UNIVERSIDAD AUTÓNOMA DEL ESTADO DE HIDALGO

INSTITUTO DE CIENCIAS BÁSICAS E INGENIERÍA

INSTITUTO DE CIENCIAS AGROPECUARIAS

INSTITUTO DE CIENCIAS DE LA SALUD

OBTENCIÓN DE CARNE DE CORDERO ENRIQUECIDA CON ÁCIDOS GRASOS OMEGA-3 MEDIANTE LA INCORPORACIÓN EN LA DIETA ANIMAL

TESIS

QUE PARA OBTENER EL GRADO DE:

DOCTOR EN CIENCIAS DE LOS ALIMENTOS Y SALUD HUMANA

PRESENTA:

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M. en A. JULIO CESAR LEINES MEDÉCIGO
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Por este medio se informa que el comité tutorial asignado al M. en C. José Enrique Javier Olloqui Pang con número de cuenta 342908, estudiante del Doctorado en Ciencias de los Alimentos y Salud Humana dio terminación al trabajo de tesis titulado "Obtención de carne de cordero enriquecida con ácidos grasos omega-3 mediante la incorporación en la dieta animal" y por lo tanto se autoriza la impresión del documento de tesis en extenso propuesto por el estudiante.

Lo anterior, en función de que, el estudiante realizó todas las correcciones, adiciones y/o modificaciones sugeridas por el comité en la revisión previa con fecha 19 de junio 2019.
Por tal motivo, solicito a usted tenga a bien permitir al doctorando dar continuidad al proceso necesario que conlleve a la obtención del grado de Doctor en Ciencias de los Alimentos y Salud Humana.

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PREFACIO

Los cambios en los estilos de vida han generado que la población mundial demandeproductos de mejor calidad y el área de los alimentos no es la excepción. En este sentido, se ha preocupado por adquirir alimentos que además de satisfacer el hambre y aportar los nutrientes necesarios, también impacten de manera positiva en la salud ya sea previniendo eldesarrollo de ciertas enfermedades o mitigando las ya existentes. Para dar respuesta a esas necesidades la comunidad científica se ha dado a la tarea de desarrollar alimentos que contengan ciertos principios bioactivos que aporten beneficios a la salud surgiendo así lo queactualmente se conoce como alimentos funcionales.

Al respecto, existen evidencias científicas que demuestran que el consumo de ácidos grasos omega-3 como el Eicosapentaenoico (EPA) y docosahexaenoico (DHA) puede ayudar a disminuir el desarrollo o el progreso de enfermedades cardiovasculares y ciertos tipos de cánceres. Por lo anterior, se han realizado esfuerzos ya sea mediante procesos tecnológicos o por suplementación animal para ampliar la gama de alimentos que contengan estos tipos de ácidos grasos.

Para contribuir en este aspecto del desarrollo de la ciencia, en el presente trabajo se realizaron estudios para mediante la suplementación animal con aceite de pescado tratar de obtener

carne de borrego con EPA y DHA y ayudar así a incrementar la gama de productos con ácidos grasos saludables.

Para plasmar lo realizado, en el documento se presenta la información por capítulos y en forma progresiva. Partiendo con el estudio del estado del arte de los alimentos funcionales, la suplementación animal e insumos utilizados en la misma, así como los objetivos del trabajo (Capítulo 1. Antecedentes generales). En los siguientes capítulos se exponen en forma de artículos científicos las diferentes fases de las investigaciones efectuadas. En el capítulo dos (Estudio de la composición de una nueva variedad no tóxica de *Jatropha curcas* como un posible ingrediente en la formulación de dietas) se realizaron estudios a la semilla y el residuo de *Jatropha curcas* para determinar la viabilidad de utilizarlos como una alternativa nutritiva para disminuir los costos que pudieran generar la inclusión del aceite de pescado utilizada como fuente de EPA y DHA. Posteriormente, se muestran los resultados relacionados a la encapsulación del aceite (Capítulo 3. Encapsulación de aceite de pescado con alginato y la liberación de EPA y DHA en un modelo de digestión rumino-intestinal *in vitro*) como un mecanismo para evitar los procesos de biohidrogenación ruminal de los ácidos grasos. La siguiente etapa consistió en corroborar en un sistema *in vivo* la protección ruminal de los encapsulados (Capítulo 4. Rendimiento en el crecimiento, perfil de ácidos grasos y metabolitos sanguíneos de corderos suplementados con aceite de pescado encapsulado). Posteriormente, se realizó la suplementación en los borregos durante un periodo de veinticinco días, después del cual fueron sacrificados para obtener y analizar la carne (Capítulo 5. Características físico-químicas en la canal y en carne de cordero enriquecida con aceite de pescado encapsulado en la dieta). Por último, se presentan las conclusiones generales del estudio (Capítulo 6. Conclusiones generales) y los anexos (Capítulo 7) en los

que destacan las estancias realizadas, así como las presentaciones en congresos internacionales de los trabajos derivados de las investigaciones, así como también el protocolo aprobado por el comité de bioética para la realización de los estudios en los borregos.

ÍNDICE GENERAL

Capítulo I

Antecedentes generales

1.1. Alimentos funcionales

En los últimos años, se ha desarrollado el interés de las personas por un estilo de vida saludable lo que ha generado cambios en diferentes aspectos de la vida, donde el área de los alimentos no es la excepción (Verplanken *et al*., 2005). Al respecto, el sector de los alimentos funcionales ha generado gran expectativa. En ese sentido, la acción concertada de laComisión Europea en la ciencia de los alimentos por sus siglas en inglés (FuFoSE), refiere aun alimento funcional como un producto alimenticio que además del impacto nutricional básico tiene efectos benéficos en una o más funciones del organismo humano, pueden mejorar la condición general y física y reducir el riesgo de evolución de una enfermedad (Martirosyan & Singh, 2015).

La investigación en el campo de los alimentos funcionales se ha enfocado en los siguientes ámbitos (Martirosyan & Singh, 2015):

(a) Crecimiento y desarrollo.

(b) Metabolismo de sustancias.

(c) Defensa contra el estrés oxidativo.

(d) Enfermedades cardiovasculares.

(e) Funcionamiento del tracto intestinal.

(f) Funciones psicológicas y conductuales.

En el caso de las enfermedades cardiovasculares, se ha evaluado el efecto de ácidos grasos que mitigan y disminuyen la incidencia de este tipo de padecimientos. En este campo, los ácidos grasos omega-3 han acaparado el interés de la comunidad científica por todos los efectos positivos asociados a su consumo (Narayan *et al.,* 2006).

1.2. Ácidos grasos omega-3 y efecto en la salud

Los omega-3 son ácidos grasos polinsaturados (PUFAs) que se caracterizan porque presentan dos o más insaturaciones, siendo el que se encuentra en el carbono tres contando a partir del grupo metilo terminal (carbono omega) el que le otorga la categoría de omega 3 o n-3 (Figura 1).

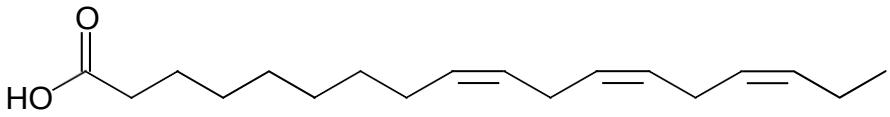
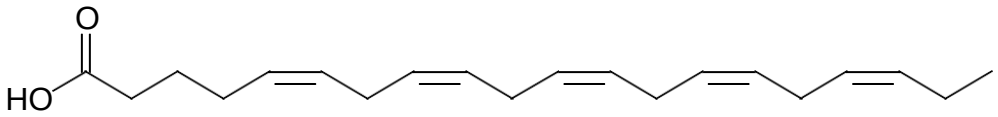


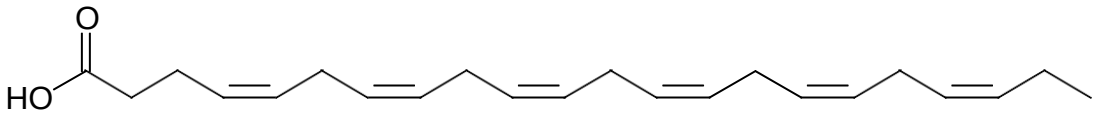
Fig. 1. Estructura molecular de los ácidos grasos omega 3. (Narayan *et al.,* 2006)

En este contexto, se ha demostrado que son precisamente la estructura molecular y la configuración espacial las que le otorgan efectos fisiológicos importantes a los PUFAs (Calder, 2015). De tal manera que, los ácidos grasos PUFAs n-3 como el eicosapentaenoico (EPA) y el docosahexaenoico (DHA) (Figura 2) intervienen directamente en la fluidez de la

membrana celular, en los sitios de receptor de membranas y actúan como sustrato para otras funciones importantes (Narayan *et al.*, 2006).



EPA



DHA

Fig. 2. Estructuras moleculares de los ácidos eicosapentaenoico (EPA) y docosahexaenoico (DHA) (Narayan *et al.*, 2006).

Así, los principales efectos de los PUFA n-3 en la salud humana se pueden dividir en tres categorías:

1. La importancia en órganos específicos, como el caso de DHA en las funciones de retina, membranas celulares y el sistema nervioso central.
2. El rol significativo en el equilibrio de lípidos en sangre.
3. La función como precursor para respuestas bioquímicas y fisiológicas, como en el caso de cáncer, diabetes, desordenes neuropsiquiátricos, enfermedades autoinmunes y particularmente efectos cardioprotectores (Tabla 1).

Tabla 1. Efectos cardioprotectores asociados con EPA y DHA y sus posibles mecanismos.

Efecto antiarrítmico	Reduce arritmias ventriculares por la modificación de lípidos cardiacos
Incremento en la viabilidad de la tasa cardiaca	Incrementa el tono parasimpático, inhibe y/o altera los niveles de citoquinas, alterando los niveles de mitógenos y otros factores.
Efecto antitrombótico	Inhibe la vía del tromboxano A2 en la cascada del ácido araquidónico, reduciendo la actividad plaquetaria e incrementando la producción de prostaciclinas (pro-vasodilatadoras).
Efecto inhibitorio en la arterioesclerosis	Regula las concentraciones de colesterol plasmático, inhabilitando la migración de monocitos y estimulando la producción endotelial de óxido nítrico.
Efecto hipolipidémico	Reduce el colesterol plasmático y triglicéridos por la disminución de las lipoproteínas de muy baja (VLDL), baja (LDL) y mediana densidad (IDL) en hígado.
Efecto antiinflamatorio	Inhibe la proliferación de células lisas, altera la síntesis de eicosanoides: prostaglandinas y leucotrienos (principalmente PGE_2 y LTB_4 respectivamente) y reduce la expresión de moléculas de células de adhesión a nivel endotelial.

1.3. Fuentes de EPA y DHA

Los organismos marinos (peces de agua fría), son la principal fuente de EPA y DHA en la dieta humana (Narayan *et al.,* 2006). Lo anterior, debido a que las algas marinas y el fitoplancton, que son las fuentes más importantes de EPA y DHA, son consumidos por los peces de agua fría y algunos animales marinos por lo que estos ácidos grasos son absorbidos a través de la cadena alimenticia (Boelen *et al*., 2017). En los productos de origen vegetal y de animales superiores no existe de manera natural la presencia de EPA y DHA, encontrándose únicamente el ácido alfa linolénico como el representante de la familia omega-3 y precursor de EPA y DHA. Sin embargo, la tasa de bioconversión de ácido alfa linolénico es muy baja del 0.1 y 0.03% para EPA y DHA, respectivamente (Hussein *et al.,* 2005). Debido a lo limitado de las fuentes naturales de EPA y DHA, así como al efecto benéfico para la salud que ha mostrado su consumo se han buscado alternativas para incorporarlos a productos alimenticios de consumo habitual. Lo anterior se ha realizado por medios tecnológicos y por la suplementación en la dieta animal (Toral *et al*., 2018).

1.4. Suplementación de EPA y DHA

Además de proporcionar energía, la suplementación con lípidos en la dieta modifica el perfil de ácidos grasos (Ghasemi *et al*., 2016). En este sentido, Manso *et al*. (2016) indicaron que la inclusión de PUFAs en la dieta animal, con el objetivo de obtener un alimento funcional, proporcionó un perfil lipídico saludable en leche y carne. Al respecto, se han realizado diversos estudios para incorporar ácidos grasos omega-3 principalmente mediante la utilización de harinas y aceites de pescados provenientes de aguas muy frías

como el arenque (*Clupea harengus*), la caballa (*Scomber scombrus*), el salmón (*Salmo salar*) y el bacalao (*Gadus morhua*) (Calder, 2015).

Sin embargo, al tratarse de ácidos grasos polinsaturados EPA y DHA son susceptibles a alteraciones por diferentes factores como la luz, oxígeno y biohidrogenación (Gawad *et al.*, 2015). Por lo anterior, se buscan diversas alternativas para conservar su estructura y sus propiedades.

1.4.1. Suplementación de EPA y DHA en rumiantes

Para lograr la incorporación de EPA y DHA en los alimentos de los rumiantes se evalúan diversas metodologías con la finalidad de protegerlos (Toral *et al.*, 2016). Lo anterior, debido a que, en uno de los cuatro compartimentos del estómago de los rumiantes se encuentran bacterias celulolíticas, hemicelulolíticas, amilolíticas, sacarolíticas, proteolíticas y lipolíticas que facilitan la digestión de celulosa, hemicelulosa y xilano (Bessa *et al.* 2015); sin embargo, estos microrganismos pueden alterar los ácidos grasos de la alimentación y modificar las funciones gastrointestinales (Encina *et al.*, 2016).

Por ello, Scott *et al.* (1970) usaron proteína y formaldehído, para producir enlaces intra e intermoleculares con una susceptibilidad reducida a la degradación ruminal. Este método presentó resultados favorables en la deposición de ácidos grasos en carne y leche (Ashes *et al.*, 1992). Sin embargo, el uso del formaldehído no está permitido por muchos organismos regulatorios, por consiguiente, este procedimiento no es aceptado ni usado de forma comercial (Scollan *et al.*, 2014).

Otra metodología usada es la técnica de saponificación, ésta envuelve la adición de cloruro de calcio a sales de sodio fundido de los lípidos. Las sales de calcio resultantes son insolubles en el rumen, pero se disocian en el abomaso por su ambiente ácido (Wu *et al.*, 1991). No obstante, la protección en esta técnica solo ocurre a bajas concentraciones de ácidos grasos insaturados, por lo que es un factor limitante en su uso (Oliveira *et al.*, 2012).

Una de las opciones que mejores resultados ha mostrado es la de encapsulación con polisacáridos; en este sentido, Tan *et al.* (2009) microencapsularon ácidos grasos con alginato y almidón obteniendo resultados favorables en la protección de estos en condiciones gastrointestinales. En tanto que, Gawad *et al.* (2015) encapsularon aceite de linaza con alginato y carragenina concluyendo que, es una opción viable para proteger a los PUFAs de la hidrogenación ruminal.

El encapsulamiento del aceite con alto contenido de ácidos grasos polinsaturados tiene gran relevancia principalmente cuando ésta se pretende incorporar en la dieta de animales rumiantes (Olloqui *et al.*, 2018). La encapsulación ha permitido en el área de nutrición animal, la entrega de moléculas bioactivas de rápida degradación como antioxidantes, minerales, vitaminas, fitoesteroles, luteína, ácidos grasos, licopeno, prebióticos, aminoácidos, enzimas, así como de células vivas (probióticos), esto a través de los alimentos, agua de bebida o suplementación oral. El principal objetivo es la protección de estas moléculas y células, así como la entrega controlada a un sitio específico del tracto gastrointestinal (Augustin & Hemar, 2009; Betoretel *et al.*, 2011; Nedovic *et al.*, 2011). Sin embargo, no es un proceso sencillo debido a las características anatomofisiológicas del sistema digestivo de este tipo de animales.

Por tal motivo, la encapsulación es una opción viable para evitar posibles alteraciones como la biohidrogenación y lograr el transporte de los PUFAs al abomaso para que puedan ser digeridos en el intestino delgado.

Tabla 2. Estudios en suplementación de aceite de pescado en rumiantes

Referencias	Estudios
Kitessa *et al.*, (2001)	Probaron la suplementación de aceite de atún protegido (7% en la dieta) durante 12 días y obtuvieron un incremento tres veces mayor de EPA y DHA en la grasa intramuscular de corderos suplementados con aceite de atún protegido con respecto al grupo testigo.
Scollan *et al.,* (2001)	Suplementación a bovinos con 1.4% de aceite de pescado en la dieta por 14 días, obteniendo un incremento tres veces mayor de EPA y DHA en los músculos del grupo tratado con respecto al grupo testigo.
Ponnampalam *et al.,* (2002)	En este estudio se suplementaron con aceite de pescado (168 g) por 56 días, logrando un incremento del 300% de EPA y DHA con respecto al grupo testigo en el músculo *longissimus thoracis* de corderos.
Najafi *et al.*, (2012)	Suplementación de cabritos con 2% de aceite de pescado por 84 días, resultando en un incremento cuatro veces mayor de EPA y DHA en el músculo *longissimus lumborum*.

Urrutia *et al.*, (2016)	Suplementaron corderos con 5% de linaza y 3.89% de microalgas marinas (*Schizochytrium spp*) desde el destete hasta que alcanzaron el peso comercial de 24 a 28 kg. Obteniendo un aumento de tres veces de EPA y veinte veces de DHA en el tejido subcutáneo.
Jaworska *et al.*, (2016)	Suplementación a corderos con 1% de aceite de pescado y 2% de aceite de colza por 35 días, obtuvieron un aumento de 85% de DHA en el músculo *longissimus* con respecto al grupo testigo.

1.5. Utilización de subproductos agroalimentarios

La incorporación de productos marinos, como es el caso de encapsulados de aceite de pescado con alto contenido en EPA y DHA puede incrementar los costos de producción de los hatos ganaderos, por tal motivo se han buscado alternativas que permitan mitigar dichos efectos sin descuidar el balance de todos los nutrientes de la dieta (Dunne *et al.*, 2011). Muchas de ellas están relacionadas con subproductos agroalimentarios.

Una opción pueden ser los residuos generados en la industria de procesamiento de aceites como la de *Jatropha curcas.* Ésta tiene diversos usos medicinales y alimenticios cobrando mayor importancia la obtención del aceite para la generación de biocombustible, aunque, también se ha empleado para la elaboración de jabones insecticidas, barnices, sustituto de parafina, repostería, así como combustible para la iluminación de hogares rurales y abono. En Europa, también se ha utilizado en el hilado de la lana y manufactura textil. De

tal manera que, debido a este potencial industrial, actualmente es cultivada en muchas regiones de Sudamérica, África y Asia principalmente (Divakara *et al*., 2010).

Los residuos generados en esta industria a nivel mundial son la cáscara (62 400 000 toneladas) y la pasta (93 600 000 toneladas) en las cuales se reporta una composición de nutrientes (proteína, lípidos, fibra y cenizas), sin menospreciar la posible presencia de metabolitos tóxicos, la hacen una buena opción para suplementar la dieta de rumiantes de forma económica (Contran *et al.,* 2013).

Distintos estudios alrededor del mundo reportan variaciones en los contenidos de proteína cruda (19-31%), lípidos (43-59%), fibra (3.5-6.1%) y cenizas (3.4-5%). En diversas regiones de México (trópico a semiárida), Martínez-Herrera *et al*. (2006) reportaron concentraciones nutricionales que oscilan entre 31-34.5% de proteína, 55-58% de lípidos, 2.8-3.4% de fibra y 3.0-5.1% de cenizas. Makkar *et al*. (1997) y Martínez-Herrera *et al*. (2006) encontraron resultados similares en el contenido de aminoácidos esenciales de la semilla de *Jatropha curcas*; sin embargo, los aminoácidos esenciales (lisina, triptófano, metionina y cisteína) no cumplen con los requerimientos para la alimentación humana establecidos por la Organización para la Alimentación y la Agricultura (FAO).

Por otro lado, el perfil graso en las semillas de *Jatropha curcas* está compuesto principalmente por ácido palmítico (C16:0), esteárico (C18:0), oleico (C18:1) y linoleico (C18:2). En menor cantidad, se reporta el ácido mirístico (C14:0), ácido palmitoleico(C16:1), ácido linolénico (C18:3), ácido cis-11-eicosanoico (C20:1cis), ácido cis-11- eicodienoico (C20:2cis), ácido araquídico (C20:0), ácido araquidoleico (C20:1) y el ácido behénico (C22:0) (Abedowale & Adedire, 2006; Martínez-Herrera *et al*., 2006).

Sin embargo, las semillas de *J. curcas* pueden ser tóxicas y es una limitante para el consumo humano y animal, ya que pueden ocasionar mareo, vómito y diarrea (Levin *et al*., 2000). el potencial tóxico es proporcionado por diterpenos tetracíclicos característicos de la familia *Euphorbiacaea*, denominados como ésteres de forbol. Su acción se deriva de la activación de la Proteína C kinasa, lo que afecta la síntesis de proteínas, ADN, poliaminas, diferenciación celular y la expresión de genes (Goel *et al*., 2007). La planta se considera tóxica a partir de una concentración de 0.11 mg/g de ésteres de forbol (Makkar *et al*., 1998),

Por otro lado, también contienen metabolitos secundarios con efectos metabólicos en humanos y animales (Ennekin & Wink, 2000). Algunos de estos compuestos son el ácido fítico, inhibidores de tripsina, lectinas, saponinas, alcaloides y taninos. Estos compuestos no fungen precisamente como factores antinutricionales, debido a que pueden ser utilizados como tratamientos paliativos en diversos padecimientos. Los glucósidos cianogénicos son compuestos con la capacidad de liberar cianuro y provocar envenenamiento al consumidor. En trabajos realizados con semillas de *J. curcas* de otras regiones, no se ha detectado estos compuestos. Sin embargo, es importante su determinación y cuantificación (Colomé *et al*., 1993).

Con base en esta información, es de interés caracterizar y evaluar el posible uso de la semilla y pasta de *Jatropha curcas L. var. Sevangel* para su inclusión en la dieta animal como fuente de ácidos grasos omega.

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Objetivo General:

Obtener carne de cordero enriquecida con EPA y DHA mediante la incorporación de aceite de pescado encapsulado en la dieta para diversificar las fuentes de ácidos grasos omega-3.

Objetivos Específicos:

- Evaluar el perfil químico, ácidos grasos, compuestos secundarios y propiedades antioxidantes de la semilla y pasta de *Jatropha curcas var. Sevangel*, para conocer el potencial como ingrediente en una dieta de engorda de cordero.
- Elaborar el registro de los cambios en el tiempo en el perfil de los ácidos grasos omega-3, durante la digestión ruminal *in vitro* de las distintas concentraciones de alginato como matriz de encapsulación del aceite de pescado.
- Analizar las variables de crecimiento en los corderos, el contenido de ácidos grasos en plasma sanguíneo, así como los niveles de glucosa, colesterol total, triglicéridos, HDL y LDL en suero sanguíneo de corderos suplementados con aceite de pescado.
- Evaluar las características de la canal de cordero y el análisis fisicoquímico de la carne obtenida de los corderos suplementados con omega-3.

Capítulo II

Estudio de la composicion de una nueva variedad no tóxica de *Jatropha curcas* como un posible ingrediente en la formulación de dietas

Study of composition of a new non-toxic variety of *Jatropha curcas* as a possible ingredient in diet formulations

Non-toxic variety of *Jatropha curcas* as a new source of high-quality vegetal protein

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Abstract

The seeds and cake of a new variety of Jatropha *(J. curcas L. var. Sevangel*) were characterized through the quantification of their nutrients and minority components (phorbol esters and secondary compounds) to evaluate their possible use in diets. The dietary fiber was the main nutritional component in both samples (29.4-33.4%). The new variety of jatropha is a source of protein (18% in the seeds, and 32.2% in the cake) with high values ofglutamine, histidine, and tyrosine (13.09, 20.74 and, 10.17 g 100^{-1} protein). The most abundant minerals in both samples were Ca, K and Mg. The main fatty acids detected were palmitic and linoleic, and the total content of unsaturated fatty acids was 304.94 mg/100 g and 61.46 mg/100 g in the seed and cake, respectively. Phorbol esters were not detected, so the analyzed plant can be considered as harmless, compared to other toxic varieties. The content of phytic acid was higher in the seeds than in the cake (8.1 vs 5.0 mg mL^{-1}); which may favor the potential as a functional ingredient. The tannins content, trypsin inhibitors andsaponins with hemolytic activity do not represent a risk for consumption of this plant. *Jatropha curcas L. var. Sevangel* is a rich source in dietary fiber, protein, some essential amino acids, and calcium, which gives it properties suitable for its use in diets.

Keywords: jatropha, phorbol esters, phytic acid, dietary fiber, non-toxic.

Abbreviations

ADF	acid detergent fiber	ICP-OES	Inductively coupled plasma optical emission spectrometry
AE	aqueous extract	TI	Trypsin inhibitors

AOAC	Association of Analytical Communities	NDF	neutral detergent fiber
CHO	Carbohydrates	PA	phytic acid
CP	crude protein	PE	phorbol esters
DF	dietary fiber	PMA	phorbol 12-myristate-13-acetate
DM	dry matter	PUFA	Polyunsaturated fatty acid
FAs	fatty acids	SFA	saturated fatty acid
CG	Cyanogenic glucosides	Tan	Tannins
HS	hemolytic saponins	TI	trypsin inhibitors

Introduction

Jatropha curcas is a plant belonging to family *Euphorbiaceae* and it is native to Central America and Mexico [1]. This plant has a good adaptability to different climates (tropics and arid), so it can be planted in inhospitable areas for other types of crops [2]. There are two genotypes of *J. curcas,* one toxic and the other non-toxic. Jatropha toxicity is due to the content of phorbol esters (PE), which are diterpenes that cause toxic symptoms when toxic plants are eaten [3].

Besides, *Jatropha* seeds also contain some secondary compounds that can act as antinutritional, such as tannins (Tan), trypsin inhibitors (TI), lectins (Lec), phytic acid (PA), and saponins [4].

Some studies have reported index of mortality in mice, broilers, goats and pigs that were fed by a toxic seed [1,3]. In humans, accidental poisoning with *Jatropha* seeds can lead to dizziness, vomiting and diarrhea [5].

On the other hand, non-toxic varieties of *Jatropha* have been used to obtain oil for biofuel production [5]; and this waste extraction (cake) has been proposed for using in the animal diets formulation [4,6] or for human consumption [2]. In Mexico, the seed of the non-toxic variety is toasted and used for the elaboration of traditional dishes [4].

Non-toxic *Jatropha* seeds can be an important source of energy, because have a high oil content (48-64%) [4]. In addition, these seeds contain important bioactive compounds, as phenolics, which can give antioxidant activity [7].

Jatropha curcas L. var. Sevangel has not been previously studied; however, it has been registering by a Mexican Authority (SIAP-SAGARPA, register number: 1461). Therefore, the aim of this study was to elucidate the nutritional and antioxidant properties, and PE content of seed and cake from this plant. These results will give insight into the possible use of seed and cake as an ingredient in the human and/or animal diet.

Materials and methods

Sample.

The seeds were collected from all the trees of an experimental forest located in Yautepec, Morelos, Mexico, during November-December 2016, and were natural dried in the open air, and stored in a sealed container at room temperature until their analysis. Two random seed samples (4 kg) were taken. One sample was subjected to oil extraction by pressing (7111.5 psi, T=80°C) for obtaining the cake.

Proximate analysis.

The milled samples (particle size of 100 μm) were treated following the AOAC official methodologies [8]: moisture (925.09B), crude fat (CF, 920.39), ash (942.05), crude protein (CP, 954.01), neutral detergent fiber and acid detergent fiber (NDF and ADF, 973.18), and dietary fiber (DF, 985.29). Carbohydrates (CHO) content was estimated by weight difference. All determinations were performed by triplicate.

Amino acid compositions.

The samples (seed and cake) were hydrolyzed in a reflux system at 100°C with hydrochloric solution (6 mol L^{-1}) containing 0.1% (w/v) phenol. Mixtures were kept for 16 h, cooled at room temperature, and then pH was adjusted at 6.8. Solutions were filtered through Whatman filter paper grade 1, and transferred to 50 mL volumetric flasks, norleucine was used as internal standard (2.5 mL, 5mmol L^{-1}) and fitted with Milli-Q water. Finally, 3 mL was filtered through a Waters 0.2 μm nylon acrodisc filter (Milford, MA, USA).

Derivatization was carried out using Waters AccQ-Fluor reagent kit™ (Milford, MA, USA), and following manufacturer´s methodology.

The amino acid separation by HPLC was performed on Agilent 1100 series, equipped with a diode array detection (DAD) system. An AccQ-Tag amino acids column (3.9 mm x 150 μmm i.d., 4 μm, Waters) was used for the analysis, and all separations were detected at 254 nm. The flow rate was 1 mL min^{-1}, the injection volume was 20 μL, and the column temperature was kept at 37°C.

Two mobile phases were used: phase A was a mixture of acetonitrile:10% AccQ-Tag Ultra® concentrate (93.5:6.5); and phase B was an acetonitrile:water mixture (60:40). The following

gradient elution was used: 0-0.5 min, 98% A-2% B; 15 min, 93% A-7% B; 19 min, 90% A-10% B; 32-35 min, 67% A-33% B; 36-41 min, 100% B.

Tryptophan determination was performed separately. Samples were hydrolyzed, neutralized and diluted in a sodium borate buffer solution (pH 9), following the methodology proposed by Yust et al. [9].

Quantification of minerals.

Samples were subjected to accelerated reaction by microwave system and diluted with deionized water. All solutions (including standards) were analyzed by ICP-OES. The content of the elements was calculated by interpolation in the calibration curve.

Fatty acids profile.

The lipid extraction and fatty acids (FAs) derivatization were performed following the Añorve-Morga et al. method [10]. FAs identification was performed by comparing retention times of a standard (FAME Mix C4-C24, Supelco®, USA). FAs quantification was performed interpolating from a standard curve.

Antioxidant activity.

The extraction process of antioxidant compounds, and all determinations of this section (except ABTS assay) were performed according to Ramírez-Godínez et al. [11].

ABTS assay was performed following the methodology described previously [7] with modifications. ABTS reagent was dissolved in water (7 $\mu molL^{-1}$). $ABTS^{\bullet+}$ radical was produced by reacting a stock solution with $K_2S_2O_8$ (2.45 $\mu molL^{-1}$) and kept under darkness condition at room temperature for 12-16 h before use. The $ABTS^{\bullet+}$ solution was diluted with

water until an absorbance of 0.7-0.8 at 734 nm was obtained. For the test, 3 mL of the diluted $ABTS^{\bullet+}$ solution was added to 30 µL of AE, solution was kept a rest for 6 min and then, the absorbance was measured at 734 nm. The results were expressed as mg equivalents of ascorbic acid/100 g of sample.

Analysis of toxic and secondary compounds.

PE were analyzed as toxic components, whereas, PA, TI, lectins (Lec), hemolytic saponins (HS), cyanogenic glycosides (CG), and tannins (Tan) were determined as secondary compounds. PE were extracted by Saetae and Suntornsuk methodology [12] and were analyzed by HPLC according to Haas and Mittelbach with modifications [13]. A C18 Nova-Pak column (150x3.9 mm; 4 µm, Waters, USA) with column guard SB-C18 (12.5x4.6 mm, 5 µm; Agilent, USA) was used. The column temperature was controlled at 25°C. Mobile phase was a mixture of acetonitrile:water (80:20 v/v) at flow rate of 1 $mLmin^{-1}$. The detection was at 254 nm. The phorbol 12-myristate-13-acetate (PMA) was used as an external standard. PA was determined by a colorimetric method described by Martínez-Herrera et al [4]. The results were expressed in percentage of phytic acid equivalent (w/w). The TI activity was determined by a modification of a method reported previously [14].

Hemagglutinating activity of Lec was determined by the method of serial dilutions using human positive O-type erythrocytes [15]. The determination of soluble protein in the sample was also performed by the Lowry method.

The activity of HS was performed by the method of serial dilutions using washed and trypsinized erythrocytes. The endpoint was determined by a visual estimate of the red blood cells hemolysis [16]. Saponin from Quillaja bark extract was used as an external standard.

CG were determined by the qualitative picrate paper strip test of Guignard and the Gettler-Goldbaum semi-quantitative method [15]. Tan analysis was performed by the vanillin-HCl method modified by Chavan et al. [16] using (+)-catechin as standard.

Statistical analysis.

All measurements were done by triplicate and expressed as means±standard deviation. The results were analyzed by analysis of variance (ANOVA) and a means comparison by a Tukey test using Statgraphics Centurion XVI.I software.

Results and discussion

Proximate composition.

The chemical composition of the cake and seeds is showed in Table 1. The dry matter (DM) content was about 93% with no significant difference between the samples (seeds and cake). High contents of CP were found in both samples; besides, there were significant differences between samples. The high protein content is very important, because proteins allow the formation of bioactive peptides. In fact, *in vitro* studies have reported that antioxidant peptides of different molecular weights can be produced by gastrointestinal enzymes action [18].

On the other hand, it is known that human protein malnutrition produces negative impacts on physical growth and neurological reflexes [19]; therefore, the consumption of protein rich ingredients avoids this detrimental effect produced by a low consumption of protein. So that, an alternative is the search for new protein sources that are profitable and that can be incorporated into the diets.

The fat content was higher in seeds than in the cake. The fat content was like that reported in the cake of a toxic variety [12]; however, other authors have determined lower fat values in cake from 1.5% [5] to 8% [20]. Higher fat values in the cake can be attributed to an inefficient oil extraction process.

The seeds and cake showed a high content of DF, NDF and ADF. DF is beneficial for human health, because its consumption is associated with risk reduction of some chronic diseases [21]. These results obtained in DF, NDF and ADF were similar with the results reported for other varieties of *Jatropha curcas* [5,6,11]. These components are other factors that can be an excellent option to increase the consumption of these components in the diet. Ash and total CHO contents were slightly higher for seeds than cake. The proximate composition results were like reported previously for other varieties of *Jatropha curcas* [4,5].

Amino acid compositions

The amino acid composition of the seed and cake are showed in Table 2. The seed and cake of Jatropha curcas presents a wide variety of essential, conditional and non-essential amino acids. The major amino acids were histidine, glutamine and tyrosine in both samples (seed and cake). In the seed, values of some amino acids (lysine, glutamine and, alanine) were similar with other reports of different varieties in Thailand, Cape Verde, Nicaragua and Mexico [22,23]. However, in Mexican varieties it is the first report in which tryptophan was detected [4]. These results showed that the amino acid composition of both samples were affected by variety, agronomic and climatic conditions.

The essential amino acids of the seed and cake, except valine, leucine, phenylalanine and, lysine, were higher than that reported by Makkar et al. [22] and Martínez-Herrera et al. [4]

in seed and cake, respectively (Table 2). Another hand, the seed of *Jatropha curcass L. var. Sevangel* has a higher concentration of methionine, isoleucine, histidine, threonine and, tryptophan compared to quinoa (*Chenopodium quinoa*), recognized by a spectrum of balanced amino acids [24].

In addition, the asparagine values were lower compared to other varieties of *Jatropha curcas*, which contributes to decrease the synthesis of acrylamide (carcinogenic compound) by Maillard reaction [25].

Mineral composition.

Table 3 shows the mineral composition of the seeds and cake. Calcium was the most abundant element in both samples. *Jatropha* can be considered as an important source of minerals (Ca, Cu, P, Fe, K, Mg, Mn, Na, and Zn), especially Ca. However, it is necessary to carry out *in vitro* assay to evaluate the bioavailability of all these minerals. Al, Ba and Ni were determined to ensure that their contents do not cause any damage to the consumer. According with the results, these are lower than necessary amount to generate observable adverse effects in humans [26].

Fatty acid profile.

FAs profile of seed and cake of *Jatropha curcas L. var. Sevangel* is presented in Table 4. Lauric (C12:0), myristoleic (C14:1), palmitic (C16:0), stearic (C18:0), linoleic (C18:2n6c), and linolenic (C18:3n3) acids were identified in seeds; while, C16:0, oleic (C18:1n9c), C18:2n6c, and C18:3n3 were found in cake.

The main FA detected in seeds was C16:0, which caused that content of saturated fatty acids (SFA) be higher in the seeds than in the cake. SFA detected in seeds (C12:0, C16:0, C18:0)

are commonly found in many diets [10]. Despite the SFA can have negative effects on human health, other animals can use them as energy source; e.g., ruminants have presented greater digestibility of SFA when the chain length is shorter [27].

The FAs profile of the new *Jatropha* variety was different compared to previous report [4]; in fact, the type and content of the FAs was varied. The changes in nutrimental profile are caused by the variety and the soil characteristics (organic matter, minerals, pH, among others). Cake had low FA content; however, the main FA found in this sample were unsaturated fatty acids (UFA). World Health Organization (WHO) has recommended that the SFA is replaced by UFA consumption in the diet, due to the adverse effects on human health [28]; in this sense, the cake of *Jatropha curcas L. var. Sevangel* is a viable source of UFAs.

Total phenolic compounds and antioxidant activity.

Total phenolic content and antioxidant activity (FRAP, DPPH and ABTS assays) of the AE are presented in Table 5. Total phenols content was higher in the seeds than in the cake. The antioxidant activity by ABTS method was higher in the seeds than in the cake. Likewise, DPPH and FRAP results were no statistical difference between both samples ($P \geq 0.05$).

The antioxidant activity of the seeds and cake were lower than reported in other varieties of *Jatropha curcas* [29]. Phenolic compounds are important phytoconstituents that have significant potential against some diseases, due to their antioxidant activity. However, the results showed that total phenolic contents were not directly related with the antioxidant activity, and suggest that there are other compounds (tocopherols, vitamin C and carotenoids) that contribute in the antioxidant activity of the seeds and cake [30].

Toxic and secondary compounds.

Table 6 shows the content of some secondary compounds present in the seeds and cake. PE were not detected, indicating absence of toxicity in this variety. PEs are listed as toxic substances in animal feed of monogastric and ruminants by EFSA [3].

PA contents were higher in both samples and exceeded 2%, which are the limit of concentration to consider the PA as antinutrient, because forms complexes with some metals (Zn, Ca, Fe and Mg) and decreases the proteins digestibility [4]. However, the PA consumption has also been recognized by the benefits in human health [31], due to this compound and derivate have shown several functions: preventive for the formation of kidney stones, selective anti-inflammatory inhibitor, energy storage, vesicular regulator via binding to various proteins, and reducer of the risk of colon cancer [32].

PA content of other jatropha varieties has only reported in seeds, and the results found for seeds were within the percentages previously reported (7.07-12%) [4].

TI were found in both samples. TI affects the proteins digestibility; however, they can be inactivated by heating processes [4]. Also, these compounds have been reported to have inhibitory effects on carcinogenic cells of prostate and mammary gland [33]. The Lec and CG were not detected, and these results were agreed with other reports [12,34].

HS were found in both samples; however, these concentrations were lower in comparison to quinoa content (64 UH mg^{-1}) [35], which is a grain of habitual consumption. Saponins decrease the bioavailability of nutrients by reduction of the activity of digestive enzymes, such as trypsin and chymotrypsin [36]. Nevertheless, *in vitro* and *in vivo* studies have demonstrated beneficial effects of these compounds; moreover, some of their biological

activities reported are: anticarcinogenic, antimutagenic, hypoglycemic, hepatoprotective, immunomodulatory, neuroprotective, anticoagulant, anti-inflammatory and antioxidant [37]. The content of Tan in the cake is lower than those previous studies [38]. Tan are polyphenolic compounds that form insoluble complexes with proteins and essential nutrients, decreasing food intake, growth rates and food efficiency in animals [36]. However, in the last years, it has been found that tannins promote human health benefits as immunomodulatory, anticancer, antioxidant, anti-inflammatory, vasodilator, cardioprotective, antithrombotic and ultra violet protective [39].

The results obtained of secondary components were like data previously reported for other varieties of the plant [20].

Recently, dietary supplementation of goats during gestation and lactation was tested. No animal showed any signs of toxicity and was corroborated by liver toxicity tests (data not published).

Conclusions

The cake of *Jatropha curcas L. var. Sevangel* is a rich source of DF, protein (mainly methionine, isoleucine, histidine, threonine and, tryptophan), and Ca (components of great nutritional value). PE were not contained in this variety, which are the main indicative of the toxicity. Besides, other detrimental components were not found in the samples (heavy metals [Pb and Cd], Lec, and CG). PA, HS, and Tan were found in both samples; however, recent advances in the study of these components have demonstrated that they can have beneficial effects on human and/or animal health.

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Compliance with ethical standards

Conflict of interest. The authors declare that they have no conflict of interest.

Ethical approval. This article does not contain any studies with human or animal subjects.

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Table 1. Proximate composition in seed and cake of *Jatropha curcas L. var. Sevangel.* Results are expressed in dry basis as mean ± standard deviation.

Component	**Seed**	**Cake**	**Component**	**Seed**	**Cake**
DM, %	93.2±0.2	93.1±0.1	NDF, %	37.92±0.06	39.44±0.03
CP, %	18.03±1.61[a]	32.23±3.60[b]	ADF, %	29.88±0.40[a]	30.07±0.80[b]
Fat, %	29.24±0.30[a]	13.53±0.40[b]	DF, %	29.47±1.80[a]	33.47±0.10[b]
Ash, %	7.37±0.20	6.99±0.20	CHO	13.27	8.01

Different literal between samples indicate statistically significant difference ($P \leq 0.05$).

Table 2. Amino acid digestible composition in seed and cake of *Jatropha curcas L. var. Sevangel* (g 100^{-1} protein). Results are expressed in dry basis as mean ± standard deviation.

Amino acids	**Seed**	**Cake**	**Non-toxic seed**[c]	**Defatted seed**[d]	**Quinoa (Salta)**[e]
Essential					
Methionine	2.57±0.003[a]	0.63±0.002[b]	1.76	1.38 - 1.58	1.22
Valine	2.65±0.005[a]	3.22±0.06[b]	5.3	3.79 - 4.35	3.89
Isoleucine	5.24±0.01[a]	4.78±0.17[b]	4.85	3.08 - 3.93	3.23
Leucine	3.31±0.03[a]	2.92±0.05[b]	7.5	5.92 - 6.55	6.21
Phenylalanine	1.14±0.03[a]	1.61±0.03[b]	4.89	3.82 - 4.2	4.85
Histidine	20.74±0.20[a]	15.51±0.51[b]	3.08	2.65 - 2.89	3.46
Lysine	2.69±0.009[a]	3.23±0.05[b]	3.4	3.49 - 3.63	4.63
Threonine	7.36±0.04[a]	4.21±0.05[b]	3.59	3.15 - 3.33	3.28
Tryptophan	0.86±0.004[a]	0.37±0.005[b]	ND	ND	ND
Conditionally Essential					
Arginine	1.62±0.04[a]	5.12±0.26[b]	12.9	10.4 - 11.8	9.21
Cysteine	0.70±0.005[a]	0.36±0.004[b]	1.58	1.60 - 1.81	0.79
Glycine	1.68±0.003[a]	0.64±0.016[b]	4.61	4.16 - 4.4	5.77
Proline	8.10±0.04[a]	8.07±0.06[a]	3.8	3.86 - 4.21	3.76
Tyrosine	10.17±0.05[a]	19.21±0.09[b]	3.78	2.45 - 3.37	5.06
Non-essential					
Asparagine	7.33±0.05[a]	4.20±0.06[a]	9.92	11.4 - 12.2	8.72
Serine	7.36±0.04[a]	0.87±0.02[b]	4.82	4.59 - 4.91	4.29
Glutamine	13.09±0.05[a]	18.80±0.06[b]	15.91	14.7 - 16.7	16.88
Alanine	3.80±0.007[a]	3.88±0.04[a]	4.94	4.26 - 4.51	4.24

Different literal between samples indicate statistically significant difference ($P \leq 0.05$).

ND: No detected

[c] Makkar et al. (1998)

[d] Martínez-Herrera et al. (2006)

Table 3. Mineral composition in seed and cake of *Jatropha curcas L. var. Sevangel* (mg/100 g). Results are expressed in dry basis as mean ± standard deviation.

Element	Seed	Cake	Element	Seed	Cake
Al	0.99±0.02[a]	1.19±0.04[b]	K	246.6±1.4[a]	778.91±5.90[b]
Ba	0.50±0.05[a]	0.92±0.03[b]	Mg	139.38±0.80[a]	367.22±1.10[b]
Ca	974.60±2.43[a]	829.25±2.00[b]	Mn	1.10±0.10[a]	3.65±0.04[b]
Cu	35.27±1.10[a]	32.65±0.20[b]	Na	13.21±1.00[a]	8.45±1.30[b]
P	0.37±0.01[a]	0.52±0.03[b]	Ni	0.73±0.10[a]	0.91±0.04[b]
Fe	0.10±0.04[a]	1.7±0.3[b]	Zn	0.19±0.00[a]	1.80±0.01[b]

Different literal between samples indicate statistically significant difference (P≤0.05).

Table 4. Fatty acid profile in seed and cake of *Jatropha curcas L. var. Sevangel* (mg/100 g). Results are expressed in dry basis as mean ± standard deviation.

SFAs	Seed	Cake	PUFAs	Seed	Cake
C12:0	513.76±0.20	ND	C14:1	1157.19±1.53	ND
C16:0	13985.73±21.37[a]	800.70±15.81[b]	C18:1n9c	398.93±1.67[a]	149.48±1.01[b]
C18:0	27.82±0.61	ND	C18:2n6c	4306.77±14.37[a]	1018.11±1.01[b]
			C18:3n3	235.84±0.86[a]	61.56±0.90[b]
ΣSFA	14527.31	800.70	ΣUFA	6098.73	1229.15

Different literal between samples indicate statistically significant difference (P≤0.05). ND: not detected.

Table 5. Antioxidant capacity in seed and cake of *Jatropha curcas L. var. Sevangel*. Results are expressed in dry basis as mean ± standard deviation.

Sample	Total phenols	FRAP	DPPH	ABTS
Seed	1690.99±2.70[a]	440.56±8.10	317.61±1.90	101.81±3.30[a]
Cake	961.04±13.40[b]	441.18±4.00	312.39±1.90	76.62±3.30[b]

Total phenols mg gallic acid/100 g; FRAP mg Fe^{2+}/100 g; DPPH* mg Trolox 100 g; ABTS mg AA/100 g). Different literal rows indicate significant statistical difference (P≤0.05).

Table 6. Secondary compounds on seed and cake of *Jatropha curcas L. var. Sevangel*. Results are expressed in dry basis as mean ± standard deviation.

Component	**Seed**	**Cake**	**Component**	**Seed**	**Cake**
Tannins	10.39±0.10	11.94±0.40	Saponins	14.77±0.10[a]	10.39±0.10[b]
TI	24.03±0.01[a]	12.85±0.01[b]	Cyanogenic glycosides	ND	ND
Lectins	ND	ND	PE (mg/g)	ND	ND
PA	8.11±0.40[a]	5.09±0.30[b]			

Tannins mg EC/100 g; TI mg/g; lectins mg/50 µL protein; PA %; saponins UH/mg; cyanogenic glycosides mg HCN/100 g; PE mg/g. ND: not detected. Different literal columns indicate statistically significant difference ($P \leq 0.05$).

Capítulo III

Encapsulación de aceite de pescado con alginato y la liberación de EPA y DHA en un modelo de digestión rumino-intestinal *in vitro*

Capítulo IV

Rendimiento en el crecimiento, perfil de ácidos grasos y metabolitos sanguíneos de corderos suplementados con aceite de pescado encapsulado

Growth performance, fatty acids profile and blood metabolites of lambs supplemented with encapsulated-fish oil

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Lambs supplemented with fish oil

Title of the manuscript: Growth performance, fatty acids and blood metabolites of lambs supplemented with encapsulated-fish oil

ABSTRACT

Objective: This experiment was conducted to evaluate the productive performance, fatty acids profile and metabolites in blood of growing lambs fed a concentrated diet and supplemented with fish oil encapsulated in alginate beads.

Methods: Eighteen Suffolk×Pelibuey lambs of 5 months old and 30 kg of body weight were randomly assigned to one of three treatments: control= Concentrated diet (C) (Metabolizable energy [ME]= 2.78 Mcal/kg and, Crude Protein [CP]= 11.64%), unencapsulated fish oil= C + fish oil (3.75 g/kg LW) and, Encapsulated= C + encapsulated fish oil (18.75 g / kg LW of fish oil beads). Growth performance: Dry matter intake, daily weight gain, and feed efficiency for 25 days period was evaluated. Biochemical profile at day 25 (glucose, triglycerides, cholesterol, HDL-cholesterol and, LDL-cholesterol) as well as temporal changes on day 0, 7, 14 and 25 of fatty acids, glucose, triglycerides, cholesterol, HDL-cholesterol and, LDL-cholesterol were performed by means of analysis of variance (ANOVA).

Results: Growth performance parameters were not affected ($p \geq 0.05$) by fish oil addition. Glucose and triglycerides were decreased ($p \geq 0.05$) at day 25 in supplemented groups respect to control. HDL-cholesterol concentration was higher ($p \geq 0.05$) at 25 days in lambs supplemented with encapsulated fish oil. Supplemented unencapsulated fish oil in the diet increased the proportion of C20:5, C22:5 and C22:6 from the third week of supplementation

($p \leq 0.05$). In the same way, a decrease of C18:0 was observed in the supplemented groups ($p=0.001$).

Conclusion: Inclusion of low concentrations of fish oil modifies the serum concentrations of glucose, triglycerides, HDL as well as plasma fatty acid profile. However, not modifies the growth performance of supplemented lambs. The process of encapsulation showed a better efficiency for the transfer of C20: 5n3, C22: 5n3 and C22: 6n3 in blood plasma.

Keywords: Fish Oil; PUFA; Growth performance; Biochemical; Fatty acids; Sheep.

INTRODUCTION

In the sheep industry, one of the main objectives is to obtain high meat quality to satisfy market consumption [1]. Lambs feeding in intensive and semi-intensive production systems is based on corn, soybean, sorghum, canola, and other grains [2], which contribute with a low content of polyunsaturated fatty acids (PUFA) and increase of saturated fatty acids in meat [3]. However, lamb meat is characterized by its high content of omega-3 polyunsaturated fatty acids (PUFA n-3) with respect to omega-6 fatty acids in a ratio of 3:1 [4].

Currently, the nutritional recommendations are to increase the consumption of PUFA n-3 and a lower consumption of saturated fatty acids [5]. Lamb meat has fatty acids such as rumenic and vaccenic acids (C18: 2 *cis9 trans11* and C18: 1 *trans11*, respectively), which have beneficial effects on consumer health, but these fatty acids are biohydrogenated in the rumen from linoleic and linolenic acid [6].

In addition to rumenic and vaccenic acid, there are PUFA n-3 such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) that have beneficial effects on human health [7]. However, EPA and DHA are candidates for biohydrogenation, decreasing absorption in the small intestine and consequently on meat deposition [8]. Besides, fatty acid supplementation modifies ruminal microorganism populations, producing detrimental effects in ruminal fermentation [8] and, reduce feed intake [9]. For this reason, the encapsulation of these fatty acids can act as a bypass in the rumen and be included in the meat without causing detrimental effects. This form of nutritional supplementation is an easy, practical, safe and inexpensive way, resulting in a meat with a greater added value [10].

The fatty acid profile of the meat can be manipulated nutritionally so to reach values closer to the optimum in the human diet [9]. Fish oil is a very important source of EPA and DHA, that can be used in animal feed to increase PUFA in foods of animal origin. However, the oil concentrations supplemented in the animal diet must be controlled, to not transfer the sensory characteristics of the fish oil to the product generated [11]. Therefore, this study was carried out to investigate the effects of supplementation at low concentration of encapsulated fish oil on growth performance, fatty acids profile and metabolites in the blood of lambs.

MATERIALS AND METHODS

Animal care

The experimental protocol was approved by the "Internal Ethics Committee for the care and use of laboratory animals" of the Autonomous University of the State of Hidalgo, Mexico (PROY 060917), according to recommendations for the World Organisation for Animal Health (OIE) [12].

Localization

The study was carried out in an ovine farm located in Pachuca, Hidalgo State, Mexico. The climate of the region is temperate sub humid with rain in summer, a pluvial rainfall of 576 mm and an average annual temperature of 14.5 ° C [13].

In a previous study, the encapsulated release of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was determined using different concentrations of alginate in an *in vitro* ruminal digestion study. The results of this test simulated encapsulated EPA and DHA release in rumen, abomasum and small intestine of the sheep. The formulation used for

the preparation of the beads was 1% of sodium alginate (encapsulation matrix), 20% glucose syrup (stabilizer agent) and, 20% of fish oil [14]. The fatty acid composition of fish oil is presented in Table 2.

Animals and diets

Eighteen male lambs crossbreed Suffolk-Pelibuey of 5 months of age and an initial average weight of 30 $\pm$ 3.2 kg, managed in intensive system were used for the study. Animals were assigned randomly to one of three treatments (n = 6): C= Concentrated diet (C), O= C + unencapsulated fish oil and, E= C + encapsulated fish oil (Table 1). Diet was elaborated for growing lambs of 30 kg BW (70% of concentrate and 30% of forage) to obtain a daily weight gain of 250 g [15]. The experimental period of the animal performance test was carried out during 24 days with a period of adaptation to the experimental diet of 15 days.

At the beginning of the study, lambs were vitaminized (Vigantol ADE, Bayer; Mexico City, Mexico 0.5 mL / lamb, intramuscularly via) and dewormed (Ivomec F, Merial, Mexico City, Mexico) 0.2 mg/kg BW, subcutaneous via, and a second dose at 9 days later. Chemical analysis of diet was carried out following the methodology of the AOAC [16]: dry matter (AOAC 925.09B), crude fat (AOAC 920.39), ash (AOAC 942.05), crude protein (AOAC 954.01), neutral detergent fiber and acid detergent fiber (AOAC 973.18). All determinations were analyzed in triplicate. The chemical analysis and ingredients of the diet for the lambs are shown in Table 1. The feed was offered twice a day in equal parts (7:00 and 17:00 h) and the water was offered *ad libitum*.

Growth performance

Lambs were weighted weekly during the study. Initial and final weights of lambs were obtained when started and finishing the experimental stage. The daily weight gain was calculated based on the average of the gain obtained during three weighing of the lambs made during the experimental stage, performed every 7 days. Dry matter intake was considered as the difference between feed offered and feed rejected daily. Feed efficiency was calculated as the relationship between daily weight gain and daily dry matter intake.

Collection of blood samples

Blood samples were collected in all experimental lambs by jugular venipuncture before providing them with feed (17:00 h). The samples were taken on day 0, 12 and 21 in two vacutainer tubes (10 mL) (BD Vacutainer K2 EDTA) for fatty acid profile and without anticoagulant (BD Vacutainer Serum) for blood metabolites. After that, samples were placed immediately at 4°C for 10 min, then were centrifuged (Sigma, 2-16k, Germany) at 3500 g during 20 min. The plasma and serum were separated and conserved in Eppendorf tubes at -20°C until analysis.

Blood metabolites

Glucose (Glucosa LQ), triglycerides (Trigliceridos LQ), cholesterol (Colesterol LQ; oxidase-peroxidase), and HDL-cholesterol (HDL Colesterol D) concentrations in blood serum were determined by enzymatic methods, with specific kits acquired from Spinreact (Barcelona, Spain) according to the manufacturer's recommendations. Readings were measured by a UV light spectrophotometer (PowerWave HT microplate BioTek, USA) at

505 nm for glucose, triglycerides, cholesterol, and 550 nm for HDL-cholesterol. These evaluations were carried out on 0, 7, 14 and 25 days. Ten microliters of serum from each sample, standard and distilled water (blank) were taken and pipetted into 1 mL of reagent in a test tube (15 mL), respectively, and incubated for 10 min at 21°C. LDL cholesterol (mg/dL) was calculated using the equation of Friedewald [18].

$$LDL_{chol} = Cholesterol - (\frac{HDL_{chol} + Triglycerides}{5})$$

Fatty acids profile in plasma

All samples were subjected to the extraction and derivatization of fatty acids according to Añorve-Morga *et al.* [19] method with some modifications. Blood plasma (50 μL) was taken from each sample and 1 mL of 10% solution of sulfuric acid in methanol (v/v; JT Baker, USA) was added to perform the transesterification of the methyl esters of fatty acids. The mixture was stirred in a vortex (Labnet, USA) and maintained 10 min at 100°C in a water bath. The sample was cooled for 5 min, previously adding 1 mL of water saturated with hexane and, 1 mL of hexane (JT Baker, USA); the sample was then stirred in a vortex and was placed at -20°C. The supernatant was transferred using 1 mL V-Vials (Wheaton, USA) and, evaporated to dryness with nitrogen gas. The sample was adjusted at 0.5 mL with dichloromethane (JT Baker, USA) before injecting into the gas chromatography.

The analysis was performed by Auto System XL gas chromatography (Perkin-Elmer, USA), with a Supelco SP TM-2560 (75 m x 0.18 mm, 0.14 μm) capillary column with a

spitless mode injection volume (1 μL). Nitrogen was used as carrier gas at flow rate of 1 mL/min. Injector and detector temperatures were kept at 230 and 250°C, respectively. The following conditions were used during gradient: an initial temperature of 150°C, increasing 4°C/min to 214°C, holding for 2 min; then, it was increased 2.5°C/min to 244°C and finally, this was kept for 5 min.

Fatty acids identification was performed by comparing retention times of the standards (FAME Mix C4-C24, Supelco, USA; all-*cis*-7,10,13,16,19-Docosapentaenoic acid, Sigma, USA). Their quantification was performed interpolating from standard curves.

Statistical analysis

Data were analyzed in a completely random design with 6 replications per treatment for growth performance and, 3 replications per treatment for blood analysis. The growth performance variables and, serum metabolites concentrations (Triglycerides, HDL, LDL) were performed by single factor analysis of variance (ANOVA) and *post hoc* comparison by Tukey test using Statgraphics Centurion XVI.I statistical software (Statpoint Technologies Inc; VA, USA, 2009). For the changes in the biochemical profile and fatty acids variables an analysis of variance of repeated measurements and a *post hoc* with the Bonferroni test were performed. All tests were performed with a significance ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Growth performance

Data on dry matter intake, daily weight gain and feed efficiency are shown in Table 3. The results of current study showed that fish oil supplementation had no effect negative on growth performance ($p \geq 0.05$) among control group and the supplemented treatments. Unencapsulated and encapsulated fish oil no modified the growth performance of lamb during supplementation.

These results agree with those of Ferreira et al. [9] who used diets different degrees of inclusion of fish oil with soybean oil in diet lambs, concluding that animals supplemented with fish oil not modifies dry matter intake, average daily gain, feed efficiency and final body weight. In the case of beef cattle, Scollan et al [7] examined the effects of different sources of dietary PUFA n-3 on the performance and fatty acid composition of *longissimus thoracis* muscle and associated subcutaneous adipose tissue, where not found changes in the feed intake and growth rate, despite the implied modification to on rumen metabolism.

However, Parvar et al. [20] performed a supplementation with various sources of oils including fish oil (3%) for 84 days, observed that fish oil and its combinations with other kinds of oils decrease daily weight gain ($p \leq 0.05$). This weight loss can be attributed to high oil consumption that decreases digestibility, in addition, oils with high contents of polyunsaturated fatty acids increase cholecystokinin, decreasing ruminal movements and, consequently, food intake [20]. It is important to note that the concentrations, time of supplementation and the type of oil supplemented are important factors that can influence these parameters.

These results support that the inclusion of this concentration of fish oil used in the present study is not associated with detrimental effects in ruminal fermentation and, consequently, in the growth performance of supplemented lambs.

On the other hand, supplementation with unencapsulated fish oil and fish oil encapsulated increased the concentration of glucose in serum of lamb respect to control group ($p=0.002$) there was a linear effect ($p\leq0.05$) (Figure 1A). At the end of the experiment (day 25), the glucose concentrations in serum were decreased in supplemented groups respect to the control group ($p\leq0.001$) (Table 4). Consistent with our findings, previous studies showed that feeding oil increased serum glucose concentration via oil supplementation [21-22]. However, some studies reported that feedings oil had no effect on concentrations of serum glucose [20, 23]. The increase in serum glucose could be due to an increase in the concentrations of total fatty acids (mainly propionate) by oil supplementation [22], which leads to an increase in the synthesis of hepatic glucose in ruminants [24].

At the 7 days, a quadratic decrease ($p=0.008$) in the total serum cholesterol concentrations of the oil supplemented groups were observed with respect to in comparison with the control group (Figure 1B). There are other reports where cholesterol levels increase in dairy cows supplemented with vegetable oil [24]. Bianchi et al. [25] suggest that ruminants fed with fats have an increase in cholesterol in bloodstream and is related to an increase in the absorption of fatty acids.

Regarding to concentrations of triglycerides, HDL and LDL in serum, these were not affected ($p\geq0.05$) by the treatment (Figure 1C) and this was consistent with other reports [20-21]. However, at the end of the study, serum HDL concentrations were higher in the group supplemented with unencapsulated fish oil than control group ($p=0.02$), while serum LDL concentrations in the supplemented groups were lower than in the control group ($p=0.03$) (Table 4).

The composition of the major fatty acids in plasma is shown in Table 5. Due to individual differences and the different microbial consortium of each animal, different concentrations fatty acid composition was observed in plasma (coefficient of variation= 20.01%), despite this, fatty acids showed the same tendency. The inclusion of supplemented fish oil in the diet increased the proportion of C20:5, C22:5 and C22:6 from the third week of supplementation ($p \leq 0.05$), while a decrease of C18:0 was observed in the supplemented groups ($p=0.001$). In the case of C20:4n6, there was no change during the supplementation in evaluated groups ($p \geq 0.05$).

According to the results obtained, concentration of saturated fatty acids in blood plasma presented a decrease in supplemented groups (unencapsulated fish oil and encapsulated) (C18:0) ($p \leq 0.05$). The concentrations of C18: 0 decreased ($p \leq 0.05$) 900 and 400% at 14 and 21 days in the unencapsulated fish oil and encapsulated groups, respectively, compared to the start of supplementation. In control group, concentrations of C18: 0 showed no significant difference ($p \geq 0.05$) during the whole supplementation. In this sense, only control group presented concentrations of C20:0 in plasma, while were not detected in the supplemented groups. The results are relevant, since, the intake of products of animal origin rich in saturated fatty acids is recognized as a predisposing factor of cardiovascular diseases [26]. The biosynthesis of fatty acids in tissues is closely related to the composition of ingested lipids [27]. Therefore, the decrease in plasma concentrations of C18:0 indicates a possible reduction in the accumulation of saturated fatty acids in lamb meat, resulting in a product with a healthy lipid profile [27].

It is known that diets rich in monounsaturated fatty acids have shown an improvement in vascular function [26]. In this study, an increase ($p \leq 0.01$) in plasma concentrations of

C18:1n9 during fish oil supplementation was observed. In contrast, the plasma concentrations of C18:3n3 were lower ($p \leq 0.05$) in the unencapsulated fish oil group compared with control group. These changes may be due to an increase in the biohydrogenation of fatty acids present in fish oil during ruminal fermentation [8]. In the case of plasma concentrations of C16:0, C18:1n9, C18:2n6, and C20:4n6, there were no differences ($p \geq 0.05$) between the evaluated groups.

The concentrations of C20:5n3, C22:5n3 and C22:6n3 were higher in groups supplemented with unencapsulated fish oil and encapsulated with respect to control group ($p \leq 0.05$). The increase in PUFA n-3 concentrations, particularly EPA and DHA (C20:5n3 and C22:6n3, respectively) is associated with a low risk of developing cardiovascular diseases, blood pressure and cardiovascular function [26]. Therefore, these changes in the fatty acid profile of supplemented lambs may result in the improvement of the lipid composition of products of animal origin.

CONCLUSIONS

The inclusion of low concentrations of fish oil rich in n-3 PUFA (3.75 g/kg live weight) modifies the serum concentrations of glucose, triglycerides and, HDL as well as plasma fatty acid profile. However, it not alters the growth performance of supplemented lambs. The encapsulation process showed a better efficiency for the transfer of C20:5n3 (EPA), C22:5n3 (DPA) and C22:6n3 (DHA) on lamb meat.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Table 1. Ingredients and chemical composition of experimental diets.

Ingredient	Control (C)	Unencapsulated fish oil (UFO)	Encapsulated (E)
Fish oil (g/kg LW)	-	3.75	3.75
Oat hay (%)	25.00	25.00	25.00
Maize (%)	42.00	36.33	36.33
Soy bean (%)	26.00	27.53	27.53
Molasses (%)	5.00	5.00	5.00
Mineral premix (%)	2.00	2.00	2.00
Chemical composition (%)			
DM	91.48	91.36	91.59
CP	11.64	11.96	11.80
EE	1.48	1.48	1.47
Ash	6.07	6.18	5.98
NDF	35.41	37.04	36.23
ADF	22.43	21.48	21.95
TDN	76.99	78.07	77.54
DE (Mcal/kg DM)	3.39	3.44	3.41
ME (Mcal/kg DM)	2.78	2.82	2.80

*3.75 g of oil fish is equivalent to 18.75 g of fish oil beads.

DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; TDN: total digestible nutrients; DE: digestible energy; ME: metabolism energy. TDN, DE and, ME were calculated according to Alves et al. [17].

[a] TDN = 102.56 - (1.14 x ADF)

[b] DE = TDN x 0.044

[c] ME = DE x 0.82

Table 2. Fatty acids profile of fish oil used in the study (mg/100 g). Date are represented as mean ± standard deviation

Fatty acids	Fish oil	Control diet
C6:0	4683.64±16.40	ND
C8:0	3563.11±12.52	ND
C11:0	429.39±2.10	ND
C12:0	343.05±18.13	ND
C14:0	901.17±4.51	30.61±0.51
C15:1	3189.53±135.27	ND
C16:0	1359.74±15.46	3872.92±105.70
C17:0	ND	30.94±1.46
C17:1	ND	9.75±0.02
C18:0	1725.68±12.39	2557.31±21.21
C18:1n9c	4277.27±35.76	9653.17±319.64
C18:2n6c	1663.17±4.44	12530.20±16.01
C18:3n3	815.81±29.91	1677.83±15.87
C20:1n9	2371.80±64.63	94.35±2.37
C20:2	1125.99±14.74	ND
C20:3n3	5597.35±125.75	ND
C20:4n6	1634.91±9.08	ND
C20:5n3	1628.23±8.89	ND
C22:0	ND	213.96±1.99
C22:5 n3	493.63±17.58	ND
C22:6n3	1086.86±0.70	ND
C24:0	ND	62.52±0.58

ND: Not detected

Table 3. Growth performance of lambs supplemented with unencapsulated and encapsulated fish oil Date are represented as mean ± standard deviation.

Variable	Control	Unencapsulated oil	Encapsulated oil
IW (kg)	27.36±4.11	29.77±4.39	29.56±4.20
FW (kg)	33.29±4.48	36.47±4.76	34.78±4.21
DMI (kg)	1.52±0.35	1.55±0.20	1.57±0.17
DWG (kg)	0.26±0.07	0.29±0.08	0.23±0.05
FE	0.17±0.05	0.19±0.05	0.15±0.03

IW: initial weight; FW: final weight; DMI: dry matter intake; DWG: daily weight gain; FE: feed efficiency. Different literal in rows indicate differences ($P \leq 0.05$).

Table 4. Serum chemistry at 25 d in lambs of groups: control, unencapsulated and encapsulated fish oil. Date are represented as mean ± standard deviation.

Serum chemistry (mg/dL)	Control	Unencapsulated Oil	Encapsulated oil	P
Glucose	95.63 ± 2.23^{a}	72.01 ± 2.74^{bc}	76.18 ± 4.93^{bc}	≤ 0.001
Cholesterol	76.37 ± 4.97^{a}	92.50 ± 3.88^{ab}	93.31 ± 6.19^{bc}	0.05
Triglycerides	28.81 ± 0.62^{a}	14.51 ± 0.57^{b}	18.87 ± 1.10^{c}	≤ 0.03
HDL cholesterol	35.51 ± 1.98^{a}	44.97 ± 2.87^{ab}	52.06 ± 8.29^{b}	0.02
LDL cholesterol	32.12 ± 1.22^{a}	39.09 ± 4.19^{b}	37.99 ± 0.87^{ab}	0.03

HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

Different literal in rows indicate differences ($P \leq 0.05$).

Table 5. Major fatty acids profile (mg/dL) in blood plasma of lambs supplemented with unencapsulated and encapsulated fish oil. Date are represented as mean ± standard deviation.

Fatty acids	**Control**				**Unencapsulated oil**				**Encapsulated oil**			
Days	0	7	14	25	0	7	14	25	0	7	14	25
C16:0	21.6	22.2	20.9	16.6	20.4	20.1	14.5	16.9	28.8	22.4	13.9	13.6
C18:0	19.3^{a}	17.3^{a}	22.0^{a}	19.1^{a}	18.9 ^{ab}	12.5 ^{ab}	8.6^{b}	2.2^{b}	18.3^{ab}	6.4^{ab}	6.9^{ab}	4.1^{ab}
C18:1n9	0.9	0.9	0.6	1.0	1.1	2.7	4.7	5.3	1.6	2.1	3.8	4.0
C18:2n6	20.9	15.1	11.1	10.6	18.8	11.4	6.3	7.6	20.8	12.0	6.9	5.0
C20:0	23.4	22.1	20.1	21.3	ND	ND	ND	ND	ND	ND	ND	ND
C18:3n3	7.0^{a}	5.1^{a}	5.8^{a}	9.6^{a}	7.3^{a}	3.6^{a}	1.2^{a}	1.7^{b}	7.8^{a}	4.0^{a}	7.8^{a}	8.2^{a}
C20:4n6	3.2	3.0	3.3	3.4	3.3	6.8	4.0	5.1	2.0	3.5	1.3	2.5
C20:5n3	0.2^{ab}	0.2^{ab}	0.5^{ab}	0.8^{ab}	0.2^{ab}	0.3^{ab}	0.7^{ab}	2.2^{ab}	0.5^{ab}	2.3^{ab}	7.7^{bc}	11.2^{c}
C22:5n3	0.2^{ab}	0.3^{ab}	0.2^{ab}	0.1^{ab}	0.3^{ab}	4.6^{ab}	8.6^{ab}	13.0^{b}	0.3^{ab}	0.5^{ab}	4.5^{c}	18.1^{c}
C22:6n3	0.3^{ab}	0.5^{ab}	0.9^{ab}	1.0^{ab}	0.2^{ab}	0.9^{ab}	1.7^{ab}	3.3^{ab}	0.4^{ab}	0.7^{ab}	10.5^{ab}	11.2^{c}
∑ n-3	7.7	6.1	7.4	11.5	8.1	9.5	12.2	20.2	9.0	7.5	30.5	48.6
∑ n-6	24.0	18.2	14.4	14.0	22.0	18.2	10.3	12.7	22.8	15.5	8.2	7.5
SFA	64.3	61.6	63.0	56.9	39.4	32.5	23.1	19.1	47.1	28.8	20.8	17.6

∑ n-3: Sum of n-3 fatty acids; ∑ n-6: Sum of n-6 fatty acids; SFA: Saturated fatty acids.

Different literal in rows indicate differences (P≤0.05).

Coefficient of variation= 20.01%.

ND: Not detected.

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Fatty acids	**Control**				**Unencapsulated oil**				**Encapsulated oil**			
Days	0	7	14	25	0	7	14	25	0	7	14	25
C16:0	21.6	22.2	20.9	16.6	20.4	20.1	14.5	16.9	28.8	22.4	13.9	13.6
C18:0	19.3^{a}	17.3^{a}	22.0^{a}	19.1^{a}	18.9^{ab}	12.5^{ab}	8.6^{b}	2.2^{b}	18.3^{ab}	6.4^{ab}	6.9^{ab}	4.1^{ab}
C18:1n9	0.9	0.9	0.6	1.0	1.1	2.7	4.7	5.3	1.6	2.1	3.8	4.0
C18:2n6	20.9	15.1	11.1	10.6	18.8	11.4	6.3	7.6	20.8	12.0	6.9	5.0
C20:0	23.4	22.1	20.1	21.3	ND	ND	ND	ND	ND	ND	ND	ND
C18:3n3	7.0^{a}	5.1^{a}	5.8^{a}	9.6^{a}	7.3^{a}	3.6^{a}	1.2^{a}	1.7^{b}	7.8^{a}	4.0^{a}	7.8^{a}	8.2^{a}
C20:4n6	3.2	3.0	3.3	3.4	3.3	6.8	4.0	5.1	2.0	3.5	1.3	2.5
C20:5n3	0.2^{ab}	0.2^{ab}	0.5^{ab}	0.8^{ab}	0.2^{ab}	0.3^{ab}	0.7^{ab}	2.2^{ab}	0.5^{ab}	2.3^{ab}	7.7^{bc}	11.2^{c}
C22:5n3	0.2^{ab}	0.3^{ab}	0.2^{ab}	0.1^{ab}	0.3^{ab}	4.6^{ab}	8.6^{ab}	13.0^{b}	0.3^{ab}	0.5^{ab}	4.5^{c}	18.1^{c}
C22:6n3	0.3^{ab}	0.5^{ab}	0.9^{ab}	1.0^{ab}	0.2^{ab}	0.9^{ab}	1.7^{ab}	3.3^{ab}	0.4^{ab}	0.7^{ab}	10.5^{ab}	11.2^{c}
∑ n-3	7.7	6.1	7.4	11.5	8.1	9.5	12.2	20.2	9.0	7.5	30.5	48.6
∑ n-6	24.0	18.2	14.4	14.0	22.0	18.2	10.3	12.7	22.8	15.5	8.2	7.5
SFA	64.3	61.6	63.0	56.9	39.4	32.5	23.1	19.1	47.1	28.8	20.8	17.6

∑ n-3: Sum of n-3 fatty acids; ∑ n-6: Sum of n-6 fatty acids; SFA: Saturated fatty acids.

Different literal in rows indicate differences (P≤0.05).

Coefficient of variation= 20.01%.

ND: Not detected.

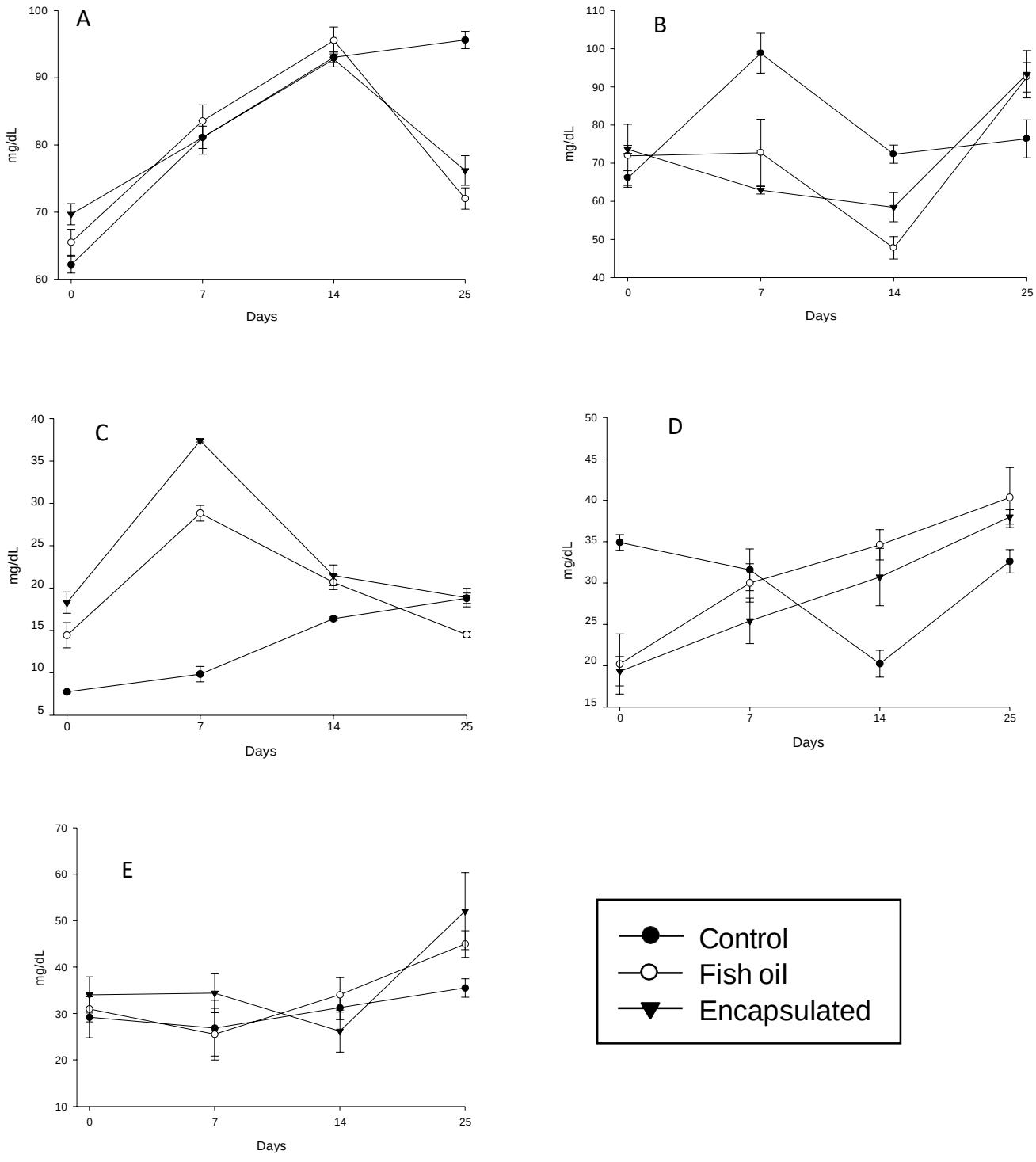

Figure 1. Temporal changes in serum metabolites in lambs fed without lipid supplementation (control) and, supplemented with 3.75 g/kg LW (fish oil unencapsulated or encapsulated fish oil). Values are the mean ± SEM. * Significant difference between diet groups ($P \leq 0.05$).

(A) Glucose (B)Cholesterol (C) Tryglicerides (D)LDL-chol and, (E)HDL-chol

Capítulo V

Características físico-químicas en la canal y en carne de cordero enriquecida con aceite de pescado encapsulado en la dieta

Physical-chemical characteristics of the carcass and lamb meat enriched with fish oil encapsulated in the diet

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Abstract

Objective: This study was conducted to evaluate the physical-chemical and mechanical properties of meat (*Longissimus dorsi, Triceps brachii,* and *Semimembranosus*), with emphasis on the profile of fatty acids, from lambs fed with EPA and DHA encapsulated with alginate.

Methods: Eighteen male lambs crossbreed Suffolk×Dorper (30.5 kg LW) were randomly assigned to one of three assigned groups (control (C), unencapsulated fish oil (FO) and, encapsulated fish oil (E)) and supplemented with 3.75 g / kg LW of fish oil for 25 days. At the slaughter, were evaluated morphometric measures of the carcass and, characteristics physic-chemical of the lamb meat (Color, pH, Capacity of retention of water, the texture profile analysis and Shear Force Testing, proximate analysis. The thiobarbituric acid reactive substances test was performed and fatty acids profile.

Results: The proportion of EPA, DPA and, DHA resulted higher in all muscles from the supplemented group than in the control group. Besides, the content of C18:0 and C16:0 decreased in E group compared to FO and control groups in the Ld, Sm and Tb muscles ($P \leq 0.05$). Chemical composition of the meat was modified in the three muscles evaluated by supplementation with fish oil. The intramuscular fat content was lower in the supplemented groups of the Ld and Tb muscles compared to the control group.

In general, fish oil supplementation does not interfere with pH, WHC and, postmortem examination of lamb carcasses. However, in color parameters the b* values in Ld decreased as a consequence of supplementation with fish oil.

Conclusion: Supplementation with fish oil encapsulated in the diet modifies the physical-chemical parameters of lamb meat, causing the meat to have a beneficial fatty acid profile and a higher protein content.

Keywords: Fish oil, Carcass, encapsulation, PUFA, EPA, DHA

1. Introduction

Meat is an important source of highly digestible protein, fatty acids, vitamin B12, iron and zinc, essential for the optimal development of the individual (Jiménez-Colmenero et al., 2012). However, high consumption of red meat has been related to the development of cardiovascular diseases and colon cancer, which resulted in a lack of consumer confidence (McAfee et al., 2010).

Like other red meats, lamb meat has a low profile of polyunsaturated fatty acids (PUFA) with respect to saturated fatty acids (SFA) (Komprda et al., 2012). The profile of fatty acids can be affected by breed, sex, age, diet and within the cut of meat. However, the main factor that affects fatty acids in meat are changes in the animal's diet (Díaz et al., 2011). For this reason, there has been an increase in the interest to develop nutritional strategies for the manipulation of the fatty acid profile to reduce SFA and increase PUFA. Lamb meat has PUFAs that are beneficial in health, such as rumenic and vaccenic fatty acids (C18:2 *cis9 trans11* and C18:1 *trans11*, respectively) (Tsiplakou and Zervas, 2008). In addition to these fatty acids, there are EPA and DHA that also have benefits in consumer health, however, they are only foundin considerable concentrations in cold water fish and some algae (Boeckaert *et al.*, 2007; Strobel *et al.*, 2012).

On the other hand, EPA and DHA are compounds highly susceptible to lipid oxidation, particularly in meat products, so many parameters must be considered before the addition of PUFA to food in order to produce stable products that are acceptable by the consumers (Tan *et al.*, 2009). In addition to this, the incorporation of PUFA into the lamb's diet is not a simple process, due to the hydrogenation of PUFA by ruminal microorganisms (Harfoot & Hazlewood, 1997). For this reason, the protection of these compounds supplied in the diet of

the lamb may be of interest in this context, in order to obtain a meat enriched directly in the diet (Olmedillo-Alonso *et al.,* 2013). This increase in the nutritional quality and nutritional value of the meat will lead to an increase in the consumption of lamb products (Pethick *et al.*, 2011).

The challenge that involves the incorporation of PUFAS n-3 into meat products consists of maintaining the technological and sensory properties of the product. The objective of the present study was to evaluate the physical-chemical and mechanical properties of meat (*Longissimus dorsi, Triceps brachii,* and *Semimembranosus*), with emphasis on the profile of fatty acids, from lambs fed with EPA and DHA encapsulated with alginate.

2. Materials and methods

The experimental protocol and lamb slaughter were approved by the "Internal Ethics Committee for the care and use of laboratory animals" of the Autonomous University of the State of Hidalgo (PROY-060917), according to recommendations for the World Organisation for Animal Health (OIE).

2.1 Formulation of beads

In a study previous, Olloqui et al. (2018) determinate the release of EPA and DHA from beads with different concentration of alginate in a process ruminal digestion simulated. This manuscript shows the results of release in rumen, abomasum and intestinal simulated digestion in sheep. The formulation used for elaboration of beads was 1% of alginate follows reported by Olloqui *et al.* (2018) with a 24G needle.

2.2 Animals and diets

Eighteen male lambs crossbreed Suffolk and Dorper were used in the experiment, with an average initial live weight of 30.5 kg. All animals were randomly assigned to one of three

assigned groups (control, oil and, encapsulated) for obtained 6 lambs per treatment. The treatments were: control (C) with a diet of 75:25 (concentrate y forage, respectively) and supplemented (FO) was also provided 3.75 g / kg LW of fish oil. The ingredients and nutritional composition of both diets are shown in Table 1. The two diets were formulated to be isoenergetic and isonitrogenous, in order to have similar fatty acids contents. All lambs were housed in pens for each treatment (8 m^2) with individual feeders and water *ad libitum* and were fed by 10 days with an adaptation diet. Experimental diets were offered for 25 days.

2.3 Lamb slaughter and morphometric measures

Lambs were then transported and slaughtered in a commercial abattoir according to standard commercial practices. the head was removed at the atlanto-occipital joint and fore and hind feet removed at the carpal and tarsal joints, respectively. The animals were partially skinned lying on their back on the floor. Thereafter, the animals were suspended by the hind legs for further skinning. Carcass and non-carcass components were weighed immediately after slaughter and carcasses were chilled at 4°C. Lungs, trachea and heart were weighed as one piece and designated as pluck. Non-carcass components included head, skin, feet, digestive tract, liver, spleen, pancreas and pluck. Weight of digestive contents was computed as the difference between full and empty digestive tract. The empty live weight (ELW) was computed as the difference between slaughter weight and weight of digesta content. Kidney, omental, pelvic and mesenteric fats were separated and weighed. Hot carcass weight was recorded with weight immediately after slaughter.

After a 24-h chilling period (4 °C) cold carcass weight was recorded and measured leg length, carcass length, thorax depth and, rump perimeter (Cañeque & Sañudo, 2005). Carcass

compactness index (kg cm^{-1}) was calculated as the ratio of cold carcass weight and length of the carcass (from cranial edge of the symphysis pelvis to the cranial edge of the first rib).

Muscles were obtained by manual dissectionated (*longissimus dorsi* (Ld), *triceps brachii* (Tb) *and semimembranous* (Sm)), then subcutaneous fat and epimysium were removal of from the muscles separated. The samples were placed in polyethylene bags and transported to laboratory at refrigeration temperature.

2.4 Characteristics physic-chemical

2.4.1 Color

Cut surface of each muscle was exposed to the air at ambient temperature for 30-40 min and color of meat was determination using Minolta Chroma meter (Models CR-300 and CR400) set on the L^*, a^* and b^* system. (where L^* relative lightness, a^* relative redness, and b^* relative yellowness) (Annon, 1998)

2.4.2 pH

The pH of the Ld, Tb and Sm were recorded at 24 h after slaughter using a potentiometer Hanna Instruments Hi 9023 calibrated at chiller temperatures (3-4 °C).

2.4.3 Water-Holding Capacity

The capacity of retention of water by measurement by the loss by compression by Torres, Cañeque and Sañudo, (2005) with some modifications. It was carried out taking 3 g of each muscle (Ld, Tb and Sm) and it was maintained in the refrigeration for 24 h after the sacrifice. The filter paper was folded in half, so that the sample is completely covered by the filter paper so that it absorbs completely in liberated water. Subsequently, each of the samples was

subjected to a pressure of 2.25 kg for 5 min. The result of the test was expressed as percentage of expelled water.

2.4.4 Texture Profile Analysis

The texture profile analysis (TPA) was performed on the meat samples (1 cm^3). The samples (five repetitions) were cut in parallel to the direction of the muscle fiber and TPA parameters, including hardness, cohesiveness and springiness were determined in a texturometer (Brookfield, CT3 Texture Analyzer, USA) according to the procedure suggest by Bourne (2002).

2.4.5 Shear Force Testing.

Frozen dice meat samples (Ld, Tb and Sm) were maintained for 24 h at 2 ° C. All samples were dissected and cooked in an electric oven at 20 ° C until the internal temperature of the meat reached 70 ° C, they are left to cool for 1 h and, each sample of each muscle is cut in the direction of the fibers, to obtain subsamples of 1 cm2 and 2 cm in length. The cutting force was using a Warner-Bratzler strategy, mounted on a texturometer (Brookfield, CT3 Texture Analyzer, USA). The texturometer was used with a compression load of 25 kg at a crosshead speed of 2 mm/s by up to 25 mm. The data was collected using software (). The measurements of the nuclei of each muscle are presented as the average of 6 subsamples of the maximum force required to cut the sections perpendicular to the axis in the direction of the muscle fiber (Francisco *et al.,* 2015).

2.4.6 T-BARS

Thiobarbituric acid reactive substances (TBARS) were determined using the method of Chirinos *et al.,* (2015). Sample absorbance was measured at 532 nm in a spectrophotometer

PowerWave XS (Biotek, USA). Results were expressed as mg malonaldehyde (MDA)/kg meat using a standard curve developed with 1,1-3,3 tetrametoxypropane.

2.4.7 Proximate analysis

The meat samples were treated following the AOAC official methodologies (AOAC, 2005): moisture (AOAC 925.09B), crude fat (AOAC 920.39), ash (AOAC 942.05), crude protein (AOAC 954.01). The carbohydrate content was estimated by difference. All determinations were performed by triplicate.

2.4.8 Chromatographic determination

All samples were subjected to the extraction and derivatization of EPA and DHA according to Añorve-Morga *et al.* (2015) method. The analysis was performed by gas chromatography, with a Supelco® SP TM-2560 (75mx0.18 mm, 0.14 μm) capillary column with a spitless mode injection volume (1 μL). Nitrogen was used as carrier gas at flow rate of 1 mL min^{-1}. Injector and detector temperatures were kept at 230 and 250°C, respectively. The following conditions were used during gradient: an initial temperature of 150°C, increasing 4°C min^{-1} to 214°C, holding for 2 min; then, it was increased 2.5°C min^{-1} to 244°C and finally, this was kept for 5 min.

All fatty acids identifications were performed by comparing retention times of a standard (FAME Mix C4-C24, Supelco®, USA). Their quantification was performed interpolating from standard curves.

2.5 Statistical analysis

All tests were performed in triplicate. Data were expressed as mean ± standard deviation. Statistical design was randomized. Evaluation was performed by one-tailed analysis of

variance (ANOVA) and a media comparison by Tukey test for a significant result (α= 0.05) using Statgraphics Centurion XVI.I statistical software.

3. Results and discussion

3.1 Carcass characteristics

Postmortem examination of lamb carcasses did not show any marked abnormality. The yields of different quantitative carcass characteristics are presented in Table 2 ($P \geq 0.05$). These data are consistent with the data obtained in breed of meat sheep F1 katahdin×dorper and in pure meat breeds (Vazquez-Soria *et al*., 2011; López-Velázquez *et al*., 2016). These parameters indicate that there is no difference between the quality of the channels with respect to supplementation.

3.2 Values of pH, WHC% and color parameters.

The effects of fish oil supplementation on pH, WHC (%) and meat color are summarized in Table 3. In general, fish oil supplementation does not interfere with pH. The pH at 24 h post mortem of three tested muscles ranged from 5.70-5.82 (Table 3), there was no significant difference ($P \geq 0.05$), indicating that animals were not stressed at the time of slaughter. Meat quality traits were within the range of commercially good quality meats in all groups. The pH range is acceptable in agreement with Yarali *et al*., (2014). In the same way, similar results were found for WHC ($P \geq 0.05$).

The instrumental color parameters lightness (L*), redness (a*) and yellowness (b*) were obtained in lamb meat samples: Ld, Sm and Tb. There was a significant decrease of b* values as a consequence of supplementation with fish oil in the samples Ld (C vs E) y Sm (C vs O and E) ($P \leq 0.05$). This was most likely due to the formation of metmyoglobin, giving rise to

a brownish color. These results differ from Ponnampalam *et al.*, (2016) by supplementation lambs with seaweed and flaxseed as a source of omega-3.

Higher b* values as a consequence of supplementation with fish oil have not been detected by another author (de la Fuente-Vazquez *et. al.*, 2014; Ponnampalam *et al.*, 2016).

3.3 Instrumental texture parameters

Obtained values for the different textural variables included in the TPA analysis and for shear force (SF) of lamb muscles (Ld, Sm and Tb) are shown in Table 4. Springiness, cohesiveness and chewiness were not significantly affected by supplementation with fish oil ($P \geq 0.05$). On the other hand, the Warner Bratzler shear force values were affected by each treatment in some cases, Ld (FO) was higher compared with Ld (C) and Ld (E) ($P \leq 0.05$). Furthermore, force values of Tb (E) was lower related with Tb (C) and Tb (FO) ($P \leq 0.05$).

3.4 T-BARS

The formation of oxidative substances determined from TBARS values in meat post slaughter are reported to range from 0.89-1.10 mg of MDA per kg of meat, no significant difference between groups of all muscles ($P \geq 0.05$). The lipid oxidation in muscles will be increased as the PUFA concentration in muscle systems increases (Ponnampalam *et al.*, 2016). Despite this increase in the concentrations of polyunsaturated fatty acids in meat, it did not show an increase in the concentrations of substances reactive to thiobarbituric acid.

3.5 Proximate analysis

Proximate composition was influenced by muscle source ($P \leq 0.05$). A greater ($P \leq 0.05$) dry matter (Ld and Sm) and lower ($P \leq 0.05$) ash (Ld and Tb) contents were observed in supplemented than in control group. These results agree with the data obtained by some

authors (de la Fuente-Vazquez et al., 2014; Jaworska et al., 2016), resulting in a change in the chemical composition of the meat obtained in the three muscles evaluated.

The intramuscular fat content was lower in the supplemented groups of the Ld and Tb muscles compared to the control group ($P \leq 0.05$). However, in Sm muscle and intramuscular fat content was higher in the group supplemented with encapsulated (E) compared to the control and direct supplementation (FO) groups ($P \leq 0.05$). This may be due by the effect of peroxisome proliferator-activated receptors system (PPAR), which involves in the regulation of the adipogenesis, energy balance, lipid biosynthesis, participates in fatty acid oxidation, mostly in skeletal and cardiac muscles (Grygiel-Górniak, 2014).

In general, in all muscles the protein content was higher in the groups supplemented with fish oil compared to the control group. This protein increase in ruminant muscles has not been reported previously and can be caused by PPAR system, because it plays an important role in glucose homeostasis (Grygiel-Górniak, 2014). Glucose is a major energy-substrate for glycolytic fibers and the precursor of glycogen in fibers and of fatty acids in intramuscular adipocytes, due to this it can be used in liver by increasing protein levels in the muscle (Hocquette *et al*., 2001).

3.6 Fatty acid composition

The fatty acid composition of Ld, Sm and, Tb muscles from each dietary treatment is presented in Table 6-10. The proportion of eicosapentaenoic acid (EPA, C22:6n-3) resulted higher in muscles from the supplemented group than in the control group, and the highest proportion of docosahexaenoic acid (DHA, C22:6n-3) was observed in Ld muscle from the E group, followed by the FO group ($P \leq 0.05$). Moreover, LD muscle from the E group had intermediate proportions of docosapentaenoic acid (DPA, C22:5n-3) with the highest in the

E group and the lowest in the C group ($P \leq 0.05$). Another hand, the content of C18:0 and C16:0 decreased in E group compared to FO and control groups in the Ld, Sm and Tb muscles ($P \leq 0.05$). These results are attributed to effect of the supplementation during the feed period, due to the fact the proportion of these fatty acids increased during this period.

These results might be possible as a consequence of a higher numerical content of the aforementioned fatty acids at the beginning of storage period in LD muscle from the E group resulting in a significantly lower n-6/n-3 ratio (Table 10) in the E group compared with meat from the C group. In fact, Muiño *et al.* (2014) reported a greater total PUFA in the muscle of Ld for fish oil, linseed and antioxidants supplemented lambs compared with muscle from animals that did not receive supplemental dietary. In contrast, the n-3 PUFA proportions showed stable values for all supplemented groups.

4. Conclusions

The proportion of EPA, DPA and, DHA resulted higher in all muscles from the supplemented group than in the control group. Besides, the content of C18:0 and C16:0 decreased in E group compared to FO and control groups in the Ld, Sm and Tb muscles ($P \leq 0.05$)

Chemical composition of the meat was modified in the three muscles evaluated by supplementation with fish oil. The intramuscular fat content was lower in the supplemented groups of the Ld and Tb muscles compared to the control group.

In general, fish oil supplementation does not interfere with pH, WHC and, postmortem examination of lamb carcasses. However, in color parameters the b* values in Ld decreased as a consequence of supplementation with fish oil.

Supplementation with fish oil encapsulated in the diet modifies the physical-chemical parameters of lamb meat, causing the meat to have a beneficial fatty acid profile and a higher protein content.

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Compliance with ethical standards

Conflict of interest. The authors declare that they have no conflict of interest.

Ethical approval. The experimental design was approved by the "Internal Ethics Committee for the care and use of laboratory animals" of the Autonomous University of the State of Hidalgo (PROY-060917), according to recommendations for the World Organisation for Animal Health (OIE).

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Table 1. Ingredients and chemical composition of experimental diets

Ingredient	**Control (C)**	**Fish Oil (FO)**	**Encapsulated (E)**
Fish oil (g/kg LW)	---	3.75	3.75
Oat hay (%)	25.00	25.00	25.00
Maize (%)	42.00	36.33	36.33
Soy bean (%)	26.00	27.53	27.53
Molasses (%)	5.00	5.00	5.00
Mineral premix (%)	2.00	2.00	2.00
Chemical composition (%)			
DM	91.48	91.36	91.59
CP	11.64	11.96	11.80
EE	1.48	1.48	1.47
Ash	6.07	6.18	5.98
NDF	35.41	37.04	36.23
ADF	22.43	21.48	21.95
TDN	76.99	78.07	77.54
DE (Mcal/kg DM)	3.39	3.44	3.41
ME (Mcal/kg DM)	2.78	2.82	2.80

*3.75 g of oil fish is equivalent to 18.75 g of fish oil beads.

DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; TDN: total digestible nutrients; DE: digestible energy; ME: metabolism energy. TDN, DE and, ME were calculated according to Alves et al. (2011).

[a] TDN = 102.56 - (1.14 x ADF)
[b] DE = TDN x 0.044
[c] ME = DE x 0.82

Table 2. Parameters and yields in the carcass of lambs supplemented with direct or encapsulated fish oil and the control group. Results expressed as mean ± standard deviation.

Variables	**Control (C)**	**Fish Oil (FO)**	**Encapsulated (E)**
Slaughter weight (kg)	35.50±2.29	34.93±4.30	33.78±4.15
Empty live weight (kg)	29.08±2.11	28.51±4.12	27.36±4.01
Hot carcass weight (kg)	16.79±0.64	15.89±1.26	15.49±1.74
Cold carcass weight (kg)	16.21±0.83	15.30±1.25	14.98±1.70
Yield hot carcass	47.34±1.23	45.69±2.68	45.91±2.04
Yield cold carcass	45.69±0.90	43.97±2.18	44.39±1.76
Biological yield of hot carcass	57.85±2.30	56.20±4.79	56.91±3.03
Biological yield of cold carcass	55.83±1.73	54.08±4.19	55.02±2.61
Carcass length (cm)	67.33±1.53	65.17±6.33	63.98±5.21
Long leg length (cm)	30.00±1.73	29.83±0.29	29.57±1.04
Pelvic width (cm)	18.67±1.53	24.00±1.00	23.15±1.43
Maximum width between ribs (cm)	20.67±1.26	21.17±1.61	20.38±1.28
Minimum width between ribs (cm)	15.83±0.29	16.50±0.50	16.10±0.70
Carcass compactness index	0.53±0.02	0.54±0.09	0.53±0.06

Table 3. Water holding capacity (compression) and pH in muscles: *Longissimus dorsi* (Ld), *Triceps brachi* (Tb) and, *Semimembramosus* (Sm) of lambs supplemented with direct or encapsulated fish oil and the control group. Results expressed as mean ± standard deviation.

	Control (C)			**Fish Oil (FO)**			**Encapsulated (E)**		
pH 24 h cold carcass									
Ld	5.82±0.08			5.73±0.09			5.70±0.04		
Sm	5.73±0.17			5.84±0.09			5.81±0.05		
Tb	5.76±0.14			5.71±0.31			5.86±0.05		
WHC (%)									
Ld	26.03±0.89			27.19±0.69			27.29±0.86		
Sm	25.47±0.82			26.51±0.44			25.91±0.71		
Tb	27.78±0.91			29.21±0.77			28.55±0.89		
Color parameters	*L**	*a**	*b**	*L**	*a**	*b**	*L**	*a**	*b**
Ld	42.83	26.67	18.72[a]	44.07	26.57	19.00[ab]	45.14	27.51	21.64[b]
Sm	43.18	25.46	22.70[a]	46.85	22.33	18.00[b]	41.91	24.44	18.03[b]
Tb	42.60	24.22	19.62	50.07	26.95	19.43	48.27	22.86	17.84

Different literal in rows indicate statistically significant difference (P≤0.05).

L*: lightness, a*: redness and, b*: yellowness

Table 4. Texture profile analysis (TPA) and shear force testing in muscles: *Longissimus dorsi* (Ld), *Triceps brachi* (Tb) and, *Semimembramosus* (Sm) of lambs supplemented with direct or encapsulated fish oil and, control group. Results expressed as mean ± standard deviation.

Muscle	***Hardness (N)***	***Cohesiveness***	***Springiness***	***Shear force (N)***
Ld (C)	1.52±0.58	0.63±0.10	0.66±0.11	38.12±0.97[a]
Ld (FO)	1.15±0.19	0.63±0.02	0.66±0.06	44.53±2.09[b]
Ld (E)	1.43±0.1	0.66±0.07	0.75±0.06	37.83±2.13[a]
Sm (C)	1.34±0.14	0.65±0.09	0.75±0.11	26.50±0.78
Sm (FO)	2.51±0.32	0.66±0.01	0.78±0.04	28.30±2.86
Sm (E)	1.90±0.91	0.60±0.06	0.71±0.06	27.28±0.43
Tb (C)	1.15±0.48	0.66±0.09	0.72±0.08	52.75±0.77[a]
Tb (FO)	1.02±0.16	0.72±0.09	0.78±0.02	51.68±3.65[a]
Tb (E)	1.24±0.36	0.70±0.09	0.74±0.07	42.98±1.84[b]

Different literal in columns in each muscle indicate statistically significant difference ($P \leq 0.05$).

Table 5. Proximate analysis in muscles: *Longissimus dorsi* (Ld), *Triceps brachi* (Tb) and, *Semimembramosus* (Sm) of lambs supplemented with direct or encapsulated fish oil and, control group. Results expressed as mean ± standard deviation.

	DM	**Ash**	**EE**	**%N**
Ld (C)	30.89±0.02[a]	0.50±0.07[a]	7.51±0.18[a]	8.38±0.55[a]
Ld (FO)	32.13±0.2[b]	1.19±0.03[b]	6.44±0.12[b]	10.09±0.67[a]
Ld (E)	32.58±0.42[b]	1.24±0.08[b]	6.20±0.14[b]	13.05±0.12[b]
Sm (C)	32.60±0.28[a]	1.23±0.08	7.37±0.21[a]	9.37±0.11[a]
Sm (FO)	33.03±1.03[b]	1.22±0.03	7.23±0.26[a]	12.05±0.10[b]
Sm (E)	30.93±0.26[b]	1.15±0.05	9.77±0.06[b]	11.49±0.11[c]
Tb (C)	31.49±1.00	1.61±0.09[a]	7.56±0.32[a]	9.48±0.57[a]
Tb (FO)	37.37±0.33	1.12±0.04[b]	5.09±0.05[b]	12.50±0.35[b]
Tb (E)	28.25±1.03	0.91±0.01[b]	6.09±0.10[c]	12.00±0.08[b]

Different literal in columns in each muscle indicate statistically significant difference ($P \leq 0.05$).

Table 6. Fatty acids composition and TBARS of meat from *Longissimus dorsi* muscle in lambs supplemented with fish oil direct (FO), encapsulated (E) and, control group (C). Results are expressed as mean ± standard deviation.

Fatty acids	C	FO	E	**Fatty acids**	C	FO	E
C8:0	10.86±0.12	ND	ND	**C18:1n9c**	ND	123.66±5.37	11.19±0.81
C10:0	16.78±0.71	7.99±0.01	ND	**C18:2n6t**	605.57±17.42	233.83±3.18	149.61±12.82
C11:0	4.54±0.37	ND	8.42±0.64	**C18:2n6c**	ND	872.15±15.70	633.79±24.12
C12:0	4.89±0.05	8.10±0.40	7.74±0.47	**C18:3n6**	ND	20.17±0.68	36.50±5.15
C13:0	6.02±0.34	ND	ND	**C20:0**	27.32±0.65	ND	ND
C14:0	0.62±0.01	0.84±0.01	0.57±0.01	**C20:1n9**	ND	1.63±0.09	104.41±20.90
C14:1	72.33±0.50	72.25±3.33	12.78±1.95	**C20:2**	ND	ND	5.66±0.22
C15:0	11.24±0.54	ND	5.22±0.48	**C20:3n3**	60.06±2.54	5.38±0.01	67.44±4.07
C15:1	4.41±0.18	16.86±0.82	90.92±7.48	**C20:5n3**	ND	2.73±0.26	3.17±0.14
C16:0	355.71±11.78	414.02±8.05	15.49±0.13	**C21**	ND	6.37±0.10	6.24±0.56
C16:1	3.44±0.04	ND	271.09±24.76	**C22:1n9**	ND	ND	6.71±0.79
C17:0	21.85±0.54	20.07±1.15	6.11±0.75	**C22:2n6**	ND	3.71±0.22	75.18±16.19
C17:1	11.71±0.85	ND	ND	**C22:5n3**	ND	ND	2368.01±151.35
C18:0	9.72±1.28	12.50±0.31	17.01±0.87	**C22:6n3**	ND	2.29±0.22	6.16±0.43
C18:1n9t	288.37±11.54	7.14±0.79	35.29±4.92	**TBARS**	0.89±0.01	0.96±0.05	1.01±0.03

Table 7. Fatty acids composition and TBARS of meat from *Semimembranosus* muscle in lambs supplemented with fish oil direct (FO), encapsulated (E) and, control group (C). Results are expressed as mean ± standard deviation.

Fatty acids	C	FO	E	**Fatty acids**	C	F	E
C8:0	20.23±0.42	ND	ND	**C18:2n6c**	35.91±0.65	362.45±63.44	574.66±35.86
C10:0	7.64±0.45	9.19±0.16	ND	**C18:3n3**	2.98±0.11	12.41±0.06	9.20±0.34
C12:0	9.48±0.54	7.80±0.22	4.85±0.50	**C18:3n6**	3.71±0.11	ND	ND
C13:0	3.73±0.08	ND	ND	**C20:0**	ND	565.50±11.62	ND
C14:0	ND	1.15±0.05	ND	**C20:1n9**	13.78±1.38	8.03±0.09	17.17±0.61
C14:1	66.56±2.99	ND	ND	**C20:2**	ND	ND	4.84±0.13
C15:0	12.77±0.85	ND	10.01±0.88	**C20:3n3**	ND	ND	26.45±1.10
C15:1	497.23±18.52	11.08±0.75	12.45±1.25	**C20:3n6**	ND	ND	7.13±0.77
C16:1	21.57±0.60	352.22±7.13	304.00±20.26	**C20:4n6**	ND	10.09±0.10	39.52±5.13
C17:0	10.21±0.43	ND	ND	**C21**	7.31±0.27	ND	ND
C17:1	8.71±0.59	ND	ND	**C22:2n6**	ND	73.74±7.23	ND
C18:0	145.74±9.94	22.20±2.70	7.79±0.22	**C22:5n3**	ND	12.40±3.28	73.58±13.90
C18:1n9t	678.45±45.95	21.06±1.35	9.51±0.72	**C22:6n3**	ND	2.38±0.11	3.65±0.23
C18:1n9c	ND	10.42±0.10	6.58±0.55	**TBARS**	1.10±0.06	1.09±0.02	1.02±0.02
C18:2n6t	ND	79.05±5.68	115.52±4.42				

Table 8. Fatty acids composition and TBARS of meat from *Triceps brachii* muscle in lambs supplemented with fish oil direct (FO), encapsulated (E) and, control group (C). Results are expressed as mean ± standard deviation.

Fatty acids	C	FO	E	**Fatty acids**	C	FO	E
C10:0	10.53±0.28	ND	6.53±0.56	**C18:2n6c**	455.06±21.39	652.88±55.43	ND
C11:0	7.56±0.36	12.12±0.06	3.68±0.38	**C18:3n3**	2.78±0.02	ND	ND
C12:0	5.38±0.01	7.03±0.35	6.19±0.36	**C18:3n6**	34.02±1.25	36.15±5.00	17.79±1.82
C13:0	ND	ND	4.24±0.30	**C20:0**	ND	222.64±15.21	ND
C14:0	0.79±0.01	0.92±0.02	1.43±0.08	**C20:1n9**	ND	19.76±0.90	22.82±1.63
C14:1	ND	ND	9.68±1.56	**C20:2**	4.60±0.04	11.28±1.32	ND
C15:0	48.26±0.26	62.02±5.79	32.40±3.00	**C20:3n3**	12.58±0.92	9.74±0.15	10.35±1.02
C15:1	12.16±0.19	12.98±0.86	18.77±0.94	**C20:3n6**	7.55±0.32	ND	12.64±0.80
C16:0	ND	356.87±5.28	ND	**C20:4n6**	37.89±3.06	19.38±1.80	30.79±3.27
C16:1	174.21±7.91	252.12±12.11	353.46±54.38	**C21**	13.88±0.03	ND	ND
C17:0	ND	14.61±0.47	3.35±0.29	**C22:5n3**	17.39±1.28	46.07±3.13	95.89±0.29
C18:0	20.89±2.07	14.51±0.45	7.14±1.19	**C22:6n3**	1.79±0.01	2.84±0.09	5.72±0.15
C18:1n9t	9.15±0.62	13.30±0.45	17.62±0.63	**C24:0**	10.35±0.68	ND	ND
C18:1n9c	13.53±0.45	240.89±19.49	8.52±0.51	**TBARS**	1.07±0.03	0.96±0.02	0.93±0.05
C18:2n6t	149.00±3.92	ND	14.17±2.65				

Table 9. Total fatty acids, saturated (SFA), monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids of meat from *Longissimus dorsi, Semimembranosus and, Triceps brachii* muscles in lambs supplemented with fish oil direct (FO), encapsulated (E) and, control group (C). Results are expressed as mean ± standard deviation.

∑ TFA	**Control (C)**	**Fish Oil (FO)**	**Encapsulated (E)**
Ld	1515.44	1831.69	3944.71
Sm	1555.51	1561.16	1226.93
Tb	1049.36	2008.08	743.31
∑ SFA			
Ld	469.55	469.89	66.8
Sm	226.62	605.82	22.65
Tb	117.65	690.70	64.94
∑ MUFA			
Ld	665.63	1140.26	3345.52
Sm	42.60	540.12	780.98
Tb	722.66	778.33	247.50
∑ PUFA			
Ld	665.63	1140.26	3345.52
Sm	42.60	540.12	780.98
Tb	722.66	778.33	247.50

Table 10. Sum of n-6 and n-3 fatty acids, PUFAs:SFA ratio and FA n-6:FA-3 ratio of meat from *Longissimus dorsi, Semimembranosus and, Triceps brachii* muscles in lambs supplemented with fish oil direct (FO), encapsulated (E) and, control group (C). Results are expressed as mean ± standard deviation.

∑ FA n-6	**Control (C)**	**Fish Oil (FO)**	**Encapsulated (E)**
Ld	605.57	1129.86	900.74
Sm	39.63	525.33	741.68
Tb	688.11	719.68	75.39
∑ FA n-3			
Ld	60.06	10.40	2444.78
Sm	2.98	14.79	39.30
Tb	34.55	58.65	172.11
PUFAs : SFA ratio			
Ld	1.42	2.43	50.08
Sm	0.19	0.89	34.47
Tb	6.14	1.13	3.81
FA n-6 : FA n-3			
Ld	10.08	108.64	0.37
Sm	13.32	35.53	18.87
Tb	19.92	12.27	0.44

Capítulo VI

Conclusiones generales

- La pasta de *Jatropha curcas L. var. Sevangel* es una fuente rica en fibra, aminoácidos esenciales (metionina, isoleucina, histidina, treonina y triptófano) y calcio, componentes de gran valor nutricional. También carece de ésteres de forbol en esta variedad, los cuales son el principal indicativo de toxicidad. Además, no se encontraron otros componentes perjudiciales en las muestras (plomo y cadmio, lectinas y glucósidos cianogénicos). Además, se encontraron ácido fítico, hemolíticas saponinas y taninos en ambas muestras; sin embargo, los avances recientes en el estudio de estos componentes han demostrado que pueden tener efectos beneficiosos en la salud humana y/o animal. Sin embargo, el principal ácido graso detectado en las semillas fue el C16:0, un ácido graso saturado con efectos negativos en la salud, pero puede ser una fuente de energía en rumiantes. En este sentido, la pasta de *Jatropha curcas L. var. Sevangel* es una fuente viable de ácidos grasos insaturados, pero no de ácidos grasos omega 3, como se plantea en el objetivo de este trabajo.

  Debido a esto, la búsqueda de fuentes ricas de EPA y DHA como el aceite de pescado sigue vigente. No obstante, la búsqueda de alternativas para transferir EPA y DHA y sean absorbidas en el intestino nos lleva a la necesidad de planificar un método de encapsulación que sea práctico, económico y se adapte a las condiciones fisiológicas de los rumiantes.

- Las características morfológicas de las cápsulas de alginato están relacionadas con la eficiencia de encapsulación y el patrón de liberación de EPA y DHA. El número de poros en la superficie de los encapsulados de 1.0% de alginato permiten una liberación satisfactoria de EPA y DHA durante la digestión posruminal. El potencial

de la encapsulación de EPA y DHA a través de alginato fue demostrado en este estudio mediante un modelo de digestión *in vitro*. Sin embargo, es necesario realizar investigaciones *in situ* o *in vivo* para probar el potencial tecnológico de mejorar la biodisponibilidad de la digestión de los lípidos encapsulados (PUFA) con alginato.

- La inclusión de bajas concentraciones (3.75 g/kg de peso vivo) de aceite de pescado rico en PUFA n-3 modifica las concentraciones séricas de glucosa, triglicéridos y HDL, así como el perfil de ácidos grasos en plasma. Además, el proceso de encapsulación mostró mayor eficiencia con la posibilidad de transferir EPA, DPA y DHA en la carne de cordero.
- La proporción de EPA, DPA y DHA resultaron mayores en todos los músculos de los animales suplementados con aceite de pescado. Además, el contenido de C18:0 y C16:0 disminuyó en todos los músculos del grupo de encapsulados comparado con los grupos control y sin encapsular.

  El contenido de grasa intramuscular fue menor en los grupos suplementados con aceite de pescado del músculo Ld y Tb comparado con el grupo testigo.

  De forma general, la suplementación realizada con aceite de pescado no interfiere en el pH, capacidad de retención de agua y la examinación post mortem de las canales de corderos.

  La suplementación con aceite de pescado encapsulado en las dietas modifica los parámetros fisicoquímicos de la carne de cordero, provocando un perfil de ácidos grasos benéfico y mayor contenido de proteína.

ANEXO

Constancias y Estancias

Research Article

Accepted Article

Encapsulation of fish oil into low-cost alginate beads and EPA-DHA release in a rumino-intestinal *in-vitro* digestion model†

EPA-DHA release in *in-vitro* ruminal digestion

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Certifica

que el trabajo:

CARNE DE CORDERO ENRIQUECIDA CON EPA Y DHA: CARACTERÍSTICAS LIPÍDICAS EN LOMO

José Enrique Javier Olloqui Pang, Aracelí Castañeda Ovando, Juan Carlos Ramírez Orejel, Esther Ramírez Moreno, Judith Jaimez Ordaz, Javier Añorve Morga

Ha sido presentado
en las actividades científicas del evento,
realizado en La Habana, Cuba,
del 15 al 17 de mayo del 2019

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UNIVERSIDAD DE BURGOS
DEPARTAMENTO DE BIOTECNOLOGÍA Y CIENCIA DE LOS ALIMENTOS
ÁREA DE TECNOLOGÍA DE LOS ALIMENTOS

Isabel Jaime Moreno, Directora del Área de Tecnología de los Alimentos, perteneciente al Departamento de Biotecnología y Ciencia de los Alimentos de la Universidad de Burgos, España.

A quien corresponda,

Por medio de la presente certifico que M.C. José Enrique Javier Olloqui Pang (CVU: 298335), estudiante del Doctorado en Ciencias de los Alimentos y Salud Humana impartido por Universidad Autónoma del Estado de Hidalgo, México, ha realizado una estancia de investigación durante los meses de septiembre, octubre y noviembre de 2018, bajo mi supervisión en Área de Tecnología de los Alimentos perteneciente al Departamento de Biotecnología y Ciencia de los Alimentos de la Universidad de Burgos.

Durante la estancia ha realizado trabajos de investigación con la finalidad de conocer la influencia de distintas dietas que difieren en el contenido de ácidos grasos omega-3 sobre el contenido de compuestos volátiles de muestras de carne provenientes de corderos sometidos a las dietas referidas.

Para ello se ha realizado la cuantificación de los compuestos volátiles de las muestras de carne mediante cromatografía de gases con detector de masas y se ha determinado el perfil olfativo global de las muestras mediante nariz electrónica.

La estancia se ha desarrollado con plena satisfacción y se han obtenido resultados muy interesantes.

A petición del interesado y los efectos oportunos, firmo en Burgos, España a 23 de noviembre de 2018.

Dra. Isabel Jaime Moreno

Facultad de Ciencias. Plz. Misael Bañuelos s/n. 09001 Burgos. España. Telf.: 947 25 88 14 Fax: 947 25 88 31 email: ijaimc@ubu.es

Universidad Autónoma del Estado de Hidalgo
Instituto de Ciencias de la Salud
Doctorado en Ciencias de los Alimentos y Salud Humana
Área Académica de Nutrición

OTORGA LA PRESENTE

CONSTANCIA

A: ENRIQUE JAVIER OLLOQUI PANG

Por su participación como **ASISTENTE del**
«CURSO DE CROMATOGRAFÍA»
Llevado a cabo del 6 al 10 de noviembre de 2017, con duración de 40 h en las instalaciones del Instituto de Ciencias de la Salud.

MC. Esp. Adrián Moya Escalera
Director del ICSa

M. en NH Arianna Omaña Covarrubias
Jefa del Área Académica de Nutrición

Dra. Esther Ramírez Moreno
Coordinadora Doctorado en
Ciencias de los Alimentos y Salud Humana

La Universidad Autónoma de Guadalajara

a través del

Decanato de Diseño, Ciencia y Tecnología

otorga el presente

Reconocimiento

a

Olloqui-Pang, E., Añorve-Morga, J., Castañeda-Ovando, A., Evangelista-Lozano, S., Alanís-García, E., Ramírez-Moreno, E., y Valadez-Vega, M.

Por la presentación en formato Oral del trabajo

CARACTERIZACIÓN QUÍMICA DE LA SEMILLA Y PASTA DE JATROPHA CURCA L. CAR. SEVANGEL PARA SU USO COMO POSIBLES INGREDIENTES FUNCIONALES EN LA DIETA.

durante el 6° Congreso Internacional de Biología, Química y Agronomía **"Ciencia e innovación para la Salud"**, llevado a cabo del 27 al 29 de septiembre.

Zapopan, Jalisco, México, 29 de septiembre de 2017.

MSC. Tomas Urmeias Salas

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A Quien Corresponda:

Por medio de la presente me permito informarle que el **M.C. José Enrique Javier Olloqui Pang**, alumno del Doctorado en Ciencias de los Alimentos y Salud Humana de la Universidad Autónoma del Estado de Hidalgo ha cubierto satisfactoriamente la estancia de investigación programada durante el periodo del 11 de julio al 19 de diciembre del 2016 en un horario de 8:00 a 15:00 horas.

El M.C. Olloqui Pang estuvo bajo supervisión general del que suscribe, Dr. David Hernández Sánchez y de la Dra. María Magdalena Crosby Galván, adscritos al Posgrado de Ganadería del Colegio de Posgraduados, Campus Montecillo, ubicado en Texcoco, Estado

UNIVERSIDAD AUTÓNOMA DEL ESTADO DE HIDALGO
CGSA- BIOTERIO
CIECUAL
DICTAMEN DE LA EVALUACIÓN DEL CIECUAL

INSTITUCIÓN: Área Académica de Química (ICBI)	FECHA SOLICITUD: 04/09/2017
PROYECTO : "Efecto de la carne de cordero enriquecida con ácidos grasos omega-3 en adultos con dislipidemias"	Responsable del Proyecto: Dr. Javier Añorve Morga

ASPECTOS A EVALUAR:	RESULTADO: El proyecto		
	ACEPTABLE	NO ACEPTABLE	SUGERENCIAS
JUSTIFICACIÓN DEL USO DE ANIMALES DE LABORATORIO	X		
BIENESTAR Y ESTRÉS ANIMAL	X		
PROCEDIMIENTOS Y CUIDADOS APLICADOS EN EL ANIMAL	X		
ASPECTOS ÉTICOS DEL PROTOCOLO	X		
PUNTO FINAL Y EUTANASIA	X		

OBSERVACIONES GENERALES:

El proyecto cumple con la NORMA Oficial Mexicana NOM-062-ZOO-1999, Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio. Y además, este proyecto se justifica el uso de los animales para experimentación científica por tener como fin mejorar la alimentación de los humanos.

FECHA DE REVISIÓN:	RESULTADO	SI	NO	OBSERVACIONES:
06/10/2017	APROBADO	X		Ver observaciones generales

INVESTIGADOR	PRESIDENTE DEL CIECUAL
Dr. Javier Añorve Morga	Dr. Héctor A. Ponce Monter.