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Analysis of Fibrous Compounds Using a Pressurized and Non-pressurized Conditions

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Abstract

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were evaluated in pressurized and unpressurized conditions using samples of roughage and concentrates. In summary, the samples were dried, processed in a knife mill, weighed in nonwoven bags (100g/m²), placed in a container and treated with neutral or acid detergents. Extractions of NDF and ADF content were carried out in a non-pressurized condition at temperature of 100°C for 60min and in pressurized condition using different temperatures of 100 and 110°C for 60min. Results of the different temperatures using the pressurized procedure were compared to those obtained with the pressurized through the linear regression analysis. The method with the temperature 110°C for 60 min had a high level of agreement. Was not observed a bias potential of proportion (P>0.05). There was not a systematic inclination of the methods to overestimate or underestimate errors. This methodology can be carried out with roughage and concentrate feedstuffs simultaneously.

Keywords: acid detergent fiber, autoclave, feedstuffs, neutral detergent fiber, temperature

1. Introduction

The concentration of structural carbohydrates has been used to determine the nutritional and digestible quality of food, as well as being a predictor in mathematical models to estimate energy. Since 1960s the analytical methods to determine fiber content have been used and improved by researchers. The fiber detergent analysis system was initially proposed by Van Soest in 1963 to determine neutral detergent fiber (NDF) and acid detergent fiber (ADF). Initially, the proposed w to apply this methodology in forage, however it can be extended to analyze concentrated foods. Is well know that starch can contaminate samples, causing an overestimation of fiber values (Van Soest et al., 1991). Thus, the method to determine NDF and ADF has been improved to reduce the amounts of starch by using α-amylase, sodium sulfite, or 8M urea solution (Van Soest et al., 1991). Other adaptations have been proposed as an alternative to the original test, including an application of the filter bag procedure and a replacement of the conventional digester such as the use of pressurized equipment, e.g., an autoclave. (Pell and Schofield, 1993; Deschamps, 1999; Ferreira and Mertens, 2007; Senger et al., 2008).

The technique to determine NDF and ADF requires: (a) specialized equipment, which is very expensive and usually is not available in all laboratories, there may still be the formation of air bubbles inside the bags, at the time of the detergent boiling, which compromises its contact with the sample reducing the extraction efficiency of the non-fibrous components of the food (Gomes et al., 2011), or (b) requires pressurized equipment or an autoclave, which is more common in laboratories. According to Senger et al. (2008), the use of filter bags and autoclave treatment for the analysis of NDF or ADF results in a more practical and rapid test when compared with the conventional method using Gooch crucibles. In addition, it can be cheaper than using the ANKOM® fiber analyzer. However, there is no agreement about treatment duration and temperature described in literature.

The objective of this study was to test the accuracy when using pressurized equipment for the

analysis NDF and ADF by means of evaluating two temperatures in a pressurized environment and comparing them to the non-pressurized equipment (fiber analyzer) operated at a temperature recommended by the manufacturer.

2. Method and Methods

2.1 Food Sampling and Location

The experiment was performed at the Laboratory of Forage and Animal Nutrition at the Federal University of Pampa - Uruguaiana Campus/ RS. For this study, were used different samples from roughage and concentrate food to determine NDF (n=19 samples) and ADF (n=13 samples). The samples used in this study were: *Avena sativa L.* (oats, oats with husks and oats bran), *Lolium multiflorum L.* (Italian ryegrass), *Pennisetum purpureum Schum* (elephant grass, BRS Kurumi), *Glycine max L.* (soybean husks, crushed soybean, soybean plant, soybean meal, and soybean pie), *Medicago sativa L.* (alfalfa hay), native grass, *Manihot esculenta* (cassava silage and cassava root silage), *Olea europaea* (olive silage), *Sorghum bicolor* (sorghum), *Zea mays L.* (maize), *Cynodon spp.* (Tifton 68 e Tifton 85), *Oryza sativa* (rice bran, rice bran with husks, and broken rice), *Zea mays L.* (corn bran, wet corn grain, and ground corn), *Helianthus annuus L.* (sunflower and sunflower seed) (Table 1). Additional information about samples and fiber content (NDF and ADF) can be found in Table 1.

Table 1. Neutral (NDF) and acid (ADF) detergent fiber concentrations (g/kg dry matter) in experimental feedstuffs

Feedstuff	NDF		
	Not Pressurized	Pressurized at 100°C	Pressurized at 110°C
White oats w/ husks I	312..82	325.80	250.23
Oats III	309..93	326.23	252.88
Oats bran	321.90	292.03	276.40
Ryegrass	330.35	385.38	365.85
Elephant grass Kurumi	424.78	454.37	454.87
Elephant grass dwarf	446.40	454.28	365.40
Elephant grass common	413.20	469.15	441.92
Soybean bran	452.02	481.48	441.23
Alfalfa hay	467.97	500.03	482.17
Native grass	460.95	488.20	483.23
Cassava silage	404.20	424.42	380.22
Olive silage	302.22	341.80	341.37
Soybean silage	286.53	335.47	306.12
Soybean plant	393.93	429.52	409.95
Sorghum plant	433.80	468.78	466.05
Teosinto	377.33	397.28	389.48
Tifton 68	459.55	492.52	505.25
Tífton 85	462.66	498.95	483.73
Soybean pie	184.93	219.22	221.92

		ADF	
Rice bran III	171.58	171.85	145.70
Rice bran w/husks II	329.62	337.77	408.18
Rice bran w/husks IV	267.15	289.97	292.85
Rice bran w/husks I	226.70	236.87	236.52
Corn bran II	172.30	168.47	138.10
Corn bran III	106.47	129.23	130.58
Soybean bran I	128.35	142.00	81.33
Sunflower II	236.63	311.75	284.48
Sunflower seed	322.58	321.83	298.52
Ground corn	87.07	75.17	81.95
Wet corn grain	43.15	46.53	56.43
Broken rice	40.63	38.82	45.70
Cassava root silage	334.75	309.90	290.28

Averages in g/NDF and ADF

2.2 Fiber Analysis in Pressurized and Non-pressurized Conditions

The samples used in this study were pre-dried in an oven with forced ventilation at 60°C for 72 hours and processed in a knife mill using a sieve with a porosity of 1 mm. To carry out the analyses, nonwoven bags (100 g/m²) were made, with approximately 25cm², heat sealed, and dried in the oven for 12 hours at temperature of 105°C. After this procedure, the bags were weighed on an analytical balance and properly labeled. The samples were placed in the nonwoven bags, respecting the ratio of 20 mg of dry matter per cm² (Nocek, 1997).

The extraction of the detergent fiber was carried out in triplicate for each sample and both were arranged in a device with a pressurized and non-pressurized condition, following the detergent to sample ratio of approximately 100 ml/g. A repetition of the all analytical execution was performed. For the concentrated samples, 8M urea and heat-stable α-amylase were used (Termamyl 120L, Novozymes Latin América ltda.) To substantially reduce the amounts of starch, samples were submerged in a 1L beaker for 4 hours (VAN SOEST et al., 1991). To assess the fiber content in neutral detergent (NDF) and acid detergent (ADF), the detergent was prepared according to the recommendations of AOAC 2002.04; MERTENS, 2002.

To determine NDF and ADF in a non-pressurized condition, an equipment model TE-149, manufactured by Tecnal®, with a capacity of 30 tests separated into 10 perforated discs, was used. The foods were separated into concentrates foods and roughage and, subjected to a temperature of 100 ºC for 60 minutes. After the procedure, the bags were washed sequentially, at least three times, with hot distilled water and soaked with acetone to remove the remaining detergent. Bags were dried in an oven with forced ventilation at 60ºC for 24h.Subsequently, bags were dried again in an unventilated oven at 105ºC for 2h. Then, the bags were placed in a desiccator until they reached room temperature and afterwards, weighed in a precision analytical balance

To determine the NDF and ADF in a pressurized condition, model AV 18L equipment, manufactured by Phoeniz luferco, was used. The samples were placed in a Becker containing a solution of neutral or acid detergent. The Becker was properly sealed to prevent the entry of steam, and placed in a vertical autoclave for 60 minutes. This procedure was tested using two diferent temperatures: 100ºC with pressure at 0 Kgf / cm² and 110 ° C 0.33 Kgf / cm². As in the previous method, the samples were also separated into roughage foods and concentrated. After treatment, the bottles were removed from the autoclave and the bags followed the same procedure describe previously for non-pressurized condition.

2.3 Statistical Analysis

The data were analyzed using the IBM SPSS Statistics software version 20.0. Shapiro – Wilk test was used to verify the normality of the data and Levene's test was used to check the homogeneity of variances. To assess whether there are differences between the variables, where the hypothesis of bias being zero or not was tested by the two-tailed t test, where there is agreement by P> 0.05. A simple linear regression equation was performed for the values obtained in a pressurized environment (Y) over the values obtained in a non-pressurized environment (X). the statistical evaluation being conducted under the following assumptions: H0: $\beta 0 = 0$, and $\beta 1 = 1$; vs. Ha: not H0, where the regression slope deviation of 1 was assessed using a two-tailed t-test. For the case of non-acceptance of the null hypothesis, it was concluded that the extraction environments are different.

3. Results

The difference in NDF and ADF values was evaluated between the non-pressurized environment and the two temperatures in a pressurized environment (Table 2). In the NDF analysis, we observed a significant difference between the pressurized at 100 °C and non-pressurized environments (P<0.05), and there was an agreement (p- value = 0.59) between the pressurized at 110 °C and non-pressurized environments. In the ADF analysis, there was an agreement between the non-pressurized environment and both pressurized environments at 100 °C (p-value = 0.31) and 110 °C (p-value = 0.87).

Table 2. The analysis of the difference between pressurized and non-pressurized environments in the determination of neutral detergent fiber (NDF) and acid detergent fiber (ADF) at different temperatures

Difference	Average Bias (g/kg dry matter)	SEM	P- Value[2]	Regression coefficient	
				β_1[3]	P-value[4]
NDF					
Pressurized at 100°C - Not Pressurized	28.39	4.17	0.01	-0.032	0.547
Pressurized at 110°C - Not Pressurized	3.83	7.11	0.59	-0.124	0.164
ADF					
Pressurized at 100°C - Not Pressurized	8.70	8.36	0.31	0.081	0.966
Pressurized at 110°C - Not Pressurized	1.81	11.02	0.87	-0.105	0.307

SEM: standard error mean. [1] According to the Student's *t* test. [2] β1 refers to the slope of the

linear regression model y= β0+ β1x. [3] P-value of the predictor variable. NDF: neutral detergent fiber. ADF: acid detergent fiber.

The Table 2 also shows that a potential proportion bias was not observed (P<0.05) in either of the methods, i.e., there was no tendency for differences to be concentrated above or below the mean. Therefore, there was no systematic inclination of the methods to overestimate or underestimate errors. In the evaluation of potential proportion bias, it was observed that the methods had evenly distributed values (P<0.05). The linear regression analysis used the difference between methods as the dependent variable and the mean between the methods as the independent variable (Table 2).

The slope of the regression for both NDF and ADF did not differ from 1 (P> 0.05). The treatment with autoclave for NDF using the temperature of 110 ºC had the lowest coefficient of determination R^2= 0.76. For the ADF analysis, the highest coefficient of determination was for the temperature of 100 ºC (R^2 85%), however, for the temperature of 100 the R^2 was 78% (Table 3).

Table 3. Relationship between neutral detergent fibre (NDF) concentrations (g/kg dry matter) in feed samples as analysed using not pressurized method (X) vs. pressurized (Y)

Comparison	Regression	Slope S.E[b]	R^2
NDF			
Pressurized at 100°C - Not Pressurized	*Y=35.82+0.98X*	0.054	0.90
Pressurized at 110°C - Not Pressurized	*Y=12.04+0.97X*	0.091	0.76
ADF			
Pressurized at 100°C - Not Pressurized	*Y=24.00+0.91X*	0.078	0.85
Pressurized at 110°C - Not Pressurized	*Y=6.40+0.98X*	0.105	0.78

letter lowercase in X from the equation slope different from 1 (P<0.05). [b]Slope standard error where *n* = 38 per treatment from NDE and n= 26 per treatment from ADF.

4. Discussion

The use of pressurized equipment, such as autoclave allows for a greater number of samples to be processed simultaneously. Deschamps (1999), tested a similar approach that we used in this study, using filter bags and a pressurized equipment at 120 °C for 40 minutes. He described that autoclave had a greater productivity since it allows to use 120 samples per operation and generated considerable reagent savings. Cordeiro et al. (2007) compared the contents of NDF and ADF using the conventional method described by Pell and Schofield (1993), which uses digesting blocks/filter crucibles and a temperature of 105 °C for 60 minutes.

There was no difference (p-value = 0.12) between the methods analyzed in this study, which proved the effectiveness to use pressurized equipment's to analyze fiber content, such as autoclave. To find an alternative procedure that was not different from the conventional method, Senger et al. (2008) evaluated different autoclave durations and temperatures in the NDF and ADF analyses. These authors concluded that the autoclave stated at 110 ºC for 40

minutes did not differ (P<0.05) from the conventional method, that uses a temperature at 105 °C for 60 minutes. Additionally, the analysis of forages and concentrates could be performed simultaneously. Gomes et al. (2011) utilized filter bags and compared a pressurized conditiosn with a non-pressurized conditions with effective extraction time of one hour at a temperature of 100ºC, observing differences between two conditions.

The results obtained in this experiment for NDF, showed to be sensitive to a temperature of 100 ° C. However, at a temperature of 110 °C, the NDF proved to be accurate for treatment using non-pressurized equipment. In the FDA tests, all temperatures tested were using a non-pressurized method and were consistent with the non-pressurized physical condition. Although the coefficient of determination, it is lower, both for NDF and FDA, using a temperature of 110ºC, its use is recommended, as it is closer to the differences between the environments. According our findings we can recommend the use of pressurized equipment, such as the autoclave to determine fiber content. Other observations include: (a) the pressurization to avoid accumulation of gas in the bags, which can compromise the action of the detergent, and (b) the nitrogen content of the waste can be analyzed in a subsequent step by the Kjeldhal method (Senger et al., 2008; Gomes et al., 2011).

5. Conclusions

The analysis of fiber in neutral and acid detergent in pressurized condition at a temperature of 110 ºC for 60 min was consistent with the non-pressurized method, just as roughage and concentrated foods can be performed simultaneously.

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