

Relationship between forage neutral detergent fiber and non-fibrous carbohydrates on ruminal fermentation products and neutral detergent fiber digestibility in goats

Relación entre la fibra detergente neutra del forraje y los carbohidratos no fibrosos sobre los productos de la fermentación ruminal y digestibilidad de la fibra detergente neutra en cabras

Efeito da relação da fibra em detergente neutro advindo de forragem e carboidratos não fibrosos sobre os produtos da fermentação ruminal e digestibilidade da fibra em detergente neutro em caprinos

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Abstract

Background: There is a comprehensive understanding of the role of dietary fiber as a nutrient and its function during digestion in cattle. On the other hand, the role of fiber digestion in goats has not received similar attention. **Objective:** To evaluate the effects of different ratios of forage neutral detergent fiber (fNDF)

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and non-fibrous carbohydrates (NFC) on ruminal fermentation products, and *in vitro* neutral detergent fiber (NDF) digestibility in goats. **Methods:** A 3×5 factorial arrangement in a completely randomized design with three fNDF levels (100, 190, and 290 g/kg) and five NFC levels (350, 400, 450, 500, and 550 g/kg) was used. The experiment was performed *in vitro*. Two ruminally fistulated goats were used as rumen liquid donors. **Results:** The ratios between fNDF and NFC affected all ruminal parameters ($p < 0.05$). Increasing NFC levels in diets containing 100 and 290 g/kg fNDF resulted in linear increase ($p < 0.05$) in ammonia concentration after 48 h fermentation. There was no adjustment of linear models ($p > 0.05$) for pH values. Total volatile fatty acids (VFA) and their individual molar proportions were affected ($p < 0.05$) at all fNDF and NFC levels. The NDF digestibility was not affected ($p > 0.05$) by fNDF or NFC levels, except for diets containing 290 g/kg fNDF, which were fit to a quadratic model ($p < 0.05$). **Conclusion:** The relationship between fNDF and NFC concentrations affect the fermentation end products and *in vitro* NDF digestibility. NFC plays a more consistent role than fNDF in the ruminal microbial ecosystem of goats during *in vitro* fermentation.

Keywords: digestion, energy, microbial protein, physically effective fiber, rumen.

Resumen

Antecedentes: Aunque hay una amplia comprensión del papel que juega la fibra dietética como nutriente y su función durante la digestión en el ganado vacuno, el papel de la digestión de la fibra en las cabras no ha recibido una atención similar. **Objetivo:** Evaluar los efectos de diferentes proporciones de fibra detergente neutra proveniente del forraje (fNDF) y carbohidratos no fibrosos (NFC) sobre los productos de la fermentación ruminal y la digestibilidad *in vitro* de la fibra detergente neutra (NDF) en cabras. **Métodos:** Se utilizó un arreglo factorial de 3×5 , distribuidos en un diseño completamente aleatorizado con tres niveles de fNDF (100, 190 y 290 g/kg) y cinco niveles de NFC (350, 400, 450, 500 y 550 g/kg). El experimento se realizó *in vitro*. Se utilizaron dos cabras fistuladas en rumen como donantes de líquido ruminal. **Resultados:** La relación entre fNDF y NFC afectó todos los parámetros ruminales estudiados ($p < 0,05$). El aumento de los niveles de NFC en dietas con 100 y 290 g/kg de fNDF resultó en aumento lineal ($p < 0,05$) de la concentración de amoníaco después de 48 h de fermentación. No hubo ajuste de modelos lineales ($p > 0,05$) para valores de pH. Los ácidos grasos volátiles totales y sus proporciones molares individuales se afectaron ($p < 0,05$) en todos los niveles de fNDF y NFC. La digestibilidad de NDF no fue afectada ($p > 0,05$) por los niveles de fNDF ni NFC, con excepción de las dietas con 290 g/kg fNDF, que se ajustaron a un modelo cuadrático ($p < 0,05$). **Conclusión:** La relación entre las concentraciones de fNDF y NFC afecta los productos finales de fermentación y la digestibilidad *in vitro* de NDF. Los niveles de NFC juegan un papel más consistente que fNDF en el ecosistema microbiano ruminal de la cabra durante la fermentación *in vitro*.

Palabras clave: digestión, energía, fibra físicamente efectiva, proteína microbiana, rumen.

Resumo

Antecedentes: Embora exista um compreensível entendimento do papel que a fibra dietética desempenha como nutriente e sua função durante a digestão em bovinos, o papel da digestão das fibras em caprinos não recebeu atenção semelhante. **Objetivo:** Avaliar os efeitos de diferentes relações da fibra em detergente neutro advindo de forragem (fNDF) e carboidratos não-fibrosos (NFC) sobre produtos da fermentação ruminal e digestibilidade *in vitro* da fibra em detergente neutro (NDF) em caprinos. **Métodos:** Utilizou-se um esquema fatorial de 3×5 distribuídos em um delineamento inteiramente casualizado com três níveis de fNDF (100, 190 e 290 g/kg) e cinco níveis de NFC (350, 400, 450, 500 e 550 g/kg). O experimento foi realizado *in vitro*. Duas cabras fistuladas no rúmen foram utilizadas como doadoras de líquido ruminal. **Resultados:** As relações entre fNDF e NFC impactaram todos os parâmetros ruminais estudados ($p < 0,05$). O aumento dos níveis de NFC em dietas contendo 100 e 290 g/kg de fNDF resultou em um aumento linear ($p < 0,05$) na concentração de amônia após 48 h de fermentação. Não houve ajuste de modelos lineares ($p > 0,05$) para valores de pH. Os ácidos graxos voláteis totais e suas proporções molares individuais foram afetados ($p < 0,05$) em todos os níveis de fNDF e NFC. A digestibilidade NDF não foi afetada ($p > 0,05$) pelos níveis de fNDF e NFC, com exceção de dietas experimentais contendo 290 g/kg fNDF, que foram ajustadas a um modelo quadrático ($p < 0,05$). **Conclusão:** A relação entre as concentrações de fNDF e NFC afeta os produtos finais de fermentação e a digestibilidade *in vitro* de NDF de dietas experimentais. Os níveis de NFC desempenham um papel mais consistente do que o fNDF no ecossistema microbiano ruminal de cabras durante a fermentação *in vitro*.

Palavras-chave: digestão, energia, fibra físicamente efetiva, proteína microbiana, rúmen.

Introduction

Recommendations on the minimum total dietary neutral detergent fiber (NDF) and the minimum level of forage NDF (fNDF) for cows are 250 g/kg of total NDF, and 190 g/kg of fNDF, respectively. These values have been defined through a compilation of research studies evaluating the performance of dairy cows under specific feeding conditions (NRC, 2001).

While there is a comprehensive understanding of the role that dietary fiber plays as a nutrient and its function during digestion in cattle, the role of fiber digestion in goats has not received similar attention (Lu *et al.*, 2008). Goats differ from cattle in their feeding behavior, the level of fiber they ingest and their dietary selections (Reid *et al.*, 1990). Based on voluntary dry matter intake (DMI), it is clear that the demand for energy and the physical capacity of the digestive tract direct the level of intake for a particular animal. Therefore, information obtained from other ruminant species cannot be simply extrapolated to goats.

Among the various lines of research related to ruminant nutrition, the study of the relationship between NDF and NFC is of great importance. Suitable NDF levels in a ruminant's diet during production are usually adjusted in order to maintain normal function of the rumen, maintain microbial protein synthesis (Xu *et al.*, 2014), and optimize its intake and digestibility (Oliveira *et al.*, 2011). Similarly, the NFC content has been considered in ruminant ration formulations as a nutritional criterion that is adjusted to obtain the appropriate fiber fraction in the feed (NRC, 2001). However, it is not known which of these components (fNDF or NFC) most affects the rumen microorganisms of goats.

For goats, there are no recommendations on the maximum and minimum values of fiber and NFC in the diet to maximize intake, animal performance and/or maintenance of normal conditions in the rumen. Thus, the evaluation of diets with different fNDF and NFC ratios under *in vitro* fermentation conditions can be used to determine the minimum and maximum quantities of fNDF and NFC that should be included in goat feed, since information on this topic is scarce.

Therefore, this study aimed to evaluate the effects of different forage neutral detergent fiber (fNDF) and non-fibrous carbohydrates (NFC) ratios on ruminal fermentation products and *in vitro* neutral detergent fiber (NDF) digestibility in goats.

Material and methods

Ethical considerations

The experimental procedures were approved by the Committee of Ethics on Animals at the Federal University of Paraiba (approval number: 2105/2013).

Location

In vitro experiments were conducted in the municipality of Areia, Paraiba (Brazil), located at an average altitude of 623 m, with a 6° 57' 46" S latitude and 35° 41' 31" W longitude.

Animals

Two ruminally fistulated goats, fed a total mixed ration (TMR) consisting of 60:40 forage to concentrate ratio on a DM basis, were used as rumen liquid donors. The animals were fed corn silage and a concentrate composed of ground corn, soybean meal and a mineral supplement.

Rumen fluid was collected 2 h after the morning feeding, filtered through four layers of cheesecloth, packed in a thermal bottle and immediately taken to the laboratory. The rumen fluid was transferred to 500 mL Erlenmeyer flasks, which were gassed for 20 s with anaerobic-grade CO₂. Then, they were sealed with plastic wrap and placed at rest for 30 min in an environmental chamber SP LABOR (SP-500 model, Presidente Prudente, SP, Brasil) at 39°C. After formation of liquid interfaces, the intermediate liquid was withdrawn in order to obtain an inoculum containing the most active microbial population (Oliveira *et al.*, 2012).

Experimental design

The experiment consisted of a completely randomized design in a 3 × 5 factorial scheme with three levels of fNDF (100, 190, and 290 g/kg) and

five NFC levels (350, 400, 450, 500, and 550 g/kg), with three replicates each. The experimental diets were formulated using Tifton-85 hay, ground corn, soybean meal, wheat bran, urea and starch (Table 1).

Table 1. Chemical composition of ingredients used to formulate the experimental diets.

Item	Ingredients (g/kg)					
	Tifton hay	Ground corn	Soybean meal	Wheat bran	Urea	Starch ²
DM	858.8	876.4	883.7	880.0	975.7	887.7
Organic matter	946.0	988.4	944.1	946.8	-	91.3
Ash	54.0	11.6	55.9	53.2	-	8.7
Crude protein	88.0	95.2	464.1	160.0	2810	4.9
Ether extract	7.7	38.0	15.6	35.3	-	2.6
NDF ¹	731.0	283.3	133.6	440.0	-	-
NDFap ¹	712.5	247.5	96.1	394.3	-	-
NFC ¹	3.61	582.0	300.0	303.6	-	990.6

DM = dry matter; g/kg on a fresh matter basis; ¹NDF = Neutral Detergent Fiber; NDFap = Neutral Detergent Fiber corrected for ash and protein; NFC = Neutral Fiber Carbohydrate. ²Data from Brazilian Tables of Food Composition for Cattle (Valadares Filho *et al.*, 2015).

Different combinations of fNDF and NFC levels were achieved through the use of experimental diets formulated to be isonitrogenous according to (NRC, 2007) (Tables 2 and 3).

Samples of Tifton-85 hay, ground corn, soybean meal and wheat bran used in the formulation of experimental diets were dried in a forced-air oven at 55°C for 72 h, ground in a Wiley mill (Wiley mill, Arthur H. Thomas, PA, USA) with a 1-mm screen, and stored in plastic containers for further laboratory analyses. Dry matter (DM) was determined with method 934.01. Organic matter (OM) was determined by the ash method (method 924.05). Crude protein (CP) was calculated by determining the total nitrogen content using the micro-Kjeldhal technique (method 920.87), and using a fixed conversion factor (6.25). Ether extract (EE) was determined gravimetrically after extraction with petroleum ether in a Soxhlet apparatus (method 920.85), and acid detergent fiber (ADF) (method 973.18) as described by the AOAC

Table 2. Proportion of ingredients used to formulate the experimental diets (g/kg DM).

Ingredients (g/kg)	fNDF (g/kg)	NFC (g/kg)				
		350	400	450	500	550
Tifton hay		133.2	133.2	133.2	133.2	133.2
Ground corn		223.8	223.8	223.8	223.8	200.0
Soybean meal	100	0.00	0.00	0.00	0.00	16.0
Wheat bran		643.0	590.0	525.0	440.0	364.0
Urea		0.00	0.00	4.0	8.0	10.0
Starch		0.00	53.0	114.0	195.0	276.8
Tifton hay		253.1	253.1	253.1	253.1	253.1
Ground corn		250.0	250.0	250.0	250.0	250.0
Soybean meal	190	35.0	35.0	40.0	40.0	40.0
Wheat bran		451.9	380.0	296.0	216.9	140.0
Urea		0.00	1.9	5.9	10.0	14.0
Starch		10.0	80.0	155.0	230.0	302.9
Tifton hay		386.3	386.3	386.3	386.3	386.3
Ground corn		290.0	290.0	290.0	125.0	0.00
Soybean meal	290	63.0	63.0	63.0	105.0	108.0
Wheat bran		228.7	147.7	70.0	20.0	0.00
Urea		0.00	5.0	8.7	10.0	14.5
Starch		32.0	108.0	182.0	353.7	491.2

fNDF = forage neutral detergent fiber; NFC = non-fibrous carbohydrates.

(1990). Neutral detergent fiber (NDF) was determined as described by Mertens (2002), and sulfuric acid lignin as described by Robertson and Van Soest (1981). The NDF content was corrected for ash and protein, and performed according to Licitra *et al.* (1996) and Mertens (2002).

The NFC were calculated according to Detmann and Valadares Filho (2010): $NFC (g/kg) = 1000 - [(CP - CPU + U) + NDF + EE + ash]$, where: CPU is the CP content from urea (%), and U is the urea content (%).

Aliquots of the experimental diets (350 mg of DM) were packed in 50 mL glass bottles. McDougall's buffer (McDougall, 1948) was prepared in Erlenmeyer

Table 3. Chemical composition of the experimental diets.

Item (g/kg)	fNDF (g/kg)	NFC (g/kg)				
		350	400	450	500	550
DM		873.7	829.7	877.6	878.6	882.2
Organic matter		953.2	905.8	957.2	957	960.5
Crude protein		135.4	127.4	128.8	126.9	126.4
Ether extract	100	32.1	30.4	28.1	25.1	21.8
Ash		43.8	41.2	38.8	35	32.5
NDF		442.4	420.4	391.8	354.4	317.6
NFC		384	417	454	503	542
DM		873.9	874.7	875.6	876.6	877.6
Organic matter		957.3	958.6	958.1	957.6	957
Crude protein		134.7	128.8	129.3	128.6	127.9
Ether extract	190	27.9	25.4	22.5	19.7	17
Ash		42.7	39.5	36	32.4	29
NDF		459.3	427.7	391.4	356.6	322.8
NFC		349	401	445	490	535
DM		871.2	872.3	873.2	875.4	878.3
Organic matter		959.8	958.4	958.2	957.5	955.1
Crude protein		127.6	129.1	127.4	127.7	127.7
Ether extract	290	23.1	20.2	17.4	10.1	4.7
Ash		40.2	36.6	33.1	32.5	31.4
NDF		473.6	437.9	403.7	340.6	296.9
NFC		311	389	434	485	537

DM = dry matter; g/kg on a fresh matter basis; fNDF = forage neutral detergent fiber; NFC = non-fibrous carbohydrates; NDF = neutral detergent fiber.

flasks, and the pH was adjusted to 6.8 by saturating it with CO₂ gas. To each glass bottle, 28 mL of McDougall's buffer and 7 mL of strained ruminal fluid were added. Bottles containing all experimental ingredients were immediately gassed for 10 s with anaerobic-grade CO₂ and then sealed. The final composition of the treatments was 100 mg of forage (DM) in a 10 mL final solution, and 1 mL of ruminal fluid in 4 mL of buffer solution (Tilley and Terry, 1963). Bottles were incubated at 39°C for 48 h. The incubation was conducted according to the partial methodology described by Tilley and Terry (1963). The incubation times were 0, 3, 6, 12, 24 and 48 h. The incubation process was repeated three times, generating

three evaluations per incubation time for each treatment. Once the fermentations were complete, 1.0 mL aliquots were collected from each bottle using a syringe with a 0.80 × 25 mm needle in order to maintain anaerobic conditions within the tubes (Lana *et al.*, 2000). The pH was measured using a digital pH meter (TECNOPON, Piracicaba, SP, BRA). Aliquots were acidified with meta-phosphoric acid (one part acid to one part sample) and centrifuged (Hettich Mikro 200 Model, Tuttlingen, BW, GER) at 5200 × g for 10 min. The supernatant was frozen (−20°C) and subsequently analyzed for volatile fatty acids (VFA) by high performance liquid chromatography (HPLC) according to Siegfried *et al.* (1984). The HPLC (SPD-10 AVP, Shimadzu Corporation, Osaka, OS, Japan) apparatus was equipped with a refractive index detector and used an Aminex HPX-87H column (BIO-RAD, Hercules, CA, USA) with a mobile phase containing 5 mM H₂SO₄ and a flow rate of 0.6 mL/min at 50°C. The VFAs were measured at 0 and 48 h. Molar proportions of VFA were calculated by dividing the concentration of individual acids by the total concentration of VFA. Ammonia was determined by the colorimetric method of Chaney and Marbach (1962). The resulting pellets were washed with a 0.9% (w/v) NaCl solution, centrifuged at 5200 × g for 10 min, resuspended in a 0.9% (w/v) NaCl solution and frozen at −20°C. Microbial protein was estimated using the method of Bradford (1976).

At the end of the incubation (48 h), the residue obtained from each bottle was vacuum filtered in a gooch crucible (gross porosity) and conditioned in bottles (120 mL) with the addition of 50 mL of a neutral detergent solution (Mertens, 2002). After sealing, the polyethylene bottles were autoclaved at 105 °C for 1 h in order to extract all of the soluble components (neutral detergent micro-extraction method (Pell and Schofield, 1993). After this procedure, the gooch crucibles were again submitted to vacuum filtration followed by sequential washing with hot distilled water and acetone. The NDF residue obtained was dried at 105°C for 16 h. The NDF digestibility was calculated using the following equation: NDF digestibility = (amount of NDF present in extracted sample bottles) – (amount of NDF in sample after washing)/(amount of NDF present in extracted sample bottles) × 100.

Statistical analysis

Data were analyzed using the GLM procedure of SAS software, version 9.4 (SAS Institute, Inc, Cary, NC, USA) (2012). Regression models from different fNDF levels were submitted to a model identity test. The choice of regression equation was based on the determination coefficient and the significance of regression coefficients using the t test, where $\alpha = 0.05$.

Results

The ammonia concentration varied throughout the fermentation process as a function of NFC levels in a similar pattern for the three fNDF levels studied, all reaching maximum concentrations after 48 h (Figures 1a, 2a, and 3a). Microbial protein concentration had a decrease after 24 h of incubation (Figure 1b, 2b and 3b), except in the diet containing 29% fNDF with 45 and 55% NFC.

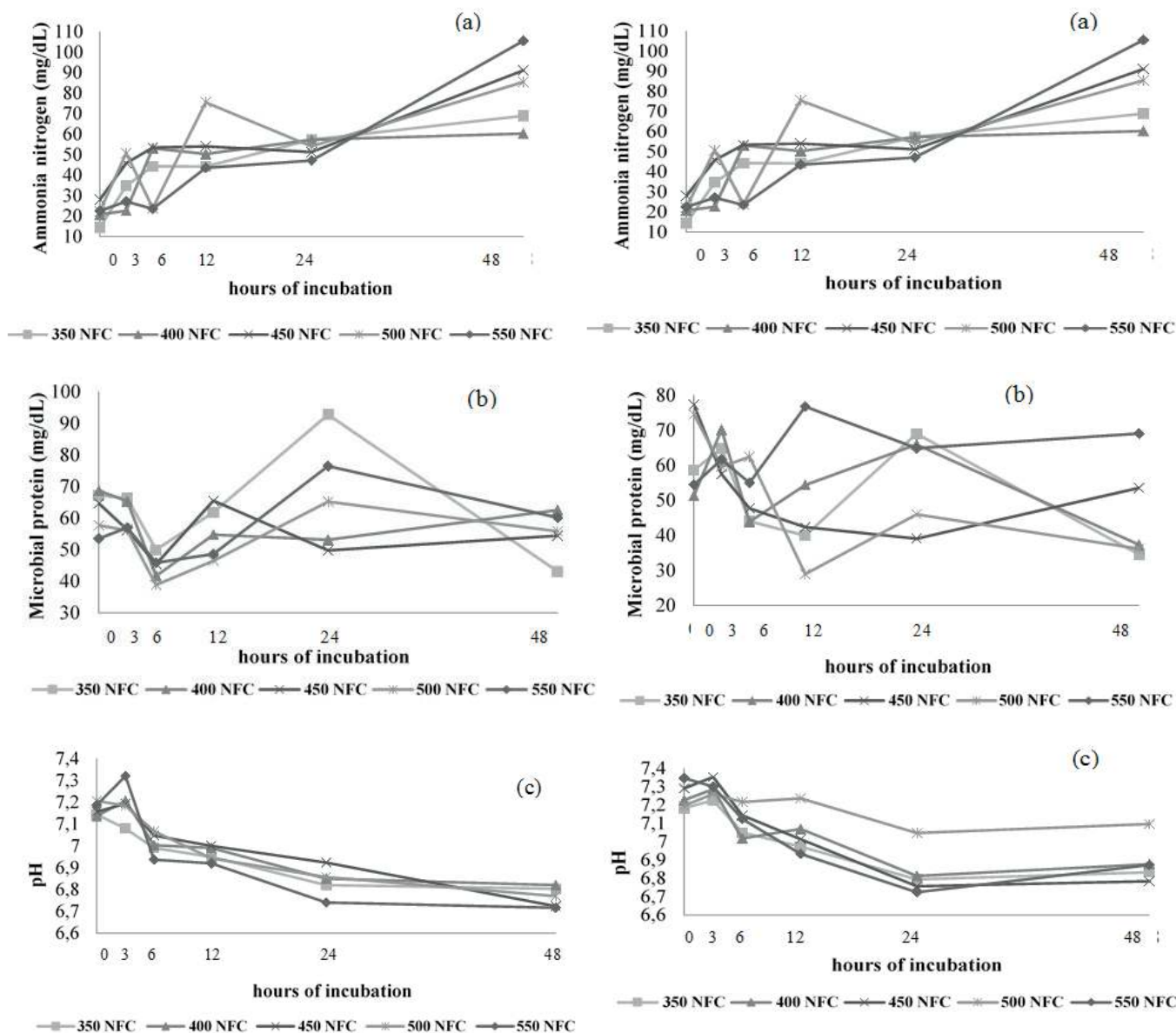


Figure 1. Effect of NFC levels on ammonia (a) and microbial protein (b), and changes in acidity (c) throughout the fermentation time by ruminal microorganisms in diets containing 100 g/kg fNDF.

Figure 2. Effect of NFC levels on ammonia (a) and microbial protein (b) concentration, and changes in acidity (c) throughout the fermentation time by ruminal microorganisms in diets containing 190 g/kg fNDF.

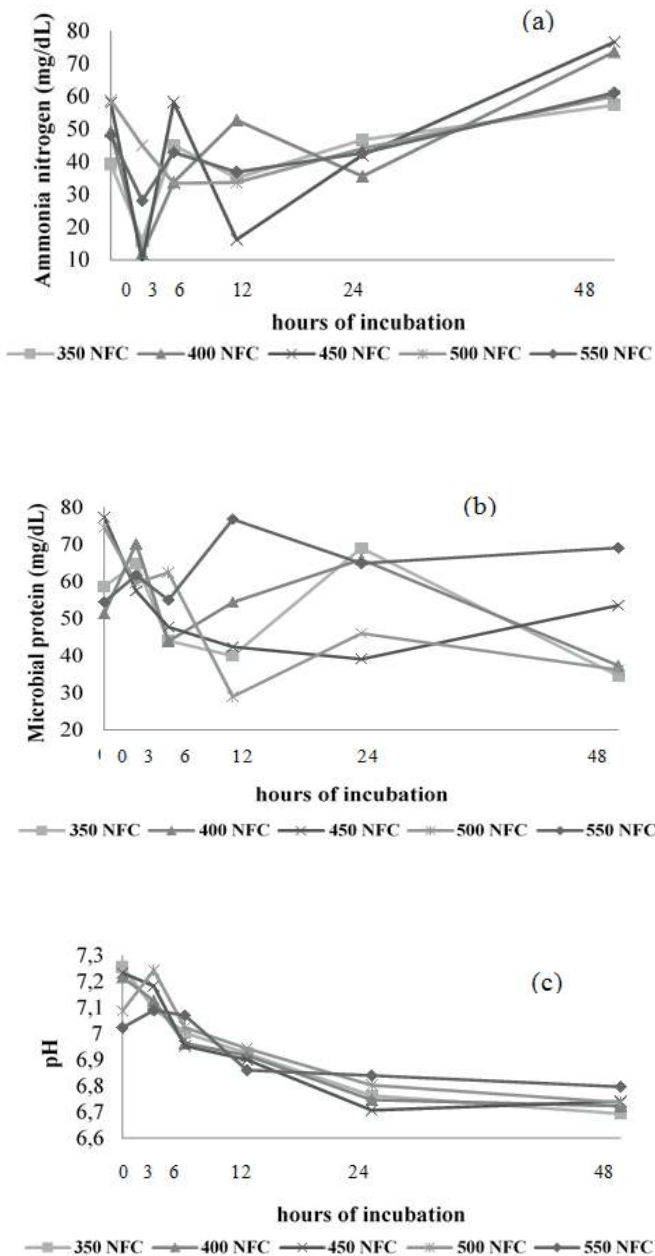


Figure 3. Effect of NFC levels on ammonia (a) and microbial protein (b) concentration, and changes in acidity (c) throughout the fermentation time by ruminal microorganisms in diets containing 290 g/kg fNDF. W

Microbial protein concentration after 48 h of fermentation had a linear crescent effect ($p < 0.05$) on all fNDF levels as a function of increased NFC levels, except for diets containing 190 g/kg fNDF (Table 4). When the model identity test was carried out, the

linear model of NFC levels for the fNDF levels of 100 and 290 g/kg were similar ($p > 0.05$).

Increasing NFC levels in media containing 100 and 290 g/kg fNDF had a linear decrease and increase ($p < 0.05$) in ammonia concentration until 48 h of fermentation, respectively (Table 4). The experimental diet containing 190 g/kg fNDF resulted in a quadratic increase ($p < 0.05$) in ammonia concentration as a function of NFC levels. When the model identity test was carried out, the linear models chosen among fNDF levels differed according to the NFC levels ($p < 0.05$). Thus, NFC levels act differently among the fNDF levels in ammonia production. With the exception of media containing 100 g/kg fNDF, the increase in NFC levels promoted an increase in the ammonia concentration (Table 4).

Different combinations of fNDF and NFC levels had no effect ($p > 0.05$) on the pH (Table 4).

Even with significant increases in total VFA production at 48 h of fermentation (Table 5), the amount of acid was not enough to decrease the pH.

Total VFA and individual molar ratios were different ($p < 0.05$) among fNDF and NFC levels (Table 5). Total VFA concentrations at 48 h were higher than at 0 h for all combinations of fNDF and NFC. The diet containing 190 g/kg fNDF quadratically increased ($p < 0.05$) total VFA with the addition of NFC; however, a linear increase ($p < 0.05$) was observed in diets containing 290 g/kg of fNDF. When the model identity test was carried out, the models of fNDF as function of NFC levels were different ($p < 0.05$). Thus, the change in NFC levels acted differently among the fNDF levels for total VFA production.

In general, significant changes were not observed between the individual molar ratios of acetate, propionate, and butyrate with the addition of NFC at each fNDF level (Table 5).

Table 4. Effect of forage neutral detergent fiber (fNDF) and non-fibrous carbohydrates (NFC) levels on microbial protein (MP), ammonia concentration and pH during *in vitro* fermentation.

Item	fNDF (g/kg)	NFC (g/kg)					CV (%)	P-value	
		350	400	450	500	550		L	Q
MP 0h, mg/dL	100	577.9	712.4	692.4	522.9	585.9		-	-
	190 ¹	672.9	686.4	646.6	596.6	535.4	6.11	<0.0001	-
	290 ²	586.1	513.0	773.5	745.7	544.4		0.0409	<0.0001
MP 48h, mg/dL	100 ³	283.6	435.6	526.8	588.6	657.8		<0.0001	-
	190	430.8	625.8	544.5	558.1	601.8	14.29	-	-
	290 ⁴	345.8	373.8	535.8	561.8	690.8		<0.0001	-
Ammonia 0h, mg/dL	100	329.4	407.7	309.1	507.3	396.4		-	-
	190 ⁵	392.0	492.9	581.3	584.3	582.0	6.63	<0.0001	<0.0001
	290 ⁶	123.7	207.9	280.1	222.5	225.1		<0.0001	<0.0001
Ammonia 48h, mg/dL	100 ⁷	893.8	791.2	859.1	791.2	662.4		<0.0001	-
	190 ⁸	573.2	735.8	765.8	600.9	610.6	7.66	-	0.0004
	290 ⁹	689.6	603.2	910.9	855.0	1,056		<0.0001	-
pH 48h	100	6.69	6.72	6.74	6.74	6.74		-	-
	190	6.80	6.82	6.72	6.77	6.72	0.58	-	-
	290	6.83	6.87	6.78	7.09	6.87		-	-

MP = microbial protein; fNDF = forage neutral detergent fiber; NFC = non-fibrous carbohydrates.

$$^1\hat{Y} = 955.917 - 0.7297\text{NFC}$$

$$r^2 = 0.8681$$

$$^2\hat{Y} = -2575.323 + 14.3044\text{NFC} - 0.156\text{NFC}$$

$$r^2 = 0.4118$$

$$^3\hat{Y} = 312.8 + 1.8029\text{NFC}$$

$$r^2 = 0.9572$$

$$^4\hat{Y} = 238.28 + 87.72\text{NFC}$$

$$r^2 = 0.5158$$

$$^5\hat{Y} = -1543.843 + 8.4442\text{NFC} - 0.0833\text{NFC}^2$$

$$r^2 = 0.9881$$

$$^6\hat{Y} = -1638.181 + 7.9736\text{NFC} - 0.0837\text{NFC}^2$$

$$r^2 = 0.8529$$

$$^7\hat{Y} = 1216.093 - 0.9256\text{NFC}$$

$$r^2 = 0.6826$$

$$^8\hat{Y} = -2114.360 + 12.7561\text{NFC} - 0.1431\text{NFC}^2$$

$$r^2 = 0.6016$$

$$^9\hat{Y} = -63.840 + 1.9708\text{NFC}$$

$$r^2 = 0.7508$$

CV= Coefficient of variation; L= linear; Q= quadratic.

Table 5. Effect of forage neutral detergent fiber (fNDF) and non-fibrous carbohydrates (NFC) levels on volatile fatty acids (VFA) concentration and individual molar proportion during *in vitro* fermentation.

Item	fNDF (g/kg)	NFC (g/kg)					CV (%)	P-value	
		350	400	450	500	550		L	Q
Total VFA 0h, mM	100	498.9	499.7	481.9	510.4	512.7		-	-
	190 ¹	563.2	509.7	514.2	528.1	612.8	4.40	0.0094	<0.0001
	290 ²	586.3	549.1	516.0	498.9	500.4		<0.0001	-
Total VFA 48h, mM	100 ³	1,761	1,744	1,803	1,707	1,577		<0.0001	0.0008
	190 ⁴	1,666	1,550	1,664	1,787	1,884	2.60	<0.0001	-
	290 ⁵	1,796	1,771	1,660	1,476	1,473		<0.0001	-
Acetate 0h, mol/100 mol	100	522.3	531.2	528.7	528.1	527.7		-	-
	190 ⁶	549.0	626.8	617.1	613.0	644.7	3.68	0.0001	-
	290 ⁷	638.5	627.5	657.6	607.9	570.8		0.0005	0.0084
Acetate 48h, mol/100 mol	100 ⁸	739.3	727.3	734.1	734.8	715.2		0.045	-
	190	696.7	692.5	696.9	715.8	691.7	1.50	-	-
	290	698.7	712.2	695.6	693.1	698.0		-	-
Propionate 0h, mol/100 mol	100	280.0	260.9	269.5	271.7	272.1		-	-
	190	248.5	230.2	248.5	237.7	213.4	7.02	-	-
	290 ⁹	190.3	221.0	218.4	237.9	206.3		-	0.0086
Propionate 48h, mol/100 mol	100	190.5	188.7	184.7	183.0	181.9		-	-
	190	207.8	213.2	212.5	199.9	219.0	4.03	-	-
	290 ¹⁰	231.0	221.0	235.1	210.6	208.7		0.0011	-
Butirate 0h, mol/100 mol	100	197.6	207.8	201.8	200.1	200.1		-	-
	190 ¹¹	202.6	143.0	134.3	149.3	141.9	7.24	<0.0001	<0.0001
	290 ¹²	171.3	151.4	124.0	154.2	222.9		<0.0001	<0.0001
Butirate 48h, mol/100 mol	100 ¹³	70.1	840	8.2	82.2	102.9		<0.0001	-
	190	95.5	94.2	90.6	84.3	89.4	8.10	-	-
	290 ¹⁴	70.3	66.8	69.3	96.3	93.3		<0.0001	-

VFA = volatile fatty acids; fNDF = forage neutral detergent fiber; NFC = non-fibrous carbohydrates.

$${}^1\hat{Y} = 2053.089 - 7.1166\text{NFC} + 0.0817\text{NFC}^2 \quad r^2 = 0.9746$$

$${}^2\hat{Y} = 730.013 - 0.4441\text{NFC} \quad r^2 = 0.8839$$

$${}^3\hat{Y} = -62.033 + 8.9612\text{NFC} - 0.1085\text{NFC}^2 \quad r^2 = 0.8991$$

$${}^4\hat{Y} = 1104.5 + 1.346\text{NFC} \quad r^2 = 0.6877$$

$${}^5\hat{Y} = 2482.087 - 1.8815\text{NFC} \quad r^2 = 0.9161$$

$${}^6\hat{Y} = 450.443 + 0.3549\text{NFC} \quad r^2 = 0.5983$$

$${}^7\hat{Y} = 14.720 + 3.0860\text{NFC} - 0.0377\text{NFC}^2 \quad r^2 = 0.8333$$

$${}^8\hat{Y} = 766.800 - 0.0815\text{NFC} \quad r^2 = 0.4695$$

$${}^9\hat{Y} = -407.814 + 2.7344\text{NFC} - 0.0293\text{NFC}^2 \quad r^2 = 0.7864$$

$${}^{10}\hat{Y} = 270.757 - 0.1099\text{NFC} \quad r^2 = 0.5433$$

$${}^{11}\hat{Y} = 980.342 - 3.5230\text{NFC} - 0.0366\text{NFC}^2 \quad r^2 = 0.8220$$

$${}^{12}\hat{Y} = 1394.071 - 5.8248\text{NFC} - 0.0671\text{NFC}^2 \quad r^2 = 0.9417$$

$${}^{13}\hat{Y} = 26.830 + 0.1273\text{NFC} \quad r^2 = 0.7236$$

$${}^{14}\hat{Y} = 11.220 + 0.1511\text{NFC} \quad r^2 = 0.6938$$

CV = Coefficient of variation; L = linear; Q = quadratic.

Changes in fNDF and NFC levels resulted in interaction ($p < 0.05$) on *in vitro* NDF digestibility. Only diets containing 290 g/kg fNDF had a quadratic

relationship ($p < 0.05$) to increasing NFC levels and *in vitro* NDF digestibility with maximum values estimated at 659.6 g/kg for 373.2 g/kg NFC (Table 6).

Table 6. Effect of forage neutral detergent fiber (fNDF) and non-fibrous carbohydrates (NFC) levels on *in vitro* NDF digestibility.

Item	fNDF (g/kg)	NFC (g/kg DM)					CV(%)	p-value	
		350	400	450	500	550		L	Q
NDF digestibility (g/kg)	100	635.3	646.4	648.5	647.4	658.1	2.65	ns	ns
	190	652.1	679.1	660.6	657.6	680.5		ns	ns
	290	658.4	651.6	649.1	607.4	565.0		0.0001	0.0056

fNDF = forage neutral detergent fiber; NFC = non-fibrous carbohydrates.

$$\hat{Y} = 212.67 + 23.763\text{CNF} - 0.315\text{CNF}^2$$

$$r^2 = 0.9845$$

CV = Coefficient of variation; L = linear; Q = quadratic.

Discussion

As *in vitro* fermentation experiments take place in closed environments, and no ammonia is absorbed through the rumen walls as it occurs in *in vivo* experiments, the ammonia concentration was directly related to microbial growth since it is used as a nitrogen source for microbial protein synthesis (Russell, 2002; Seo *et al.*, 2010; Seo *et al.*, 2013) (Figures 1a, 2a and 3a).

Thus, we observed that the microbial standard in goats varied more with changes in NFC concentration than changes in fNDF concentration (Figures 1b, 2b and 3b). This difference in the rumen environment of goats must be associated with their feeding behavior as they are concentrate pickers and much of their diet comes from less coarse foods. Thus, it is possible that changes in the fNDF concentration less significantly modify the ruminal environment relative to variations in NFC concentration. Similar behavior observed in the three fNDF levels studied reinforces this hypothesis.

Thus, NFC levels affected microbial protein production similarly, regardless of the amount of fNDF contained in the media (Table 4). Thus, we concluded that microbial protein concentration in goats is influenced more by NFC levels than by fNDF levels. Providing a greater amount of rapidly fermentable carbohydrates in the form of NFC created an environment favorable for bacterial growth and increased the amount of energy available to the rumen microorganisms for multiplication

(Russell *et al.*, 1992; Costa *et al.*, 2008; Ma *et al.*, 2014; Ma *et al.*, 2015). Coupled with an increase in energy levels for microbial protein synthesis, ruminal ammonia levels greater than 5 mg/dL should be an adequate nitrogen source for rumen microorganisms (Satter and Styler, 1974).

The increase in the ammonia concentration, promoted by the increase in NFC levels, may be occur due to the increased amounts of urea added to the experimental diets, as ammonia is produced both by deamination of amino acids and hydrolysis of nonprotein nitrogenous compounds (Valadares Filho and Pina, 2011) (Table 4).

The increase in total VFA was not enough to decrease the pH, probably due to the strong buffering capacity of McDougall's solution (Table 5). Several *in vitro* studies using this solution reported minor variations in the pH, regardless of the substrate used and whether its degradation was slow or rapid (Costa *et al.*, 2008; Costa *et al.*, 2009; Berthiaume *et al.*, 2010; Purcell *et al.*, 2014).

We expected an increase in the concentration of total VFA as NFC levels increased; however this was not observed for the fNDF levels 100 and 290 g/kg (Table 5). Under ideal conditions with excess fNDF, providing more rapidly fermentable carbohydrates promoted the growth of fermenting NFC microorganisms and increased VFA concentrations in the media (Vieira *et al.*, 2008). Berthiaume *et al.* (2010) and Ma *et al.* (2015) observed similar

increases in total VFA concentration when the NFC/NDF ratio was improved. However, increased NFC concentrations in the media may not influence or even decrease the total VFA concentration due to a simultaneous increase in fNDF concentration or total NDF in the diet (Archimede, 1996; Cantalapiedra-Hijar *et al.*, 2009).

The absence of significant changes in individual molar ratios of acetate, propionate and butirate may have occurred due to small variations on H⁺ concentrations, which affect pH and the metabolic pathways of some microorganisms. In this case, the metabolic fermentation pathways of microorganisms were not modified, which would have interfered with the production of acetate and propionate (Russel, 2002; Jiang *et al.*, 2013); thus the proportions of these metabolites were similar in the different combinations of fNDF and CNF studied. Russel (1988) reported that some ruminal microorganisms are capable of producing acetate and propionate in combination with other products such as formate, ethanol and CO₂, and that a change in acidity is capable of modifying the fermentation metabolic pathways of microorganisms (Aschenbach *et al.*, 2011; Castillo-González *et al.*, 2014). As an example, when fermentation is slow and extracellular pH is near neutral, *Streptococcus bovis* produces acetate, formate and ethanol, which are products of primary fermentation when growth rate is slow (Russel, 1988; Castillo-González *et al.*, 2014). These products were produced by the enzyme pyruvate formate lyase. On the other hand, if extracellular and intracellular pH declines, as is the case with more rapidly fermentable carbohydrates available than what is needed to increase growth rate, the pyruvate produced is converted into lactate. In this specific case, the change in metabolic pathways and the change in fermentation end products happens because pyruvate lyase becomes inactive at low pH (Russel, 2002).

Studies reported changes in the proportions of the main VFA, where low and high NFC/NDF ratios are evaluated in sheep (Ma *et al.*, 2015), and goat (Cantalapiedra-Hijar *et al.*, 2009). In contrast, Costa *et al.* (2008) did not find changes in the proportions of the main VFA. In this last study, authors noted that there was no rapid degradation of carbohydrates, which was indicated by the high pH. Thus, pH is an

important variable that responds to variations of the main VFA in the rumen. As observed in this study, the absence of pH variation did not cause significant changes in VFA molar ratios, even at high NFC/NDF ratios.

Diets with high NFC/NDF ratio resulted in lower NDF digestibility, probably due to the decline in pH (Cantalapiedra-Hijar *et al.*, 2009; White *et al.*, 2010; Ma *et al.*, 2015) that directly impacted how different microbial groups ferment carbohydrates (Homen Júnior *et al.*, 2015). Increased VFA production caused a decrease in pH and inhibited growth of bacteria that ferment fibrous carbohydrates, thus compromising NDF digestibility (Van Soest, 1994; Krajcarski-Hunt *et al.*, 2002; Sung *et al.*, 2007; Cerrato-Sánchez *et al.*, 2008; Russell *et al.*, 2009). However, in the present study, the minimum pH value observed was approximately 6.70 (Figure 1c). It likely did not decrease significantly due to the buffering capacity of the media, and there was not a decrease in NDF digestibility when higher NFC concentrations were used (Table 6).

In conclusion, the relationship between fNDF and NFC concentrations affects fermentation end products and *in vitro* NDF digestibility. The NFC levels appear to play a more consistent role over rumen microbial ecosystem during *in vitro* fermentation. Changes in the microbial standard of goats during *in vitro* fermentation are more affected by changes in NFC concentrations than fNDF concentrations. Thus, a reduction in NFC levels will allow goat diet formulations with minimum levels of NDF.

Conflict of interest

The authors declare that they have no conflict of interests with regard to the work presented in this report.

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