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Kinetics *in vitro* of ruminal fermentation of cocoa husks subjected to alkali and heat treatment

Cinética de fermentação ruminal *in vitro* da casca de cacau submetida a tratamento alcalino e térmico

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Abstract

The objective was to evaluate the parameters of kinetics of ruminal fermentation of cocoa husks (CH) treated with alkali and thermal agents, using the semi-automated in vitro gas production technique. Cocoa husks samples were subjected to alkali and thermal methods (effect of time of exposure) treatment, as follows: control; alkaline treatment with calcium hydroxide ($(Ca(OH)_2$) and calcium oxide (CaO), both doses of 15.0; 30.0 and 45.0 g kg^{-1} of CH; heat treatment in an autoclave at a pressure of 1.23 kg cm^{-2} (15 psi) and a temperature of 123°C for 30, 60 and 90 minutes. For statistical analysis, orthogonal contrasts and regression. The degradation rate and the final volume of gases of non-fiber carbohydrates decreased with the addition of $Ca(OH)_2$ and CaO, however, for fibrous carbohydrates effects were positive. For each percentage of $Ca(OH)_2$ and CaO included, it is estimated an increase of 5.74 and 2.9% in the final volume of the fiber, respectively. When the heat treatment, a decrease in all parameters was estimated. For each minute of exposure to heat, there was a decrease of 0.4% in total final volume of gases. The alkali treatment can be an efficient alternative for improving the digestibility of fibrous fractions of CH.
Key words: Alternative feed, chemical treatment, residue, ruminants

Resumo

Objetivou-se avaliar os parâmetros da cinética de fermentação ruminal da casca de cacau (CC) tratada com agentes alcalinos e térmicos, utilizando a técnica in vitro semi-automática de produção de gases. Amostras da CC foram submetidas aos métodos de tratamento alcalino e térmico (efeito do tempo de exposição), assim distribuídos: controle; tratamento alcalino com hidróxido de cálcio ($(Ca(OH)_2$) e óxido de cálcio (CaO) ambos nas doses de 15,0; 30,0 e 45,0 g kg^{-1} da CC; tratamento térmico em autoclave

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com pressão de 1,23 kg cm^{-2} (15 psi) e temperatura de 123º C durante 30, 60 e 90 minutos. Para análise estatística, foram utilizados contrastes ortogonais e regressão. A taxa de degradação e o volume final dos gases dos carboidratos não fibrosos decresceram com a inclusão de $Ca(OH)_2$ e CaO, entretanto, para os carboidratos fibrosos os efeitos foram positivos. Para cada percentual de $Ca(OH)_2$ e CaO incluídos, estima-se um acréscimo de 5,74 e 2,9 % no volume final dos carboidratos fibrosos respectivamente. Quanto ao tratamento térmico, houve decréscimo em todos os parâmetros estimados. Para cada minuto de exposição ao calor, houve diminuição de 0,4% no volume final total dos gases. O tratamento alcalino pode ser uma alternativa eficaz para a melhoria da digestibilidade das frações fibrosas da CC.
Palavras-chave: Alimentos alternativos, resíduo, ruminantes, tratamento químico

Introduction

Verticalization in agriculture has resulted in greater resource generation for the entire production chain. By processing commodities, the agricultural industries in Brazil increase their final product's value and thereby strengthen the country's position as a major food producer. However, a large amount of agro-industrial waste is generated in the process that must be dealt with in accordance with environmental laws.

Using byproducts left from processing cocoa almonds (cocoa husks) in feed for ruminants has already been a matter of study for some time (ALY, 1981). This byproduct is composed of the integument that surrounds the cocoa almond's core and its chemical composition depends a lot on the different types industrial processing machinery used, which cause the amount of crude protein (CP) to vary from 135.0 (MARCONDES et al., 2009) to 169.0 g kg^{-1} of dry matter (DM) (OLIVEIRA et al., 2010), this is due to the greater or lesser presence of NIBS (fragmented cotyledons from the cocoa seed), which is the raw material used during chocolate manufacturing.

Cocoa Husks (CH) are considered byproducts that have a relatively low nutritional value (TDN of 588.3 g kg^{-1} DM) and high cell-wall content, which is associated with a high lignin content (185.4 g kg^{-1} DM) (AZEVÊDO et al., 2011). Ruminant diets with CH can have a negative impact on intake, this is because a portion of the ingested fiber that is resistant to fermentation by ruminal microorganisms accumulates in the rumen compared with the potentially digestible fraction (ALLEN; MERTENS, 1987) and consequently, has a direct effect in terms of reducing animal performance (ALY, 1981; AREGHEORE, 2002; CARVALHO et al., 2004; MARCONDES et al., 2009; AZEVÊDO et al., 2011). Thus, alternatives designed to increase the potentially digestible fraction of fiber or its digestion rate can have positive impacts on the animals' intake and performance. In this sense, alkalizing agents can be used to improve the digestibility of agro-industrial byproducts (PINA et al., 2011).

During digestibility studies in cattle, results obtained in vivo have always been more realistic, however they are limited due to the fact that they require a representative number of animals, in addition to needing large quantities of feeds that may be used during the adjustment and experimental periods.

In vitro methodologies for evaluating feeding have been used to determine the nutritional value of forage, with a high correlation between consumption and in vivo digestibility being registered (ØRSKOV, 2002). This study aimed to evaluate the effects of alkaline and thermal treatments in cocoa husks on the kinetic parameters of in vitro ruminal fermentation.

Material and Methods

Location

The experiment was conducted on the premises of the Animal Nutrition Laboratory under the administration of the Department of Agricultural and Environmental Sciences at the State University of Santa Cruz located in Ilhéus, Southern Bahia. The

cocoa husks (CH) were obtained from a city known as Arataca – Bahia and consist of the integument that surrounds the almond core.

In order to evaluate how effective the treatments were, 50 samples, each with 250 grams of ground CH, were placed into a 1 mm sieve and distributed in a completely randomized design, with five repetitions, and were subjected to 10 treatments in accordance with the alkaline and thermal treatment methods as follows: untreated control; alkaline treatment with calcium hydroxide $Ca(OH)_2$ and calcium oxide (CaO), diluted in water (1:10), in doses of 15.0, 30.0 and 45.0 g kg^{-1} of CH, base of the natural matter; heat treatment in an autoclave with 1.23 kg cm^{-1} pressure (15 psi) and a temperature of 123°C for 30, 60 and 90 minutes. After being exposed to hydrolysis for a 24-hour period, the material was taken to a forced ventilation oven set at 55°C for 24 hours.

Bromatological analyses

All samples were analyzed in accordance with procedures adopted by Detmann et al. (2012) for contents of DM, CP, organic matter (OM), ether extract (EE) and acid detergent fiber (ADF). During the neutral detergent fiber (NDF) analyses, the samples were corrected for ash and nitrogen. Non-fibrous carbohydrate (NFC) content, expressed as a % in the DM, was calculated in accordance with Hall (2000), namely: 100- (%NDF +%CP +%EE +%MM). These values can be seen in Table 1.

Table 1. Bromatological chemical composition of cocoa husks exposed to alkaline and thermal treatments.

Item	Control	Alkaline treatment						Heat treatment		
		$Ca(OH)_2$			CaO			Time (min)		
		15.0	30.0	45.0	15.0	30.0	45.0	30	60	90
DM[1]	964.0	945.0	963.0	940.0	930.0	937.0	924.0	942.0	948.0	948.0
OM[2]	929.0	905.0	904.0	891.0	913.0	898.0	893.0	922.0	928.0	932.0
CP[2]	152.0	146.0	149.0	148.0	155.0	153.0	154.0	155.0	153.0	154.0
EE[2]	92.0	98.0	92.0	105.0	117.0	107.0	122.0	154.0	132.0	124.0
NDFap[2]	517.0	559.0	568.0	560.0	538.0	541.0	543.0	514.0	521.0	519.0
ADFap[2]	489.0	500.0	457.0	462.0	451.0	495.0	491.0	485.0	508.0	479.0
NFC[2]	167.0	102.0	94.0	78.0	102.0	97.0	73.0	99.0	121.0	136.0
NDICP[3]	344.0	371.0	362.0	370.0	361.0	367.0	353.0	358.0	365.0	383.0
ADICP[3]	274.0	282.0	245.0	247.0	281.0	267.0	273.0	358.0	255.0	254.0

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDFap, ash and protein-free neutral detergent fiber; ADFap, ash and protein-free acid detergent fiber; NFC, non-fibrous carbohydrates; NDICP, neutral detergent insoluble crude protein; ADICP, acid detergent insoluble crude protein.
[1] g/kg natural matter
[2] g/kg dry matter
[3] g/kg crude protein.

Kinetics of in vitro ruminal fermentation

In vitro incubations by the semi-automatic gas production technique were performed in accordance with that as described by Santos et al. (2010). Were weighed 300 mg samples of each treatment, in four repetitions, and deposited in 50 mL glass flasks.

The ruminal inoculant was obtained from a rumen fistulated bovine male, which after filtering was put in a thermos that had been pre-heated to 39°C and immediately taken to the laboratory for incubation in the flasks.

The pressure from the accumulated gases at the top of the flasks was measured using a "Press DATA 800" pressure transducer attached to a 6 mm syringe, at 1, 2, 3, 4, 6, 12, 14, 8, 10, 17, 20, 24, 28, 36, 48, 60, 72, 84, 96, 108 and 120 hours after incubation.

The regression equation used to convert pressure (P) to volume (mL) = 0.04755 + 1.9754P + 0.01407P^2, (R^2 = 0.99), was standardized in accordance with Santos et al. (2010) following the methodology proposed by Maurício et al. (2003). A bicompartimental model was used which was fitted to cumulative gas production curves (SCHOFIELD et al., 1994) with the following equation.

V = FVNFC / (1 + exp (2 – 4*kdNFC*(T – L))) + VFFC / (1 + exp(2 – 4*kdFC*(T – L))), where: FVNFC is equivalent to the maximum volume of gases from the NFC fraction; kdNFC is the degradation rate (h^{-1}) of this fraction; VFFC is the maximum volume of gas from the fibrous carbohydrates fraction (FC); kdFC is the degradation rate (h^{-1}) of the FC; T and L are the incubation (hours) and latency (hours) times, respectively.

Statistical analyses

The data obtained on the gas production parameters from the NFC and FC was fitted by nonlinear regression using the Gauss-Newton method, which is implemented in the Statistical Analysis System software. Analyses of variance were performed and, when significant, were compared to the means between treatments using orthogonal contrasts (Table 2).

Table 2. Distribution of coefficients for the orthogonal contrasts.

Item	Control	$Ca(OH)_2$			CaO			Heat (min)		
		15	30	45	15	30	45	30	60	90
1	+3	-1	-1	-1	0	0	0	0	0	0
2	+3	0	0	0	-1	-1	-1	0	0	0
3	+3	0	0	0	0	0	0	-1	-1	-1
4	0	1	1	1	-1	-1	-1	0	0	0
5	0	1	1	1	0	0	0	-1	-1	-1
6	0	0	0	0	1	1	1	-1	-1	-1

Whenever significance was present, regression analysis was performed on the treatment levels or time. The models were selected on the basis of the coefficients of determination and the significance of the regression coefficients.

Results and Discussions

Degradation curves

In the curves from the kinetics of in vitro ruminal fermentation, a higher total final volume (TFV) was observed for CH treated with 15.0 g of CaO. Considering the linear relationship between the disappearance of total carbohydrates (TC) and the TFV (PELL et al., 1994), it can be inferred that this treatment was the one that made a greater disappearance of TC possible compared with the others (Figures 1, 2 and 3).

It is possible that there might have been greater ester type ruptures in the links between the lignin, the cellulose structural carbohydrates and the hemicellulose, which in turn provides these components in greater quantity when compared to other treatments. Macedo et al. (2011) suggested that adding CaO improves the degradability of the dry matter and the fibrous fraction, suggesting that its use is advantageous. Less TVF was observed in the thermally treated samples, indicating a lower utilization of carbohydrates (Figure 3).

Figure 1. Gas production from in vitro ruminal fermentation depending on the incubation times of cocoa husks treated with 0; 1.5; 3.0 and 4.5% of $Ca(OH)_2$.

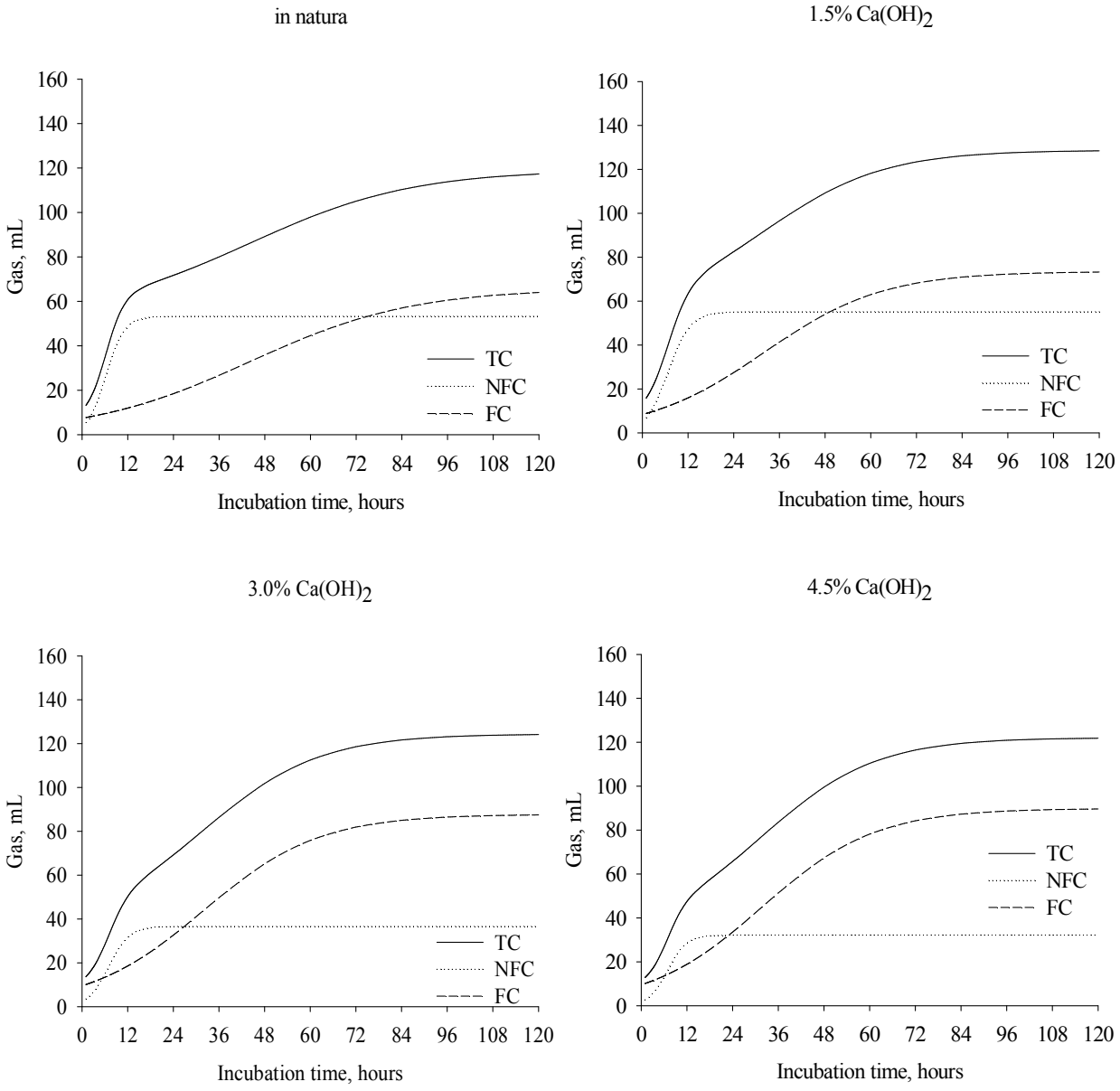


Figure 2. Gas production from in vitro ruminal fermentation depending on the incubation times of cocoa husks treated with 0; 1.5; 3.0 and 4.5% of CaO.

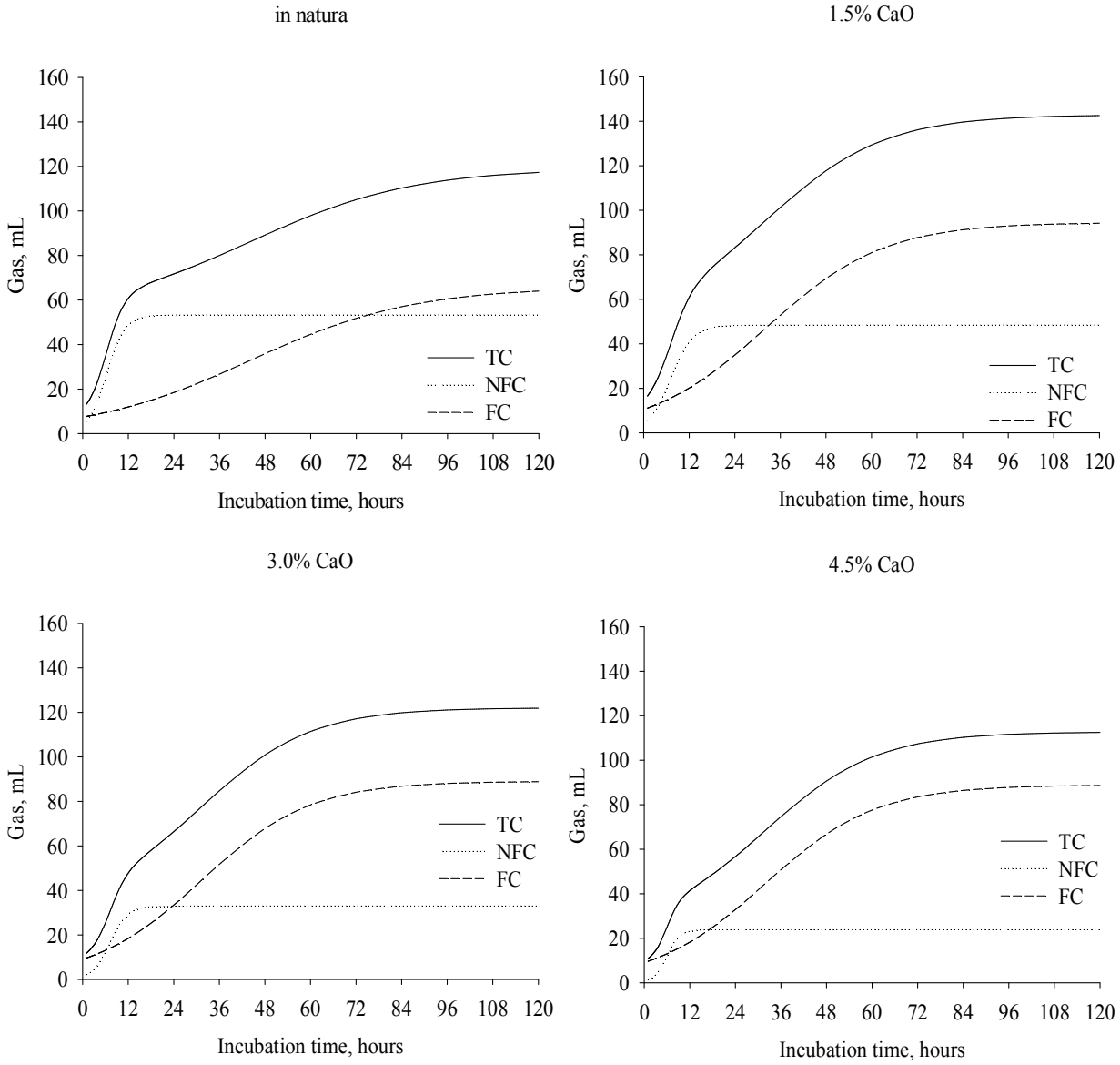
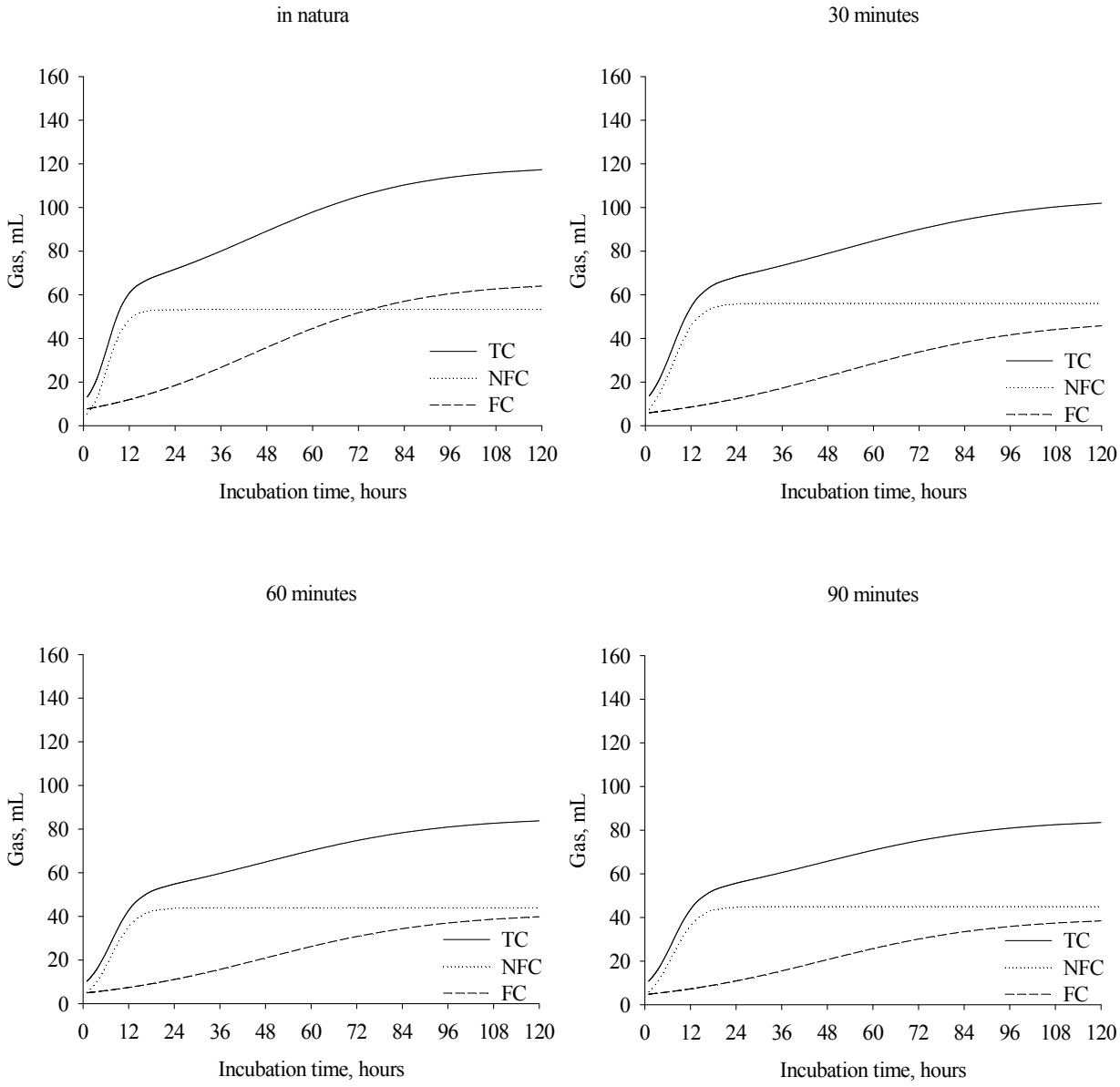


Figure 3. Gas production from in vitro ruminal fermentation depending on the incubation times of cocoa husks exposed to 1.23 kg/cm pressure (15 psi) and a 123°C temperature for 0, 30, 60 and 90 minutes.



Result from the contrasts

Mean estimates of the kinetic parameters of in vitro ruminal fermentation of the non-fibrous carbohydrates (NFC) and fibrous carbohydrates (FC) (Table 3) indicate differences ($P<0.05$) between the samples analyzed for the contrasts 1, 2, 4, 5 and 6 (Table 4). However, in contrast 3 no effect was observed ($P>0.05$) for the latency time (*L*) parameter, and in contrast 5 no effect was observed ($P>0.05$) for the FC degradation rate (kdFC) or TFV parameters.

Table 3. Kinetic parameters of in vitro ruminal fermentation from the non-fibrous and fibrous carbohydrates of cocoa husks treated with alkaline agents, and of those exposed to pressure and temperature.

Item	Parameters					
	fvNFC[1]	kdNFC[2]	L[3]	fvFC[1]	kdFC[2]	fvT[1]
Control	53.300	0.104	1.424	65.872	0.012	119.171
			$Ca(OH)_2$			
15.0g	55.226	0.085	0.817	73.512	0.016	128.738
30.0g	36.661	0.093	1.644	87.758	0.017	124.419
45.0g	32.224	0.101	1.948	89.862	0.017	122.085
Mean	41.370	0.093	1.470	83.711	0.016	125.081
			CaO			
15.0 g	48.420	0.085	1.228	94.507	0.016	142.928
30.0 g	33.017	0.108	2.579	89.050	0.017	122.067
45.0 g	23.843	0.146	2.555	88.880	0.017	112.723
Mean	35.093	0.113	2.121	90.813	0.017	125.906
			Heat treatment			
30 min	56.185	0.075	0.487	48.927	0.009	105.111
60 min	43.970	0.077	0.981	41.634	0.010	85.605
90 min	45.073	0.075	0.625	40.007	0.010	85.080
Mean	48.409	0.076	0.698	43.523	0.010	91.933

fvNCF, final volume of the non-fiber carbohydrates; kdCNF, degradation rate of the non-fiber carbohydrates; L, lag time; fvFC, final volume of the fiber carbohydrates; kdNFC, degradation rate of the fiber carbohydrates; fvT, final volume total.
[1]ml [2](h^{-1}) [3]hours.

Table 4. Error type I probability (P $Value^{-1}$) associated with the t test for the orthogonal contrasts.

Item	Contrasts					
	1	2	3	4	5	6
fvNFC	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
kdNFC	0.0261	<0.0001	0.0141	<0.0001	<0.0001	<0.0001
L	<0.0001	<0.0001	0.7151	<0.0001	<0.0001	<0.0001
fvFC	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
kdFC	<0.0001	<0.0001	<0.0001	<0.0001	0.0531	<0.0001
fvT	<0.0001	<0.0001	<0.0001	<0.0001	0.0714	<0.0001

fvNCF, final volume of the non-fiber carbohydrates; kdCNF, degradation rate of the non-fiber carbohydrates; L, lag time; fvFC, final volume of the fiber carbohydrates; kdNFC, degradation rate of the fiber carbohydrates; fvT, final volume total.

Control contrast: $Ca(OH)_2$

There was a reduction in the NFC degradation rate (kdNFC) and in the NFC final volume (FVNFC), with the inclusion of $Ca(OH)_2$ for the alkaline treatment of cocoa husks. This result can be attributed to lower gas production in the early times, with this being the most representative period for the bicompartimental model, with regards to the NFC fermentation parameters.

According to Pell et al. (1997), the contribution from the high quality voluminous soluble fraction is responsible for the volume of gases produced during the first 15 fermentation hours. During this period, microbial mass production occurs practically independent of the fibrous carbohydrates. However, cocoa husks have high lignin content and low soluble carbohydrate content, therefore the soluble fraction may not have been representative when compared to the high quality forage.

For the levels of $Ca(OH)_2$ inclusion, there was an observed decrease of 5.4% in the FVNFC for each unit percentage when $Ca(OH)_2$ was added to the CH (Table 5). The kdNFC and L showed quadratic behavior with a minimum point estimated at 2.24 and 1.46% of $Ca(OH)_2$ in cocoa husks, respectively. It is possible that adding an alkaline agent such as $Ca(OH)_2$ in the CH raised the pH of the in vitro environment and did not provide suitable conditions for developing a microbial population that was able to degrade the NFC, which resulted in decreased FVNFC and the minimum point kdNFC behavior, as was measured when $Ca(OH)_2$ was added to CH.

Table 5. Solution of the fixed effects regression equations for the kinetic parameters of in vitro ruminal fermentation, and their respective coefficients of determination for $Ca(OH)_2$ inclusion levels in cocoa husks.

Parameters	fvNFC	kdNFC	L	fvFC	kdFC	fvT
Intercept	56.62	0.102	13.26	66.31	0.0119	119.96
SE	13.05	0.03	0.11	0.86	0.02	0.65
P^{-1}	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Level of $Ca(OH)_2$	-54.53	-0.01	-0.29	57.47	0.003	62.45
EP	0.465	0.003	0.121	0.3097	0.003	0.701
P^{-1}	<0.0001	0.001	0.020	<0.0001	<0.0001	<0.0001
Level of $Ca(OH)_2$		0.003	0.101		-0.004	-13.22
SE		0.08	0.25		0.01	0.14
P^{-1}		0.009	0.004		<0.0001	<0.0001
r^2	0.83	0.85	0.72	0.94	0.96	0.74

fvNCF, final volume of the non-fiber carbohydrates; kdCNF, degradation rate of the non-fiber carbohydrates; L, lag time; fvFC, final volume of the fiber carbohydrates; kdNFC, degradation rate of the fiber carbohydrates; fvT, final volume total; SE, standard error.

Whereas for the FC, it was observed that for each increase in the unit percentage of $Ca(OH)_2$, the VFC increased by 5.75%. The kdFC and TFV had quadratic behavior with a maximum point estimated at 3.42 and 2.36% $Ca(OH)_2$, respectively.

The increase in the $Ca(OH)_2$ concentration may have resulted in the pH being maintained near neutral for a longer period of time compared to the control treatment. This phenomenon may have been favorable for fibrocystic microorganisms, which, according to Dijkstra et al. (2012), the majority of cellulase are highly sensitive to variations of ruminal pH, and that none of the ruminal cellulolytic bacteria grow at pH values that are significantly below 6.0.

It is possible to infer that in spite of this reduction in kdNFC and FVNFC, including this alkali provided a greater extent and speed in FC degradation which resulted in a greater extent in TC degradation and nutrient availability to the population of in vitro microorganisms.

Control contrast: CaO

There was an increase ($P < 0.05$) in kdFC and in VFFC, which was detrimental to kdNFC and in the FVNFC. The results found for the CH that were treated with CaO pointed out the best use of the CH fibrous fraction.

For each percentage unit of CaO in the CH, there was an estimated decrease of 6.9% in the FVNFC and an increase of 0.3% in the L (Table 6). The kdNFC presented quadratic behavior with a minimum point estimated at 1.45% of CaO in the cocoa husk. For the FC, the kdFC and the vfFC were observed to have quadratic behavior with a maximum point estimated at 2.91 and 3.37% of CaO, respectively. The TFV had quadratic behavior with a maximum point estimated at 1.88% of CaO in the CH.

Table 6. Solution of the fixed effects regression equations for the kinetic parameters of in vitro ruminal fermentation, and their respective coefficients of determination for CaO inclusion levels in cocoa husks.

Parameters	fvNFC	kdNFC	L	fvFC	kdFC	fvT
Intercept	55.21	0.10	1.23	67.84	0.01	121.97
SE	0.80	0.04	0.11	1.64	0.02	2.17
P^{-1}	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Level of CaO	-6.91	-0.01	0.31	18.64	0.03	13.87
EP	0.01	0. 04	0.04	1.75	0.01	2.33
P^{-1}		0.0003	<0.0001	<0.0001	<0.0001	<0.0001
Level of CaO		0.01		-3.20	-0.01	-3.67
SE		0.01		0.37	0.01	0.49
P^{-1}		<0.0001		<0.0001	<0.0001	<0.0001
r^2	0.97	0.98	0.72	0.99	0.99	0.69

fvNCF, final volume of the non-fiber carbohydrates; kdCNF, degradation rate of the non-fiber carbohydrates; L, lag time; fvFC, final volume of the fiber carbohydrates; kdNFC, degradation rate of the fiber carbohydrates; fvT, final volume total; SE, standard error.

There were differences between the samples for the latency period, at which time no degradation of the substrate was verified. The samples that were subjected to alkaline treatment with CaO at levels of 30.0 and 45.0 g kg^{-1} were those that provided the greatest estimates for latency period, namely 2.57 and 2.55 hours, respectively, compared with the other samples. It is possible that the highest levels of CaO kept the pH high inside the flasks, thus not providing an ideal environment for microorganism activity in the early incubation days which involve substrate moisture, microbial colonization and adhesion, and may not have had full availability of rapidly fermentable components thereby resulting in slow gas production.

Control contrast: thermal

The CH submitted to pressure and temperature at the 90 minute time showed the worst results for all the evaluated parameters, with the exception of the Latency period. These results may be related to lesser availability of substrate capable of microbial fermentation that result from the negative effects caused by the Maillard reaction, since, in addition to the thermal exposure that occurred in the industry during cocoa processing, these samples were exposed for longer periods to temperature and heat in the autoclave.

The Maillard reaction occurs among reducing sugars and proteins and leads to Brown pigments being formed, which results from the reaction between the glucose and glycine. The reaction between the amino group and the carbonyl or ketone groups from the reducing carbohydrates occurs even at relatively low temperatures, this is due to the high energy from this type of reaction's activation (SHIBAO; BASTOS, 2011).

Despite there being good protein content (approx 150.0g kg^{-1} of DM), the cocoa husk has low protein utilization, which is a result of high ADIN levels (PIRES et al., 2009). This protein fraction corresponds to proteins associated with lignin, tannin-protein complexes and products derived from the Maillard reaction.

For heat exposure levels at the time slots, there was an observed decrease of 0.12% in the FVNFC and an increase of 0.63% in L during each minute of treatment exposure for the cocoa husks (Table 7). The kdNFC presented quadratic behavior with a minimum point estimated at 64.06 minutes.

Table 7. Solution of the fixed effects regression equations for the kinetic parameters of in vitro ruminal fermentation, and their respective coefficients of determination for time exposed to temperature and pressure for cocoa husks.

Parameters	fvNFC	kdNFC	L	fvFC	kdFC
Intercept	55.16	0.10	65.67	0.01	117.00
SE	10.17	0.01	0.65	0.01	13.68
P^{-1}	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Time	-0.12	-0.01	-0.66	-0.01	-0.40
SE	0.02	0.01	0.03	0.01	0.02
P^{-1}	<0.0001	<0.0001	<0.0001	0.0016	<0.0001
$Time^2$		0.01	0.01	0.01	
SE		0.01	0.01	0.01	
P^{-1}		<0.0001	<0.0001	0.0027	
r^2/R^2	0.62	0.90	0.99	0.64	0.91

fvNCF, final volume of the non-fiber carbohydrates; kdCNF, degradation rate of the non-fiber carbohydrates; L, lag time; fvFC, final volume of the fiber carbohydrates; kdNFC, degradation rate of the fiber carbohydrates; fvT, final volume total; SE, standard error.

The kdFC and the VFFC had quadratic behavior with a minimum point estimated at 49.7 and 78.3 minutes of exposure to heat, respectively (Table 7). At every minute of exposure to heat, there was a 0.4% decrease in the TFV.

$Ca(OH)_2$ contrast: Cao

The FVNFC was higher ($P < 0.05$) for CH treated with $Ca(OH)_2$ when compared with those treated with CaO. Whereas the kdNFC and L showed higher values ($P<0.05$) for the CH treated with CaO. Despite the TFV that were estimated from the samples treated with the two sources being very close, there was no difference ($P<0.05$) between the means. The results for the CH treated with CaO were greater than those treated with $Ca(OH)_2$, indicating that the CH alkaline treatment with CaO can be more efficient than when treated with $Ca(OH)_2$.

Thermal Ca $(OH)_2$ contrast:

According to Table 3, the estimated means for all the parameters behaved negatively for the samples subjected to heat when compared to the estimated averages of the parameters for the CH treated with $Ca(OH)_2$.

CaO contrast: thermal

Estimated means between the treatment parameters compared in this contrast can be found in Table 3. The alkaline treatment may have provided most of the fiber for fermentation, with it being more effective when compared to the thermal treatment associated with nutrients being unavailable due to excessive heat during the thermal treatment.

Conclusion

Treating cocoa husks with calcium hydroxide and calcium oxide improves the gastrointestinal kinetic parameters of the cocoa husks, this is primarily as it improves the fermentation pattern of the fibrous fractions. Alkaline treatments which use calcium oxide are better than those using calcium hydroxide. Thermally treating cocoa husks is not recommended.

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