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Effect of *Pouteria sapota* kernel meal on *in vitro* ruminal fermentation, nutrient degradability, and protozoa population

Adriana Sánchez-Zárate[1a], Alfonso Juventino Chay-Canul[2], Edgar Aguilar-Urquizo[1b], Jorge Canul-Solis[1c], German Giácoman-Vallejos[3a], Avel González-Sánchez[3b], Emanuel Hernández-Núñez[4], Alfonso Pérez-Gutiérrez[1d], San German Bautista-Parra[1e], Einar Vargas-Bello-Pérez[5], and Angel Trinidad Piñeiro-Vázquez[1f]*

[1a] Tecnológico Nacional de México, Campus Conkal, Yucatán, Mexico
https://orcid.org/0000-0003-4412-4947
[1b] Tecnológico Nacional de México, Campus Conkal, Yucatán, Mexico
https://orcid.org/0000-0003-4727-7894
[1c] Tecnológico Nacional de México, Campus Tizimín, Yucatán, Mexico
https://orcid.org/0000-0001-9934-4302
[1d] Tecnológico Nacional de México, Campus Conkal, Yucatán, Mexico
https://orcid.org/0000-0001-9838-2030
[1e] Tecnológico Nacional de México, Campus Conkal, Yucatán, Mexico
https://orcid.org/0009-0001-8655-0884
[1f] Tecnológico Nacional de México, Campus Conkal, Yucatán, Mexico
https://orcid.org/0000-0002-8400-4046
[2] Universidad Juárez Autónoma de Tabasco, División Académica de Ciencias Agropecuarias, Tabasco, Mexico
https://orcid.org/0000-0003-4412-4972
[3a] Facultad de Ingeniería. Universidad Autónoma de Yucatán. Industrias no Contaminantes S / N por Periférico Norte. CP. 97310, Mérida, Yucatán, Mexico
https://orcid.org/0000-0002-9792-6904
[3b] Facultad de Ingeniería. Universidad Autónoma de Yucatán. Industrias no Contaminantes S / N por Periférico Norte. CP. 97310, Mérida, Yucatán, Mexico
https://orcid.org/0000-0003-4727-7894
[4] Departamento de posgrado e investigación, Instituto Tecnologico de Calkiní. Av. Ah Canul S/N por carretera federal, 24930. Calkiní, Campeche, México.
https://orcid.org/0000-0002-7467-7538
[5] Facultad de Zootecnia y Ecología, Universidad Autónoma de Chihuahua, Periférico R. Aldama Km 1, 31031 Chihuahua, Mexico
https://orcid.org/0000-0001-7105-5752
* Corresponding author:pineiroiamc@gmail.com

ABSTRACT

This study determined the effect of different levels of mamey kernel meal (MKM) as a substitute for ground corn and soybean meal on *in vitro* gas production, nutrient degradability, fermentation, and protozoan population. The study was carried out in a randomized block design repeated over time. The treatments consisted of seven levels of mamey kernel meal, namely 0, 5, 10, 15, 20, 25, and 30% dry matter (DM), replacing ground corn and soybean meal. The inclusion of MKM had a linear effect on *in vitro* gas production (P=0.03); the highest production was at 30% inclusion and the lowest at 0%, with 381 and 291 mL g^{-1}, respectively. No differences (P>0.05) were found in gas production,

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fermentation rate, and lag time between treatments. The maximum volume of gas production (397.43 mL g^{-1}) was at 30%, while the lowest (291.40 mL g^{-1}) was observed at 0% inclusion of MKM. No differences were found in nutrient degradability, protozoan population, and volatile fatty acids (P>0.05). The genera of protozoa that were identified were Entodinium and Holotrich. The different inclusion levels of MKM did not affect the total protozoa population (P>0.05). In conclusion, MKM can be included up to 15% under *in vitro* conditions without negative effects on fermentative parameters. However, further *in vivo* studies are needed.

Keywords: byproduct, *Pouteria sapota*, gas production, seed meal, oleic acid.

INTRODUCTION

Feeding ruminants with agro-industrial byproducts can be an alternative for contributing to the sustainable development of livestock activity. Agro-industrial byproducts can have fair amounts of nutrients (14% CP and 40% EE), making them suitable for inclusion in ruminant diets (Silva et al., 2015).

In tropical areas of Mexico, the production and processing of mamey (*Pouteria sapota)* generates residual biomass and the kernel is the main byproduct (Solís-Fuentes et al., 2015). The chemical composition of the mamey kernel varies and depends on the fruit growth stage, as well as agroecological and climatic conditions. Mamey kernels have a high content of lipids (40% DM) and protein (14% DM) (Moo-Huchin et al., 2013) and, consequently, it could be used in animal feed as a source of energy and protein to partially replace common feedstuffs such as soybean and corn grain.

Previous studies that have investigated oilseeds, including avocado and sunflower, as unconventional feed sources have reported that it is possible to include them in ruminant diets from moderate to high amounts. These studies have recommended that up to 10% of oilseeds can be included without affecting nutrient degradability (Herrera-Pérez et al., 2018; Lemus-Flores et al., 2020).

In the tropics, mamey kernel meal (MKM) can be an alternative to traditional ingredients in ruminant diets that are used to increase energy density and improve animal performance. However, there is limited evidence of the use of this byproduct in ruminant feed. Therefore, in this study, we hypothesized that the high lipid content from MKM could affect nutrient degradability, fermentation kinetics, and population of protozoa at the ruminal level. Hence, the objective of this study was to determine the effect of different levels of MKM as a substitute for ground corn and soybean meal on *in vitro* gas production, nutrient degradability, fermentation kinetics, and protozoan population.

MATERIALS AND METHODS

The study was conducted at the Digestive Physiology Laboratory of the Technological Institute of Conkal, Yucatán, municipality of Conkal (21°, 05', N; 89°, 32', W, with an altitude of 9 masl). With an average annual temperature of 26.6 °C and an average rainfall of 469 mm. Animals were handled according to the standards and procedures for handling experimental animals approved by the National Technological Institute of Mexico in the project "Productive behavior and meat quality of lambs fed with increasing levels of ground *Pouteria sapota* kernel" (ID 13289.21-P).

Pouteria sapota kernel meal

The mamey kernel used in this study was obtained from the mamey plantations of the Magaña I and Magaña II varieties, which were donated by the mamey pulp processing plant of the Huertas Magaña company, located in Akil, Yucatan (20°,16' 01.7, N, 89°, 20'56.0, W), during the harvest period of June 2020. Kernels were collected after processing the mamey pulp and transferred to the facilities of the Technological of Conkal, where they were washed, to later manually extract the kernels for their subsequent dehydration at 60 °C in an oven with forced air circulation. Finally, they were ground in a hammer mill to obtain a particle size of 3 mm in a Thomas Wiley® mill.

Chemical analysis

Dry matter (DM) was determined by drying samples in an oven at 105 °C for 16 h. Ash content was determined by incineration at 550 °C for 6 h, while ether extract (EE), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) were measured according to the Association Official Analytical Chemists (AOAC, 1990).

The chemical composition from MKM on a dry basis was 41.8% DM, 95.9% OM, 14.6% CP, 45.2 EE, 26.90% NDF, 12.6% ADF, and 7.9% lignin. The fatty acid composition of mamey kernel meal was mostly composed of 48 g 100 g^{-1} of C18:1cis9,

15 g 100g^{-1} of C18:2 n6 cis, 12 g 100 g^{-1} of C18:3 n3, and 6 g 100 g^{-1} of C16:0.

Dietary treatments

The treatments consisted of seven diets, based on straw, molasses, wheat bran, ground corn, and soybean meal with different levels of mamey kernel meal inclusion: 0, 5, 10, 15, 20, 25, and 30%, formulated to replace the ground corn and soybean meal. The chemical composition of the treatments is shown in Table 1.

Ruminal inoculum and in vitro incubations

The ruminal inoculum used for *in vitro* incubations was collected from four hair sheep with an average live weight of 31±1 kg. Animals were housed in a roofed pen of 5 × 6 m with straw bedding. The animals were fed twice a day for three weeks with a diet based on 40% forage and 60% concentrate with a crude protein content of 16% per kg of DM. Water was provided *ad libitum*. Ruminal fluid was extracted by esophageal tube (Ramos-Morales et al., 2014); samples were collected 2 h after morning feeding (09:00 h), placed in an airtight thermos at 39 °C under anaerobic conditions, and subsequently transported to the laboratory to be filtered with double gauzes and immediately saturated with carbon dioxide (CO_2).

In vitro incubations were carried out in amber flasks with a capacity of 120 mL. The content of each bottle was composed of 1 g of substrate, with a particle size of 1 mm, corresponding to the inclusion levels (0 to 30%) of dietary MKM. Subsequently, 90 mL of mixed ruminal inoculum was added in a 1:9 ratio, with a reduced mineral solution according to the method proposed by (Menke and Stengass, 1988). As the inoculum was added, the continuous flow of CO_2 was constant to maintain anaerobic conditions. The bottles were hermetically sealed with rubber stoppers and aluminum rings. Four bottles were included as blanks, which only contained ruminal inoculum. The flasks were placed in a water bath at a constant temperature of 39 °C. Subsequently, gas production was measured with a manometer with a scale from 0 kg cm^2 to 1 kg cm^2 with a hypodermic needle at 2, 4, 6, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60 and 72 h of incubation, which was transformed into gas volume using the following logistic regression equation: V = (P + 0.019) (0.024) −1 where V = volume of gas produced and P = pressure that is generated in each bottle.

***In vitro* gas fermentation kinetics**

The maximum volume (Vm, mL g^{-1}), the fermentation rate (S, h^{-1}) and the lag phase (L, h^{-1}) of gas production were estimated in the

Table 1. Ingredients and chemical composition of the experimental diets with increasing levels of mamey kernel meal.

	Mamey kernel meal (%)						
	0	**5**	**10**	**15**	**20**	**25**	**30**
Ingredients (g kg^{-1})							
Straw	200	200	200	200	200	200	200
Molasses	100	100	100	100	100	100	100
Wheat bran	100	100	100	100	100	100	100
Ground corn	364	321	278	234	191	0	0
Mamey kernel meal	0	15	100	150	200	250	300
Calcium carbonate	10	10	10	10	10	10	10
Soybean meal	205	198	191	185	178	319	269
Vitamin and mineral mix	21	21	21	21	21	21	21
Chemical composition (g kg^{-1} DM)							
Dry matter (*g kg^{-1}*)	963	900	928	900	902	896	896
Organic material	909	903	913	913	915	903	902
Crude protein	139	139	138	151	139	195	193
Ether extract	21	51	81	91	122	160	179
Neutral detergent fiber	246	229	211	248	253	310	310
Acid detergent fiber	142	128	124	113	131	175	187
Lignin	78	70	70	61	55	86	90
Non-fibrous carbohydrates	50.3	48.5	48.2	42.34	40.1	23.8	21.8
Metabolic energy (*MJ kg^{-1} DM*)	239	276	262	272	286	286	279

PROC GLM of SAS (2012) using the following logistic model: $V = Vm /1+ \exp^{(2-4*s*(t-L)}$ (Pell and Schofield, 1993), where V = volume of gas at time (t), Vm =volume maximum gas production, t = time, s = specific fermentation rate (similar to the rate of degradation) and L = colonization time of micro-organisms. Cumulative volumes of gas were also obtained for the intervals from 0 h to 8 h (Vf0 -8), from 8 h to 24 h (Vf8-24), and from 24 h to 72 h (Vf24-72) of incubation. With each accumulated volume, the fractions of rapid fermentation (RF), medium fermentation (MF), and slow fermentation (SF), were estimated according to the linear regression equations proposed by Miranda-Romero et al. (2020): RF=Vf0-0/0.427, MF=Vf8-24/0.615 and SF=Vf24-72/0.345. The sum of the three fractions was used to calculate the total fermentable fraction (TFF).

Likewise, the metabolizable energy (ME) was estimated from the following equation proposed by (Menke et al., 1979):

$$EM = (1.1456 * GP\ 24) + (0.07675 * CP) + (0.1642 * EE) + 1{,}198.$$

Where, ME= metabolizable energy (MJ kg^{-1} DM), GP24= gas production at 24 h of incubation (mL), CP= crude protein (%DM), and EE=ether extract (%DM).

pH and volatile fatty acids (VFA)

The pH was measured with a portable potentiometer (HANNA®, Woonsocket, USA), calibrated with reference buffers of pH 4, 7, and 10, and recorded obtaining the sample to determine VFA. For VFA analysis, 4 mL of ruminal fluid was placed in a tube containing 1 mL of 25% (w/v) metaphosphoric acid and frozen until later analysis according to the method described by Erwin et al., (1961), using a gas chromatograph (GC Trace 1310, Thermo Scientific, Waltham, MA USA), equipped with a flame ionization detector (FID). The type of column used was Elite-1730 m x 0.25 mm x 0.25µm, Perkin Elmer; and the injector and detector temperatures were 200 °C and 250 °C, respectively.

***In vitro* degradability of dry matter and organic matter**

In vitro degradability of DM (IVDM) was estimated by drying the residual material at 55 °C until a constant weight was achieved. The *in vitro* degradability of OM (IDOM) was estimated by combustion in a muffle at 550 °C (AOAC, 1990). Subsequently, IVDM (%) was calculated based on initial and residual DM (Monforte-Briceño et al., 2005). Similarly, IDOM (%) was calculated based on initial and residual OM (Schneider and Flatt, 1975).

Protozoa count

The protozoa in the ruminal fluid samples were counted according to the procedures described by Rosales (1989). The sample was mixed in a 1:1 ratio with a methyl green solution (35 Ml L^{-1} formaldehyde, 0.14 Mm NaCl, 0.92 Mm methyl green) and centrifuged at 2000 rpm for 20 min, kept refrigerated and in the dark. Protozoa count was enumerated microscopically (Leica-DM500 at 40x) using a counting chamber (Neubauer Improved Bright-Line counting) cell, with 0.1 mm 157 depth (Hausser Scientific, Horsham, PA, USA), and the genus was identified as indicated by Ogimoto and Imai, (1981). The number of protozoa was expressed as log10/ml and estimated with the following formulas: Number of cells per ml = [(n1 + n2 + n3 + n4 + n5) / 5] /0.022 mm^3 x 10^3 × d. where: n1 … n5: number of protozoa per quadrant and d = dilution factor.

Experimental design and statistical analysis

Data on ruminal fermentation, degradability, protozoan population, pH, and volatile fatty acids were analyzed using ANOVA with repeated measures over time using the SAS PROC ANOVA procedure (SAS Inst. Inc., Cary, NC) for a completely randomized design, with the following linear model:

$$Yij = \mu + \eta i + \alpha j + \varepsilon ij$$

Where:
and ij = score of subject i under treatment j
μ = global average of all experimental data
η i = μ i – μ = effect associated with block i
αj = μj – μ = effect of treatment j
εij = experimental error associated with subject i under treatment j.

Statistical differences were considered significant at $P \leq 0.05$. To determine the linear, quadratic, and cubic effect of the treatments, a response surface test was performed (Cochran and Cox, 1990).

RESULTS

Chemical composition

Dry matter decreased as the inclusion of MKM increased (Table 1). The inclusion of the MKM increased ether extract and was in the range of 21 g kg^{-1} DM (0%) to 179 g kg^{-1} DM (30%), which represented an inclusion percentage of EE from 2.1 to 17.9%, respectively. The CP of each level was in the range of 138 g kg^{-1} DM (10%) to 195 g kg^{-1} DM (25%). The NDF content was higher for the 25 and 30% levels (310 g kg^{-1}DM), as was the case with the ADF content (175 and 187 g kg^{-1} DM, respectively).

Gas production kinetics, and *in vitro* nutrient degradability

The substitution of ground corn and soybean meal with MKM at different inclusion levels did not affect rumen fermentation (P>0.05; Table 2). The maximum volume (Vm) of gas was observed with 30% (381.45 mL g^{-1}) and the minimum with 0% (291.40 mL g^{-1}) MKM inclusion, respectively. On the contrary, the highest value of gas production rate corresponded to 20% inclusion (0.035 h^{-1}) and the lowest to 35% (0.027 h^{-1}).

The value of L for all levels was negative. The highest level of inclusion required less time (L) to start fermentation (-0.19 h), which is related to the highest maximum volume reported. Conversely, the 10% inclusion required more time (-1.56 h), but no differences were observed (P>0.05) between inclusion levels.

Table 2. ***In vitro*** **gas production and nutrient degradability from increasing levels of mamey kernel meal as substitutes for ground corn and soybean meal.**

	Mamey kernel meal (%)									
	0	5	10	15	20	25	30	SEM[a]	P-value	Linear	Quadratic
Parameters											
Vm[b] (mL g^{-1})	291	371	311	373	329	354	381	21.96	0.043	0.035	0.799
S[c] (h^{-1})	0.031	0.027	0.032	0.029	0.035	0.032	0.027	0.004	0.738	0.887	0.455
L[d](h)	-0.97	-0.57	-1.56	-0.57	-1.19	-0.83	-0.19	0.42	0.360	0.330	0.230
Fractional volumes (mL g^{-1})											
V_{0-8} [e]	109b	119ab	120ab	120ab	135ab	128ab	121ab	4.51	0.012	0.005	0.029
V_{8-24} [f]	97.5ab	118a	105ab	113ab	110ab	115ab	116a	5.49	0.126	0.054	0.528
V_{24-72} [g]	100	145	103	151	98.0	120.40	157	22.96	0.296	0.347	0.702
$DMD_{24\,h}$ (%)[h]	64.3	66.2	66.0	60.7	63.2	67.33	67.3	2.03	0.280	0.460	0.160
$DMD_{72\,h}$ (%)[i]	70.8	77.4	79.0	77.0	73.2	73.90	72.8	2.10	0.130	0.530	0.030
$DOM_{72\,h}$ (%)[j]	74.5	81.2	83.5	80.8	77.2	77.30	76.2	2.23	0.130	0.460	0.020

[a] SEM: standard error of the mean.
[b] Vm: maximum volume of gas
[c] S: gas production rate
[d] L: lag phase;
[e] V0-8: fractional volume in the rapidly fermentable fraction (0h-8h)
[f] V 8-24: fractional volume of gas in the middle fraction (8h-24h)
[g] V24-72: fractional volume of gas in the slow fermentation fraction (24h-72h)
[h] DMD 24 h: dry matter degradability at 24 h
[i] DMD 72 h: dry matter degradability at 72 h.
[j] DMO 72 h: organic matter degradability at 72 h
Different letters in the same row indicate differences (p<0.05)

Regarding fractionable volumes, differences (P<0.05) were found in fast fermentation (V 0-8) between 20% (135.12 mL g^{-1}) and 0% (109.08 mL g^{-1}) of inclusion. Medium and slow fermentation had no differences between inclusion levels; however, 0% MKM obtained the lowest volumes of 97.5 and 100 mL g^{-1}, respectively. During the first 8 h of incubation, the inclusion of MKM obtained higher values than at 0% MKM inclusion, with a linear trend (P<0.05) maintained at 24 h of incubation (P<0.0540). At 72 h, although without differences, the levels containing MKM obtained the highest volumes compared to 0% inclusion.

No effect (P>0.05) was found on the *in vitro* degradability of dry matter with the inclusion of different levels of MKM (Table 2) at 24 h and 72 h of incubation. Inclusion of 25 and 30% MKM resulted in the highest DMD at 24h (67.3% and 67.3%), while the lowest DMD (60.7%) was obtained with 15% MKM inclusion. Similarly, without differences (P>0.05), DMD 72h with the inclusion percentages from 5 to 15% had the highest values (77.05-79.0), the percentage of degradation for 0% and from 20 to 25% of inclusion of MKM had the lowest values (70.8-72.8%).

pH and end products of ruminal fermentation

The inclusion of MKM had a linear effect (P<0.0001) on pH at 24 h of incubation, with the highest value being at 0% inclusion (6.35) and the lowest being at 25% (6.05). There were no differences (P>0.05) in the pH between the different levels at 72 h, and the pattern was contrary to that at 24 h, with the lowest value at 0% (6.07) and the highest at 30% (6.15) MKM inclusion, respectively. Lastly, total VFA was not affected (P>0.05) by MKM inclusion (Table 3).

Protozoan population

The genera of protozoa that were identified were Entodinium and Isotricha. The different inclusion levels of MKM did not affect the total protozoa population (P>0.05). However, Holotrich was numerically lower than Entodinium (2.83 versus 4.04 log_{10}/ mL^{-1}) (Table 4).

DISCUSSION

In the search to produce healthy and sustainable meat, agro-industrial byproducts have emerged as an alternative to ingredients traditionally used in animal diets as corn and soybean. However, before including any ingredient in the feed, its nutritional value must be evaluated and the *in vitro* gas production technique is a procedure that complements the proximal composition information (Yánez-Ruiz et al., 2016).

Chemical composition of diets

This study was focused on the use of mamey kernel (*Pouteria sapota*) (which is a byproduct of one of the most important crops with the highest production in the south-eastern region

Table 3. ***In vitro*** **ruminal fermentation characteristics from increasing levels of mamey kernel meal as substitutes for ground corn and soybean meal.**

	Inclusion levels										
Parameters	**0%**	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**SEM**	**P-value**	**Linear**	**Quadratic**
pH											
24 hours	6.35a	6.15bc	6.15bc	6.25ab	6.10c	6.05c	6.10c	0.032	<.0001	<.0001	0.098
72 hours	6.07	6.08	6.08	6.13	6.13	6.15	6.15	0.025	0.104	0.0028	0.67
Volatile fatty acids, %											
Acetic	70.5	70.5	70.5	70.6	70.5	70.5	70.5	0.04	0.69	0.98	0.94
Propionic	29.0	28.9	28.9	29.1	29.1	28.7	28.7	0.28	0.89	0.51	0.44
Butyric	0.21	0.20	0.16	0.17	0.20	0.15	0.14	0.04	0.81	0.25	0.96
Acetate:propionate ratio	2.43	2.44	2.44	2.42	2.42	2.45	2.46	0.02	0.88	0.49	0.42

SEM: standard error of the mean. Different letters in the same row indicate differences (p<0.05).

Table 4. Population of protozoa (log_{10} mL^{-1}) at different levels of inclusion of mamey seed flour under *in vitro* gas conditions.

	Inclusion levels										
Items	**0%**	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**	**SEM**	**P-value**	**Linear**	**Quadratic**
Holotrich	2.82	2.82	2.82	2.82	2.82	2.82	2.90	0.03	0.45	0.15	0.16
Entodinium	4.14	3.89	4.15	3.99	3.99	4.02	4.11	0.07	0.19	0.94	0.20
Total protozoa	6.96	6.72	6.97	6.82	6.82	6.85	7.01	0.08	0.20	0.57	0.12

SEM: standard error of the mean; Different letters in the same column indicate differences ($p<0.05$).

of Mexico), to replace corn and soybean, which are traditional ingredients for ruminant feed. As MKM was included, the amount of corn was reduced compared to soybean meal due to the amount of EE contained in the kernel, reflected in the gradual increase in the diet. From the 25% inclusion of MKM, corn was eliminated. Likewise, the levels of NDF and ADF increased up to 26% with the addition of MKM.

Gas production kinetics, fractional volumes, and *in vitro* dry matter degradability

Gas production can be an indirectly measure from the degradation of structural and non-structural carbohydrates, which are substrates available for microbial fermentation in the rumen (Yánez-Ruiz et al., 2016). Therefore, the increasing gas production in MV as MKM was included because its carbohydrates are more digestible than those found in ground corn. This agrees with Olivares-Palma et al. (2013), who found that the inclusion of oilseed pressed cakes (from castor, cotton, moringa, palm kernel, radish, and sunflower) from bio-diesel production did not affect gas production. The highest value of S was detected at 20% inclusion, which could mean that they had a higher concentration of ruminal microorganisms. The value of L for all levels was negative, indicating that the diet was a source of rapidly degradable carbohydrates in the rumen. This fact is related to the high pressures obtained in the first hours of sampling. On the other hand, the levels of lowest inclusion (0-15%) had the lowest values, which suggests that high levels of inclusion of MKM facilitate the rapid nutrient degradation, reflected in the faster initiation of fermentation. Conversely, a previous study on the inclusion of oil and fats reported negative effects on ruminal fermentation and gas production (Morais et al., 2023). In the present study, the inclusion of MKM, which is rich in oil, did not affect the ability of some bacteria to adhere to the substrate particles. Likewise, other compounds of MKM (i.e., carotenoids) could be affecting fermentation rates in conjunction with the carbohydrate content and as a result, the highest fermentable fractions were obtained.

The physical form of lipids (oil vs. seed) and concentration are determining factors that regulate their effect of dietary inclusion on fiber digestibility and OM in ruminants (John et al., 2021) son). The inclusion of lipids above 7% promotes the death of bacteria, mainly cellulolytic bacteria, responsible for fiber degradation because it inhibits enzymatic activity and decreases cell wall digestion (Vargas et al., 2020).In the present study, replacing ground corn with 30% MKM inclusion increased the EE concentration to 17.9% compared to the control with an EE concentration of 2.1% (0% MKM inclusion). However, no negative effects were found ($P>0.05$) when obtaining similar degradability without inclusion and with increasing levels of MKM, with levels above 60.7%, which is within the accepted range for this parameter (Delgado et al., 2020). This is related to the fractional volumes at 8 and 24 h, which indicate a higher composition of non-fibrous carbohydrates. Likewise, OMD at 72 h was not affected by inclusion. This contrasts with the findings of Herrera-Pérez et al. (2018), who conducted *in vitro* studies including lipids in a diet for ruminants and reported a decrease in degradability.

Characteristics of ruminal fermentation

The inclusion of MKM reduced the pH values linearly ($P<0.05$) at 24 h of incubation, while no differences were found between levels at 72 h. The reported values are within normal physiological values ranging from 5.8 to 7.0 (Ramírez-Bribiesca, 2018). The decrease in pH is related to the inclusion of the kernel and its rapid degradability since having more available substrate or rapidly degradable sugars results in more VFA in the first hours of incubation (Judd and Kohn, 2018).

Volatile fatty acids were not altered by the inclusion of MKM, and neither acetic: propionic ratio. This agrees with Sun et al., (2022), who reported that the inclusion of ingredients rich in polyunsaturated fatty acids does not affect the

pH or the total production of VFA at the rumen level. However, the very small values of butyric acid that may be related to the inclusion of MKM, which is rich in EE, are striking. Contrary to this, previous research has shown that the inclusion of vegetable oils increases the molar proportion of propionic acid in the rumen, which plays an important role due to its energetic nature and effect on the production of hydrogen metabolites for the formation of CH_4 (Vargas et al., 2020).

Protozoan population

Research on the effect of oilseeds on the population of protozoa is scarce. The factors that seem to influence the concentration and composition of the protozoan fauna are the composition of the diet, pH, and the inclusion levels of dietary lipids (Vargas et al., 2019). Oleic and linoleic fatty acids have been reported to have antimicrobial activity in the rumen (Atikah et al., 2018). In the present study, the high oleic acid content of MKM (48 g 100 g^{-1}) did not negatively affect (P>0.05) the population of protozoa compared to the diet without inclusion, with no reduction in the populations as the level of inclusion of MKM increased. Conversely, Szumacher-Strabel et al. (2004) found that vegetable oil supplementation, including rapeseed, sunflower and flaxseed oils, had a negative effect on protozoa counts and population. Differences in the reported effects may be due to the degree of unsaturation of the fatty acids, with oleic acid having one unsaturated double bond compared to the two double bonds of linoleic acid found in sunflower and flaxseed (Hristov et al., 2005).

CONCLUSIONS

The inclusion of mamey kernel meal as substitute for ground corn and soybean meal in a traditional sheep diet could be up to 15% without negative effects on *in vitro* degradability, fermentation kinetics, and gas production. However, high levels of mamey kernel meal inclusion could affect the degradability of organic matter. *In vivo* studies involving the rumen microbiome (protozoa, methanogenic archaea, and bacteria) are required to accompanied these findings.

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Author contributions

The authors present in this manuscript actively participated in the development of this study: Adriana Sánchez-Zárate, Alfonso Juventino Chay-Canul, Edgar Aguilar-Urquizo, Angel Trinidad Piñeiro-Vázquez. The following participated in the methodological design: Jorge Canul-Solis, German Giácoman-Vallejos, Avel González-Sánchez, Emanuel Hernández-Núñez, Alfonso Pérez-Gutiérrez, San German Bautista-Parra, Einar Vargas-Bello-Pérez. The following participated in the writing, revision and discussion of the manuscript, as well as the approval of the final version of the document: Adriana Sánchez-Zárate, Alfonso Juventino Chay-Canul, Edgar Aguilar-Urquizo, Einar Vargas-Bello-Pérez, Angel Trinidad Piñeiro-Vázquez.

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