Revista Brasileira de Zootecnia
© 2012 Sociedade Brasileira de Zootecnia
ISSN 1806-9290
www.sbz.org.br

R. Bras. Zootec., v.41, n.1, p.225-231, 2012

Technical Note

Evaluation of sodium sulfite and protein correction in analyses of fibrous compounds in tropical forages[1]

Daiany Íris Gomes[2], Edenio Detmann[3], Sebastião de Campos Valadares Filho[3], Rafael Mezzomo[2], Natália Krish de Paiva Souza[2], Augusto César de Queiroz[3], Kelly da Silva Coutinho Detmann[4]

[1] This paper had financial support from CNPq, FAPEMIG (PPM) and INCT - Ciência Animal.
[2] Programa de Pós-Graduação em Zootecnia, UFV, Viçosa-MG.
[3] Departamento de Zootecnia, UFV, Viçosa-MG. Researcher 1A from CNPq and researcher from INCT-Ciência Animal.
[4] Departamento de Biologia Vegetal, UFV, Viçosa-MG.

ABSTRACT - The objective of this study was to evaluate the contents of fibrous compounds in tropical grasses and legumes according to utilization of sodium sulfite in the neutral detergent solution or using a procedure for contaminant protein correction. Samples of ten grasses and ten legumes were used. The contents of neutral detergent fiber were decreased when sodium sulfite was used; however, more prominent reductions were verified in legumes. Sodium sulfite decreased the acid detergent fiber content in both forage groups. The contents of neutral and acid detergent insoluble protein and lignin were reduced by sodium sulfite in legumes, but no effect was observed in grasses with regard to these variables. The decrease in fiber contents in legumes could be explained by the solubilization of lignin and decrease in insoluble nitrogen. However, the decreases in fiber in grasses could not be solely explained by the decrease in contaminant protein and solubilization of lignin, and loss of other fibrous compounds probably occurred. The utilization of sodium sulfite compromises the accuracy of the estimates of fibrous compounds contents in tropical forages. The precision of the estimates were not relevantly increased by sodium sulfite. The correction of insoluble fibrous compounds for protein is suggested instead of using sodium sulfite because there are no modifications on neutral detergent solution or undesirable solubilization of fibrous compounds.

Key Words: acid detergent fiber, acid detergent insoluble protein, lignin, neutral detergent fiber, neutral detergent insoluble protein

Introduction

The content and the diversity of insoluble fibrous compounds present a main role in the evaluation of tropical forages in ruminant nutrition because they directly influence several characteristics of the diets, such as the rumen fill effect and the energy and nitrogenous compounds availability (Detmann et al., 2008). Nevertheless, the concepts of fiber applied in animal nutrition are essentially defined by the analytical method. Thereby, any variation in the analytical procedures may potentially produce results that will differ from the original proposition. As a consequence, this can compromise the rusticity and accuracy of the method and the comparison of results obtained in different experimental or productive conditions (Detmann, 2010).

Sodium sulfite has been suggested as a component of the solution used to analyze the neutral detergent insoluble fiber (NDF). The addition of sodium sulfite to neutral detergent solution was proposed aiming at the decrease of the contamination with protein in the gravimetrically measured residue (Mertens, 2002). However, its utilization was also associated with the undesirable solubilization of fibrous compounds, such as lignin (Hartley, 1972; Van Soest et al., 1991; Dorleans et al., 1996). From this, it could be assumed that sodium sulfite utilization in NDF analysis is controversial.

Nitrogenous compounds are supposed to be the main contaminant of gravimetrically measured fiber (Van Soest, 1994; Detmann & Valadares Filho, 2010). To avoid biases on insoluble fiber and non-fibrous carbohydrates estimates, it has been suggested that crude residue of NDF be corrected for contaminant protein and ash (Detmann & Valadares Filho, 2010; Detmann et al., 2010). Particularly, protein correction is an uncomplicated procedure because it is based on routine analyses such as the Kjeldahl

Received July 25, 2011 and accepted October 13, 2011.
Corresponding author: detmann@ufv.br

technique. When compared with sodium sulfite, protein correction could present two probable analytical advantages. First, the analysis of crude residue of fiber for protein content would not promote the undesirable solubilization of any fibrous compounds as highlighted before for sodium sulfite utilization. Second, protein correction allows taking into account the total contaminant nitrogen in the fiber residue whereas sodium sulfite decreases, but not eliminate such contamination. However, an accurate comparison of the estimates of fibrous compounds considering the use of sodium sulfite or protein correction in tropical forages still needs to be established.

Thereby, the objective of this study was to evaluate the effects of using sodium sulfite in neutral detergent solution and protein correction on estimates of insoluble fibrous compounds in tropical grasses and legumes.

Material and Methods

The experiment was conducted at the Laboratório de Nutrição Animal of the Departamento de Zootecnia of the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

Ten grasses (*Pennisetum purpureum*, *Brachiaria decumbens*, *Panicum repens*, *Brachiaria humidicula*, *Andropogon gayanus*, *Panicum maximum* cv. Aruana, *Panicum maximum* cv. Mombaça, *Brachiaria brizantha* cv. Xaraés, tifton 85 bermudagrass (*Cynodon* sp.) and *Panicum maximum* cv. Massai) and ten legumes (*Arachis pintoi*, *Medicago sativa*, *Leucaena leucocephala*, *Galactia striata*, *Dolichos lablab*, *Centrosema pubescens*, *Glycine weghtii*, *Gliricidia sepium*, *Stylosantes guianenensis* and *Cajanus cajan*) were evaluated. The forages were cultivated in 2- × 4-m plots. All samples were cut at ground level in December 2008. The plants had approximately 45 days of regrowth.

Samples were oven-dried at 60°C and processed in a knife mill (1-mm). After that, the dry matter (DM, index no. 934.01) contents of the samples were analyzed according to the methods of the AOAC (1990).

For the NDF analysis, twelve aliquots of each forage (1 g) were put in 120-mL polyethylene pots, and 100 mL of neutral detergent were added (Mertens, 2002). Sodium sulfite was added in six pots (0.5 g/100 mL). Heat-stable alpha amylase was used in all aliquots (Termamyl 2X, Novozymes). After sealing, pots were autoclaved at 105 ºC for 1 h (Pell & Schofield, 1993). The neutral-detergent-insoluble residue was retained by vacuum filtration in filter crucibles, washed sequentially with hot distilled water and acetone, oven-dried at 105 ºC for 16 h, put in a dissecator and weighed. Four aliquots (two with sodium sulfite and two without sodium sulfite) were separated and analyzed for neutral detergent insoluble protein (NDIP) contents (Licitra et al., 1996).

The eight crucibles remaining were conditioned in 120-mL polyethylene pots, and 100 mL of acid detergent were added (Van Soest & Robertson, 1985). After sealing, the pots were autoclaved at 105 ºC for 1 h (Pell & Schofield, 1993). The acid-detergent-insoluble residue was retained by vacuum filtration in the filter crucibles, washed sequentially with hot distilled water and acetone, and oven-dried and weighed as described before. Similarly, four aliquots (two with sodium sulfite and two without sodium sulfite) were separated and analyzed regarding acid detergent insoluble protein (ADIP) contents (Licitra et al., 1996).

Both NDIP and ADIP contents were obtained by multiplying the contents of insoluble nitrogen in NDF and ADF by 6.25 (Licitra et al., 1996).

The four crucibles remaining were used to quantify lignin contents. The filter crucibles were put in 120-mL polyethylene pots, and 30 mL of 12 M sulfuric acid were added and homogenized with a glass rod. Subsequently, 12 M sulfuric acid was added up to half the height of the crucible, and the samples were homogenized after 30 minutes (Van Soest & Robertson, 1985). After 3 h, the crucibles were subjected to vacuum filtration and washed with hot distilled water to completely remove the acid. The material was oven-dried at 105 ºC for 16 h and then weighed to obtain the mass of the residue composed of lignin and minerals. Then, the crucibles were transferred to a muffle furnace at 500 °C, where they remained for 3 h. They were weighed again, and the mass of lignin was calculated by the weight loss after incineration.

The contents of NDF, NDF corrected for protein (NDFp), acid detergent fiber (ADF), ADF corrected for protein (ADFp), lignin, NDIP, and ADIP obtained with or without using sodium sulfite were directly compared between the different species groups (grasses or legumes) according to the model:

$$Y_{ijk} = \mu + G_i + S_{(i)j} + M_k + GM_{ik} + \varepsilon_{ijk} \quad (1),$$

where μ = general constant; G_i = effect of the species group i (grass or legume; fixed effect); $S_{(i)j}$ = effect of species j nested within group i (random effect); M_k = effect of the method of analysis k (with or without sodium sulfite; fixed effect); GM_{ik} = interaction effect of the species group i and the method k; and ε_{ijk} = random error. The mean square of $S_{(i)j}$ was used to test the species group effect. On the other hand, the sodium sulfite and interaction effects were evaluated using random error mean square.

Some relationships between variables evaluated with or without sodium sulfite were evaluated by adjusting a simple linear regression equation (Table 1). These statistical

Table 1 - Description of variables evaluated in the equality test using linear regression analysis

	X		Y	
Relationship	Variable	Description	Variable	Description
1	Δ_{NDF}	Difference between NDF contents estimated with or without sodium sulfite	Δ_{N}	Fractions of NDIP and lignin solubilized by sodium sulfite
2	Δ_{ADF}	Difference between ADF contents estimated with or without sodium sulfite	Δ_{A}	Fractions of ADIP and lignin solubilized by sodium sulfite

NDF = neutral detergent fiber; ADF = acid detergent fiber; NDIP = neutral detergent insoluble protein; ADIP = acid detergent insoluble protein.

analyses were conducted independently for grasses and legumes under the null hypothesis:

$H_0 : \beta_0 = 0$ and $\beta_1 = 1$ (2).

Variables X and Y were considered to be similar when the null hypothesis was not rejected.

All statistical procedures were performed using the PROC MIXED and the PROC REG of SAS (Statistical Analysis System, version 9.1) adopting $\alpha = 0.05$.

Results

Except for ADF and ADFp contents (P>0.05), there was interaction (P<0.05) of sodium sulfite utilization and forage group on the other variables (Table 2).

The ADF and ADFp contents were decreased (P<0.05) by using sodium sulfite (Table 2). The NDF and NDFp contents were decreased (P<0.05) by using sodium sulfite in both forage groups. However, the decrease was more prominent in legumes compared with grasses (Table 2). The NDIP, ADIP and lignin contents were decreased by sodium sulfite in legume samples (P<0.05), but such effect was not observed (P>0.05) in grass samples (Table 2).

The decreases in NDF and ADF contents caused by sodium sulfite in legumes were found to be equivalent (P>0.05) to the sum of solubilized lignin and NDIP, for NDF, and solubilized lignin and ADIP, for ADF (Table 3). As there was no decrease in lignin and insoluble nitrogen in grasses

Table 2 - Estimates of fibrous compounds contents according to species groups and utilization of sodium sulfite

	Grasses		Legumes				P Value[6]		
Item	WSS	SS	WSS	SS	SDap[5]	SDwp[5]	F	S	F × S
NDF[1]	730.2a	710.1b	551.0	458.2b	98.1	14.1	<0.001	<0.001	<0.001
NDFp[1]	696.3a	680.3b	458.1a	417.1b	92.5	11.9	<0.001	<0.001	0.004
ADF[1]	377.2	354.4	350.6	305.7	76.7	21.6	0.149	<0.001	0.133
ADFp[1]	371.0	349.0	320.2	294.0	72.0	15.4	0.037	<0.001	0.675
NDIP[1]	33.9a	29.8a	92.9a	41.1b	28.1	9.3	0.001	<0.001	<0.001
NDIP[2]	47.1a	42.4a	158.7a	86.1b	45.1	17.9	<0.001	<0.001	<0.001
ADIP[1]	6.2a	5.4a	30.3a	11.7b	12.9	7.1	0.002	<0.001	0.001
ADIP[3]	16.8a	15.0a	84.3a	41.8b	31.3	9.6	<0.001	<0.001	<0.001
Lignin[1]	44.9a	34.2a	114.4a	80.6b	41.6	13.3	<0.001	<0.001	0.017

NDF = neutral detergent fiber; NDFp = NDF corrected for protein; ADF = acid detergent fiber; ADFp = ADF corrected for protein; NDIP = neutral detergent insoluble protein; ADIP = acid detergent insoluble protein; WSS = without sodium sulfite; SS = using sodium sulfite; SDap = standard deviation among plots; SDwp = standard deviation within plots; F = effect of forage group; S = effect of sodium sulfite; F × S = interaction of forage group and sodium sulfite.
a, b: means in same row within grasses or legumes followed by different letters differ at P<0.05.
[1] g/kg DM.
[2] g/kg NDF.
[3] g/kg ADF.

Table 3 - Estimates of regression parameters for different relationships according to utilization of sodium sulfite

Variable[1]		Parameter Estimate[2]				
X	Y	Intercept	Slope	s_{XY}	r^2	P Value[3]
			Grasses			
Δ_{NDF}	Δ_{N}	17.22±3.68	-0.116±0.138	7.58	0.082	<0.001
Δ_{ADF}	Δ_{A}	-3.64±3.30	0.667±0.132	4.27	0.760	<0.001
			Legumes			
Δ_{NDF}	Δ_{N}	-56.67±39.54	1.555±0.420	25.42	0.634	0.379
Δ_{ADF}	Δ_{A}	21.59±12.79	0.689±0.211	25.75	0.603	0.292

[1] See details of variables in Table 1.
[2] Estimate ± standard error.
[3] See equation 2 for details.

(Table 2), such equivalence could not be observed in this forage group (P<0.05; Table 3).

Discussion

The recommendation for using sulfite in neutral detergent solution is based on its ability to decrease contaminant protein in the insoluble residue (Mertens, 2002). Those contaminant nitrogenous compounds can be originated from four potential sources: nitrogen that is naturally associated with the cell wall, nitrogen attached to artifacts (non-enzymatic products), nitrogen linked to tannins (proanthocyanidins), and keratins of animal origin (Van Soest, 1994). The last source is not applicable to the results of this study.

Part of the protein contamination could be attributed to the formation of artifacts by the non-enzymatic reactions. However, their formation occurs mainly during drying at temperatures of at least 65 ºC, which were avoided in this study (samples were dried at 60 ºC). The use of adequate temperatures and ventilated ovens reduces the formation of these artifacts by accelerating the removal of humidity from the material, which is necessary for non-enzymatic reactions to occur (Van Soest, 1994).

It has been speculated that protein contamination would be more prominent in tannin-rich material or samples with higher crude protein content (Hintz et al., 1996; Krueger et al., 1999; Pagán et al., 2009). Considering this, it will be expected that legumes show higher protein contamination than grasses due to their greater tannin content, which would result in the formation of insoluble complexes with the protein components of forages (Van Soest, 1994; Krueger et al., 1999). Such statement seems to support the results obtained in this study, where sodium sulfite caused more prominent decrease in NDF and ADF contents in legumes when compared with grasses (Table 2; Figure 1).

On the other hand, the utilization of sodium sulfite has been associated with a partial solubilization of lignin (Hartley, 1972; Van Soest et al., 1991), which was observed for legume samples (Table 2). Such pattern indicates that lignin estimates can be less reliable in this type of forage when sodium sulfite is used. However, the decreases of NDF and ADF contents in grasses could not be solely explained by the decrease in contaminant protein and solubilization of lignin (Tables 2 and 3).

The decreases in NDF and ADF contents caused by protein correction or sodium sulfite were approximately equivalent to each other in legume samples (Figure 1). Despite this, those decreases present different components. The utilization of sodium sulfite in legumes decreased, but did not obliterate the contaminant protein (Table 2; Figure 1), which allowed verifying an additional decrease in fiber contents when protein correction was applied to samples previously treated with sodium sulfite (Figure 1). Thereby, NDF and ADF decreases were at least partially caused by the solubilized portion of lignin (Tables 2 and 3). In this way, despite providing results similar to sodium sulfite (Figure 1), it can be assumed that protein correction provides more accurate estimates of fibrous compounds in legumes under a chemical point of view.

Actually, the decreases in NDF and ADF contents caused by sodium sulfite or protein correction were approximately additive to each other in grass samples (Figure 1). This can be affirmed because sodium sulfite did not promote any significant decrease in the insoluble protein included in fiber residues (Table 2). Moreover, the sodium sulfite did

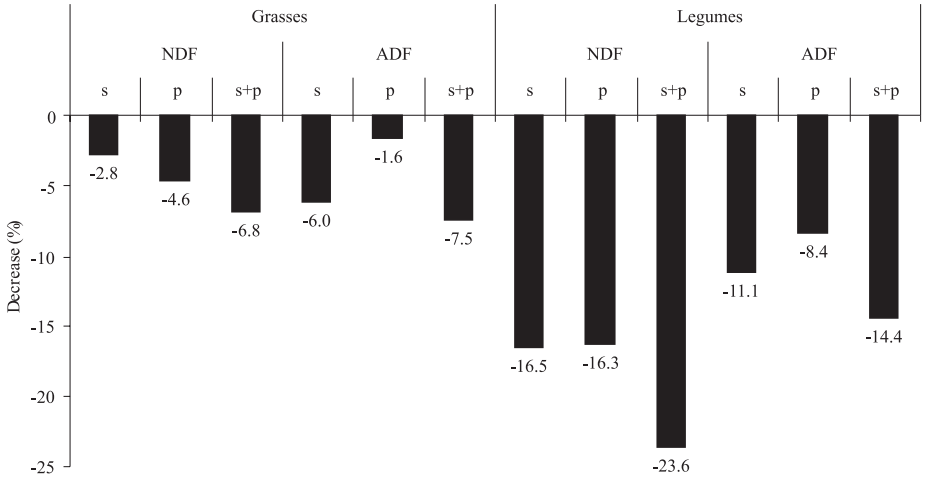

Figure 1 - Decrease of fiber contents in relation of analytical procedure without using sodium sulfite or protein correction (s = using sodium sulfite; p = considering protein correction). NDF = neutral detergent fiber; ADF = acid detergent fiber.

not cause significant decrease in lignin content in grasses (Table 2). In this context, the actual cause of the decreases in NDF and ADF contents (Figure 1) could not be entirely explained by decrease in insoluble nitrogen or solubilization of lignin (Table 3). Such pattern stresses that sodium sulfite can solubilize other components of the cell wall (Hintz et al., 1996). When compared with the control method (without sodium sulfite or protein correction), about 26 and 49% of the decreases caused by sodium sulfite in NDF and ADF could not be identified from results obtained in this study (Figure 2), which implicates lack of accuracy on fiber content estimates.

Considering the results obtained with grass samples, the protein correction could be assumed as an alternative to avoid the bias caused by sodium sulfite. This statement is based on the fact that protein correction does not demand any modification in neutral detergent solution and avoids the undesirable loss of known or unknown fibrous compounds. Moreover, considering the results obtained with legume samples, the partial solubilization of insoluble nitrogen and lignin could compromise the estimates of these compounds when they are imputed in feed evaluation models.

Oven-drying procedures of forages can cause the formation of nitrogen-containing artifacts, which seem to be originated from oxidative polymerization of tannins and protein (Pagán et al., 2009) or through non-enzymatic reactions between sugars and free amino acids (Van Soest, 1994). Those compounds can noticeably increase the protein contamination of neutral detergent insoluble residue, but they would be not necessarily eliminated by sodium sulfite. Such pattern could be more relevant by considering that different oven-drying procedures could cause different increases in insoluble nitrogen contents (Pagán et al., 2009; Pelletier et al., 2010), which would obviously decrease the reproducibility of the analytical method. Nevertheless, Terrill et al. (1994) affirmed that protein correction is able to eliminate the variation associated with drying procedure on insoluble fibrous residue.

A possible problem associated with protein correction procedure is the conversion of insoluble nitrogen contents into equivalent protein. According to Hintz et al. (1996), the conversion factor of nitrogen into protein of a simple Maillard artifact (amino acid plus sugar) would be around half of that one applied for true protein (6.25). The probable bias caused by uncorrected conversion of nitrogen into equivalent protein could be increased in digestion assays because little true protein is found in feces. A great part of fecal nitrogenous compounds presents nitrogen content around 70-110 g/kg (Van Soest, 1994). This would demand a conversion factor varying from 14.3 to 9.1. Thereby, despite of the 6.25 factor being recommended in the evaluation of nitrogenous compounds associated with fiber (Licitra et al., 1996), its utilization could underestimate the actual mass of contaminant nitrogenous compounds and overestimate the mass of insoluble fiber. However, evaluations with regard to the true nitrogen content in the nitrogenous compounds associated with insoluble fiber remains to be performed. Despite this, it must be emphasized that bias observed with protein correction would be less relevant when compared with effective loss of nitrogenous and fibrous compounds caused by sodium sulfite utilization (Table 2; Figures 1 and 2).

According to Hintz et al. (1996), the sodium sulfite utilization could be advantageous because it would increase the precision of fiber estimates. The benefit of using sodium sulfite in NDF analysis is that it would make some samples easier to filter, especially those that are high in protein or those that have been heated or fermented. In these cases, sulfite would greatly reduce variability among replicates and improve repeatability as well.

However, using sodium sulfite caused higher precision only on lignin contents (Figure 3). It probably occurred due

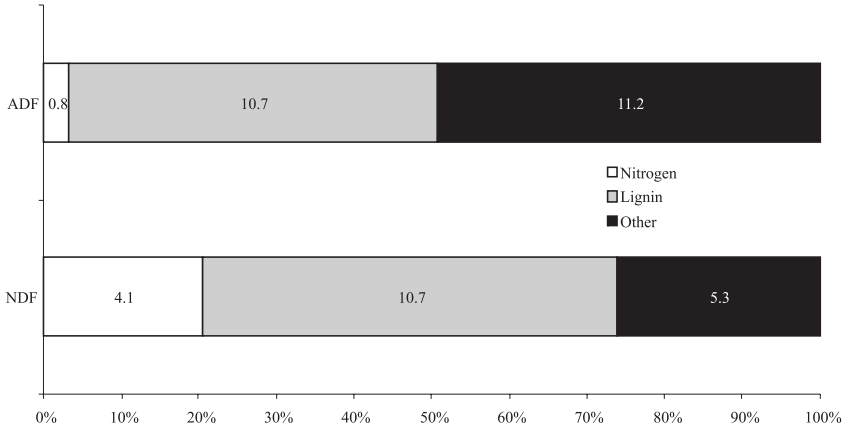

Figure 2 - Descriptive evaluation of the average decrease in neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents caused by sodium sulfite in grass samples (g/kg DM).

to decrease in the contaminant protein of lignin residue. The lignin estimated by solubilization of cellulose in sulfuric acid presents significant contamination by nitrogenous compounds (Henriques et al., 2007; Gomes et al., 2011). Nonetheless, the increased precision (Figure 3) may be associated with decreased accuracy (Table 2), which would not be a good analytical advantage.

Considering the NDF contents, the sodium sulfite utilization produced results with lower precision when compared with control method or protein correction. If the filtration problems are more prominent in NDF analysis than in ADF or lignin analyses, the analytical advantage pointed out by Hintz et al. (1996) for using sodium sulfite seems not to be relevant for tropical forages analyses.

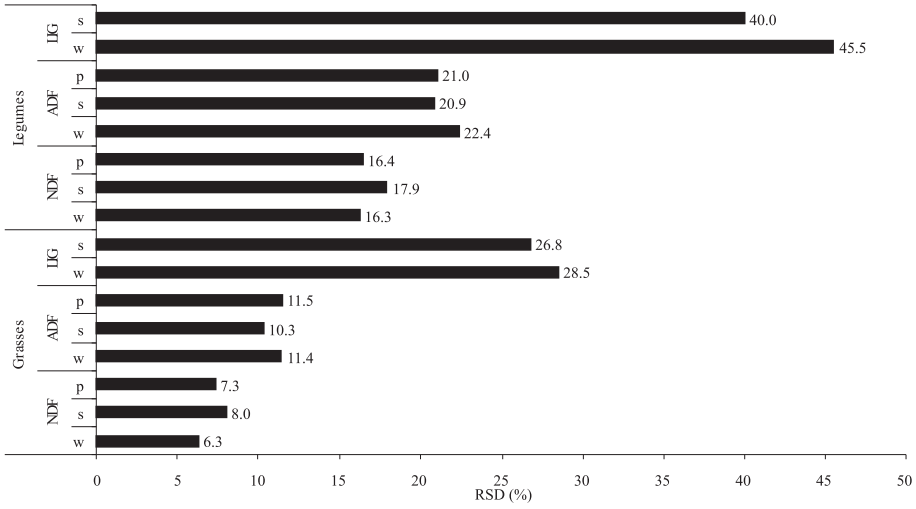

Figure 3 - Relative standard deviation (RSD) for estimates of fiber compounds according to utilization of sodium sulfite or protein correction (w = without sodium sulfite or protein correction; s = using sodium sulfite; p = considering protein correction). NDF = neutral getergent fiber; ADF = acid detergent fiber; LIG = lignin.

Conclusions

The utilization of sodium sulfite compromises the accuracy of the estimates of fibrous compounds contents in tropical forages. The procedure for contaminant protein correction is suggested as alternative to sodium sulfite because it does not encompass any modification in neutral detergent solution or cause any undesirable solubilization of fibrous compounds.

References

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTRY – AOAC. **Official methods of analysis**. 15.ed. Arlington: AOAC International, 1990. paginação descontínua.

DETMANN, E. Fibra na nutrição de novilhas leiteiras. In: PEREIRA, E.S.; PIMENTEL, P.S.; QUEIROZ, A.C. et al. (Eds.). **Novilhas leiteiras**. Fortaleza: Graphiti, 2010. p.253-302.

DETMANN, E; VALADARES FILHO, S.C. On the estimation of non-fibrous carbohydrates in feeds and diets. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.62, p.980-984, 2010.

DETMANN, E.; PAULINO, M.F.; VALADARES FILHO, S.C. Avaliação nutricional de alimentos ou de dietas? Uma abordagem conceitual. In: SIMPÓSIO SOBRE PRODUÇÃO DE GADO DE CORTE, 6., 2008, Viçosa, MG. **Anais...** Viçosa, MG: DZO-UFV, 2008. p.21-52.

DETMANN, E.; VALADARES FILHO, S.C.; PAULINO, M.F. Prediction of the energy value of cattle diets based on chemical composition of the feeds. In: VALADARES FILHO, S.C.; MARCONDES, M.I.; CHIZZOTTI, M.L. et al. (Eds). **Nutrient requirements of zebu beef cattle BR-Corte**. 2.ed. Viçosa, MG: UFV, 2010. p.45-60.

DORLEANS, M.; MANDRAN, M.; SAUVANT, D. Study of the use of a protease with the Van Soest procedure. **Animal Feed Science and Technology**, v.61, p.129-136, 1996.

GOMES, D.I.; DETMANN, E.; VALADARES FILHO, S.C. et al. Evaluation of lignin contents in tropical forages using different analytical methods and their correlations with degradation of insoluble fiber **Animal Feed Science and Technology**, v.168, p.206-222, 2011.

HARTLEY, R.D. p-Coumaric and ferulic acid components of cell walls of ryegrass and their relationships with lignin and digestibility. **Journal of the Science of Food and Agriculture**, v.23, p.1347-1354, 1972.

HENRIQUES, L.T.; DETMANN, E.; QUEIROZ, A.C. et al. Frações dos compostos nitrogenados associados à parede celular em forragens tropicais. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.59, p.258-263, 2007.

HINTZ, R.W.; MERTENS, D.R.; ALBRECHT, K.A. Effects of sodium sulfite on recovery and composition of detergent fiber and lignin. **Journal of the AOAC International**, v.79, p.16-22, 1996.

KRUEGER, C.G.; ALBRECHT, K.A.; REED, J.D. et al. Sodium sulfite effects on recovery and composition of detergent fibre and lignin from forage legumes varying in levels of

proanthocyanidins. **Journal of the Science of Food and Agriculture**, v.79, p.1351-1356, 1999.

LICITRA, G.; HERNANDEZ, T.M.; Van SOEST, P.J. Standardization of procedures for nitrogen fractionation of ruminant feeds. **Animal Feed Science and Technology**, v.57, p.347-358, 1996.

MERTENS, D.R. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. **Journal of the AOAC International**, v.85, p.1217-1240, 2002.

PAGÁN, S.; WOLFE, R.M.; TERRILL, T.H. et al. Effect of drying method and assay methodology on detergent fiber analysis in plants containing condensed tannins. **Animal Feed Science and Technology**, v.154, p.119-124, 2009.

PELL, A.N.; SCHOFIELD, P. Computerized monitoring of gas production to measure forage digestion *in vitro*. **Journal of Dairy Science**, v.76, p.1063-1073, 1993.

PELLETIER, S.; TREMBLAY, G.F.; BERTRAND, A. et al. Drying procedures affect non-structural carbohydrates and other nutritive value attributes in forage samples. **Animal Feed Science and Technology**, v.157, p.139-150, 2010.

TERRILL, T.H.; WINDHAM, W.R.; EVANS, J.J. et al. Effect of drying method and condensed tannin on detergent fiber analysis of *Sericea lespedeza*. **Journal of the Science of Food and Agriculture**, v.66, p.337-343, 1994.

Van SOEST, P.J. **Nutritional ecology of the ruminant**. 2.ed. Ithaca: Cornell University Press, 1994. 476p.

Van SOEST, P.J.; ROBERTSON, J.B. **Analysis of forages and fibrous foods**. Ithaca: Cornell University, 1985. 202p.

Van SOEST, P.J.; ROBERTSON, J.B.; LEWIS, B.A.S Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. **Journal of Dairy Science**, v.74, p.3583-3597, 1991.