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

EVALUACIÓN DEL POTENCIAL DE MORINGA (*Moringa oleifera*) COMO ALIMENTO FUNCIONAL Y SUSTITUTO DE ANTIBIÓTICO PROMOTOR DE CRECIMIENTO EN CODORNIZ JAPONESA (*Coturnix coturnix japonica*)

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"Que se eduque a los hijos del labrador

y del barrendero como a los del más rico hacendado"

Generalísimo Don José María Morelos y Pavón (1765 – 1815)

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“Casi todo lo que realice será *insignificante*,

pero es muy *importante* que lo haga”

Mahatma Gandhi (1869-1948)

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RESUMEN

Moringa oleifera es un árbol con muchos usos, y de gran importancia económica, que se encuentra en la mayor parte de los trópicos. Ha sido incorporada en la dieta para examinar sus efectos en conejos destetados, asimismo en alimento para tilapias para medir su desempeño productivo. Las variedades de *Moringa oleifera* producida en localidades de Culiacán, Sinaloa, ha demostrado contener componentes bioactivos antioxidantes y antimicrobianos, además de proveer una fuente viable de compuestos nutricionales. Algunas leguminosas y plantas tropicales se introdujeron en las dietas de aves de corral como fuentes de proteínas para disminuir el coste de la alimentación. Estudios recientes muestran que algunas hierbas, especias y extractos, pueden tener efecto antimicrobiano, coccidiostático, y antihelmíntico, que pudiesen reemplazar a los antibióticos promotores de crecimiento (APC).

Los sectores de la producción de aves de corral, en los países en desarrollo. se enfrentan a algunos problemas, uno de los cuales es el aumento en el costo del alimento, debido a los altos precios de las fuentes de proteínas y energía. Además, se enfrentan con el problema del desarrollo de patógenos resistentes a los antibióticos debido al uso imprudente y excesivo de APC. En la actualidad, un alimento ideal sería aquel que aporte con fuentes alternativas baratas, disponibles y seguras de proteínas y energía, qué a su vez, disminuyan el requerimiento de antibióticos (APC) y agentes químicos para el control microbiano. Comité Swann de la Unión Europea considera estos como innecesarios y que contribuyen a generar costos excedentes en la producción animal, denunciando que se posterga la salud humana en favor de los intereses económicos, volviéndose un problema de salud pública.

En este trabajo, se evaluó harina y extractos hoja de *Moringa oleifera* de dos variantes existentes en la localidad de Sinaloa; se determinó *in vitro,* su contenido nutrimental aplicando la metodología recomendada por la AOAC en sus diferentes apartados, encontrando valores de proteína de 31.69 a 36.83% (con perfil adecuado de aminoácidos esenciales limitantes como metionina, treonina y

lisina, entre 1 y 1.5 g AAS•100g^{-1}); minerales, destacando Calcio (13.37 - 16.78 g•Kg^{-1}) y Hierro (111.78 - 128.6 ppm); ácidos grasos esenciales (linolénico 62.72 - 66.19%, linoleico 7.64 - 9.65% y oleico 2.14 - 3.24%), así como compuestos fenólicos totales (71.08 - 76.63 mg EAGg^{-1}); su potencial antioxidante mediante la inhibición del radical DPPH (968.97-981 µmol ET•g^{-1}), ORAC (154.71 -182.31 µmolTEg^{-1}); el perfil de compuestos fenólicos por cromatografía UPLC-DAD (gálico 1.193-1.238 mg•g^{-1}, clorogénico 0.568 - 0.585 mg•g^{-1}, cafeíco 0.485 - 0.503 mg•g^{-1}, cumárico 1.090-1.114 mg•g^{-1} y ferúlico 0.518 - 0.536 mg•g^{-1}); y la actividad antimicrobiana (14.36-22.54 mm) mediante método de Kirby-Bauer (técnica de difusión de disco en Agar). Mediante una prueba *in vivo* del consumo de piensos suplementados con harina de hoja de *Moringa oleífera*, en codorniz japonesa, se determinó su potencial nutrimental y como sustituto de APC de *Moringa oleifera*, encontrándose niveles productivos similares (ganancia de peso 89.81 - 133.76 g•ave^{-1}, índice de conversión alimenticia 2.02 -4.63 g•g^{-1} y eficiencia alimenticia, 0.217-0.502 g•g^{-1}) a las dietas tradicionales (29.89% de maíz blanco y 65.80% de pasta de soya para la fase de iniciación y 43.89% de maíz blanco 51.74% de pasta de soya, que incluyen el uso de APC).

El estudio estableció que la harina de hojas de *Moringa oleífera,* son una alternativa viable para su inclusión hasta un 21% en dietas tradicionales de aves y que ofrece una opción para el reemplazo de APC, sin comprometer la salud del animal y por ende la productividad.

Palabras claves: nutrición, nutracéuticos, compuestos fenólicos, antioxidantes, antimicrobianos, APC

ABSTRACT

Moringa oleifera is a tree with many uses, and of great economic importance, found in most of the tropics. It has been incorporated into the diet to examine their effects on weaned rabbits also food for tilapia production to measure their performance. *Moringa oleifera* varieties produced in cities of Culiacan, Sinaloa, has been shown to contain antioxidants and antimicrobial bioactive compounds, as well as providing a viable source of nutritional compounds. Some legumes and tropical plants were introduced in the diets of poultry as protein sources to reduce the cost of food. Recent studies show that some herbs, spices and extracts, may have antimicrobial, coccidiostatic, and anthelmintic effect, which could replace antibiotic growth promoters (AGP).

Sectors of poultry production in developing countries. They face some problems, one of which is the rising cost of food due to high prices of sources of protein and energy. In addition, they faced with the problem of development of antibiotic - resistant due to the reckless and excessive use of AGP pathogens. Currently, an ideal food would be one that brings with cheap, available and safe alternative protein and energy sources, which in turn, reduce the requirement for antibiotics (AGP) and chemical agents for microbial control. Swann Committee of the European Union considers these as unnecessary and that contribute to cost overruns in animal production, denouncing human health in favor of economic interests is delayed, becoming a public health problem.

In this paper, flour and *Moringa oleifera* leaf extracts of two existing variants in the town of Sinaloa we were assessed; was determined in vitro, its nutritional content applying methodology recommended by the AOAC in its different sections, finding protein values of 31.69 to 36.83% (with suitable profile of essential amino acids limiting as methionine, threonine and lysine, between 1 and 1.5 gAAS •100g^{-1}); minerals, highlighting Calcium (13.37 - 16.78 g•kg$^{-1)}$ and Iron (111.78 - 128.6 ppm); essential fatty acids (linolenic 62.72 - 66.19%, linoleic 7.64 - 9.65%, and oleic 2.14 - 3.24%) and total phenolics (71.08 - 76.63 mg EAGg^{-1}); its antioxidant potential by inhibiting the radical DPPH (968.97 - 981 ET•gmol^{-1}), ORAC (154.71 - 182.31 µmolTEg $^{-1}$); the profile of phenolic compounds by chromatography UPLC-DAD (gallic 1.193-1.238 mg•g^{-1}, chlorogenic 0.568-0.585 mg•g^{-1}, caffeic 0.485-0.503 mg•g^{-1}, coumaric 1.090 - 1.114 mg•g $^{-1}$ and ferulic 0.518 - 0.536

mg•g^{-1}); and antimicrobial activity (14.36 - 22.54 mm) by Kirby-Bauer method (disk diffusion method in agar). Through an *in vivo* test consumption of feed supplemented with leaf meal *Moringa oleifera,* in Japanese quail, it determined its nutritional potential and as a substitute for AGP *Moringa oleifera,* finding similar production levels (weight gain 89.81 - 133.76 g•bird^{-1}, feed conversion ratio 2.02 - 4.63 g•g^{-1} and feed efficiency, 0.217 - 0.502 g•g^{-1}) to traditional diets (29.89% of white corn and 65.80% of soybean meal for the initiation phase 43.89% and 51.74% white corn soybean meal, including the use of AGP).

The study found that the leaf meal Moringa oleifera, are a viable alternative for inclusion up to 21% on traditional diets of birds and provides an option for replacing APC without compromising animal health and hence productivity.

Keywords: *Moringa oleifera*, nutraceuticals, phenolics, antioxidants, antimicrobials.

CAPÍTULO I: INTRODUCCIÓN GENERAL

Las hojas de *Moringa oleífera* poseen propiedades nutricionales y nutracéuticas (Makkar & Becker, 1996) ya que se caracterizan por su alto contenido de proteínas, vitaminas y minerales, y bajos niveles de sustancias antinutricionales, por lo que puede considerarse libre de dosis letales o efectos adversos; por ello, es utilizada tradicionalmente en Asia, África y Nicaragua en alimentación animal, ya que presenta una alta productividad de materia verde comparada con otros pastos, como la alfalfa, y los valores más elevados se alcanzan con una densidad de siembra de un millón de plantas por hectárea (Makkar & Becker, 1996; Fahey, 2005; Singh, 2009; Padilla *et al.*, 2014). En una investigación realizada en el Instituto de Producción Animal en los Trópicos y Subtrópicos (en Hohenheim, Alemania), se demostró que la composición de aminoácidos de las hojas de Moringa es comparable con la soya (Makkar & Becker, 1996). Adicionalmente, se ha evaluado la seguridad y eficacia nutricional de la hoja de Moringa en pollitos White-Leghorn de siete días hasta cinco semanas pudiendo sustituir hasta el 10% de inclusión de harina de hojas de Moringa en el pienso comercial, sin comprometer la ganancia de peso. Además, el grupo control tenía niveles significativamente más altos de colesterol; triglicéridos y ácido úrico. Infiriéndose que la harina de hojas de Moringa oleifera puede ser fuente de sustitución de soya y confiere un efecto nutracéutico en el modelo animal (Ashong y Brown, 2011). Por otro lado, el contenido de fenoles totales (105.04 mg EAG•g^{-1}) y su capacidad antioxidante (85.77%) de extractos metanólicos de hoja de Moringa demuestran sus propiedades antioxidantes (Singh *et al*., 2009). Diversos estudios de extractos acuoso, etanólicos, metanólicos y extractos de éter de petróleo de las hojas de *Moringa oleífera*, mostraron actividades antimicrobianas contra los organismos clínicos seleccionados incluyendo especies de *Staphylococcus aureus* y *Streptococcus*. El resultado de este estudio es esta actividad está relacionada con los compuestos fenólicos presentes (Ajayi & Fadeyi, 2015).

Los aditivos alimentarios como los antimicrobianos o antibióticos promotores del crecimiento (APC) desempeñan un rol esencial en el desarrollo económico de la producción avícola moderna, lo que se traduce en beneficio para los productores y consumidores de los productos de origen animal (Brizuela *et al*., 2009). Los aditivos se utilizan en la alimentación animal con tres fines fundamentales: mejorar las características fisicoquímicas de las materias primas y piensos o productos animales; prevenir enfermedades (principalmente Infecciones bacterianas y coccidiosis) y aumentar la eficiencia de producción de los animales con respecto a la conversión y eficiencia alimenticia. Sin embargo, debido al riesgo que presentan los APC de crear resistencia cruzada con los antibióticos utilizados en medicina humana y por la presencia de estos compuestos en los productos de origen animal, su uso se ha reducido drásticamente y prohibición en algunos casos para la formulación de piensos para la crianza animal (Gauthier *et al*., 2011).

No obstante, algunos investigadores han sugerido que la supresión de estas sustancias puede provocar un aumento en la incidencia de infecciones bacterianas (diarreas, coccidiosis, necrosis intestinal, entre otras) (Ramírez *et al*., 2013). Por lo que existe la necesidad de encontrar alternativas al uso de antibióticos (Gauthier *et al*., 2011). Entre estas alternativas, las más utilizadas son los probióticos, prebióticos, enzimas, aceites esenciales, hierbas, especias y extractos vegetales (Brizuela *et al*., 2009).

En este sentido, existen reportes de que los extractos de hojas de *Moringa oleifera* (Moringa) poseen actividad antimicrobiana sobre bacterias Gram positivas y Gram negativas.

Por lo anterior, el objetivo de este estudio es determinar el efecto del consumo de piensos suplementados con hojas de Moringa (*Moringa oleifera*) sobre el estado fisiológico de codorniz japonesa (*Coturnix coturnix japonica*), de acuerdo a sus características nutrimentales, nutracéuticas y antimicrobianas.

Los objetivos específicos de esta investigación, en primer término, fue la caracterización nutrimental, nutracéutica y antimicrobiana de dos variantes de

Moringa oleifera con el fin de determinar sus diferencias y potencialidades como fuente de antioxidantes, así como de proteína (aminoácidos), grasas (ácidos grasos poliinsaturados), cenizas (minerales) y fibra dietética. En segundo término, probar el efecto nutrimental, nutracéutico y antibiótico promotor de crecimiento (APC) de *Moringa oleifera* sobre el estado fisiológico de la codorniz japonesa (*Coturnix coturnix japonica*).

A continuación, se muestra una breve sinopsis de los capítulos que integran el presente proyecto de investigación.

El Capítulo I se presenta una introducción donde se describe la importancia nutricional de las hojas de *Moringa oleífera* y sus principales usos en algunas regiones del mundo, debido a su contenido principalmente de proteína. Enfatizando el potencial nutracéutico de los compuestos fenólicos contenidos en las hojas de *moringa oleifera*. Por otra parte, se destaca sus propiedades antimicrobianas debido estos compuestos, así como los diversos estudios donde se aprobado su seguridad y su eficacia en la nutrición en aves. También se aborda, los antibióticos promotores de crecimiento (APC), sus principales características e importancia en la industria avícola; así como, los principales motivadores del por qué están siendo prohibidos en su inicio por la Unión Europea y sus principales socios comerciales con la misma. Esto provoca que se busquen nuevas alternativas y/o sustitutos de APC, donde se destacan el uso de materiales vegetales, ya sea de alguna de sus partes o extractos de estas. En este sentido, se propone *Moringa oleifera* como solución completa, ya que no solo es una alternativa a los APC, sino además provee la parte nutrimental y nutracéutica en la alimentación de dicho sector.

En el Capítulo II, se plantea el problema de investigación, iniciando con una revisión de la literatura, respecto en primer término, al origen, taxonomía, clasificación del árbol *Moringa oleífera*, así como su composición e importancia nutricional y usos principales de sus hojas. También platea los beneficios nutrimentales que ofrece el uso de la harina de hojas de Moringa, enfatizando el

potencial nutracéutico de los compuestos fenólicos contenidos en los extractos metanólicos de estas harinas, así como de su actividad antimicrobiana. Finalmente, aborda la importancia actual con la definición y usos de los APC, así como sus repercusiones positivas y efectos adversos en la industria avícola y su prohibición por causa de estas últimas. En este sentido, en este capítulo se describe cómo nace la necesidad intrínseca de buscar alternativas naturales y viables para la sustitución de los APC, donde se plantea el uso de la harina de hojas de *Moringa oleifera*. Asimismo, se planea que Moringa no solo puede sustituir a los APC, sino que también puede ser una solución a usarla como fuente proteica y sustituir parcialmente el uso de la pasta de soya, debido a que su hoja de Moringa se caracteriza por tener un alto contenido de proteína de buena calidad, por contener los principales aminoácidos esenciales. Adicionalmente, compuestos como los ácidos grasos y los compuestos fenólicos pueden conferir un efecto nutracéutico al ser consumidos.

En Capítulo III, se refiere al primer artículo que consiste en el primer reporte formal de la caracterización nutricional y de compuestos fenólicos de las hojas de dos variantes de *Moringa oleifera* producidas en Sinaloa, una de vaina larga y otra de vaina corta; la primera parte consistió en determinar, en harina de hojas de Moringa, sus componentes nutricionales (contenido proximal, aminoácidos, minerales y ácidos grasos). La segunda parte en la determinación del contenido total de compuestos flavonoides y fenólicos libres, y su perfil de la fracción de fenólicos libres de extractos de hoja de *Moringa oleifera*, así como de su potencial antioxidante; el cual fue evaluado por dos métodos colorimétricos *in vitro*: inhibición del radical DPPH y ORAC. Esto con el fin de determinar si existe diferencia entre las dos variantes de las hojas de Moringa en cuanto a propiedades nutrimentales y nutracéuticas.
Este artículo fue aceptado en mayo de 2016, en la revista Pakistan Journal of Botany (Print ISSN 0556-3321, Online ISSN 2070-3368).

El Capítulo IV, se refiere al segundo artículo, donde se presenta la revisión de la literatura relacionada con alternativas naturales como antibióticos promotores de crecimiento (APC) en la producción animal, el uso de estos compuestos a nivel mundial; así como su propósito principal: producir carne de la mejor calidad, indistintamente de su origen (aves, cabras, vacas, cerdos, etc.), libres de residuos de fármacos, bacterias causantes de toxiinfecciones alimentarias y agentes contaminantes, garantizando al consumidor productos de alta calidad; sus efectos secundarios adversos como APC, cuya tendencia mundial es prohibir su uso, debido al riesgo de desarrollo de resistencia bacteriana, su transmisión vertical y horizontal, impactando en la salud humana, animal y su productividad. Por ello, en esta revisión, se plantea la necesidad de buscar nuevas alternativas de origen vegetal que sustituyan a los APC tales como, hierbas, especias, extractos vegetales y/o aceites esenciales que funjan como antimicrobianos y coadyuven a la nutrición animal.
Este artículo fue enviado el 24 de diciembre del 2015, a The Journal of Animal and Plant Sciences (ISSN: 1018-7081). Será publicado en JAPS 26 (5) 2016 October issue.

El Capítulo V. Se presenta el tercer artículo que contiene un estudio experimental *in vivo* enfocado a la inclusión de harina de hojas de Moringa (*Moringa oleifera*) en piensos para alimentación de codorniz japonesa (*Coturnix coturnix japonica*), como promotor de crecimiento. Como estrategia inicial se llevó a cabo una caracterización nutrimental y nutracéutica de los componentes principales de los piensos para alimentación de las aves: pasta de soya y maíz, así como a la harina y extractos de hoja de Moringa. Esto con la finalidad de conocer sus aportes principales sobre todo a los que se refiere a contenido de proteína, minerales, aminoácidos y ácidos grasos por la parte nutrimental. Así como, el contenido total de compuestos flavonoides y fenólicos, incluyendo el perfil de estos compuestos, la determinación de su capacidad antioxidante por los dos métodos colorimétricos *in vitro*: inhibición del radical DPPH y ORAC. Como parte central de este artículo fue la prueba *in vivo* de la sustitución de harina de Moringa por la pasta de soya,

para lo cual se desarrolló un diseño experimental, donde se emplearon codornices de 0 días de edad, sin sexar (hembras y machos). Donde se realizaron dietas con 0, 7, 14 y 21% de sustitución de harina de hoja de Moringa por pasta de soya, con y sin APC, con dos medidas repetidas en el tiempo (Periodo de Iniciación (1 a 14 d) y un periodo de engorde (15 a 35 d), 16 tratamientos en total, los cuales se replicaron cinco veces, tomando como unidad experimental una jaula con 12 codornices. Para las variables productivas se empleó un diseño con dos factores cruzados: APC y Moringa, mediante medidas repetidas en el tiempo. Para las variables nutracéuticas se realizó un diseño de un factor (dietas), totalmente al azar, tomando como unidad experimental, la codorniz seleccionada al azar de cada jaula para un total de cinco réplicas por tratamiento. Asimismo, se realizaron pruebas de química sanguínea y biometría hemática a fin de comprobar la no alteración de los parámetros fisiológicos del modelo animal por el consumo de hoja de *Moringa oleifera* y corroborar el efecto nutracéutico de la misma, en función de los niveles de ácido úrico, colesterol y triglicéridos.
Este artículo se planea enviar a Poultry Science (Print ISSN 0032-5791- Online ISSN 1525-3171).

Capítulo V. Finalmente, este capítulo expone los aportes de esta investigación y su relevancia en la nutrición en aves y por ende a la salud tanto animal como humana. Así como las perspectivas de la presente investigación.

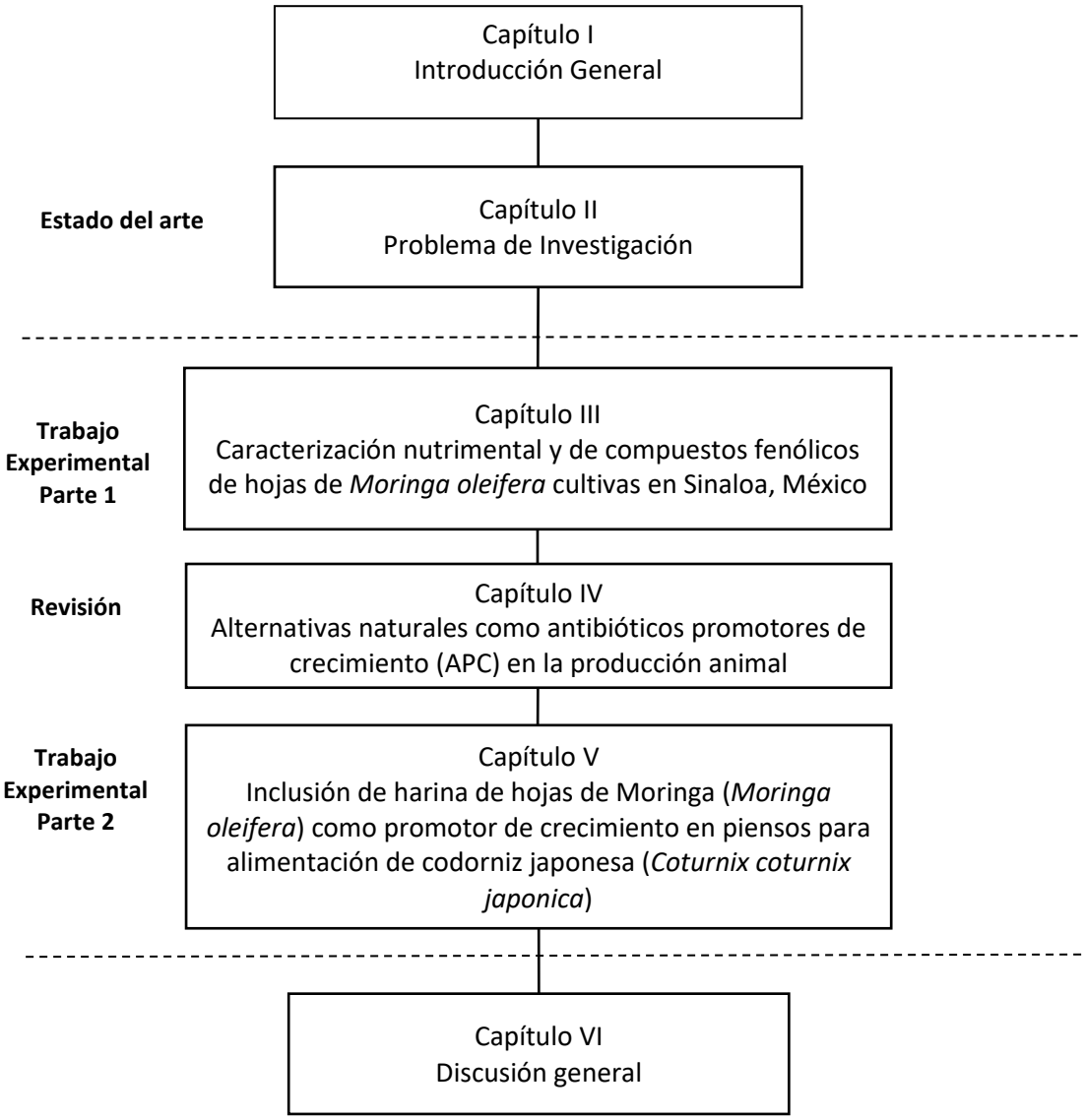

Figure 1. Esquema general que muestra la planificación seguida en la investigación, así como la estructura general de la tesis.

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CAPÍTULO II: PROBLEMA DE INVESTIGACIÓN

Revisión de la Literatura

Uso Potencial de *Moringa oleifera*

Moringa oleifera es la especie más conocida del género Moringa, un grupo pequeño de plantas dentro del orden *Brassicales* que incluye la familia de la col y del rábano, junto con la familia del mastuerzo y de las alcaparras (APG, 2009). La familia más cercanamente emparentada con *Moringaceae* es *Caricacea*e, la de la papaya, con la cual comparte la característica de presentar glándulas en el ápice del peciolo (Olson, 2002). *Moringaceae* comprende únicamente un género, Moringa.

Moringa cuenta con 13 especies: *arborea, concanensis, drocanensis, drouhardii, hildebrandtii, pygmeae, peregrina, ovalaifolia rospoliana, stenopetala, rivae, oleifera* y *borziana,* las cuales abarcan una gama muy diversa de hábitos o formas de crecimiento, desde hierbas y arbustos hasta árboles grandes (Adams, 1972; Olson & Razafimandimbison, 2000; Olson, 2001a y 2001b). Si bien su forma es muy variada, es fácil distinguir un miembro de Moringa de cualquier otra planta.

Es un árbol originario del sur del Himalaya, el nordeste de la India, Bangladesh, Afganistán y Pakistán. Se encuentra diseminado en una gran parte del planeta y en América Central; se conoce con diversos nombres comunes: Moringa, benzolivo, mlonge, mulangay, palillo, kelor, marango, resedá, nébéday, saijhan y sajna, entre otros.

En Sinaloa, Moringa existe de manera silvestre y como cultivos de baja tecnificación con posible potencial agronómico (Pérez *et al.*, 2010). Detectándose dos variantes: una variante corresponde a plantas que poseen frutos cuya

longitud oscilan entre 15 y 25 cm, conocida como Moringa de vaina corta (MVC) y otra cuyo fruto es de longitud entre 30 y 80 cm, denominada Moringa de vaina larga (MVL). Sin embargo, no existen estudios sobre Moringa de vaina corta; la gran mayoría, cuando describe el fruto de *Moringa oleifera,* se refiere a Moringa de vaina larga o bien, se da por sentado que se trata de esta variante. Falasca y Bernabé (2008; 2009) describen los frutos con longitudes de 20 a 45 cm de largo para la variedad hibrida de Moringa PKM-1 y vaina extra larga que pueden alcanzar alrededor de 125 cm de largo, de la variedad hibrida PKM-2, pero no menores a 20 cm. Existen otros autores que describen los frutos silvestres de Moringa usualmente, de 20 a 45 cm de largo (Parrota, 1993), sin que hasta el momento existan reportes de Moringa de vaina corta.

Indistintamente de la morfología del fruto de *Moringa oleifera*; la hoja, ya sea en fresco o deshidratada, se está constituyendo en un recurso de primer orden y bajo costo de producción, que ha demostrado riqueza nutritiva y nutracéutica (Fuglie 2001; Foidl et al., 2001; Fahey, 2005; Olson & Fahey 2011); su principal utilidad es como suplemento alimenticio. Además de sus nutrientes, posee propiedades antioxidantes, antimicrobianas, anti-inflamatorias, anti-envejecimiento y elementos que contribuyen al mejoramiento y prevención de problemas de la salud cardiovascular y salud endocrina; de reparación y sanación de tejidos, y de mejora de los procesos digestivos, entre otras (Fahey, 2005). Debido a esto, en los últimos años, ha crecido el interés por esta planta, a raíz de descubrimientos científicos que destacan sus propiedades nutricionales y medicinales.

También se ha descrito ampliamente su utilidad como producto no alimentario (por ejemplo, madera, carbón vegetal, aceite lubricante, clarificador de agua, etc.) (Foidl *et al*., 2001; Falasca y Bernabé, 2008; Muñoz *et al*., 2008). En muchos países asiáticos y africanos ha sido tradicionalmente usada como alimento humano y animal (Pérez *et al*., 2010). En este contexto, un árbol que ha recibido mucha atención en los últimos años (Fuglie, 2001; Fahey, 2005; Ferreira *et al*.,

2008) es *Moringa oleifera*. Este árbol tiene un gran potencial para su cultivo en México, así como en muchas partes de América tropical por su combinación singular de propiedades.

Una de las características más atractivas de la Moringa es el alto contenido de proteína en sus hojas, alcanzando un 28.7% (Teixeira *et al.*, 2014). Las investigaciones de Fuglie (2001) sobre casos en África occidental donde la adición de Moringa a la dieta rescató a personas en desnutrición extrema se han tomado como evidencia del extraordinario valor del contenido proteínico de la planta. En este sentido, sus beneficios nutricionales son tan ampliamente reconocidos que hay poco lugar para dudar del impacto positivo del consumo de harina de hoja de Moringa en situaciones de inanición inminente. Sin embargo, el desarrollo de un mayor número de pruebas clínicas bien controladas y documentadas con claridad sería de inmenso valor. Los análisis del contenido proteínico de las hojas secas muestran que hasta el 30% de su peso está formado por proteína (la leche en polvo contiene 35%) y que la mayor parte de ésta parece ser directamente asimilable. Además, las hojas contienen todos los aminoácidos esenciales (las unidades de las proteínas que el cuerpo no puede sintetizar) en un perfil alto y bien balanceado (Freiberger *et al*., 1998).

Por todo esto, es claro que la Moringa es un alimento importante, un hallazgo que ha sido comprobado de manera repetida (Richter *et al*., 2003). Muchas plantas muestran estructuras ricas en proteínas, por ejemplo, los frijoles. Sin embargo, mientras la mayoría de ellas, producen estas proteínas en sus frutos. La Moringa se destaca por contener las proteínas en sus hojas, las cuales están presentes en el árbol prácticamente todo el año (Alfaro, 2008).

Durante siglos, la gente en muchos países ha utilizado las hojas de Moringa como medicina tradicional para sus dolencias comunes. Los estudios clínicos han empezado a sugerir que al menos algunas de estas afirmaciones son válidas. Los beneficios que se pueden percibir en cuanto al tratamiento o la prevención

de enfermedades, en cuanto al tratamiento de infecciones a través de la aplicación de preparados de Moringa, no están tan bien entendidos como sus beneficios nutritivos (Palada, 1996).

Si bien existe una tradición extensa y los testimonios sobre sus beneficios médicos son voluminosos, estos beneficios han recibido relativamente poca investigación científica. Por lo tanto, se propone revisar algunos de los principales beneficios que se le han atribuido a la planta, la calidad y naturaleza de la evidencia disponible. Existen documentos recientes que equilibran la evidencia derivada de la medicina no convencional, tales como la medicina tradicional, el conocimiento tribal y testimonios personales, con las pruebas científicas, las cuales son necesarias para tomar decisiones sobre la eficacia de estas prácticas (Sampson, 2005; Talalay & Talalay, 2001).

Estudios preliminares de *Moringa oleifera* han encontrado que estimula el sistema inmune, actuando a través de la inmunidad celular y humoral en modelos experimentales de inmunidad. Sin embargo, a bajas dosis se encontró que era más eficaz que la dosis alta. Esto podría ser debido a la presencia de agente tóxico tal como isotiocianato y cianuros glicósido que pueden representar el estrés en una concentración elevada y, por tanto, reduciendo el potencial antioxidante de *Moringa oleifera* (Das *et al.*, 2012). De igual manera, existen reportes de uso de la hoja de Moringa para el tratamiento de diabetes mellitus, lo cual fortalece las teorías del potencial de Moringa como suplemento nutracéutico (Jaiswal *et al*., 2009).

A pesar de que la Moringa se caracteriza por su alto contenido de proteínas y vitaminas, contiene muy bajos niveles de sustancias antinutritivas (Makkar & Becker, 1997). Sin embargo, ese tipo de sustancias no se encuentran en dosis letales o que provoque efectos secundarios o adversos, es decir que se en encuentran en cantidades insignificantes (Olson & Fehey, 2011). Makkar & Becker (1996) mostraron que las hojas de Moringa contenían cantidades

despreciables de taninos; asimismo, sus análisis no arrojaron indicios ni de lectinas ni de inhibidores de tripsinas. Se encontraron saponinas, pero en cantidades bajas, equivalentes a los niveles registrados en los frijoles de soya, es decir, en niveles inocuos y no encontraron actividad hemolítica (Makkar & Becker, 1997; Gidamis *et al*., 2003).

Foidl *et al.*, 2011, estimaron el contenido de minerales de hoja de Moringa en base seca, reportando 175 ppm de Fe, 51.8 ppm de Mn, 13.7 ppm de Zn, 7.1 ppm Cu, 26.4 $g \bullet Kg^{-1}$ de Ca, 1.36 $g \bullet Kg^{-1}$ de P, 0.11 $g \bullet Kg^{-1}$de Mg, 2.37 $g \bullet Kg^{-1}$ de Na y 21.7 $g \bullet Kg^{-1}$ de K. Concluyeron que *Moringa oleifera* es una fuente biodisponible de hierro para paliar las deficiencias nutricionales en los trópicos. Por otro lado, Jideani & Diedericks, 2014, estudiaron las propiedades nutricionales, terapéuticas y profilácticas de extracto de la *Vigna subterranea y Moringa oleifera*. Determinaron que los ácidos: gálico, clorogénico, elágico, ferúlico, kaempferol, quercetina y vainillina estaban presentes en los extractos. Una ingesta diaria de esta planta podría inhibir significativamente el daño provocado por los radicales libres.

Además de sus atributos físicos y químicos, la calidad nutracéutica de la Moringa se vuelve importante al incrementarse los estudios donde se demuestra su potencial para algunos padecimientos crónico degenerativos. Dentro de los compuestos nutracéuticos presentes en Moringa se encuentran los compuestos fenólicos, tales como ácido benzoico, zeatina, quercetina, beta-sitosterol, ácido cafeoilquinico, kaempferol y principalmente bencil isocianato (Atawodi *et al*., 2010).

Compuestos Fenólicos

Los compuestos fenólicos o polifenoles provienen del metabolismo secundario de las plantas. Químicamente, son compuestos que tienen al menos un anillo aromático al que están unidos uno o más grupos hidroxilo. Existe una gran

variedad de compuestos fenólicos, y se clasifican en flavonoideos, formados por dos anillos aromáticos unidos por un heterociclo oxigenado y que dependiendo del grado de hidrogenación y de la sustitución del heterociclo son, flavonoles, flavonas, isoflavonas, antocianos, proantocianidinas, flavanonas, etc. (Figura 1) y se encuentran generalmente en forma de glicósidos, y los no flavonoideos, compuestos benzoicos y cinámicos, llamados comúnmente ácidos fenólicos, que contienen un anillo aromático con diferentes grupos funcionales, y que pueden estar formando ésteres con los ácidos orgánicos (Figura 2). Otros compuestos de naturaleza polifenólica son estilbenos, taninos, ligninas y lignanos. Algunas de las propiedades de los productos de origen vegetal, como color, astringencia y aroma son debidas a la presencia de compuestos de este tipo.

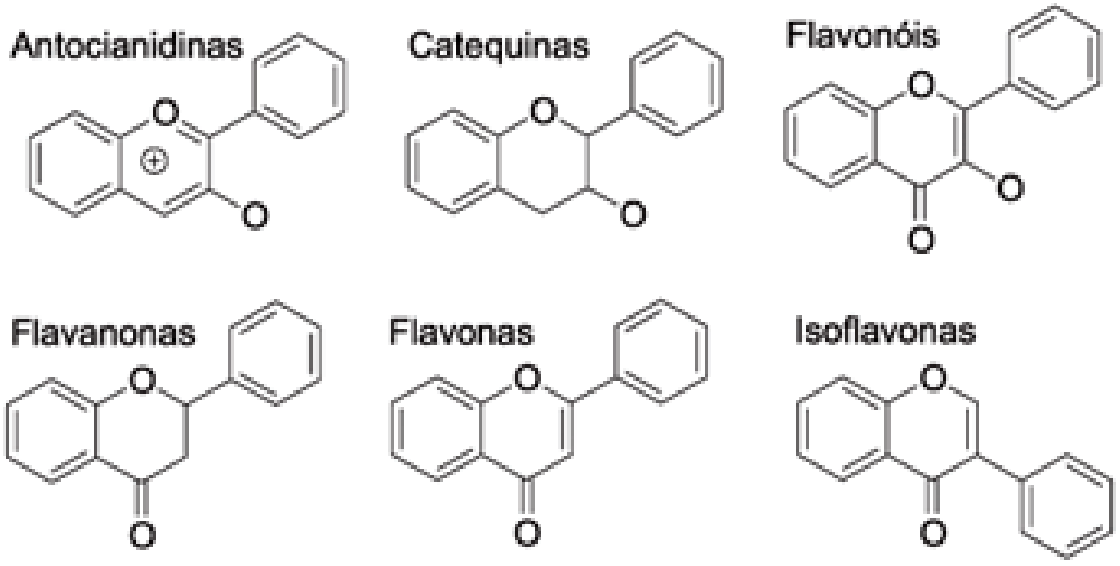

Figure 2. Estructura química de los principales compuestos flavonoides.

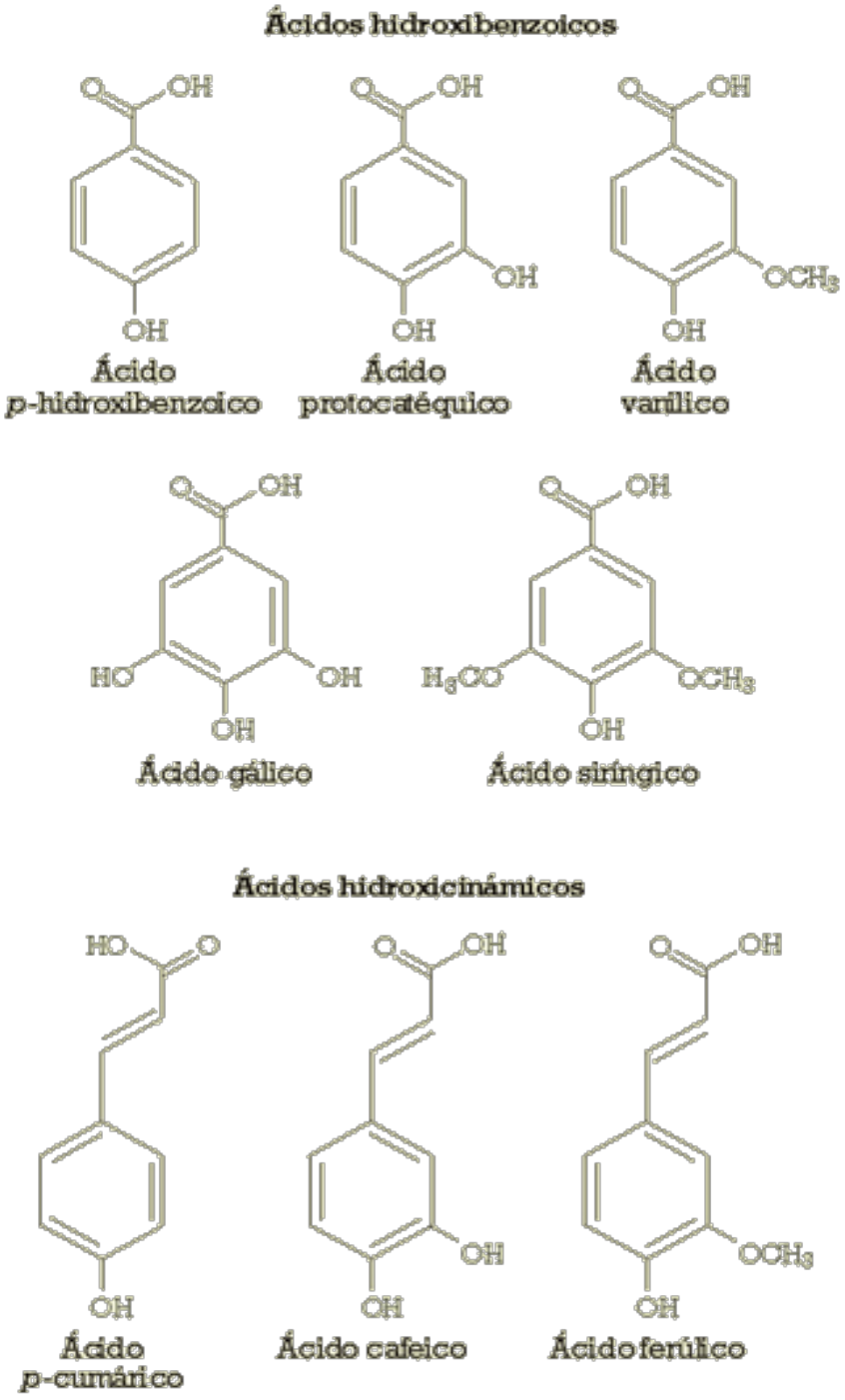

Figure 3. Estructura química de los principales ácidos fenólicos.

En los últimos años, los polifenoles han cobrado gran interés por sus propiedades benéficas para la salud, sobre todo, como agentes antioxidantes (Duran y Borja, 1993). Un antioxidante es un agente que presenta capacidad de donar electrones a agentes oxidantes o radicales libres. Los radicales libres, a su vez, son moléculas que poseen un electrón desapareado y que para encontrar estabilidad toman electrones de otras moléculas, tales como proteínas, lípidos o ADN, dando lugar al proceso deteriorativo denominado oxidación (Figura 3) (Rao & Agarwal, 2000).

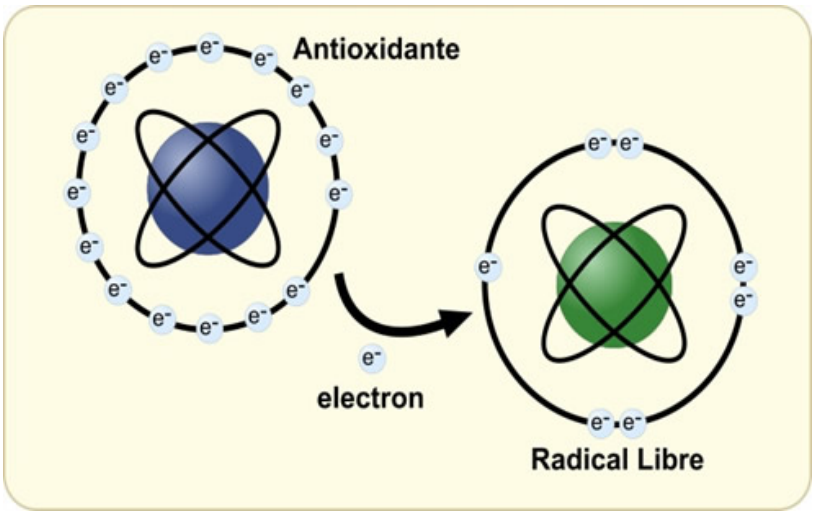

Figure 4. Acción de los antioxidantes. Los antioxidantes pueden detener la reacción en cadena dañina al organismo provocada por los radicales libres.

Se han de considerar dos conceptos de antioxidantes: por un lado, las sustancias que añadidas a los alimentos son capaces de preservar estos, retardando su deterioro, ranciedad o decoloración, debido a la oxidación; y por otro, los compuestos originalmente presentes en los alimentos y que, como consecuencia de sus propiedades antioxidantes, tienen efectos benéficos para la salud (Paladino, 2008).

La industria de los alimentos usa antioxidantes para prevenir el deterioro de la calidad de algunos productos, sobre todo los de alto contenido en grasas y

lípidos, y mantener así su valor nutritivo. Estos antioxidantes, principalmente de naturaleza fenólica, son la mayoría sintéticos, como terbutil-hidroxitolueno (BHT), terbutil-hidroxianisol (BHA), galato de propilo (PG), galato de dodecilo (DG) y terbutil-hidroquinona terciaria (TBHQ) (Duran y Borja, 1993).

En la actualidad, existe un mayor interés de la industria alimentaria por los antioxidantes naturales, que son componentes naturales de los alimentos de origen vegetal, principalmente polifenoles o compuestos fenólicos, que están de forma natural en los productos iniciales, o que se forman como consecuencia de su procesado. Los flavonoides y los ácidos fenólicos son los que reciben mayor atención como agentes potenciales antioxidantes, debido fundamentalmente a su amplia presencia en un alto número de alimentos de gran consumo (García *et al*., 2012).

La actividad antioxidante de los polifenoles se debe a su facilidad para reducir la producción de radicales libres, bien por inhibición de las enzimas que intervienen, o por quelación con los metales de transición, responsables de la generación de los radicales libres. Además, los flavonoides por su bajo potencial re-dox, son capaces de reducir las especies de oxígeno reactivo altamente oxidadas. En general los compuestos polifenólicos como antioxidantes, son multifuncionales y actúan según la mayoría de los mecanismos mencionados. Los polifenoles de tipo flavonoideo, como flavonoles, flavonas, isoflavonas, antocianos, flavanonas, catequinas y proantocianidinas, son los antioxidantes más potentes presentes en los alimentos vegetales (Verma *et al*., 2009).

Uno de los factores más importantes que determina la actividad antioxidante de los polifenoles es su grado de hidroxilación y la posición de los hidroxilos en la molécula. Los flavonoideos debido a su heterociclo oxigenado muestran mayor actividad que los no flavonoideos. A su vez, la solubilidad y los efectos estéricos de cada molécula pueden verse afectados por el tipo de estructura de dicha molécula, como es el caso de los derivados glicosilados y otros aductos, lo que

puede aumentar o disminuir la actividad antioxidante. Los compuestos flavonoideos se suelen encontrar en los vegetales en forma de glicósidos, pero la acción de enzimas o de algunos procesos puede liberar la correspondiente aglicona (Adisakwattana & Chanathong, 2011). La actividad de los ácidos fenólicos está también en función de los grupos hidroxilo del anillo aromático y de la unión de estos compuestos a ácidos orgánicos y/o a azúcares para formar ésteres. Los mecanismos por los que actúan todos estos compuestos varían dependiendo de su concentración y tipos de compuestos presentes en los alimentos (Zapata *et al*., 2007).

El estrés oxidativo y la peroxidación lipídica son los causantes de un gran número de enfermedades crónicas que incluyen: cáncer, enfermedades cardiovasculares, cataratas y demencia. Algunos estudios han demostrado que el consumo de frutas y hortalizas puede reducir la incidencia y mortalidad de estas enfermedades y, hasta donde se conoce, este efecto protector está determinado por la presencia de agentes antioxidantes en estos alimentos, principalmente polifenoles. Un antioxidante previene el daño oxidativo inhibiendo la generación de especies reactivas, capturando los radicales libres o aumentando el nivel de antioxidantes endógenos protectores (Sreelatha & Padma, 2009).

El hecho conocido como “Paradoja Francesa” reconoce la baja incidencia de episodios cardiovasculares entre la población francesa, a pesar de una dieta rica en grasas saturadas, lo que se atribuye a un consumo regular y moderado de vino tinto, que contiene una considerable concentración de compuestos fenólicos antioxidantes (Paladino, 2008).

En relación a la naturaleza de los antioxidantes, existen diversos factores que influyen en la concentración de estos; la composición de la hoja, así como la producción de una variedad de compuestos antioxidantes, pueden verse afectados por diversos aspectos asociados a la fenología de la planta como son:

la genética y las condiciones ambientales (suelo y clima), entre otros (Shih *et al.,* 2011).

En los últimos años, diversos trabajos realizados sobre los efectos *in vivo* de estos compuestos han probado que una pequeña fracción de los polifenoles ingeridos en la dieta se absorben en su forma inicial, aglicona o glicósido, mientras que la mayor parte se degradan a diferentes metabolitos. Tanto los compuestos absorbidos como los metabolitos a que dan lugar muestran capacidad antioxidante *in vivo* lo que indica la existencia de una especie de reacciones en cascada en las que intervienen los antioxidantes de forma diferente (Sreelatha & Padma, 2009).

Estudios *In Vivo* de *Moringa oleifera*

Las características nutricionales de *Moringa oleifera* la convierten en una opción viable como forraje, ya es utilizada de esta manera a gran escala en varios países africanos y en Nicaragua (Padilla *et al.*, 2014). Presenta una alta productividad de materia verde comparada con otros pastos, como la alfalfa, y los valores más elevados se alcanzan con una densidad de siembra de un millón de plantas por hectárea (Makkar & Becker, 1996).

Sus hojas han sido utilizadas en la formulación de raciones para la alimentación animal (Reyes, 2006; Pérez *et al.,* 2010). En una investigación realizada en el Instituto de Producción Animal en los Trópicos y Subtrópicos (en Hohenheim, Alemania), se demostró que la composición de aminoácidos de las hojas de Moringa es comparable con la de la soya; se comprobó que el índice de proteína digerible de sus hojas en los intestinos (PDI) es superior al de varios suplementos proteínicos convencionales, como las tortas de coco y las semillas de algodón, maní, sésamo y girasol (Makkar & Becker, 1996).

Adicionalmente, se ha evaluado la seguridad y eficacia nutricional de la hoja de Moringa en pollitos White-Leghorn de siete días hasta cinco semanas. Con niveles de sustitución de harina de Moringa de 0% (grupo de control), 10%, 20% y 30%; se confirmó que hasta un 10% de inclusión de harina de hojas de Moringa en el pienso comercial, presentó el más bajo consumo de alimento con ganancia de peso por encima de los pollitos alimentados con la dieta control. Además, el grupo control tenía niveles significativamente más altos de colesterol; triglicéridos y ácido úrico. Infiriéndose que la harina de hojas de *Moringa oleifera* puede ser fuente de sustitución de soya y confiere un efecto nutracéutico en el modelo animal (Ashong y Brown, 2011)

Otro estudio, probó piensos con inclusión de harina de *Moringa oleifera* como fuente de proteína para pollos de engorda, adicionalmente, comprobar si el perfil de aminoácidos de la proteína de Moringa era capaz de proveer los niveles requeridos de metionina y lisina por el ave. Probaron niveles de sustitución de harina de Moringa de 0%, 7.5%, 7.5% (sin metionina y lisina), 15% y 30%. Pudiendo remplazar hasta en un 7.5% el formulado comercial sin afectar, ganancia de peso, utilización de nutrientes y consumo de alimento; sin comprometer el nivel de digestibilidad. Observando que ha este nivel de sustitución, la harina de hoja de Moringa provee lo requerimientos necesarios de metionina y lisina. Esta evidencia da pauta a que la harina de hoja de Moringa se puede utilizar con éxito en modelos animales, con la seguridad de que provee los aminoácidos que requiere los organismos como las aves, así como de proporcional los requerimientos nutricionales, con el objetivo de sustituir total o parcialmente la pasta de soya, en la dieta de la codorniz japonesa (*Coturnix coturnix japonica*), cuyo modelo es de los más utilizados debido a su fácil manejo, baja variabilidad genética, crecimiento rápido y maduración sexual temprana (35 d) (Vásquez-Romero & Ballesteros-Chavarro, 2008).

Planteamiento del Problema

En Sinaloa, Moringa existe de manera silvestre y se han identificado algunos cultivos de baja tecnificación con posible potencial agronómico. Existiendo dos variantes: una de vaina corta y otra de vaina larga. La harina de sus hojas se comercializa y se consume como suplemento alimenticio; sin embargo, no está caracterizada su composición química y propiedades bioactivas. Asimismo, sus propiedades antimicrobianas y los efectos que puede producir en aves suplementadas con harina de la hoja de *Moringa oleifera*. Debido a las exigencias nacionales e internacionales de producir proteína de origen animal de buena calidad y libre de sustancias químicas o dañinas a la salud tanto animal como humana, en este último caso, como pueden ser los antibióticos promotores de crecimiento.

Preguntas de Investigación

1. ¿Existe diferencia en la composición nutrimental (proteína, grasa, fibra dietética, carbohidratos, minerales, ácidos grasos, aminoácidos) de la hoja de dos variantes de *Moringa oleifera* colectada en Sinaloa?

2. ¿Cuál es el contenido de compuestos fenólicos y flavonoides totales presentes en extractos de hoja de dos variantes de *Moringa oleifera* colectada en Sinaloa

3. ¿Existe diferencia en la actividad antioxidante de los extractos fenólicos de la hoja de dos variantes de *Moringa oleifera* colectada en Sinaloa?

4. ¿Cuáles son los perfiles de compuestos fenólicos de la hoja de dos variantes de *Moringa oleifera* colectada en Sinaloa?

5. ¿Cuáles son los efectos nutricionales del consumo de piensos con inclusión de harina de hojas de *Moringa oleifera*, con la mejor composición nutrimental y nutracéutica, en codorniz japonesa?

6. ¿Cuáles son los efectos nutracéuticos y fisiológicos del consumo de piensos con inclusión de harina de hojas de *Moringa oleifera*, con la mejor composición nutrimental y nutracéutica, en codorniz japonesa?

7. ¿Cuál es el efecto como promotor de crecimiento que presenta la hoja de *Moringa oleifera*, con la mejor composición nutrimental y nutracéutica, al ser consumida como suplemento por codorniz japonesa?

Hipótesis

1. Las hojas de Moringa de vaina corta tienen un mayor contenido nutrimental (proteína, grasa, fibra dietética, carbohidratos, minerales, ácidos grasos, aminoácidos) que la Moringa de vaina larga.

2. Las hojas de Moringa de vaina larga presentarán mayor contenido de compuestos fenólicos y flavonoides totales.

3. Las hojas de Moringa de vaina larga presentarán mayor capacidad antioxidante.

4. Las hojas de las dos variantes de *Moringa oleifera* contienen: ácido gálico, ácido clorogénico, ácido elágico, ácido ferúlico, kaempferol y quercetina como los compuestos fenólicos de mayor proporción a fin de que ejerzan un efecto nutracéutico y antimicrobiano.

5. El consumo de hoja de *Moringa oleifera* genera una ganancia de peso, conversión alimenticia y rendimiento de canal similar a la pasta de soya en codorniz japonesa.

6. Los parámetros hematológicos y fisiológicos de las codornices con dietas con inclusión de hoja de *Moringa oleifera* se presentan dentro de los rangos considerados normales para codornices sanas.

7. El consumo de hoja de *Moringa oleifera* genera efecto similar al antibiótico promotor de crecimiento (Virginiamicina).

Problema de Investigación

Esta propuesta, se realizó en dos fases la primera corresponde a una investigación descriptiva de extractos de hoja de dos variantes de *Moringa oleifera* producidas en localidades de Culiacán, Sinaloa; donde se caracterizaron los componentes nutricionales y bioactivos antioxidantes mediante ensayos *in vitro*. En la segunda fase, se desarrolló una investigación experimental, donde se determinó el efecto del consumo de piensos suplementados con hojas de Moringa (*Moringa oleifera*) sobre el estado fisiológico de codorniz japonesa (*Coturnix coturnix japonica*). Estas actividades se llevaron a cabo en el Laboratorio de Ciencia y Tecnología de Alimentos de CIAD y en Facultad de Medicina Veterinaria y Zootecnia de la Universidad Autónoma de Sinaloa.

En la fase descriptiva se realizó la caracterización química mediante la extracción y purificación de compuestos con potencial nutracéutico de las hojas de dos variantes de *Moringa oleifera,* mediante las determinaciones de ácidos grasos, aminoácidos, minerales, proteínas, grasas, fibra dietética (AOAC, 1998; Folch *et al*., 1957). El material se recolectó en el mes de noviembre de 2013 y de enero a agosto de 2014. Adicionalmente se determinó la capacidad antioxidante y antimicrobiana de los extractos, y se determinó el perfil de las dos variantes de *Moringa oleifera.*

En el mes de octubre del 2015 se determinó el efecto del consumo de piensos suplementados con hojas de Moringa (*Moringa oleifera*) sobre el estado fisiológico de codorniz japonesa (*Coturnix coturnix japonica*).

Objetivos

Objetivo General:

Determinar el efecto del consumo de piensos con la inclusión de harina de hojas de Moringa (*Moringa oleifera*) sobre la respuesta productiva, el estado fisiológico, y su efecto como APC en codorniz japonesa (*Coturnix coturnix japonica*).

Objetivos Específicos.

1. Caracterizar nutrimentalmente (proteína, grasa, fibra dietética, carbohidratos, minerales, ácidos grasos y aminoácidos) la hoja de dos variantes de *Moringa oleifera* colectada en Sinaloa.

2. Determinar los compuestos fenólicos y flavonoides totales de extractos de hoja de dos variantes de *Moringa oleifera* colectada en Sinaloa.

3. Determinar la capacidad antioxidante de extractos de hoja de dos variantes de *Moringa oleifera* colectada en Sinaloa.

4. Determinar el perfil de compuestos fenólicos de extractos de la hoja de dos variantes de *Moringa oleifera* colectada en Sinaloa.

5. Determinar el efecto sobre la respuesta productiva (ganancia de peso, conversión alimenticia y rendimiento de canal) del consumo de piensos con inclusión de harina de hoja *Moringa oleifera* en codorniz japonesa.

6. Determinar el efecto nutracéutico (colesterol sérico, triglicéridos y ácido úrico) y fisiológico (biometría hemática, funcionamiento hepático y renal) del consumo de piensos con inclusión de harina *Moringa oleifera* en codorniz japonesa.

7. Determinar el efecto como promotor de crecimiento (APC) *in vivo* del consumo de piensos con inclusión de harina de hoja *Moringa oleifera* en codorniz japonesa.

Justificación

Moringa oleifera se está constituyendo en un recurso de primer orden y bajo costo de producción, que ha demostrado riqueza nutritiva y nutracéutica. Su principal utilidad es como suplemento alimenticio. Hasta el momento solo se ha reportado estudios sobre la seguridad y su efecto hasta ciertos niveles de consumo de piensos suplementados con hojas de Moringa (*Moringa oleifera*) sobre el estado fisiológico de aves. Teniendo como reto, buscar mayores niveles de sustitución y si estos niveles son suficientes para cubrir los requerimientos de aminoácidos, sobre todo en aquellos que son limitantes como metionina, treonina y lisina, para su el desarrollo de musculo, así como el aporte necesarios de calcio, fosforo y magnesio para la formación de huesos y plumas, Adicionalmente, es necesario explorar, si los compuestos fenólicos, así como el tipo de compuestos que constituyen estos, son capaces de desarrollar tal actividad bacteriana que pueda tener un efecto similar a la antibiótico promotores de crecimientos (APC´s), así como un efecto nutracéutico en el estado fisiológico de las aves. Con ello, se generaría, una alternativa nutricional, nutracéutica y sustituto de APC´s, en un solo material vegetal. Adicionalmente, se podría corregir la desertificación y beneficiar la reconversión de cultivos. Por todo lo anterior, surge la necesidad de investigar las propiedades nutricionales y nutracéuticas y antimicrobianas de la hoja de *Moringa oleifera.*

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CAPÍTULO III: NUTRITIONAL AND PHENOLIC CHARACTERIZATION OF *Moringa oleifera* LEAVES GROWN IN SINALOA, MÉXICO

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Abstract

The present study shows the results of a research done on the chemical composition, minerals, fatty acid (FA) and phenolic compounds contents, and antioxidant capacity in two variants of *Moringa oleifera* leaves grown in Sinaloa, Mexico. The leaves of the two variants of *Moringa oleifera* revealed high protein content (31.69-36.83%, db). Dried leaves presented the following minerals content: calcium=15.08-15.58 g•kg^{-1}, magnesium=3.55-3.62 g•kg^{-1}, sodium=1.42-1.54 g•kg^{-1}, potassium=2.69-3.01 g•kg^{-1}, iron=120.19-105.31 ppm, manganese=54.5-59.77 ppm, zinc=56.48-46.89 ppm and copper=10.92-7.96 ppm. The main fatty acids were linolenic acid (62.72-66.19%) having the highest value, followed by palmitic (17.13-17.26 %), linoleic (9.65-7.64%), oleic (3.24-2.14%)**,** and stearic acid (2.71-2.66%). The total dietary fiber (TDF) content was found at levels of 29.16-29.11% (db). On average, the three most abundant amino acids in both variants were tyrosine, glutamate, aspartate, histidine, phenylalanine and leucine acid. The two Moringa variants showed total phenolic and flavonoids contents of 71.08-76.63 mg EAG•g^{-1} (db) and 55.7-60.3 mg QE•g^{-1} (db), respectively. The antioxidant capacity of the ORAC assay was 154.71-182.31 µmol ET•g^{-1} (db), while the DPPH assay value was 86.82-87.92%. Finally, gallic, chlorogenic, caffeic, coumaric and ferulic acids were found in a concentration range of 1.238-1.193, 0.585-0.568, 0.503-0.485, 1.090-1.114 and 0.536-0.518 mg•g^{-1}, respectively. Our results show that *Moringa oleifera* leaves are an important source of protein, fatty acids, minerals and phenolic compounds that could be used in food, nutraceutical and pharmaceutical industry.

Introduction

Moringa oleifera is the most widely known and studied species of the genus Moringa. Taxonomic classification indicates it belongs to the *Moringaceae* family and *Capparidales*, *Magnoleopsida* class. The Moringa genre includes 13 species: *arborea*, *concanensis*, *drocanensis*, *drouhardii*, *hildebrandtii*, *pygmeae pilgrim*, *rospoliana, ovalifolia*, *stenopetala*, *rivae*, *oleifera***,** and *borziana* (Olson, 2002). This tree is native to the Himalayas, India northeast, Bangladesh, Afghanistan**,** and Pakistan. It is spread out around the planet such as tropical and subtropical weather in Central America. It is known by various common names: Benzolivo, Mlonge, Mulangay, Stick, Kelor, Moringa, Reseda, Nébéday, and Sajna Saijhan, etc. (Pérez *et al.*, 2010). Moringa is quickly gaining attention because it is a nutritional and caloric source and has a low cost of production. It has been used as Food Supplement because of its proven nutritional and nutraceutical wealth. Recently, there has been a lot of interest in this plant due to the results of plenty research and relevant publications on the topic that highlights its nutritional and medicinal properties (Olson & Fahey, 2011). Furthermore, it has been evaluated as a useful food ingredient and as a product such as wood, charcoal, lubricating oil**,** and water clarifier (Folkard & Sutherland, 1996).

Moringa tree has a high potential for cultivation in Mexico and many parts of tropical America because of its unique combination of characteristics (Pérez *et al.*, 2010). One of the most attractive features of Moringa is the high protein content in their leaves (Yang *et al.*, 2006). Moyo *et al.* (2011) highlighted the protein content of 30.3% in dry sheets; most of this seems to be directly assimilated into the human body. Furthermore, the amino acid content demonstrates a desirable nutritional balance. These results show that the leaves contain a substantial amount of nutrients and can be included in diets as a supplement to our daily nutritional requirements (Oduro *et al.*, 2008). Although Moringa is characterized by its high content of protein and vitamins, it also has light levels of antinutritional substances (Makkar & Becker, 1996). Nevertheless, such substances are not found in lethal doses; therefore, they cannot cause

negative side effects (Olson & Fahey, 2011). Makkar and Becker (1996) showed that Moringa leaves do not contain significant levels of antinutritional substances; in this sense, the leaves can be considered free of lethal doses or adverse effects on human health. However, the content of phenolic compounds is affected by environmental conditions, the variety of the plant, and the ripening stage of the leaves. In Sinaloa, Moringa exists in the wild and as low technology crops with possible agronomic potential (Pérez *et al.*, 2010). Two variants have been detected: plants whose fruit ranges in size from 15 to 25 cm recognized as Moringa short pod (SPM); and another one, whose fruit varies from 30 to 80 cm called Moringa long pod (LPM). However, nutritional composition, nutraceutical and antioxidant capacity of these materials are unknown. Therefore, additional analysis are needed to identify significant genotypes in the production and quality of the leaves in order to reach further growth of these plants by clonal reproduction and genetic improvement of species (Steinitz *et al.*, 2009). The identification, propagation, selection and domestication of materials with features of interest are necessary to determine the agronomic potential of Moringa (Pérez *et al.*, 2010). Thus**,** the objective of this study was to characterize the nutraceutical and nutritional properties of two variants of Moringa grown in Sinaloa: The long pod (LPM) and the short pod (SPM).

Materials and Methods

Plant Material. Leaves from long pod and short pod trees of two variants of *Moringa oleifera* were collected in November 2013 in Imala, Culiacan, Mexico (24 ° 51' 23" N, 107 ° 12' 56" W, at 160 m ASL). Plant material was washed in a 150 ppm chlorine solution and then dried in an electric oven at 55-60 ºC for 6 h to a constant weight for moisture determination. Finally**,** pulverized in a fine mill to get Moringa leaves flour.

Preparation of Methanolic Extract. It was made using 1g of a powdered sample of dried leaves mixed with 10 mL of methanol. The mixture was homogenized in a tissue homogenizer Ultra-Turrax for one minute and incubated

at 200 rpm for 2 h, centrifuged at 8000 g for 20 min and 4 °C. The supernatant (extract) was recovered and stored at 8 °C for their subsequent analysis.

Chemical Composition. The methods used for Analysis of total crude protein, moisture, fat, carbohydrates, ash, and crude fiber followed the recommendations of The Official Methods of Association of Official Analytical Chemists (AOAC) (Anon., 1998). The total carbohydrate was determined by the difference method [100 − (proteins + fats + moisture + ash in percentage)] (Valdez-Solana *et al.,* 2015).

Mineral Analysis. Minerals content was quantified according to the official AOAC method No. 955.06 (Anon., 2005). After acid digestion of the ash, the sample was filtered and reached to 100 mL with deionized water. Using an atomic absorption spectrophotometer, the absorbance for each mineral was measured at specific wavelengths: Ca (422.7nm), Na (589.6nm), K (769.9nm), Mg (285.2nm), Mn (279.5nm), Fe (248.3nm), Cu (324.7nm) and Zn (213.9nm). A calibration curve of reference standards of known concentration was used for each mineral. The concentration of each of the minerals was calculated in ppm.

Determination of Amino Acids Composition. The composition of amino acids was determined by HPLC according to Vázquez *et al.* (1995) with minimal variations. Sample Preparation: Hydrolysis**;** 3 mg of Moringa flour defatted were weighed into hydrolysis tubes and 3 mL of 6M HCl were added. Then the tubes were sealed under vacuum for 3 minutes. Subsequently, the tubes were placed in a dry bath for hydrolysis at 120 ° C for 24 h. Extraction**;** The hydrolysate was evaporated at 65 ° C and washed using 3 mL of distilled water to remove HCl; then amino acids were recovered using 1 mL of sodium citrate buffer pH 2.2 and stored at 0 ° C until derivatization and chromatographic quantification. **Derivatization of the Sample**. Aliquots of 100 µL of hydrolysate, 40 µL of internal standard 100 mM were mixed and filled to a volume of 1 mL with sodium citrate buffer pH. Subsequently, 250 µL of these dilutions were withdrawn and mixed with 250 µL of OPA (O-phthalaldehyde) in a syringe for liquid chromatography,

followed by filtration (nylon 0.2 um). A 10 µL of the derivative was injected into chromatograph (HPLC) model 9012 (Varian, Palo Alto, CA). Timing from derivatization to sampling for injection should not exceed 2 min.

Amino Acids Profile Analysis by RP-HPLC. The amino acid profile was performed by liquid chromatography high-resolution reversed phase model 9012 (Varian, Palo Alto, CA); Varian fluorescence detector, injector capacity 10 µL and column (Restek Pinnacle II, C18, 5 µm 150 mm x 4.6 mm). The mobile phase was solvent A: sodium acetate buffer (0.1 M, pH 7.2); methanol and tetrahydrofuran were used as an organic modifier (900: 95: 5 v/v/v) (Sigma Chemical Co.), solvent B: methanol (Sigma Chemical Co.). The gradient flow was 1.5 $mL \cdot min^{-1}$ (min/A%: B%): 0/100: 0, 0.5/80: 20, 7.5/80: 20, 10/50: 50, 15/50: 50, 18/20: 80, 20/20: 80, 23/0: 100, 25/100: 0, 30/100: 0. The detection was by fluorescence using the wavelengths of emission 455 nm and excitation 340 nm. The column heater was maintained at 30 °C. The identification and quantification of amino acids were performed by comparing the retention time of the sample against amino acid standards of known concentration using the computer program (version 4.0 Chromatography Varian Star).

Determination of Fatty Acids Composition. Fatty acids were determined as reported by Folch *et al.* (1957) and the AOAC 963.22 (Anon., 1998) standard method with some modifications. Fat removal. 10 g of sample were weighed, placed in a 250 mL Erlenmeyer flask, mixed with 60 mL of Folch reagent (1 volume of methanol plus two volumes of chloroform) and homogenized. Subsequently, vacuum filtered on a Buchner funnel, the residue was mixed with 50 mL of Folch reagent and homogenized again. The residue was washed with 50 mL of Folch reagent, the flask was cleaned and vacuum filtered again. The filtrates (60 + 50 + 50 mL) were mixed in a dropping funnel and added to 40 mL of 0.73% sodium chloride, stirred vigorously and allowed to settle overnight. After 24 hours**,** the lower phase (organic) (F1) was decanted and filtered through anhydrous sodium sulfate. The filtrate was recovered in a round flat bottom flask. The upper phase (F2) was washed with 50 mL of a mixture of 20% NaCl (0.58%)

and 80% of Folch reagent. It waved and left to stand for 2 hours. Then it was decanted and filtered through anhydrous sodium sulfate to get F3. F1 and F3 were mixed, evaporated and dried in the rotary evaporator. Methylation: after evaporating the chloroform, 0.5 g of sodium hydroxide and three glass beads were added to methanol. The flask was placed in a cooling rosary and refluxed for 10 min. Subsequently, Boron trifluoride (BF3) was added to the top of the condenser and refluxed for another 5 min. Then, 4 ml of heptane were added underwent reflux for 2 min. The ball flask and the contents were added in a test tube, and a saturated NaCl was added (stir gently) until it changed its milky white color. After that, a pinch of sodium sulfate was added to remove the fatty acids. The upper phase was taken and filtered through a Pasteur pipette previously packed with fiberglass**,** and the filtrate was recovered in a vial of 2 ml. The vial was kept in a nitrogen atmosphere and was later placed in the freezer. The organic phase (1 mL) was filtered through a 0.45 µm membrane. A sample (1 µL) was injected into the gas chromatography system. All samples were analyzed in triplicate. The equipment used was a gas chromatograph (Varian CP-3800, USA) with a flame ionization detector (FID) equipped with a 30 m x 0.32 mm ID, 0.25 mm Omegawax 320 column (Supelco, USA). Helium was used as carrier gas at a flow rate of 3 $mL \bullet min^{-1}$. The oven temperature was kept at 140 °C for 5 min, preset at a maximum temperature of 240 °C at a rate of 4 °C for 1.5 min. Both the injector and detector temperature were set at 260 °C. For identification and quantification of fatty acids, the retention times of the sample were compared with those of a standard mixture consisting of 37 fatty acid methyl esters (Supelco, Bellefonte, USA). The results were expressed in percentage of fatty acid contained in the sample.

Total Soluble Phenolics. The method developed by Folin and Ciocalteu (Swain & Hillis, 1959) was used. The extract was oxidized with Folin-Ciocalteu reagent and the reaction was quenched after 3 minutes with sodium carbonate. The absorbance of the resulting blue was measured at 725 nm after 120 min

incubation protected from light. Gallic acid was used as standard at concentrations of 0 to 0.4 mg•mL^{-1} to calculate the results.

Total Flavonoids. The method of aluminum chloride was used to determine the total flavonoid content of the extracts, as reported by Ebrahimzadeh *et al.* (2009), with slight adjustments. An aliquot of 20 µL of the prepared extract was taken and placed in a 96-well plate. Subsequently added 112 µL of distilled water plus 60 µL of methanol. Next, 4 µL of 10% aluminum chloride were added plus 4 µL of 1 M potassium acetate. Incubation of the sample was performed in the dark during 30 min. After that, absorbance was read at 415 nm using a Synergy HT Microplate Reader (BioTek, Inc., USA). A standard calibration curve was generated using known concentrations 415 nm quercetin. The concentration of flavonoids in the test samples was calculated using a standard curve and expressed as mg equivalent•g^{-1} quercetin sample.

Antioxidant Capacity by DPPH Method. The antioxidant capacity was measured using the percentage of inhibition of the DPPH radical determined by the DPPH (1,1-diphenyl-2-picrylhydrazyl) method developed by Brand-Williams *et al.* (1995), using a microplate reader Synergy HT (BioTek, Inc. EEU), measuring absorbance at 515 nm. The results were expressed as a percentage of DPPH radical inhibition.

Antioxidant Capacity by ORAC Method. 96-well dark-microplate (Costar, USA), which was added to 25 µL of extracts dilutions (in phosphate buffer) of *Moringa oleifera* leaf, 25 µL of a target were used, and 25 µL of the Trolox standard curve. The plate was placed in a microplate reader model Synergy HT (BioTek, Inc., USA) which was at 37 °C in the time of incubation. Microplate reader dispensed in each of the plate 200 µL of 0.96 mM fluorescein and 75 µL of 2,2'-azobis, 2-amidino-propane dihydrochloride (AAPH) 95.8 mM. The reaction started after the last reagent was added measuring the fluorescence at 70 sec intervals for 70 min at a wavelength of 485 nm for excitation and 580 nm emission. The calculations were performed using the linear regression equation of the standard

curve and the area under the curve fluorescence loss. Results are expressed as µmol Trolox equivalent (mol TE)•g^{-1} (Huang *et al.*, 2002).

Phenolics UPLC Profile. Moringa components based on different types of free or conjugated chemical interactions by UPLC chromatography with diode array detector (DAD) were separated by liquid chromatography (Corral-Aguayo *et al.*, 2008). Samples (1 mL) of the extract were homogenized in sodium phosphate buffer 50 mM (10 mL) with an ULTRA-TURRAX® T 25 digital (IKA Works, North Carolina, USA). The homogenate was centrifuged at 10000 rpm for 10 min at 4 ° C on a Thermo Scientific 120 centrifuge (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and the supernatant to a separatory funnel Kimax® No. 22 was transferred. The residue was resuspended in 10 mL of ethyl acetate, homogenized, and centrifuged again under the same conditions and the supernatant was transferred to the funnel. This procedure was repeated two more times until the residue has no coloration. The various supernatants were mixed and the time needed to phase separate in the funnel, and then the upper phase was collected and expected, the little flow solvent evaporated nitrogen and stored at -20 °C until analysis. For analysis of UPLC-DAD**,** a sample of the extract obtained above was added to 4 ml of ethyl acetate. The supernatant was filtered through a Sep-Pak C18 cartridge. An aliquot of 20 uL was injected into a liquid chromatograph (Acquity-UPLC) (Water Inc, USA) with a diode array detector (PDA). An Acquity UPLC BEH C18 column of 100 mm length x 2.1 mm in diameter with a particle size of 1.7 microns and a pore size of 100 Å was used. The mobile phase "A" used was a mixture of 95% Water, Methanol 2% and 3% formic acid, and phase "B" was a mix of 95% Methanol, Water 2% and 3% formic acid , using a gradient: 0 min, 90% A, 10% B (0.3 mL • min); 3 min, 75% A, 25% B (0.3 mL • min); 5 min, 70% A, 30% B (0.25 mL • min); 9 min, 60% A, 40% B (0.3 mL • min); 11 min, 50% A, 50% B (0.3 mL • min); 12 min, 0% A, 100% B (0.3 mL • min); 13 min, 0% A, 100% B (0.3 mL • min); 15 min, 90% A, 10% B (0.3 mL • min); 16 min, 90% A, 10% B (0.3 mL • min) with a flow of 0.3 mL•min^{-1}. The reading was performed at 190 and 420 nm. Quantification of phenol was conducted using

standard calibration curves of chlorogenic acids, gallic, ferulic, coumaric, and t-cinnamic acid (Sigma Chemical Co., USA) using concentrations from 5 to 50 µg • mL^{-1}.

Statistical Analysis. A completely two-factor randomized experiment with ten replicates, and three replicates for each variable, was used. Data were analyzed in Minitab 16.

Results and Discussion

Chemical Composition. Table 1 shows the values of the proximate composition (moisture, lipid, total ash, protein, crude fiber and carbohydrates) for both long and pod Moringa. Highlights its protein content and ash, being considerably higher in LPM regarding SPM. These values are similar to those reported by Alfaro (2008) in leaves of Moringa, 33.50 ± 1.10% protein and 8.78% ash. These results support the potential of Moringa as a source of dietary protein described by other authors. Although crude protein levels found in this study are higher than those reported by other authors Moringa plants from other places in Mexico (the states of Sonora, Michoacan, and Coahuila) (Sánchez-Machado *et al.*, 2010; Valdez-Solana *et al.*, 2015). These variations can be caused by weather variations, crop management, if they cultivate or wild, the state of maturity of the plant at the time of collection, and the type of post-collection processing. Therefore, Moringa leaves of both variants are a good potential for additional protein source in the human diet.

Amino Acids Profile. The amino acids profile in both variants of *Moringa oleifera* (Table 2) shows that the total amino acid concentrations are in the range of 0.98% to 3.95%. SPM and LPM had similar levels of amino acid profile and no significant differences. The total amino acid content revealed that the essential amino acids represented 40% of LPM and 51 % of SPM. The amino acids that were concentrated in a higher proportion in both variants are glutamic acid, aspartic acid, histidine, tyrosine, leucine, and arginine; while the lowest concentrations are methionine serine and lysine. The amino acid profile in

Moringa oleifera leaves were tested in earlier studies (Makkar & Becker 1996; Sánchez-Machado *et al.*, 2010; El-Massry *et al.*, 2013). The composition of amino acids as aspartic acid, glutamic acid, histidine, glycine, arginine, alanine, tyrosine, methionine, valine, phenylalanine, isoleucine, and leucine, except lysine, show a variation of the published data by at least one of the mentioned researchers. Both variants of *Moringa oleifera* contain high percentages of essential amino acids except for methionine, commonly deficient in green leaves. It could be possible that the variations in the amino acid composition of the leaves are influenced by the quality of the protein and the origin of the plant (cultivated or wild).

Lysine content of Moringa leaves of both variants contains an acceptable level of lysine amino acid that is frequently found in low concentrations in vegetables, legumes and cereals exception.

In general, the content of essential amino acids of the evaluated materials cover the requirements recommended by WHO and FAO to a child (3-10 years), vulnerable population group daily intake when it comes to availability of quality protein. Therefore, Moringa could be incorporated into the human diet, particularly for children to prevent or cut malnutrition.

Mineral Composition. The content of eight essential minerals, i.e. iron (LPM: 120.19 ± 8.41 ppm and SPM: 105.31 ± 8.89 ppm), zinc (LPM: 56.48 ± 5.13 ppm and SPM: 46.89 ± 4.88 ppm)**,** and copper (LPM: 10.92 ± 0.91 ppm and SPM: 7.96 ± 0.34 ppm) in Moringa leaves are shown in Table 3. Potassium, calcium, magnesium, and sodium that are nutritionally important, levels of daily intake requirements of the general population. LPM and SPM showed significant differences in micronutrients (i.e., Cu, Zn and Fe) but not in macronutrients. Meanwhile, SPM is higher in Mn concentration than LPM. From these results, Moringa could help to prevent diseases related to malnutrition.

Fatty Acids Content. 14 fatty acids were identified in both variants (Table 4). Linolenic acid was found in high amount followed by palmitic acid; both represent 80% of total fatty acids, similar to that presented by Sánchez-Machado

et al. (2010). The material showed the presence of linoleic acid and linolenic acid as essential fatty acids. The occurrence of polyunsaturated fatty acids was increased by 96% as compared to monounsaturated fatty acids. The consumption of polyunsaturated fatty acids caused decreased levels of total and LDL cholesterol, having a cardioprotective role of these compounds. That effect is because they are antiarrhythmic agents that improve vascular endothelial function and descend blood pressure, which inhibits platelet aggregation. That is associated with an impediment to the formation of plaques on the inside of blood vessels and adherence to endothelium. It has been observed that people whose diets are rich in polyunsaturated fatty acids show a low incidence of cardiovascular disease.

Total Soluble Phenolics and Flavonoids. SPM and LPM had similar levels of total phenols and flavonoids (Table 5), and there were no significant differences ($P>0.05$) being SPM and LPM presented. The phenol content was higher than previously reported for Moringa (45.21 mg GAE•g^{-1}) (Adisakwattana & Chanathong, 2011). In the case of total flavonoids, results were greater than those reported in 37.0 mg GAE•g^{-1} (Saikia, 2011). The values indicate that 78% of total phenolic compounds corresponded to flavonoids. Phenolic compounds or polyphenols are derived from the secondary metabolism of plants. These compounds are commonly found in plants and have been extensively exploited because of its multiple biological activities, including antioxidant effects. Flavonoids and phenolic acids are receiving increased attention as potential antioxidants, mainly due to its strong presence in a significant number of consumer foods (García-Cruz, 2012). In phenolics and flavonoids, at least, one hydroxyl ion is substituted with an aromatic ring forming chelate complexes with metal ions thus are readily oxidized. They, therefore, serve as great units to donate electrons. The antioxidant activity of the phenolic compounds in the above reports is shown to be mainly due to its redox properties, allowing them to act as reducing agents, hydrogen donors, or singlet oxygen quenchers (Sankhalkar, 2014). It turns out that most researches done conclude there is a correspondence

between phenolic compounds and antioxidant activity in plants. This study confirms the antioxidant potential in vitro of crude methanolic extracts, whose activity is likely to be due to phenolic compounds and flavonoids sample**;** therefore, *Moringa oleifera* leaves can be considered as a source of antioxidant compounds with activity sufficient to reduce the activity of free radicals and reactive oxygen species.

Antioxidant Activity. The results of DPPH and ORAC antioxidant activity were similar between LPM and SLM with no significant ($P<0.05$) differences observed. As seen in Table 5, extracts of both variants showed ORAC antioxidant capacity similar to the 121 µmol TE•g^{-1} reported by Yang *et al.* (2006). Also, the DPPH free radical protocol was used to evaluate the ability of the extracts of the leaves of the two Moringa variants to eliminate free radicals, forming stable diamagnetic molecules (Table 5) (Singh *et al.*, 2009). The DPPH assay was expressed in terms of antiradical power and values ranged from 86.82% for LPM to 87.92% for SPM, finding in both methanol extracts of *Moringa oleifera* a good scavenger of free radicals similar to the 86.77% DPPH reported by Singh *et al.* (2009). It was observed the relationship between antioxidant activity and phenolic compounds in both variants. One of the most important factors that determine the antioxidant activity of the polyphenols is the degree of hydroxylation and the position of the hydroxyls in the molecule. The flavonoids due to oxygen heterocycle are more active than non-flavonoid molecules. In turn, solubility and steric effects of each molecule may be affected by the type of structure of such molecule**s**, as glycosylated derivatives of other adducts which can increase or decrease of antioxidant activity (Jahan *et al.*, 2015). The flavonoid compounds commonly found in plants as glycosides, but the action of enzymes or some processes can release the corresponding aglycone. The activity of phenolic acids is also based on the hydroxyl groups of the aromatic ring and the binding of these compounds to organic acids and sugars to form esters. The mechanisms by which these compounds act vary depending on the concentration and types of compounds present in foods (Zapata, 2007; Jahan *et al.*, 2015). Our results

suggest the potential of Moringa as a functional ingredient in foods that may also aid in the prevention of illnesses related to oxidative stress.

Phenolic Acids UPLC Profile. The Phenolic acids profile **in** both variants of the *Moringa oleifera* (Table 6) shows that the total phenolic acid concentrations are in the range of 0.485 mg•g^{-1} to 1.238 mg•g^{-1}. In this study**,** it was confirmed the presence of phenolic compounds (Figure 1)**:** gallic, chlorogenic, caffeic, coumaric**,** and ferulic, which peaks occurred in 1,099, 3,074, 3,492, 4,905 and 5,726 min for LPM, respectively. For SPM, these compounds**,** whose peaks presented at 1.099, 3.064, 3.484, 4.896, and 5.717 min respectively**,** were also confirmed (Figure 2). The concentrations of phenolic acids are within the ranges reported by Prakash *et al.* (2007), except ferulic acid. The difference can be explained since chelates plant produces a variety of secondary metabolites, defense mechanisms against pests, predators and different levels of water stress. Moreover, Leone *et al.* (2015) report the presence of ferulic acid at levels of 0.0661 to 0.0969 mg•g^{-1} lower concentration tan that found in this study. Similarly, the amount of ferulic acid found in the leaves of *Moringa oleifera* was comparable with the amount found in some whole grains like brown rice and cornmeal, but far below the amounts found in various grains, fruit, and vegetables such as peanuts, orange, eggplant, and spinach (Zhao & Moghadasian, 2008). Therefore, Moringa can be considered a product with possible application in the food, nutraceutical and pharmaceutical industries.

Conclusion

The *Moringa oleifera* leaves variants show potential to be used as functional ingredients for human food; this is given by its protein content and amino acids profile, also both variants have a high content of dietary fiber and low lipid content. Unsaturated fatty acids are present in both variants. The study showed that the concentration of phenolic compounds in the extracts *Moringa oleifera* is enough to be considered as a potential antioxidant supplement source. Therefore, Moringa leaves can be considered a product with potential application in the food,

nutraceutical and pharmaceutical industries, which can have positive financial and social benefits to the population.

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Abbreviations

LPM, Moringa Long Pod; SPM, Moringa Short Pod; DPPH, 1, 1-difenil-2-picrilhidrazil; ORAC, Oxygen radical absorbance capacity.

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Table 1. Proximal content of *Moringa oleifera* leaf.

Determination (%)	Moringa Variants	
	Long pod	Short pod
Protein	36.83±2.16[b]	31.69±2.25[a]
Fat	8.16±0.50[a]	7.57±0.96[a]
Crude Fiber	3.37±1.36[a]	4.03±2.14[a]
Ash	6.56±0.57[b]	8.03±0.48[a]
Moisture	3.79±0.48[a]	3.88±0.18[a]
Total solids	96.21±0.48[a]	96.12±0.18[a]
Carbohydrates	41.29±0.54[a]	44.79±4.10[a]

Different letter in the same row indicates significant difference (P>0.05).

Table 2. Amino acids content of *Moringa oleifera* (g AAS•100g^{-1}).

Amino acid (%)	Moringa Variants	
	Long pod	Short pod
Aspartate	2.04±0.79[a]	3.12±0.67[a]
Glutamate	2.64±0.71[a]	3.53±0.72[a]
Serine	1.88±1.48[a]	0.98±0.23[a]
Histidine *	2.59±1.14[a]	2.94±0.47[a]
Glycine + Treonina	1.64±0.49[a]	2.30±0.20[a]
Arginine	1.53±0.54[a]	0.91±0.13[a]
Alanine	0.90±0.25[a]	1.58±0.37[a]
Tyrosine *	3.95±0.99[a]	3.21±1.09[a]
Methionine *	1.45±0.89[a]	1.05±0.39[a]
Valine *	1.79 ±0.56[a]	1.42±0.24[a]
Phenylalanine *	2.29±0.30[a]	1.69±0.04[a]
Isoleucine *	1.48±0.81[a]	1.09±0.20[a]
Leucine *	2.06±0.68[a]	2.18±0.51[a]
Lysine *	1.37±1.14[a]	0.98±0.41[a]

Different letter in the same row indicates significant difference ($\alpha<0.05$).
* Essential amino acid

Table 3. Mineral contents of dried *Moringa oleifera* leaves.

Mineral	Moringa Variants	
	Long pod	Short pod
Na*	1.42±0.12[a]	1.54±0.15[a]
K*	2.69±0.29[b]	3.01±0.30[a]
Mg*	3.55±0.31[a]	3.62±0.34[a]
Ca*	15.08±1.71[a]	15.58±1.16[a]
Cu**	10.92±0.91[a]	7.96±0.34[b]
Mn**	54.50±5.52[b]	59.77±4.42[a]
Zn**	56.48±5.13[a]	46.89±4.88[b]
Fe**	120.19±8.41[a]	105.31±8.89[b]

Different letter in the same row indicates significant difference (P>0.05).
* Macro-elements ($g \bullet kg^{-1}$).
** Micro-elements (ppm).

Table 4. Fatty acid composition (percent of total fatty acids).

Fatty acid (%)	Moringa Variants	
	Long pod	Short pod
Lauric (C12:0)	0.16±0.03[a]	0.18±0.09[a]
Myristic (C14:0)	0.78±0.07[a]	1.05±0.41[a]
Palmatic (C16:0)	17.13±0.77[a]	17.26±0.11[a]
Palmitoleic (C16:1)	0.27±0.03[a]	0.23±0.01[a]
Heptadecanoic (C17:0)	0.18±0.00[a]	0.19±0.01[a]
Stearic (C18:0)	2.71±0.34[a]	2.66±0.16[a]
Oleic (C18:1 c+t)	3.24±0.99[a]	2.14±0.50[a]
Linoleic (C18:2 c+t)	9.65±1.51[a]	7.64±1.06[a]
Linolenic (C18:3 n3)	62.72±3.31[a]	66.19±1.23[a]
Arachidic (C20:0)	0.32±0.05[a]	0.37±0.11[a]
Arachidonic (C20:4)	0.18±0.04[a]	0.22±0.05[a]
Behenic (C22:0)	0.48±0.07[a]	0.57±0.21[a]
Tricosanoic(C23:0)	0.20±0.04[a]	0.25±0.06[a]
Lignoceric (C24:0)	0.74±0.06[a]	0.80±0.21[a]
Satured	22.70±0.44[a]	23.59±1.42[a]
Monounsaturated	3.51±1.01[a]	2.37±0.50[a]
Polyunsaturated	72.55±1.78[a]	74.05±1.42[a]

Different letter in the same row indicates significant difference ($\alpha<0.05$).

Table 5. Contents of phenolic compounds and antioxidant activity of methanolic leaf extracts of *Moringa oleifera.*

Determination	Moringa Variants	
	Long pod	Short pod
Flavonoids total (mg EQ•g^{-1})	60.26±7.21^{a}	55.703±7.00^{a}
Total phenolic (mg GAE•g^{-1})	76.63±10.63^{a}	71.08±12.05^{a}
ORAC (µmol ET•g^{-1})	154.71±36.95^{a}	182.31±32.68^{a}
DPPH (µmol TE•g^{-1})	981.22±7.58^{a}	968.97±23.87^{a}
DPPH (%)	87.92±2.15^{a}	86.82±0.68^{a}

Different letter in the same row indicates significant difference (P>0.05).

mg EQ•g^{-1}	mg Quercetin equivalents per g dry weight
mg GAE•g^{-1}	mg gallic acid equivalents per g dry weight
µmol ET•g^{-1}	µmol Trolox equivalents per g dry weight
% Inhibition	Inhibition of DPPH radical

Table 6. Phenolic acid profile ($mg \bullet g^{-1}$).

Phenolic acid	Moringa Variants	
	Long pod	Short pod
Gallic	1.238±0.011[a]	1.193± 0.042[b]
Chlorogenic	0.585±0.006[a]	0.568± 0.019[b]
Caffeic	0.503±0.005[a]	0.485± 0.017[b]
Coumaric	1.090± 0.019[a]	1.114± 0.035[a]
Ferulic	0.536±0.006[a]	0.518± 0.016[b]

Different letter in the same row indicates significant difference ($\alpha<0.05$).

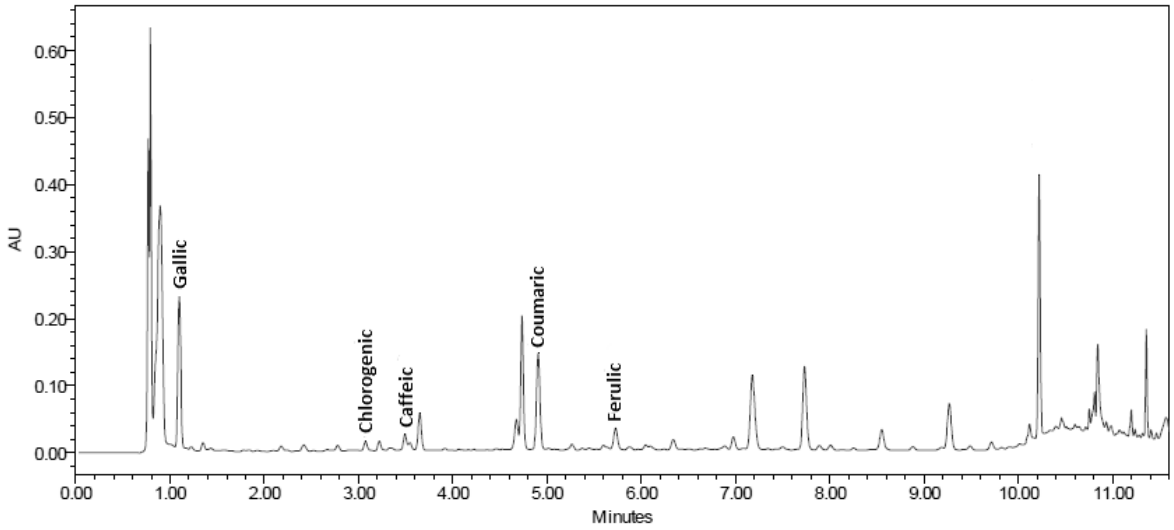

Figure 5. UPLC-DAD chromatogram of methanolic extracts of *Moringa oleifera* leaves (LPM).

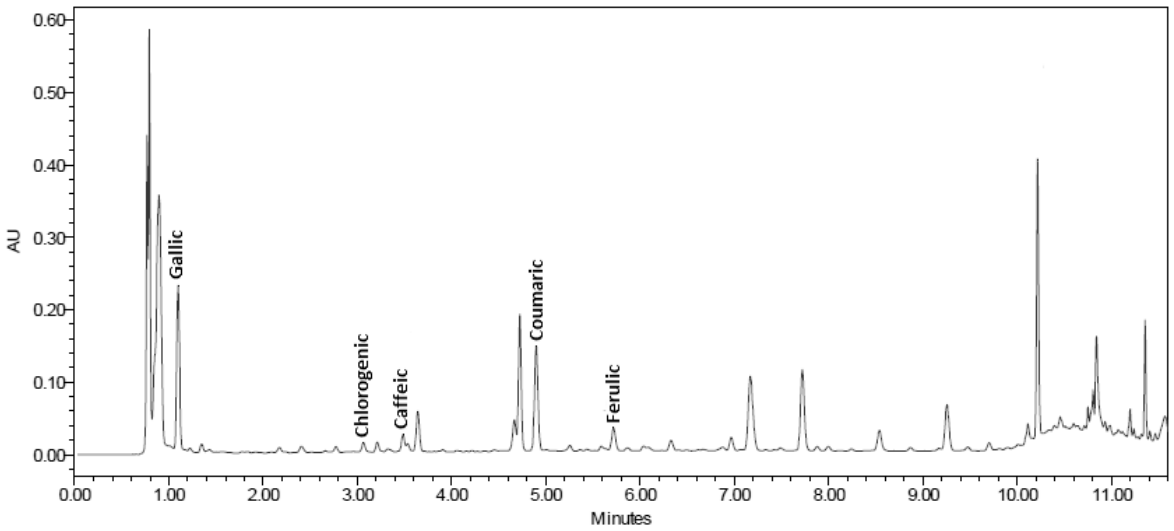

Figure 6. UPLC-DAD chromatogram of methanolic extracts of *Moringa oleifera* leaves (SPM).

CAPÍTULO IV: NATURAL ALTERNATIVES TO ANTIBIOTICS GROWTH-PROMOTING (AGP) IN ANIMAL PRODUCTION

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Abstract

There is a worldwide tendency to produce the best meat regardless of their origin (i.e., poultry, goats, cows, and pigs) free of drug residues, bacteria causing food toxicity, and contaminants (chemicals), ensuring consumers high-quality products. These pollutants are used in animal feed to improve the characteristics of raw materials, fodders, and/or animal products, to prevent diseases, and to increase production. The most commonly used additives are antibiotics or antimicrobial agents used as growth promoters (AGP) whose global trend is to avoid their use due to the risk of developing bacterial resistance, and vertical and horizontal transmission that can impact on human health, on animal wellness, and on productivity. Therefore, the search for new plant origin alternatives to replace the AGP's such as herbs, spices, plant extracts and/or essential oils to be used as antimicrobials and to also make them available to contribute to animal nutrition.

Keywords: antibiotic (antimicrobial) growth promoters, additives, plant extracts, essential oils.

Introduction

The use of additives in animal feed began in the forties to improve the organoleptic characteristics of raw materials, fodders, and/or animal products, to prevent diseases and to improve the of production efficiency by decreasing the mortality, and stimulating the weight gain in the forties (Dibner and Richards, 2005; Castanon, 2007; Upadhayay and Vishwa, 2014).

Additives used in animal feed are diverse and heterogeneous. Different categories are found depending on their properties and functions (Marroquin-Cardona *et al*., 2010; Upadhayay and Vishwa, 2014). The European Union (EU) classifies them as follows (Ministry of Agriculture, Fisheries, and Food, 2000):

- Antibiotics
- Antioxidants`
- Aromatics and flavorings
- Coccidiostats and other medicinal substances
- Emulsifiers, stabilizers, thickeners, and gelling
- Colorants including pigments
- Preservatives
- Vitamins, provitamins, and other chemically well-defined substances with similar effect.
- Trace elements (oligo elements)
- Binders, anti-caking agents, and coagulants
- Acidity Regulators
- Enzymes
- Microorganisms
- Radionuclide binders

There are antimicrobial nature additives in this classification; that is to say, antibiotic additives or antimicrobial agents used as growth promoters (AGP) in

animals (Casewell *et al.*, 2003; Allen *et al*., 2013) also known as "digestive modifiers" (Singh, 2015).

Antimicrobial or antibiotic growth promoters (AGP) played an essential role in the economic development of modern poultry production that benefitted producers and consumers of animal products (Upadhayay and Vishwa, 2014). Nevertheless, due to the risk posed by AGPs to create cross-resistance to antibiotics used in human medicine and the presence of these compounds in animal products, their use has dropped dramatically, and it has been banned in some cases on the formulation of fodders for animal husbandry (Gaucher *et al.*, 2015). In 1969, The Swann Committee recommended restrictions on the use of antimicrobials in animal fodders and allowed only those not used as therapeutics in human and veterinary medicine. In 1993, the first studies showing a relationship between the use of avoparcin and an increment and transmission of vancomycin-resistant enterococci, the same antibiotic group (glycopeptides), arises. Later in 1998, the EU prohibits ardamicina as AGP because of the risk of generating cross-resistance, and since 1999, another four antibiotics (virginiamycin, bacitracin zinc, tylosin phosphate, spiramycin) as a precaution. In the same year, The US Permanent Scientific Committee recommends abandoning the AGP that can be used in human and veterinary medicine or those who promote cross-resistance. It is prohibited the use of inhibitors (carbadox and olaquindox) for reasons of occupational health. In 2006, the use of AGPs (avilamycin, flavophospholipol, salinomycin, monensin) was prohibited. The last two AGPs could be used in chickens as coccidiostats until January 2012 (Livermore, 2005; Wise, 2005; Aminov, 2010; Gaucher *et al*., 2015).

Nonetheless, some researchers have suggested that the removal of these substances is causing an increase in the incidence of bacterial infections (i.e., diarrhea, coccidiosis and intestinal necrosis) (Castanon, 2007: Allen *et al.*, 2013). These prohibitions impact the livestock sector economically because it leads to increased production costs. American industry shows that the use of GPS in

poultry production is associated with losses for producers (Dibner and Richards, 2005; Graham *et al*., 2007).

So, there is the need to find alternatives to the use of antibiotics (Gaucher *et al*., 2015). Among these alternatives, the most used are probiotics, prebiotics, enzymes, essential oils, herbs, spices and vegetable extracts (Table 7) (Huyghebaert *et al*., 2011; Upadhayay and Vishwa, 2014).

In this last category, the use of AGP substitutes or replacements focuses on the control of the intestinal and/or bacterial flora, particularly the pathogen type (Casewell *et al*., 2003; Dibner, and Richards, 2005). Herbs, plant extracts, and essential oils used as food additives include different bioactive ingredients such as alkaloids, flavonoids, tannins, and saponins that are expected to act on the appetite of the animal and gut microflora, on the stimulation of production of digestive enzymes. Also, they can act on the intensification of endogenous enzymatic activity and immune system along with a wide antimicrobial and antioxidant properties that can benefit health and weight gain of farm animals (Umar Lule and Xia, 2005; Hervert-Hernández and Goni, 2011; Cicerale *et al.*, 2012). The primary action mode of growth promoting additives can be started in stabilizing fodders hygiene and beneficially affecting the ecosystem of the gastrointestinal microflora by controlling potential pathogens. This applies primarily to the critical stages in the development of animals when having a high susceptibility to digestive disorders (Platel *et al.*, 2004).

In 1945, Sanders *et al*. they reported more than 120 species of plants exhibiting inhibitory properties against the growth of *Bacillus subtilis* and *Escherichia coli*. Recently, many scientific studies have focused on the antimicrobial effects of herbs and plant extracts (Dorman and Deans, 2000).

Effect of the Compound Type and Structure in Bacterial Activity.

The effect and the structural requirements are not fully defined for antimicrobial activity. Studies have proved there must be, at the least, hydroxyl (OH) and methoxy (-OCH_3) groups, and some degree of lipophilicity (Mandalari *et al*., 2007;

Sánchez-Maldonado *et al*., 2011). These groups yield an oxidative phosphorylation, causing a rising of pH, and hence, toxicity. Sanchez-Maldonado *et al*., (2011) found that in phenolic acids such as benzoic acid, cinnamic acid, hydroxybenzoic acids (p- hydroxybenzoic, protocatechuic, gallic acid, and syringic), and hydroxycinnamic acids (p-coumaric, caffeic and ferulic), the antimicrobial activity of the hydroxycinnamic acids was comparable or higher than that of hydroxybenzoic acids with the same number of hydroxyl groups.

Polyphenolic compounds are more efficient uncoupling as long as they have a higher number of hydroxyl groups per molecule transferring more protons, which increases their degree of lipophilicity (Mandalari *et al*., 2007; Sánchez-Maldonado *et al*., 2011).

As in polyphenolic compounds, in essential oils, there is no absolute definition of antimicrobial activity, which in this case, is attributed to different mechanisms ranging from damage to the cytoplasmic membrane, proteins, and cell wall. Consequently, there is cell content filtration driving force reduction (Lambert *et al*., 2001; Cava-Roda *et al.*, 2012.). This effect is due to the high degree of hydrophobicity or lipophilicity that allows the separation of the lipid structure of the cell membrane and mitochondria, disordering the structure. Therefore, it affects their permeability, allowing the migration of ions and other compounds, and resulting in a homeostatic imbalance (Bajpai *et al.*, 2013) that leads to a cytotoxic effect on the cells (Bakkali *et al*., 2008).

Some plant materials that can be considered as substitutes for AGPs because of their antimicrobial activity by the presence of polyphenolic compounds and essential oils would be garlic (*Allium sativum*), oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), and Moringa (*Moringa oleifera*), which are addressed in this review as proposals.

1. Garlic (*Allium sativum*) and Antibiotics Growth-Promoting (AGPs)

Garlic is a crop that is considered one of the most studied natural alternatives to be used as AGP. Several potential antimicrobial compounds can be extracted

from Garlic. Garlic extracts have been studied as effective against organisms as *Helicobacter pylori*, *Mycobacterium tuberculosis, Shigella dysenteriae 1, Shigella flexneri Y, Shigella sonnei, Escherichia coli, Staphylococcus aureus, Streptococcus, Viridins, Streptococcum hemolyticus, Llebsiella pneumonae* and *Proteus vulgaris* (Table 8) (Cellini *et al.*, 1996; Chowdhury *et al.*, 1991; Santhosha *et al.*, 2013; Uchida *et al.*, 1975). Extracted garlic compounds have broad-spectrum antibacterial properties acting against Gram (+) and Gram (-). The garlic compound whose antimicrobial activity is attributed to is known as allicin, an organosulfur compound from which the metabolites diallyl sulfide and diallyl disulfide are formed. The action mechanism of allicin is given by its chemical reaction with thiol groups of various enzymes (alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase); it also inhibits the formation of enzyme complexes as acetyl-CoA whose process is reversible and noncovalent (Ankri and Mirelman, 1999; Rabinkov *et al*., 1998).

1.1. Using Garlic as a Growth-Promoting Antibiotic

It has been found that garlic supplementation of $1g \bullet kg^{-1}$ for 35 days did not have a significant effect on weight gain, feed rate, feed efficiency, plasma cholesterol, triglycerides, and glucose (Horto *et al*., 1991). Nevertheless, reported that using garlic in chicken diets reduced cholesterol biosynthesis by inhibiting lipogenic enzymes (Cross *et al.,* 2011). Also, Cross *et al*. (2011) supplemented diets of broiler chickens with garlic and reported a significant weight gain after seven days of being fed. However, they reported that supplementation with garlic affects the flavor intensity of the meat, either unusual taste or garlic flavor.

It has also been reported that the total plasma cholesterol concentration, dried fecal matter, and relative weights of organs such as heart, pancreas, liver, and spleen are not significantly affected by the garlic diet. However, compared with the group of chickens supplemented with commercial antibiotic that exhibited reduced weight in the small intestine than those animals fed with garlic (Sarica *et al*., 2005).

Additionally, the use of garlic in the diet of broiler chickens has proven to lower the lipid content and cholesterol in plasma and liver (Konjufca *et al*., 1997; Sarica *et al*., 2005). In another study, Carreño-Botía and Hortúa-Lopez (2013) evaluated the use of garlic extract as an alternative to growth promoters in broilers. They reported that the inclusion of garlic extracts in diets improved weight gain and feed conversion rate; on the other hand, no effect was observed on the parameters of mortality and presence of endoparasites. In contrast, Toghyani *et al.*, (2011) used powdered garlic supplement in diets for broilers. They reported Feed intake, feed efficiency, internal organ weights and carcass characteristics were not significantly influenced by consuming de garlic, similarly, serum protein, albumin, triglyceride and Serum glutamic pyruvic transaminase were not affected by dietary treatments. Garlic powder significantly increased red blood cell count, hemoglobin concentration, and hematocrit percentage compared to the control group. Sensory evaluation of thigh meat displayed no abnormal odor or flavor in the meat.

In conclusion, the main advantage of using garlic as AGP strives on its effect without harmful side effects for broiler chickens and consumers. However, the main disadvantage is reported in some cases modifying the flavor to the meat. These aspects should be studied further in experiments with better control and/or inter-laboratory experiments.

2. Oregano (Origanum vulgare)

Oregano is the common name used to define a characteristic aroma and taste derived from a broad range of plant genera and species used as a spice. At least 61 species and 17 genera from six plant families are known as oregano being the most important *Verbenaceae* and *Lamiaceae* (Kintzios, 2012). Besides being used as condiments to flavor foods, oregano has been attributed to other properties such as antioxidant, anti-inflammatory, and antimicrobial. These properties are related to the presence of various types of phytochemicals such as phenolic compounds, flavonoids, and terpenoids among others (Baratta *et al*., 1998; Loizzo *et al.*, 2009; Kogiannou *et al.*, 2013.). Although a broad range of

active compounds of oregano has been isolated, the most important group because of their commercial and industrial applications are essential oils consisting mainly of terpenoids (Kintzios, 2012).

Oregano has brought great interest as an alternative to the use of Growth-Promoting Antibiotic because its essential oil is rich in monoterpenoids, carvacrol, and thymol that exhibit antioxidant and antimicrobial properties in vitro and in vivo along with animal digestion stimulation (Hernández *et al*., 2004). Various studies have been conducted to determine the antimicrobial properties of essential oil of oregano and evaluate their complementary use in animal diets. Table 9 shows some studies that have evaluated the antimicrobial activity of oregano extracts. The incorporation of dried oregano leaves in the diet of sheep was tested. The carcasses of lambs fed with the supplement of dried oregano leaves had a similar productive performance to the control; i.e., no significant differences between the final carcass weight and the conversion of food (Bampidis *et al*., 2005). The same research group determined the effect of including dried oregano leaves to feed turkeys by detecting the body weight of the animal, feed conversion efficiency, the characteristics of the channel, and the concentration of cholesterol in blood serum. This study showed that adding oregano to diet for turkeys does not significantly affect the parameters mentioned in these animals at 42 days of age. However, the inclusion of oregano improved feed conversion efficiency in animals at 43 to 84 days of age. These researchers suggest oregano growth promoter in turkeys (Bampidis *et al*., 2005). Meanwhile, Soultos *et al*. (2009) analyzed two diets supplemented with oregano essential oil at concentrations of 100 and 200 $mg \bullet kg^{-1}$ for feeding rabbits. It was observed that the performance parameters, the end of the channel, and feed conversion ratio of weight were not affected significantly; while the population count of *Pseudomonas spp.* and *Enterobacteriaceae* in the channels was significantly decreased compared to the control (standard diet without an inclusion of essential oil) (Soultos *et al*., 2009). Mohiti-Asli *et al*. (2015) evaluated the effectiveness of essential oil of oregano on growth and prevention of coccidiosis, an intestinal disease caused by *Eimeria*

coccidiosis in broilers. The inclusion of essential oils at 500 ppm in the diet of broilers mitigates the negative effects of coccidiosis without affecting weight gain of chickens (Mohiti-Asli, 2015).

These studies suggest that the addition of oregano essential oil in the diet for animal feeding does not affect performance parameters, but has an effect on microbial growth; so oregano is an excellent choice for use as an alternative to Growth-Promoting Antibiotic.

3. Thyme (Thymus vulgaris L.)

Thyme is an aromatic annual herb belonging to the *Lamiaceae* family that can be used fresh or dried as a spice and has various biological activities as antiseptic, expectorant, and antioxidant. It has also been reported to possess antibacterial activity against a broad number of pathogen microorganisms (Vincent, 2000). Furthermore, it has been reported beneficial effects in controlling coccidosis in chickens (Allen *et al*., 1998). These activities are related to the presence of phenolic compounds and terpenoids as thymol and carvacrol, constituting between 40 to 50% of its essential oil (Siatis *et al*., 2005).

In Table 10 some studies with thyme, extracts or essential oils on the activity and survival of microorganisms related to birds' microbiota.

There have been several studies to evaluate the use of thyme and its essential oil in the diet of poultry and determine their antimicrobial activity. Rahim *et al*. (2001) studied the effect of aqueous extracts of thyme used as an alternative to antibiotics for chickens' growth, in the efficiency of feed conversion, and blood factors. The extract showed no significant effect of the above factors.

Denli *et al*. evaluated in 2004 the effect of thyme essential oil (60 $mg \bullet kg^{-1}$ diet) assessing the parameters of growth, carcass characteristics, and organ weight in quail (*Coturnix coturnix japonica*) compared with commercial antibiotic (flavomycyn). No significant effect was observed on weight gain related to the control diet, a decrease in the percentage of abdominal fat and intestine weight,

the weight, and length of the carcass, and gizzard weight were not affected either. This group concluded that the use of essential oil of Thymus improves feed conversion efficiency and tends to decrease the percentage of abdominal fat quail; therefore, thyme is suggested as a growth promoter in quail.

Bölükbaşi *et al*., 2008 studied the effect of adding thyme at levels of 0.1, 0.5 and 1% of the total weight of the basal diet for laying hens. They found an improvement in the efficiency of feed conversion and production eggs was significantly improved with supplementation of thyme to levels of 0.5% that significantly reduced the presence of *Escherichia coli* in feces compared to the basal diet. This study agrees with the results of the study by Sarıca *et al*. (2005) who found that broiler chickens fed with 0.1% of thyme in the basal diet had a significant effect in reducing the count of Escherichia coli in the small intestine compared to the control diet.

Several studies have shown that essential oils and herbal extracts improve animal performance and have antibacterial and anticoccidial effects, but other authors report that these additives are not effective in this regard. Still they have received considerable attention as replacements to Growth-Promoting Antibiotics.

4. Moringa (Moringa oleifera)

Moringa oleifera is the best-known species of the genus Moringa, a small group of plants within the order *Brassicales*, a family that includes cabbage and radish along with the family of cress and capers (APG, 2009). The most closely related family to *Moringaceae* is *Caricaceae*, which includes papaya, sharing both, the characteristic of glands at the apex of the petiole (Olson, 2002). *Moringaceae* comprises only one genus, Moringa. Moringa embraces 13 species; *arborea, concanensis, drocanensis, drouhardii, hildebrandtii, pygmeae, pilgrim, rospoliana ovalaifolia, stenopetala, rivae, oleifera*, and *borziana*, which cover a diverse range of habits or growing ways from sorts of herbs and shrubs to large trees (Olson and Razafimandimbison, 2000; Olson, 2001; Atawodi, *et al.*, 2010). While varying greatly in form, it is very easy to distinguish a member of Moringa from any other

plant. It is a tree from the southern Himalayas, northeast India, Bangladesh, Afghanistan, and Pakistan. It is widely distributed over a large part of the planet and in Central America. It is known by several common names: Moringa, benzolivo, mlonge, mulangay, stick, kelor, Moringa, Reseda, nébéday, saijhan, and sajna among others. Its main use is as a nutritional supplement using flour Moringa leaves for this purpose. Moreover, the leaves, either ground into flour or extract have anti-inflammatory and antimicrobial antioxidant properties (Olson and Fehey, 2011). Their antimicrobial property is possibly linked to different phenolic compounds such as Benzoic acid, zeatin, quercetin, beta-sitosterol, caffeoylquinic acid, kaempferol, and especially benzyl isocyanate (Prakash *et al.,* 2007; Atawodi, 2010; (Jideani and Diedericks, 2014).

In this regard, it has been reported that leaves extracts of *Moringa oleifera* (Moringa) have antimicrobial activity against Gram (+) and Gram (-) (Viera *et al*., 2010). On the other hand, Nkukwana *et al.*, (2014) evaluated flour fodders of Moringa leaves as food sources for broiler chickens as a partial replacement of protein in a commercial fodder. The replacement of commercial feed within levels t levels ranging between 1 and 25 g per kg of feed in the starter, grower and finisher diets; did not alter the nutrient composition of the diets. Moringa flour did not reveal differences in weight gain, nutrient utilization, feed intake, and digestibility as from 2.5% level of substitution. This evidence allows to consider the Moringa leaf meal as a substitute for AGP in the production system of animal protein from birds. Further study is needed, at inclusion levels higher than 2.5% are recommended to determine nutrient flow and retention directly from digestibility.

Although the vast majority of studies have focused on Moringa leaf, there is also evidence of the antimicrobial activity of extracts of other plant organs such as fruit, bark, seeds, and roots (Table 11) (Nikkon *et al.*, 2003; Chuang *et al.*, 2007; Singh *et al.*, 2013; Arora and Onsare, 2014; Ndhlala *et al.*, 2014; Rim Jeon *et al.*, 2014; Elumalai *et al.*, 2015). These studies have shown results from mild to very high inhibition effects of different microorganisms; thus, becoming a potential source

of AGP. It is only pending studies on their cytotoxic and aggregate level in consumers to establish their degree of substitution and safety.

Other Plant Sources with Potential as Antimicrobials and/or AGP.

Herbs, extracts, and essential oils obtained from plants have been used as alternatives to antibiotics, but with contradictory results. Therefore, it is ever more important to study them for their possible antimicrobial effects and the stimulatory effect on the animal digestive System.

Table 12 shows other sources of plant extracts, essential oils and/or components of their oils.

Cinnamon extracts with methylene chloride inhibit *Helicobacter pylori* to a concentration range similar to those of common antibiotics. These properties are primarily related to the content of cinnamaldehyde and eugenol followed by carvacrol (Tabak *et al.*, 1999).

The addition of 200 $mg \bullet kg^{-1}$ and 500 $mg \bullet kg^{-1}$ of rosemary essential oil in a mixture of wheat-corn-soybean diet does not improve the overall weight gain, nor the feed conversion efficiency compared to the control diet and diet with antibiotic avilamycina (Hernández *et al.*, 2004). Furthermore, Spernakova *et al.*, (2007) reported that the addition of 500 $mg \bullet kg^{-1}$ rosemary powder sample in broiler chickens' diets shows a high gain in weight compared with the control group.

300 $g \bullet t^{-1}$ of a commercial preparation of a natural blend of essential oils from basil, caraway, laurel, lemon, oregano, sage, tea, and thyme (Tecnaroma Herbal Mix PL) were added to the basal diet of broilers. It was found that the improvement in growth performance was not dosed dependent (Khattak *et al.*, 2014).

On the other hand, Yurtseven *et al.*, (2008) found that there is no significant effect on the addition of 7.5 mL of sage extract per kilogram of food in weight gain, feed intake, feed conversion, and carcass weight compared to diets containing 0.1% of flavomycin antibiotic.

Sage essential oil has shown an inhibitory effect to *Escherichia coli* (Rahimi *et al.*, 2011).

Previous authors evaluated the effects of *Echinacea purpurea* extracts added to drinking water in the growth, immune system, blood factors, and intestinal population in broilers. They concluded that the weight of these chickens was lower as compared with the antibiotic virginiamycin, feed conversion rate is higher when the extracts are used, and the inhibition of *Escherichia coli* does not have a difference between the control diet and when extracts are added.

Roth-Maier et al., (2005) reported that the use of 10 $mg \bullet kg^{-1}$ of *Echinacea purpurea* added to the broilers diet produced a fall in food consumption and weight when comparing with the utilization of the antibiotic flavomycin. In this study, they also added the aerial parts of *Echinacea* in the diet of healthy broilers and laying hens, which did not give any beneficial effect on feed intake and animals' growth.

These authors concluded that *Echinacea purpurea* should not be considered as an alternative to antibiotics as growth promoters in animal feed.

Most essential oils exert their antimicrobial activity affecting bacterial cell walls by breaking them given the lipophilic character of oils, which accumulate themselves in membranes, and coagulating proteins. On the other hand, the outer membrane of a gram (+) contains lipopolysaccharide that forms a hydrophilic surface by creating a barrier to permeation of hydrophobic substances such as essential oils (Dorman and Deans, 2000).

These studies have shown that several herbs, herbal extracts, and essential oils have different effects on the performance of broilers, which seems related to the composition of phenolic compounds and terpenoids. The inclusion of a simple herb extract or essential oil does not always have a similar effect on bird performance. The quality of diet and environmental conditions are important in testing the inclusion of bioactive diets. So, herbs, extracts, and essential oils can influence the performance and production of secretions in broilers.

The results of previous studies warrant further research in this area to determine optimal levels of inclusion in the diet of animals and their mode of action for optimal growth and digestion.

Conclusions and Future Trends in the Field

Notwithstanding the existence of different plant constituents that can replace the use of AGPs, replacing these remains partial and its effectiveness has not been entirely convincing. It should, therefore, be complemented with other hygiene measures and animal management practices. In Sweden, ten years after the banning of AGPs, poultry production doubled reaching 68 million per year (more than 15% to export). At present, nutritional diseases are rare in Sweden (Wierup, 2001).

The banning of AGPs continues to gain ground worldwide and its trend is increasing, especially in the demand for "free" or free of contaminants, drug residues, and bacteria that cause food toxic infections ensuring consumers high-quality products at "acceptable" costs. Educating consumers to "eat more healthy foods does not necessarily cost more» (Torres and Zarazaga, 2002).

Besides, it leads to a change of approach on the sight of producers not focusing solely on lowering feed costs (IC) and compromising the quality of the final product, but also adding value to their products by guaranteeing their customers an almost zero risk to consumption.

Additionally, these actions should focus directly on animal welfare with better breeding, fattening, and products derived from them.

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Table 7. Alternatives to AGPs.

Additive type	**Possible action mechanism**
Organic acids	Inhibition of bacterial growth
Prebiotics	Stimulus of desirable bacteria in the intestinal tract
Probiotics	Introduction of desirable bacteria in the intestinal tract
Enzymes	Removing enzymes antinutritional effects of non-starch polysaccharides (NSP)
Herbs, species, extracts vegetables, Essential Oils	Multiple depends on the composition

Table 8. Antimicrobial properties of Garlic.

Extract type	**Microorganism**	**Reference**
Aqueous	*Shigella dysenteriae, Shigella flexneri* Y*, Shigella sonnei, Escherichia coli*	Chowdhury *et al.*, 1991
Aqueous (10 mM, pH 7.0, phosphate buffer)	*Helicobacter pylori*	Cellini *et al.*, 1996
Purified Aline	*Mycobacterium tuberculosis*	Uchida *et al.*, 1975

Table 9. Antimicrobial activity of essential oils extracted from oregano.

Extract type	**Chemical components**	**Microorganism/bacteria**	**Reference**
Distilled with vapor	Essential oils: Carvacrol, *p*-Cimeno	*Listeria monocytogenes* and *Salmonella enteritidis*	Pesavento *et al.*, 2015
Hidrodistilled	Carvacrol, thymol, and terpineol	*Staphylococcus aureus*	Marques *et al.*, 2015
-	Essential oils	*Listeria monocytogenes*, *Escherichia coli* and *Salmonella enteritidis*	Siroli *et al.*, 2015
Orego-Stim®	Essential oil	Sporulated oocysts of *Eimeira acervulina*, *Eimeira máxima* and *Eimeira tenella*	Mohiti-Asli and Ghanaatparast-Rashti 2015
Orego-Stim®	Essential oil	*Pseudomonas* spp., *Enterobacteriaceae*, *Brochothrix thermosphacta*, Yeast and fungi.	Soultos *et al.*, 2009

Table 10. Antimicrobial activity of essential oils extracted from thyme polyphenolic compounds.

Material or extract	Chemical composition	Strain or microorganism	Reference
Thyme powder basal diet	Flavonoids Phenolic Acids	*Escherichia coli*	Sarıca *et al.*, 2005 Bölükbaşi *et al.*, 2008
Essential Oil	Thymol Carvacrol	*Clostridium perfringens* *Escherichia coli*	Acamovic and Cross, 2007. Mitsch *et al*., 2004.

Table 11. Antimicrobial activity of phytochemicals extracted from Moringa.

Part of the plant	Chemical component (s) Component group (s)	Bacterial strain/ microorganism	Extract type	Reference
Leaves	Alkaloids, Flavonoids Phenolics compounds	*Staphylococcus aureus* *Bacillus subtilis* *Pseudomonas aeruginosa* *E. coli* *Proteus mirabilis Candida albicans Candida tropicalis*	Aqueous	Elumalai *et al.,* 2015
	Flavonoids Phenolic compounds	*Klebsiella pneumoniae* *Staphylococcus aureus* *Candida albicans*	Ethanol 70%	Ndhlala *et al*., 2014
	Essential oils	*Trichophyton rubrum* *Trichophyton mentagrophytes* *Epidermophyton Xoccosum* *Microsporum canis*	Ethanol	Chuang *et al.*, 2007
Pod/ Fruit	-	*Staphylococcus aureus* *Staphylococcus epidermidis* *Escherichia coli,*	Acetone	Arora and Onsare, 2014
Seeds	Gallic acid Catechin Epicatechin p-Coumaric acid Ferulic acid Vanillin Caffeic acid Protocatechuic acid Cinnamic acid Quercetin	*Staphylococcus aureus* *Bacillus cereus* *Escherichia coli Yersinia enterocolitica*	Ethanol Methanol Acetone Hexane Chloroform (10:1(v/w))	Singh *et al.*, 2013
	4-(α-L-rhamnosyloxy)-benzyl isothiocyanate	*Staphylococcus aureus* *Escherichia coli* *Pseudomonas aeruginosa* *Candida albicans* *Aspergillus niger*	Ethanol 70% Methanol	Rim Jeon *et al*., 2014
root bark	Desoxy-niazimicin aglycone	*Shigella boydii* *Shigella dysenteriae* *Staphylococcus aureus* *Bacillus megaterium* *Candida albicans* *Aspergillis flavus*	Chloroform	Nikkon *et al.*, 2003.

Table 12. Antimicrobial activity of vegetable extracts.

Plant/Extract	**Chemical composition**	**Microorganism**	**Reference**
Cinnamon (*Cinnamomum verum*) Extract with methylene chloride	Cinnamaldehide Eugenol Carvacrol	*Helicobacter pylori*	Tabak *et al.,* 1999. Mitsch *et al*., 2004
Cinnamon (*Cinnamomum verum*)	Cinnamaldehide	*Escherichia coli*	Chang *et al.,* 2001
Echinacea (*Echinacea purpurea*)		*Escherichia coli*	Rahimi *et al*., 2011
Sage (*Salvia officinalis*)	α-pinene 1,8-cineole	*Escherichia coli*	Tzakou *et al.*, 2001

CAPÍTULO V: INCLUSION OF MORINGA LEAF MEAL (*Moringa oleifera*) IN FODDER FOR FEEDING JAPANESE QUAIL (*Coturnix coturnix japonica*) AS GROWTH PROMOTER

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Abstract

This research evaluated the nutritional, nutraceutical, antimicrobial, as well as the growing promoter effect of *Moringa oleifera* (MOR) leaves flour in fodders for fattening Japanese quails. The antimicrobial activity was measured using the method of Kirby-Bauer (disk agar diffusion technique) and its nutrient composition was also determined. A completely random design with 4 x 2 factorial arrangement was used, fodders included 0, 7, 14 or 21% of MOR, and 0 or 100 ppm of Virginiamycin (Antibiotic Growth Promoter, AGP) during 35 d of fattening, 480 one-day old unsexed quails were used, each treatment had 5 replicates with 12 quails in each cage. Body weight and consumption of food were measured at 14 and 35 days of experimentation. Characteristics of blood in males and of carcass in both sexes were measured at the end of the experiment. MOR inhibited the growth of bacteria gram (+) and gram (-). The inclusion of MOR in the period from 1 to 14 d inhibited the weight gain ($P < 0.001$), increased feed conversion ($P < 0.059$), without affecting the feed intake; however, in the period of 15 to 35 d MOR did not affect weight gain and the feed intake; the hematological and biochemical profile were within the normal range for quails. The inclusion of MOR decreased ($P \leq 0.001$) cholesterol and triglycerides concentrations. Levels of aspartate aminotransferase (AST), alanine transferase (ALT), and creatinine decreased ($P \leq 0.001$) when the amount of substitution of MOR was 21%. The carcass weight and its performance with MOR up to 14% was similar ($P > 0.001$). The results of this experiment showed that flour from leaves of *Moringa oleifera* is a viable alternative to be included up to 21% in commercial diets of birds offering an option for AGP replacement without compromising the health of the animal and therefore its productivity.

KEYWORDS. *Moringa oleifera*, nutriments, antioxidants, phenolics.

Introduction

Food additives as antimicrobials or antibiotics growth promoters (AGP) play an essential role in the economic development of modern poultry production, which yields into benefits for producers and consumers of animal products (Brizuela et al., 2009). Additives are used in animal feed with three fundamental aims: to improve features in raw materials and fodders or animal products; to prevent diseases, and to increase the efficiency of animal production. However, due to the risk that AGP create cross-resistance with antibiotics used in human medicine and by the presence of these compounds in animal products, its use has been drastically reduced and prohibited in some cases for the formulation of fodders animal breeding (Gauthier et al., 2011). However, some researchers have suggested that the removal of these substances may cause an increase in the incidence of microbial infections (diarrhea, intestinal necrotic enteritis, and coccidiosis) (Ramírez et al., 2013). Thus, there's been the need of finding alternatives to the use of growth promoting antibiotics (Gauthier et al., 2011). Among these alternatives, the most used are the probiotics, prebiotics, enzymes, essential oils, herbs, spices, and vegetable extracts (Brizuela et al., 2009). In this sense, there are reports that extracts from leaves of *Moringa oleifera* (Moringa) possess antimicrobial activity on Gram positive and Gram negative bacteria (Devendra et al., 2011). In addition, the leaves have nutritional and nutraceutical properties (Makkar y Becker, 1996), since they are characterized by their high content of proteins, vitamins and minerals, and low levels of anti-nutritional substances, so it can be considered free of lethal doses or adverse effects; For that reason, they are traditionally used in Asia and Africa in animal feed (Makkar and Becker, 1996; Singh et al., 2009; Fahey, 2005). On the other hand, the content of total phenols (105.04 mg EAG/g) and its antioxidant capacity (85.77%) of methanolic extracts of Moringa leaves show their antioxidants properties. Therefore, the objective of this study is to determine the effect of the consumption of feed supplemented with Moringa leaves (*Moringa oleifera*) on the physiological state of Japanese quail (*Coturnix coturnix japonica*), according to its antimicrobial, nutritional and nutraceutical features.

Materials and Methods

Materials and Chemical Analysis

Plant Material. Mature leaves of *Moringa oleifera* (Moringa) collected during the month of March 2015 from a crop located in the town of "La Campana", Culiacán, Sinaloa, Mexico (24 ° 59'21. 17 ' N, 107 ° 34'27 25 "W, at 120 m above sea level). Material plant was washed with a solution of 150 ppm of sodium hypochlorite, for its later drying in an electric oven at 55-60 °C during 6 to 8 h until a constant weight to determine its humidity. Finally, it was smashed through a fine mill to obtain a homogenous particle size Moringa leaf flour. White corn (Maize) and soybean (Soya) intended for human and animal food paste was purchased in a local marketing company. Before developing the experimental diets, broken, damaged by insects, and immature grains were pulled out from the white corn, as well as of impurities. It was subsequently processed with a Thomas-Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ, USA) with two mm mesh.

Chemicals Analysis. The nutrient content and energy were analyzed according to the recommendations of The Official Methods of the Official Analytical Chemists Association (AOAC, 2000): moisture (method 934.01), fat (EE) (method 920.39), protein (CP) (method 960.52), dietary fiber (DF) (method 985.29), crude fiber (CF) (978.10 method), ashes (method 942.05) and total carbohydrate (TC), were determined by the method of difference of [100 - (CP + EE + humidity + ash)] in percentage. The content of metabolizing energy was calculated through the formula EM (Mcal/kg) = 3.75 x CP + 8.09 x EE - 6.95 x CF + 3.94 x TC (Moir et al, 1980). The concentration of calcium (Ca) and phosphorus (P) in Moringa, corn and soybeans were quantified in accordance with the official method of the AOAC # 955.06 (2005). After an acid digestion of their ashes, the sample was filtered and diluted to 100 mL with deionized water, an atomic absorption spectrophotometer (AA system serie200 and GTA120 of Agilent Technologies, USA) was used to read the absorbance for each mineral in specific wavelengths: Ca (422.7 nm), Na (589.6 nm), K (769.9 nm), Mg (285.2 nm), Mn (virus infection 279.5 nm), faith (248.3 nm), Cu (324.7 nm) and Zn (213.9 nm). A reference of

known concentration standards calibration curve was used for each mineral. The concentration of each of the minerals is calculated in ppm for microelements and g/kg for macroelements.

The amino acids profile was performed using the technique for the detection and quantification of amino acids by high-performance liquid chromatography (HPLC) according to Vázquez et al. (1995), with minimal variations. These were determined in Moringa, corn, and soybean used to develop the experimental diets. To do so, the Varian liquid chromatography system was used (Palo Alto, CA), high resolution 9012 model, adapted with a fluorescent 9075 Varian detectors, a 10 µL volume injector, a PDS RPC column C18 10 cm x 4.6 mm, ID 3 µm, 100 A; and for cysteine, a Phenomenex Gemini column 5µ C18 110A 150 x 4.6 mm, ID 5 µm. Hydrolysis. Approximately, 3 g of sample were weighed whose moisture was removed and degreased by the methods previously described. Sample preparation: after the sample degreasing, 3 mg of it were taken for those in which the protein content was lower than 40%, and 1 mg for those whose protein content was more than 40%. The samples were placed in tubes for hydrolysis (Pierse 29560) and 3 mL of HCL 6M. Vacuum was applied for 3 min to later place the tubes in a dry toilet at 110 °C for 12 hours. To remove the HCL 6M and obtain the sample, this was put in rota-evaporation at a temperature of 65 °C (Brinkmann Buchi RE 121), through 3 washed with distilled water by adding the same volume of HCL (3 mL). Then samples were collected with a 0.2 sodium citrate buffer pH 2.2, N. The samples were immediately labeled and tested or stored at 0 °C. Derivatization. An aliquot of 100 µl of the hydrolysate was taken and added with 40 µL of internal standard 2.5 µmol/mL, this was diluted to 1 mL with sodium citrate buffer pH 2.2. 250 µL of the dilution and 250 µL of OPA (Ortho-phthalaldehyde) solution were taken into a syringe for chromatography. The mixture of these two solutions lasts 2 minutes to immediately undergo a filter (0.2 µm) separation. 10 µL of the derivative were taken and injected into the chromatograph. Oxidation. For the determination of cysteine as cystic acid, the samples were subjected to a prior oxidation to the hydrolysis. The oxidation

consisted in the use of performyc acid (90%) and peroxide (30%) as oxidizing agents (9:1 v/v). The oxidizing solution was prepared and maintained at room temperature for 1 hour; subsequently, it was submitted to a cold bathroom at a temperature of 4 °C for 15 min; then 1 mL of oxidizing solution was added to the tubes of hydrolysis with 1 mg of sample previously weighed; these were then submitted to a dry bath at a temperature of 50 ° C during 15 min. For the removal of the oxidant solution, a lyophilisation with a Labconco Freezone 6 plus serial 051044488A number freeze-drying was applied. Then it was followed a hydrolysis with HCL 6N for 12 hours at 110 °C. A mobile phase with solvent A was used: methanol and the solvent B: sodium acetate buffer (0.1 M, pH 7.2), methanol and tetrahydrofuran, which are used as an organic modifier (900:95:5 v/v/v), (Sigma Chemical Co.). The identification and quantification of amino acids were carried out by comparing the retention time of the control sample with the standard. To do so, the chromatography system was connected to a software (Barian Star Chromatography version 4.0) where the readings of the peaks in areas at wavelengths of EX = 340 nm and EM = 455 nm were reported.

The determination of the composition of fatty acids was analyzed by the methods of Folch et al. (1957) and the standard method of the AOAC 963.22 (1998) with some modifications. Previously, the following reagents were prepared: Folch reagent: NaCl 0.73%. Weigh 7.3 g of NaCl and filling to 1 liter, and NaCl 0.58%. Weigh 5.8 g of NaCl and diluting to 1 liter. Removal of fat. Firstly, 10 g of the sample were weighed and placed in an Erlenmeyer flask of 250 mL, mixed with 60 mL of the reagent of Folch (1 volume of methanol plus 2 volumes of chloroform), and finally homogenized. Subsequently, it was vacuumed with a Buchner funnel, the residue was mixed with 50 mL of the Folch reagent and homogenized again. It was vacuum filtered again. The residue was washed with 50 mL of Folch reagent, the flask cleaned, and vacuum filtered once more. The filtrates (60 + 50 + 50 mL) were mixed in a separating funnel and added with 40 mL of sodium chloride 0.73%, stirred vigorously, and left decanting overnight. The next morning, the lower phase (organic) (F1) was decanted and filtered through

anhydrous sodium sulfate. The filtering was recovered in a flat bottom round flask. The upper phase (F2) was washed with 50 mL of a mixture of 20% NaCl (0.58%) and 80% of Folch reagent, stirred up, and left to rest for 2 hours, then decanted and filtered on anhydrous sodium sulfate getting F3. F1 and F3 were mixed, dry evaporated in the rota evaporator. Methylation: After evaporating the chloroform, sodium hydroxide 0.5 N in methanol and 3 glass pearls were added. The flask was connected to a Rosario refrigerant and subjected to reflux for 10 minutes. After, through the top of the condenser boron trifluoride (BF3) was added and refluxed for another 5 minutes, then 4 ml of heptane were added and refluxed for 2 minutes. The ball flask was removed from the heat and its content added to a test tube. Saturated NaCl was added and gently shaken until its milky white color changed. Subsequently, a piece of sodium sulphate was added to separate the fatty acids. The top of the mixture was taken out and filtered by a Pasteur pipette previously packed with glass fiber, and the filtering was recovered in a 2 mL vial. The vial was saved in a nitrogen atmosphere and was subsequently placed in the freezer. 1 µL of the sample was injected into a gas chromatograph. The equipment used was a gas chromatograph (Varian CP-3800, USA) with a flame ionization detector (FID) equipped with a column Omegawax 320 30 m x 0.32 mm ID, 0.25 mm (Supelco, USA). Helium was used as the carrier gas at a flow rate of 3 mL/min. The oven temperature was maintained at 140 °C for 5 min, preset at a maximum temperature of 240 °C at a speed of 4 °C during 1.5 min. Both the temperature of the injector and detector were set at 260 °C. For the identification and quantification of fatty acids, the retention times of the sample were compared with those of a standard mixture that consists of 37 fatty acid methyl esters (Supelco, Bellefonte, USA). The results were expressed in percentage of fatty acid contained in the sample.

Characterization Nutraceuticals

Total Free and Bound Phenols. The content of total phenols both free as linked was determined following the methodology of Swain and Hillis (1959). **Free Phenolic Compounds Extraction.** 1.0 g of sample was weighed, later added

with 10 mL of cold methanol 80%, and mixed using the Ultraturrax (brand IKA Works, model T25, North Carolina, USA) tissue homogenizer. The mixture is agitated at 200 rpm for 2 hours at room temperature in protected from the light. Subsequently, the sample was centrifuged at 10 000 rpm for 15 min at 4 ºC. Finally, the supernatant was collected, which was the extract to be used in the quantification assay. The precipitated material (pellet) should not be discarded since it would be used for the removal of the linked phenols. **Phenolic Bound Compounds Extraction.** The pellet obtained in the previous step was added with 10 mL of NaOH 2N and shaken in a vortex. The mixture obtained was exposed to a flow of nitrogen to remove the oxygen, and subsequently put in a water bath for 30 min at 95°C with agitations every 10 min. Next, the sample was maintained in agitation during 1 h at 25°C. Afterward, 2 ml of concentrated HCl were added to the sample and homogenized in a vortex. Then 10 ml of hexane were added to the sample by the walls of the tubes and was centrifuged at 10,000 rpm for 10 min at 10°C. The supernatant was then discarded and the pellet was washed 5 times with 10 mL of ethyl acetate each time. The ethyl acetate is evaporated completely in the rota-evaporator using the vacuum at a temperature of 35 °C. To reconstitute the linked phenols 2 mL of methanol 80% were added. **Folin-Ciocalteu Test**. A 15 µL aliquot of the obtained extracts in the previous stages was taken of and placed in a microplate of 96 holes (Cost, USA), diluted with 240 µL of distilled water and added with 15 µL of Folin-Ciocalteu reagent 2N. then it was incubated for 3 min at room temperature (25 °C). To suspend the generated reaction and reveal its color 30 µL of Na_2CO_3 4N was added, subsequently incubated for 2 hours in the absence of light. A reader of Microplates Synergy HT (BioTek, Inc, USA) was used to Measure the absorbance at 725 nm using methanol as white test. The total free phenols and bound were determined from a standard curve of gallic acid at concentrations of 0 to 0.4 $mg \bullet mL^{-1}$ and the results expressed in equivalents mg of gallic $acid \bullet g^{-1}$.

Determination of Free and Total Linked Flavonoids. It was performed according to the methodology described by Ebrahimzadeh et al. (2009), with slight

modifications. From the methanolic extracts obtained for free and linked phenols above, 20 µL were taken and put in a plate of 96 holes. Subsequently, 112 µL of distilled water, 60 µL of methanol, 4 µL of aluminum chloride to 10%, plus 4 µL of potassium acetate 1M were added. Finally, the samples were incubated in the dark for 30 min, then placed on the Microplate Reader Synergy HT (BioTek, Inc, USA), and the absorbance read at 415 nm. The total content of flavonoids (TFC), both free as linked are expressed as milligrams equivalent of quercetin per gram of dry extract (mg EQ/g of dry extract).

Analysis of Antioxidant Capacity (Oxygen Radical Absorbance Capacity, ORAC). From the methanolic extracts obtained for free and bound phenols, an aliquot of 25 µL of the supernatant (if necessary, dilutions would be made with phosphate buffer 75mM), 25 µL of a white test (phosphates buffer), and 25 µL of a standard curve of Trolox were taken using a microplate of 96 holes with dark walls and light bottom (Cost, USA). The plate was filled and placed in a reader of Microplates Synergy model HT (BioTek, Inc, USA) which was set a temperature of 37°C of incubation. The Microplate device reader displayed in each hole of the plate 200 µL of fluorescein 0.96 µM and 75 µL 2,2' - Azobis, 2-amidino-propane dihydrochloride (AAPH) 95.8 µM, initiating the reaction once added this latter reagent, measuring the fluorescence for 70 min with intervals of 70 s to a wavelength for excitation of 485 nm and 580 nm for emission. The calculations were made using the linear regression equation of a standard curve of Trolox (6.25, 12.5, 25, 50, 75 and 100 µM) and the area under the curve of the loss of fluorescence. The results are expressed in mol Trolox equivalent•g^{-1} (Huang et al., 2002).

Analysis of Antioxidant Capacity (Method DPPH)**.** From the methanolic extracts obtained for free and linked phenols. In the first three cells on the plate, 20 µL of methanol 80% (white) were added. Using a microplate of 96 transparent holes, an aliquot of 20 µL of the extracts was taken. Subsequently, using a multi-channel micropipette 280 µL of DPPH (2,2-diphenyl-1-picrilhidrazil 200 µM) (including white cells) were added. After filling the plate in, this one was roofed and left to

incubate for 30 min in the dark. When the time went over, the plate was put into the Microplate Reader Synergy HT (BioTek, Inc, USA) to read the absorbance at 540 nm. The calculations were made using the linear regression equation of a standard curve of Trolox (6.25, 12.5, 25, 50, 75 and 100 M) and the area under the curve of the loss of fluorescence. The results are expressed in Moles Trolox equivalent•g^{-1} (Karadag et al., 2009).

Phenolics UPLC-DAD. Main diet components based on different types of free or conjugated chemical interactions by UPLC chromatography with diode array detector (DAD) were separated by liquid chromatography (Corral-Aguayo et al., 2008). Of the methanol extracts obtained for free and conjugated was filtered through to Sep-Pak C18 cartridge. An aliquot of 20 uL was injected into a liquid chromatograph (Acquity UPLC) (Water Inc, USA) with a diode array detector (PDA). An Acquity UPLC BEH C18 column of 100 mm length x 2.1 mm in diameter with a particle size of 1.7 microns and a pore size of 100 Å was used. The mobile phase "A" used was a mixture of 95% water, methanol 2% and 3% formic acid, and phase "B" was a mix of 95% methanol, water 2% and 3% formic acid , using a gradient: 0 min, 90% to 10% B (0.3 mL•min); 3 min, 75% to 25% B (0.3 mL•min); 5 min, 70%, 30% B (0.25 mL•min); 9 min, 60% to 40% B (0.3 mL•min); 11 min, 50% to 50% B (0.3 mL•min); 12 min, 0% to 100% B (0.3 mL•min); 13 min, 0% to 100% B (0.3 mL•min); 15 min, 90% to 10% B (0.3 mL • min); 16 min, 90% A, 10% B (0.3 mL • min) with a flow of 0.3 mL/min. The reading was performed at 190 and 420 nm. Quantification of phenol was conducted using standard calibration curves of chlorogenic acids, gallic, ferulic, coumaric, and t-cinnamic acid (Sigma Chemical Co., USA) using concentrations from 5 to 50 µg/mL. The chromatographic profile was originated by readings at a λ of 320 for all standards, except for gallic acid (λ=271 nm). The results were expressed as mg/g, the corresponding phenolic compound in dry base.

Profile of Flavonoids by UPLC-DAD. Of the methanol extracts obtained for free and conjugated was filtered through to Sep-Pak C18 cartridge. An aliquot of 20 uL was injected into a liquid chromatograph (Acquity UPLC-) (Water Inc, USA)

with a diode array detector (PDA). An Acquity UPLC BEH C18 column of 100 mm length x 2.1 mm in diameter with a particle size of 1.7 microns and a pore size of 100 Å was used. The binary mobile phase was prepared by (a) water acidified with phosphoric acid to 0.05% and (B) methanol HPLC grade to 80%, which were previously gas-free and filtered, using the following gradient 0 min 80% and 20% of B; 2.5 min 60% of A and 20% of B; 6 min 40% of A and 60% of B; 7 min 20% of A and 80% of B; 8 min 20% of A and 80% of B; 9 min 80% and 20% of B; 12 Min 80% and 20% of B, with a flow of 0.167 mL/min. The quantification of flavonoids was performed using calibration curves of standards of quercetin, kaemferol, catechin, apigenin, routine, naringin and hesperidin (sigma Cheical Co, USA) at concentrations of 5 to 50 µg/mL. The chromatographic profile was originated by readings at a λ=280 nm. The results were expressed as mg/g of the corresponding flavonoid compound in dry base.

Antimicrobial Activity. Antimicrobial activity was evaluated by the paper diffusion method described by Prabuseenivasan et al. (2006) with modifications. *Escherichia coli, Staphylococcus aureus, Salmonella Typhimurium, Candida albicans, Pseudomonas Aeruginosin* and *Listeria monocytogenes*. A bacterial suspension was prepared was obtained, two or three previously selected colonies isolated and placed into tubes containing 108 mL of sterile solution to 0.87%. Proceeded to adjust the suspension to a concentration of 10 CFU/mL. Of each bacterial suspension samples with a sterile swab was taken, was rotated against the wall of the tube to remove excess inoculum and spread *Moringa oleifera* were diluted with 10% dimethylsulphoxide (DMSO) containing 0.5% Tween 80 (v/v) and sterilized by filtration through a membranous filter of 0.45 µm. The dilutions of the methanol extracts were 1: 1 (6.6 mg/disc), 1: 5 (2.6 mg/disc) and 1:10 (1.3 mg/disc), and added 5 µL of each dilution on filter paper discs sterile (5 mm diameter Whatman # 1), were placed in the center of the plates and incubated at 35 °C for 24 h. This was carried out in triplicate. Was used as blank 5 µL DMSO (solvent) and positive control as ampicillin (Sigma-Aldrich cas 49975) at the same concentrations as methanolic extracts. After the incubation time, and with the help

of a calibrated digital vernier caliper diameter halo of inhibition of bacterial growth for each disk was measured in mm. The results reported were based on the proposal by escal Ponce et al. (2003) is not sensitive, if the total diameter was less than 8.0 mm; sensitive to 9-14 mm; highly sensitive to 15-19 mm; and extremely sensitive to inhibition diameters greater than 20 mm.

Diets

Fodders Formulation and Preparation of Diets. Fodders were made according to the nutritional requirements for Japanese quails and the standard NCR (1994) guidelines of the Council of production for these organisms. Four corn-based with soybean paste diets with different substitutions of 0, 7, 14, and 21% per Moringa flour without and with APC (0-100 ppm of Virginiamycin, Eskalin, PB Animal Health of Mexico S. de R.L. de C.V.) in the same proportions of Moringa were elaborated. The rest of the diets ingredients (white maize, soybean oil, sea salt, methionine, limestone, orthophosphate, vitamins for fattening and minerals) were mixed in equal amounts with the protein sources to meet the quails' requirements according to the growing and fattening stages (Table 13).

Birds Housing

Location and climate. The productive response test was performed in the Poultry Unit and in the Food Analysis Laboratory of the Veterinary Medicine and Zootechnics Faculty of the Autonomous University of Sinaloa, located in the city of Culiacán, Sinaloa, Mexico. The test was performed from February to March, with an average temperature and relative humidity of 30 °C and 67%, respectively.

Animals and management. The institutional ethics Committee for the care and use of experimental animals of the Veterinary Medicine and Zootechnics Faculty of the Autonomous University of Sinaloa approved the experimental protocol of this investigation. The feeding test lasted 35 days. 480 unsexed three day old quail chicks (12.13± 0.14 g) were used. The chicks are distributed randomly in 40 (90 cm x 90 cm x 60 cm high) metal cages placed over 60 cm from the floor (12 birds per cage). They were provided with heat (35 to 38 °C) using incandescent

light bulbs during the first three days; from 32 to 35°C until the seventh day. On the second week temperature was reduced at a rate of 5 °C (Lucotte 1990). To ensure that birds had a suitable environment blue towel bed 75147 (Scott, KIMBERLY-CLARK, USA) was placed on the cages floor. The walls and ceilings were covered with plastic sheeting during the first 10 days. These devices were gradually removed according to the increasing birds age. During the first three days a 25 x 18 x 2.5 cm dish type feeder and a 1/2 L glass barrel sprue were placed in each cage. On the fifth day, a 25 x 17.5 x 25 cm semi-automatic floor chute type feeder and a 1 L glass barrel sprue were placed. From the second week on, the chute type feeders were risen 2.5 cm by means of steel profiles, while preserving the same sprues. Within two weeks and a half, the feeders were raised 5 cm with steel profiles and a 2 L glass barrel sprue. To promote the animals' welfare, the cages were placed in a conventional booth, equipped with adjustable in height black plastic curtains, according to the room temperature, air currents, and sunlight. The food was served at 7:00 p.m. and the provided amount was recorded. At the end of the week, the food consumption per bird (offer less rejection of food between the number of animals) was estimated. During the course of the experiment weightings at the end of each period (14 d and the 35 d) were made to register the gain of weight (initial weight – final weight), and feed efficiency (gained weight between feed intake). Mortality was daily accounted and recorded. Dead or discarded birds were not replaced. Within two weeks, the food and the birds were weighed with digital scales (OhausMR, capacity of 2 610 g and precision 0.1 g). At the end of the experiment, the quails were weighed with digital scales (TorreyMR), with a capacity of 20 kg and precision of 1 g. To know the weight of the carcass and its performance percentage, all 35-day-old survived animals were sacrificed on the basis of the established procedures by the Official Mexican Norm NOM-033-ZOO-2015. They were processed according to the Genchev and Mihaylov protocol (2008) with slight modifications.

Experimental Procedure

Nutritional Effect**:** To assess productive response the following indicators were used: feed intake (FI), weight gain (GP), and feed conversion rate (FCR); to do so, a design with two crossed factors was used: AGP and Moringa through repeated measures in time, which were measured at the end of each phase. For live weight at slaughter (LWS), weight of the hot carcass (WHC), and yield carcass (YC) a design with two factors was used: AGP and Moringa, measured at day 35. The birds were sacrificed with 3 h of fasting.

Nutraceuticals Effect**.** On day 35, from the jugular vein of each decapitated quail blood samples were extracted and put into test tubes with and without anticoagulant. Subsequently, blood chemistry analysis (serum cholesterol, triglycerides, uric acid, ALT, AST, creatinine, and glucose) was performed, and for hematic biometry (hematocrit, hemoglobin, leukocytes, proteins, halterophiles, and lymphocytes) birds were made to go on a diet for 8 hours. There was a design of a factor (diets), completely at hazard, taking as experimental unit the selected quail at random from each cage for a total of 5 replicates per treatment.

Statistical analysis.

Data from the experiment was analyzed as a completely random 4 x 2 factorial arrangement design. Two nest factors: MOR: 0, 7, 14 and 21%, and AGP: 0-100 ppm. For the productive response, a crossed factor of the feeding periods was included: 1 to 14 d initiation and completion of 15 to 35 d, the experimental unit was every cage, and this was considered as a random effect (Table 14). The main effects and interactions of the first and second order were tested. For hematologic variables the main effects and interaction of the first order were also proven, the experimental unit was every sample of blood. For carcass data, the effect of sex was included in the analysis, as well as all the possible interactions, the unit of observation was each carcass. All the collected data was analyzed using the statistical package Minitab v. 17. The Declaration of statistical difference was based on a value of $P \leq 0.05$. When some main effect was significant, the Tukey multiple comparison test was used.

Results and Discussion

Chemical and Nutritional Composition of the Main Components of the Diet.

The results of the proximal composition of the main components used in the feeding test of japanese quail are shown in Table 15. The contents of the PC (28.90±0.42%), EE (12.63±0.27%), and ash (0954±0.66%) for Moringa are similar to values reported by Yaméogo et al. (2011), 27.2±0.8 PC, 17.1±4.5 EE and 11.1±4.1 of ashes, in plants with similar climatic conditions and management; highlighting the high content of PC; due to this, it is a viable option for its use in the replacement of the PC of Moringa leaves' flour by the PC of the soybean meal due to the fact that both cover the requirement of 24% PC (NRC, 1994). On the other hand, the levels of FD are similar in their content. Therefore, it is expected that a similar effect in quail by this fact since this should be limited to the same as the FC (Fracanzani, 1996). At the same time the value of metabolizing energy (3485.15± 42.51) of Moringa is similar compared to the value of metabolizing energy (3597.87±30.40) of the soybean paste that has previously been referred (Ríos-Rincón, 2014). This would ensure its energy value on the general balance of the formulation of the diet coupled with the contributions of maize. For this reason, it is no longer necessary to add soy oil to the formulation of diets, which in addition to reduce powders, gives them energy. Being the established values of EM for quail on initiation diet (2800 and 2900 Kcal/Kg), and for fattening diet (2900 and 3100 Kcal/Kg) (NRC, 1994).

The recommendations of the NRC (1994) are not only that the amount of PC must be around 24% but also this PC must provide the limiting amino acids as methionine (0.28%), threonine (1.02%), lysine (1.30%), and cysteine (0.50%). In that order, as you can see Moringa provides alone the requirements of these amino acid with the exception of cysteine (Table 16). However, in the balance of the diets (Table 13) the requirements of these amino acids are covered, not having the need to add them to the formulated diets.

The mainly limiting minerals established by the NRC (1994) are calcium (0.80%) and phosphorus (0.30%) present in Moringa above these requirements.

Although it also covers the needs of other minerals such as in the case of iron, manganese, potassium, and zinc (Table 17).

Linoleic acid is the essential metabolically fatty acid. Its deficiency can easily give signs of the loss of the integrity of the membrane in the cells. Its requirement is even greater to maintain a satisfactory egg's weight. The requirements are 1% of linoleic acid (NRC, 1994). As noted (Table 18) this requirement is covered six times more by Moringa, having the security that there will be no affectations by linoleic acid deficiency.

The results of phenols content and total flavonoids in Moringa are present from 8 to 18 times higher than that in soybean paste and white maize, according to the antioxidant activity (Table 19). These effects are associated principally to compounds such as phenolic acids (chlorogenic, caffeic, and cumaric), and flavonoids (Kaemferol and routine) (Table 20) present in Moringa mainly in greater quantity. Because of this, it is expected that the nutraceutical and antibacterial effect came mainly from Moringa.

Antimicrobial activity

This effect can be seen in the evaluation of the antimicrobial activity of Moringa extracts, soybean meal, and white corn where the only material that had activity was the Moringa extracts. Table 21 shows that the highest inhibition was observed in Gram (+) bacteria, followed by the Gram (-) and fungus (Figure 1). This activity is related to the phenolic compounds and flavonoids present in Moringa (Devendra et al., 2011); Ndhlala et al., 2014). This effect is mainly associated to compounds such as phenolic acids (gallic acid, chlorogenic acid, ferulic acid and ellagic acid) and flavonoids (quercetin and kaemferol) present in Moringa (Prakash et al., 2007); Jideani and Diedericks, 2014). Because of this, the nutraceutical and antibacterial effect occurs primarily due to the extracts of Moringa. These effects and the structural requirements are not fully defined for the antimicrobial activity. There are studies that show there must be at least hydroxyl (-OH) and methoxy (-OCH3) groups, and some degree of lipophilicity

(Modak et al., 2002; Sánchez-Maldonado et al., 2011) as it is the case of phenolic compounds found in Moringa. These groups provoke an oxidative phosphorylation, causing a pH elevation; and therefore, toxicity. Although these groups are in greater proportion in flavonoids, in particular in flavones (Mukne et al., 2011), Sánchez-Maldonado et al., (2011) found that phenolic acids such as benzoic acid, cinnamic acid, hydroxybenzoic acids (p-hydroxybenzoic, protocatechuic, gallic and syringic) and hydroxycinnamic acids (p-coumaric, caffeic and ferulic), the antimicrobial activity of hydroxycinnamic acids was comparable or greater than the hydroxybenzoic acids with the same number of hydroxyl groups. The greater number of hydroxyl groups polyphenolic compounds have, the more efficient uncoupling compounds they become, transferring more protons per molecule (Omojate et al., 2014), raising their level of lipophilicity (Modak et al., 2002; Mukne et al., 2011). This effect is due to the high grade of hydrophobicity or lipid solubility, allowing with this, the separation of the lipidic structure of the membrane cell and mitochondria, messing its structure what causes its permeability, letting the migration of ions and another compounds to happen, resulting in an imbalance homeostatic (Rosas-Gallo and Lopez-Malo, 2011); and therefore, exercising a cytotoxic effect in the cells (García-García and Paulo-Garcia, 2008).

Productive Response of the Japanese Quail in Fattening

The substitution of soybean meal by Moringa (MOR) flour strongly affected the weight, and therefore the weight gain in the period of initiation of 1-14 d, the MOR factor is statistically significant ($P<0.001$). The more the inclusion of MOR increased at 0, 7, 14, and 21% and soybean meal dropped by about 4% in the experimental diets, it was observed a progressive reduction in weight, which resulted in lower weight gain at 14 days regarding the control of 4.72%, 8.76, and 17.93%, respectively. this effect is mainly due to MOR, since food consumption was kept at similar values ($P>0.05$), although a statistical significance was appreciated in diets containing AGP ($P<0.050$). The conversion rate increased as the inclusion of MOR increased in the diet during the 14- day experimental period

(P<0.001). In 35-day-old quails, the replacement of the 0, 7, 14 and 21% of MOR affected significantly the weight (P<0.001); however, the gain of weight was similar, having significant difference between the weight at 0% (235.70 g) and 21% (218.26 g) of replacement of MOR, not showing significant difference for AGP (P>0.05). Due to the previous effects, feed conversion increased significantly at 35 days of age (P< 0.05). Food intake was not affected by the replacement of MOR flour (P>0.05), but it was by the presence of AGP in the diet. The highest weight gain was observed at 0, 7, and 14% of inclusion of MOR, and the lowest was obtained by the inclusion of 21% of MOR although the latter had greater weight recovery in the same period while diets with and without AGP were kept in intermediate weights not presenting significant differences between them (P=0.080) although a tendency to be higher with AGP due to its nature as an antibiotic promoter of growth (Table 22). The fact that there is no significant difference in feed intake among the treatments with the inclusion of Moringa suggests that it did not affect the energy and nutrient contribution of diets, since the feed intake of any animal species is determined by food requirements, and an increase in feed intake is observed when the contribution of the diet is low in terms of nutritional quality and low dietary energy density (Aami-Azghadi et al., 2014). On the other hand, if feed intake decreases this can be due to the high content of CF and DF. According to Obregón et al. (2012), there is an inverse relationship between feed intake and retention time of dry matter (DM) in the upper digestive organs of the digestive tract when fibrous feed are included in diets for monogastric organisms. Apparently, diets with a high content of CF and DF remain longer in the gizzard, which reduces this indicator. The results differ from Ashong and Brown, (2011), who when using diets with different levels of inclusion of Moringa leaves flour in White Leghorn chickens from 7 days up to 5 weeks. With substitution levels of Moringa flour at 0% (control group), 10%, 20% and 30%, significant differences in feed intake were found, since as the percentage of inclusion increased, feed intake decreased significantly, since the nutrient and energy intake increased, and therefore caused greater satiety in birds, although it could be due to the fiber effect discussed above and not by what they claim. The

Moringa factor was statistically highly significant in weight gain, as shown in Figure 8. The gain of weight in the period from 1 to 14 d decreased when the level of Moringa increased. This is also in line with the results of a study conducted by Olugbemi et al. (2010) when including Moringa up to 5% in diets based on cassava in broilers. In addition, it coincides with a study reported by Gadzirayi et al. (2012), where they replaced soybean meal with Moringa (0%, 25%, 50%, 75% and 100%), and where the average weight of broilers was maintained until a replacement of 25% of Moringa. They attributed this effect to the high levels of fiber in diets. Results according to the literature that monogastric cannot use diets with a high content of crude fiber in an efficient way as previously discussed. There is another possibility that the digestive apparatus in quails could be passing by a period of adaptation to the antioxidant and antibacterial components; this effect is progressive and not immediately as the AGP. The antioxidant and antimicrobial effects of Moringa oleifera have been mainly studied in vitro (Prakash et al., 2007; Saikia et al., 2011; Sankhalkar, 2014; Adline et al., 2014; Ajayi and Fadeyi, 2015). Its effects and mechanisms of action in vivo models are still being studied (Amer and Khan, 2012; Okorondu et al., 2012). This effect of adaptation to the components of Moringa is inferred from the field observation that quail's feces in the period from 1 to 14 d were less consistent than those from the period of 15 to 35 d and it is conclusive under the weight gain response at 35 d, where there is no significant difference in this variable. The analysis of variance for the feed conversion of Japanese quails showed statistically significant differences of Moringa and AGP. Feed conversion is defined as the amount of food required to achieve a kilogram of final product; end therefore, a treatment with lower feed conversion is better in terms of production (Mora-Brautigan, 1991). Thus, a lower value food conversion therapy is better in terms of production. The feed conversion in the first period was due to the effect of the weight gain in the period of initiation. But for the completion of the experiment, feed conversion had no significant difference (Table 22). This performance coincides with the one found by El-Faham, (2014) when assessing the productive performance of chickens; they observed that the pace of growth with respect to

the body mass and the most efficient food usage occurs during the first weeks of life.

Blood characteristics

The hematological results of this investigation reflect the inclusion of Moringa in diets did not contain substances that could alter some hematological parameters, since there only were significant differences in values regarding leucocytes, lymphocytes, ALT, and AST; in spite of this, they are within the recommended range for Japanese quails (Table 23 and 24) (Woodard et al., 1973; Itoh et al., 1998; Uyanik et al., 2005; Asrani, 2006, Ayoola et al., 2015). Concerning hematocrit, heterophyles, and hemoglobin parameters, there are not significant differences between diets with MOR meaning that there wasn't any type of infection or inflammatory process in birds. In addition, Onibi et al. (2011) reported that the decrease of red blood cells is mainly associated with the low quality of food and protein deficiency suggesting that Moringa has good quality and does not affect the physiological development of birds. The replacement of MOR meal in diets suggests antimicrobial effect in diets (Devendra et al., 2011; Ndhlala et al., 2014) due to the only significant concentration of leukocytes observed ($P<0.001$) having a greater concentration in the diet of 0% of MOR, values within the acceptable range for this variable. This can be supported with the results of the concentration of lymphocytes, since their lower concentration occurs in diets with AGP, fulfilling its antimicrobial function. The hematological variables are commonly altered by the influence of different dietary treatments (Aletor and Edberongbe, 1992). With respect to the variables of kidney and liver failure, the inclusion of MOR in diets is significant for the case of ALT ($P< 0.00$) and creatinine ($P<0.001$). The higher this inclusion is, the lower these variables get, avoiding therefore the liver and kidney failure respectively by the presence of xenobiotics in the diets that could damage them. Regarding the AST, it was significant the presence of the APC ($P<0.05$) resulting in the increase of this variable from 221. 75 to 223.45 u/L. The use of Virginiamycin as AGP could result into renal damage since the Japanese quails can be sensible to this antibiotic (Reece, 1988).

Nutraceutical Activity

Quails' serum cholesterol concentration (Table 24) presented significant differences among the used diets ($P<0.05$). When increasing the percentage of MOR in diets, cholesterol values tend to decrease; this can be due to the nutraceutical effect provoked by the antioxidant capacity of Moringa (Prakash, et al., 2007; Ebrahimzadeh, et al., 2009; Ashong and Brown, 2011). This is supported with the concentration of triglycerides where there are significant differences ($P < 0.001$); when increasing the inclusion of Moringa, this decreases inversely to the concentration of HDL, which indeed increases, presenting significant difference in the study by the inclusion of MOR ($P<0.001$). Portomicrons (lipoproteins) in birds are transported by via porta vein, and not lymphatic as chylomicrons in mammals. The lipoproteins metabolism, plasma lipids levels, and lipid accumulation differs between males and females and between blood or genetic lines, as well as the type of diet and physical activity (Osorio et al., 2011). It is very common these values increase in commercial birds in cages due to the diet and to their reduced physical activity, especially in quails, that along with this, are of fast growth, being more prone to this condition.

Carcass yield

Since weight gain parameters ($P<0.001$) resulted with significant differences in the variables of live weight at slaughter (LWS) and hot carcass weight (HCW) present significant differences ($P<0.001$, $P<0.05$, respectively), due to the main effect provoked by MOR without affecting significantly (Table 25) the hot carcass weight being consistent with the efficiency of 61% submitted by Obregón (2012) and Aybar (2011).

Conclusions

Fattening quails showed a weight decrease when the level of Moringa flour was increased in the diet from 0 to 21% in the period from 0 to 14 days, recovering this weight in a period of 35 days without showing significant differences in gain and feed conversion rate. Feed intake and the inclusion of Moringa remained without significant variation in the diets; this was observed throughout all the experiment. Blood characteristics were kept within acceptable ranges for quails, the inclusion of Moringa decreased cholesterol and triglyceride concentrations. Levels of aminotransferase aspartate, alanine transferase and creatinine were significantly reduced when the amount of Moringa replacement was 21%. Similarly, to the gain parameters, live weight at slaughter (LWS) and carcass yield (CY) variables presented significant differences due to the main effect provoked by MOR, not significantly affecting the hot carcass weight. The inclusion of Moringa leaves flour has a similar effect to growth promoting agents (AGP) without causing hematological harm in Japanese quails during the fattening period and providing a nutraceutical effect. *Moringa oleifera* is an appropriate source of protein, amino acids, volatile fatty acids and vegetable antoxidantes in the formulation of balanced food for birds.

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Table 13. Composition and calculated analysis of the basal diets.

	Diet of started (1 to 14 d)				Diet of finisher (15 to 14 d)			
	Moringa leaf powder				Moringa leaf powder			
Item (%)	Control	(7%)	(14%)	(21%)	Control	(7%)	(14%)	(21%)
Corn	29.89	27.71	25.57	23.39	43.89	41.56	39.23	36.68
Soybean	65.80	61.29	56.82	52.30	51.74	47.39	43.06	39.00
Soybean oil	-	-	-	-	-	-	-	-
Moringa	-	7.00	14.00	21.00	-	7.00	14.00	21.00
Sugar	-	-	-	-	-	-	-	-
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.30
DL-Methionine	0.34	0.34	0.34	0.34	0.40	0.40	0.40	0.40
L-Lysine	-	-	-	-	-	-	-	-
L-Threonine	-	-	-	-	-	-	-	-
Limestone	1.40	1.10	0.70	0.40	1.40	1.08	0.74	0.40
Mono-dicalcic phosphate	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Vitamins premix[1]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Minerals premix[2]	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Probiotic	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Adsorbent[3]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Phytase[4]	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Calculated Analysis								
CP%	28.18	28.18	28.13	28.10	24.00	24.02	24.04	24.15
EM (kcal/kg)	3111	3122	3135	3145	3148	3158	3169	3181
Lysine %	1.99	1.94	1.90	1.86	1.60	1.56	1.52	1.49
Methionine %	0.56	0.55	0.55	0.55	0.56	0.56	0.56	0.56
Cysteine %	0.38	0.37	0.37	0.36	0.32	0.31	0.31	0.31
Threonine %	1.07	1.07	1.06	1.06	0.89	0.88	0.88	0.88
Tryptophan %	0.33	0.33	0.32	0.32	0.27	0.27	0.27	0.26
Calcium %	0.95	0.97	0.94	0.95	0.91	0.92	0.91	0.91
Phosphorus %	0.38	0.37	0.37	0.36	0.36	0.35	0.35	0.34
Crude fiber %	2.49	2.89	3.29	3.69	2.36	2.76	3.16	3.56
Ethereal extract %	2.47	3.13	3.78	4.44	2.69	3.35	4.00	4.65
Ac. Linoleic	1.28	1.24	1.21	1.18	1.35	1.32	1.29	1.25
Dry matter %	88.31	88.68	89.06	89.43	88.42	88.79	89.17	89.54

[1]Vitamin premix provided the following per kg of diet: 3.75 mg retinol; 112 mg cholecalciferol; 30 mg tocopherol acetate; 3 mg Menadione sodium bisulfide; 1.5 mg thiamin; 6 mg riboflavin; 3 mg pyridoxine; 15 mg cyanocobalamin; 1.5 mg folic acid; 55 mg niacin; 15 mg Ca pantothenate; 180 µg biotin; 600 mg choline; 120 mg Banox (BHA + BHT).
[2]Mineral premix provided the following per kg of diet: 75 mg Mn; 75 mg Zn; 75 mg Fe; 900 mg Mo; 750 ug Co; 105 mg Se.
[3]Aluminosilicate, Zeolex.
[4]Natuphos* 5000 GP Fitasa, Basf Mexicana, S.A. de C.V.

Table 14. Design of experiment.

AGP	Moringa (%inclusion inclusion)	Arrangement (45 Cages 12 quails per cage)	Time (days)	
			0 to 14 (phase 1)	15 to 35 (phase 2)
Without APC (0)	0	1 a 5	Initiation diet	Finalization diet
	7	6 a 10		
	14	11 a 15		
	21	16 a 20		
With AGP (1)	0	21 a 25	Initiation diet	Finalization diet
	7	26 a 30		
	14	31 a 35		
	21	36 a 40		

Table 15. Proximate composition of the main components of the diets.

Determination (%)	Moringa	Soybean meal	White corn
Protein	28.90±0.42	43.97±0.69	9.82±0.29
Grease	12.63±0.27	2.37±0.60	4.15±0.14
Dietary fiber	21.97±0.91	21.43±0.58	9.03±0.31
Ashes	9.54±0.66	6.44±0.12	1.80±0.08
Humidity	6.27±0.25	12.26±1.32	11.52±0.08
Carbohydrates	21.00±0.38	14.11±0.62	64.48± 2.48
Crude fiber	8.49±0.32	3.28±0.05	2.17±0.14
Calculated analysis ME (Kcal/Kg)	3485.15± 42.51	3597.87±30.40	3857.23±19.00

Table 16. Amino acid content (g AAS/100 g).

Mineral	Moringa	Soybean meal	White corn
Aspartate	2.18±0.06	5.29±0.17	0.49±0.01
Glutamate	2.83±0.00	9.19±0.04	1.58±0.01
Threonine *	1.04±0.00	1.75±0.06	0.24±0.00
Tryptophan *	0.28±0.00	0.54±0.01	0.07±0.00
Serina	1.13±0.01	2.10±0.03	0.31±0.00
Histidine *	0.84±0.02	1.10±0.05	0.20±0.00
Glycine	1.32±0.03	2.03±0.08	0.30±0.00
Arginine	1.32±0.00	3.75±0.06	0.36±0.00
Alanine *	1.17±0.01	1.28±0.05	0.43±0.01
Tyrosine	0.74±0.00	1.22±0.00	0.22±0.01
Methionine *	0.23±0.01	0.42±0.02	0.09±0.00
Valine *	0.88±0.01	2.06±0.07	0.33±0.00
Phenylalanine *	1.22±0.02	2.25±0.07	0.33±0.01
Isoleucine *	0.61±0.00	1.93±0.09	0.19±0.00
Leucine *	1.95±0.00	3.71±0.12	1.06±0.04
Lysine *	1.43±0.02	3.35±0.15	0.19±0.01
Proline	1.09±0.05	2.10±0.06	0.74±0.00
Cysteine	0.31±0.01	0.60±0.02	0.12±0.00

* Essential amino acid

Table 17. Mineral content of the main components of the diet.

Mineral	Moringa	Soybean meal	White corn
Ca*	21.51±1.54	3.65±0.13	0.15±0.02
Mg*	3.38±0.07	3.05±0.07	1.01±0.06
Na*	0.55±0.04	0.26±0.03	0.18±0.06
K*	5.23±0.15	3.64±0.08	2.45±0.23
P*	0.62±0.04	2.07±0.13	0.51±0.04
Fe**	42.39±2.49	15.14±0.21	21.13±1.02
Mn**	4.12± 0.28	4.02± 0.11	0.30±0.01
Zn**	17.63±0.84	19.99±0.14	110.28±9.72
Cu**	5.94±0.28	12.21±0.37	2.07±0.11

* Macro-elements (g/kg), ** Micro-elements (ppm)

Table 18. Fatty acid composition.

Mineral (%)	Moringa	Soybean meal	White corn
Lauric (C12: 0)	0.27±0.02	-	-
Myristic (C14: 0)	0.44±0.08	0.10±0.03	0.07±0.03
Palmitic (C16: 0)	17.29±0.65	16.17±0.23	13.82±0.23
Palmitoleic (C16: 1)	0.21±0.02	0.14±0.02	0.13±0.02
Heptadecanoic (C17: 0)	0.89±0.10	0.15±0.01	0.10±0.01
Stearic acid (C18: 0)	2.54±0.42	4.13±0.23	2.99±0.23
Oleic acid (C18: 1 c + t)	2.25±0.78	12.62±0.90	34.45±0.90
Linoleic acid (C18: 2 c + t)	6.97±0.98	56.13±0.54	46.31±0.54
Linolenic acid (C18: 3 n3)	65.26±1.43	9.70±0.04	1.20±0.04
Arachidic (C20: 0)	0.51±0.03	0.23±0.07	0.48±0.07
Arachidonic acid (C20: 4)	0.28±0.06	-	-
Behenic (C22: 0)	0.84±0.08	0.30±0.00	-
Tricosanoic (C23: 0)	0.31±0.01	0.12±0.00	-
Lignoceric (C24: 0)	0.89±0.09	-	-
Saturated	25.01±0.65	26.74±0.98	15.62±0.98
Monounsaturated	2.46±0.87	4.27±0.25	3.11±0.25
Polyunsaturated	72.52±1.34	68.98±1.20	81.25±1.20

Table 19. Phenolic content and antioxidant activity of methanolic extracts of the major components of the diet.

Material	Phenolic compounds	Total phenol content (mg GAE/g)	Total flavonoid content (mg QE/g)	Antioxidant activity	
				ORAC (µmol TE/g)	DPPH (Mol TE/g)
Moringa	Free	16.08±0.82	17.81±0.46	486.25±22.49	107.00±14.23
	Conjugated	6.58±0.35	10.12±0.75	245.97±8.77	33.29±3.44
Soybean meal	Free	2.24±0.18	0.45±0.09	100.03±12.43	3.24±0.14
	Conjugated	0.55±0.07	1.20±0.01	54.72±0.50	1.14±0.08
White corn	Free	0.81±0.01	0.52±0.08	21.78±1.87	2.73±0.31
	Conjugated	3.08±0.23	1.03±0.14	93.78±9.41	9.42±0.70

mg QE/g	mg Quercetin equivalents per g dry weight
mg GAE/g	mg Gallic acid equivalents per g dry weight
µmol TE/g	µmol Trolox equivalents per g dry weight
Mol TE/g	mol Trolox equivalents per g dry weight

Table 20. Phenolics profile ethanolic extracts of the major components of the diet.

Compound type	Compound	Concentración (mg/g)					
		Moringa		Soybean meal		White corn	
		Free	Conjugated	Free	Conjugated	Free	Conjugated
Phenolic acids	Chlorogenic	0.69±0.02	N.D.	ND	ND		
	Caffeic	0.96±0.04	2.04±0.00	ND	ND		
	Coumaric	1.37±0.04	ND	ND	ND	0.05±0.00	0.09±0.00
	Ferulic	ND	ND	ND	ND	0.02±0.00	0.30±0.00
	Benzoic	ND	ND	ND	ND	0.17±0.01	0.20±0.01
Flavonoids	Kaempferol	0.25±0.05	15.10±0.32	ND	ND	ND	ND
	Routine	6.21±0.20	1.72±0.06	ND	ND	ND	ND
	Quercetin	ND		ND	ND	ND	ND

ND: Not detected

Table 21. Antimicrobial activity by inhibition halo major component of the diet.

Extract	*Staphylococcus aeureus*	*Salmonella*	*Escherichia coli*	*Candida albicans*	*Pseudomona aeuginosa*	*Listeria monocytogenes*
Moringa	22.54±2.15	17.03±1.23	12.42±0.83	14.36±0.78	14.81±1.17	23.63±1.67
Soybean	-	-	-	-	-	-
Corn	-	-	-	-	-	-
Ampicillin	31.41±1.47	26.51±0.96	15.01±0.67	14.36±0.57	16.21±1.08	30.16±0.82
MeOH (80%)	-	-	-	-	-	-

Units in m

Table 22. Effect of inclusion of Moringa, with and without AGP by weight, weight gain, feed intake and feed conversion rate in Japanese quail from 1 to 35 days old.

		Weight (g)			WG (g)			FI (g)			FCR (g/g)		
Ítem	Level	0 d	14 d	35 d	0-14 d	14-35 d	0-35 d	0-14 d	14-35 d	0-35 d	0-14 d	14-35 d	0-35 d
Main effect													
MOR	0	12.13	101.94^{a}	235.70^{a}	89.81^{a}	133.76	223.57^{a}	163.43	624.5	789.4	1.82^{c}	4.68	3.54^{b}
	7	12.20	97.12^{ab}	232.29^{a}	84.92^{ab}	135.17	220.09^{a}	163.26	615.8	779.1	1.93^{bc}	4.56	3.54^{b}
	14	12.16	93.00^{b}	227.51^{ab}	80.84^{b}	134.51	215.35^{ab}	166.51	625.5	792.0	2.06^{b}	4.67	3.68^{ab}
	21	12.10	83.66^{c}	218.26^{b}	71.56^{c}	134.60	206.16^{b}	162.26	620.5	782.8	2.27^{a}	4.61	3.80^{a}
SEM		0.16	1.61	2.54	1.56	2.04	2.52	4.56	11.5	14.1	0.05	0.10	0.06
APC	Without	12.22	92.49	226.26	80.24	133.77	214.02	168.52^{a}	632.68	801.93^{a}	2.12^{a}	4.73^{a}	3.75^{a}
	With	12.05	95.37	230.62	83.32	135.25	218.57	159.21^{b}	610.51	769.72^{b}	1.93^{b}	4.52^{b}	3.53^{b}
SEM		0.11	1.14	1.80	1.10	2.04	1.78	3.23	8.10	9.99	0.03	0.07	0.04
Interaction effect													
0	Without	12.22	99.49	234.44	87.27	134.95	222.22	168.42	654.0	825.4	1.93	4.85	3.72
0	With	12.04	104.39	236.96	92.35	132.57	224.92	158.44	595.0	753.5	1.71	4.50	3.35
7	Without	12.23	95.75	228.00	83.52	132.25	215.77	164.97	606.9	771.9	1.98	4.60	3.58
7	With	12.17	98.49	236.57	86.32	138.08	224.41	161.55	624.8	786.4	1.87	4.53	3.50
14	Without	12.26	92.10	226.53	79.84	134.43	214.27	173.08	647.6	820.7	2.17	4.8	3.83
14	With	12.06	93.89	228.49	81.83	134.60	216.43	159.95	603.4	763.3	1.95	4.51	3.53
21	Without	12.27	82.61	216.07	70.34	133.46	203.80	167.63	622.2	789.8	2.39	4.66	3.87
21	With	11.94	84.71	220.46	72.77	135.74	208.52	156.89	618.8	775.7	2.15	4.56	3.72
SEM		0.23	2.28	3.59	2.20	2.89	3.56	6.45	16.2	20.0	0.07	0.14	0.08
Source of variation							*P*-value						
MOR		0.978	0.000	0.000	0.000	0.970	0.000	0.921	0.930	0.911	0.000	0.832	0.006
APC		0.233	0.083	0.096	0.057	0.476	0.080	0.050	0.062	0.029	0.000	0.045	0.000
MOR*APC		0.949	0.903	0.791	0.898	0.545	0.797	0.890	0.850	0.138	0.786	0.679	0.244

Different letters in column indicate significant differences between samples ($P \leq 0.05$).

SEM: standard error of the mean.

Table 23. Results of the hematic biometry of Japanese quail with diets 0, 7, 14 and 21 % Moringa, without and with AGP.

Ítem	level	Hematocrit 31-41 %	Hemoglobin 12.9-14.5 g/dL	Leukocytes 14.7 - 30.7 M/mm3	Heterophil 50-52 %	Lymphocytes 40-46 %	Glucose 93.60- 141.50 (mg/dL)	Protein 64-83 g/L
Main effect								
MOR	0	40.6	12.55	23.04[a]	53.5	44.6	96.7	65.77
	7	39.6	13.07	21.72[a]	50.9	43.9	98.4	64.45
	14	39.1	12.93	19.63[b]	52.4	42.7	100.7	63.40
	21	39.0	13.11	18.41[b]	53.6	42.8	101.6	62.80
SEM		0.660	0.226	0.380	0.845	0.896	1.38	0.915
APC	Without	39.95	12.80	20.81	52.3	45.35[a]	99.8	64.24
	With	39.20	13.03	20.59	52.9	41.65[b]	98.9	63.97
SEM		0.466	0.160	0.269	0.597	0.633	0.979	0.647
Interaction effect								
0	Without	40.80	12.02	23.16	54.8	48.0	95.4	65.74
0	With	40.40	13.08	22.92	52.2	41.2	98.0	65.80
7	Without	39.40	13.12	21.81	50.0	45.8	97.4	64.48
7	With	39.80	12.03	21.62	51.8	42.0	99.4	64.42
14	Without	39.60	12.93	19.70	51.6	44.4	102.6	63.76
14	With	38.40	12.94	19.56	53.2	41.0	98.8	63.04
21	Without	40.00	12.94	18.56	52.8	43.2	103.8	62.98
21	With	38.20	13.09	18.26	54.4	42.4	99.4	62.62
SEM		0.933	0.319	0.538	1.19	1.27	1.96	1.29
Source of variation	*P*-value							
MOR		0.314	0.293	0.000	0.106	0.389	0.071	0.130
APC		0.264	0.313	0.572	0.483	0.000	0.520	0.770
MOR*APC		0.667	0.231	0.999	0.209	0.153	0.168	0.991

Different letters in the line indicate significant differences between the samples ($P \leq 0.05$).

SEM: standard error of the mean.

Table 24. Results of blood chemistry markers of liver and kidney function Japanese quail with inclusión diets of Moringa 0, 7, 14 and 21%, without and with AGP, to 35 days.

Ítem	Level	Uric acid 4.4-10.1 mg/dL	Cholesterol <5.3 mmol/L	LDL 3.90-2.22 mmol/L	HDL 2.38-3.92 mmol/L	Triglycerides <105 mg/dL	ALT 10.73-16.87 U/L	AST 214.88-230.72 U/L	Creatinine 0.25-0.35 mg/dL
Main effect									
MOR	0	3.980	5.846^{a}	4.190	1.3060^{b}	92.90^{a}	16.40^{a}	228.80^{a}	0.3430^{a}
	7	4.111	5.553^{ab}	3.810	2.0230^{a}	89.90^{a}	15.30^{b}	223.80^{b}	0.3170^{b}
	14	4.088	5.330^{ab}	3.770	2.1400^{a}	83.40^{b}	14.40^{bc}	220.30^{c}	0.2930^{c}
	21	3.721	5.262^{b}	3.730	2.2320^{a}	77.30^{c}	13.50^{c}	217.50^{d}	0.2840^{c}
SEM		0.168	0.141	0.156	0.0603	1.40	0.245	0.530	0.0053
APC	Without	4.132	5.374	3.570^{a}	1.9475	87.050	15.10	221.75^{b}	0.3015^{b}
	With	3.828	5.622	4.180^{b}	1.9030	84.700	14.70	223.45^{a}	0.3170^{a}
SEM		0.119	0.100	0.110	0.0426	0.986	0.173	0.375	0.0038
Interaction effect									
0	Without	4.132	5.880	3.700	1.4200	92.80	16.80	227.80	0.3420
0	With	3.828	5.812	4.680	1.1920	93.00	16.00	229.80	0.3440
7	Without	4.124	5.306	3.500	2.0060	91.60	15.40	223.20	0.3060
7	With	4.098	5.800	4.120	2.0400	88.20	15.20	224.40	0.3280
14	Without	3.762	5.144	3.540	2.1400	84.80	14.60	219.40	0.2760
14	With	4.414	5.516	4.000	2.1400	82.00	14.20	221.20	0.3100
21	Without	3.660	5.164	3.540	2.2240	79.00	13.60	216.60	0.2820
21	With	3.782	5.360	3.920	2.2400	75.60	13.40	218.40	0.2860
SEM		0.237	0.200	0.221	0.0852	1.97	0.346	0.750	0.0076
Source of variation					*P*-value				
MOR		0.349	0.027	0.158	0.000	0.000	0.000	0.000	0.000
APC		0.513	0.089	0.000	0.466	0.102	0.112	0.003	0.007
MOR*APC		0.251	0.534	0.544	0.387	0.767	0.801	0.956	0.127

Different letters in the line indicate significant differences between the samples ($P \leq 0.05$).

SEM: standard error of the mean.

Table 25. Effect of inclusion of Moringa, with and without AGP on carcass characteristics in Japanese quail at 35 days of age.

	Main effect						Interaction effect								Source of Variation		
	MOR (%)				AGP		MOR (%)-AGP								Valor P		
Ítem	0	7	14	21	Without	With	0-Without	0-With	7- Without	7- With	14- Without	14- With	21- Without	21- With	MOR	AGP	MOR*AGP
n	100	101	89	94	201	183	40	49	49	51	48	53	46	48			
LWS (g)	223.47[a]	225.04[a]	224.31[a]	206.33[b]	218.61	220.96	224.70	222.25	222.01	228.08	221.98	226.64	205.77	206.89	0.000	0.364	0.654
SEM	2.73	2.43	2.33	2.82	1.82	1.84	3.89	3.83	3.33	3.54	3.40	3.18	3.87	4.10			
WHC (g)	130.26[a]	131.22[a]	133.25[a]	124.30[b]	129.27	130.25	131.46	129.06	129.14	133.30	132.26	134.23	124.21	124.40	0.000	0.509	0.454
SEM	1.57	1.39	1.34	1.62	1.04	1.06	2.23	2.20	1.91	2.03	1.95	1.82	2.22	2.35			
YC (g)	58.64[b]	58.50[b]	59.55[ab]	60.32[a]	65.10	64.51	58.75	58.53	58.39	58.61	59.68	59.42	60.45	60.20	0.001	0.719	0.947
SEM	0.371	0.330	0.317	0.383	0.247	0.250	0.53	0.52	0.45	0.48	0.46	0.43	0.53	0.56			

Different letters in the line indicate significant differences between the samples ($P \leq 0.05$).

SEM: standard error of the mean.

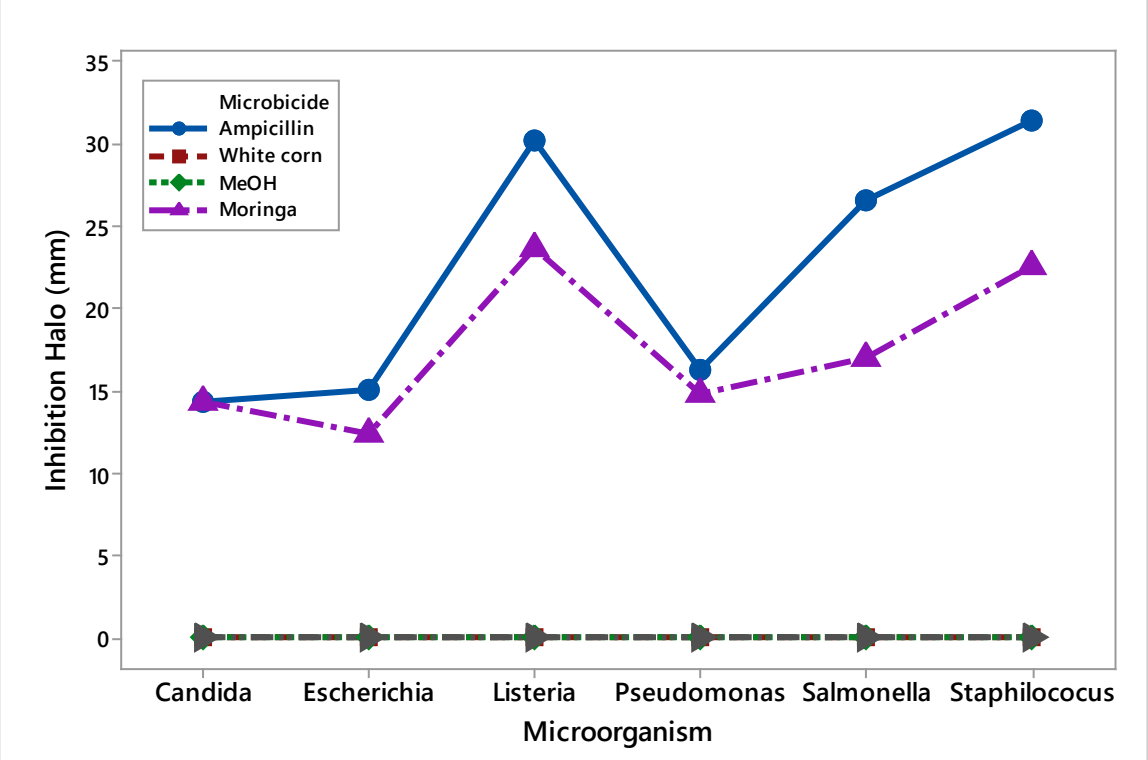

Figure 7. Effect of antimicrobial activity of extracts of Moringa, soybean meal and white corn.

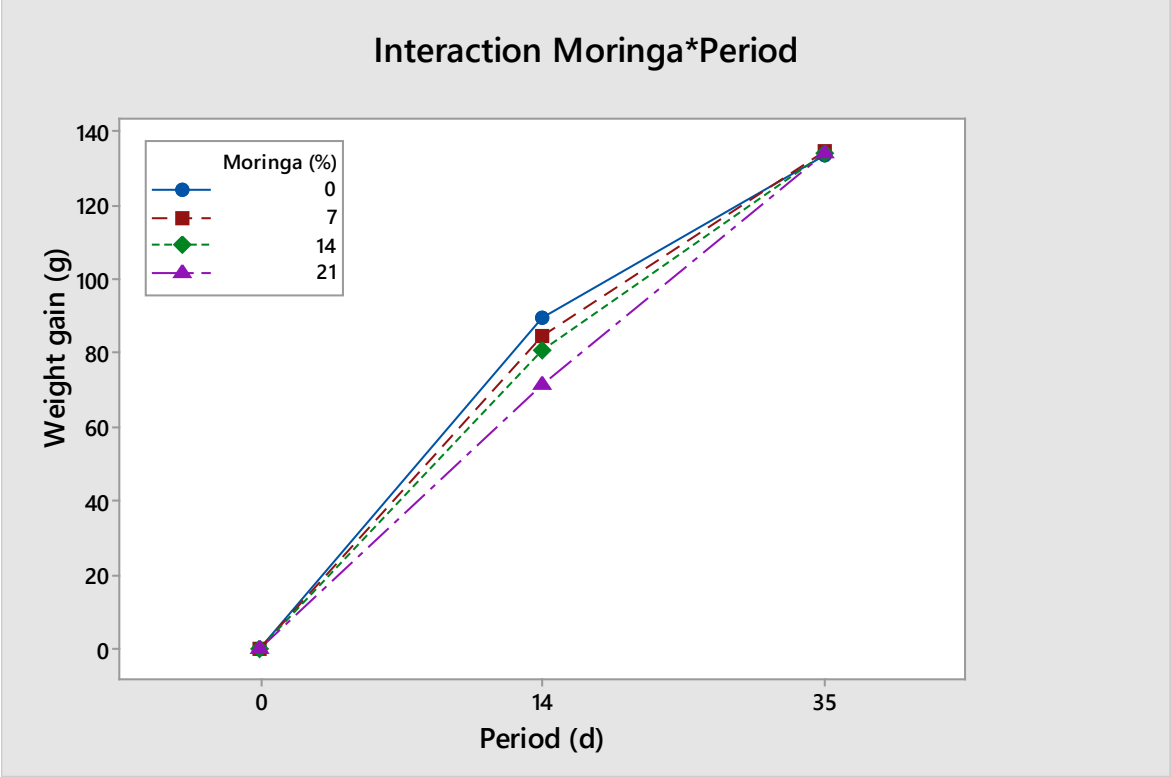

Figure 8. Weight gain, interaction Moringa * Period

CAPÍTULO V. DISCUSIÓN GENERAL

En este capítulo se presenta una integración de los resultados y conclusiones reportados en los Capítulos III y V. Así mismo, se sugieren aspectos relacionados con la problemática investigada que pueden ser temas para posteriores investigaciones.

Propiedades nutrimentales

Los resultados de los análisis nutrimentales indicaron que proteínas, grasas y minerales de las harinas cumplen con los requerimientos nutrimentales especificados por la NRC (1994), lo cual indica que pueden ser utilizadas en la elaboración de dietas para aves. Ambas variantes de *Moringa oleifera* contienen altos porcentajes de los aminoácidos esenciales, en particular de metionina, treonina y lisina que son los aminoácidos limitantes en la nutrición de las aves. La grasa es de calidad alta en ambos materiales debido a que aproximadamente el 75 % está constituida por ácidos grasos poliinsaturados, incluyendo ácidos esenciales como oléico, linolénico y linoléico este último tiene un efecto protector contra la oxidación de membranas celulares. Por último, los minerales presentes en las variantes de la harina de hoja de Moringa como el calcio, fósforo, sodio y potasio se encuentran dentro de los rangos aceptables para la nutrición de las aves. Debido a que el calcio de la dieta contribuye al crecimiento, la eficiencia alimenticia, el desarrollo óseo, la salud de las piernas, el funcionamiento de los nervios y el sistema inmune de las aves es vital aportar el calcio en las cantidades adecuadas y en forma consistente. De igual forma, el fósforo se requiere en la forma y la cantidad correctas para la estructura y el crecimiento óptimos del esqueleto. En relación al sodio y potasio, estos minerales se requieren para las funciones metabólicas generales, por lo que su deficiencia puede afectar el consumo de alimento, el crecimiento y el pH de la sangre.

Actividad Antioxidante

Los valores de actividad antioxidante de los ácidos fenólicos y flavonoides totales encontrados en las muestras están dentro de los valores reportados por otros autores. Los principales compuestos fenólicos y flavonoides encontrados fueron gálico, clorogénico, cafeíco, cumárico, ferúlico, kaempferol y rutina. La concentración de compuestos fenólicos en los extractos se relaciona con su capacidad antioxidante. Por lo anterior, la harina a base de hojas de Moringa puede ser considerada un producto para la elaboración de alimentos funcionales

y productos nutracéuticos con posible aplicación en la industria alimentaria, nutracéutica y farmacéutica.

Actividad Antimicrobiana

Las pruebas de actividad antimicrobiana del extracto metánolico de la hoja de Moringa revelaron que la mayor inhibición de microorganismos se obtuvo en las bacterias Gram (+), seguidas de las Gram (-) y hongo. Esta actividad está relacionada a los compuestos fenólicos y flavonoides. Este resultado prueba que los extractos de Moringa pueden constituirse en una fuente de principios activos que contribuyan al descubrimiento de antimicrobianos de origen natural, los cuales pueden ser utilizados como base para la síntesis de moléculas viables a nivel farmacéutico o bien de uso veterinario.

Efecto Nutracéutico

La inclusión de harina de hoja de Moringa en dietas para aves provee un efecto nutracéutico disminuyendo en mayor grado triglicéridos en codorniz japonesa.

Respuesta productiva

Las dietas con inclusión de harina de hoja de Moringa generan una respuesta productiva similar a las que contienen pasta de soya como insumo proteico para engorda de codorniz japonesa constituyendo una fuente apropiada de proteína, aminoácidos, ácidos grasos y antioxidantes de origen vegetal en la formulación de alimentos balanceados para aves.

Agente Promotor de Crecimiento

la inclusión de harina de hoja de Moringa tiene un efecto similar a los agentes promotores de crecimiento (APC) y no ocasiona daño hematológico en codorniz japonesa durante el periodo de engorda. Esto implica que el engorde de la codorniz japonesa con la inclusión de harina de hoja de Moringa se traduce en una opción viable con la plusvalía del efecto APC, que desde el punto de vista económico para el productor el disminuir costos de producción.

Implicaciones Científicas y Tecnológicas

Una contribución científica importante de este trabajo es el establecimiento del efecto nutrimental, nutracéutico y como APC de la harina de Moringa. Estos resultados proporcionan una alternativa para los productores, a fin de que no se centren únicamente en la disminución costos de alimentación, comprometiendo la calidad del producto final con las alternativas hasta hoy utilizadas, sino que puedan adoptar esta alternativa que agrega valor a sus productos y garantiza a sus consumidores un riesgo casi nulo al consumirlos. Adicionalmente, estas acciones deben enfocarse directamente al bienestar de los animales con mejores condiciones de crianza, engorde y productos derivados de ellos.

Perspectivas del Trabajo

Los resultados de esta investigación soportan de manera significativa propuestas para darle valor agregado a productos derivados de la planta *Moringa oleifera*, en este caso la hoja. Dando pauta, para la realización de validaciones de los productos funcionales y nutracéuticos derivados de hoja de Moringa, bajo el cumplimiento de la normatividad de COFEPRIS para contar con análisis de biodisponibilidad, estabilidad, farmacocinética, estableciendo con ello protocolos que puedan desarrollar cadenas de valor cuyos eslabones los conforman los productores de la materia prima, la validación y desarrollo de proceso de separación y de formulación de productos funcionales y nutracéuticos a partir de estos materiales y las validaciones clínicas por parte de los centros de investigación, la gestión y posicionamiento del producto por intermediarios o empresarios; soportado todo esto en buenas prácticas de manufactura, convirtiéndose en un negocio rentable para todos, no solo monetariamente sino incidiendo de manera directa en la salud de las personas, en el reforzamiento de las políticas públicas y la economía de un país.