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Reutilization of chemical waste in methodologies for analyses of neutral detergent fiber and acid detergent fiber

Maria do Socorro Nahuz Lourenço[1*], Telma Teresinha Berchielli[2], Ana Paula de Oliveira Sader[2], Euclides Braga Malheiros[3], Juliana Duarte Messana[4] and Roberta Carrilho Canesin[4]

*[1]Departamento de Quimica e Biologia, Universidade Estadual do Maranhão, Cidade Universitária Paulo VI, Cx. Postal 9, 65055-970, Tiririca, São Luís, Maranhão, Brazil. [2]Departamento de Zootecnia, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, São Paulo, Brazil. [3]Departamento de Ciências Exatas, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, São Paulo, Brazil. [4]Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, São Paulo, Brazil. *Author for correspondence. E-mail: snahuz@hotmail.com*

ABSTRACT. The disposal of chemical waste and the precision of analyses of the neutral (NDF) and acid (ADF) detergent fiber contents were evaluated utilizing conventional (Van Soest) and alternative methods of analyses. The recovery of acetone promoted both economic and environmental gains, with a recovery rate of 84.12%. The precision of the analyses was not observed in most of the determinations with reutilization of chemical waste in all the analytical methods tested, in spite of promoting decrease in cost, time invested in the preparation of solutions and the disposal of chemical waste.

Keywords: animal nutrition, chemical analysis, sustainability.

Reutilização de resíduos químicos em metodologias para análises de fibra em detergente neutro e fibra em detergente ácido

RESUMO. Foram avaliados descarte de resíduos químicos em análises dos teores de fibra em detergente neutro (FDN) e fibra em detergente ácido (FDA), utilizando métodos convencional (Van Soest) e alternativos de análises. A recuperação da acetona promoveu ganhos tanto econômico como ambiental, observando-se taxa de recuperação de 84,12%. A precisão das análises não foi observada na maioria das determinações com reutilização de resíduos químicos, em todos os métodos analíticos testados, apesar de promover redução no custo, no tempo investido no preparo das soluções e no descarte de resíduos químicos.

Palavras-chave: nutrição animal, sustentabilidade, análise química.

Introduction

The global question about the damage generated to the planet by the human activities has been an outstanding object in the media. In this context is the chemical waste generated by the industries, chemical laboratories and research institutes (BENDASSOLLI et al., 2005; GERBASE et al., 2006). In the routine of the animal nutrition laboratories we can observe a large-scale use of detergent solutions and acetone during the chemical analyses, especially for the determination of NDF and ADF. After bring used, these products generate chemical waste that is disposed in the environment *in natura*.

The application of the Principles of Analytical Chemistry and Green Chemistry concerning the precision of analyses and reutilization of chemical reagents in the same process they were generated, respectively, is an essential tool for the researchers who perform these analyses and are concerned with the sustainability of the planet (LENARDÃO et al., 2003).

The neutral and acid detergent solutions, utilized in these analytical procedures, are prepared by mixing several chemical reagents, as follows: ethylenediaminetetraacetic acid (EDTA), sodium tetraborate decahydrate ($Na_2B_4O_7$ $10H_2O$), sodium lauryl sulfate (detergent), disodium hydrogen phosphate (Na_2HPO_4) and triethylene glycol ($C_3H_8O_2$), used in the preparation of the first solution; and cetyltrimethylammonium bromide (detergent CTAB) and sulfuric acid, in the preparation of the second solution. The sodium lauryl sulfate is a detergent that has been used as chemical reagent for being an anionic surfactant whose hydrophobic fatty acid chain is attached to a negatively-charged hydrophilic group and because of its detergent properties: wetter, foam booster, emulsifier and solubilizer.

Cetyltrimethylammonium bromide (detergent CTAB) in turn is a cationic surfactant that presents a polar group with positive charge (BRANCINI et al., 1983). Detergent solutions promote the extraction of the particles (SILVA; QUEIRÓZ, 2002) through the phenomenon of adsorption, in which the hydrophobic part, which is also lypophilic, binds to the fat, and the hydrophilic part binds to the water. Thus, the hydrocarbon chain works as a bridge that has one of its ends bound to the fat and another (polar group) to the water.

The disposal of these detergent solutions contributes significantly to environmental pollution, impairing the water quality and leaving it too alkaline, in addition to forming an insoluble layer on the surface of water bodies which reduces the penetration of oxygen from the air into the water, thus decreasing the oxygen available for the respiration of aquatic organisms (BORSATO et al., 1999).

The use of acetone (propanone) and its consequent discard are also part of the laboratory routine in analyses of NDF and ADF. When it is discarded on the soil, acetone is biodegradable and does not bioaccumulate, but it is known to be a toxic product to marine life, especially fish (MOZETO; ZAGATTO, 2006).

Based on the reutilization of chemical reagents (Principle of Green Chemistry) we proposed the validation of the analytical results using alternative methods that prove the possible reutilization of these detergent solutions as well as the reutilization of acetone, without the loss of analytical precision. Given that detergent solutions act as extractors, we proposed the recovery through simple filtering followed by monitoring of the pH in each analysis, for both the original and recovered solutions. The filtering process is usually employed to remove small amounts of solids from large volumes of liquids where neither the solid nor the liquid have a high unit value and when the solid product should not be recovered, while the pH measure is a parameter for controlling the acidity of the medium commonly utilized to monitor physicochemical analyses.

The process of simple distillation was performed in the recovery of acetone with the aid of a rotary evaporator or rotavap (TE-210 – Tecnal), which enabled the separation of the liquid (acetone) from the nonvolatile substances (residues).

The conventional method of Van Soest (VAN SOEST 1963; VAN SOEST et al., 1991) has the precision of results as its main advantage. However, the laboratory routine is quite slow, due to the manual stages, which require a long time of execution, in addition to being a method of elevated costs and that generate chemical waste in significant levels[7]. Several alternative methods have been adopted, but many times without ensuring the precision and accuracy of the analyses, as in the case of the methodologies which utilize autoclaves in substitution of the conventional digester (DESCHAMPS, 1999, PELL; SCHOFIELD, 1993; SENGER et al., 2008). The digestion of the samples is done collectively, and samples can be weighed both in filter crucibles and in small bags. In most of the times, TNT (non-woven textile) bags have been preferentially utilized, so as to reduce the cost of the analysis (CASALI et al., 2009).

The objective of this study was to evaluate the analytic precision of the determinations of the NDF and ADF contents utilizing conventional (Van Soest) and alternative methods of analyses with recovery and reutilization of the chemical waste generated in the process.

Material and methods

The experiment was conducted in the Laboratory of Animal Nutrition of UNESP, Campus Jaboticabal, in São Paulo State, Brazil. Six feedstuffs of current use in animal nutrition were utilized, among which five were roughages: Tifton 85 hay (*Cynodon* spp.), sugarcane (*Saccharum officinarum* L.), corn silage (*Zea mays L.*), xaraes grass (*Brachiaria brizantha cv. Xaraés)*, marandu grass (*Brachiaria brizantha cv. Marandu*) and a protein concentrate, babassu meal *(Orbignya phalerata)*. Except for the babassu meal and the Tifton 85 hay, all the samples were pre-dried according to the procedure described by Silva and Queiroz (2002).

At the preparation of the detergent solutions (neutral and acid), the procedures described by Van Soest et al. (1991) were adopted, without the use of decalin and sodium sulfite, utilizing the enzyme thermostable alpha-amylase (Termamyl) in the determinations of the NDF contents in samples of corn silage. In each method of analysis adopted, 1.5 liters of each solution were prepared separately and at once, aiming at precision of the results and minimization of possible errors in the quantitative determinations. In the procedure of washing the samples, acetone PA was utilized as solvent and all the water utilized in the analytical procedures was deionized.

Both the first step of the analyses, developed with original reagents, and the second step, developed with recovered reagents, utilized four methodologies, determined as follows: CMT – Conventional Method (digestor/Filter crucibles),

AMT_1 – Alternative Method 1 (autoclave/ANKOM), AMT_2 – Alternative Method 2 (autoclave/TNT) and AMT_3 – Alternative Method 3 (autoclave/Filter crucibles).

For the development of the analyses with recovered reagents, both the solutions of neutral and acid detergent were filtered, always after their initial use. The pH value of the solutions was also monitored in each analysis, both for the original and for the recovered solutions; there was no significant variation in the pH values.

All the acetone utilized during the process of washing the samples in the analyses, utilizing the original detergent solutions, was conditioned in amber glass bottle from the same manufacturer, and then recovered in rotary evaporator.

The conventional method followed the procedures described by Van Soest et al. (1991). In the determination in the non-sequential form, about half a gram of the sample was dissolved in 100 mL acid or neutral detergent solution, followed by digestion. In the filtering step, the detergent solution reused was discarded and the samples were transferred to glass filter crucibles with sintered plate.

The acetone recovered in the last washing was used twice. The average volume of acetone utilized in each washing was of 30 mL per sample, discarded right after the process. Next, samples were dried at 105ºC and weighed.

The analytical results were obtained through the formula defined in (1), in the determination of the NDF and ADF contents:

$$\% \text{ NDF or } \% \text{ ADF} = \frac{(WC - T)}{WS} \times 100 \quad (1)$$

In which: WS = weight of the dry matter of the sample in grams; WC = weight, in grams, of the crucible plus residue of the detergent fiber after digestion and drying; T = tare (initial weight) of the crucible in grams.

Afterwards, the calcination step was conducted in a muffle furnace at 550ºC, for three hours.

The analyses of determination of the NDF contents in the sequential form followed the same analytical procedures previously described (VAN SOEST et al., 1991). The crucibles with the residues from the NDF analyses were reutilized in the sequential analysis of ADF, by coupling each one to beakers containing 100 mL recovered acid detergent solution, and then subjected to the same stages of the non-sequential analyses (VAN SOEST et al., 1991).

In all the analytical determinations, the detergent solutions and acetone were discarded after the analyses with recovered reagents.

In the analyses developed through alternative methods 1 and 2, the samples were conditioned in small bags (KOMAREC, 1993; SENGER et al., 2008) and the digestion process was done in an autoclave (DESCHAMPS, 1999; PELL; SCHOFIELD, 1993; SENGER et al., 2008). The time and temperature utilized in the autoclave were adjusted according to the best performance observed by Senger et al. (2008).

The ANKOM bags (alternative method 1) were obtained ready for use. The TNT (non-woven textile) ones, in turn, were conditioned (SENGER et al., 2008) manually with the same dimensions as the ANKOM bags, identified, sealed and weighed on a digital analytical scale.

During digestion, in each replication, 19 sealed bags (blank and triplicates of the six feedstuffs) were immersed in 600 mL recovered detergent solution.

The washing step occurred collectively: bags were subjected to three rinses with warm deionized water and then drained and immersed in recovered acetone. Bags were dried, chilled, weighed and calcinated. For the calculation of the NDF and ADF concentrations, the formula defined in (2) was utilized.

$$\% \text{ NDF or } \% \text{ ADF} = \frac{[WE - (T \times B)]}{WS} \times 100 \quad (2)$$

In which: WS = dry matter weight of the sample in grams; WE = weight of the bag plus residue from the detergent fiber after digestion and drying in grams; T = tare (initial weight) of the bag in grams; B = blank value (final weight of the bag after drying/initial weight of the bag), in grams.

In the sequential form, the relative determinations of the NDF contents were developed similarly to what was reported in the non-sequential analyses, excluding the calcination step. The ADF analyses started with the reutilization of the bags employed in NDF. At the end of the analysis, both the detergent solution and the acetone reused were discarded.

The combination of weighing the samples in glass filter crucibles with sintered porous plate no. 02 with the autoclave digestion (DESCHAMPS, 1999, PELL; SCHOFIELD, 1993; SENGER et al., 2008) is the basis of alternative method 3. The time and temperature utilized in the autoclave were the same utilized in the alternative methods which utilized bags in the conditioning of samples (SENGER et al., 2008).

In the non-sequential analyses, about half a gram of each sample was weighed in triplicate, in filter crucibles, conditioned in individual plastic beakers. About 600 mL recovered detergent solution were distributed in the 18 beakers for the digestion of samples. The washing of the crucibles was done through rinsing with warm deionized water and recovered acetone.

The steps of drying, chilling, weighing and calcination of the samples were conducted similarly. The NDF and ADF contents were calculated by the difference between the tare of the crucible and its weight plus the NDF or ADF residue after digestion and drying, utilizing the formula defined in (1).

The same practical procedure in the determinations of non-sequential NDF is the one adopted in the sequential determinations, with the elimination of the calcination step. At the determination of the ADF contents, the filter crucibles from the NDF analysis were reutilized.

The design adopted was completely randomized, in a 2 x 4 x 2 factorial arrangement (2 utilizations, 4 methodologies and 2 forms of analysis).

The statistical model utilized was:

$$Y_{ijkl} = \mu + UT_i + MT_j + FM_k + (UT*MT)_{ij} + (UT*FM)_{ik} + \\ + (MT*FM)_{jk} + (UT*MT*FM)_{ijk} + \varepsilon_{ijkl}$$

in which: Y_{ijkl} NDF and ADF contents; μ = mean overall effect; UT_i = effect of utilization i; MT_j = effect of method j; FM_k= effect of form k; $(UT\star MT)_{ij}$ = effect of the interaction between utilization i and method j; $(UT\star FM)_{ik}$ = effect of the interaction between utilization i and form k; $(MT\star FM)_{jk}$ = effect of the interaction between method j and form k; $(UT\star MT\star FM)_{ijk}$ = effect of the interaction between utilization i, method j and form k; ε_{ijkl} = residual error.

The data were subjected to analysis of variance, through command General Linear Models (GLM) of software Statistical Analysis System (SAS 9.1®). Means were compared by the test of Dunnet test, having the conventional method as "control" (α = 5%).

Results and discussion

The recovery of the detergent solutions minimized the expenditure of chemical reagents and the discard of the waste generated in the analyses. In the development of the 1st step of the analyses (1440 analyses), utilizing the original detergent solutions, it was necessary to prepare 60 liters of each detergent solution (neutral and acid). After the development of these analyses, the 120 liters of detergent solutions were recovered and reutilized in the conduction of more than 1440 analyses (2nd step). Thus, the expenses with chemical reagents were reduced in 50%. After surveying the market values of the chemical reagents necessary for the preparation of the detergent solutions, in Brazilian reals, an economy of about R$147.90 was observed in the analyses of NDF and ADF, which utilized recovered reagents. It is worth stressing that the costs referring to glassware, equipment, electric power and labor force were not inserted in this study.

Concerning the environmental conservation, the decrease of 50% in the discard of the detergent solutions *in natura* contributed significantly.

The mean volumes of the acetone spent in each methodology utilized were: conventional method – 520 mL, alternative method 1 – 250 mL, alternative method 2 – 260 mL and alternative method 3 – 510 mL.

In the first step of the analyses, developed with original acetone, 30.80 liters of acetone were necessary for the determinations of NDF and ADF, in the four methods utilized. It was observed that the mean volume of acetone recovered in all the process reached 420.6 mL, which corresponds to 84.12% of t he recovery rate. After surveying the market value of the 30.80 liters of acetone at R$ 355.74, it was possible to verify that the inclusion of the step of recovery of acetone in the analytical procedures enabled a gain of R$ 299.25. As for the environmental gain, it is undeniable that the reduction of 84.12% in the discard considerably minimized the discard of this solvent in the environment.

The F values and the coefficients of variation (CV) obtained in the analysis of variance of the NDF contents in the feedstuffs studied are presented in Table 1.

There was a significant difference ($p < 0.01$) in the utilization and in the methods in all the feedstuffs utilized. Concerning the form adopted (non-sequential or sequential), the latter differed significantly ($p < 0.01$) only for babassu meal and xaraes grass.

There was significant difference ($p < 0.01$) between the utilization and the method of analysis (UT⋆MT) in all the feedstuffs. Regarding the interactions utilization and form

(UT⋆FM) and utilization, method and analytical form (UT⋆MT⋆FM), the latter was not significant ($p \geq 0.05$) in all of the feedstuffs studied. The method and form interaction (MT⋆FM) was significant ($p < 0.05$) only for babassu meal.

The means concerning the NDF contents in the six feedstuffs studied, considering the utilization and the analytical methods, are shown in Table 2.

Table 1. F values with respective probabilities p and CV obtained in the analysis of variance of the NDF content in the feedstuffs studied.

STATISTICS	FEEDSTUUFS					
	Tifton hay	Sugarcane	Corn silage	Babassu meal	Xaraes grass	Marandu grass
F p/ UT[(1)]	55.74($p < 0.01$)	19.31($p < 0.01$)	47.63($p < 0.01$)	25.51($p < 0.01$)	22.17($p < 0.01$)	21.77($p < 0.01$)
F p/ MT[(2)]	81.05($p < 0.01$)	184.14($p < 0.01$)	75.63 $p < 0.01$)	24.43($p < 0.01$)	30.10($p < 0.01$)	27.30($p < 0.01$)
F p/ FM[(3)]	2.23($p > 0.10$)	1.10($p > 0.10$)	0.21($p > 0.10$)	13.38($p < 0.01$)	14.21($p < 0.01$)	3.25($p = 0.05$)
F p/ UT ⋆ MT	25.46($p < 0.01$)	9.62($p < 0.01$)	7.75($p < 0.01$)	7.25($p < 0.01$)	25.78($p < 0.01$)	18.90($p < 0.01$)
F p/ UT ⋆ FM	0.00($p > 0.10$)	0.18($p > 0.10$)	2.50($p > 0.10$)	5.44($p = 0.05$)	1.95($p > 0.10$)	1.22($p > 0.10$)
F p/ MT⋆FM	1.33($p > 0.10$)	2.12($p = 0.05$)	2.41($p = 0.05$)	18.35($p < 0.01$)	1.78($p > 0.10$)	2.62($p = 0.05$)
F p/ UT⋆ MT⋆FM	2.29($p > 0.10$)	0.45($p > 0.10$)	0.42($p > 0.10$)	0.67($p > 0.10$)	1.04($p > 0.10$)	0.50($p > 0.10$)
CV	2.01	2.83	7.64	5.92	2.49	2.34

(1) utilization; (2) method; (3) form.

Table 2. Means obtained in the analysis of variance in the feedstuffs, utilizations and methods studied in the evaluation of the NDF contents.

FEEDSTUFF	UTILIZATION	METHODS				OVERALL
		CMT [(1)]	AMT_1[(2)]	AMT_2[(3)]	AMT_3[(4)]	
	OS [(5)]	8.98b(II)	78.84a	79.11a	81.81⋆b	79.62
TIFTON HAY	RS [(6)]	83.22a	79.05⋆(I)a	78.70⋆a	84.05a	81.19
	OVERALL	81.10	78.94	78.91	82.95	
	SO	53.48b	50.23⋆a	49.73⋆a	53.68b	51.78
SUGARCANE	SR	55.98a	50.29⋆a	49.65⋆a	54.55⋆a	52.62
	OVERALL	54.73	50.26	49.69	54.12	
	SO	44.90b	55.10⋆b	48.21⋆b	48.53⋆a	49.20
CORN SILAGE	SR	49.87a	59.01⋆a	53.85⋆a	47.92a	52.66
	OVERALL	47.39	57.06	51.03	48.23	
	SO	67.79b	63.88⋆b	67.42a	67.69b	66.71
BABASSU MEAL	SR	74.06a	65.95⋆a	66.87⋆a	70.52⋆a	69.35
	OVERALL	70.95	64.91	67.14	69.31	
	SO	68.64b	71.81⋆a	69.98⋆a	71.39⋆b	70.43
XARAES GRASS	SR	72.87a	71.96a	68.68⋆b	72.73a	71.57
	OVERALL	70.72	71.89	69.34	72.09	
	SO	72.51b	76.37⋆a	74.36⋆a	74.93⋆a	74.70
MARANDU GRASS	SR	76.18a	76.02a	73.77⋆a	75.55a	75.73
	OVERALL	74.34	76.20	74.07	76.31	

(I) Means of the methods (AMT1),(AMT2) and (AMT3) (rows) followed by (⋆) differ from control by the Dunnett test ($\alpha = 0.05$). (II) Means of the utilizations (OS) and (RS) (columns) followed by the same letter do not differ by the F test ($\alpha = 0.05$); (1) CMT – Conventional method; (2) AMT1 – Alternative method (Autoclave/ANKOM); (3) AMT2 – Alternative method (Autoclave/TNT); (4) AMT3 – Alternative method (Autoclave/Filter crucibles); (5) OS – Utilization of the original solution; (6) RS – Utilization of the recovered solution.

Considering that there was significant interaction ($p < 0.01$) between the utilization and the method (UT⋆MT) in all the feedstuffs, different results were obtained concerning the precision of the alternative methods when compared with the conventional method and concerning the utilization of the detergent solutions (original and retrieved) in the analyses.

The results from the NDF content obtained for Tifton hay indicated that AMT_3 was the only one to differ ($p < 0.05$) from CMT. There were also alterations when the alternative methods were compared with the conventional one, because AMT_1 and AMT_2 differed ($p < 0.05$) from CMT. It could also be observed that in CMT and AMT_3, the utilizations of the detergent solutions (original and recovered) differed ($p < 0.05$), whereas in AMT_1 and AMT_2, the utilizations of the detergent solutions (OS and RS) did not differ ($p > 0.05$), but there was loss of the analytical precision of the methods in relation to CMT, so the analysis with reutilization of these solutions was not indicated.

The data on sugarcane showed that at the first utilization of the reagents (Original Solution), the means obtained in AMT_1 and AMT_2 differed ($p < 0.05$) from CMT, while at the second utilization (Recovered Solution), all the alternative methods differed ($p < 0.05$) from CMT. There was also difference ($p < 0.05$) in CMT and AMT_3, while in AMT_1 and AMT_2, there was loss of analytical precision when compared with CMT and the utilizations of the detergent solutions (OS and RS) did not differ ($p > 0.05$), so the analytical procedures with the reutilization of the detergent solutions were not recommended.

For corn silage, significant difference ($p < 0.05$) of all the alternative methods could be verified in relation to CMT in the analyses with original detergent solution (OS), and AMT_1 and AMT_2 differed ($p < 0.05$) from CMT in the analyses with recovered detergent solution (RS). Regarding the utilizations of the detergent solutions (OS and RS), the latter differed ($p < 0.05$) in CMT, AMT_1 and AMT_2, whereas in AMT_2, the analyses developed with recovered detergent solution (RS) did not differ ($p > 0.05$) from the analyses with original detergent solution (OS), so no loss of precision of this method in comparison with the conventional method (CMT) was observed. Therefore, the development of analyses utilizing detergent solution recovered by AMT_3 becomes viable.

In babassu meal, it could be observed in the first utilization (OS) that AMT_1 differed ($p < 0.05$) from CMT. In the second utilization (RS), all the alternative methods differed ($p < 0.05$) from CMT. The utilizations of detergent solutions (OS and RS) differed ($p < 0.05$) in CMT, AMT_1 and AMT_2. Despite differing ($p < 0.05$) as for its precision in relation to CMT, AMT_2 did not demonstrate difference ($p > 0.05$) concerning the reutilization of detergent solutions (OS and RS). Due to the loss of precision, it is not indicated to reutilize detergent solutions as analytical reagents.

In the analyses performed on xaraes Grass, with original detergent solution (OS), all the alternative methods differed ($p < 0.05$) from CMT. With the reutilization of the detergent solution (RS), only AMT_2 differed ($p < 0.05$) from CMT. The utilizations of the detergent solutions (OS and RS) differed ($p < 0.05$) from CMT, AMT_2 and AMT_3. Thus, the analyses with recovered detergent solutions can be developed through AMT_1 for no loss is observed in the analytical precision of this method. Agrupe resultados iguais e apresente-os de forma conjunta para permitir leitura mais objetiva.

As for marandu Grass, it could be observed that in the first utilization (OS), all the alternative methods differed ($p < 0.05$) from CMT. In the second utilization (RS), only AMT_2 differed ($p < 0.05$) from CMT. Concerning the utilizations of the detergent solutions (OS and RS), difference was observed ($p < 0.05$) only in CMT. No difference was observed ($p > 0.05$) in any of the alternative methods between the utilizations of detergent solutions (OS and RS), but only in methods AMT_1 and AMT_3 there was no loss in analytical precision in comparison with the conventional method (CMT). Therefore, analyses with recovered detergent solution can be developed through AMT_1 and AMT_3.

Table 3 presents the F values and coefficients of variation (CV) obtained at the analysis of variance of the ADF contents, in the feedstuffs studied.

At the determinations of the ADF contents, there was significant difference ($p < 0.01$) in the two types of utilization of the detergent solution and also in the four methods utilized ($p < 0.01$) in the analytical determinations in all the feedstuffs studied. Table 4. F values with respective probabilities p and CV obtained in the analysis of variance of the ADF contents in the feedstuffs studied.

Table 3. F values with respective probabilities p and CV obtained in the analysis of variance of the ADF content in the feedstuffs studied.

STATISTICS	FEEDSTUFF					
	Tifton hay	Sugarcane	Corn silage	Babassu meal	Xaraes grass	Marandu grass
F p/ UT[(1)]	87.52($p < 0.01$)	47.44($p < 0.01$)	42.04($p < 0.01$)	48.41($p < 0.01$)	72.00($p < 0.01$)	60.23($p < 0.01$)
F p/ MT[(2)]	130.02($p < 0.01$)	52.53($p < 0.01$)	74.35($p < 0.01$)	58.59($p < 0.01$)	87.84($p < 0.01$)	77.11($p < 0.01$)
F p/ FM[(3)]	5.17($p = 0.05$)	0.16 ($p > 0.10$)	0.98($p > 0.10$)	2.03($p > 0.10$)	8.82 ($p < 0.01$)	4.48 ($p = 0.05$)
*F p/ UT * MT*	71.01($p < 0.01$)	31.72($p < 0.01$)	32.99($p < 0.01$)	16.61($p < 0.01$)	43.31($p < 0.01$)	32.29($p < 0.01$)
*F p/ UT * FM*	2.94($p = 0.05$)	1.23($p > 0.10$)	0.06 ($p > 0.10$)	2.23($p > 0.10$)	0.04($p > 0.10$)	0.01($p > 0.10$)
*F p/ MT*FM*	29.25($p < 0.01$)	9.47($p < 0.01$)	13.12($p < 0.01$)	11.54($p < 0.01$)	7.57($p < 0.01$)	5.93($p < 0.01$)
*F p/ UT*MT*FM*	10.24($p < 0.01$)	1.75($p > 0.10$)	5.53($p < 0.01$)	0.82($p > 0.10$)	0.42($p > 0.10$)	0.06($p > 0.10$)
CV	8.65	9.23	8.56	11.75	10.21	11.42

(1) utilization; (2) method; (3) form.

Table 4. Means obtained in the analysis of variance in the feedstuffs, utilizations and methods studied in the evaluation of the ADF contents.

FEEDSTUFF	UTILIZATION	METHODS				OVERALL
		CMT [(1)]	AMT_1[(2)]	AMT_2[(3)]	AMT_3[(4)]	
	OS [(5)]	40.05[b(II)]	45.61*[(I)a]	64.29*[a]	45.77*[a]	48.12
TIFTON HAY	RS [(6)]	42.69[a]	42.35[b]	46.36*[b]	44.79*[a]	44.05
	OVERALL	41.37	43.98	54.33	45.28	
	SO	31.03[b]	33.81*[a]	42.90*[a]	34.44*[a]	35.54
SUGARCANE	SR	33.11[a]	30.94*[b]	33.98[b]	32.93[a]	32.74
	OVERALL	32.07	32.37	38.44	33.69	
	SO	26.10[b]	30.28*[a]	37.76*[a]	30.81*[a]	31.11
CORN SILAGE	SR	28.30[a]	28.03[b]	30.52*[b]	29.38[b]	29.06
	OVERALL	27.20	29.15	34.05	30.07	
	SO	40.30[a]	42.33[a]	56.84*[a]	44.77*[a]	46.06
BABASSU MEAL	SR	40.35[a]	37.24*[b]	44.78*[b]	43.38*[a]	41.44
	OVERALL	40.32	39.78	50.81	44.07	
	SO	36.93[a]	44.06*[a]	57.65*[a]	41.26*[a]	44.97
XARAES GRASS	SR	38.24[a]	40.22*[b]	42.26*[b]	40.13*[a]	40.21
	OVERALL	37.58	42.13	49.95	40.70	
	SO	39.06[a]	46.42*[a]	60.97*[a]	43.24*[a]	47.42
MARANDU GRASS	SR	39.40[a]	42.91*[b]	44.81*[b]	42.03*[a]	42.29
	OVERALL	39.23	44.67	52.89	42.64	

(I) Means of the methods (AMT_1),(AMT_2) and (AMT_3) (rows) followed by (*) differ from control by the Dunnett test ($\alpha = 0.05$); (II) Means of the utilizations (OS) and (RS) (columns) followed by the same letter do not differ by the F test ($\alpha = 0.05$); (1) CMT – Conventional method; (2) AMT1 – Alternative method (Autoclave/ANKOM); (3) AMT2 – Alternative method (Autoclave/TNT); (4) AMT3 – Alternative method (Autoclave/Filter crucibles); (5) OS – Utilization of the original solution; (6) RS – Utilization of the recovered solution.

As to the form adopted (non-sequential or sequential), there was no significant difference ($p \geq 0.05$) in any of the feedstuffs, except for xaraes grass ($p < 0.01$).

As for the interactions, the differences were significant ($p < 0.01$) for utilization and method (UT*MT) and for method and form (MT*FM), in all feedstuffs. No significant difference ($p \geq 0.05$) in the interaction between utilization and form (UT*FM) was observed in any of the feedstuffs. The interaction between utilization, method and form (UT*MT*FM) was significant ($p < 0.01$) in the samples of Tifton hay and corn silage.

Table 4 are presented the means for the ADF contents in all the feedstuffs studied, considering the utilization of the reagent and the method of analysis.

In the Tifton sample, it could be observed that in the utilization of original detergent solution (OS), all the alternative methods differed ($p < 0.05$) from CMT. In the utilization of recovered detergent solution (RS), in turn, AMT_2 and AMT_3 differed ($p < 0.05$) from CMT. In CMT, AMT_1 and AMT_2, differences ($p > 0.05$) between the utilizations of detergent solutions (OS and RS) were observed.

In AMT_3, however, there was loss of analytical precision in relation to the conventional method (CMT) and the utilizations of detergent solutions did not differ ($p > 0.05$). Therefore, analyses with recovered detergent solution are not recommended.

At the analytical determinations in sugarcane, it could be observed that in the first utilization (OS), all the alternative methods differed ($p < 0.05$) from CMT. Different behavior was observed after the reutilization of the detergent solution (RS), since only AMT_1 differed ($p < 0.05$) from CMT.

There was difference ($p < 0.05$) between the utilizations of the detergent solutions (OS and RS) in CMT, AMT_1 and AMT_2. Thus, the analyses with reutilization of detergent solutions are viable through AMT_3 because of the absence of loss in analytical precision.

In the corn silage, it could be verified that in the analyses with original detergent solution (OS), all the alternative methods were different ($p < 0.05$) from CMT. When the detergent solution (RS) was reutilized, only AMT_2 differed ($p < 0.05$) from CMT. In all the methods, there was difference ($p < 0.05$) between the utilizations of the detergent solutions (OS and RS), so their reutilization is not recommended by any method studied, in this feedstuff.

The results obtained in the sample of babassu meal revealed that, at the first utilization (OS), AMT_2 and AMT_3 differed ($p < 0.05$) from CMT. At the second utilization (RS), all the alternative methods presented significant difference ($p < 0.05$) in relation to CMT. The analyses utilizing original solution (OS) differed from the analyses with recovered solution (RS) in AMT_1 and AMT_2, whereas in AMT_3 there was loss in analytical precision, and no difference was observed ($p > 0.05$) between the utilizations of the detergent solutions (OS and RS), so the analysis with reutilization of these solutions is not recommended.

The same behavior in the methods was observed with xaraes and marandu grasses, for all the alternative methods differed ($p < 0.05$) from CMT in the two utilizations of the detergent solutions (OS and RS). Identical behavior was also observed in AMT_1 and AMT_2, in which the two utilizations of the detergent solutions (OS and RS) differed ($p < 0.05$). In AMT_3, there was loss in analytical precision and no difference was observed ($p < 0.05$) between the utilizations of detergent solutions (OS and RS). Therefore, the analysis with reutilization of these solutions is not recommended.

In all the determinations of the ADF contents in the six feedstuff studied, the greatest means observed were always obtained through AMT_2, which is possibly related to the non-uniform porosity of the TNT (DESCHAMPS, 1999) (non-woven textile) fabric.

Only one study describing the report of the National Meeting on Laboratory Methodologies (MET) concerning the use of detergent solutions in analyses of the NDF and ADF contents was found in the literature. This meeting was held by the group of applied instrumental analysis "Pecuária Sudeste", of the Chemistry Institute of São Carlos. The authors cite that the samples utilized were from forages and concentrate feeds; the study only mentions that no significant differences ($p < 0.05$) were observed between the results found in the three extractions and also between the contents obtained in the first and the other extractions. There is also no information as to the application of any procedure for the recovery of the detergent solutions before they were utilized. Thus, there are no reports of the NDF and ADF contents obtained in analyses developed with reutilization of detergent solutions.

The results obtained in this study concerning the possibility of reutilizing detergent solutions in analyses of the NDF and ADF contents, through the analytical methods studied in the six feedstuffs analyzed, indicated loss of analytical precision of the analyses. This behavior can be related to the saturation of the detergent solutions. The neutral detergent solution solubilizes the cell content and pectin whereas the acid detergent solution solubilizes the cell content and also

the fraction hemicellulose. All these soluble components in detergent solutions form a set of diverse macromolecules that differ according to size, mass, shape, density and reactivity. Thus, after the digestion of samples, the detergent solutions possess a set of dissolved macromolecules that are diversified in their interior, which indicates that it is possible that there was loss of solubilization of the detergent solution since it reached the saturation point for the molecules in question.

Conclusion

The inclusion of the stages of recovery and reutilization of the solvent acetone and of the detergent solutions (neutral and acid) in the analytical procedures promoted reduction in the cost necessary for the development of the analyses, thus enabling, in particular, reduction in the disposal of chemical waste in the environment. However, the precision of the analyses was not observed in the determinations with reutilization of acid and neutral detergent solutions in any the analytical methods tested, so the association between the methodology and the feedstuff analyzed must be better observed.

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