UNIVERSIDAD AUTÓNOMA DE SINALOA
COLEGIO DE CIENCIAS AGROPECUARIAS
DOCTORADO EN CIENCIAS AGROPECUARIAS

TESIS:

"Evaluación de una fuente estandarizada de suplementos de aceites esenciales (crina®rumiantes) en comparación con la suplementación de ionóforos en corderos alimentados con dietas altas en energía en la fase de finalización: respuesta productiva, energética de la dieta, características de la canal y digestión de nutrientes"

Que para obtener el grado de
Doctor en Ciencias Agropecuarias

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Culiacán. Sinaloa, México; noviembre de 2022

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UNIVERSIDAD AUTÓNOMA DE SINALOA

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FACULTAD DE MEDICINA VETERINARIA Y ZOOTECNIA

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AGRADECIMIENTO

A **Dios** por darme sabiduría, fortaleza y entendimiento para llegar hasta aquí.

A la **Facultad de Medicina Veterinaria y Zootecnia de la Universidad Autónoma de Sinaloa FMVZ-UAS**, por abrir sus puertas a los estudiantes foráneos y no hacer menos ninguna profesión muchas gracias.

Al **Consejo Nacional de Ciencia y Tecnología (CONACYT)** por el apoyo económico recibido durante los estudios del Doctorado en Ciencias Agropecuarias.

A la **Unidad de Engorda Experimental de Pequeños Rumiantes (UEEPR),** por permitir realizar los estudios de campo para el presente trabajo.

A la planta docente que integra este equipo de trabajo liderado por el **Dr. ALFREDO ESTRADA ANGULO, Dra. BEATRIZ ISABEL CASTRO PEREZ, Dr. FRANCISCO GERARDO RÍOS RINCÓN, Dr. ALEJANDRO PLASCENCIA JORQUERA** y **Dr. LUIS CORONA GOCHI,** infinitas gracias por su amistad, ayuda y conocimiento adquirido por cada uno de ustedes para la realización del presente trabajo, mi admiración y respeto para cada uno de ustedes.

DEDICATORIA

A mis padres **IRENE WENCES LAGUNAS** y **SALVADOR ARTEAGA REYNA** mi motor más grande para salir a delante, una vez más misión cumplida mis amores nunca me cansaré de agradecerles todo lo que han hecho por mí, son el claro ejemplo de lo que yo quiero para mi futuro, con todo el amor y cariño esto es para ustedes.

A mi única hermana, mi confidente y mejor amiga **ANABELY ARTEAGA WENCES** por todo el apoyo en este proceso te quiero muchísimo, gracias por hacerme tía de dos princesas hermosas **VALENTINA** y **JULIET** que han llegado para hacer de nuestros días los más felices y también gracias al cuñado **JUAN PEDRO** que juntos han creado un gran equipo y esperemos que sigan así por muchos años.

A mi chaparrito **JORGE LUIS RAMOS MENDEZ** por todas esas veces que quise abandonar el barco y siempre estabas ahí para levantarme y seguir adelante, ambos sabemos que no fue nada fácil pero aquí seguimos y hasta ahorita somos el mejor equipo TU Y YO CONTRA EL MUNDO. Te amo jamás lo olvides.

A mis abuelitos que físicamente ya no están conmigo, pero los llevo en el corazón por siempre **MARCELINA LAGUNAS TORRES†** y **LEONARDO WENCES MORALES†**, **VALENTINA REYNA VILLALVA†** y **ANTONIO ARTEAGA TRUJILLO†.**

A **Dra. ALMA BEATRIZ PÉREZ FERNÁNDEZ** por convertirse en parte de nuestra familia, por todos los consejos, regaños y risas que hacen que nos duelan hasta las costillas, por esta aventura que seguimos escribiendo a lado suyo infinitas gracias.

A las familias **ARTEAGA WENCES Y RAMOS MENDEZ** por todas las muestras de cariño y ánimo en esta aventura.

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RESUMEN

"Evaluación de una fuente estandarizada de suplementos de aceites esenciales (crina®rumiantes) en comparación con la suplementación de ionóforos en corderos alimentados con dietas altas en energía en la fase de finalización: respuesta productiva, energética de la dieta, características de la canal y digestión de nutrientes"

M.C. Yesica Janeth Arteaga Wences

Se desarrollaron tres experimentos para evaluar los efectos de la suplementación de una fuente estandarizada de aceites esenciales en la respuesta productiva, la densidad energética de la dieta, las características de la canal, los cortes primarios, la masa visceral y la digestión de tracto total de ovinos durante la fase de finalización en condiciones de clima subtropical. Para el desarrollo del primer experimento se utilizaron 36 corderos machos sin castrar de raza Pelibuey × Katahdin (28.5 ± 3.5 kg) durante 56 días bajo un diseño de bloques completos al azar. Los corderos fueron alimentados con una dieta de finalización a base de maíz (13.8% de PC y 2.14 Mcal ENm/kg de MS) suplementada con 1) ningún aditivo (Testigo), 2) 30 mg MON/cordero/d, y 3) 150 mg AE/cordero/d. Resultados: el consumo de agua de los corderos alimentados con AE y Testigo fue similar. Los corderos alimentados con MON consumieron un 18.1% menos ($P < 0.01$) agua comparada con el Testigo y AE. La suplementación de AE comparado con el grupo Testigo, mejoró ($P < 0.05$) la eficiencia alimenticia y la energía neta de la dieta (EN). En comparación con MON, AE aumentó ($P < 0.05$) el consumo de materia seca (CMS), la ganancia diaria de peso (GDP) y la eficiencia alimenticia, y tendió a incrementar ($P=0.09$) la EN de la dieta. Se observó una diminución en el CMS y la GDP, pero no, en la EA ni en la EN de la dieta de los corderos alimentados con MON comparados con el Testigo. Con excepción del peso de la canal caliente (los corderos alimentados con MON registraron un menor PCC que el Testigo y AE), no hubo efecto entre tratamientos en el resto de las variables de características de la canal. La suplementación de AE y MON disminuyó los pesos (como proporción respecto al peso de cuerpo vacío) del intestino y de la grasa omental comparados con el grupo Testigo. En comparación con MON, AE disminuyó el peso relativo de la grasa mesentérica. Se concluye que, en comparación con los corderos del grupo Testigo (no suplementados), la suplementación de AE mejora la EA y la EN de la dieta. Al compararlo con MON, el suplemento de AE mejora la GDP. Sin embargo, los efectos de la MON y AE sobre la eficiencia alimenticia y la energía neta de la dieta no son apreciables para observar diferencia estadística entre los tratamientos. El suplemento de AE no afectó las características de la canal en la masa visceral. Por lo tanto, AE es una alternativa viable natural al antibiótico monensina sódica para mejorar la eficiencia de los corderos en finalización. Para el segundo experimento de respuesta productiva, así como el de digestión en tracto total, se evaluó una mezcla suplementaria de aceites esenciales solos (AE) o combinada con una enzima exógena (AE+ENZ) frente a virginiamicina (VM). Resultados: los corderos fueron alimentados con una dieta de finalización alta en energía suplementada con: (1) ningún suplemento (Testigo); (2) 150 mg de suplemento de AE; (3) 150 mg de AE suplementario más 560 mg de alfa-amilasa (AE+ENZ); y 4) 25 mg de virginiamicina (VM). Resultados: en comparación con el Testigo, la respuesta productiva de AE y VM fue similar, mejorando (5.7%, P<0.05) la eficiencia alimenticia y la energía neta de la dieta. En comparación con el Testigo, la suplementación con AE+ENZ tendió a aumentar (P=0.09) el consumo de materia seca (6.8%), mejorando (P<0.05) la ganancia de peso y la eficiencia alimenticia (10.4 y 4.4%, respectivamente). La utilización de la energía de la dieta fue mayor (2.7%, P<0.05) para AE y VM que para AE+ENZ. No se encontró efecto de los tratamientos sobre la canal y la masa visceral, pero la suplementación de los aditivos alimenticios disminuyó (P<0.03) el peso de los intestinos. No hubo efecto en las características de la digestión de tracto total ni en la energía digestible de la dieta, por lo cual se concluye que, la suplementación de AE puede ser una alternativa eficaz a la VM en las dietas de finalización con alta energía para corderos. La combinación de AE+ENZ puede mejorar el consumo de materia seca, promoviendo una mayor ganancia de peso.

Palabras clave: corderos; aceites esenciales; monensina; respuesta productiva, características de la canal, virginiamicina.

ABSTRACT

"Evaluation of a standardized source of essential oil supplementation (crina®rumiantes) compared to ionophore supplementation in lambs fed high energy diets in the finishing phase: productive response, diet energetics, carcass characteristics and nutrient digestion"

M.C. Yesica Janeth Arteaga Wences

Three experiments were developed to evaluate the effects of supplementation of a standardized source of essential oils on productive response, diet energy density, carcass characteristics, primal cuts, visceral mass and total tract digestion of sheep during the finishing phase under subtropical climate conditions. For the development of the first experiment, 36 uncastrated male lambs of Pelibuey × Katahdin breed (28.5 ± 3.5 kg) were used for 56 days under a randomized complete block design. Lambs were fed a corn-based finishing diet (13.8% CP and 2.14 Mcal ENm/kg DM) supplemented with 1) no additive (Control), 2) 30 mg MON/lamb/d, and 3) 150 mg AE/lamb/d. Results: Water intake of lambs fed AE and Witness was similar. Lambs fed MON consumed 18.1% less ($P < 0.01$) water compared to the Control and AE. Supplementation of AE compared to the Control group improved ($P < 0.05$) feed efficiency and net dietary energy (NE). Compared to MON, AE increased ($P < 0.05$) dry matter intake (DM), daily weight gain (DWG) and feed efficiency, and tended to increase ($P = 0.09$) dietary NE. A decrease was observed in CMS and GDP, but not in feed efficiency or diet EN of lambs fed MON compared to the control. With the exception of warm carcass weight (MON-fed lambs recorded a lower CCW than the Control and AE), there was no effect between treatments on the remaining carcass trait variables. Supplementation of AE and MON decreased the weights (as a proportion of empty body weight) of intestine and omental fat compared to the Control group. Compared to MON, AE decreased the relative weight of mesenteric fat. It is concluded that, compared to lambs in the Control group (unsupplemented), AE supplementation improves AE and EN of the diet. When compared to MON, AE supplementation improves the GDP. However, the effects of MON and AE on feed efficiency and diet net energy are not appreciable to observe statistical difference between treatments. AE supplementation did not affect carcass characteristics in visceral mass. Therefore, AE is a viable natural alternative to the antibiotic monensin sodium for improving the efficiency of finishing lambs. For the second production response experiment, as well as the total tract digestion experiment, a supplemental mixture of essential oils alone (AE) or combined with an exogenous enzyme (AE+ENZ) was evaluated against virginiamycin (VM). Results: lambs were fed a high-energy finishing diet supplemented with: (1) no supplement (Control); (2) 150 mg of supplemental AE; (3) 150 mg of supplemental AE plus 560 mg of alpha-amylase (AE+ENZ); and (4) 25 mg of virginiamycin (VM). Results: Compared to the Control, the productive response of AE and VM was similar, improving (5.7%, $P<0.05$) feed efficiency and net energy of the diet. Compared to the Control, supplementation with AE+ENZ tended to increase ($P=0.09$) dry matter intake (6.8%), improving ($P<0.05$) weight gain and feed efficiency (10.4 and 4.4%, respectively). Dietary energy utilization was higher (2.7%, $P<0.05$) for AE and VM than for AE+ENZ. No effect of treatments on carcass and visceral mass was found, but feed additive supplementation decreased ($P<0.03$) gut weight. There was no effect on total tract digesta characteristics or digestible energy of the diet, therefore it is concluded that, AE supplementation can be an effective alternative to VM in high energy finishing diets for lambs. The combination of AE+ENZ can improve dry matter intake, promoting higher weight gain.

Keywords: lambs; essential oils; monensin; productive response; carcass characteristics; virginiamycin.

CAPÍTULO 1: INTRODUCCIÓN Y REVISIÓN DE LITERATURA

1.1 INTRODUCCIÓN

Desde la aprobación de monensina (MON) a mediados de los años 70, la suplementación con ionóforos (especialmente MON) y algunos antibióticos (como virginiamicina VM) en las dietas para promover el crecimiento animal se ha convertido en una práctica de alimentación convencional en varios países, con la expectativa de mejorar la eficiencia de ganancia del 8 al 12 %. La mejora de la eficiencia energética se ha atribuido a los cambios en las relaciones molares de los AGV, a la disminución de la producción de metano y a la reducción de la degradación ruminal de la proteína dietética proteína de la dieta (Tedeschi y Gorocica-Buenfil, 2018).

Baran *et al.* (1986), concluyeron que, en los corderos el efecto de MON es mayor en las dietas de menor densidad de energía (dietas con alto contenido de forraje). Spires *et al.* (1990), observaron que a medida que la densidad energética de la dieta aumenta más allá de 2.00 Mcal NEm/kg, la magnitud de la mejora en la eficiencia de la ganancia debido a la suplementación de MON comienza a disminuir, y no se espera ninguna mejora en la eficiencia con la suplementación de ionóforos en una dieta que contenga más de 2.23 Mcal NEm/kg.

Duffield *et al.* (2012), señalaron que, en los últimos 40 años, el impacto de MON en la eficiencia alimenticia ha disminuido del 8.1 al 3.5%. Este cambio podría ser atribuido en parte, por el aumento de la densidad energética de la dieta de las actuales formulaciones para corderos de engorda, y ganado vacuno. Es por ello que, a medida que aumenta la digestión ruminal del almidón como ocurre con las dietas altas en cereales, los patrones de fermentación ruminal cambian para dirigir el carbono y el hidrógeno lejos de la metanogénesis hacia la producción de propionato (Wang *et al.,* 2018). La reducción de la pérdida de energía por metano se ha planteado como la base principal de los efectos de los ionóforos sobre la eficiencia energética (Gibb *et al.,* 2001).

Actualmente existe un gran interés por limitar el uso de antibióticos convencionales como aditivos alimenticios en la producción ganadera, esto ha llevado a la búsqueda de alternativas de aditivos "generalmente reconocidos como seguros". En sustitución al uso de los antibióticos. La suplementación con compuestos de aceites esenciales (AE; como timol, limoneno, eugenol, piperina, entre otros) ha mostrado características similares a los ionóforos con propiedades antimicrobianas que pueden ralentizar la tasa de digestión ruminal del almidón, aumentar la proporción ruminal de propionato acetato y reducir el grado de degradación de la proteína del alimento en el rumen (Koyunco y Canbolat, 2010; Samii *et al.,* 2016; Meschiatti *et al.,* 2016).

Hasta donde se sabe, no hay información disponible que evalúe los efectos comparativos de la suplementación de AE comparado con MON en corderos de finalización alimentados con dietas altas en energía (es decir, >2.10 Mcal ENm/kg MS); dietas en las que monensina suplementaria ha mostrado efectos moderados.

1.2 REVISIÓN DE LITERATURA

1.2.1 Aditivos alimenticios

Los aditivos para alimentación animal son tan numerosos y heterogéneos, que es difícil hacer una definición precisa. Sin embargo, se puede definir un aditivo alimenticio como un producto incluido en la formulación a un nivel bajo de inclusión cuyo propósito es incrementar la calidad nutricional del alimento, el bienestar o la salud del animal. De acuerdo a la comunidad científica, la definición más aceptada del término aditivo para la alimentación animal es la emitida en el Reglamento (CE) No. 1831/2003 del Parlamento Europeo, donde se refiere a sustancias, microorganismos y preparados distintos de las materias primas para dietas y de las premezclas, que se añaden intencionadamente al alimento o al agua para influir favorablemente en: 1) las características de las dietas o de los productos de origen animal, 2) las consecuencias ambientales de la producción, 3) los rendimientos productivos, el bienestar, la salud, mediante su influencia en el perfil de la flora microbiana intestinal o la digestibilidad de los alimentos, o 4) por su efecto coccidiostático o histomonostático (Ravindran, 2010).

Durante décadas se han utilizado los aditivos en la producción animal con el objetivo de mejorar los rendimientos productivos, no solo incrementando los niveles de producción sino también mejorando los parámetros reproductivos y el estado sanitario de los animales. De esta forma, se logran disminuir los costos e incrementar la eficiencia en los sistemas productivos (García y García, 2015).

1.2.1.1 Uso de antibióticos como promotores de crecimiento

El uso de diversos antibióticos y productos antimicrobianos como promotores del crecimiento animal se convirtió en una práctica común después de su descubrimiento en los años cuarenta. Los antibióticos promotores de crecimiento (APC) son los aditivos más utilizados en la alimentación animal, ya que provocan modificaciones de los procesos digestivos y metabólicos de los animales, que se traducen en aumentos de la eficiencia de utilización de los alimentos y en mejoras significativas de la ganancia de peso. También producen modificaciones en el tracto digestivo, que suelen ir acompañadas de cambios en la composición de la flora digestiva (disminución de agentes patógenos), reducciones en el ritmo de tránsito de la digestión, aumento en la absorción de algunos nutrientes (vitaminas) y reducciones en la producción de amoníaco, aminas tóxicas y alfa toxinas (Rosen, 1995).

Carro y Ranilla (2002), establecen que el empleo de los APC reducen la incidencia de enfermedades en el ganado, dado que mejoran la digestión y utilización de los alimentos, y reducen la cantidad de gases y excretas producidas por los animales. Lo cual se puede traducir en beneficios para el consumidor, como consecuencia de una reducción del precio de venta de los productos de origen animal. Hoy en día, los antimicrobianos más utilizados en la producción de ganado son los ionóforos.

1.2.1.1.1 Virginiamicina

Es un antibiótico que pertenece al grupo de los estreptograminos originados de la bacteria *Streptomyces virginiae,* cuya actividad es preferentemente sobre microorganismos gram-positivos como lo son los cocos, en general, Clostridium y algunos microorganismos gram-negativos como *Leptospiras, Treponema hyodisenteriae* y *Haemophilus*, este antibiótico actúa en el rumen uniéndose a los ribosomas de los microorganismos gram-positivos, inhibiendo la síntesis de proteínas de estos y así se incrementa la población de gram-negativas, formadores de ácido propiónico, que al unirse dos moléculas son las precursoras de la formación de glucosa en rumiantes que están en pastoreo (Terencio, 2011). Este antibiótico a nivel ruminal únicamente ataca a bacterias del género gram-positivas, puesto que las gram-negativas poseen una bicapa lipidica protectora. La inhibición de bacterias gram-positivas genera una fermentación más eficiente debidoa que el substrato de la dieta pasa a ser fermentado por bacterias que favorecen el acumulo del sub-producto ácido propiónico disminuyendo la proporción de ácido acético y butírico, los cuales generan energía en forma de grasa (Goulart, 2010).

En un estudio realizado por Tedeschi *et al.* (2003), demostraron que el uso de virginiamicina aumenta la eficiencia del uso de nitrógeno por rumiantes y reduce la producción de gas metano en el rumen, pudiendo contribuir con la mitigación de la emisión de este gas de efecto invernadero.

A nivel productivo, los factores que mejora el uso de virginiamicina son: perfil de ácidos grasos volátiles (AGV´S), aumento de la digestibilidad, reducción de la desaminación por bacterias ruminales, control de coccidiosis, y reducción de casos de acidosis ruminal porque se disminuye la producción de lactato, también se ha comprobado que la inclusión de virginiamicina en la dieta a razón de 20 a 40 gramos por tonelada de alimento ha mejorado la eficiencia de conversión alimenticia de un 10-12%, al incrementar las ganancias de peso diarias en un 4% al mismo tiempo que se reduce el consumo de alimento en un 7%. El rendimiento en canal de los animales se aumenta en 1 a 1.5 unidades porcentuales, lo que representa aproximadamente 4-6 kilos más por canal en un animal de 420 kilos al sacrificio (Goulart, 2010).

1.2.1.2 Antibióticos poliéter carboxílicos (ionóforos)

Los ionóforos son antibióticos que se usan como coccidiostáticos y promotores del crecimiento en la práctica veterinaria. Forman complejos con cationes mono y divalentes y facilitan el movimiento de iones metálicos al proporcionar canales lipofílicos a través de las membranas lipídicas hidrofóbicas (Kart y Ali, 2008). De acuerdo con la Administración de Alimentos y Medicamentos de los Estados Unidos (FDA, por sus siglas en inglés) existen al menos, cuatro ionóforos aprobados por dicho órgano regulatorio y que son empleados de manera comercial en la alimentación de rumiantes. Lasalocida sódica, laidlomicina, salinomicina y monensina sódica, este último, el más utilizado en los corrales de finalización. Todos parecen tener los mismos efectos sobre la fermentación ruminal. Uno de los más importantes mecanismos es la inhibición de la producción de metano durante la fermentación y un aumento en la producción de ácido propiónico en el rumen, lo que resulta en una reducción en la relación molar de acetato a propionato (Spears y Schicker, 1989).

Esta propiedad de transportar iones disipa los gradientes de iones y desacopla los gastos de energía del crecimiento en bacterias susceptibles, matando a estas bacterias. Estos antimicrobianos se dirigen específicamente a la población bacteriana ruminal y alteran la ecología microbiana, lo que resulta en una mayor retención de carbono y nitrógeno por parte del animal, aumentando la eficiencia de producción (Callaway *et al*., 2003).

Los ionóforos hacen que el ganado crezca de manera más eficiente, pero originalmente se usaron para controlar los parásitos intestinales (coccidiostato) en aves de corral. La monensina se ha comercializado para el ganado como inhibidor en la producción de metano y potenciador del propionato (el AGV [gluconeogénico] más eficientemente utilizado). Los beneficios adicionales del uso de monensina incluyen una reducción de la desaminación de proteínas en la dieta, lo que resulta en una menor excreción urinaria de amoníaco, y una disminución en la producción de ácido láctico que da como resultado una reducción en el ruminal acidosis y abscesos hepáticos. Los aumentos en la disponibilidad de energía y la retención de nitrógeno mejoran la eficiencia de la utilización del alimento por parte del animal rumiante y, por lo tanto, mejoran la productividad animal y la rentabilidad de la producción (Brown *et al.,* 1974).

1.2.1.2.1 Mecanismo de acción de ionóforos

Los ionóforos son generalmente bacteriostáticos y no bactericidas y sus mecanismos de acción son su capacidad para alterar el flujo de cationes a través de la membrana (Gonçalves *et al*., 2012). Las membranas bacterianas son relativamente impermeables a los iones, lo que permite utilizar gradientes iónicos como fuerza impulsora para la absorción de nutrientes. Las bacterias ruminales mantienen altas concentraciones de potasio intracelular y bajas concentraciones de sodio intracelular y, por el contrario, el ambiente ruminal contiene altas concentraciones de sodio y bajas concentraciones de potasio. Por lo tanto, las bacterias ruminales dependen en gran medida de los gradientes de iones (gradientes de K^+ y Na^+) para absorber nutrientes y establecer una fuerza motriz de protones. El pH ruminal es algo ácido debido a las concentraciones de AGV, sin embargo, el pH intracelular de muchas bacterias ruminales es casi neutral, creando así un gradiente de protones dirigido hacia adentro (Russel y Strobel, 1989).

Monensina es un anti portador de metal / protón que puede intercambiar iones de H^+ por Na^+ o K^+. Una vez insertado en la membrana, monensina intercambia iones de potasio intracelulares por protones extracelulares, o sodio extracelular por protones intracelulares (Figura 1). Debido a que el gradiente de potasio es mayor que el gradiente de sodio, los protones se acumulan dentro de la bacteria. La bacteria reacciona a esta acidificación citoplasmática activando una ATPasa reversible para bombear estos protones fuera de la célula. Además, otras bombas primarias que utilizan ATP para la eliminación de Na^+ y la absorción de K^+ se activan para restablecer los gradientes de iones; resultando en el desacoplamiento de la hidrólisis de ATP del crecimiento, disminuyendo así los conjuntos de ATP intracelular, lo que lleva a la muerte celular (Elsasser, 1984).

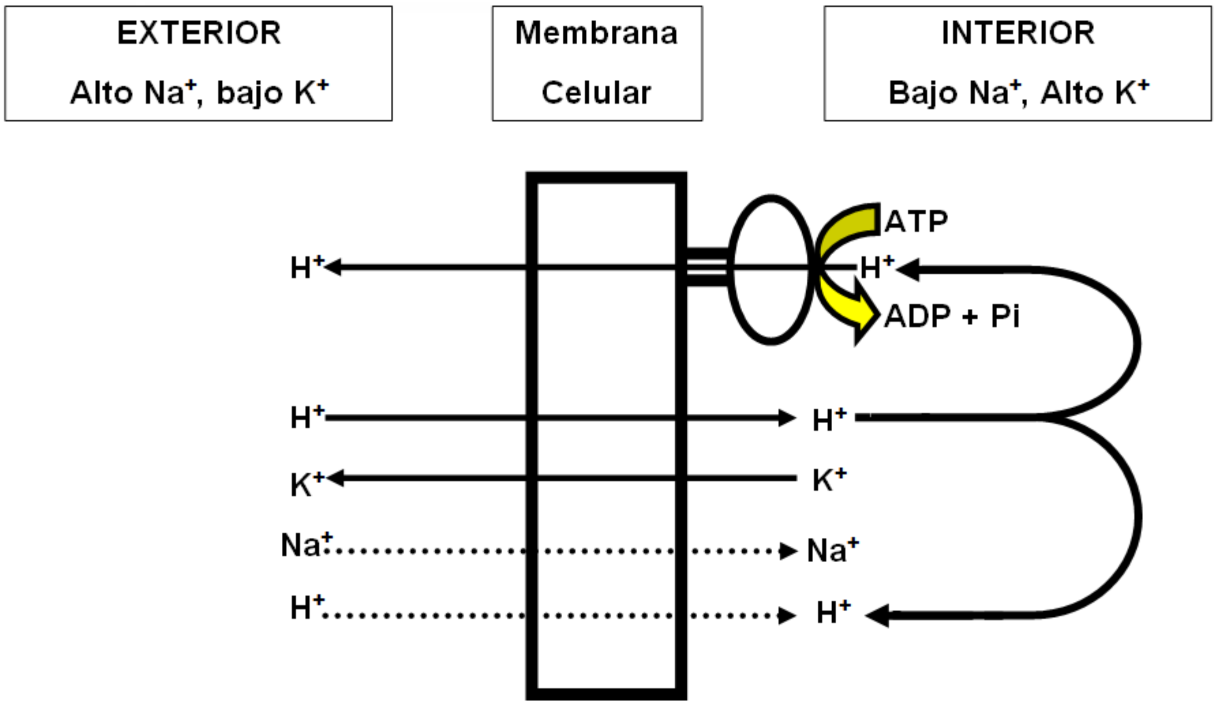

Figura 1. Efecto de monensina (M) en el flujo de iones (Russell, 1987), obtenido de Castro, 2010.

1.2.1.2.2 Efecto sobre la población ruminal y metabolismo energético

MON es producida por la fermentación de *Streptomyces cinnamonensis*, y promueve la desestabilización iónica de bacterias Gram positivas por medio del flujo de iones Na^+ en la célula, comprometiendo el equilibrio osmótico y electrolítico de los microorganismos (Neumann *et al*., 2018). En el ambiente ruminal, actuará sobre las bacterias del ácido láctico (*Streptococcus bovis* y *Lactobacillus spp*.), reduciendo la incidencia de enfermedades metabólicas y ruminales, como la acidosis láctica, causado por una alta proporción de concentrado en el alimento, y favoreciendo una reducción en la producción de metano por la reducción de bacterias Gram positivas que tienen hidrógeno como producto final (Nagaraja *et al.,* 1982).

Por lo tanto, hay un aumento en la participación de bacterias Gram negativas, como las productoras de propionato y las utilizadoras de lactato en el rumen, modificando la proporción de ácidos grasos volátiles en el rumen, aumentando la producción de ácido propiónico y reduciendo los porcentajes molares de ácidos butírico y acético (Duffield *et al*., 2012). Una producción mejorada de propionato en el rumen puede aumentar la disponibilidad de glucosa para el huésped a través de la gluconeogénesis. La glucosa derivada del propionato producido en el rumen constituye una parte importante (24-61%) de la producción total de energía (Markantonatos y Varga, 2017). El aumento de la producción de ácido propiónico proporciona así más energía del alimento al animal a través del aumento del suministro de glucosa y aumentando la energía metabolizable debido a una reducción en la producción de acetato (Chen y Wolin, 1979).

Además, Existe una mayor disponibilidad de proteínas derivadas de alimentos en el intestino delgado, debido a una disminución de las bacterias proteolíticas y fermentadoras de aminoácidos, lo que favorece el metabolismo de las proteínas (Bergen y Bates 1984).

1.2.1.2.3 Efecto sobre la productividad en rumiantes en Crecimiento- Finalización

Duffield *et al*. (2012), mencionan que, los impactos reportados de monensina desde su aprobación para el ganado de engorda a mediados de la década de 1970 en la eficiencia de la alimentación, la ganancia diaria promedio y el consumo de materia seca no siempre han sido consistentes. Por ejemplo, muchos estudios han encontrado que monensina mejora significativamente la eficiencia alimenticia, mientras que algunos estudios no han encontrado dichos efectos. En 2012, se realizó un metaanálisis (Cuadro 1) con el objetivo de evaluar el impacto de MON en el crecimiento y la finalización del ganado bovino productor de carne; los resultados mostraron que monensina redujo el CMS ($P <0.001$) y mejoró tanto la GDP ($P <0.001$) como la EA ($P <0.001$). La concentración promedio de monensina en el alimento entre estudios fue de 28.1 mg / kg de alimento (100% MS) y esto resultó en un aumento de aproximadamente 6.4% (pero solo 2.5 a 3.5% en las últimas dos décadas) en EA, disminución de 3% en CMS, y un aumento del 2.5% en GDP.

Cuadro 1. Resumen de las estimaciones del tamaño del efecto de monensina sobre los resultados de rendimiento en el ganado en crecimiento y acabado, derivado del meta-análisis.

Medidas de resultados	Diferencia de ẋ ponderada para el control de MON (IC 95%)[5]	Cambio, %	Corrales o ganado por tratamiento	Pruebas	Tamaño del efecto *P*
CA[1], kg	-0.53	-6.4	634	130	<0.001
		(-0.61, -0.45)			
CMS[2], kg	-0.268	-3.1	854	151	<0.001
		(-0.32, -0.21)			
GDP3, kg/d	+0.0291	+2.5	799	156	<0.001
		(0.019, 0.040)			
EA[4], kg	+0.0021	+1.3	186	32	0.048
		(-0.0001, 0.0043)			

1CA= Conversión alimenticia
2CMS= Consumo de materia seca
3GDP= Ganancia diaria de peso
4EA= Eficiencia alimenticia
5La diferencia de medias ponderada es la estimación del efecto real del tratamiento en unidades de medida; IC=Intervalo de confianza

1.2.1.2.4 Restricción sobre el uso de antibióticos como aditivos alimenticios

A pesar de los beneficios evidentes, en la Unión Europea, el uso de antibióticos como promotores del crecimiento (APC) en dietas se ha asociado con la resistencia a los antibióticos en humanos que consumen los productos y coproductos de estos animales, presumiblemente debido a la presencia de residuos, y ha sido prohibido desde 2006 (Neumann *et al*., 2018). La prohibición del uso de APC se basa, esencialmente, en la peligrosidad de estas sustancias por su capacidad para crear resistencias cruzadas con los antibióticos utilizados en medicina humana. Sin embargo, desde algunos sectores se apuntan otras razones, como son la existencia de intereses comerciales y la posibilidad de bloquear así la importación de productos animales procedentes de países en los que el uso de estas sustancias está permitido (Carro y Ranilla, 2002).

1.2.2 Alternativas al uso de antibióticos

Después de las medidas tomadas por la Comisión Europea sobre la prohibición del uso de antibióticos como aditivo en los alimentos para animales, se produjo una problemática de urgente y difícil solución en la práctica. Esto es debido a que, el empleo de muchos aditivos y entre ellos los antibióticos, además de justificarse por razones económicas inmediatas, tiene en numerosos casos una justificación razonable debido a la mejora de la eficacia de los procesos metabólicos y de la salud de los animales. El reto actual, para el sector ganadero, es conseguir hacer rentables los sistemas de producción más extensivos, que no hagan necesario el uso de los antiguos aditivos que podían suponer un riesgo para la salud del consumidor o para el medio ambiente, o conseguir efectos semejantes con el uso de productos naturales, nuevos y sin riesgo (Caja *et al*., 2003).

Es por ello por lo que, parece oportuna la investigación de sustancias alternativas para modificar la fermentación en el rumen y mejorar la digestibilidad del alimento, lograr mayores ganancias de peso o producciones de leche, reducir la eliminación al medio de productos de desecho que pueden llegar a resultar contaminantes y aliviar los efectos de algunos procesos mejorando el estado de bienestar de los animales. Todo ello de forma segura, sin comprometer la salud del consumidor. Entre estas sustancias, se propone el uso de plantas medicinales o de sus extractos o aceites esenciales (López *et al.,* 2002).

1.2.2.1 Extractos vegetales

Los organismos vegetales producen una gran diversidad de compuestos químicos, muchos de ellos desempeñan una importante función en las interacciones entre las plantas y el medio que las rodea, además tienen aplicaciones en el campo de la medicina, industria aromática e industria alimentaria (Martínez, 2008).

La utilización de los extractos de plantas se plantea como una de las alternativas más naturales a los antibióticos promotores de crecimiento. Los mecanismos de acción de estos compuestos, no se conocen totalmente, y varían según la sustancia de la que se trate, pero algunos de los mecanismos propuestos mencionan que mantienen efectos como la disminución de la oxidación de aminoácidos, ejercen una acción antimicrobiana sobre algunos microorganismos, estimulan la secreción de enzimas digestivas, aumentan la palatabilidad de los alimentos, estimulan su consumo y mejoran el estado inmunológico del animal (Carro y Ranilla, 2002).

Se debe destacar que los aditivos a base de plantas presentan una posible ventaja respecto a los tratamientos químicos. En las plantas, los principios activos siempre están biológicamente equilibrados por la presencia de sustancias complementarias que van a potenciarse biológicamente entre sí, no se acumulan en el organismo y sus efectos indeseables son limitados. Por lo anterior, los extractos vegetales suponen una alternativa confiable al uso de antibióticos para mejorar los índices productivos en rumiantes, tanto como promotores de crecimiento como para la mejora de la producción (Pereira *et al*., 2017).

1.2.2.2 Taninos

Los taninos son sustancias de origen vegetal, no nitrogenadas de estructura polifenólica, solubles en agua, alcohol y acetona. Estas sustancias son de sabor astringente y tienen la propiedad común de curtir la piel, haciéndola resistente a la putrefacción e impermeable al fijarse sobre sus proteínas (Lizcano y Vergara, 2008).

Existen dos tipos de taninos: hidrolizables (TH) y condensados (TC), siendo estos últimos los que poseen mayor capacidad de interacción con otras moléculas, mejorando la producción animal. En la actualidad, existe interés en TC como integrantes de dietas en rumiantes, debido a los beneficios potenciales sobre el valor nutritivo de la dieta y la salud animal. Los TC se unen con la proteína de la dieta, formando complejos estables e insolubles que se disocian en el abomaso a pH < 3.5, por este motivo, los taninos pueden reducir la degradabilidad de la proteína y el amoniaco en el rumen, y pueden aumentar el flujo de proteína hacia el intestino delgado, mejorando así el rendimiento de los rumiantes. Además, se observó que los taninos redujeron la producción de metano, debido a la reducción de la población protozoaria asociada a la metanogénesis y a la disminución de la degradación de la fibra (Mencio *et al*., 2014).

Sin embargo, se ha demostrado también que una dosis alta de taninos puede afectar el consumo de alimento y la digestibilidad, lo que probablemente tenga consecuencias en la productividad de los animales (Frutos *et al*., 2004). En el cuadro 2 se muestran los resultados de las investigaciones en las que se ha evaluado la inclusión de taninos en las dietas de rumiantes.

Cuadro 2. Resultados sobre los efectos de los taninos en la función del rumen.

Tipo	Fuente (planta)	Dosificación	Sistema/ Huesped	Efecto	Referencia
Taninos condensados	Quebracho	0.1 y 0.2%/kg de materia seca	*in vivo* (novillos), *in vitro*	*Aumento de la GDP en ambos niveles de suplementación (2.25 kg/d a 2% y 2.09 kg/d a 1%, comparado con 1.82 kg/d en el control). *Disminución en la producción de metano ruminal al aumentar la suplementación.	Min *et al.* (2016)
Taninos condensados	Tanino de quebracho secado y pulverizado	0.1, 0.2 y 0.4 mg/mL de medio	*in vitro*	*Ningún efecto sobre el nivel de AGVs pero la proporción molar de propionato fue significativamente mayor en casi un 10% y el butirato en un 13% en comparación con el control. *Reducción de la concentración de NH3 en un 47%, aunque la DMS no se vio afectada significativamente.	Min *et al.* (2016)
Taninos condensados	Quebracho, Silvateam, Ontario, CA	0, 0.2, 0.4 y 0.6% en base materia seca	*in vivo* (novillos Holstein)	*La GDP aumentó en un 6.5%, con una tendencia (efecto lineal) para aumente con el nivel de suplementación. *El CMS también tendió a aumentar (efecto lineal) con el nivel de suplementación.	Rivera *et al.* (2017)
Taninos condensados e hidrolizables	Quebracho, Silvateam, Ontario, CA Castaño, Silvateam, Ontario, CA	1)0, 0.6% taninos condensados 2)0.6% taninos hidrolizables 3)0.3% taninos condensados y 0.3% taninos hidrolizables combinados	*in vivo* (novillos Holstein)	*Los taninos (0.6%) aumentaron la GDP en 6.8%. Esta respuesta no fue afectada por la fuente de taninos. *El CMS mejoró en un 4%. Sin embargo, en comparación con los controles, el CMS fue mayor en un 7.1%, para los novillos alimentados con las combinaciones 50:50 de los taninos condensados e hidrolizables que cuando las fuentes de taninos se ofrecieron individualmente (2.4%).	Rivera *et al.* (2017)
Taninos condensados e hidrolizables	Extracto de tanino	0,2,4 Y 6 g/kg de MS	Ovinos	*La adición de tanino en la dieta no afectó la GDP, CMS, EA. Sin embargo, disminuyó la EN de la dieta y aumentó el CMS observado respecto al esperado. * Las respuestas fueron máximas cuando se suplemento con 4 g/kg de tanino. *No hubo efectos de los tratamientos sobre las características de la canal o la composición química de la paleta.	Rojas *et al.* (2017).

CMS= Consumo de materia seca; GDP= Ganancia diaria de peso; AGVs= Ácidos grasos volátiles; NH3= Amoniaco; EA= Eficiencia alimenticia; DMS= Digestibilidad de la materia seca

1.2.2.3 Derivados isoquinolínicos

Los alcaloides son compuestos derivados de microorganismos, organismos marinos y plantas. Son metabolitos secundarios con estructuras complejas nitrogenadas que han sido usadas en la medicina como agentes anticancerígenos, para combatir la malaria y como analgésicos, entre otras enfermedades. Además de sus propiedades medicinales, también se ha reportado la actividad antimicrobiana de los alcaloides, tanto para inhibición de bacterias como hongos. Además de su variedad en cuanto a actividad biológica, los alcaloides presentan elevada diversidad estructural y diversos orígenes de acuerdo con su ruta, de biogénesis. Una de las clasificaciones más destacadas, es la de los alcaloides isoquinolínicos, los cuales se denominan así, por tener como base de su estructura, la isoquinolina. Los derivados isoquinolínicos, han sido reportados como los metabolitos secundarios de mayor actividad antimicrobiana en plantas superiores. Estos alcaloides han sido aislados de plantas del género *Siparuna, como S. arianae, V. Pereira y S. tanduziana* (Peña, 2011).

En el cuadro 3 se muestran los resultados de las investigaciones en las que se ha evaluado la inclusión de derivados isoquinolínicos en las dietas de rumiantes.

Cuadro 3. Resultados sobre los efectos de los derivados isoquinolínicos en la función del rumen.

Fuente	Dosificación	Sistema/ Huesped	Efecto	Referencia
Sangrovit-RS (mezcla de alcaloides cuaternarios de benzofenantridina y alcaloides protopina)	0, 2, 4 y 6 g/d (0, 6, 12 y 18 mg de principio activo respectivamente)	*in vivo* (novillos)	*La eficiencia microbiana ruminal (N microbiano duodenal; g/kg MO fermentada en el rumen) y la eficiencia de la proteína (N no amoniacal duodenal; g/g ingesta de N) aumentó a medida que aumentó el nivel de suplementación. *La energía digestible de la dieta tendió a aumentar con la suplementación con Sangrovit-RS. *El pH ruminal y las concentraciones molares totales de AGVs no fueron diferentes entre los tratamientos, sin embargo, la proporción molar ruminal de acetato aumentó a medida que aumentaba el nivel de suplementación.	Aguilar *et al.* (2016)
Macleaya cordata (sanguinarina)	0 y 4 g/d (0 y 6 mg de principio activo respectivamente)	*in vivo* (toretes)	*El CMS, la GDP y la CA fueron similares entre los tratamientos. *Los animales que consumieron sanguinarina tuvieron un mayor rendimiento en canal y fueron más eficientes en la transformación de materia seca consumida a kilogramos de canal que los testigos.	Michels *et al.* (2018)

CMS= Consumo de materia seca; GDP= Ganancia diaria de peso; CA= Conversion alimenticia; AGVs= Ácidos grasos volátiles; N= Nitrógeno; MO= Materia orgánica

1.2.2.4 Aceites esenciales

Los aceites esenciales (AE) son componentes secundarios de las plantas, generalmente de naturaleza volátil. El término esencial deriva de la palabra “essentia”, lo cual significa que se puede oler o degustar. Se caracterizan de acuerdo con sus múltiples composiciones químicas, naturaleza y propiedades bio-activas. La función principal de los aceites esenciales es brindarle a la planta protección contra agentes estresantes abióticos y bióticos, y en algunas ocasiones atraer a otros organismos para favorecer la polinización y dispersión de sus semillas. Los principios activos que se encuentran en los aceites esenciales se clasifican dentro de dos grupos químicos; terpenoides (monoterpenoides y sesquiterpenoides) y fenilpropanoides. Estos dos grupos se originan de diferentes precursores del metabolismo primario y son sintetizados por vías metabólicas diferentes en las plantas.

De acuerdo con Polin *et al*. (2014), establecen que, los terpenoides son el grupo más numeroso y diversificado se han descrito aproximadamente 15,000 compuestos. Se denominan así porque derivan de una estructura básica de cinco carbonos (C_5H_8), comúnmente denominada unidad isopreno. Los fenilpropanoides son menos comunes, pero algunas plantas los contienen en cantidades altas, poseen cadenas de tres carbonos ligados a anillos aromáticos de seis carbonos y derivan en su mayoría de la fenilalanina. Los isoprenos (C_5) son los más comúnmente presentes en las plantas; isopentenil difosfato y dimetilamina difosfato, básicamente constituidos por terpenos y pertenecen al grupo de los terpenoides. Ejemplo de isoprenos son el limoneno, timol, carvacrol, linalol, carvon.

1.2.2.4.1 Uso de aceites esenciales en la alimentación animal

Los aceites esenciales se han usado para el tratamiento o prevención de enfermedades de los animales y en los últimos años se han evaluado como una alternativa en la nutrición de aves (principalmente en pollos de engorda), cerdos, peces y recientemente en rumiantes.

Entre los beneficios que tiene el uso de aceites esenciales en la alimentación animal se encuentran sus propiedades antimicrobianas, antioxidantes, antiparasitarias, antiinflamatorias, antidiarreicas y antimicóticas. Se ha observado que el suministro de aceites esenciales en la alimentación animal mejora la conversión alimenticia, estimulan enzimas digestivas y dan mejor sabor a los alimentos.

Martínez *et al.* (2015), señalan particularmente para el caso de su uso en rumiantes, los aceites esenciales tienen propiedades que son capaces de modificar la fermentación ruminal, haciendo más aprovechables los nutrientes de los alimentos, con lo que mejora la eficiencia de producción de leche y de carne en bovinos y ovinos, obteniendo mayor ganancia de peso y mejor conversion alimenticia. Otro uso que se ha dado a los aceites esenciales ha sido el de reducir o inhibir la metanogénesis en el rumen al inhibir el crecimiento de algunas bacterias metanogénicas del rumen (*Methanobrevibacter smithii, M. ruminantium. Methanosphaera stadtmanae*).

1.2.2.4.2 Mecanismo de acción de aceites esenciales

Los factores que determinan la actividad de los aceites esenciales son la composición, los grupos funcionales presentes en los componentes activos y sus interacciones sinérgicas. El mecanismo de acción antimicrobiano varía con el tipo de aceite esencial o la cepa del microorganismo utilizado. Es bien sabido que, en comparación con las bacterias Gram negativas, las bacterias Gram positivas son más susceptibles a los aceites esenciales. Esto se puede atribuir al hecho de que las bacterias Gram negativas tienen una membrana externa que es rígida, rica en lipopolisacáridos (LPS) y más compleja, y la resistencia limita la difusión de compuestos hidrófobos a través de ella, mientras que esta membrana extra compleja está ausente en bacterias Gram positivas que, en cambio, están rodeadas por una gruesa pared de peptidoglucano que no es lo suficientemente densa como para resistir pequeñas moléculas antimicrobianas, lo que facilita el acceso a la membrana celular. Además, las bacterias Gram-positivas pueden facilitar la infiltración de compuestos hidrófobos de los aceites esenciales debido a los extremos lipofílicos del ácido lipoteicoico presente en la membrana celular.

Se ha demostrado en varios informes que los componentes bioactivos presentes en los aceites esenciales podrían unirse a la superficie de la célula y, a partir de entonces, penetrar en la bicapa de fosfolípidos de la membrana celular. La integridad estructural de la membrana celular se ve alterada por su acumulación, que puede influir perjudicialmente en el metabolismo celular causando la muerte celular. Además, se ha informado que la acción de los aceites esenciales sobre la integridad de la membrana celular cambia la permeabilidad de la membrana, lo que conduce a la pérdida de contenido intracelular vital como proteínas, reduciendo azúcares, ATP y ADN, al tiempo que inhibe la generación de energía (ATP) y las enzimas relacionadas a la destrucción de la célula y la fuga de electrolitos (Chouhan *et al.,* 2017).

1.2.2.4.3 Efecto de los aceites esenciales en la población microbiana ruminal

Se ha reportado que los aceites esenciales inhiben a las bacterias productoras de nitrógeno amoniacal, decreciendo así la desaminación de los aminoácidos principalmente en dietas que contienen cantidades no muy altas de proteína. También se ha observado que el número total de bacterias viables no es afectado, es decir cambian solamente las proporciones de grupos bacterianos, pero en estudios in vitro donde se han incluido dosis altas, sí provocan descenso en el número total de microorganismos. Por otro lado, los cambios en las poblaciones de protozoarios dependen del aceite esencial y la dosis utilizada, ya que los resultados de los experimentos realizados son muy variables, pudiendo no haber cambios en la población de protozoarios, provocando una disminución en el número total de protozoarios o afectando solo a ciertos grupos de protozoarios ruminales (Polin *et al*., 2014).

1.2.2.4.4 Aceites esenciales y fermentación ruminal

Algunos investigadores reportan una pequeña disminución en la concentración total de ácidos grasos volátiles al incluir aceites esenciales en las dietas de rumiantes, otros mencionan disminuciones significativas especialmente cuando se emplean concentraciones altas de aceites esenciales y sólo unos cuantos estudios mencionan ligeros aumentos en la concentración total de AGV´s con inclusión de cinamaldehído en dosis de 200 mg/kg de MS (Chaves *et al*., 2008b), y en dosis de 250 mg/kg de MS de aceite de orégano (Wang *et al*., 2009). También se ha reportado que el uso de mezclas de AE (timol, limoneno y guayacol) en dosis de 1.5 ml/L (*in vitro*), incrementan los AGV´s totales (Castillejos *et al*., 2005). La respuesta a la inclusión de los aceites esenciales sobre la concentración total de ácidos grasos volátiles depende del tipo de dieta o substrato, lo cual puede deberse a las diferentes características nutrimentales de los insumos utilizados; otro factor que modifica la respuesta a la inclusión de aceites esenciales en dietas es el pH ruminal, esto debido a que a un pH ligeramente ácido los aceites esenciales se encuentran en un estado no disociado y por lo tanto en forma hidrofóbica, lo cual les permite interactuar de forma más fácil con las membranas microbianas y por tanto modificar la población ruminal y la proporción y producción de ácidos grasos volátiles; además, el perfil de los principios activos que contenga el aceite esencial influye en la cantidad y proporción de los ácidos grasos volátiles producidos (Polin *et al*., 2014).

Las modificaciones en el total y proporción de los ácidos grasos volátiles impactan sobre los perfiles de producción de metano, sobre todo si reduce la proporción de acetato y se incrementa la proporción de propionato, por lo tanto, cambios en estos repercutirán positiva o negativamente en la producción de metano.

Como ya se ha mencionado, algunos aceites esenciales inhiben a las bacterias generadoras de nitrógeno, por tanto, la desaminación de las proteínas decrece, y se ha reportado hasta un 25 % en la reducción de estas bacterias cuando se utilizó aceite de orégano en dosis de 30 y 300 mg/L (Newbold *et al*., 2004; Cardozo *et al*., 2005). Es así como decrece la concentración de nitrógeno amoniacal en el rumen con el uso de aceites esenciales, y puede favorecer a un flujo mayor de las proteínas al intestino delgado. Cabe mencionar que los efectos sobre las bacterias productoras de nitrógeno son dependientes del tipo y cantidad de proteína en la dieta. Aunque este tipo de bacterias representa sólo el 1 % del total de la población bacteriana en el rumen, poseen una actividad alta de desaminación (Polin *et al*., 2014).

Los resultados en los que se ha evaluado la mitigación del metano como objetivo de la inclusión en las dietas de aceites esenciales son pocos e inconsistentes. Se ha observado que el timol, mayor componente derivado del *Thymus v. y Origanum v*., a dosis de 400 mg/L inhibe consistentemente el metano *in vitro* (Evans y Martin, 2000). En otros trabajos *in vitro* también con timol a dosis de 900 mg/L, observaron una mitigación en la producción del metano hasta un 99 % en relación con el tratamiento testigo (Chaves *et al*., 2008a). El principal componente activo del aceite de canela es el cinamaldehído, el cual a dosis de 660 mg/L disminuye la producción de metano hasta en un 94 % (Macheboeuf *et al*., 2008). El aceite de eucalipto inhibe la producción de metano en un 58 % con dosis de 1.66 ml/L (Kumar *et al*., 2009), 90.3 % a 2 ml/L (Sallam *et al*., 2009), y 70 % a 330 mg/L el aceite α-ciclodextrinaeucalipto (Sallam *et al*., 2009).

Estudios realizados con bovinos productores de carne en los que se usó una mezcla comercial de aceites esenciales (timol, eugenol, vainillina y limoneno) a 1 g/d, durante 25 días, no encontraron diferencias en la producción de metano en comparación con el tratamiento control (Beauchemin *et al*., 2009), y tampoco se encontraron diferencias en estudios con aceite de pino a dosis de 8 mg/L (Soliva *et al*., 2008). En el cuadro 4 se muestran los resultados de las investigaciones en las que se han evaluado in vivo la inclusión en las dietas con diferentes aceites esenciales y dosis en vacas lecheras, sobre la respuesta en la producción de metano y AGV´s totales.

Cuadro 4. Efecto de la adición de diferentes aceites esenciales y dosis sobre el consumo, producción de leche, metano y ácidos grasos volátiles totales en vacas lecheras, en diferentes estudios. Estimación de las diferencias porcentuales con respecto al tratamiento testigo.

Aceite esencial	Dosis g/d	CMS (%)	Producción de leche (%)	Producción de metano1 (%)	AGV´s Totales (%)	Referencia
	0.32	-1.52	-3.76	ND	ND	
MAE	0.64	-1.01	-2.13	ND	ND	Santos *et al.* (2010).
	0.96	-0.50	-2.25	ND	ND	
MAE	2.0	-2.62	-0.87	-2.74	-7.63	Benchaar *et al.* (2006b).
MAE	0.75	-0.57	-5.9	5.20	5.09	Benchaar *et al.* (2007).
	0.75	-1.12	-0.5	-1.32	-10.31	
Cinemaldehído	1.0	-0.43	1.78	0.89	0.49	Benchaar *et al.* (2008).
Aceite de ajo	5.0	-1.44	3.10	-2.24	-1.47	Yang *et al.* (2007).
Aceite de enebro	2.0	-0.96	1.37	2.82	-0.62	
	13.88	1.71	-2.31	ND	ND	
Dialil disulfuro	10.02	1.29	-2.64	ND	ND	Zijderveld *et al.* (2011).
	3.36	-5.08	1.63	ND	ND	
Carvacrol	6.35	-2.62	1.46	0.60	-2.49	Tekippe *et al.* (2011).
MAE	1.2	7.19	4.77	ND	ND	Kung *et al.* (2008).
	1.87	-3.84	1.03	-7.27	2.48	
MAE	3.96	-3.84	-3.67	-6.13	2.90	Mossaad *et al.* (2009).
	6.13	-2.19	-1.37	-1.17	0.77	
MAE	1.0	-5.10	0.08	ND	ND	Santos *et al.* (2010).

MAE= Mezcla de aceites esenciales; CMS= Consumo de materia seca; AGV´s= Ácidos grasos volátiles; ND= No determinado; 1= CH_4 = 0.45 (acetato) – 0.275 (propionato) + 0.4 (butirato) (Moss *et al.,* 2000). Adaptado de Polin *et al.*, (2014).

1.2.2.4.5 Efecto en el comportamiento productivo de rumiantes

Los efectos positivos de la inclusión de aceites esenciales sobre la digestibilidad del alimento se dan por dos razones principalmente; primero, se reduce la degradación de la proteína en el rumen al inhibir la proliferación de bacterias productoras de nitrógeno amoniacal o proteolíticas, y segundo, se reduce la degradación de almidones como respuesta a la inhibición de microorganismos amilolíticos, favoreciendo en cantidad el flujo de estos dos nutrientes al intestino (Meyer *et al*., 2009)

En muchos de los estudios la digestibilidad del alimento no se modificó por la inclusión de aceites esenciales (Malecky *et al*., 2009). Sin embargo, otros estudios muestran que dosis de 500 mg de aceite de orégano en ovinos repercute en una alta concentración de proteína a nivel ruminal, pero por otro lado se afectó la digestibilidad total de nutrientes (Kozelov *et al*., 2001). Reportes *in vivo* en vacas lecheras con dosis de aceite esencial de enebro de 2 g/d, mostraron un aumento en la digestibilidad de la materia seca en un 13 % utilizando dietas con 40:60 forraje-concentrado; estos investigadores explican que el efecto puede ser debido a que se incrementó la digestibilidad de la proteína de manera significativa en un 11 %, pero también puede deberse a un ligero incremento de digestibilidad de otros nutrientes. Sin embargo, dosis altas de aceites esenciales pueden afectar la digestibilidad de MS, atribuible esto a la disminución de la digestibilidad de la fibra a nivel ruminal (Beauchemin *et al*., 2009; Yang *et al*., 2010a).

Al igual que en muchos otros casos, la respuesta de la adición de aceites esenciales en dietas para rumiantes sobre el consumo de materia seca depende también del tipo de aceite esencial y la dosis (Cuadro 5). Estudios realizados con altas dosis de cinemaldehido (500 mg/d) en vacas productoras de leche, o una mezcla de cinemaldehido 500 mg/d y eugenol 90 mg/d en bovinos productores de carne, afectó el consumo de alimento, lo cual puede ser atribuido a la palatabilidad que adquieren el alimento cuando se les adicionan cantidades altas de aceite esencial (Santos *et al*., 2010).Otro estudio demostró claramente que el cinemaldehido tiene efecto positivo en el consumo de alimento en 10.3 % con dosis bajas de 400 mg/d, pero que dosis altas de 1.6 g/d el consumo se mantiene igual en comparación con el tratamiento control en novillos (Yang *et al*., 2010b).

Cuadro 5. Efecto de la adición de diferentes aceites esenciales y dosis sobre respuesta productiva en rumiantes

Especie animal y fin zootécnico	Fuente (planta)	Dosificación	Efecto	Referencia
Bovinos en finalización	Mezcla de Timol (50%), carvacrol (50%)	*All Sacch Beef (Saccharomyces cerevisiae 5×108 UFC/g) *MAE (1.17 g/kg of thymol and 1.17 g/kg)	*Sin efecto sobre las variables de respuesta productiva ni las características de la canal.	Missi *et al.* (2022)
Ovinos Producción de proteína	Mebogrow® (*Terminalia bellirica, Azadirachta indica* y *Chebulic myrobalan*) y hojas de Neem (*Azadirachta indica*).	*0.0, 2.0 % Mebogrow® Y *0.5 y 1.0 % Hojas de Neem	*No se observó ningún cambio en las variables productivas ni ruminales, sin embargo, al incluir el poliherbal se observó un efecto benéfico en el metabolismo de proteínas. *La inclusión de hojas de *Azadirachta indica* (Neem) en dosis de 1.0% mejoró la digestibilidad de la materia seca y se observó que la inclusión incrementa el conteo de neutrófilos favoreciendo así la inmunidad de ovinos en etapa de finalización de los ovinos.	Rodríguez (2021).
Ganado Bovino en finalización	Mezcla de aceites esenciales (CRINA®) (Limoneno, vainillina, timol, eugenol) + enzima amilasa (AM)	*Monensina sódica (26 mg/kg); *MAE (90 mg/kg); *MAE+MON (90 mg/kg + 26 mg/kg); *MAE+ α-amilasa exógena (90 mg/kg + 560 mg/kg) *MAE+AM+PRO (90 mg/kg + 560 mg/kg + 840 mg/kg)	*Se observó un mayor consumo de alimento del ganado con respecto a la dieta control. *No hubo efecto sobre la eficiencia alimenticia. *La combinación de MAE+AM no afecto la eficiencia ni las características de la canal.	Meschiatti *et al.* (2019)
Novillas en finalización	Mezcla de aceites esenciales (CRINA®) (Limoneno, vainillina, timol, eugenol)	*Monensina sódica (26 mg/kg) *MAE (90 mg/kg)	*Se observó un mayor consumo de alimento del ganado con respecto a la dieta con MON. *Sin efecto sobre la GDP, PVF, EA. *Mayor rendimiento de canal	Acedo *et al*. (2017)

MAE= Mezcla de aceites esenciales; CMS= Consumo de materia seca; GDP= Ganancia diaria promedio; PVF= Peso vivo final; EA= Eficiencia alimenticia.

Al igual que en el consumo de materia seca, la respuesta en cuanto a ganancia diaria de peso es inconsistente y depende del tipo de aceite esencial y la dosis. En estudios en los que se evaluó la ganancia diaria de peso en ovinos con inclusión en la dieta de hojas de orégano (144 o 280 mg/kg MS) no se observaron diferencias con respecto al tratamiento control (Bampidis *et al*., 2005); tampoco en estudios realizados con bovinos productores de carne, con dosis de 2 o 4 mg/d de una mezcla de aceites esenciales (timol, eugenol, vainillina y limoneno), ni en la media de ganancia diaria de peso con respecto al tratamiento control (Benchaar *et al*., 2006a). En el cuadro 6 se muestran los resultados de las investigaciones en las que se han evaluado la inclusión de diferentes aceites esenciales y dosis, en bovinos y ovinos, sobre el consumo y la ganancia promedio de peso. Existen reportes en los que la adición de mezclas comerciales de aceite esencial (timol, eugenol, vainillina y limoneno) ha incrementado la producción en ganado lechero (Kung *et al*., 2008).

También que la alimentación con una mezcla de aceite esencial conteniendo eugenol, extracto de geranio y aceite de cilantro, como el mayor componente este último y en una dosis de 500 mg por vaca por día incrementa el porcentaje de grasa en la leche (Santos *et al*., 2010). Esto puede deberse al cambio en la proporción de ácidos grasos volátiles, acetato o proporción de acetato-propionato, que pueden modificarse con la inclusión de aceites esenciales, o por el cambio de aporte energético y el mejoramiento de la condición corporal por la mejoría en la alimentación (Polin *et al*., 2014).

Cuadro 6. Efecto de la adición de diferentes aceites esenciales y dosis sobre el consumo, promedio de ganancia diaria, metano y ácidos grasos volátiles totales en ovinos, en diferentes estudios. Estimación de las diferencias porcentuales con respecto al tratamiento testigo.

Tratamiento	Dosis g/d	CMS (%)	GDP (%)	Producción de metano1 (%)	AGV´s Totales (%)	Referencia
Cinamaldehído	0.238	4.64	15.40	-17.04	16.04	Chaves *et al.* (2008b).
Aceite de ajo	0.227	-0.31	1.19	-7.35	24.44	Chaves *et al.* (2008a).
Aceite de enebro	0.24	5.11	17.34	-20.69	25.43	Chaves *et al.* (2008c).
Dialil disulfuro	4.61	0.26	ND	3.13	-3.65	
Lovastatina	0.092	0.34	ND	2.48	-5.43	Klevenhusen *et al.* (2011b).
Aceite de ajo	5.31	-5.85	ND	1.29	-9.30	
Dialil disulfuro	2.19	-2.92	ND	-0.40	-9.30	
	0.5*	ND	ND	-4.445	-0.66	
Aceite de ajo	0.5*	ND	ND	-4.97	-0.49	Anassori *et al.* (2011).
	0.75*	ND	ND	-5.40	-0.66	
MAE	0.11	ND	ND	2.43	-7.02	Castillejos *et al.* (2007).
Carvacrol	0.2	-4.48	7.18	-9.59	13.68	
Cinamaldehído	0.2	0.41	8.35	-10.58	19.03	Chaves *et al.* (2008b).
Carvacrol	0.2	5.24	1.61	-3.58	5.94	
Cinamaldehído	0.2	13.59	3.29	11.28	10.72	
	0.1	0.19	-0.12	8.91	-8.68	
Cinamaldehído	0.2	3.32	6.78	-1.56	-22.08	Chaves *et al.* (2011).
	0.4	1.81	-2.17	-2.30	-27.46	

MAE= Mezcla de aceites esenciales; CMS= Consumo de materia seca; GDP= Ganancia diaria de peso; AGVs= Ácidos grasos volátiles; * g/kg de materia seca; ND= No determinado; 1= CH_4 = 0.45 (acetato) – 0.275 (propionato) + 0.4 (butirato), (Moss *et al., 2000*). Adaptado de Polin *et al.*, (2014).

1.3 CONCLUSIÓN

Los antibióticos carboxilados (ionóforos) se han utilizado por los últimos 40 años mostrando efectos positivos en la respuesta productiva, así como una reducción en la formación de metano, debido a sus características de modulación de la fermentación ruminal. Actualmente se reconoce que el uso masivo de antibióticos ha llevado a un mayor problema de resistencia, y la presencia de residuos de antibióticos en los alimentos y el medio ambiente esto compromete la salud humana y la de los animales. En este sentido, el uso de aceites esenciales en la nutrición animal ha demostrado tener propiedades como la capacidad de modificar la fermentación ruminal, logrando un mayor aprovechamiento de los nutrientes de los alimentos, con lo que mejora la eficiencia de producción de leche y de carne en rumiantes. Además, muestran efectos positivos en la digestibilidad del alimento ya que reducen la degradación de la proteína en el rumen al inhibir la proliferación de bacterias productoras de nitrógeno amoniacal o proteolíticas, y segundo, se reduce la degradación de almidones como respuesta a la inhibición de microorganismos amilolíticos, favoreciendo en cantidad el flujo de estos dos nutrientes al intestino. Por lo cual Influyen en el consumo voluntario del animal, dicho efecto dependerá del tipo de aceite esencial y la dosis que se suministre en la dieta.

ARTÍCULO 1

The effects of feeding a standardized mixture of essential oils vs monensin on growth performance, dietary energy and carcass characteristics of lambs fed a high-energy finishing diet

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Publicado en: Small Ruminant Research (2021)

DOI: https://doi.org/10.1016/j.smallrumres.2021.106557.

Small Ruminant Research 205 (2021) 106557

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

The effects of feeding a standardized mixture of essential oils vs monensin on growth performance, dietary energy and carcass characteristics of lambs fed a high-energy finishing diet

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A R T I C L E I N F O

Keywords:
Lambs Essential
oilsMonensin
Growth performance
Dietary energy Carcass

A B S T R A C T

Thirty-six Pelibuey × Katahdin crossbred intact male lambs (28.5 ± 3.5 kg) were used in a 56-d experiment in a randomized complete block design to evaluate the effects of a standardized mixture of essential oils (EO) versus monensin sodium (MON) on growth performance, dietary energy, and carcass characteristics. Lambs were fed a corn-based finishing diet (13.8 % CP and 2.14 Mcal NE_m/kg DM) supplemented with: 1) no additive (Control), 2) 30 mg MON/lamb, and 3) 150 mg EO/lamb. Water consumption of EO and Control lambs was not different. In contrast, lambs fed MON consumed 18.1 % less ($P < 0.01$) water than Controls and EO groups. Compared to Controls, EO improved ($P < 0.05$) gain efficiency, estimated dietary net energy (NE). Compared to MON, sup- plemental EO increased ($P < 0.05$) dry matter intake (DMI), average daily gain (ADG) and gain efficiency, and tended ($P = 0.09$) to increase estimated dietary NE. Compared to Controls, lambs fed MON decreased DMI and ADG but without showing difference on gain efficiency and estimated dietary NE. With the exception of carcass weight (lambs fed MON had lower hot carcass weight than Control and EO), there were no treatments effects on carcass composition. Compared with Controls, EO and MON supplementation decreased relative weights (as a proportion of empty body weight) of intestine and omental fat. Compared with MON, EO decreased relative weight of mesenteric fat. We conclude that compared with Control (non-supplemented) lambs, supplemental EO enhances feed efficiency, and dietary net energy. Compared with MON, supplemental EO enhances ADG. However, effects of MON and EO on feed efficiency and dietary net energy are not appreciable different. Sup- plemental EO did not negatively affect carcass characteristics or visceral organ mass. As such, supplemental EO is a viable alternative to the antibiotic monensin for enhancement of feed efficiency of finishing feedlot lambs.

1. Introduction

Since approval of the antibiotic monensin (MON) in the mid-1970's, ionophore supplementation (particularly MON) in growing-finishing diets has become the conventional feeding practice in several coun- tries, with expectation of improved gain efficiency from 8 to 12 %. Improved energetic efficiency have been attributed to changes in VFA molar ratios, decreased methane production, and decreased ruminal degradation of dietary protein (Tedeschi and Gorocica-Buenfil, 2018; da Fonseca et al., 2019). In an earlier report, Baran et al. (1986) concluded that in lambs, the effect of MON is greater in diets of lesser energy density (high roughage diets). Spires et al. (1990) observed that as theenergy density of the diet increases beyond 2.00 Mcal NE_m/kg, the magnitude of improvement in gain efficiency due to MON

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https://doi.org/10.1016/j.smallrumres.2021.106557
Received 19 June 2020; Received in revised form 15 September 2021; Accepted 10 October 2021
Available online 12 October 2021
0921-4488/

supplementation starts to decrease, and no improvement in feed effi-ciency would be expected with ionophore supplementation of a diet containing greater than 2.23 Mcal NE_m/kg. Their prediction equation was later verified by the meta-analysis performed by Duffield et al. (2012), noting that in the last 40 years, the impact of MON on feed ef- ficiency has decreased from 8.1 to 3.5%. This change may be explained,in part, by increases in diet energy density in current finishing diet formulations for feedlots lambs (Leite et al., 2021) and cattle (Hales, 2019; Pinto and Millen, 2019). The basis of this effect is not fully un- derstood. A popular explanation is that as ruminal starch digestion in- creases, as occurs with high-cereal diets, ruminal fermentation patterns shift to direct carbon and hydrogen away from methanogenesis and toward propionate production (Wang et al., 2018). Reduced methaneenergy loss has been put forth as the primary basis for ionophores effectson energetic efficiency (Gibb et al., 2001). Current interests in limiting the use of conventional antibiotics as feed additives in livestock pro- duction, has led to the search for “generally-recognized-as-safe” additive alternatives. Dietary supplementation with essential oil compounds (**EO**; such thymol, limonene, eugenol, piperine, among others) has exhibited ionophore-like characteristics with antimicrobial properties that may slow the rate of ruminal starch digestion, increase ruminal propionate: acetate molar ratios, and reduce extent of ruminal feed protein degra- dation. (Koyunco and Canbolat, 2010; Samii et al., 2016; Meschiatti et al., 2016). Supplemental essential oils (Smeti et al., 2015; Parvaret al., 2018) and MON (Safaei et al., 2014) have both enhanced growth performance in small ruminants fed finishing diets of moderate energy density (i.e. < 2.0 Mcal NE_m/kg). To our knowledge, no information is available that evaluates the comparative effects of supplemental EO vs MON in finishing lambs fed high-energy finishing diets (i.e. > 2.10 Mcal NE_m/kg DM); diets in which supplemental MON has shown modest ef- fects. The objective of this experiment was to compare the influence of supplementation with a standardized mixture of essential oils (EO) vs monensin sodium (MON) on growth performance, dietary energetic, and carcass characteristics in lambs fed a corn-based high-energy finishing diet. A non-supplemented treatment was included as a negative control.

2. Material and methods

This experiment was conducted at the Universidad Autónoma de Sinaloa Feedlot Lamb Research Unit, located in the Culiacán, México (24 ° 46′ 13′′ N and 107 ° 21′ 14′′W). Culiacán is about 55 m above sea level, and has a tropical climate. During the course of the experiment, air temperature averaged 20.9 °C (minimum and maximum of 15.5 and 26.3 °C, respectively) and relative humidity averaged 71.8 % (minimum and maximum of 58.4 and 85.2 %, respectively). All animal manage- ment procedures were conducted within the guidelines of locally-approved techniques (NOM-062-ZOO-1999) for animal use and care.

2.1. Animal, diets, and samples analyses

Thirty-six Pelibuey × Katahdin crossbred intact male lambs (165 ± 20 d age; 28.5 ± 3.5 kg initial weight) were used in a 56-d growth-performance experiment to compare the effects of supple- mentation of a mixture of essential oils (EO) vs sodium monensin (MON) on growth performance, dietary energetic, and carcass characteristics in lambs fed a corn-based high-energy finishing diet. Two weeks before initiation of the experiment the lambs were treated for parasites (Albendaphorte 10 %, Animal Health and Welfare, México City, México), injected with 1 × 10^6 IU vitamin A (Synt-ADE®, Fort Dodge, Animal Health, México City, México), and vaccinated for *Mannheimia haemolityca* (One shot Pfizer, México City, Mexico). Upon initiation of the experiment, lambs were weighed just prior to the morning meal (electronic scale; TORREY TIL/S: 107 2691, TORREY Electronics Inc., Houston TX, USA), blocked by initial weight and assigned within blocksto 18 pens, two lambs per pen. Dietary treatments were randomly assigned to pens within blocks, resulting in 6 replicates per treatment.

Pens were 6 m^2 with overhead shade, automatic waterers and 1 m fence- line feed bunks. Lambs were fed with cracked corn-based finishing diet(Table 1) and 3 treatments were tested as follows: 1) non supplemented (**Control**), 2) a daily supplementation with 30 mg MON/lamb (**MON**; Rumensin 90, Elanco Animal Health, Indianapolis, IN), and 3) daily supplementation with150 mg EO/lamb (**EO**, CRINA-Ruminants, DSM Nutritional Products, Basel, Switzerland, containing a standardized mixture of essential oils including thymol, eugenol, vanillin, guaiac, andlimonene). The daily dose of 150 mg EO was estimated based on a previous report where ingestion of 100–200 mg EO/day resulted in maximal enhancements in ruminal fermentation and feed efficiency in lactating ewes (Giannenas et al., 2011). The dosage of 30 mg MON/dayis the average of the recommended daily dosage for finishing lambs of 20–40 mg MON (Elanco, AF1404). Lambs were weighed just prior to the morning feeding on days 1 and 56 (final day). Live weights (LW) on days 1 was converted to shrunk body weight (SBW) by multiplying LW by 0.96 to adjust for the gastrointestinal fill (Cannas et al., 2004). Lambs were fasted for 18 h before recording the final LW. Additives were premixed with ground rice hulls (Powder mixer, JETENGE-L, Mod 2002, Guadalajara, Jalisco, Mexico) to provide the desired dosage of MON (30 mg) or EO (150 mg) in 10 g of final premix. The respective premix treatments were hand-weighed using a precision balance (Ohaus, mod AS612, Pine Brook, NJ, USA) and premixed for 5 min with minor in- gredients (urea, limestone and trace mineral salt) before incorporation into complete mixed diets using a 2.5 m^3 capacity paddle mixer (model30910-7, Coyoacán, México). To avoid contamination, the mixer was thoroughly cleaned before elaboration of each dietary treatment. To ensure additive consumption, the total daily dosage per lamb was concentrate in 300 g of diet provided in the morning feeding (all lambs were fed the basal Control diet in the afternoon feeding). Thus, lambs were provided fresh feed twice daily at 0800 and 1400 h, in which morning feed was offered constant, while afternoon feed was offered ad libitum to allowing for a feed residual of refusal of ~50 g/kg daily feed offering. Residual feed was collected between 0740 and 0750 h each morning and weighed. Adjustment to either increase or decrease daily feed delivery, was provided at the afternoon feeding. Water consump- tion was measured daily at 0700 h by dipping a graduated rod into the tank drinker (one watering tank for each pen). Once the measure was taken, the remaining water was drained, and the tanks were refilled with fresh water.

Feed samples were taken from each elaborated batch, while feed refusal were collected daily and composited weekly for DM analysis

Table 1
Composition of basal diet fed to lambs (DM basis).[1]

Item	% DM
Ingredient	
Corn grain cracked	64.50
Soybean meal	10.50
Sudan grass hay	10.00
Molasses cane	9.00
Yellow grease	3.50
Urea	2.10
Minerals supplement[1]	0.40
Nutrient composition (DM basis)[2]	
Net energy (Mcal/kg)	
Maintenance	2.14
Gain	1.47
Crude protein (%)	13.80
NDF (%)	15.53
Ether extract (%)	6.43

[1] Minerals supplement contained (%): $CoSO_4$, 0.068; $CuSO_4$, 1.04; $FeSO_4$, 3.57; ZnO, 1.24; $MnSO_4$, 1.07; KI, 0.052; limestone, 56.96 %; urea, 18 %, and NaCl, 18 %.
[2] Based on tabular values for individual feed ingredients (NRC, 2007) with the exception of CP and NDF, whichwere determined in our laboratory.

(oven drying at 105 °C until no further weight loss; method 930.15, AOAC, 2000). Feed samples were subjected to the following analyses:DM (oven drying at 105 °C until no further weight loss; method 930.15; AOAC, 2000); CP (N × 6.25, method 984.13; AOAC, 2000), and NDF [Van Soest et al., 1991, corrected for NDF-ash, incorporating heat stable α-amylase (Ankom Technology, Macedon, NY).

2.2. Calculations

Estimates of daily weight gain (ADG), and dietary net energy were based on shrunk body weight (SBW; 96 % of full live weight, Cannaset al., 2004). Average daily gain was computed by subtracting initial SBW from final SBW and dividing the result by the number of days on feed. Gain efficiency was computed as ADG/ daily DMI. One approachfor evaluation of the efficiency of dietary energy utilization in growth-performance trials is the ratio of observed-to-expected DMI and observed-to-expected dietary NE. Based on diet NE concentration and measures of growth performance, there is an expected energy intake.This estimation of expected DMI is performed based on observed ADG,
average SBW, and NE values of the diet (Table 1): expected DMI, kg/d = $(EM/NE_m) + (EG/NE_g)$, where EM (energy required for maintenance, Mcal/d) = $0.056 \times SBW^{0.75}$, EG (energy gain, Mcal/d) = 0.276× ADG × SBW 0.75, and NEm and NEg are corresponding NE values based on the ingredient composition of the experimental diet (Table 1, NRC, 2007). The coefficient (0.276) was taken from NRC (1985) assuming a mature weight of 113 kg for Pelibuey × Katahdin male lambs (Cantonand Quintal, 2007). Observed dietary net energy was calculated using EM and EG values, and DMI observed during experiment by means of the quadratic formula: $x = (-b - \sqrt{b^2 - 4ac})/2c$, where $x = NE_m$ (Mcal/kg), $a = -0.41$ EM, $b = 0.877$ EM + 0.41 DMI + EG, and $c = -0.877$ DMI (Zinn et al., 2008).

2.3. Carcass characteristics and whole cuts

All lambs were harvested on the same day and were slaughtered by disgorging after they were stunned by mechanical procedure. After slaughter, lambs were bled and skinned, the gastrointestinal organs were separated and weighed, the omental and mesenteric fat were weighed, as well hot carcass weight (HCW) was registered. After carcasses (with kidneys and internal fat included) chilled in a cooler at −2 to 1 °C for 24 h, the following measurements were obtained: 1) cold carcass weight (CCW); 2) body wall thickness (distance between the 12th and 13th ribs beyond the ribeye, five inches from the midline of the carcass); 3) sub-cutaneous fat (fat thickness) was taken over the 12th to 13th thoracic vertebrae; 4) LM surface area, measure using a grid reading of the cross-sectional area of the *longissimus muscle* between 12th and 13th rib, and 5) kidney, pelvic and heart fat (KPH) was removed manually and af-terward weighed and reported as a percentage of the cold carcass weight (USDA, 1982). Each carcass was split into two halves. The left side was fabricated into wholesale cuts, without trimming, according to the North American Meat Processors Association guidelines (NAMP, 1997). Rack, breast, shoulder and foreshank were obtained from the foresaddle, and the loins, flank and leg from the hindsaddle. Weight of each cut wassubsequently recorded. The tissue composition of shoulder was assessed using physical dissection by the procedure described by Luaces et al.(2008).

2.4. Visceral mass data

Components of the digestive tract (GIT), including tongue, esoph- agus, stomach (rumen, reticulum, omasum, and abomasum), pancreas, liver, gall bladder, small intestine (duodenum, jejunum, and ileum), and large intestine (caecum, colon, and rectum) were removed and weighed. The GIT was then washed, drained, and weighed to get empty weights. The difference between full and washed digesta-free GIT was subtracted from the SBW to determine empty body weight (EBW). All tissue weights are reported on a fresh tissue basis. Organ mass is expressed as grams of fresh tissue per kilogram of final EBW, where final EBW represents the final full live weight minus the total digesta weight. Full visceral mass was calculated by the summation of all visceral components (stomach complex + small intestine + large intestine + liver + lungs + heart), including digesta. The stomach complex was calculated as the digesta- free sum of the weights of the rumen, reticulum, omasum and abomasum.

2.5. Statistical analyses

Growth performance (ADG, DMI, gain efficiency), estimated dietary NE and DMI, and carcass data were analyzed as a randomized complete block design, using pen as the experimental unit (SAS, 2007) accordingto the statistical model: $Y_{ij} = \mu + B_i + T_j + \epsilon_{ij}$, in which μ is the common experimental effect, B_i represents initial BW block effect (df = 5), T_j represents dietary treatment effect (df 2), and ϵ_{ij} represents the re- sidual error (df = 10). Water intake was analyzed as a completely ran- domized design using linear mixed model for analysis of repeated measures (SAS, 2007).

Visceral organ mass data was analyzed using the MIXED procedure (SAS, 2007), with treatment and pen as fixed effects and interaction treatment × pen and individual carcasses within pen by treatment subclasses as random effects. Treatment effects were considered significant when the *P*-value was ≤ 0.05, and tendencies were identified when the *P*-value was > 0.05 and ≤ 0.10.

Results

Dietary additive intakes averaged 4 mg/kg LW and 0.80 mg/kg LWfor EO and MON, respectively.

2.3. Growth performance and dietary energy

Water consumption between EO and Control lambs was very similar. In contrast, lambs fed MON consumed 18.1 % less ($P < 0.01$) water than Controls and EO groups (Table 2).
Average daily gain was similar for Controls and EO supplemented lambs. However, supplemental EO tended ($P = 0.09$) to decrease DMI. Consequently, gain efficiency for EO supplemented lambs was greater (4.7 %, $P < 0.05$) than that of Control lambs.
Compared to MON, EO supplementation increased ($P < 0.05$) DMI(9.3 %), ADG (13.2 %) and gain efficiency (4.7 %, $P < 0.05$). Comparedto Controls, MON supplementation decreased ($P < 0.05$) DMI (11.6 %) and ADG (11.7). However, gain efficiency was not different.
Compared with Control lambs, EO supplementation increased (4 %,$P < 0.05$) estimated dietary NE. Compared with MON, supplemental EO tended (2.2 %, P = 0.09) to improve dietary NE. Compared to Controls, differences in dietary NE due to MON supplementation was not appre- ciable ($P = 0.18$).

2.4. Carcass characteristics and visceral mass

With exception of carcass weight and weight of the intestine and visceral fat depots expressed as g/kg EBW, treatment effects on carcass characteristics were small and not appreciable (Tables 3 and 4). Consistent with slower ADG, lambs fed MON had lower (4.6 %, $P < 0.01$) HCW than lambs receiving EO, and tended ($P = 0.08$) to have lower HCW than Control lambs. Compared with Controls, EO and MON supplementation decreased ($P < 0.05$) relative weight of intestines (3.8 %) and omental fat (9.7 %). Relative weight of visceral fat was lower to EO than Controls (9.1 %, P < 0.05). EO supplemented lambs had lower relative weight of mesenteric fat than lambs receiving MON (21.7 %,$P < 0.05$).

Table 2
Treatments effect on growth performance in finishing lambs.

	Treatments[1]				P-value		
Item	Control	MON	EO	SEM	MON vs Control	EO vs Control	EO vs MON
Live weight (kg)[2]							
Initial	28.49	28.40	28.61	0.157	0.91	0.60	0.37
Final	44.74	42.74	45.12	0.458	0.02	0.57	0.01
Water consumption (L/d)	2.55	2.10	2.58	0.032	<0.01	0.99	<0.01
Daily gain (kg)	0.290	0.256	0.295	0.008	<0.01	0.62	<0.01
Dry matter intake (kg/d)	1.190	1.052	1.160	0.027	<0.01	0.09	0.02
Gain to feed (kg/kg)	0.244	0.244	0.256	0.002	0.98	<0.01	<0.01
Observed dietary NE (Mcal/kg)							
Maintenance	2.16	2.19	2.23	0.017	0.18	<0.01	0.09
Gain	1.48	1.51	1.55	0.015	0.18	<0.01	0.09
Observed to expected dietary NE							
Maintenance	1.01	1.02	1.04	0.007	0.19	<0.01	0.09
Gain	1.01	1.03	1.05	0.010	0.19	<0.01	0.09
Observed to expected DM intake	0.99	0.97	0.95	0.009	0.19	<0.01	0.09

[1] MON = Sodium monensin fed at dose of 30 mg/lamb/day (Rumensin 90, Elanco Animal Health, Indianapolis, IN); [2] EO = a mixture of essential oils (CRINA® Ruminants, DSM Nutritional Products, Basel, Switzerland) fed a dose of 150 mg/lamb/day.
[2] Live weights (LW) on days 1 was converted to shrunk body weight (SBW) by multiplying LW by 0.96 to adjust for the gastrointestinal fill (Cannas et al., 2004). Alllambs were fasted (drinking water was not withdrawn) for 18 h before recording the final LW.

Table 3
Treatments effect on carcass characteristics and whole cuts of lambs.

Treatments[1]					P-value		
Item	Control	MON	EO	SEM	MON vs Control	EO vs Control	EO vs MON
Hot carcass weight (kg)	26.71	25.97	27.23	0.26	0.08	0.19	<0.01
Dressing percentage	59.70	60.72	60.35	0.48	0.16	0.36	0.60
Cold carcass weight (kg)	26.37	25.63	27.00	0.24	0.06	0.09	<0.01
Longissimus muscle area (cm^2)	15.90	15.51	15.77	0.16	0.12	0.57	0.28
Kidney-pelvic-heart fat (%)	3.78	4.16	3.95	0.20	0.22	0.57	0.48
Back fat thickness (cm)	2.26	2.43	2.46	0.11	0.31	0.25	0.87
Wall thickness (mm)	11.60	12.64	12.68	0.42	0.12	0.11	0.94
Leg circumference (cm)	45.08	45.46	46.42	0.83	0.76	0.28	0.42
Shoulder composition (%)							
Muscle	63.59	64.04	64.50	0.80	0.71	0.44	0.69
Fat	15.13	15.83	15.53	0.85	0.57	0.75	0.81
Muscle to fat ratio	4.20	4.05	4.15	0.16	0.55	0.89	0.66
Whole cuts (as percentage of CCW)							
Forequarter IMPS202	39.71	39.10	39.42	0.23	0.11	0.41	0.36
Hindquarter IMPS230	35.53	35.70	35.31	0.25	0.63	0.54	0.29
Shoulder IMPS206	14.15	14.11	14.15	0.14	0.83	0.98	0.86
Shoulder IMPS207	8.27	7.92	7.97	0.19	0.23	0.29	0.88
Rack IMPS204	6.55	6.63	6.48	0.18	0.76	0.79	0.58
Breast IMPS209	3.75	3.45	3.55	0.20	0.32	0.50	0.72
Loin IMPS231	6.46	6.52	6.44	0.14	0.74	0.95	0.70
Flank IMPS232	5.41	5.43	5.43	0.15	0.92	0.92	0.99
Leg IMPS233	23.66	23.69	23.38	0.22	0.93	0.37	0.33

CCW = cold carcass weight.
[1] MON = Sodium monensin fed at dose of 30 mg/lamb/day (Rumensin 90, Elanco Animal Health, Indianapolis, IN); [2] EO = a mixture of essential oils (CRINA® Ruminants, DSM Nutritional Products, Basel, Switzerland) fed ay dose of 150 mg/lamb/day.

3. Discussion

The relative average ingestion of 3.5 mg EO/kg LW (same blendedoils than we used in this experiment) resulted in improved feed effi-ciency in lactating ewes (Giannenas et al., 2011). It has been determined that the effects of essential oils on ruminal fermentation and growthperformance are dose-dependent and that these compounds are more effective when administered at high doses than at low doses (Benchaaret al., 2006; Giannenas et al., 2011). The recommended daily dose ofMON for increased feed efficiency in finishing lambs are between 20 y 40 mg MON (Elanco, AF1404). Therefore, the final doses ingested in both experiments should not represent a limiting factor for the responsesevaluated.

Similarly to our results, it has been reported absence of effects on water intake in finishing feedlot cattle that daily received up to 20 mg eugenol or cinnamaldehyde/kg LW (Ornaghi et al., 2017). On the other hand, reduced water consumption with MON supplementation has been previously reported in non-ruminant species (EFSA, 2008). The basis for this effect is uncertain, and appears to be unrelated to differences onDMI. Water consumption per kg DMI averaged 2.00, 2.11 and 2.22 for MON, Control, and EO treatments, respectively.

The effects of supplemental essential oils on lamb growth perfor- mance have been inconsistent. Moura et al. (2017) observed that sup- plementation with 500 mg copaiba essential oils/kg DM (equivalent to 370 mg/lamb/day; terpene class, primarily caryophyllene and colave- nol) did not affect DMI, but markedly increased ADG (14.7 %) and gain efficiency (3.4 %) compared to a non-supplemented group. In contrast, Chaves et al. (2008) reported that supplementing a high-energy diet for growing lambs with cinnamaldehyde or carvacrol (200 mg/kg DMI) had no effect on DMI, gain, feed efficiency. Likewise, Simitzis et al. (2014) observed that cinnamon oil supplementation (1 mL/kg diet DM) did not affect lamb growth performance or meat quality characteristics. Parvar

Table 4
Treatments effect on visceral mass characteristics of lambs.

	Treatments[1]				P-value		
Item	Control	MON	EO	SEM	MON vs Control	EO vs Control	EO vs MON
EBW (percentage of full weight)	90.82	91.13	90.29	0.77	0.78	0.64	0.46
Organs (g/kg of EBW)	59.70	60.72	60.35	0.48			
Stomach complex[2]	29.23	28.96	28.52	0.58	0.75	0.36	0.88
Intestines[3]	46.53	44.06	45.06	0.44	<0.01	0.04	0.15
Heart/lungs	24.45	23.73	24.99	0.62	0.43	0.55	0.18
Liver/spleen	20.08	20.17	20.48	0.58	0.91	0.64	0.71
Kidney	2.81	2.60	2.91	0.12	0.26	0.59	0.12
Omental fat	30.87	27.45	28.32	0.75	<0.01	0.04	0.43
Mesenteric fat	7.29	8.15	6.38	0.46	0.21	0.19	0.03
Visceral fat	38.16	35.60	34.70	1.00	0.11	0.04	0.54

EBW = empty body weight.
[1] MON = Sodium monensin fed at dose of 30 mg/lamb/day (Rumensin 90, Elanco Animal Health, Indianapolis, IN); [2] EO = a mixture of essential oils (CRINA® Ruminants, DSM Nutritional Products, Basel, Switzerland) fed ay dose of 150 mg/lamb/day.
[2] Stomach complex = (rumen + reticulum + omasum + abomasum), without digesta.Small and large[3] intestines without digesta.

et al. (2018) observed that supplementation with essential oils (250–750 mg/kg DM) from *Ferulago angulata* (containing a mixture of α-pinene and α-ocimene) decreased DMI, ADG and diet digestibility.

Although supplemental essential oils may be grouped together as a class, their chemical structure and composition vary (Dhifi et al., 2016). Consequently, their effects on DMI and animal performance may like- wise vary. de Souza et al. (2019), evaluating 4 distinct EO blends in heifers, observed that in comparison with non-supplemented lambs, some EO blends (eugenol + thymol + vanillin + clove) supplemented at 4 g/heifers/day, enhanced ADG and gain efficiency, whereas others (eugenol thymol vanillin) only affected DM intake.

At the time of writing this report, there is no published research evaluating effects of the EO (CRINA-Ruminants) on performance and feed efficiency in finishing lambs. In lactating ewes, Giannenas et al. (2011) evaluated EO (comparable blend to that of the present study) at levels of 0, 50 or 150 mg/kg of concentrate (equivalent to 100 and 200 mg EO/day). Supplementation did not affect DMI, but enhanced feed efficiency. Lin et al. (2013) observed that supplementation with 500 mg EO/d (comparable blend to that of the present study) increased ruminal propionate and decrease protein degradation without detri- mental effects on nutrient digestion in cannulated sheep.

Compared with a non-supplemented high energy finishing diet, supplementation with 2–8 mg EO/kg BW (comparable blend to that of the present study) did not affect DMI, but tended to increase (4 %) feed efficiency of feedlot cattle (Meyer et al., 2009). Supplementation of afinishing diet with 6 or 12 mg EO/kg BW (comparable blend to that of the present study) likewise did not affect DMI, but markedly enhanced16 %) feed efficiency of Nellore heifers (de Souza et al., 2019). Benchaar et al. (2006) conducted two trials evaluating EO blend (comparable to that of the present study) supplemented at 0, 4.7, or 9.4 mg EO/day. Inthe first trial EO did not affect DMI. Whereas, in the second trial, EO blend increased DMI. There is no published research that directly compares effects of the supplemental EO (CRINA-Ruminant) vs MON on growth performance of finishing lambs. Ribeiro et al. (2020) compared thyme essential oil (1.25, 2.50, or 3.75 g/kg DM) vs MON (25 mg/kg DM) in cannulated lambs fed with high-energy diet. Apparent total tract digestion, N metabolism, and ruminal fermentation were similar for the two addi- tives. In a 56-d growth-performance study involving Dorper lambs (22 kg) fed a moderately low-energy finishing diet (forage:concentrate ratio of 53:47, 1.83 Mcal NE_m/kg DM), Moura et al. (2017) observed that compared with MON, supplementation with 500 mg copaiba essential oils/kg DM (equivalent to 370 mg/lamb/day) numerically increased (11 %) DMI, but markedly enhanced ADG (19 %), and feed efficiency (10.3 %). However, they observed that EO supplementation at 1000 or 1500 mg/kg DM (equivalent to 800 and 1240 mg EO/lamb/day, respectively) depressed lamb growth performance.

Several studies have been conducted comparing the effects of sup- plemental EO (similar to that of the present study) vs MON and feedlot cattle growth performance. Meyer et al. (2009), observed that compared with MON, supplementation of feedlot steers with 2.5 mg EO/kg LW numerically increased ADG (2.8 %) and gain efficiency (4 %). Meschiatti et al. (2019) observed that compared with MON, supplementation of feedlot bulls with 834 mg EO/d increased DMI and ADG (6.9 and % 5.7 %, respectively), although gain efficiency was not affected. In contrast, supplementation with 4000 mg EO/day vs MON did not affect growth performance of steers and heifers fed a low-energy silage-based diet (1.50 Mcal NE_m/kg). Araujo et al. (2019) did not detect differences in 208-day growth performance of feedlot steers fed corn-silage-based growing-finishing diet supplemented with 33 mg MON/kg diet DM vs150 mg EO/kg diet DM (mixture of carvacrol + thymol + eugenol).

In as much as supplemental EO may enhance metabolizable protein supply to the small intestine (Samii et al., 2016; Soltan et al., 2018), variation in growth performance responses to supplemental EO may be due, in part, to adequacy of the basal diet in meeting the increased metabolizable protein requirements during the initial start-up phase. Compared with MON (there were no control group in their experiment), supplementation with EO (blend comparable to that of the present study) enhancements in DMI, ADG and/or gain efficiency were most apparent during the initial 19–30 days on feed (Meschiatti et al., 2016; Acedo et al., 2018).

Observed-to-expected dietary NE and the observed-to-expect DMI ratio for the lambs fed the control diet was 0.99 (DMI was consistentwith expectations based on observed ADG and formulated NE value of the diet, Table 1). This close agreement is supportive of the practicalityof prediction equations for the estimation of DMI in relation to SBW and ADG in feedlot lambs. A dietary NE ratio (observed-to-expected dietary NE) of 1.0 is indicative that daily weight gain was consistent with observed DMI and tabular NE value of the diet (NRC, 2007). If the ratiois greater than 1, the observed dietary NE (estimated dietary NE basedon growth-performance) is greater than expected based on growth performance and diet formulation, indicative of enhanced metaboliz- able energy utilization for maintenance and gain (the reverse being the case when the ratio is less than 1). As stated above, compared with Control lambs, EO supplementation enhanced (4 %, $P < 0.05$) estimated dietary NE, and compared with MON, supplemental EO tended (2.2 %, P 0.09) to enhance dietary NE. The basis for improved dietary energy utilization for growth due to supplemental EO is not clear, but could due to effects of supplemental EO toward decreased ruminal acetate:propi- onate molar ratio, and enhanced N and starch utilization (Lin et al., 2013; Khiaosa and Zabelli, 2013; Samii et al., 2016; Meschiatti et al., 2016). Accordingly the time length of the trial could affect the overall responses to EO supplementation. To our knowledge no information is available regarding the interaction of level and duration of EO supple- mentation in lamb performance, although as stated previously, the response to EO supplementation appears more pronounced during the early phase of supplementation (Meschiatti et al., 2016; Acedo et al., 2018).

Decreased on DMI and enhanced gain efficiency of feedlot cattle as a result of MON supplementation is well-documented (Duffield et al., 2012). Decreases on DMI in cattle fed MON has been attributed to taste preference (Erickson et al., 2004). Decrease in ADG observed in lambs fed MON is more directly related to decrease in DMI. The effect of supplemental MON on gain efficiency in feedlot cattle has been variable, ranging from nil to greater than 18 % (Barreras et al., 2013). In a meta-analysis, Duffield et al. (2012) observed that, during the past 40 years, the impact of MON on gain efficiency decreased from an average of 8.1–3.5 %. This change may be attributable to increases in NE value of the finishing diet. Accordingly, the effect of MON was optimal at energy levels under 1.37 Mcal NE_g/kg diet DM, becoming negligible at dietary energy densities of ≥ 1.55 Mcal NE_g/kg (Barreras et al., 2013). Considering the observed NE_g of the basal diet (1.48 Mcal NE_g/kg; Table 2), less appreciable gain efficiency response to supplemental was expected.

Lack of treatment effects on carcass cutout and tissue composition is consistent with previous studies (Salinas-Chavira et al., 2010; Koyunco and Canbolat, 2010; Moura et al., 2017; Parvar et al., 2018). The lower HCW observed to lambs fed MON is consistent with the decrease ADG resulted in lower final weight.

Increased FT with essential oils supplementation has been reportedin lambs fed diets of moderate energy density (Soares et al., 2012; Moura et al., 2017). However, supplemental essential oils has not affected FT in feedlot lambs fed high-energy diets (Chaves et al., 2008; Biricik et al., 2016). Likewise, supplemental MON did not affect FT in either, Pelibuey lambs (Salinas-Chavira et al., 2005; daili ingestion of 22 mg MON) or Pelibuey x Dorper crossbreed (Salinas-Chavira et al., 2010; daily ingestion of 28 mg MON).

Both MON and EO supplementation decreased the proportion of in- testine as a percentage of EBW. The basis for this is not certain, but may be attributable to antibiotic-like effects on epithelial thickness (Gha- zanfari et al., 2015). The effects of supplemental EO on fat distribution among depots is uncertain. It has been proposed that supplemental EO may have potential as an energy "repartitioning" agent, affecting net fat deposition and distribution (Kuester, 2016).This can partially explained the changes promote in meat quality of lambs by EO supplementation (Parvar et al., 2018; García-Galicia et al., 2020). To the extent that EO reduces ruminal acetate:propionate ratio (Meyer et al., 2009; Koyunco and Canbolat, 2010; Wanapat et al., 2013), the associated increase in propionate production lends to decreased visceral fat deposition (Smith and Crouse, 1984).

4. Conclusions

We conclude that compared with Control (non-supplemented) lambs, supplemental EO (blend of thymol, eugenol, vanillin, guaiac, and limonene) enhances feed efficiency, and dietary net energy. Compared with MON, supplemental EO enhances ADG. However, effects of MONand EO on feed efficiency and dietary net energy are not appreciable different. Supplemental EO did not negatively affect carcass character- istics or visceral organ mass. As such, supplemental EO is a viable alternative to the antibiotic monensin for enhancement of feed effi- ciency of finishing feedlot lambs.

Declaration of Competing Interest

Author declare no conflict of interest.

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ARTÍCULO 2

Blend of essential oils supplemented alone or combined with exogenous amylase compared with virginiamycin supplementation on finishing lambs: performance, dietary energetics, carcass traits, and nutrient digestión

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Publicado en: ANIMALS August - 2021 (MDPI)

DOI: https://doi.org/10.3390/ani11082390.

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Blend of Essential Oils Supplemented Alone or Combined with Exogenous Amylase Compared with Virginiamycin Supplementation on Finishing Lambs: Performance, Dietary Energetics, Carcass Traits, and Nutrient Digestion

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Citation: Estrada-Angulo, A.; Arteaga-Wences, Y.J.; Castro-Pérez, B.I.; Urías-Estrada, J.D.; Gaxiola- Camacho, S.; Angulo-Montoya, C.; Ponce-Barraza, E.; Barreras, A.; Corona, L.; Zinn, R.A.; et al. Blend of Essential Oils Supplemented Alone orCombined with Exogenous Amylase Compared with Virginiamycin Supplementation on Finishing Lambs:Performance, Dietary Energetics, Carcass Traits, and Nutrient Digestion. *Animals* **2021**, *11*, 2390. https://doi.org/10.3390/ani11082390

Academic Editors: Marta I. Miranda Castañón, Einar Vargas-Bello Perez and Manuel Gonzalez

Received: 14 July 2021
Accepted: 10 August 2021
Published: 13 August 2021

Simple Summary: Antibiotics have been extensively used as growth promoters in livestock, but current interests are focused on limiting the use of conventional antibiotics as feed additives in livestock production. Essential oil compounds belong to a "generally-recognized-as-safe" category of feed additives that may serve as alternatives to conventional antibiotics used as growth promoters. In this study, dietary supplementation of finishing lambs with essential oils alone, or combined with exogenous enzymes, improved dietary energy utilization and meat production in a manner comparable to that of the antibiotic virginiamycin.

Abstract: Two experiments were conducted to compare a supplemental blend of essential oils alone (EO) or combined with enzymes (EO + ENZ) versus virginiamycin (VM), on characteristics of growth performance (Exp. 1) and digestion (Exp. 2) in finishing lambs. Lambs were fed a high-energy finishing diet supplemented with: (1) no supplement (control); (2) 150 mg supplemental EO; (3) 150 mg supplemental EO plus 560 mg alpha-amylase (EO + ENZ); and 4) 25 mg VM. Compared with the control, growth performance response to EO and VM were similar, enhancing (5.7%, $p < 0.05$) feed efficiency and observed dietary net energy. Compared with control, supplementation with EO + ENZ tended ($p = 0.09$) to increase dry matter intake (6.8%), improving ($p < 0.05$) weight gain andfeed efficiency (10.4 and 4.4%, respectively). Dietary energy utilization was greater (2.7%, $p < 0.05$) for EO and VM than EO + ENZ. Treatment effects on the carcass and visceral mass were small, but additive supplementation decreased ($p \leq 0.03$) the relative weight of the intestines. There were no treatment effects on measures of digestion nor digestible energy of the diet. Supplemental EO may be an effective alternative to VM in high-energy finishing diets for feedlot lambs. CombinationEO + ENZ may further enhance dry matter intake, promoting increased weight gain.

Keywords: lambs; essential oils; exogenous amylase; virginiamycin; growth performance; digestion

1. Introduction

Virginiamycin (VM) is an antimicrobial (peptolide antibiotic) that inhibits growth of Gram-positive bacteria [1]. At supplementation levels of 22 to 28 mg/kg diet DM, VM enhances average daily gain (ADG), feed efficiency (gain-to-feed ratio, GF), anddiet energy utilization in feedlot cattle [2,3]. As well, it reduces the incidence of liver abscess and ruminal lactate accumulation [4]. In a meta-analysis, comparing VM with the ionophore monensin (MON), Gorocica and Tedeschi [5] observed that whereas VM resulted in greater ADG than MON, both additives resulted in similar enhancements in feed efficiency. Current interests in limiting the use of conventional antibiotics as feed additives in livestock production, has led to the search for “generally-recognized-as-safe” additive alternatives. Previous reports [6–8] indicate that mixtures of essential oils may result in similar or even greater enhancements in growth performance than MON. These effects may not be attributable to changes in digestion, as the effects of supplemental essential oils on total tract digestion are not appreciable [9,10]. Comparisons of essential oils vs. VM in ruminants is limited. In poultry, growth performance response and gastrointestinal tract health were similar for supplemental peppermint oil vs. VM [11]. Due to its high starch content, corn grain is extensively used as a feed energy source for livestock. Most of the starch in corn grain is within endosperm of the kernel in a granular form; the tight intermolecular bonding between starch molecules, along with the compact nature of the starch granule, impedes rapid moisture uptake (rehydration) and thus, ruminal starch digestion [12]. Because of its hydrolytic action, supplemental α-amylase may increase the availability of starch hydrolysis products in the rumen [13]. Therefore, exogenous α-amylase supplementation may enhance ruminal digestion of starch in cracked corn-based diets [14]. The combination of EO with exogenous enzyme α-amylase has enhanced both DMI and ADG of feedlot cattle that were fed with finishing cracked corn-based diets [15]. We hypothesized that mixture of essential oils may enhance growth performance, dietary energetics, and carcass characteristics of finishing feedlot lambs in a manner comparable to that of virginiamycin, and that the combination of essential oils with exogenous α-amylase might further potentiate that effect. Accordingly, two experiments were conducted to compare the effects of supplemental essential oils alone or combined with enzymes versus virginiamycin on growth performance, dietary net energy, carcass characteristics, visceral mass (Exp. 1) and measures of total tract digestion (Exp. 2).

2. Materials and Methods

This experiment was conducted at the Universidad Autónoma de Sinaloa Feedlot Lamb Research Unit, located in Culiacán, México (24°46′13” N and 107°21′14” W). Culiacán is about 55 m above sea level, and has a tropical climate. During the course of the experiment, ambient air temperature averaged 31.5 °C (minimum and maximum of 26.1 and 34.5 °C, respectively), and relative humidity averaged 36.0% (minimum and maximum of 29.7 and 52.8%, respectively). All animal management procedures were conducted within the guidelines of federal-locally-approved techniques for animal use and care [16] and approved by the Ethics Committee of Faculty of Veterinary Medicine and Zootechnics from the Autonomous University of Sinaloa (Protocol #1422019).

2.1. Exp. 1. Growth Performance and Carcass Traits

2.1.1. Animal, Diet, Treatments, and Samples Analyses

Forty-eight Pelibuey × Katahdin crossbred intact male lambs (27.87 ± 4.71 kg initiallive weight (LW)) were used in an 87-d experiment to evaluate treatment effects on growth performance and carcass characteristics. Two weeks before initiation of the experiment lambs were treated for parasites (7.5 mg/kg LW; Closantel Panavet 15%, Panamericana Veterinaria de México City, México), injected with 2 mL vitamin A (500,000 UI, 75,000 IU vitamin D_3, and 50 IU vitamin E; Synt-ADE®, Zoetis México, México City), and vaccinated for Mannheimia haemolityca (One Shot Ultra, Zoetis México, México City). Upon initiation of the experiment, lambs were weighed before the morning meal (electronic scale; TORREY

TIL/S: 107 2691, TOR REY Electronics Inc, Houston, TX, USA). Lambs were blocked by initial weight (6 blocks) and assigned to 24 pens, two lambs/pen. Dietary treatments were randomly assigned to pens within blocks, 6 replicas per treatment. Pens have 6 m^2 with overhead shade, automatic waterers and 1 m fence-line feed bunks. Composition of the cracked corn-based basal diet is shown in Table 1. Corn grain was prepared by passing whole regional white corn through rollers (cylinder rollers of 46 cm diameter × 61 cm length, 5.5 corrugations/cm; Memco, Mills Rolls, Mill Engineering & Machinery Co., Oklahoma, CA, USA). Roll pressure was adjusted so that the kernels were broken to produce a bulk density of approximately 0.60 kg/L. Sudangrass hay was ground in a hammer mill (Azteca 20, Molinos Azteca, Guadalajara, México) with a 3.81 cm screen before incorporation into total mixed ration. Treatments consisted of basal diet supplemented with: (1) No additives (control); (2) 150 mg/d of a standardized source of a mixture of essential oils (EO); (3) 150 mg/d EO plus 560 mg/d alpha-amylase (EO + ENZ); and (3) 25 mg/d virginiamycin (VM; Stafac 500, Phibro Animal Health, Ridgefield Park, NJ, USA). The blend of essential oils used contains thymol, eugenol, limonene and vanillin on an organic carrier (CRINA-Ruminants, DSM Nutritional Products, Basel, Switzerland), and the exogenous α-amylase used was produced by *Bacillus licheniformis* (Ronozyme RumiStar, DSM Nutritional Products, Basel, Switzerland). The daily dose of 150 mg EO used was chosen based on a previous report where ingestion of 100 to 200 mg EO/d resulted in maximal enhancements on ruminal fermentation and feed efficiency in lactating ewes [17], and improved feed efficiency and observed dietary net energy in feedlot lambs [8]. The dose of 560 mg ENZ/day was estimated from data publishing from Meschiatti et al. [15]. Level of VM was based on recommended drug label dosage. The treatments (complete mixed diets) were prepared using a 2.5 m^3 capacity paddle mixer (model 30910-7, Coyoacán, México). To avoid contamination between treatments, the mixer was thoroughly cleaned between each elaborated batch. To ensure additive consumption, the total daily dosage per lamb was mixed in 300 g of basal diet provided in the morning feeding (all lambs were fed the basal control diet in the afternoon feeding). Thus, lambs were provided fresh feed twice daily at 800 and 1400 h. Whereas the amount of feed provided in the morning feeding was constant, feed offered in the afternoon feeding was adjusted daily, allowing for a feed residual ~50 g/kg daily feed offering. Residual feed was collected between 0740 and 0750 h each morning and weighed. The adjustments to either increase or decrease daily feed delivery were provided in the afternoon feeding. Water consumption was measured daily at 700 h by dipping a graduated rod into the tank drinker (one watering tank for each pen). Once the measure was taken, the remaining water was drained, and the tanks were refilled with fresh water. Lambs were weighed just prior to the morning feeding on days 1 and 87 (final day). Live weights (LW) on day 1 was converted to shrunk body weight (SBW) by multiplying LW by 0.96 to adjust for the gastrointestinal fill [18]. All lambs were fasted for 18 h before recording the final LW.

Table 1. Composition of basal diet fed by lambs in Exp. 1 and Exp. 2 †.

	Treatments §			
Item	**Control**	**EO**	**EO + ENZ**	**VM**
Ingredient composition, % DM basis				
Dry-rolled corn	67.00	67.00	67.00	67.00
Sudangrass hay	9.00	9.00	9.00	9.00
Soybean meal	10.00	10.00	10.00	10.00
CRINA-Ruminants®	0	+++	0	0
RONOZYME Rumistar®	0	0	+++	0
Stafac 500®	0	0	0	+++

Table 1. *Cont.*

Item	Treatments §			
	Control	EO	EO + ENZ	VM
Molasses cane	9.00	9.00	9.00	9.00
Urea	0.50	0.50	0.50	0.50
Tallow	2.00	2.00	2.00	2.00
Trace mineral salt *	2.50	2.50	2.50	2.50
Chemical composition (%DM basis) ‡				
Dry matter	88.60	88.60	88.60	88.60
Starch	54.00	54.00	54.00	54.00
Neutral detergent fiber	13.50	13.50	13.50	13.50
Crude protein	13.66	13.66	13.66	13.66
Ether extract	5.10	5.10	5.10	5.10
Calculated net energy (Mcal/kg) ⁋				
Maintenance	2.08	2.08	2.08	2.08
Gain	1.43	1.43	1.43	1.43

† Chromic oxide (0.30%) was added, replacing dry-rolled corn, as digesta marker in Exp. 2. § Control = non-supplemented; doses per lamb, EO = a standardized source of a mixture of essential oils compounds at dose of 150 mg EO (CRINA®Ruminants, DSM Nutritional Products, Basel, Switzerland)/day; EO + ENZ = 150 mg EOC plus 560 mg alpha-amylase (RONOZYME Rumistar®, DSM Nutritional Products, Basel, Switzerland)/day; VM = a peptolide antibiotic virginiamycin (Stafac 500, Phibro Animal Health, Ridgefield Park, NJ) at dose of 25 mg virginiamycin/day. * Mineral premix contained: limestone, 50%; urea 20%; NaCl, 15%; MgO, 5%; phosphate rock, 9.06%; CoSO4, 0.01%; CuSO4, 0.14%; FeSO4, 0.47%; ZnO, 0.16%; MnSO4, 0.14%; KI, 0.008%. ‡ Average dietary composition was determined by analyzing subsamples collected and composited throughout the experiment. Accuracy was ensured by adequate replication with acceptance of mean values that were within 5% of each other. ⁋ Calculated from NRC [19] tabular values.

Feed samples were collected for each elaborated batch. Feed refusal was collected daily and composited weekly for DM analysis (oven drying at 105 °C until no further weight loss; method 930.15) [20]. Feed samples were subjected to the following analyses: DM (oven drying at 105 °C until no further weight loss; method 930.15); CP (N 6.25, method 984.13) according to AOAC [20]; and neutral detergent fiber (NDF) following procedures described by Van Soest et al. [21] (corrected for NDF-ash, incorporating heatstable α-amylase using Ankom Technology, Macedon, NY, USA).

1.1.1. Calculations

Estimates of ADG, and dietary net energy are based on initial SBW and final (d 87) fasted SBW. Average daily gain was computed by subtracting initial SBW from final SBW and dividing the result by the number of days on feed. Feed efficiency was computed as ADG/ daily DMI. One approach for evaluation of the efficiency of dietary energy utilization in growth performance trials is the ratio of observed-to-expected DMI and observed-to-expected dietary NE. Based on diet NE concentration and measures of growth performance, there is an expected energy intake. This estimation of expected DMI is performed based on observed ADG, average SBW, and NE values of the diet (Table 1): expected DMI, kg/d = (EM/NE_m) + (EG/NE_g), where EM (energy required for maintenance, Mcal/d) = $0.056 \times SBW^{0.75}$, EG (energy gain, Mcal/d) = $0.276 \times ADG \times SBW^{0.75}$, and NE_m and NE_g are corresponding NE values based on the ingredient composition [19] of the experimental diet (Table 1). The coefficient (0.276) was taken from NRC [22] assuming a mature weight of 113 kg for Pelibuey × Katahdin male lambs [23]. The observed dietarynet energy was calculated using EM and EG values, and DMI observed during experimentby means of the quadratic formula:

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2c}$$

where x = NE_m, Mcal/kg, a = −0.41 EM, b = 0.877 EM + 0.41 DMI + EG, and c = −0.877 DMI [24].

1.1.1. Carcass Characteristics, Whole Cuts, and Tissue Shoulder Composition

All lambs were harvested on the same day. Lambs were stunned (captive bolt), exsanguinated and skinned. Gastrointestinal organs were separated and weighed, the omental and mesenteric fat were weighed, and hot carcass weight (HCW) was registered. After carcasses (with kidneys and internal fat included) chilled in a cooler at 2–to 1 °C for 24 h, the following measurements were obtained: (1) cold carcass weight (CCW); (2) body wall thickness (distance between the 12th and 13th ribs beyond the ribeye, five inches from the midline of the carcass); (3) subcutaneous fat (fat thickness) was taken over the 12th to 13th thoracic vertebrae; (4) LM surface area, measured using a grid reading of the cross-sectional area of the *longissimus muscle* between 12th and 13th rib, and (5) kidney, pelvic and heart fat (KPH) was removed manually and afterward weighed and reported as a percentage of the cold carcass weight [25]. Carcass yield was estimated as (fat thickness × 0.10) + 0.40. Each carcass was split into two halves. The left side was fabricated into wholesale cuts, without trimming, according to the North American Meat Processors Association guidelines [26]. Rack, breast, shoulder and foreshank were obtained from the foresaddle, and the loins, flank and leg from the hindsaddle. Weight of each cut was subsequently recorded. The tissue composition of shoulder was assessed using physical dissection by the procedure described by Luaces et al. [27].

1.1.2. Visceral Mass Data

Components of the digestive tract (GIT), including tongue, esophagus, stomach (rumen, reticulum, omasum, and abomasum), pancreas, liver, gall bladder, small intestine (duodenum, jejunum, and ileum), and large intestine (caecum, colon, and rectum) were removed and weighed. The GIT was then washed, drained, and weighed to get empty weights. The difference between full and washed digesta-free GIT was subtracted from the SBW to determine empty body weight (EBW). All tissue weights are reported on a fresh tissue basis. Organ mass is expressed as grams of fresh tissue per kilogram of final EBW, where final EBW represents the final live weight minus the total digesta weight. Full visceral mass was calculated by the summation of all visceral components (stomach complex + small intestine + large intestine + liver + lungs + heart), including digesta. The stomach complex was calculated as the digesta-free sum of the weights of the rumen, reticulum, omasum and abomasum.

1.1.3. Statistical Analyses

Growth performance (ADG, DMI, and feed efficiency), estimated dietary NE and DMI, carcass data (characteristics, tissue composition, and whole cuts) and visceral mass were analyzed as a randomized complete block design, using pen as the experimental unit according to the following statistical model: $Y_{ij} = \mu + B_i + T_j + \epsilon_{ij}$, where μ is the common experimental effect, B_i represents initial weight block effect, T_j represent dietary treatment effect, and ϵ_{ij} represents the residual error [28]. All the data were tested for normality using the Shapiro–Wilk test. Hot carcass weight (HCW) was used as a covariate in evaluation of treatment effects on carcass characteristics. In the analysis of shoulder tissue composition, the cold carcass weight (CCW) effect was included as a covariate. Water intake was analyzed as repeated measures using SAS PROC GLM [28]. Treatment effects were considered significant when the p-value was ≤ 0.05 and Tukey's multiple comparison procedures were used.

1.2. Exp. 2. Total Tract Digestion

1.2.1. Animals and Sampling

Four Pelibuey × Katahdin crossbred intact male lambs (32.7 ± 3.64 kg) were used in 4 × 4 Latin square experiment to study treatment effects on characteristics of apparent total tract digestion. Lambs were housed in individual metabolism crates (1.5 × 1.8 × 0.7 m) in an indoor facility with access to water at all times. Treatments were the same as those used in Exp. 1 (Table 1). Respective dosage of the additives (EO, EO + ENZ, VM) were hand-weighed using a precision balance (Ohaus, mod AS612, Pine Brook, NJ, USA) and top-dressed on the basal diet at the time of feeding. Chromic oxide (3.0 g/kg diet, air-dry basis) was used as an indigestible marker to estimate digestion. Chromic oxide was mixed in a 2.5-m^3 capacity concrete mixer (model 30910-7, León Weill, SA, Coyoacán, México) for 5 min with minor ingredients (mineral supplement and urea) before being mixed with the remainder of ingredients in the basal diet. All lambs were adapted to the basal diet for 21 days before the initiation of the trial. To avoid refusals daily feed intake (as feed basis) was restricted to 1.050 kg (equivalent to the 3.2% of LW). Diets were fed in two equal proportions at 08:00 and 20:00 h daily. In order to reduce the potential for treatment carry-over effects, treatment additives were withdrawn for 7 days before initiating the next 21-day treatment period. Accordingly, experimental periods were 25 days, with 7 days of additives withdrawal (all lambs were fed the basal control diet), 18 days of adjustment to respective dietary treatments, and 3 days of sample collection. During the collection period, feces voided were collected (approximately 50 g) each day at 750, 1150 and 1550 h. Samples from each lamb and within each collection period were composited for analysis. Fecal samples were weighed, and then stored at −20 °C for subsequent analysis.

1.1.1. Laboratory Analyses

Feed and fecal samples were subjected to the same analyses as feed samples of the Exp. 1, plus analysis of starch [29], gross energy (GE) by bomb calorimeter (Parr, 6400; Illinois, USA), and chromic oxide [30]. Total fecal DM excretion was estimated by the relationship of Cr intake (g) versus concentration of Cr in fecal samples as follows: total DM output, g/day = g Cr_2O_3 intake daily/(g Cr_2O_3/g of feces). Organic matter (OM) content of feed and fecal samples was estimated as DM concentration minus ash content.

1.1.2. Statistical Analyses

Treatment effects on characteristics of digestion were analyzed as a 4 × 4 Latin square design following the MIXED procedure from SAS software [28], where fixed effect was treatment, and random effects were lamb and period according to the following statistical model:

$$Y_{ijk} = \mu + S_i + P_j + T_k + E_{ijk}$$

where Y_{ijk} is the response variable, μ is the common experimental effect, S_i is the lamb effect, P_j is the period effect, T_k is the treatment effect and E_{ijk} is the residual error.

In all cases, least squares mean and standard error are reported. Treatment effects were tested using Fisher's least significant difference method (LSD). Contrasts were considered significant when the p-value was ≤ 0.05, and tendencies are identified when the p-value was >0.05 and ≤ 0.10.

3. Results

3.1. Exp. 1. Growth Performance and Carcass Traits

Treatment effects on water consumption, growth performance and estimates of dietary energetics are shown in Table 2. Based on average LW and the additive dosage, dietary additive intakes averaged 3.65, 13.46, and 0.61 mg/kg LW for EO, ENZ, and VM, respectively.

Lambs that were fed the combination EO + ENZ drank 8.7% more ($p < 0.01$) water than lambs fed the other treatments (EO, VM, or with non-supplemented lambs). Water consumption for EO, VM, and control was similar ($p > 0.66$), averaging 4.47 L/d.

Table 2. Effect of treatments on growth performance of finishing lambs.

	Treatments †					*p*-Value					
Item	**Control**	**EO**	**EO + ENZ**	**VM**	**SEM**	**1 vs. 2**	**1 vs. 3**	**1 vs. 4**	**2 vs. 3**	**2 vs. 4**	**3 vs. 4**
Days on test	87	87	87	87							
Pen replicates	6	6	6	6							
Water intake, L/d	4.44	4.56	4.85	4.40	0.174	0.56	<0.01	0.25	<0.01	0.15	0.01
Live weight, kg/d §											
Initial	27.88	27.89	27.87	27.82	0.104	0.90	0.96	0.70	0.86	0.62	0.75
Final	52.49	54.16	55.31	54.14	0.623	0.09	<0.01	0.10	0.27	0.98	0.61
Average daily gain, kg/d	0.283	0.302	0.316	0.302	0.007	0.09	<0.01	0.09	0.21	0.99	0.21
Dry matter intake, kg/d	1.305	1.306	1.401	1.301	0.037	0.99	0.09	0.92	0.09	0.93	0.07
Feed efficiency, kg/kg	0.217	0.232	0.227	0.233	0.002	<0.01	0.02	<0.01	0.13	0.85	0.10
Diet net energy, Mcal/kg											
Maintenance	2.08	2.19	2.14	2.20	0.017	<0.01	0.04	<0.01	0.06	0.83	0.04
Gain	1.42	1.51	1.47	1.52	0.015	<0.01	0.04	<0.01	0.06	0.83	0.04
Observed-to-expected diet NE, Mcal/kg											
Maintenance	1.001	1.053	1.027	1.056	0.009	<0.01	0.04	<0.01	0.06	0.83	0.04
Gain	0.991	1.052	1.024	1.055	0.011	<0.01	0.04	<0.01	0.06	0.83	0.04
Observed-to-expected DMI	1.006	0.948	0.976	0.944	0.010	<0.01	0.04	<0.01	0.06	0.83	0.04

† Control = non-supplemented; doses per lamb, EO = a standardized source of a mixture of essential oils compounds at dose of 150 mg EO (CRINA®Ruminants, DSM Nutritional Products, Basel, Switzerland)/day; EO + ENZ = 150 mg EOC plus 560 mg alpha-amylase (RONOZYME Rumistar®, DSM Nutritional Products, Basel, Switzerland)/day; VM = a peptolide antibiotic virginiamycin (Stafac 500, Phibro Animal Health, Ridgefield Park, NJ) at dose of 25 mg virginiamycin/day.

Growth performance and dietary energetics were not different ($p > 0.97$) for EO and VM supplemented lambs. Compared with the control, lambs supplemented with EO and VM tended ($p = 0.09$) to have greater ADG (6.3%). However, DMI was not different ($p = 0.99$). Thus, compared with control, gain-to-feed ratio (GF), observed dietary net energy (NE), and observed-to-expected diet NE were greater ($p < 0.01$) for EO and VM supplemented lambs. Compared with control, EO + ENZ increased ($p < 0.01$) 10.4% ADG. This enhancement in ADG was numerically greater than that observed with EO alone. However, EO + ENZ tended ($p = 0.09$) to increase 6.9% DMI. Compared with control EO + ENZ enhanced ($p \leq 0.04$) in feed efficiency and observed-to-expected diet NE (3.3%). This enhancement in dietary energetic efficiency was 42% less ($p \leq 0.05$) than the average improvement of 5.7% observed in lambs fed EO or VM. It is important to note that the observed-to-expected dietary NE and the observed-to-expected DMI ratio for the lambs fed the control diet was 0.99 (Table 2). This indicated that DMI was consistent with expectations based on observed ADG and formulated NE value of the diet (Table 1). The agreement in observed and expected DMI is supportive of the practicality of prediction equations for the estimation of DMI in relation to SBW and ADG of feedlot lambs. A dietary NE ratio (observed-to-expected dietary NE) of 1.0 is indicative that daily weight gain was consistent with observed DMI and tabular NE value of the diet taken from tables of NRC [19]. If the ratio is greater than 1, the observed dietary NE (estimated dietary NE based on growth performance) is greater than expected based on growth performance and diet formulation, indicative of enhanced metabolizable energy utilization for maintenance and gain (the reverse being the case when the ratio is less than 1).

The treatment effects on carcass characteristics, whole cuts and visceral mass, are shown in Tables 3 and 4. Kidney-pelvic-heart fat was lower for EO vs. VM supplemented lambs (3.13 vs. 4.16%, $p < 0.01$). Kidney-pelvic-heart fat tended ($p = 0.097$) to be low for EO vs. control lambs (3.13 vs. 3.71%). However, kidney-pelvic-heart fat was not different for EO + ENZ vs. control and VM supplemented lambs. There were no treatmenteffects ($p \geq 0.37$) on whole cuts or shoulder tissue composition. Compared with control, relative weight (g/kg EBW) of intestines was lower ($p \leq 0.03$) for EO, EO + ENZ and VM supplemented lambs. This effect was more pronounced with EO supplementation. Compared to VM, supplemented EO (alone or combined with enzyme) decreased (10.1%, $p \leq 0.04$) relative weight (g/kg EBW) of visceral fat.

Table 3. Effect of treatments on carcass characteristics of finishing lambs.

	Treatments †					*p*-Value					
Item	**Control**	**EO**	**EO + ENZ**	**VM**	**SEM**	**1 vs. 2**	**1 vs. 3**	**1 vs. 4**	**2 vs. 3**	**2 vs. 4**	**3 vs. 4**
Hot carcass weight, kg	31.24	32.68	32.93	32.67	0.73	0.18	0.12	0.18	0.81	0.99	0.80
Dressing percentage	59.47	60.30	59.48	60.33	0.83	0.19	0.98	0.47	0.48	0.98	0.47
Cold carcass weight, kg	30.93	32.28	32.63	32.28	0.74	0.21	0.40	0.58	0.99	0.99	0.99
LM area, cm^2	18.98	19.58	19.55	18.78	0.49	0.39	0.42	0.78	0.97	0.27	0.29
Fat thickness, cm §	0.283	0.256	0.253	0.262	0.19	0.32	0.27	0.43	0.91	0.83	0.75
Kidney pelvic and heart fat, %	3.72	3.13	3.65	4.16	0.25	0.09	0.83	0.21	0.14	<0.01	0.15
Carcass yield *	1.52	1.41	1.40	1.43	0.07	0.31	0.27	0.42	0.93	0.83	0.76
Shoulder composition, %											
Muscle	64.86	63.72	63.64	63.60	0.55	0.17	0.14	0.13	0.92	0.88	0.97
Fat	16.22	16.99	17.39	17.32	0.60	0.37	0.18	0.25	0.64	0.79	0.84
Muscle to fat ratio	4.03	3.80	3.68	3.72	0.16	0.30	0.15	0.20	0.66	0.78	0.87
Whole cuts (as percentage of CCW)											
Forequarter	41.66	41.74	41.69	41.65	0.40	0.88	0.96	0.99	0.92	0.87	0.95
Hindquarter	37.32	38.03	37.56	37.41	0.43	0.26	0.70	0.88	0.45	0.33	0.82
Neck	7.53	7.99	7.73	7.68	0.26	0.23	0.59	0.69	0.49	0.41	0.89
Shoulder IMPS206	14.12	13.93	14.06	13.90	0.19	0.48	0.81	0.42	0.65	0.92	0.57
Shoulder IMPS207	9.42	8.97	9.18	9.38	0.23	0.18	0.47	0.89	0.53	0.23	0.56
Rack IMPS204	5.87	6.05	5.92	6.11	0.13	0.36	0.81	0.24	0.50	0.77	0.34
Breast IMPS209	5.61	5.57	5.24	5.59	0.16	0.86	0.12	0.93	0.15	0.93	0.13
Ribs IMPS209A	6.52	6.71	6.66	6.72	0.15	0.37	0.53	0.36	0.78	0.99	0.77
Loin IMPS231	7.11	7.16	7.00	7.02	0.18	0.85	0.69	0.73	0.56	0.60	0.96
Flank IMPS232	6.09	6.23	6.30	6.26	0.21	0.63	0.49	0.57	0.83	0.93	0.89
Leg IMPS233	24.39	24.58	24.16	24.08	0.33	0.68	0.62	0.51	0.37	0.29	0.86

† Control = non-supplemented; doses per lamb, EO = a standardized source of a mixture of essential oils compounds at dose of 150 mg EO (CRINA®Ruminants, DSM Nutritional Products, Basel, Switzerland)/day; EO + ENZ = 150 mg EOC plus 560 mg alpha-amylase (RONOZYME Rumistar®, DSM Nutritional Products, Basel, Switzerland)/day; VM = a peptolide antibiotic virginiamycin (Stafac 500, Phibro Animal Health, Ridgefield Park, NJ) at dose of 25 mg virginiamycin/day. § Fat thickness over the center of the LM between the 12th and 13th ribs. * Carcass yield was estimated as (fat thickness × 0.10) + 0.40.

Table 4. Effect of treatments on visceral mass of finishing lambs.

	Treatments †					*p*-Value					
Item	**Control**	**EO**	**EO + ENZ**	**VM**	**SEM**	**1 vs. 2**	**1 vs. 3**	**1 vs. 4**	**2 vs. 3**	**2 vs. 4**	**3 vs. 4**
GIT fill, kg	3.93	4.00	4.38	3.84	0.26	0.86	0.21	0.80	0.28	0.67	0.14
Empty body weight, % of full weight	92.50	92.58	92.00	92.88	0.49	0.90	0.48	0.58	0.41	0.67	0.22
Full viscera, kg	7.83	7.86	8.45	7.79	0.28	0.93	0.13	0.92	0.16	0.86	0.12
Organs, g/kg of empty body weight											
Stomach complex	26.22	26.52	27.16	26.81	0.79	0.79	0.41	0.60	0.57	0.79	0.76
Intestines	53.97	50.49	52.27	51.71	0.48	<0.01	0.03	<0.01	0.02	0.10	0.42
Liver/spleen	16.79	15.74	15.86	16.08	0.43	0.11	0.16	0.27	0.82	0.57	0.73
Heart/lungs	20.86	19.73	19.71	19.97	0.66	0.17	0.11	0.28	0.49	0.75	0.32
Kidney	2.42	2.34	2.55	2.35	0.09	0.37	0.17	0.43	0.12	0.92	0.15
Omental fat	32.05	29.51	30.89	32.22	0.96	0.08	0.40	0.91	0.32	0.06	0.34
Mesenteric fat	11.20	10.92	11.07	13.58	0.73	0.79	0.90	0.04	0.88	0.02	0.03
Visceral fat	43.26	40.39	41.96	45.81	1.16	0.11	0.44	0.14	0.36	0.01	0.04

† EO = a standardized source of a mixture of essential oils compounds at dose of 150 mg EO/kg diet DM (CRINA®Ruminants, DSM Nutritional Products, Basel, Switzerland); EOC + ENZ = 150 mg EO plus 560 mg alpha-amylase (RONOZYME Rumistar®, DSM Nutritional Products, Basel, Switzerland)/kg DM; VM = a peptolide antibiotic virginiamycin (VM, Stafac 500 Phibro Animal Health, Ridgefield Park, NJ) at dose of 25 mg virginiamycin/kg DM.

3.1. Exp. 2. Total Tract Digestion

Treatment effects on apparent total tract digestion are shown in Table 5. There were no feed refusals. Thus, nutrient intake for all treatments were equal. Average daily gain of lambs was 0.171 kg±0.031 (data not shown). Thus, in this experiment, dietary additive intakes averaged 3.76, 14.04, and 0.62 mg/kg LW for EO, ENZ, and VM, respectively.

Table 5. Effect of treatments on nutrient digestion.

	Treatments †					*p*-Value					
Item	**Control**	**EO**	**EO + ENZ**	**VM**	**SEM**	**1 vs. 2**	**1 vs. 3**	**1 vs. 4**	**2 vs. 3**	**2 vs. 4**	**3 vs. 4**
Intake, g/d											
Dry matter	924	924	924	924							
Organic matter	866	866	866	866							
Starch	499	499	499	499							
NDF	124.7	124.7	124.7	124.7							
N	20.20	20.20	20.20	20.20							
Ether extract	47.12	47.12	47.12	47.12							
Gross energy, Mcal/d	4.055	4.055	4.055	4.055							
Fecal excretion, g/d											
Dry matter	168.0	160.8	163.1	177.3	4.35	0.28	0.45	0.18	0.72	0.04	0.06
Organic matter	143.6	137.1	138.5	154.0	5.44	0.28	0.39	0.17	0.81	0.03	0.05
Starch	3.66	2.58	3.75	3.91	0.52	0.20	0.90	0.74	0.16	0.12	0.84
NDF	60.96	58.30	59.81	63.44	3.06	0.56	0.80	0.59	0.74	0.28	0.43
N	4.69	4.61	4.76	4.51	0.15	0.68	0.77	0.40	0.49	0.66	0.27
Ether extract	8.91	9.77	9.35	8.57	1.18	0.50	0.73	0.78	0.73	0.35	0.54
Gross energy, Mcal/d	0.685	0.645	0.648	0.696	0.074	0.71	0.73	0.92	0.97	0.64	0.66
Total tract digestion, %											
Dry matter	81.94	82.76	82.37	80.79	0.71	0.29	0.57	0.16	0.60	0.04	0.07

Table 5. *Cont.*

	Treatments †					*p*-Value					
Item	**Control**	**EO**	**EO + ENZ**	**VM**	**SEM**	**1 vs. 2**	**1 vs. 3**	**1 vs. 4**	**2 vs. 3**	**2 vs. 4**	**3 vs. 4**
Organic matter	83.54	84.33	84.02	82.21	0.45	0.28	0.49	0.15	0.66	0.03	0.06
Starch	99.29	99.48	99.23	99.22	0.15	0.24	0.71	0.66	0.14	0.13	0.95
NDF	51.18	53.66	52.35	49.17	2.32	0.48	0.73	0.56	0.70	0.22	0.37
N	76.99	77.26	76.42	77.73	0.76	0.81	0.62	0.52	0.47	0.68	0.27
Ether extract	82.86	81.60	82.39	83.47	0.23	0.61	0.85	0.80	0.75	0.46	0.66
Digestible energy, %	83.22	84.26	84.07	82.60	1.06	0.36	0.46	0.70	0.86	0.22	0.28
Digestible energy, cal/kg	3.65	3.70	3.69	3.63	0.033	0.36	0.46	0.70	0.86	0.22	0.28

† Control = non-supplemented; doses per lamb, EO = a standardized source of a mixture of essential oils compounds at dose of 150 mg EO (CRINA®Ruminants, DSM Nutritional Products, Basel, Switzerland)/day; EO + ENZ = 150 mg EOC plus 560 mg alpha-amylase (RONOZYME Rumistar®, DSM Nutritional Products, Basel, Switzerland)/day; VM = a peptolide antibiotic virginiamycin (Stafac 500, Phibro Animal Health, Ridgefield Park, NJ) at dose of 25 mg virginiamycin/day.

Measures of total tract digestion and digestible energy (DE; averaged 3.66 ± 0.06 Mcal DE/kg) were not different for control vs. additives. However, apparent OM digestion was greater (2.34% $p < 0.05$) for lambs supplemented with EO or EO + ENZ than for VM supplemented lambs.

3. Discussion

The average relative ingestion of EO + EZ in our experiment was consistent (1.01 and 1.05, respectively) to the relative ingestion observed in the experiments of Giannenas et al. [17] and Meschiatti et al. [15]. Relative ingestion of 3.5 to 4 mg EO/kg LW enhanced ruminal fermentation and feed efficiency in lactating ewes [17] and in finishing lambs [8]. Effective dosage level of ENZ for finishing lambs has not been established. However, ingestion of 12.9 mg ENZ/kg LW improved DMI and ADG in finishing steers [15]. Effects of essential oils on ruminal fermentation and growth performance are dose dependent, with enhancements being less apparent when supplemented at less than 3 mg EO/kg LW [31,32]. Daily weight gain, feed efficiency, and ruminal fermentation are enhanced when VM is supplemented within the range of 0.50 to 0.83 mg/kg LW [2,3,33]. The dosage levels of EO, ENZ and VM attained in the present study are within the reported effective ranges for enhanced growth performance and digestion.

3.1. Exp. 1. Growth Performance and Carcass Traits

Based on average temperature and DMI during the experiment, observed water intake for control group, EO, and VM were consistent with expected based on NRC [19], averaging 0.98, 1.01, and 0.97, respectively. The absence of effect of EO and VM on water intake has been reported previously [34,35]. In contrast, compared with the other treatments, supplementation with EO + ENZ increased (7%) water consumption. Likewise, Valdés et al. [36] observed increased (6.4%) water consumption with EO + ENZ supplementation. Consistent with the present study, the increase in water intake was largely due to increased DMI. Indeed, water consumption/kg DMI was similar across treatments, averaging 6.80, 6.98, 6.92, and 6.76 for control, EO, EO + ENZ, and VM, respectively.

Information regarding the effects of the standardized mixture of essential oils used in the present study (CRINA Ruminants) on growth performance and dietary energetics in finishing cattle and lambs fed with high-energy diets is limited. Meyer et al. [6] observed a 4% increase in efficiency of dietary energy utilization in finishing cattle fed a high-energy corn-based diet (2.20 Mcal NE_m/kg diet DM). Likewise, Plascencia et al. [8] observed that compared with control, EO supplementation enhanced both feed efficiency (4.7%) and estimated dietary net energy (3.1%) in fattening lambs fed with a cracked corn-based diet (2.14 Mcal NE_m/kg diet DM). The basis for enhanced efficiency of energy utilization with EO supplementation has not been established. Supplemental EO has altered ruminal VFA molar ratios, with increased proportion of propionate and decreased methane production [6,17,36].

In finishing lambs, GF responses to supplemental VM have been variable, ranging from nil (lambs fed a low-to-moderate energy diet (~1.80 Mcal NE_m/kg diet DM) with a dose of 17.9 mg VM/kg diet [37]) up to a 10% increase (lambs fed a moderate energy diet (~1.95 Mcal NE_m/kg diet DM) with a dose of 20 mg/kg [38]). Growth performance response to supplemental VM depends on both dosage level and dietary energy density [3]. Consistent with the present study, enhancements of 3 to 9% in ADG and 4 to 11% in feed efficiency have been reported for feedlot cattle fed high-energy corn-based diets (>2.17 Mcal NE_m/kg diet DM) supplemented with 22 to 26 mg VM/kg diet DM [2,39]. The positive effect of VM on growth performance has been associated with enhanced N utilization and reduced liver abscess incidence [39,40].

The increased ADG with EO + ENZ supplementation observed in the present experiment was due to enhanced DMI. Indeed, the efficiency of energy utilization (expected vs. observed dietary NE) was appreciably lower (2.5%) for EO + ENZ vs. EO alone. The basis for the slightly lower energetic efficiency for EO + ENZ is not certain, but may be due to changes in site of starch digestion (increased ruminal vs. postruminal digestion; Owens et al. [41]). Tricarico et al. [42] reported results of four experiments evaluating α-amylase supplementation of feedlot cattle. Consistent with the present study, they observed supplemental α-amylase increased DMI by an average of 5.2%. Meschiatti et al. [7] observed a 2.3% increase in DMI of feedlot steers supplemented with the combination of α-amylase (560 mg/kg diet DM) and essential oils (90 mg /kg diet DM). Valdés et al. [36] observed an 11% increase in DMI and a 22% increase in ADG of lambs fed a maize silage-based diet (70% maize silage and 30% concentrate) supplemented with an enzyme mixture alone (α-amylase, endoglucanase, and xylanase). The effect was further enhanced when fed in combination with essential oils (blend of salicin, myricetin, kaempferol, and quercetin). Klingerman et al. [14] observed a 5% increase in DMI of lactating cows. Responses to supplemental α-amylase may be greatest in livestock that are fed diets containing otherwise less digestible corn hybrids (i.e., flinty corn with high concentrations of vitreous endosperm).

Lack of treatment effects on carcass traits and tissue composition is consistent with previous comparisons of supplemental EO [8,43], EO + ENZ combination [44], and VM [39].

The decrease in intestinal mass with VM supplementation is consistent with studies in which VM supplementation decreased intestinal wall thickness, and hence, intestinal weight of mice [45], broilers [46], and cattle [47]. Likewise, we observed decreased intestinal mass with EO supplementation, consistent with antibiotic-like effects on intestinal epithelial thickness. Wang et al. [48] observed that supplemental essential oils (mainly thymol) decreased jejunal wall thickness of poultry, while Ghazanfari et al. [49] observed that supplementation with essential oil mixture (mixture of linalool, terpinene, and limonene) decreased wall thickness along all segments of the small intestine of poultry by an average of 30%.

The effects of supplemental EO and EO + ENZ vs. VM on mesenteric and visceral fat (g/kg EBW) is uncertain. It has been proposed that supplemental EO may have potential as an energy "repartitioning" agent, affecting net fat deposition and distribution [50]. This may partially explain changes in meat quality of lambs supplemented with EO [51,52]. To the extent that EO reduces ruminal acetate: propionate ratio [6,43], the associated increase in propionate production may lead to decreased visceral fat deposition [53].

3.1. Exp. 2. Total Tract Digestion

For the most part, supplemental EO has not appreciably affected measures of apparent total tract digestion in dairy cattle [31,54], steers [9,55,56] and in lambs [10,57,58]. In several studies [54,57,59], supplemental essential oils were found to alter ruminal fermentation without effect on total tract digestion.

Consistent with Gouvêa et al. [44], the combination EO + ENZ did not affect apparent total tract digestion of OM, starch or NDF. Lack of treatment effects on total tract starch digestion is not surprising, in as much as apparent total tract starch digestion approached 100% for all treatments. The completeness of starch digestion in our study is consistent with numerous prior studies evaluating starch digestion in feedlot lambs fed high-grain diets [60–62]. Other researchers noted that whereas amylase supplementation of lambs did not affect total tract starch digestion, it increased ruminal starch digestion [63]. Ruminal digestion of cracked corn in lambs averages 77% [64,65]. Applying the above to the daily starch intake in the present study (499 g), the estimated ruminal escape of starch to the small intestine was 115 g/d. Xu et al. [65] observed that maximum intestinal α-amylase activity is reached when rumen escape starch is about 100–120 g/d in 25–30 kg lambs. Klingerman et al. [14] observed that although supplemental amylase did not affect total tract starch digestion, it increased NDF digestion in cows fed a high-forage diet.

Supplemental VM did not affect measures of total tract digestion. In a series of experiments evaluating the effects of 0 vs. 25 mg VM/kg DM fed to feedlot lambs, Fiems et al. [66] observed that although VM did not affect apparent total tract OM digestion, it decreased apparent total tract digestion of CP and ether extract. Da Fonseca et al. [67] did not observe any effect of VM supplementation (30 mg/kg DM) on apparent total tract digestion of DM, OM, N, and NDF in steers fed 50:50 concentrate: forage diet. Salinas et al. [2] did not observe effects on measures of total tract digestion in steers receiving a steam-flaked corn-based finishing diet supplemented with 22.5 mg VM/kg DM. Feeding greater dosage levels of VM (26 to 28 mg VM/kg DM) in feedlot steers fed similar finishing diets likewise did not affect apparent total tract digestibility of OM, NDF, starch, and CP [3,39]. The lower OM digestion observed for lambs receiving VM treatment vs. control, EO and EO + ENZ treatments is not certain. In previous reports [2,3,39], supplemental VM resulted in numerically lower apparent total tract OM digestion (averaging −2.6%) than that of control steers. In the present study, average difference between EO and EO + ENZ vs. VM was 2.10%. Since there were no effects of additives on total tract digestion, digestible energy (DE) of the diet were similar ($p \geq 0.28$) between treatments, averaging 3.66 ± 0.06 Mcal NE_m/kg. Applying the relationship between ED and NE_m derived by Zinn and Plascencia ($NE_m = 0.736DE - 0.661$) [68], the NE_m value in digestion trial resulted in 2.03 Mcal NE_m/kg; this is in close agreement (0.975) with the value of 2.08 Mcal NE_m/kg observed for the basal diet in the growth performance trial (Exp. 1).

3. Conclusions

Supplemental EO may be an effective alternative to VM in high-energy finishing diets for feedlot lambs, enhancing both feed efficiency and apparent efficiency of energy utilization. Combination EO + ENZ may further enhance dry matter intake, promoting increased weight gain.

Author Contributions: Conceptualization, methodology, supervision, visualization, writing—review and editing: A.P. and A.E.-A.; Writing—review and editing final version of the manuscript: R.A.Z.; Writing—original draft preparation: J.B.L.-M. and X.P.P.-D.; Data curation, statistical analyses: A.B. and L.C.; Investigation: Y.J.A.-W., B.I.C.-P., J.D.U.-E., C.A.-M., S.G.-C. and E.P.-B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially supported by DSM Nutritional Products, México.

Institutional Review Board Statement: This experiment was conducted at the Universidad Autónoma de Sinaloa Feedlot Lamb Research Unit, located in Culiacán, México (24°46´13” N and 107°21´14” W). All animal management procedures were conducted within the guidelines of federal-locally-approved techniques for animal use and care [16] and approved (29-11-2019) by the Ethics Committee of Faculty of Veterinary Medicine and Zootechnics from the Autonomous University of Sinaloa (Protocol #1422019).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Appreciation is expressed to CONACYT, Mexico, for fellowship support (739112) to Yesica Arteaga Wences. This project was partially supported by Faculty of Veterinary Medicine and Zootechnics, Autonomous University of Sinaloa, Culiacan (Project-1759453-2).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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CAPÍTULO 4. CONCLUSIONES GENERALES

Actualmente se reconoce que el uso masivo de antibióticos ha llevado a un mayor problema de resistencia, y la presencia de residuos de antibióticos en los alimentos y el medio ambiente esto compromete la salud humana y la de los animales. Motivo por el cual, la mezcla estandarizada de aceites esenciales (AE) compuesta por timol, eugenol, limoneno y vainillina afecta positivamente el rendimiento productivo y la energética de corderos en etapa de finalización. En comparación con corderos no suplementados, la suplementación a razón de 150 mg/d a corderos en finalización incrementó en promedio 4.9% la eficiencia para la ganancia y 3.3% la EN observada de la dieta. Comparado con corderos que recibieron 30 mg/d del ionóforo monensina, la suplementación con AE mejoró 13.2% la ganancia diaria de peso como resultado de un aumento del consumo de MS (9.3%) y de una mejor utilización de la energía de la dieta (2%). Cuando AE se comparó con el antibiótico virginiamicina, no se detectaron diferencias en la ganancia y consumo. Ambos incrementaron la energía observada de la dieta en 5%. El combinar AE con enzimas exógenas incremento la ganancia diaria comparado con aquellos que solo recibieron AE, sin embargo, la magnitud de la respuesta en eficiencia energética fue menor para la combinación. Los efectos de los AE en la canal son mínimos, el principal efecto es la reducción de la grasa omental lo que indica un potencial efecto de repartición de la energía. Dados los resultados obtenidos, los AE son una alternativa al uso de antibióticos monensina y virgianiamicina en la etapa de finalización de los corderos.

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