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In vitro rumen degradation kinetics of diets containing different levels of crambe cake using gas production technique

Cinética de degradação ruminal in vitro de dietas contendo diferentes níveis de torta de crambe pela técnica de produção de gás

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Highlights

Crambe cake is an important food source for lambs.
The protein in crambe cake does not influence the carbohydrate degradation kinetics.
Crambe cake improves ruminal fermentation efficiency.

Abstract

The objective of this study was to evaluate the *in vitro* ruminal degradation kinetics of diets containing different levels of crambe cake protein as a replacement for cottonseed meal protein using the cumulative gas production technique. Foods and diets containing different levels of crambe cake (0, 250, 500, 750, and 1000 g kg^{-1}) were evaluated. In protein fractionation, cottonseed meal and crambe cake presented the majority of nitrogenous compounds in the protein fraction that were rapidly degradable in the rumen; however, crambe cake presented the highest fraction A and the lowest fraction B3 when compared to

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cottonseed meal. The experimental diets showed high proportions of "A" and B1+B2 fractions. Crambe cake had a lower fraction A+B1 and a higher fraction C value than cottonseed meal in carbohydrate fractionation. It had potentially degradable carbohydrate values higher than those of cottonseed meal. The diets showed little variation in the rapidly and slowly degraded carbohydrate fractions. A decrease in the A1+B1 fraction was observed with increasing levels of crambe cake, which replaced cottonseed meal protein. Homogeneity was observed in the cumulative production of gases during the in vitro incubation of the diets as a function of incubation time. After 144 h of incubation, the diet containing 1000 g kg^{-1} crambe cake presented the highest final volume of cumulative gas production, and the diet with 250 g kg^{-1} had the lowest volume. The final volume of gases produced by the fermentation of fibrous carbohydrates showed a linear decreasing effect ($P < 0.05$) depending on the inclusion levels of crambe cake; however, the other variables were not influenced ($P > 0.05$). The use of crambe cake protein to replace cottonseed meal protein did not influence the kinetics of carbohydrate and protein degradation in the rumen, promoting better availability in the diet. Likewise, it did not interfere with the kinetics of in vitro ruminal fermentation of non-fibrous carbohydrates.
Key words: Alternative foods. Biofuel. Degradation rate. Residue.

Resumo

O objetivo foi avaliar a cinética de degradação ruminal *in vitro* de dietas contendo diferentes níveis de proteína da torta de crambe em substituição à proteína do farelo de algodão pela técnica de produção cumulativa de gases. Foram avaliados os alimentos e as dietas contendo diferentes níveis de torta de crambe (0, 250, 500, 750 e 1000 g kg^{-1}). No fracionamento de proteínas foi observado que o farelo de algodão e a torta de crambe apresentam a maior parte dos compostos nitrogenados na fração de proteínas rapidamente degradáveis no rúmen, entretanto, a torta de crambe apresentou a maior fração A e a menor fração B3 quando comparada ao farelo de algodão. As dietas experimentais, apresentaram elevadas proporções de frações "A" e B1+B2. No fracionamento de carboidratos, a torta de crambe apresentou valor inferior da fração A+B1 e superior da fração C quando comparado ao farelo de algodão. A torta de crambe apresentou valores de carboidratos potencialmente degradáveis, superior aos do farelo de algodão. As dietas apresentaram poucas variações nas frações de carboidratos rapidamente degradáveis e na fração de degradação lenta. Observou-se uma diminuição da fração A1+B1 com o aumento dos níveis de torta de crambe em substituição à proteína do farelo de algodão. Observou-se homogeneidade na produção cumulativa de gases durante a incubação in vitro das dietas em função do tempo de incubação. Após 144 horas de incubação, a dieta contendo 1000 g kg^{-1} de torta de crambe apresentou o maior volume final da produção cumulativa de gases e a ração com 250 g kg^{-1}, o menor volume. O volume final de gases produzidos pela fermentação dos carboidratos fibrosos apresentou efeito linear decrescente ($P<0,05$) em função dos níveis de inclusão da torta de crambe, entretanto, as outras variáveis não foram influenciadas ($P>0,05$). A utilização de proteína da torta de crambe em substituição à proteína do farelo de algodão não influencia a cinética de degradação de carboidratos e proteínas no rúmen, promovendo melhor disponibilidade na dieta. Assim como, não interfere na cinética de fermentação ruminal in vitro dos carboidratos não fibrosos.
Palavras-chave: Alimento alternativo. Biocombustível. Resíduo. Taxa de degradação.

Introduction

The use of biodiesel as an energy matrix has become one of the most efficient alternatives to fossil fuels, because it is biodegradable, less toxic, and reduces greenhouse gas emissions (Gumba et al., 2016). However, the waste generated in the production chain tends to be disposed of incorrectly (Rodrigues & Rondina, 2013). Agro-industrial waste, mainly from biodiesel production, can be considered a good alternative to ruminant feed because of its nutritional value and potential to minimize animal production costs.

One of the oilseeds cultivated for biodiesel production is crambe (Crambe abssynica Hoechst), whose crushing for oil production generates a large quantity of cake, characterized by protein and energy content (Canova et al., 2015). Crambe cake represents an important source of food for ruminant animals in several regions, particularly in the dry season of the year, when forage in general is of poor quality (Mizubuti et al., 2011)

Large quantities of agro-industrial byproducts that can be used in ruminant feeding as substitutes for traditional feeds are generated annually in Brazil (Carrera et al., 2012; Rodrigues & Rondina, 2013). However, some studies are necessary to characterize the by-products in terms of chemical composition and nutritional value.

The degradation of feed in the rumen is a complex process involving interactions between microorganisms such as bacteria, protozoa, and fungi (Patel & Ambalam, 2018). This gas production technique is simple and cost-effective, making it easy to evaluate ruminant feeds with the advantage of processing a large number of samples in a short time (Barcelos et al., 2001). Moreover, it is important to identify potential co-products that can be efficiently used in ruminant diets as substitutes for conventional ingredients (Mizubuti et al., 2011).

A cumulative gas production technique was developed to predict feed fermentation in ruminants by incubating feed with buffered ruminal fluid in vitro. Degradation measurements using gas production techniques provide important information regarding the proportion of feed fermented in the rumen and the potential fraction directed toward microbial growth (Pereira et al., 2013).

This study was conducted to evaluate the in vitro ruminal degradation kinetics of diets containing different concentrations of crambe cake as a substitute for cottonseed meal protein, using the cumulative gas production technique.

Materials and Methods

The experiments were conducted at the Animal Nutrition Laboratory of the Department of Animal Science at the State University of Londrina (UEL) and were approved by the Ethics Committee CEUA_UEL under number 123/2010.

The feed ingredients used in the diets (Table 1) were pre-dried in a forced-air oven at 60°C for 72 hours, and subsequently, the bromatological analysis was performed according to the methodologies described by Association on Official Analytical Chemists [AOAC] (2016). The diets were formulated to be isoproteic with a forage ratio of 30:70 (Table 2), using Tifton-85 hay (Cynodon dactylon Tifton 85) as forage. The formulations met the requirements for early lambs, with an average daily gain of 200 g, as described by the National Research Council [NRC] (2007).

Table 1
Chemical composition of the ingredients in the experimental diets

Item	Ingredients (g kg^{-1} DM)			
	Tifton-85 Hay	Ground corn	Cottonseed meal	Crambe cake
Dry matter	900.7	901.5	912.8	917.7
Organic matter	887.6	932.8	899.0	901.8
Mineral matter	62.4	17.2	51.0	48.2
Crude protein	71.7	91.1	329.8	261.6
Ether extract	11.8	37.6	90.9	212.8
Crude fiber	309.3	32.2	319.7	252.9
Non-nitrogenous extract	544.8	821.9	208.6	224.5
Neutral detergent fiber	620.1	177.2	365.2	362.0
Acid detergent fiber	343.0	41.3	224.2	272.7
NDFap[1]	539.8	145.5	227.1	263.8
NDFn[2]	602.3	162.7	278.2	312.1
NIDN[3]	17.8	14.4	87.0	50.0
NIAD[4]	12.8	13.8	47.1	39.2
Lignin	75.5	8.60	64.4	146.9
Total carbohydrates	854.1	854.1	528.2	477.4
Total digestible nutrients	53.67	81.73	57.93	76.97

[1] Neutral detergent fiber (NDFap) corrected for ash and protein; [2] Neutral detergent fiber free of nitrogen; [3] Nitrogen insoluble in neutral detergent (NIDN); [4] Nitrogen insoluble in acid detergent (NIAD).

The total digestible nutrient (TDN) content was estimated according to the equation described by Kearl (1982). The equation of Moe and Tyrrell (1977) was used to convert TDNs into net energy for maintenance (NEm). The carbohydrate fractions were obtained using the methodology described by Sniffen et al. (1992) where:

Total carbohydrates: TC (g kg^{-1} DM) = 100 - CP (g kg^{-1} DM) - EE (g kg^{-1} DM) - Ash (g kg^{-1} DM);

Fraction A+B1 = 100 - (B2 + C);

Fraction B2 (g kg^{-1} DM) = FDNap – fraction C

Fraction C (g kg^{-1} DM) = NDF g kg^{-1} DM) × 0.01 × LIG (% NDF) × 2.4;

Non fibrer carbohydrates (NFC) = TC (g kg^{-1} DM) – fraction B2 (g kg^{-1} MS) – fraction C (g kg^{-1} DM);

where DM = dry matter; CP = crude protein (N × 6.25); EE = ether extract; NDF = neutral detergente fiber, NDFap = neutral detergent fiber corrected for ash and protein and, LIG = lignin.

Table 2
Percentage and chemical composition of diets with different levels of crambe cake as a replacement for cottonseed meal protein

Item	Replacement levels, g kg^{-1}				
	0	250	500	750	1000
Tifton-85 Hay	300.0	300.0	300.0	300.0	300.0
Ground corn	529.0	513.0	500.0	484.0	472.0
Crambe cake	0.00	50.0	99.0	152.0	199.0
Cottonseed meal	142.0	108.0	72.0	35.0	0.00
Mix of mineral*	29.0	29.0	29.0	29.0	29.0
Total	1000	1000	1000	1000	1000
Chemical composition of diets, g kg^{-1} MS					
Dry matter	877.0	877.0	878.0	878.0	878.0
Organic matter	887.0	887.0	887.0	886.0	886.0
Mineral matter	35.0	35.0	36.0	36.0	36.0
Crude protein	117.0	117.0	117.0	117.0	117.0
Ether extract	36.0	43.0	50.0	57.0	64.0
Crude fiber	155.0	156.0	157.0	158.0	158.0
Non-nitrogenous extract	657.0	648.0	641.0	632.0	625.0
Neutral detergent fiber	332.0	334.0	337.0	340.0	342.0
Acid detergent fiber	157.0	162.0	167.0	172.0	177.0
NDFap[1]	271.0	274.0	277.0	280.0	283.0
NDFn[2]	306.0	310.0	313.0	317.0	320.0
NIDN[3]	25.0	25.0	24.0	23.0	22.0
NIDA[4]	18.0	18.0	18.0	18.0	18.0
Lignin	36.0	41.0	46.0	51.0	56.0
Total carbohydratesCarboidratos totais	735.0	751.0	720.0	712.0	706.0
Total digestible nutrients	676.0	681.0	688.0	694.0	700.0
ELm (Mcal/kg)[5]	15.0	15.0	16.0	16.0	16.0

[1] Neutral detergent fiber (NDF) corrected for ash and protein (NDFap); [2] Neutral detergent fiber free of nitrogen (NDF - Free of nitrogen); [3] Nitrogen insoluble in neutral detergent (NIDN); [4] Nitrogen insoluble in acid detergent (NIAD); [5] ELm = Energy for Maintenance, * Contains the following guaranteed levels per kilogram of the product:

Nitrogenous compound fractionation was performed as described previously (Licitra et al., 1996). Fraction A, or non-protein nitrogen (NPN), was obtained after treating the sample (0.5 g) with 50 mL of distilled water for 30 min, followed by the addition of 10 mL of trichloroacetic acid (TCA) solution at 10 mL L^{-1} and incubation for another 30 min. After this period, the solution was filtered through a filter paper, and the nitrogen content of the residue was determined. Fraction A, or NPN, was calculated as the difference between the total nitrogen content and nitrogen-insoluble content in the TCA cycle. Fraction "B3" was determined by the difference between nitrogen insoluble in neutral detergent (NIDN) and nitrogen insoluble in acid detergent (NIDA). Fraction "C" was considered as the NIDA. The fraction "B1+B2" was obtained by the difference between nitrogen insoluble in TCA and NIDN, or by subtracting the sum of fractions A, B3, and C from 100.

The ruminal in vitro degradation kinetic parameters of the diets were estimated using the semiautomatic cumulative gas production method described by Pell and Schofield (1993) and adapted to the conditions at the Animal Nutrition Laboratory of UEL. The ruminal inoculum was obtained from adult cattle fistulated in the rumen and kept on a pasture supplemented with 1 kg/day of concentrate feed containing corn, soybean meal, urea, and mineral salt.

Approximately 300 mg of each sample was incubated in glass vials (50 mL) with five replicates per sample. Five glass vials without the substrate, which were considered blanks, were also incubated to account for the gas volume from the ruminal fluid and buffer solution. Subsequently, 8 mL of McDougall buffer solution (McDougall, 1948), previously reduced by spraying with CO_2, were added to each vial containing more than 2 mL of ruminal fluid to adjust the pH (8.6) to 6.8–7.0. Buffer solution and inoculum were added in a CO_2-sparing manner to ensure that anaerobic conditions were maintained. The vials were immediately covered with rubber caps and placed in a water bath at 39°C. Depressurization was performed using needles before the incubation period to ensure that the pressure inside the vials was the same as that in the initial condition.

The pressure of the gases produced by fermentation of the substrate and accumulated in the vials was measured using a manometer (after initial depressurization) at the following time points: 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 15, 24, 27, 30, 33, 36, 48, 56, 60, 72, 96, and 144 h. The pressure values were converted into volume (mL) according to a pre-established equation for local conditions: $\hat{Y} = 0.5702 + 3.2399P + 0.1074P^2$ ($R^2 = 0.99$), where $\hat{Y}$ = total gas volume and “P” is the pressure of the gases inside the fermentation vials expressed in psi (pounds per square inch), corrected to a dry basis.

For the estimation of ruminal fermentation kinetics parameters, the data were applied to the bicompartmental logistic model proposed by Schofield et al. (1994), described as follows: V(t)=VCNF/(1+exp (2-4*KdCNF*(T-L))) + VCF/(1+exp(2-4*KdCF *(T-L))), where:VCNF = maximum gas volume of the non-fibrous carbohydrate fraction (mL); VCF = maximum gas volume of the fibrous carbohydrate fraction (mL); KdCNF = degradation rate of non-fibrous carbohydrates (% h-1); KdCF = degradation rate of fibrous carbohydrates (% h-1); T and L = incubation time (h) and latency (h).

Subsequently, the values of the degradation kinetic parameters were generated using the R Statistical Program (2016) with the Gaussian-Newton algorithm and subjected to analysis of variance and regression (when necessary) according to a completely randomized experimental design with five treatments and five replicates. Statistical significance was established at $P < 0.05$.

Results and Discussion

In the protein fractionation (g kg^{-1} DM and g kg^{-1} CP), the A fraction of crambe cake was higher than that of the cottonseed meal (Table 3). According to Santo et al. (2017), Fraction A consists of proteins rapidly degraded in the rumen by fiber-fermenting bacteria that utilize the ammonia produced to meet their protein requirements. The differences observed in the A fraction of crambe cake may be due to the oil extraction process, where excessive heating can reduce the availability of this fraction due to the Maillard reaction, in addition to intrinsic variations in co-products (Van Soest, 1994).

Table 3
Protein fractions of the foods and experimental diets containing different substitution levels of cottonseed meal protein using crambe cake protein

Item	Protein fractions (g kg^{-1} DM)			
	A	B1+B2	B3	C
Tifton-85 hay	5.0	48.0	5.2	13.5
Ground corn	18.2	57.8	0.7	14.5
Cottonseed meal	11.4	226.8	42.0	49.6
Crambe cake	36.7	172.3	11.4	41.3
Experimental diets				
0	7.0	86.3	6.0	17.2
250	11.8	78.8	6.8	19.6
500	10.4	81.2	2.2	22.9
750	10.3	85.4	4.1	17.1
1000	10.0	82.9	0.8	22.9
Protein fractions (g kg^{-1} CP)				
Tifton-85 hay	69.8	666.7	72.2	188.4
Ground corn	199.3	634.2	7.4	159.1
Cottonseed meal	34.7	687.6	127.3	150.5
Crambe cake	140.3	658.5	43.5	157.7
Experimental diets				
0	59.6	740.8	51.6	148.0
250	100.8	673.9	58.1	167.2
500	89.0	695.9	19.2	195.8
750	88.2	730.7	35.1	146.0
1000	85.9	710.7	6.7	196.7

Fraction A: Non-protein nitrogen compounds; Fraction B1+B2: Rapidly degradable true protein plus the intermediate degradation fraction; Fraction B3: Protein associated with the cell wall; and Fraction C: Indigestible protein. DM: Dry matter; CP: Crude protein.

Most of the nitrogen compounds in crambe cake and cottonseed meal were found in the B1+B2 fractions, which contain proteins that are rapidly degradable in the rumen, as well as proteins with intermediate ruminal degradation (Table 3). The utilization of these fractions depends directly on the degradation of the cell wall, making it crucial to correlate the rate of passage and the availability of this protein fraction (Franco et al., 2013; Magalhães et al., 2021; Pegoraro et al., 2017).

The higher the values of fractions A and B1, there is a greater need to supply fast-degrading carbohydrates to achieve synchronization in the fermentation of carbohydrates and proteins in the rumen, thus making the most of these elements (Silva & Silva, 2013).

The levels of proteins considered insoluble (fraction B3) were lower in crambe cake than in cottonseed meal (Table 3), this fraction allows a greater flow of amino acids to the intestine, due to being a slowly degraded fraction in the rumen, thus, the observed values show the good quality of the crambe cake (Santo et al., 2017)

Crambe cake and cottonseed meal presented similar values for fraction C (protein associated with lignin), however, they were lower in relation to corn and Tifton-85 hay (Table 3), showing that there is feasibility in including cake crambe to replace cottonseed meal.

The protein fractionation values of crambe cake obtained in this study (fractions A and B1+B2) were higher than those reported by Pegoraro et al. (2017) and Poveda-Parra et al. (2021), whereas the A fraction had lower values. Fraction C had slightly lower values than those found by Pegoraro et al. (2017), but higher than those reported by Poveda-Parra et al. (2021). The observed variations in Tifton 85 hay could be due to the stage of plant development, which may have influenced the composition of the protein and carbohydrate fractions (Van Soest, 1994). The tifton-85 hay used in this work was probably harvested at an older age than those in previous works, which can be verified by the value presented for the C fraction (lignin).

Regarding the experimental diets (g kg^{-1} DM and g kg^{-1} CP), in general, high proportions of “A” and B1+B2 fractions were evaluated (Table 3), demonstrating that the experimental diets had a protein profile of rapid availability as well as rapidly degradable proteins in the rumen. However, it is necessary to emphasize that the digestion rates of food in the rumen are directly related to the synchronism that exists between the digestion rates of proteins and carbohydrates present in the foods that make up the animals' diet, exerting an important effect on the products of ruminal production and, consequently, on animal performance and production (Silva & Silva, 2013).

In the fractionation of carbohydrates (g kg^{-1} Total carbohydrates), the crambe cake presented a fraction A+B1 value lower than the cottonseed meal, however, the value of the C fraction was higher than the cottonseed meal, reinforcing that the crambe cake it is inferior in quality and availability of total carbohydrates (Table 4). Crambe cake presented highly degradable carbohydrate values (fraction B2), lower than cottonseed meal (Table 4). The differences in carbohydrate availability between the two foods can be minimized by using both ingredients in formulating diets, controlling and dosing their proportions.

Table 4
Total carbohydrate fractions (TCH) of the ingredients and experimental diets containing different substitution levels of cottonseed meal protein using crambe cake protein

Ingredients	g kg^{-1} DM			g kg^{-1} Total carbohydrates		
	A+B1	B2	C	A+B1	B2	C
Tifton-85 hay	393.8	364.3	96.0	461.0	426.6	112.4
Ground corn	704.5	146.5	3.1	824.9	171.5	3.63
Cottonseed meal	310.2	188.2	29.8	587.3	356.2	56.5
Crambe cake	302.0	114.5	60.9	632.5	239.9	127.6
Experimental diets						
0	551.0	237.5	23.5	678.6	292.5	28.9
250	538.2	239.4	26.7	669.1	297.7	33.2
500	534.5	233.4	29.7	670.1	292.6	37.3
750	532.1	224.5	33.1	637.8	284.3	41.8
1000	522.9	224.6	35.9	667.5	286.7	45.8

Soluble carbohydrates (Fraction A+B1), potentially degradable fiber (Fraction B2), and indigestible fiber (Fraction C).

According to Pegoraro et al. (2017) the amount of total carbohydrates allows us to estimate whether the food offers greater or lesser energy input to the rumen microbiota. The amount of total carbohydrates is influenced by the levels of crude protein, ether extract and mineral matter present in the food (Oliveira et al., 2012).

In the experimental diets, it was observed that in general, there were few variations in the carbohydrate rations that are quickly degradable in the rumen (A+B1) and in the slow degradation fraction (fraction B2) (Table 4). An increase in the C fraction values was also observed with the increase in the inclusion of crambe cake (Table 4), probably due to the C fraction values of the crambe cake being higher in relation to those presented by cottonseed meal. These differences in rumen rapidly degradable carbohydrates (fractions A+B1) and potentially degradable carbohydrates (fraction B2) from crambe cake and cottonseed meal are minimized by the adequate proportion of these ingredients in the diets.

Regarding cumulative gas production during in vitro incubation, the diet containing 1000 g kg^{-1} crambe cake showed the highest final gas volume after 144 h of incubation, whereas the diet with 250 g kg^{-1} crambe cake had the lowest final volume (Figure 1). These data suggest that food degradation for up to 48 h is a good indicator of fermentation quality (Mizubuti et al., 2011). The lowest gas accumulation in the first 48 h was observed with the diets with 250 g the smallest final volume (Figure 1). Considering that the average food retention time in the rumen is 48 hours and that the fermentative quality of the food depends on degradation, it can be inferred, therefore, that the greater the degradation of the food up to 48 hours, the better it is. its quality (Mizubuti et al., 2011). In this work, the diet containing 250 g kg^{-1}

of Crambe cake replacing cottonseed meal resulted in less gas accumulation in the first 48 hours of incubation. The other rations presented higher and similar values.

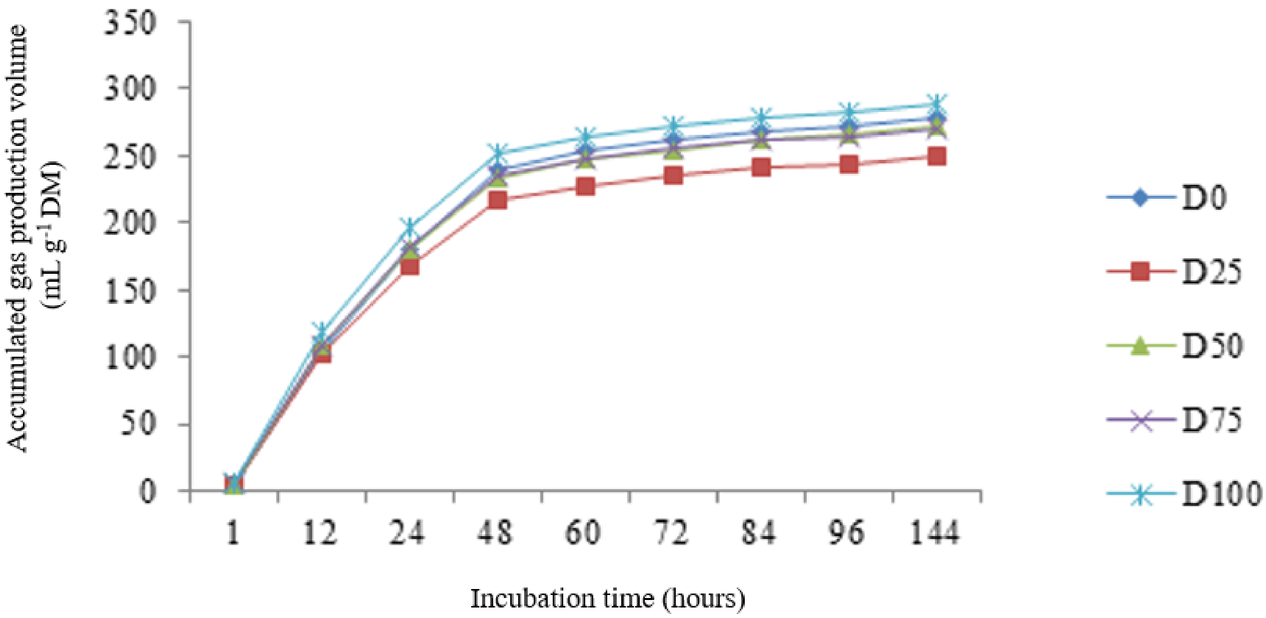

Figure 1. Accumulated gas production volume (mL g^{-1} DM) as a function of the incubation time of diets containing different levels of crambe cake as a substitute for cottonseed meal protein.

In the evaluation of the in vitro rumen fermentation kinetics (Table 5), the final volume of gases produced by the fermentation of fibrous carbohydrates (VCF) showed a decreasing linear effect ($P < 0.05$) with increasing levels of crambe cake inclusion, but the degradation rates (KdCF) were unaffected. This effect can be attributed to the decrease in total carbohydrates and the increase in lignin levels in the experimental diets. This was evident in fraction C, where values increased with substitution levels, revealing the reduced availability of carbohydrates for ruminal degradation.

Table 5
Estimation of the in vitro ruminal fermentation kinetics parameters as a function of the substitution level of cottonseed meal protein using crambe cake protein

Variables	Replacement levels (g kg^{-1})					CV (%)	Reg
	0	250	500	750	1000		
VFC (mL/g MS)	134.98	116.86	126.78	122.32	118.34	5.39	L=0.01
KdFC	0.03	0.03	0.03	0.03	0.03	5.88	NS
Lag time (h)	5.58	5.18	5.52	5.49	4.92	11.14	NS
VNFC (mL/g MS)	89.17	81.75	92.00	96.35	98.28	8.19	NS
KdNFC	0.10	0.11	0.11	0.10	0.10	11.01	NS

VFC= Final volume of gas produced from fibrous carbohydrates; KdFC = Rate of degradation of fibrous carbohydrates; VNFC = Final volume of gas produced from non-fibrous carbohydrates; KdNFC = Rate of degradation of non-fibrous carbohydrates.

As the protein from crambe cake increased in substitution for cottonseed meal in the diets, the values of nitrogen compounds in A or the potentially degradable fraction remained stable (Table 3). However, there was an increase in the slowly digestible carbohydrate fraction (B2) (Table 4), followed by a decrease in the rapidly degradable carbohydrate fraction (A+B1), which negatively affected the digestion of fibrous carbohydrates by ruminal bacteria. This characteristic may have been aggravated by the increasing levels of ether extract in the diets (Table 2), as diets with ether extract levels higher than 6% tended to reduce the activity of fibrolytic microorganisms, impairing the digestibility of the forage consumed by the animal.

In a study evaluating the fermentation kinetics of diets containing different substitution levels (0, 250, 500, 750, and 1000 g kg^{-1}) of soybean meal protein with cottonseed cake protein, Franco et al. (2013) observed a quadratic effect on KdCF, noting that these differences were insufficient to influence the final degradation of fibrous carbohydrates after 120 h of incubation. Goes et al. (2024) did not observe any effect of crambe cake inclusion (0, 50, 100, and 150 g kg^{-1}) on these parameters, which was attributed to the similar carbohydrate proportions in the diets.

The time of colonization is an important parameter related to the degradation of the fibrous fraction of food (Mertens & Loften, 1980); the longer the lag time, the longer the colonization time of the microorganisms. In this study, there was no effect of different substitution levels of cottonseed meal protein with crambe cake protein on this variable. These results were similar to those reported by Franco et al. (2013).

There was no significant effect ($P > 0.05$) of the experimental diets on the final volume of gases from non-fibrous carbohydrate fermentation (VCNF) and their degradation rates (KdCNF) (Table 5). These results indicate good synchronization between the release of energy and nitrogen, meeting the needs of microorganisms that utilize non-fibrous carbohydrates (CNF) and their degradability in the rumen. In the work of Goes et al. (2024), the total gas production showed a quadratic effect with a minimum point of 0.02 g crambe cake.

Conclusion

Using crambe cake protein as a substitute for cottonseed meal protein in isoproteic sheep diets did not affect the kinetics of carbohydrate and protein degradation in the rumen. Crambe cake protein in the diets promoted the homogenization of variations in rapidly degradable carbohydrates (A+B1) and slowly digestible fibrous carbohydrates (B2), enhancing nutrient availability in the diet. Crambe cake did not interfere with the in vitro rumen fermentation kinetics of non-fibrous carbohydrates, showing improved ruminal fermentation efficiency. However, attention should be paid to the percentage of substitutions used.

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