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The rate of fermentation and the *in vitro* degradability of palm kernel meal from *Elaeis guineensis* Jacq. when included in sheep diets

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ABSTRACT

Objective: Measure the biogas production, degradability and fermentation kinetics of diets containing different levels of palm kernel meal.

Design/methodology/approach: The treatments were 0%, 15%, 30% and 45% palm kernel meal (PKM). The nutritional and chemical content was determined, including dry matter, total protein, ethereal extract; ash; neutral and acid detergent fiber fractions. Culture vials (120 ml) were used, to which 0.5 g of the experimental diets were added, followed by 50 ml of rumen inoculum under continuous CO_2 flow. The vials were then sealed with neoprene plugs and aluminum rings and incubated at 39 °C for 72 hours. Biogas displacement was measured at 3, 6, 9, 12, 24, 48 and 72 hours.

Results: The treatment that produced less biogas ($p<0.05$) at 6 and 9 h of incubation was the one with 45% inclusion of palm kernel meal. The diet with 45% inclusion of PKM presented the lowest maximum gas volume (291.35) of the four treatments evaluated ($p<0.05$).

Study limitations/implications: Results may vary when using higher and lower PKM inclusions in experimental diets, or when combining them with one or two cereals. The fermentative and digestible behavior of the ingredients may also change.

Findings/conclusions: Under *in vitro* conditions, the inclusion of 45% palm kernel meal reduces biogas production. PKM represents an alternative use for inclusion in diets.

Key words: Palm kernel meal, biogas, degradability, diet, *in vitro*.

INTRODUCTION

Palm kernel meal (PKM) is a by-product of the *Elaeis guineensis* Jacq palm oil industry. In Mexico this crop is cultivated in the states of Chiapas, Tabasco, Veracruz and

Campeche. The national total production of fresh fruit bunches (FFB) is 1,194,210.19. In the state of Chiapas in particular, the cultivated area is 45,435.53 hectares, of which an area of 38,580.03 hectares are harvested, producing 554,519.03 tons of FFB (SIAP, 2022).

It is estimated that out of 100% of a FFB the PKM production rate is 2% to 3% (García and Yáñez 2010). This suggests an estimated annual production of 16, 635.57 tons of FFB in the state of Chiapas.

The PKM is a by-product that has been evaluated in several species of zootechnical interest such as poultry, swine, sheep, goats, cattle and fish (Zumbado *et al.*, 1992, Kperegbeyi and Ikperite, 2011; Ebrahimi *et al.*, 2012; Oladokun *et al.*, 2016; Freitas *et al.*, 2017; Mazón *et al.*, 2018).

Sheep farming now faces the dilemma of enteric fermentation, which generates methane (CH_4) emissions with a high global warming potential (IPCC, 2021).

Various feeding strategies have been implemented to reduce gas emissions from cattle and sheep, addressing this situation. One strategy has been to use agro-industrial waste, such as palm kernel cake (PKC), derived from pressing the fruit of *Elaeis guineensis* Jacq after oil extraction.

The inclusion of palm kernel meal (PKM) in cattle diets has been studied as a strategy to mitigate the production of enteric methane, which is one of the main components of biogas generated in the rumen of ruminants.

Palm kernel meal is a viable source for animal feed due to its high crude protein (CP) content (10.55%), ether extract (EE) content (7.27%), neutral detergent fiber (NDF) content (76.56%), and acid detergent fiber (ADF) content (57.20%) (España-Escobar, 2023). Including it in the diets of cattle and sheep is beneficial not only from a nutritional standpoint, but also for its potential to reduce enteric methane emissions.

However, it should be noted that including PKM in cattle rations can modify rumen fermentation and reduce methane production (Tan *et al.*, 2011). Similarly, its lipid content has a deflating effect on protozoa that are symbiotically related to methane-producing Archaea (Machmüller *et al.*, 2003).

Soltan *et al.* (2018) conducted a meta-analysis on this topic and reported that including 15% palm kernel meal in cattle diets can reduce methane emissions by 10-25%. However, the use of palm kernel meal must be balanced since excessive amounts can affect fiber digestibility and alter rumen pH.

In this regard, to evaluate the energy efficiency of a diet, the production of total biogas and methane (CH_4), which represents between 2% and 15% of the energy consumed by the animal, must be estimated (Herrera-Pérez *et al.*, 2018; Carrillo-Hernández *et al.*, 2021).

One technique for estimating the digestibility and rumen fermentation of dry matter (DM) under a kinetic model is *in vitro* gas production (IVGP), which can help establish biogas and methane production and understand the effects of a substrate under study on the total diet (Crosby-Galván & Ramírez-Mella, 2018; Sánchez-Santillán *et al.*, 2020).

Therefore, the objective of this study was to measure the biogas production, degradability, and fermentation kinetics of diets containing different levels of PKM.

MATERIALS AND METHODS

Location of the study

The experiment was carried out in the animal nutrition laboratory of the postgraduate program in Genetic Resources and Productivity-Livestock of the Colegio de Postgraduados, Campus Montecillo, Texcoco, State of Mexico.

It is located at kilometer 36.5 of the Mexico-Texcoco Highway at an altitude of 2,240 meters above sea level (asl). All procedures in this study related to the handling of cannulated animals are governed by the Regulations for the Use and Care of Animals in Research at the Colegio de Postgraduados (Reglamento para el Uso de Animales en Investigación, 2016).

Treatments and chemical analysis

Four diets were formulated to meet the nutritional requirements of fattening lambs with estimated weight gains of 250 g d^{-1} (NRC, 2007) (Table 1). The PKM was considered as a coarse ingredient and was replaced by oat hay at levels of 0%, 25%, 50% and 75%.

With this modification, the diets present a total of 0%, 15%, 30% and 45% DM.

To obtain a bromatological analysis of the mixtures, a sample of the experimental diets was collected. The sample was ground in a Thomas Wiley brand hammer mill (Model 4, Arthur H. Thomas Company, Philadelphia, PA, USA), with a 1 mm mesh size.

The dry matter (DM), total protein (TP), ethereal extract (EE) and ash contents were determined in the laboratory according to the methodology described by the AOAC

Table 1. Nutritional content of experimental diets with different levels of inclusion of palm kernel meal.

Treatments				
Ingredients	**Control**	**15% PKM**	**30% PKM**	**45% PKM**
Yellow Corn	16.5	18.5	21	23
Soybean Meal	14	12	9.5	7.5
Palm Kernel Meal	0	15	30	45
Oat Hay	60	45	30	15
Molasses	6	6	6	6
Urea	1.5	1.5	1.5	1.5
Mineral Premix[†]	2	2	2	2
Chemical composition (%)				
Dry Matter	90.44	90.82	90.91	84.67
Crude Protein	15.24	16.32	15.10	13.41
Ethereal Extract	2.35	3.21	4.08	4.94
Neutral Detergent Fiber	41.39	43.54	45.33	51.99
Acid detergent Fiber	19.48	23.07	25.71	30.97
Lignin Acid Detergent	2.25	5.33	7.27	7.47
Ash	9.96	9.55	7.82	6.04
Metabolizable Energy (Mcal kg^{-1})	2.51	2.54	2.59	3.17

PKM=Palm Kernel Meal. [†] Vitasal for sheep: Calcium 20%; Magnesium, 2%; Zinc, 5000 mg; Selenium, 30 mg; Moisture, 5%; and Ash, 95%. Metabolizable energy, calculated using JAVA Feed Tag tool, from the University of California, Davis, Department of Animal Science.

(AOAC., 2005). Neutral Detergent Fiber and Acid Detergent Fiber (NDF and ADF) according to the methodology described by (Van Soest *et al.*, 1991). Metabolizable energy (ME, Mcal kg^{-1}) was calculated with data from the chemical analysis of each of the experimental diets and crude fiber intake (calculated from tables), and with the support of the JAVA tool (Feed Tag) from the University of California, Davis, Department of Animal Science (Feed Tag: JAVA, 2021).

Anaerobic culture medium

Rumen fluid was extracted from fistulated Holstein bulls and cannulated in the rumen and filtered with a double blanket. It was then immediately mixed with a reduced mineral solution at a dilution of 1 to 1 (v/v; rumen fluid/mineral solution).

The ACM solution contained 75 mL of mineral solution I [K_2HPO_4 (6 g L^{-1} of water]; 75 mL of mineral solution II [KH_2PO_4 (6 g), $(NH_4)2SO_4$ (6 g), NaCl (12 g), $MgSO_4$ (2.45 g) and $CaCl_2$-H_2O (1.6 g L^{-1} of water)], 50 mL of 8% sodium carbonate solution [Na_2CO_3 (8 g/100 mL of water)], 20 mL of reduction solution [L-cysteine (2.5 g) at pH 10 with NaOH 2N (15 mL L^{-1}), Na2S (2.5 g) and 0.01% resazurin (2 drops of resazurin)] 100 mL of rumen fluid.

The prepared ACM was placed in a water bath at 39 °C with a continuous flow of CO_2 (Miranda-Romero *et al.*, 2020).

Biodigesters

Six 120-mL culture vials per treatment were used, to which 0.5 g of sample (experimental diets) was added, followed by 50 mL of ACM under continuous CO_2 flow. The vials were hermetically sealed with neoprene stoppers and an aluminum ring, then incubated at 39 °C for 72 hours. One vial was considered one experimental unit (Sánchez-Santillán *et al.*, 2019).

***In vitro* gas production**

The gas production technique described by (Miranda-Romero *et al.*, 2020) was used. The biogas produced was captured in a glass syringe (50 mL; BD Yale®, Brazil) for measurement. The maximum displacement of the embolus indicated the completion of biogas production (Sánchez-Santillán *et al.*, 2022).

The produced CH_4 was captured in salt traps consisting of serological vials with 90 mL of NaOH solution (2N). This solution contained 80 g of NaOH (Merk®) diluted in 1,000 mL of distilled water (Torres-Salado *et al.*, (2018).

Biogas production kinetics

The estimation parameters of the in vitro gas production kinetics were obtained using the accumulated biogas production data and the Gompertz model (Cañaveral-Martínez *et al.*, 2020).

$$Y = A * \left\{ \exp\left[-b * \exp(-\kappa * t) \right] \right\}$$

For which it is described, Y = biogas volume at time t (mL g^{-1} de DM); A = total biogas potential when $t = \infty$ (mL g^{-1} of DM); b = at the constant rate of biogas production of the potentially degradable material (mL h^{-1}); κ = at time lag (h), constant factor of microbial efficiency, defined as the intercept of the time axis of the tangent line at the inflection point; t = incubation time.

In vitro degradability

The *in vitro* degradability of dry matter (IVDDM) test was performed at the end of the biodigester incubation test (72 h). Then, the culture medium was filtered through porcelain crucibles containing 125 mm diameter Whatman 541 filter paper, which was connected to a vacuum pump (EVAR®, Model EV-40). The constant dry weight of the filter papers was previously determined and identified with respect to the corresponding biodigester that was filtered. Then, the filtered samples were dried at 60 °C for 48 hours to determine the IVDDM, using the following formula:

$$IVDDM\,(\%) = \left(\left(DSW - (RDW - BW)\right) / DSW\right) \times 100$$

Where *IVDDM* = *In Vitro* Dry Matter Degradability expressed in percentage, *DSW* = Dry Sample Weight before fermentation, *RDW* = Residual Dry Weight after fermentation, *BW* = Blank Weight samples.

Statistical analysis

Partial biogas production data, as well as *in vitro* fermentation kinetics and *in vitro* dry matter digestibility, were analyzed under a completely randomized experimental design, with four trials and six replicates (one replicate equal to one biodigester). The analysis was performed with the GLM procedure (SAS, Inc., 2011), for the difference of means the Tukey mean test was used ($p \leq 0.05$).

RESULTS AND DISCUSSION

Biogas production

Gas production with statistically different values ($p > 0.05$) was presented after 6 hours of incubation (Table 2).

The treatment with the highest PKM value (45%) registered the lowest biogas values ($p < 0.05$) during the whole study. On the other hand, the control presented the highest biogas values during incubation, and they were statistically different ($p < 0.05$).

The response obtained in the treatment with the highest PKM value could have decreased biogas production, due to the greater increase in oil residues (Escobar-España *et al.*, 2022), which could have decreased biogas production. Portela *et al.* (2022) report that the presence of palm oil in diets can decrease CH_4 production *in vitro*.

In general, a high fiber content (particularly of neutral detergent fiber and lignin, see Table 1), and a moderate proportion of fat favor rumen fermentation and concomitantly can reduce methane emissions. In fattening steers, Silva *et al.* (2016) report that including

Table 2. *In vitro* cumulative biogas production of diets with different levels of palm kernel meal inclusion.

***In vitro* biogas production**	**Time (hours)**	**Control**	**15% PKM**	**30% PKM**	**45% PKM**
(ml g^{-1} MS)	3	29.48	33.93	36.52	31.56
	6	84.90 a	79.90 a	79.19 a	63.83 a
	9	132.15 a	126.22 a	121.14 a	102.39 b
	12	172.30 a	165.81 ab	158.71 b	139.55 c
	24	263.30 a	259.50 a	237.33 ab	216.66 b
	48	342.47 a	300.15 a	300.15 ab	281.86 b
	72	372.66 a	318.75 bc	318.75 bc	300.78 c

Average values with different letters in the same column indicate significant differences ($p<0.05$).

PKM in diets decreased dry matter digestibility and microbial protein synthesis, both of which affect rumen gas production.

In other ruminants such as buffaloes, Amaral *et al.* (2008) cite decreased methane gas production when fed palm kernel cake. They attribute this effect to the modification of ruminant fermentation and a decrease in hydrogen as a precursor of methane.

The decrease in gas production is related to the high fiber content in the PKM, which limits the production of volatile fatty acids (Patra, 2017).

Conversely, Jenkins *et al.* (2008) suggest that the fat content in HP may be toxic or inhibitory to the microorganisms in the rumen that produce methane.

In general, PKM modifies rumen microbial populations and can induce decreases in methanogenic microorganisms (Belanche *et al.*, 2015).

The gas production rate shows little variation among the treatments evaluated treatments and no statistical difference. This response is due to the availability of structural and non-structural carbohydrates for microorganisms during fermentation (Elghandour *et al.*, 2016).

Regarding the lag or delay time of the gas production rate, there were no statistically significant differences ($p>0.05$) between the treatments.

The IVDMD showed significant statistical differences ($p<0.05$) among the treatments. The diet with 45% PKM inclusion (63.11%) had the lowest percentage of IVDMD at 72 hours.

Table 3. Biogas production kinetics in diets with different levels of palm kernel meal inclusion.

Variable	**Treatments**			
	Control	**15% PKM**	**30% PKM**	**45% PKM**
A (mL g^{-1} MS)	356.79^{a}	340.69^{ab}	331.47^{b}	291.35^{c}
K (h)	2.86	2.90	2.89	2.88
B (h^{-1})	0.110	0.108	0.106	0.105
DIVMS	75.83^{a}	70.08^{b}	67.35^{c}	63.11^{d}

A, maximum gas volume; *K*, gas production rate; *B*, Lag time; IVDDM, *in vitro* degradability of dry matter in % at 72 hours.

The ratio of structural to non-structural carbohydrates is linked to biogas production. The diet with 0% PKM inclusion should have a higher fermentation volume than diets including PKM due to the level of structural carbohydrates (NDF and lignin) (Sánchez-Santillán *et al.*, 2019). Estimators of fermentation kinetics demonstrate the tendency of fermentation related to the NDF and lignin content in the diet, which affects digestibility (Gómez-Trinidad *et al.*, 2023).

CONCLUSIONS

Under *in vitro* conditions, the inclusion of 45% palm kernel meal reduces biogas production. PKM represents an alternative use for its inclusion in diets.

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