 gr DM			P-value[c]	
Item	0	10	SEM[d]	0	6	SEM	ZH	SBO
Replicates	16	16	—	16	16	—	—	—
Moisture	73.33	73.53	0.83	72.83	74.04	0.85	0.81	0.17
Intramuscular fat (g/100 g)	3.20	3.61	0.56	3.15	3.66	0.59	0.64	0.55
pH	5.61	5.79	0.09	5.64	5.75	0.09	0.06	0.25
Cooking loss, %	21.88	20.04	1.49	20.96	20.95	1.50	0.23	0.99
Lightness (L*)	34.82	32.80	0.97	33.80	33.82	0.98	0.04	0.99
Redness (a*)	16.25	13.66	0.73	15.39	14.51	0.73	0.002	0.24
Yellowness (b*)	7.32	5.51	0.61	6.90	5.94	0.61	0.006	0.13
Hue angle [e]	23.99	21.65	1.30	23.73	21.91	1.31	0.09	0.17
CHROMA [f]	17.85	14.77	0.88	16.91	15.71	0.88	0.002	0.18
WHC[g]	83.83	83.99	1.49	83.53	84.29	1.50	0.91	0.61
WBSF, kg [h]	9.08	9.71	1.07	9.45	9.33	1.08	0.56	0.91

[a] ZH, zilpaterol hydrochloride; wheat grain (37.31 g/d) containing 10 mg of ZH was supplemented. SO, soybean oil.
[b] Soybean oil supplementation, ewe lambs receiving a diet without SBO (0) and ewe lambs receiving a diet containing 6% of supplemental SBO (6).
[c] Probability values associated with ZH supplementation, SBO supplementation, and ZH × SBO interactions.
[d] SEM, standard error of mean.
[e] Hue = tan−1(b*/a*) × 57.29.
[f] Chroma = (a* + b*).
[g] WHC, water holding capacity.
[h] WBSF, Warner Bratzler shear force.

Table 3. Least square mean for sensory evaluation of meat quality of ewe lambs supplemented zilpaterol hydrochloride and soybean oil.

	ZH,[a] mg/d			SBO,[b] gr/100 gr DM			P-value[c]	
Item	0	10	SEM[d]	0	6	SEM	ZH	SBO
Replicates	16	16	—	16	16	—	—	—
Overall color	8.03	8.24	0.27	8.31	7.95	0.27	0.43	0.20
Appearance	6.94	7.89	0.40	7.44	7.40	0.41	0.002	0.92
Odor	6.19	6.57	0.23	6.39	6.38	0.23	0.12	0.96
Flavor	6.09	6.11	0.32	6.14	5.97	0.32	0.75	0.60
Feeling fat	2.94	2.87	0.30	3.06	2.75	0.30	0.81	0.32
Tenderness	6.67	6.40	0.58	6.69	6.48	0.59	0.65	0.86
Juiciness	6.34	6.12	0.49	6.38	6.08	0.50	0.66	0.56
Connective tissue	2.51	2.43	0.55	2.62	2.32	0.55	0.89	0.58

It was used an unstructured 10.0-cm line scale. Line scale was anchored on the left (0 cm) with a descriptive term representing the lowest degree of odor intensity, flavor intensity, fat mouthfeeling, tenderness, juiciness, and amount of connective tissue. On the right end (10.0 cm) of the scale was a descriptive term representing the highest sensorial degree for each sensory trait.

[a] ZH, zilpaterol hydrochloride; wheat grain (37.31 g/d) containing 10 mg of ZH was supplemented. SO, soybean oil.

[b] Soybean oil supplementation, ewe lambs receiving a diet without SBO (0) and ewe lambs receiving a diet containing 6% of supplemental SBO (6).

[c] Probability values associated with ZH supplementation, SBO supplementation, and ZH × SBO interactions.

[d] SEM, standard error of mean.

Table 4. Intramuscular fat (g/100 g tissue), fatty acid profile (g/ 100 g FAME) of *longissimus thoracis* muscle from ewe lambs supplemented with zilpaterol hydrochloride and soybean oil

	ZH[a], mg/d			SBO[b], g/100 g			P-value[c]	
	0	10	SEM[d]	0	6	SEM	ZH	SBO
Replicates	16	16		16	16			
Intramuscular fat	3.20	3.61	0.56	3.15	3.66	0.59	0.64	0.55
Fatty acid								
C14:0	2.14	2.17	0.15	2.13	2.18	0.15	0.83	0.78
C15:0	0.36	0.45	0.05	0.43	0.38	0.05	0.09	0.27
C16:0	22.77	22.36	0.66	22.35	22.77	0.67	0.55	0.54
C16:1	2.54	2.58	0.12	2.62	2.50	0.13	0.76	0.37
C17:0	1.41	1.80	0.24	1.71	1.50	0.24	0.11	0.39
C18:0	13.21	13.47	0.46	13.11	13.58	0.46	0.69	0.48
C18:1, n-9t	1.89	2.13	0.58	2.35	1.68	0.41	0.59	0.27
C18:1, 9c	45.54	46.16	1.43	45.57	46.13	1.44	0.67	0.68
C18:2, n-6t	0.05	0.06	0.01	0.07	0.05	0.01	0.53	0.20
C18:2, n-6c	5.80	5.31	0.52	5.67	5.44	0.53	0.35	0.67
C:18:3, n-6	0.07	0.07	0.01	0.07	0.07	0.01	0.83	0.80
C18:3, n-3	0.43	0.39	0.03	0.40	0.43	0.03	0.16	0.37
C20:3, n-3	0.07	0.08	0.01	0.08	0.07	0.01	0.28	0.25
C20:3, n- 6	0.15	0.14	0.01	0.13	0.15	0.01	0.81	0.18
C20:4, n-6	2.28	1.74	0.29	2.14	1.88	0.29	0.08	0.39
C20:5n3	0.22	0.14	0.02	0.19	0.17	0.02	0.01	0.56
C22:6, n-3	0.13	0.08	0.02	0.11	0.11	0.02	0.01	0.84

[a] Zilpaterol hydrochloride supplementation, ewe lambs receiving 37.31 g/d of wheat grain containing 0 mg of ZH (0) and ewe lambs receiving wheat grain (37.31 g/d) containing 10 mg of ZH (10).
[b] Soybean oil supplementation, ewe lambs receiving a diet without SBO (0) and ewe lambs receiving a diet containing 6 g/100 g DM of SBO (6).
[c] Probability values associated with ZH supplementation or SBO supplementation effects.
[d] SEM, standard error of mean.

Table 5. Partial sums of fatty acid and nutritional value of intramuscular fat from *longissimus thoracis* muscle of hair ewe supplemented with zilpaterol hydrochloride and soybean oil.

	ZH,[a] mg/d			SBO,[b] gr/100 gr DM			P-value[c]	
Item	0	10	SEM[d]	0	6	SEM	ZH	SBO
Replicates	16	16	—	16	16	—	—	—
Partial sums								
Σ SFA[e]	40.58	40.88	0.86	40.37	41.09	0.87	0.73	0.41
Σ MUFA[f]	50.24	51.14	1.20	50.79	50.59	1.21	0.46	0.87
Σ PUFA[g]	9.26	8.04	0.84	8.91	8.38	0.85	0.16	0.54
Σ TFA[h]	1.95	2.19	0.58	2.41	1.73	0.58	0.68	0.25
Σ CFA[i]	51.35	51.47	1.11	51.24	51.58	1.12	0.92	0.76
Σ n-6[j]	8.37	7.31	0.80	8.11	7.58	0.81	0.20	0.52
Σ n-3[k]	0.81	0.64	0.05	0.72	0.73	0.04	0.04	0.89
Ratios								
Σ MUFA/ ΣSFA[l]	1.24	1.26	0.05	1.27	1.24	0.05	0.68	0.55
Σ PUFA/ ΣSFA[m]	0.23	0.20	0.02	0.22	0.20	0.02	0.15	0.48
n-6/n-3	10.48	11.29	0.75	11.16	10.62	0.76	0.29	0.47

[a] Zilpaterol hydrochloride supplementation, ewe lambs receiving 37.31 g/d of wheat grain containing 0 mg of ZH (0) and ewe lambs receiving wheat grain (37.31 g/d) containing 10 mg of ZH (10).
[b] Soybean oil supplementation, ewe lambs receiving a diet without SBO (0) and ewe lambs receiving a diet containing 6% of supplemental SBO (6).
[c] Probability values associated with ZH supplementation, SBO supplementation, and ZH × SBO interactions.
[d] SEM, standard error of mean
[e] SFA, Total saturated fatty acids.
[f] MUFA, Total monounsaturated fatty acids.
[g] PUFA, Total poliinsatured fatty acids.
[h] TFA, Total trans fatty acids.
[i] CFA, Total cis fatty acids.
[j] Total omega-6 fatty acids.
[k] Total omega-3 fatty acids.
[l] Monounsaturated fatty acids / saturated fatty acids.
[m] Poliinsaturated fatty acids / saturated fatty acids.

CAPÍTULO VIII

Conclusiones Generales

8.0. CONCLUSIONES GENERALES

8.1. Época de Verano

Comportamiento productivo y calidad de canal:

La suplementación de CZ redujo el contenido de grasa KPH, mientras que aumentó el rendimiento de canal, el AOC del músculo *Longissimus* y el desarrollo muscular de piernas; sin embargo, no afectó el comportamiento productivo de corderos confinados bajo condiciones de estrés por calor. Por otra parte, la suplementación de AS no mostró efecto sobre el comportamiento productivo, características de la canal ni en el rendimiento de cortes primarios de corderos de pelo confinados bajo estrés por calor.

Calidad fisicoquímica y sensorial:

La suplementación de CZ en corderos provocó una disminución en los parámetros de color a*, b*, L*, así como croma y en el contenido de grasa intramuscular. En calidad sensorial, el uso de CZ produjo valores bajos de color total y de terneza. Por otro lado, la suplementación de AS aumentó los valores de a*, Croma y CRA, sin afectar las variables sensoriales del músculo *Longissimus dorsi*.

Perfil de ácidos grasos y colesterol:

La suplementación de CZ a corderos confinados bajo estrés por calor disminuyó el contenido de grasa intramuscular del músculo *Longissimus thoracis*. Sin embargo, esta reducción no afectó el perfil de ácidos grasos. La inclusión de CZ promovió una disminución en la cantidad de MUFA y en la relación MUFA/SFA. Por otra parte, la suplementación de AS aumentó el contenido de colesterol e indujo pequeños cambios en la composición de ácidos grasos de la carne, lo cual tendría un mínimo impacto sobre la salud humana.

8.2. Época de Invierno

Comportamiento productivo y calidad de canal:

La suplementación de CZ mostró ejercer un impacto sobre las características de la canal, reduciendo la grasa KPH y aumentando el peso de la canal caliente, rendimiento de la canal, conformación, perímetro de pierna y AOC; esto sin afectar el comportamiento productivo en corderas hembras. Además, los cortes primarios de los cuartos delantero y trasero tendieron a ser mayores en las corderas suplementadas con CZ, en comparación con las no suplementadas. Por otra parte, la suplementación de AS tendió a disminuir el peso de algunos componentes de la no-canal, sin presentar efecto en comportamiento productivo, características de la canal ni en rendimiento de cortes primarios de corderas de raza de pelo.

Calidad fisicoquímica, sensorial y perfil de ácidos grasos:

La suplementación de CZ en corderas durante la temporada de invierno en el Valle de Mexicali causó una disminución en los parámetros de color (L*, a*, b* y Croma) y en el contenido de ácidos grasos totales omega-3. Por su parte, la suplementación de AS no afectó los parámetros fisicoquímicos ni sensoriales del músculo *Longissimus thoracis*; además de que no fue capaz de aumentar el contenido de PUFA ni de cualquier otro ácido graso involucrado en la salud o de importancia nutricional.

De manera general, la suplementación de CZ y AS provocó cambios modestos en algunas de las variables evaluadas de comportamiento productivo, calidad de la canal, calidad de la carne y en el perfil de ácidos grasos. Por tanto, es importante evaluar nuevas estrategias en corderos de finalización. Asimismo, la eficacia de estas nuevas estrategias tendrá que ser confirmadas en una amplia variación de condiciones ambientales y bajos otros esquemas de alimentación.