UNIVERSIDAD AUTÓNOMA DEL ESTADO DE MÉXICO
FACULTAD DE MEDICINA VETERINARIA Y ZOOTECNIA

"*Moringa oleifera* leaf meal as protein feed in goat's diets: biomethane and carbon dioxide and fermentation kinetics"

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PRESENTA

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ASESORES:

DRA.MONA MOHAMED MOHAMED YASSEEN ELGHANDOUR

Dr. ABDELFATTAH ZEIDAN MOHAMED SALEM

M.en C. JOSÉ PABLO MEDINA NAVARRO

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DEDICATORIAS

A mi Madre Maria Agustina Núñez Palma
Por su esfuerzo en ayudarme a concluir mi carrera

A mis hermanos:
Por su apoyo incondicional y darme animos para seguir adelante.

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INTRODUCCIÓN

Durante la fermentación ruminal de los piensos, se producen grandes cantidades de gases de efecto invernadero (GEI) que hace en el ganado uno de los productores de gases de efecto invernadero más importantes. La Organización de las Naciones Unidas para la agricultura y la alimentación (FAO, 2006) informó que el sector ganadero representa aproximadamente el 18% del metano (CH4) y el 9%de las producciones de dióxido de carbono (CO2). Se han hecho muchos intentos para mitigar la emisión de ch4 de rumiantes, incluyendo la inclusión de levaduras (Elghandour *et.al.*, 2017), sal de ácidos orgánicos, enzimas exógenas y aceites esenciales con resultados prometedores.

Una serie de problemas se enfrentan los productores de animales; Uno de ellos es la disponibilidad y el precio de los concentrados. En particular las fuentes de proteína, que comprometen la nutrición a buscar alimentos alternativos proteicos menos costosos (kholif *et al*, 2015).

Algunas preocupaciones deben ser consideradas para las fuentes alternativas de proteínas, incluyendo el alto contenido de proteínas y aminoácidos de perfil adecuado sin ignorar el costo. *Moringa oleífera (sinérgicos moringa pterygosperm familia moringácea)* es un arbol forrajero que crece casi todo el mundo y produce una enorme cantidad de biomasa que oscila entre 43 y 115 toneladas por hectárea (safwat *et al* 2014); con un buen contenido proteico. (kholif *et al*. 2015,2016) reportando una excelente composición química como buen alimento proteico en su contenido(kg de MS)241a 277g de proteína cruda (PC),con aproximadamente 47% disponibilidad de proteína

(Becker,1995) y un adecuado perfil de aminoácidos (Sánchez- Machado *et al*, 2010). El contenido de proteína de la harina de *Oleífera (MLM) es menor que el de los concentrados proteínicos comunes en la nutrición de los rumiantes* por ejemplo harina de soya y harina de semillas de algodón, pero comparable a la harina de sésamo se informó que el precio de las ramas con hojas y ramas blandas utilizadas como alimento para animales puede ser alrededor de 0.25 a 0.5 dólares por kilogramo de MS, lo que hace que sea más barato que los alimentos proteicos tradicionales como la harina de soya.

Poca información sobre MLM como una fuente de proteína en la dieta de los rumiantes está disponible. Sin embargo los experimentos recientes que incluyen el MLM como alimento proteico están ganando intereses cada vez mayores resultados prometedores tales como una utilización mejorada del alimento y la producción de leche de cabras (Kholif, 2015-2016).

2- REVISION BIBLIOGRÁFICA

2.1-Moringa oleifera características

Es un árbol de crecimiento rápido nativo del sur de Asia y que ahora se encuentra a lo largo de los trópicos la medicina tradicional utilizado sus hojas durante siglos a veces es descrito como el árbol milagroso el árbol baqueta o árbol de rábano picante tiene hojas pequeñas y redondeadas que están llenas de una increíble cantidad de nutrientes proteínas calcio beta caroteno y vitamina C como potasio Con razón ha sido utilizado de forma medicinal y como fuente alimenticia durante más de al menos 4000 años (Mercola, 2016).

El hecho de que la moringa crece rápido y fácilmente la hace especialmente atractiva en las áreas empobrecidas y se ha utilizado exitosamente para mejorar el consumo de nutrientes en Malawi Senegal e India. En estas áreas la moringa puede ser el alimento más nutritivo disponible localmente y puede cosecharse durante todo el año.

Las hojas de moringa son ricas en antioxidantes entre los cuales encontramos vitamina C beta carotenos quercetina y ácido cloro génico. Este último el ácido cloro génico ha demostrado disminuir la absorción de azúcar en las células y los estudios con animales han encontrado que reduce los niveles de azúcar en la sangre según afirmó el AsianPacific journal of Cáncer prevención (Mercola, 2016)

2.2- Distribución en México.

Muchos productores agrícolas en México se preguntan si la planta se puede cultivar en este país. La respuesta es que ya es parte de la horticultura tradicional desde hace mucho tiempo, principalmente con fines ornamentales: la encontramos abundantemente en los pueblos de toda la costa del Pacífico, desde el sur de Sonora hasta Chiapas, incluyendo el sur de la Península de Baja California.

Los ejemplares de moringa son especialmente abundantes y frondosos en las llanuras calientes del Sur del istmo de Tehuantepec. La planta también se cultiva en los poblados de las depresiones tropicales secas del país. Como la las balsas y la depresión central de Chiapas. La planta se encuentra en los pueblos de la zona del Infiernillo en las cercanías de Apatzingán, Mezcala y Tequesquitengo. En general próspera mejor por debajo de los 500 metros sobre el nivel del mar y crece muy poco cuando se cultiva altitudes mayores a 1500 metros.

Es probable que la planta haya llegado a territorio mexicano por primera vez gracias a marineros filipinos Durante los viajes de la Nao de China que cubría la ruta entre Manila y Acapulco.se llegó de esta manera, seguramente era utilizada como alimento por los miembros de la tripulación. Este hábito de comer la planta Se ha perdido a lo largo de los siglos, pues como se mencionó anteriormente, las plantas en cultivo informal en México tienen casi exclusivamente fines ornamentales.

Con el reciente auge mundial del cultivo de la moringa (Fuglie, 2001), el árbol A llegado a México en forma de semilla desde África y la India, Generalmente para su cultivo en Campos especializados, con la finalidad de cosechar las hojas. Soja si bien actualmente

hay en muchos países un gran interés en el aprovechamiento del árbol y la planta ha existido en México Quizás por siglos, las personas que practican el cultivo Popular de moringa oleífera usualmente desconocen el interés por el árbol, mientras los agricultores interesados en cultivar la planta a gran escala ignoran la presencia de la moringa en la horticultura tradicional mexicana (Thurber y Fahey, 2009).

2.2. a- Propiedades de moringa oleífera

Una de las características más atractivas de la moringa es el alto contenido de proteína en sus hojas. Los testimonios de (Fuglie, 2001). Sobre un sinfín de 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 proteico de la planta. En este sentido, sus beneficios nutricionales son tan ampliamente con los reconocidos. El desarrollo de un mayor número de pruebas químicas bien controladas y documentados con claridad sería de mayor valor los análisis del contenido proteico de las hojas secas muestran que hasta el 30% de su peso está formado por proteínas. Y que la mayor parte de esta Parece ser directamente asimilable además las hojas contienen todos los aminoácidos esenciales en un perfil alto y bien balanceado (Frei Berger *et al*, 1998) 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.

2.2. b- Calcio y vitamina A:

Varios estudios han indicado que la moringa es una fuente valiosa de vitamina A, Pero ha permanecido la duda de si el contenido de esta vitamina se conserva aún después del secado y molido de la hoja. Para examinar esta interrogante, (Nambiar y Seshadri 2001).

Se suele decir que las hojas de la moringa contienen altos niveles de calcio. En la literatura popular y especialmente en la publicidad se leen una y otra vez afirmaciones sobre el alto contenido de calcio de la moringa, “tales como la moringa contiene más calcio por gramo que El yogur”. Si bien es cierto que existe un alto contenido de calcio, una parte importante de este calcio está como cristales de oxalato de calcio en las células de la planta (Olson y Carlquist, 2001; Olson, 2001), una forma el cuerpo no puede asimilar y es excretada directamente (Radek y Savage, 2008), cuantificaron qué el porcentaje de calcio en la moringa en forma de oxalato, y que no puede ser absorbido, es de casi el 38%. Esta cantidad podría parecer muy alta, pero también mostraron que la moringa tiene niveles sumamente altos de calcio (>20mg/g de hoja seca), por lo que aún con la tercera parte en una forma no asimilable, la moringa ofrece cantidades notables de calcio a la dieta. La leche en polvo contiene alrededor de 13 miligramos por gramo de calcio (USAID, 2006), por lo tanto, el polvo de moringa favorablemente No sólo en cuanto a su contenido de proteína con la leche en polvo, sino también en cuanto a su contenido de calcio, a un costo de producción notablemente más bajo que la leche y con un impacto ambiental mucho menor.

2.3- IMPACTO SOBRE EL AMBIENTE

Durante la fermentación ruminal de los piensos, se producen grandes cantidades de gases de efecto invernadero (GEI) que hacen del ganado uno de los productores de GEI más importantes. Organización de las Naciones Unidas para la Agricultura y la Alimentación (FAO, 2006) informó que el sector ganadero representa aproximadamente el 18% del metano (CH4) y el 9% de las producciones de dióxido de carbono (CO2). Se han hecho muchos intentos para mitigar la emisión de CH4 de rumiantes, incluyendo la inclusión de levaduras (Elghandour *et al*., 2017), sal de ácidos orgánicos (Elghandour *et al.*, 2016), enzimas exógenas (Kholif *et al*., 2017a) y aceites esenciales (Hernández *et al*., 2017), con resultados prometedores.

Una serie de problemas se enfrentan a los productores de animales; Uno de ellos es la disponibilidad y el precio de los concentrados, en particular las fuentes de proteínas, que comprometen la nutricion a buscar alimentos alternativos proteicos menos costosos (Kholif *et al*., 2015). Algunas preocupaciones deben ser consideradas para las fuentes alternativas de proteínas, incluyendo el alto contenido de proteínas y aminoácidos de perfil adecuado sin ignorar el costo.

2.4-IMPACTO SOBRE LA DIGESTIBILIDAD

Poca información sobre MLM como una fuente de proteína en la dieta de los rumiantes está disponible; Sin embargo, los experimentos recientes que incluyeron el MLM como alimento proteico están ganando intereses cada vez mayores, con resultados prometedores tales como una utilización mejorada del alimento y la producción de leche de Cabras (Kholif *et al*., 2015, 2016). Aumentar el pH ruminal en la nutrición caprina es

un buen tema para permitir una mejor condición ruminal para la actividad de las bacterias celulolíticas.

3. JUSTIFICACIÓN

Se espera los cambios observados en la composición química en el reemplazó la harina de soya por la harina de hoja de *M. oleífera* contiene una alta fracción de fibra y menos proteína en comparación con la harina de soya. Se espera que la composición química afecte la fermentación de cada ración y la disminución de los gases de efecto invernadero, en la alimentación de cabras.

4. HIPÓTESIS

M. oleifera puede reemplazar la harina de soya como alimento proteico en las dietas de cabras por el efecto negativo en la fermentación ruminal desde el punto de vista ambiental, el reemplazo de la harina de soja por la harina de hoja de *M. oleifera* reducirá la producción de CH4, que puede utilizarse como un producto más limpio para el medio ambiente y piensos para el ganado rumiante que ayudaran a controlar la contaminación ambiental por biogases contaminantes de rumiantes.

5. OBJETIVOS

5.1- OBJETIVO GENERAL

Determinar los mejores niveles de sustitución harinas como la de soya en la utilización de piensos por la harina de hojas de *moringa oleífera* y evaluar la producción de metano en cabras lecheras.

5.2- OBJETIVOS ESPECIFICOS

- Realizar experimento para reemplazar otros alimentos proteínicos con concentrados bajos en proteínas tales como harina de soya por la harina de hojas de *moringa oleífera*

- Realizar ensayos *in vitro* como in vivo con harina de hojas *moringa oleífera* en piensos de cabras para evaluar de la inhibición de protozoos ruminales y bacterias celulíticas ruminales.

6. MATERIAL Y METODOS

MATERIAL:

1. *M. Oleifera* se preparada como composición de la biomasa de hojas y ramas pequeñas cosechada a los 40 d edad. *M. oleifera* seca al aire a 60 ° C durante 48 h, y almacenada para su uso.
2. Equipo de laboratorio
3. Dos cabras fistuladas
4. Corrales
5. Heno
6. Concentrado

TRATAMIENTOS

El total de la mezcla en la ración dieta balanceada (TMR), como sustrato contenía (/ kg de MS) 400 g de heno de alfalfa, 250 g de maíz amarillo triturado, 250 g de harina de soya y 100 g de salvado de trigo y considerado como la ración control. Las raciones se equilibraron con los minerales y las vitaminas necesarias.

En la TMR basal, el MLM seco sustituyó la harina de soya a (/ 100 g de DM): 0 g (TMR0, control), 10 g (TMR10), 20 g (TMR20), 30 g (TMR30), 40 g (TMR40) 50 g (TMR50), 60 g (TMR60), 70 g (TMR70), 80 g (TMR80), 90 g (TMR90) y 100 g (TMR100).

MÉTODO:

Determinar el contenido fenólico total Cromatográficamente como se describe por Meier *et al*. (1988). Se recolectaron inóculo ruminal de dos cabras criollas fistuladas (50 ± 2 kg LW), alojadas en corrales individuales y alimentadas ad libitum en una dieta consistente en heno de avena Y concentrado (PURINA®, Toluca, México) a razón de 60:40, con acceso libre al agua.

Los animales se alimentaron dos veces al día a las 08:00 h y 16:00 h y se manejaron bajo las condiciones estipuladas en la Norma Oficial Mexicana de Especificaciones Técnicas para la producción, cuidado y uso de animales de laboratorio (NOM-062-ZOO-1999).

Los contenidos de rumen se colocaron en un termo plástico recalentado a 39 ° C y se transportaron al laboratorio donde se enjuagaron con CO2, se mezclaron y se sometieron a filtrado a través de cuatro capas de estopilla en un matraz con O2 espacio libre de y se mantuvieron a una temperatura constante de 39 ° C y flujo continuo de CO2.

Antes del proceso de incubación, se prepararon medios de incubación que contenían solución Buffer mezclados en un matraz volumétrico con agitador magnético a 39ºC para mantener la temperatura y homogeneizar la solución. Después, el inóculo ruminal y la solución reductora se añadieron a 1: 4 vol / vol, respectivamente.

Muestras (0,5 g) del sustrato fue pesado en botellas de 120 ml. de suero. En consecuencia, se añadieron 50 ml de licor de rumen previamente preparado. Las botellas se mantuvieron a un flujo de CO2 constante durante 30 segundos, y luego se taparon con tapones de neopreno y se sellaron con anillos de aluminio. Los viales se colocaron en una incubadora (Riossa®, F-51 D, Estado de México, México) a 39 ° C durante 48 h.

Además, se incubaron tres botellas como blancos (sólo fluido ruminal) durante 48 h. Se realizaron tres pruebas de incubación en tres semanas.

Las lecturas de producción de gas (GP) se realizaron a las 2, 4, 6, 8, 10, 12, 24 y 48 h de incubación. Se utilizó un aparato de desplazamiento de agua según Fedorak y Hrudey (1983).

El aparato fue diseñado con un soporte universal, con un embudo cónico, una bureta de 100 ml y dos mangueras de látex de 0,5 y 1 m de longitud y 3/8 de pulgada de diámetro. Los viales se puncionaron con una aguja de calibre 16 colocada en el extremo de la manguera.la producción de gas (ml) fue medido por el desplazamiento de agua en la bureta**.** Después de 48 h de incubación, se recogieron 5 ml de gas y se almacenaron en los viales con solución salina saturada preparada con 400 g de NaCl en 1 L de agua destilada, y el pH ajustado a 2 y 5 ml de naranja de metilo al 20% como indicador de la determinación de las concentraciones de CH4 y CO2. La solución salina saturada se preparó previamente y se almacenó en viales serológicos de 60 ml, sin dejar espacio; Y los tapones de neopreno fueron colocados y sellados con anillos de aluminio, y almacenados lejos de la luz. Para la determinación de CH4 y CO2 de los viales con solución salina saturada, se tomó una muestra de 10 µl de la fase gaseosa y se inyectó en un cromatógrafo de gases PerkinElmer, Claurus 500 (Ciudad de México, México) con detección de ionización de llama y helio como El gas portador. Se utilizó un detector de conductividad térmica, el horno, la columna y las temperaturas de TCD fueron 80 ° C, 170 ° C y 130 ° C, respectivamente. Los tiempos de retención fueron 0,73 min y 1,05 min para CH _ {4} y CO2 respectivamente. Al final de la incubación a las 48 h, el proceso de fermentación se detuvo por remolino, las botellas en hielo durante 5 minutos, a

continuación, las botellas fueron destapadas y el pH se midió usando un medidor de pH (Thermo Scientific, Orion StarTM A121, Beverly, MA, EE.UU.). El contenido de las botellas se filtró en bolsas Ankom® Technologies F57 (a peso constante), con la ayuda de un sistema de filtración conectado a una bomba de vacío. Las botellas se enjuagaron con agua caliente 3 veces para asegurar la recuperación de todo el residuo de la fermentación. Las bolsas se colocaron en un horno de aire forzado a 55 ° C durante 48 h. La degradación de la materia seca se calculó considerando el peso inicial del sustrato y el peso del residuo.

Después de la medición del pH y filtración, se obtuvieron 4 ml del medio con una jeringa y se mezclaron con 1 ml de ácido metafosfórico al 25%, se agitaron ligeramente y Colocado bajo congelación hasta el análisis de la concentración de amoníaco-N (NH3-N). Otros 4 ml del medio se mezclaron con 1 ml de formaldehído al 10%, se agitaron ligeramente y se pusieron en refrigeración hasta el análisis del conteo bacteriano y protozoario.

ANÁLISIS ESTADÍSTICO

Para la estimación de la producción de gas cinética, se ajustaron los volúmenes de gas registrados (mL / g de MS) utilizando el procedimiento NLIN de SAS (2002) según France *et al*. (2000) como:

(1) y = b × [1 - e-c (t-L)]

Donde y es el volumen dePG en el tiempo t (h); b es asintótica de la PG (ml / g de MS; c es la velocidad fraccionaria de la fermentación (h), y L (h) es el tiempo de retardo discreto anterior al momento en que se liberó cualquier gas. La digestibilidad *in vitro* de la materia orgánica DMO,g / kg de MS) según Menke *et al*. (1979) como:

ME = 2,20 + 0,136 PG (ml / 0,5 g MS) + 0,057 PC (g / kg MS DMO = 148,8 + 8,89 PG + 4,5 PC (g / kg MS) + 0,651 Cenizas (g / kg de MS)

Donde PG es neto PG en ml de 200 mg de muestra seca después de 24 h de incubación.

El factor de reparto a las 24 h de incubación (PF24, una medida de la eficacia de fermentación) se calculó como la relación de MS Degradabilidad *in vitro* (mg) al volumen (ml) de PG a las 24 h (es decir, DMS / total PG (PG24)) según Blümmel *et al*. (1997). Se calculó el rendimiento de gas (GY24) como el volumen de gas (ml de gas / g de MS) producido después de 24 h de incubación dividido por la cantidad de DMS (g) como:

GY24 = ml de gas / g de MS / Las concentraciones de ácidos grasos (SCFA) se calcularon de acuerdo con Getachew *et al*. (2002) como:

SCFA (mmol / 200 mg MS) = 0,0222 PG - 0,00425 donde: PG es el 24 h neto PG (mL / 200 mg MS).

La producción de biomasa microbiana (MPC) se calculó (Blümmel *et al* 1997)

MPC (Mg / g MS) = Miligramos DMS - (Gas Mililitro × 2,2 mg / mL)

Donde el 2,2 mg / ml es un factor estequiométrico que expresa mg de C, H y O requeridos para El gas SCFA asociado con la producción de 1 ml de gas (Blümmel *et al*., 1997).

Los datos de cada una de las tres series dentro de la misma muestra de cada una de las tres muestras individuales de raciones se promediaron antes del análisis estadístico y se usaron los valores medios de cada muestra individual como unidad experimental.

7.1- El diseño experimental será un arreglo factorial con 3 repeticiones en un diseño de bloques completos al azar. Los datos se analizaron mediante el procedimiento GLM (SAS, 2002) con el modelo:

$Yijk = \mu + Ri + Mj + (R \times M) ij + \varepsilon ijk$

donde:

Yijk es la observación

μ es la media de la población

Ri es la fuente del inóculo Efecto

Mj es el nivel de MLM en la dieta

(R × M) ij es la interacción entre el nivel de MLM y el nivel y tipo inóculo

εijk es el error residual.

La prueba de Tukey se utilizó para comparar los medios.

LÍMITE DE ESPACIO

Posta Zootécnica de la Facultad de Medicina Veterinaria y Zootecnia del Estado de México

LÍMITE DE TIEMPO

Se realizaran tres pruebas de incubación en tres semanas. Las lecturas de producción de gas (PG) se realizaran a las 2, 4, 6, 8, 10, 12, 24 y 48 h de incubación.

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ANEXOS: ARTÍCULO

Moringa oleifera leaf meal as protein feed in goat's diets: biomethane and carbon dioxide and fermentation kinetics

Short title: *Moringa oleifera as a protein feed***.**

Abstract

Ruminal fermentation produces methane (CH_4) and carbon dioxide (CO_2) making the earth warmer. Therefore, the sustainable mitigation of CH_4 and CO_2 emissions as well as ruminal fermentation kinetics when soybean meal was replaced with *Moringa oleifera* leaf meal (MLM) as a protein source at different levels was investigated. A basal total mixed ration (TMR) containing 400 g alfalfa hay and 600 g concentrate feed mixture per kilogram with soybean meal as the sole protein source was formulated. *M. oleifera* leaf meal replaced (/100 g DM): 0 g (TMR0, control), 10 g (TMR10), 20 g (TMR20), 30 g (TMR30), 40 g (TMR40), 50 g (TMR50), 60 g (TMR60), 70 g (TMR70), 80 g (TMR80), 90 g (TMR90), and 100 g (TMR100) of soybean in the rations. Rations were incubated for 48 h using rumen inoculums from goats. Some interactions between inoculum × TMR were observed ($P<0.05$) for GP parameters, CH_4 production, and fermentation kinetics. Moreover, most determined parameters responded in different manners . Rations containing MLM decreased the asymptotic GP ($P<0.01$), while they increased ($P<0.01$) the rate of GP and lag of GP, with both inoculums. Decreased ($P<0.05$) CH_4 production and increased CO_2 production ($P<0.05$) were observed when MLM replaced soybean meal. *M. oleifera* leaf meal containing diets decreased ($P<0.05$) ruminal ammonia-N, and total protozoal number, while they increased ($P<0.05$) total bacterial number with both goat inoculums. Replacing soybean with MLM increased ($P<0.05$) fermentation pH, but decreased ($P<0.05$) organic matter degradability (OMD) with goat inoculum. On the other hand, TMR with MLM had declined ($P<0.05$) SCFA concentrations, and enhanced ($P<0.05$) OMD and DM degradability compared with the control TMR (i.e., TMR0). It is concluded that replacing soybean meal in the diets of goats negatively affected the nutritive value of the diet; however, decreased CH_4 production. From

an environmental point of view, replacing soybean with *M. oleifera* leaf meal can be used as a sustainable strategy to reduce CH_4 production from goats , and thus abate global warming.

Keywords: Greenhouse gases, *in vitro* fermentation, *M. oleifera* leaf meal, protein feeds, sustainable livestock production.

1. Introduction

During ruminal fermentation of feeds, large amounts of greenhouse gases (GHG) are produced making livestock one of most important GHG producers. Food and Agriculture Organization (FAO, 2006) reported that livestock sector accounts for about 18% of methane (CH_4) and 9% of carbon dioxide (CO_2) productions. Many attempts have been made to mitigate CH_4 emission from ruminants including the inclusion of yeast (Elghandour et al., 2017), organic acids salt (Elghandour et al., 2016), exogenous enzymes (Kholif et al., 2017a), and essential oils (Hernandez et al., 2017), with promising results.

A series of problems are facing animal producers; one of them is the availability and price of concentrates, in particular protein sources, which compel nutritionists to seek for less-expensive alternative protein feeds (Kholif et al., 2015). Some concerns should be considered for the alternative protein feeds including the high protein content and balanced amino acids profile, without ignoring suitable cost. *Moringa oleifera* Lam (syns. *Moringa pterygosperm* family *Moringaceae*) is a tree fodder growing almost worldwide and yields a huge amount of biomass ranging from 43 to 115 tons per hectare (Safwat et al., 2014), with a good protein content. Kholif et al. (2015, 2016) reported an excellent chemical composition as a protein feed containing (/kg DM) 241 to 277 g crude protein (CP), with about 47% of bypass protein (Becker, 1995) and adequate amino acid profile (Sánchez-Machado et al., 2010). The protein content of *M. oleifera*

leaf meal (MLM) is less than that of common protein concentrates in ruminant nutrition (e.g., soybean meal and cottonseed meal), but comparable to sesame meal (260 g CP/kg DM). Kholif et al. (2015) reported that the price of branches with leaves and soft twigs used as animal feed can be around 0.25 - 0.5 US$ per kg DM, making it cheaper than traditional protein feeds such as sesame and soybean meal.

Little information about MLM as a protein source in the diet of ruminants is available; however, recent experiments that included MLM as a protein feed are gaining increasing interests, with promising results such as enhanced feed utilization and milk production from goats (Kholif et al., 2015, 2016). Unfortunately, they did not study the effect of replacing soybean meal with MLM as a protein source on GHG production. Therefore, the present study aimed to evaluate the effects of replacing soybean meal at different levels with MLM in the diet for ruminant animals, as a clean feed for the environment on the sustainable mitigation of CH_4 and CO_2 production, ruminal fermentation, and CH_4 producing protozoa and bacteria using rumen inoculums from goats

2. Materials and methods

2.1. Substrate and treatments

M. oleifera leaf meal was prepared as previously described in Kholif et al. (2016). Briefly, *M. oleifera* biomass composing of leaves and small twigs was harvested at 40 d age. *M. oleifera* was air-dried at 60 °C for 48 h, and then kept for further use. The concentration of total tannins in *M. oleifera* leaves was determined according to Makkar (2003), and total phenolic content was determined chromatographically as described by Meier et al. (1988). *M. oleifera* contained 22 g/kg DM total tannins and 48 g/kg DM total phenolics. A total mixed ration (TMR) was prepared, as a substrate containing (/kg DM) 400 g alfalfa hay (*Medicago sativa*), 250 g crushed yellow corn,

250 g soybean meal, and 100 g wheat bran, and considered as a control. Rations were balanced for minerals and vitamins contents. In the basal TMR, dried MLM replaced soybean meal at (/100 g DM): 0 g (TMR0, control), 10 g (TMR10), 20 g (TMR20), 30 g (TMR30), 40 g (TMR40), 50 g (TMR50), 60 g (TMR60), 70 g (TMR70), 80 g (TMR80), 90 g (TMR90), and 100 g (TMR100). The chemical composition of ingredients and TMRs used as substrates is shown in Table 1.

2.2. In vitro fermentation and biodegradation

Rumen inoculum was collected from , two cannulated Creole goats (50 ± 2 kg LW), housed in individual pens and fed *ad libitum* on a diet consisting of oat hay and concentrate (PURINA®, Toluca, Mexico) at 60:40 ratio, with free access to water. Animals were fed twice daily at 08:00 and 16:00 h, and managed under the conditions stipulated in the Official Mexican Standard of technical specifications for the production, care and use of laboratory animals (NOM-062-ZOO-1999). Rumen contents were placed in a plastic thermo preheated at 39 °C, and transported to the laboratory where they were flushed with CO_2, mixed and strained through four layers of cheesecloth into a flask with O_2-free headspace, and maintained at a constant temperature of 39 °C and continuous CO_2 flow.

Before the incubation process, incubation medium containing buffer, macromineral, micromineral and resarzurin solutions, and distilled water were prepared according to Goering and Van Soest (1970), mixed in a volumetric flask using a platen and magnetic stirrer at 39 C to maintain the temperature and homogenize the solution. After, the ruminal inoculum and the reducing solution were added at 1:4 vol/vol, respectively.

Samples (0.5 g) of the substrate were weighed into 120 mL serum bottles. Consequently, 50 mL of previously prepared rumen liquor and the buffer were added. Bottles were maintained at

constant CO_2 flow for 30 sec, and then capped with neoprene plugs and sealed with aluminum rings. The vials were placed in an incubator (Riossa®, F-51 D, Mexico State, Mexico) at 39 °C for 48 h. Moreover, three bottles as blanks (rumen fluid only) were incubated for 48 h. Three incubation runs were performed in three weeks.

The gas production (GP) readings were performed at 2, 4, 6, 8, 10, 12, 24 and 48 h of incubation. A water displacement apparatus was used according to Fedorak and Hrudey (1983). The apparatus was designed with a universal support, with a conical funnel, a 100 mL burette and two latex hoses of 0.5 and 1 m in length and 3/8-inch diameter. The vials were punctured with a 16-gauge needle placed at the end of the hose. Gas production (mL) was measured by the displacement of water in the burette.

After 48 h of incubation, 5 ml of gas were taken and stored in the vials with saturated saline solution prepared with 400 g of NaCl in 1 L of distilled water, and the pH adjusted at 2 and 5 mL of 20% methyl orange added as an indicator for CH_4 and CO_2 concentrations determination. The saturated saline solution was previously prepared and stored in 60 mL serological vials, leaving no space; and neoprene plugs were placed and sealed with aluminum rings, and stored away from light. For the determination of CH_4 and CO_2 from the vials with saturated saline, a sample of 10 µL of the gas phase was taken and injected into a PerkinElmer, Claurus 500 gas chromatograph (Mexico City, Mexico) with a flame ionization detection, and helium as the carrier gas. A thermal conductivity detector was used, the oven, column and TCD temperatures were 80°C, 170°C and 130°C respectively. Retention times were 0.73 min and 1.05 min for CH_4 and CO_2 respectively.

At the end of incubation at 48 h, the fermentation process was stopped by swirling the bottles in ice for 5 minutes, then the bottles were uncapped and the pH was measured using a pH meter (Thermo Scientific, Orion Star™ A121, Beverly, MA, USA). The contents of the bottles were

filtered in Ankom® Technologies F57 bags (at constant weight), with the aid of a filtration system connected to a vacuum pump. The bottles were rinsed with a hot water 3 times to ensure recovery of all the residue of the fermentation. The bags were placed in a forced-air oven at 55 °C for 48 h. Dry matter degradation was calculated by considering the initial weight of the substrate and the weight of the residue.

After pH measure and filtration, 4 mL of the medium were obtained with a syringe and mixed with 1 mL of 25% metaphosphoric acid, shaken slightly and placed under freezing until analysis of ammonia-N (NH_3-N) concentration. Other 4 mL of the medium were mixed with 1 mL 10% formaldehyde, shaken slightly and placed in refrigeration until analysis of bacterial and protozoal counting.

2.3. Total bacteria and protozoa counting

The population of total bacteria was determined at 48 h of incubation using a count chamber bacterium Petroff-Hausser (Hausser Scientific®, 3900, Horsham, PA) and a phase contrast microscope (Olympus®, BX51, Mexico City, Mexico) at a magnification of 100x. Exactly, 0.5 mL of the 10% formaldehyde fixed medium sample was taken and diluted in 4.5 mL of distilled water. The concentration of bacteria per mL was determined as the average of bacteria observed in each grid, multiplied by the dilution factor and the chamber factor (2×10^7), according to the following formula: $\text{Bacterial number/mL} = \mu \times FD1 \times FD2 \times 2^7$

Where: μ is the average of bacteria in each grid per treatment, FD1 is the first dilution factor (1.25), and FD2 is the second dilution factor (10)

For the protozoal number determination, 1 mL of the 10% formaldehyde fixed sample was obtained and diluted in 1 mL of distilled water; then 0.5 mL of the mixture was taken with a Pasteur

pipette (BRAND, 7712, Wertheim, Germany) which were deposited in a Neubauer chamber (BRAND, 7178-10, Wertheim, Germany) and subsequently observed on a contrast microscope (Carl Zeiss®, Axiostar, Mexico City, Mexico) at 400× magnifications. The count of protozoa was made in eight quadrants (4 of each grid), taking as viable protozoans those that maintained their morphological integrity. The concentration of protozoa per mL of culture medium was estimated as the average of protozoa observed in each grid, multiplied by the dilution factor and the chamber factor (1×10^4), according to the following formula: $\text{Protozoal number} = \mu \times \text{FD1} \times \text{FD2} \times 10^4$

Where: = μ is the average of protozoa in each grid per treatment, FD1 is the first dilution factor (5), and FD2 is the second dilution factor (3).

2.4. Chemical analyses

Samples of the diets were analyzed for DM (#934.01), ash (#942.05), N (#954.01) and EE (#920.39) according to AOAC (1997), while the neutral detergent fiber (NDF) (Van Soest et al., 1991), and acid detergent fiber (ADF) and lignin (AOAC, 1997; #973.18) analyses were carried out using an ANKOM^{200} Fiber Analyzer Unit (ANKOM Technology Corp., Macedon, NY, USA) with the use of an alpha amylase and sodium sulfite.

The concentration of ruminal NH_3-N was determined according to Broderick and Kang (1980) methods. Samples of the incubation medium were centrifuged at 3000×g for 10 min, and 20 μL of the supernatant was mixed with 1 mL of phenol and 1 mL of hypochlorite, and the mixture was incubated at 39 °C for 30 min, after they were diluted with 5 mL of distilled water. Samples were read on a visible ultraviolet light spectrophotometer (Varian, model Cary 1E, California,

USA) at 630 nm. The resulting g/L concentration was divided by 0.8 which was the 25% metaphosphoric acid dilution factor.

2.4. Calculations and statistical analyses

For estimation of gas production (GP) kinetic, recorded gas volumes (mL/g DM) were fitted using the NLIN procedure of SAS (2002) according to France et al. (2000) model as:

(1) $y = b \times [1 - e^{-c\,(t-L)}]$

where y is the volume of GP at time t (h); b is the asymptotic GP (mL/g DM); c is the fractional rate of fermentation (/h), and L (h) is the discrete lag time prior to the time any gas was released.

Metabolizable energy (ME, MJ/kg DM) and *in vitro* organic matter digestibility (OMD, g/kg DM) were estimated according to Menke et al. (1979) as:

(2) ME = 2.20 + 0.136 GP (mL/0.5 g DM) + 0.057 CP (g/kg DM)

(3) OMD = 148.8 + 8.89 GP + 4.5 CP (g/kg DM) + 0.651 ash (g/kg DM)

where GP is net GP in mL from 200 mg of dry sample after 24 h of incubation.

The partitioning factor at 24 h of incubation (PF_{24}, a measure of fermentation efficiency) was calculated as the ratio of DM degradability in vitro (mg) to the volume (mL) of GP at 24 h (i.e., DMD/total GP (GP_{24})) according to Blümmel et al. (1997). Gas yield (GY_{24}) was calculated as the volume of gas (mL gas/g DM) produced after 24 h of incubation divided by the amount of DMD (g) as:

(4) GY_{24} = mL gas/g DM/g DMD

Short chain fatty acid concentrations (SCFA) were calculated according to Getachew et al. (2002) as:

(5) SCFA (mmol/200 mg DM) = 0.0222 GP − 0.00425

where: GP is the 24 h net GP (mL/200 mg DM).

Microbial biomass production (MCP) was calculated (Blümmel et al., 1997) as:

(6) MCP (mg/g DM) = Milligrams DMD − (Milliliter gas × 2.2 mg/mL)

where the 2.2 mg/mL is a stoichiometric factor that expresses mg of C, H and O required for the SCFA gas associated with production of 1 mL of gas (Blümmel et al., 1997).

Data of each of the three runs within the same sample of each of the three individual samples of rations were averaged prior to statistical analysis and the mean values of each individual sample were used as the experimental unit. The experimental design was a factorial arrangement with 3 replicates in a randomized complete block design. Data were analyzed using the GLM procedure (SAS, 2002) with the model: $Y_{ijk} = \mu + R_i + M_j + (R \times M)_{ij} + \varepsilon_{ijk}$ where: Y_{ijk} is the observation, μ is the population mean, R_i is the inoculum source effect, M_j is the level of MLM in the ration, $(R \times M)_{ij}$ is the interaction between MLM level and inoculum type, and ε_{ijk} is the residual error. Tukey test was used to compare means.

3. Results

3.1. Chemical composition

Replacing soybean meal with MLM gradually decreased DM, OM, CP, NSC, and hemicellulose, while it gradually increased EE, NDF, ADF, and cellulose contents of the rations (Table 1).

3.2. Gases production

Inoculum type × TMR interactions were observed ($P<0.05$) for GP parameters and CH_4 production (Table 2). Gas production parameters, CH_4 production and CO_2 production differed

(P<0.001) between goat and steer rumen liquors. Moreover, replacing soybean meal with MLM affected GP parameters and CH_4 production.

Fig. 1 and 2 show GP of TMR with different levels of MLM incubated with rumen inoculums from goats and steers at different incubation hours. With goat rumen inoculum, replacing soybean with MLM decreased the asymptotic GP (linear and quadratic effects; P<0.01), but increased the rate of GP (linear and quadratic effects; P<0.01) and lag of GP (linear effect; P<0.001). On the other hand, excluding TMR10 and TMR40 treatments, MLM containing rations quadratically decreased (P=0.011) the asymptotic GP, and linearly increased (P≤0.003) the rate of GP and the lag time of GP.

With rumen inoculum from goats, the inclusion of MLM decreased (linear and quadratic effects, P<0.05) CH_4 production and also the proportions of CH_4 production (linear effect, P<0.05), while it increased (linear effect, P=0.034) CO_2 production (Table 2).

3.3. Fermentation kinetics

Inoculum × TMR interactions were observed (P<0.05) for SCFA, NH_3-N, OMD, ME, PF24, GY24, and MCP (Table 3). All determined fermentation parameters differed goats. The ration effect on these parameters was quadratic (P<0.01).

With goats' rumen liquor, MLM increased fermentation pH (linear effect, P=0.037) and total ruminal bacteria (quadratic effect, P=0.045), but decreased ruminal NH_3-N (linear and quadratic effects, P<0.05), OMD (quadratic effect, P=0.033), and total protozoa number (P=0.015). With the steer inoculum, decreased concentrations of SCFA (quadratic effect, P=0.005), NH_3-N (linear and quadratic effects, P<0.001), and total protozoa number (quadratic effect, P=0.027) were observed when soybean was replaced with MLM. However, MLM

containing rations increased DMD (linear effect, P=0.002), OMD (quadratic effect, P=0.002), ME (quadratic effect, P=0.002), MCP (quadratic effect, P=0.005), and total bacterial number (linear effect, P=0.021).

4. Discussion

4.1. Chemical composition

The observed changes in the chemical composition when MLM replaced soybean meal were expected. *M. oleifera* leaf meal contains a high fiber fractions and less protein compared with soybean meal. In the present experiment, MLM contained 281 g CP which represents about 69% compared with soybean meal which contained 408 g CP. On the other hand, MLM contained about 345 g NDF versus 143 g for soybean meal. The chemical composition is expected to affect the fermentation of each ration, as explained later. The protein content of MLM is comparable to sesame meal (26%), however, less than soybean meal (approximately 40-44% CP), cottonseed meal (approximately 40% CP) and sunflower seed cake (approximately 35% CP), which are mostly used as protein concentrates in ruminant nutrition. Kholif et al. (2015) replaced sesame meal in the diets of goats with fresh MLM at 0, 50, 75, and 100%, and found that the CP content of the diets was not changed significantly; however, the NDF content of the diets increased. This is a result of almost equally CP content and high NDF content of MLM and sesame meal.

4.2. Gas production

The observed interactions between inoculum type and MLM level for most measured parameters of GP and fermentation reveal that the response to replacing soybean meal with MLM differed between goats These differences may be supported by the significant different response

in goat inoculums, and explain possible differences in the ruminal microbial population and the digesting capacity of ruminant species. This is an important indicator of the importance of using rumen fluid from different ruminant species to inoculate the substrates *in vitro* incubation cultures to evaluate feed nutritive value. Aderinboye et al. (2016) reported different fermentation parameters among cows, sheep and goat inoculums.

Replacing soybean with MLM affected negatively GP. Soliva et al. (2005) observed that complete replacing of soybean meal and rapeseed meal with MLM decreased *in vitro* total GP. The decreased GP with increasing lag of GP is a direct result of increased fiber concentration in MLM-containing rations. Kholif et al. (2017b) stated that the increasing fiber portion in TMR decreased the asymptotic GP. In another experiment, Elghandour et al. (2015) observed that increasing ration content of corn silage instead of concentrate feed mixture decreased GP and increased the lag time of GP. Moreover, the decreasing CP concentration in rations of MLM can partially explain the decreased GP (Elghandour et al., 2017). They showed that increasing CP content of a ration increases GP. The crude protein content is a secondary reason for the decreased GP compared with fiber content because fermentability of protein produces relatively small GP compared to carbohydrate fermentation (Makkar et al. 1995). It was expected that the observed increased bacterial number with MLM would increase GP, but this expectation did not exist. The reasons for these observations are unknown.

Plant secondary metabolites (PSM) presented in MLM can be another reason for the negatively affected ruminal fermentation. Generally, plant secondary compounds at high doses have a great antimicrobial activity against ruminal bacteria, protozoa and fungi (Bodas et al., 2012). The antimicrobial effect depends on plant species, the chemical composition of the plant, and the dose fed to animal (Bodas et al., 2012). Therefore, PSM sometimes can stimulate rumen

microbial activity (Benchaar et al., 2008), and this may explain the greater GP and shorter lag time in the case of TMR10 and TMR40 rations for steers inoculum. Ruminal microflora can tolerate and degrade low and moderate concentrations of PSM such as phenolic compounds (Varel et al., 1991) and tannins (Frutos et al., 2004), and utilize them as energy sources.

Many reports showed that goats have a high ability to tolerate high levels of tannins compared with other ruminant species (Frutos et al., 2004; Yisehak et al., 2016). In the present experiment, ruminal microflora from steers showed better response compared with that of goats, which is not in line with Frutos et al. (2004) and Yisehak et al. (2016). This may be due to the previous feed fed to the goats before starting the experiment. Ruminal microbial population depends mainly on the type of diet fed; therefore, based on the fact that goats in the present experiment were maintained on the same diet, microbial species were not expected to vary (Mould et al., 2005).

4.3. Greenhouse gases production

Reducing CH_4 production from livestock is always desirable from environmental point of view. The decreased CH_4 production with MLM-containing rations may be related to the PSM such as tannins and saponins in MLM, or the high proportion of α-linolenic acid (Machmüller et al., 2000) in MLM (Soliva et al., 2005). A decreased CH_4 production was observed by Soliva et al. (2005) from MLM compared with soybean meal. The antimicrobial and protozoal effects of PSM such as tannins can be a direct reason for the declined CH_4 production (Bodas et al., 2012). Moreover, the adverse effect of PSM on cellulolytic bacteria (Patra and Saxena, 2009) can cause a reduction in CO_2 and H_2 formation, which are required for methanogenesis, as a result of decreased SCFA production, in particular acetate (Goel and Makkar, 2012) causing a reduction in

CH_4 production. Moreover, Jayanegara et al. (2011) reported mitigating effects of phenolic compounds on CH_4 production. Goel and Makkar (2012) reported a decreased CH_4 production up to 50% in response to tannins and phenolic compounds.

4.4. Ruminal microflora

Increasing ruminal bacterial number with MLM was not expected based on the antimicrobial properties of PSM in MLM (Bodas et al., 2012). However, the reports of Varel et al. (1991) and Frutos et al. (2004) about the ability of ruminal microflora to degrade and utilize PSM as energy sources can explain the increased ruminal bacterial number. The increased bacterial populations seem to be a consequence of the observed inhibition of ruminal protozoa (Newbold et al., 1997; Goel et al., 2008), as ruminal protozoa is the main predators of bacteria in the rumen (Mathieu et al., 1996).

However, the increased bacterial number with MLM rations did not result in a greater GP or ruminal nutrient degradability or SCFA production. This may be due to the fact that not all bacteria species are affected in the same way. For example, tannins and saponins are particularly able to inhibit Gram-positive bacteria more than Gram negative bacteria (Bodas et al., 2012). Thus, the increased bacterial number might be due to increase in other species and not cellulolytic bacteria.

The decreased protozoal number when soybean was replaced with MLM is a result of the marked anti-protozoal activity of PSM such as saponins, tannins and phenolic compounds (Makkar et al., 1995; Bhatta et al., 2009; Bodas et al., 2012). Bhatta et al. (2009) reported that tannins have a clear defaunating effect, without a clear mode of action. Decreasing ruminal protozoa population is desirable because this will allow for a lower CH_4 production and higher bacterial numbers.

4.5. Fermentation kinetics

Increasing ruminal pH in goat nutrition is a good subject to allow better ruminal condition for cellulolytic bacteria activity. In the present experiment, ruminal pH values ranged between 6.42 and 6.48, and fell within the range considered acceptable for fiber digestion (Ørskov and Ryle, 1990).

In the present experiment, ruminal NH_3-N concentrations ranged between 49.0 and 69.6 g/L, and were above the range required for sufficient microbial protein synthesis (Satter and Slyter, 1974). The decreased ruminal NH_3-N with MLM is a result of the reported low degradability of MLM protein in the rumen (Kholif et al., 2015, 2016) due to tannins and phenolic compounds in MLM (Bodas et al., 2012). Tannins in feeds may reduce ruminal protein degradation because tannins have an ability to bind to dietary protein and protect it from ruminal degradation (Frutos et al., 2004). Besides, PSM such as saponins and tannins have the ability to decrease ruminal protozoa (Newbold et al., 1997) as we previously showed. Protozoa play a major role in ruminal feed protein degradation (Jouany, 1996). Another probable reason for the decreased NH_3-N is the inhibition of hyper NH_3-producing bacteria activity and their deaminase activity (Newbold et al., 2004).

The increased bacterial numbers with MLM-containing diets did not result in increased OMD or SCFA production in goat inoculum. The negatively affected nutrient degradability in MLM-containing rations may be due to the negative effects of increasing fiber concentration and the declining CP concentration on ruminal fermentation. Frutos et al. (2004) reported that less than 50 mg/g DM is the acceptable level of tannins in feeds without negative effects on digestibility. In the present experiment, tannins concentration was 22 mg/g DM, which is less the critical level that

suppresses ruminal fermentation. Therefore, tannins cannot be the main reason for the decreased degradation but the increasing fiber concentration in MLM rations, as earlier speculated. Elghandour et al. (2015) observed that increasing fiber concentration in a ration reduced nutrient degradability. In steer nutrition, the result of DMD was in contrary to the observed result in goat nutrition. Differences in ruminal microflora response to PSM might be the reason.

Decreased SCFA concentration can be interpreted as a result of declined digestion of MLM containing ration. Flatt et al. (1956) reported that the concentrations of ruminal SCFA production depend on nutrient digestibility and the activity of microbial population in the rumen. Results of decreasing NH_3-N concentration with decreasing total SCFA concentration is an evidence of improved synchronization between dietary energy and protein, which is expected to increase microbial-N production within the rumen (Seo et al., 2014). Soliva et al. (2005) compared the ruminal fermentation of MLM with soybean meal and rapeseed meal, and observed unaffected ruminal pH values and SCFA concentration, and a decreased ruminal NH_3-N concentration with MLM.

5. Conclusion

From the nutritional perspective, *M. oleifera* cannot replace soybean meal as a protein feed in diets for goats and steers because of the negative effect on ruminal fermentation. However, from an environmental point of view, replacing soybean meal with *M. oleifera* leaf meal reduced CH_4 production, which can be used as a good cleaner product for the environment and feedstuff for ruminant livestock to control the environmental contamination by biogases pollution from ruminants. More research will be desirable to determine the best levels of replacement on feed utilization and methane production in dairy and beef cattle, wool and meat sheep and dairy goats.

Besides, more experiment should be carried out to replace other protein feeds with low protein concentrates such as sesame meal and rapeseed meal with *M. oleifera* at different levels in both *in vitro* and *in vivo* trials.

Conflict of interest

All authors declare that there are no present or potential conflicts of interest between them and other people or organizations that could inappropriately bias their work.

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Table 1

Chemical composition[1] of feedstuffs and total mixed rations (TMR) with different levels of *Moringa oleifera* replacing soybean meal as a protein source

	DM (g/kg wet material)	OM	CP	EE	NSC	NDF	ADF	ADL	Cellulose	Hemicellulose
Ingredients										
Alfalfa hay	902.0	885.8	189.2	25.3	218.9	452.4	330.1	82.1	248.0	122.3
M. oleifera hay	868.2	891.0	281.1	40.9	224.4	344.6	301.0	77.6	223.4	43.6
Crushed yellow corn	866.0	890.3	90.8	45.2	540.0	214.3	88.8	10.4	78.4	125.5
Soybean meal	889.0	927.9	408.1	21.4	355.7	142.7	96.3	8.8	87.5	46.4
Wheat bran	871.4	852.2	129.7	56.2	204.4	461.9	130.6	38.0	92.6	331.3
Total mixed rations[2]										
TMR0	886.7	894.1	213.4	32.4	331.9	316.4	191.4	41.4	149.9	125.0
TMR10	886.2	893.2	210.2	32.9	328.6	321.4	196.5	43.2	153.3	125.0
TMR20	885.7	892.2	207.0	33.4	325.4	326.5	201.6	44.9	156.7	124.9
TMR30	885.1	891.3	203.9	33.9	322.1	331.5	206.7	46.6	160.1	124.8
TMR40	884.6	890.4	200.7	34.3	318.8	336.6	211.8	48.3	163.5	124.7
TMR50	884.1	889.5	197.5	34.8	315.5	341.6	217.0	50.0	166.9	124.7
TMR60	883.6	888.6	194.3	35.3	312.2	346.7	222.1	51.8	170.3	124.6
TMR70	883.1	887.6	191.2	35.8	308.9	351.7	227.2	53.5	173.7	124.5
TMR80	882.5	886.7	188.0	36.3	305.7	356.8	232.3	55.2	177.1	124.5
TMR90	882.0	885.8	184.8	36.8	302.4	361.8	237.4	56.9	180.5	124.4
TMR100	881.5	884.9	181.6	37.3	299.1	366.9	242.6	58.6	183.9	124.3

[1]ADF, acid detergent fiber; CP, crude protein; DM, dry matter; EE, ether extract; NDF, neutral detergent fiber; NSC, non-structural carbohydrates; OM, organic matter

[2]*Moringa oleifera* replaced 0% (TMR0), 10% (TMR10), 20% (TMR20), 30% (TMR30), 40% (TMR40), 50% (TMR50), 60% (TMR60), 70% (TMR70), 80% (TMR80), 90% (TMR90), and 100% (TMR100) of soybean meal, respectively.

Table 2

Biogases production (mL/g DM) of total mixed rations (TMR)[1] containing different levels of *Moringa oleifera* leaf meal replacing different levels of soybean meal, and incubated with rumen liquors from goats

	TMR	Gas production parameters[2]				CH_4 production at 48 h of incubation			CO_2 production at 48 h of incubation		
		b	*c*	*Lag*	mL gas/g degraded DM	mL CH_4/g incubated DM	Proportional CH_4 production	mL CH_4/g degraded DM	mL CO_2/g incubated DM	Proportional CO_2 production	mL CO_2/g degraded DM
	TMR0	288	0.091	1.39	369	243	85.4	315	41.4	14.6	53.7
	TMR10	291	0.096	1.91	376	245	85.4	321	42.1	14.6	55.1
	TMR20	257	0.108	2.62	336	214	83.7	282	41.5	16.3	54.4
	TMR30	235	0.106	2.77	309	194	82.9	256	39.9	17.1	52.7
	TMR40	267	0.109	2.53	357	217	81.5	291	49.3	18.5	66.3
	TMR50	280	0.116	2.56	381	228	81.7	311	51.0	18.3	69.5
	TMR60	262	0.106	2.46	343	211	80.9	278	49.7	19.1	65.5
	TMR70	268	0.118	2.48	365	219	81.9	299	48.3	18.1	66.0
	TMR80	269	0.124	2.54	356	219	81.7	291	49.3	18.3	65.2
	TMR90	260	0.113	2.38	360	208	80.3	289	51.0	19.7	70.9
	TMR100	264	0.122	2.52	363	212	80.4	292	51.7	19.6	71.2
SEM		4.3	0.0025	0.118	11.5	3.8	0.58	9.5	1.71	0.58	2.97
Linear		0.009	<0.001	<0.001	0.733	0.011	0.026	0.922	0.034	0.267	0.317

Quadratic	0.011	0.003	0.725	0.473	0.037	0.460	0.568	0.114	0.460	0.349
P value										
TMR										
Linear	0.007	<0.001	<0.001	0.019	0.076	0.218	0.036	0.068	0.218	0.022
Quadratic	0.004	0.028	0.798	0.049	0.001	0.330	0.079	0.020	0.331	0.054

[1]*Moringa oleifera* replaced 0% (TMR0), 10% (TMR10), 20% (TMR20), 30% (TMR30), 40% (TMR40), 50% (TMR50), 60% (TMR60), 70% (TMR70), 80% (TMR80), 90% (TMR90), and 100% (TMR100) of soybean meal, respectively.

[2]*b* is the asymptotic gas production (mL/g DM); *c* is the rate of gas production (/h); *Lag* is the initial delay before gas production begins (h).

Table 3

Fermentation kinetics[1] of total mixed rations (TMR)[2] containing different levels of *Moringa oleifera* leaf meal replacing different levels of soybean meal, and incubated with rumen liquors from goats

	TMR	pH	SCFA	NH_3-N	DMD	OMD	ME	PF_{24}	GY_{24}	MCP	Total bacteria × 10^8	Total protozoa × 10^5
	TMR0	6.42	5.65	69.6	771	702	10.3	5.15	194	753	9.5	5.12
	TMR10	6.44	5.78	65.3	765	712	10.5	5.13	195	765	10.3	4.27
	TMR20	6.47	5.25	58.6	763	665	9.8	5.23	191	720	13.1	3.42
	TMR30	6.45	4.78	58.8	756	629	9.2	5.35	187	681	13.5	3.96
	TMR40	6.44	5.48	60.2	745	679	10.0	5.18	193	739	11.3	3.27
	TMR50	6.46	5.81	58.4	734	708	10.5	5.12	195	767	11.1	3.44
	TMR60	6.48	5.34	53.9	759	668	9.9	5.21	192	728	11.4	3.62
	TMR70	6.44	5.59	56.6	734	685	10.1	5.16	194	748	13.2	4.10
	TMR80	6.46	5.65	49.0	755	689	10.2	5.15	194	754	11.0	3.93
	TMR90	6.40	5.37	51.0	720	667	9.8	5.21	192	730	11.3	3.92
	TMR100	6.44	5.53	52.0	727	681	10.0	5.18	193	744	11.4	2.88
SEM		0.005	0.093	1.37	15.7	7.5	0.11	0.018	0.7	7.8	0.810	0.203
Linear		0.037	0.399	<0.001	0.062	0.059	0.074	0.308	0.412	0.403	0.733	0.812
Quadratic		0.087	0.104	0.014	0.399	0.033	0.338	0.111	0.105	0.100	0.045	0.015
P value												
TMR												
Linear		0.066	0.660	<0.001	0.146	0.363	0.466	0.790	0.726	0.664	0.023	0.047
Quadratic		0.015	0.001	<0.001	0.805	0.001	0.001	0.002	0.001	0.009	0.477	0.818

[1]DMD is dry matter degradability (mg/g DM), GY_{24} is gas yield at 24 h (mL gas/g DMD), MCP is microbial protein production (mg/g DM), ME is metabolizable energy (MJ/kg DM), NH_3-N (g/L) is ammonia-N, OMD is *in vitro* organic matter digestibility (g/kg DM), PF_{24} is partitioning factor at 24 h of incubation (mg DMD/mL gas), pH is ruminal pH, SCFA is short-chain fatty acids (mmol/g DM).

[2]*Moringa oleifera* replaced 0% (TMR0), 10% (TMR10), 20% (TMR20), 30% (TMR30), 40% (TMR40), 50% (TMR50), 60% (TMR60), 70% (TMR70), 80% (TMR80), 90% (TMR90), and 100% (TMR100) of soybean meal, respectively.

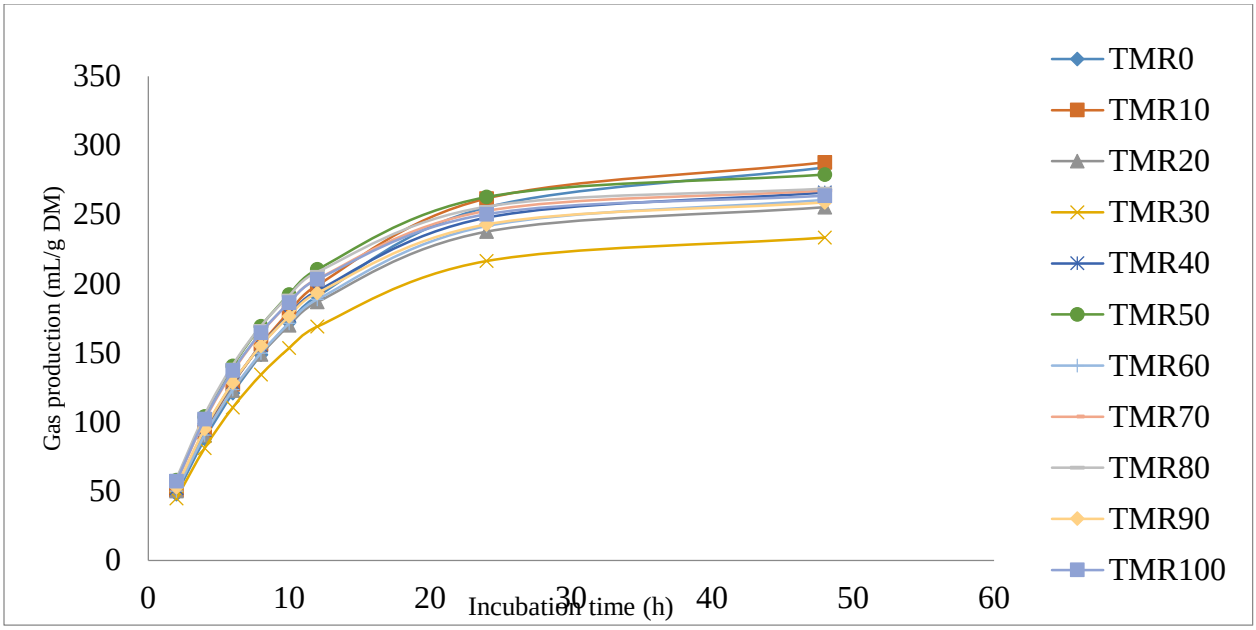

Fig. 1

Gas production (mL/g DM) of total mixed rations (TMR)[1] containing different levels of *Moringa oleifera* leaf meal replacing different levels of soybean meal, and incubated with rumen liquors from goats. *Moringa oleifera* soybean meal at (/100 g DM): 0 g (TMR0, control), 10 g (TMR10), 20 g (TMR20), 30 g (TMR30), 40 g (TMR40), 50 g (TMR50), 60 g (TMR60), 70 g (TMR70), 80 g (TMR80), 90 g (TMR90), and 100 g (TMR100).