Notas Científicas

Protein contamination on Klason lignin contents in tropical grasses and legumes

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Abstract – The objective of this work was to evaluate the extent of protein contamination on Klason lignin (KL) in tropical grasses and legumes, and to propose an equation to estimate the protein-free content of Klason lignin (KLp). Five grass (30 samples) and 12 legume species (31 samples) were evaluated. Legumes had higher KL contents. Protein contamination was significant in both grasses and legumes, but greater in legume samples. The model to predict KLp was based on KL and crude protein (CP) contents, as follows: KLp = 0.8807KL - 0.0938KL x D - 0.00338CP (R^2=0.935), in which D=0, for grasses, and D=1 for legumes.

Index terms: fiber degradation, forage analysis, nitrogen contamination.

Contaminação proteica sobre os teores de lignina Klason em gramíneas e leguminosas tropicais

Resumo – O objetivo deste trabalho foi avaliar a extensão da contaminação proteica sobre a lignina Klason (LK) em gramíneas e leguminosas tropicais, e propor uma equação para estimar o conteúdo livre de proteína da lignina Klason (LKp). Foram avaliadas cinco espécies de gramíneas (30 amostras) e 12 de leguminosas (31 amostras). As leguminosas apresentaram maiores teores de LK. A contaminação proteica foi significativa em gramíneas e leguminosas, mas maior em amostras de leguminosas. O modelo para estimar LKp foi baseado nos conteúdos de LK e de proteína bruta (PB), da seguinte forma: LKp = 0,8807LK - 0,0938LK x D - 0,00338PB (R^2=0,935), em que D=0, para gramíneas, e D=1 para leguminosas.

Termos para indexação: degradação da fibra, análise de forragem, contaminação por nitrogênio.

From a nutritional and functional perspective, methods for lignin quantification must provide information regarding the portion of feed that corresponds to deleterious effects on the digestion of fibrous carbohydrates. However, a method that accurately represents lignin and establishes the best relationship between lignin content and ruminal degradation parameters remains undefined for tropical forages (Gomes et al., 2011).

Klason lignin (KL) is strongly correlated with the rate and extent of fiber degradation of tropical forages by ruminants (Jung et al., 1997; Gomes et al., 2011). However, its estimates are believed to be biased in forages due to high contamination with nitrogenous compounds (Gomes et al., 2011). Considering the great biological relevance of KL for understanding forage utilization by cattle in the tropics, it is necessary to know how protein contamination affects the estimates of KL contents. Moreover, the possibility to predict protein contamination using variables of easier evaluation, such as crude protein, still needs to be determined.

The objective of this work was to evaluate the extent of protein contamination on KL in tropical grasses and legumes, and to propose an equation to estimate the protein-free content of KL (KLp).

The experiment was done at the Animal Nutrition Laboratory of the Animal Science Department of Universidade Federal de Viçosa, in the state of Minas Gerais, Brazil.

Thirty samples were taken from five grass species (two from *Pennisetum purpureum*, ten from *Urochloa* sp. (Syn. *Brachiaria* sp.), 12 from *Panicum* sp., two from *Andropogon gayanus*, and four from *Cynodon* sp.) and 31 from 12 legume species (three from *Arachis*

pintoi, two from *Medicago sativa*, three from *Leucaena leucocephala*, two from *Galactia striata*, one from *Dolichos lablab*, three from *Centrosema pubescens*, three from *Glycine wightii*, three from *Gliricidia sepium*, four from *Stylosanthes guianensis*, three from *Pueraria phaseoloides*, one from *Calopogonium mucunoides*, and three from *Cajanus cajan*). The forages were cultivated in 2x4 m plots.

The samples were taken from forage cuts at ground level, from December 2008 to December 2010. The plants had approximately 45 days for regrowth. All samples were oven-dried at 60°C and processed in a 1 mm knife mill. Subsequently, the contents of dry matter (DM), determined by the INCT-CA G-003/1 method; of crude protein (CP), by the Kjeldahl procedure, using the INCT-CA N-001/1 method; and of neutral detergent insoluble protein (NDIP), by the INCT-CA F-002/1 and N-002/1 methods, were quantified according to the standard analytical procedures of Instituto Nacional de Ciência e Tecnologia de Ciência Animal (Brazilian National Institute of Science and Technology in Animal Science – INCT-CA) (Detmann et al., 2012).

KL content was determined through an acid hydrolysis of the water-insoluble fraction (Theander & Westerlund, 1986). Approximately 250 mg DM were taken from the samples and conditioned in 120 mL polyethylene pots. Three milliliters of a 12 mol L^{-1} sulfuric acid solution were added to the sample, which was homogenized with a glass rod. Pots were kept in a water bath at 30ºC, for 30 min. Afterwards, 80 mL of distilled water were added to each pot, which were then sealed and autoclaved at 105°C for 1 hour. While contents were still warm, the insoluble material was quantitatively vacuum-transferred to filter crucibles, washed with hot distilled water, and dried at 105°C for 16 hours. Subsequently, the crucibles were incinerated at 500°C for 3 hours, and the ash weight was subtracted from the weight of the insoluble residue in sulfuric acid, to calculate lignin content.

To quantify the content of residual nitrogen associated with lignin, aliquots of the residues obtained after treatment with sulfuric acid were evaluated by the Kjeldahl procedure, using the INCT-CA N-001/1 method (Detmann et al., 2012). The protein content of the contaminant was obtained by multiplying the nitrogen content by 6.25.

The variables were compared between the species group (grasses or legumes), in a completely randomized design, according to the model, $Y_{ijk} = \mu + G_i + S_{(i)j}$, in which: μ is a general constant; G_i is the effect of the species group i (fixed effect); and $S_{(i)j}$ is the effect of species j nested within group i (random effect), which was assumed as a random error. After analysis of variance, a correlation analysis was performed to verify the relationship between the protein contamination in KL and the chemical characteristics of the samples. The independent variables were selected based on Pearson's correlations. After that, regression models were adjusted using a backward method (Draper & Smith, 1966). The lack of fitting of adjusted models was evaluated through the adjustment of a single linear regression of observed values (Y) on predicted ones (X). The following null hypothesis were tested: $H_0 : \beta_0 = 0$ and $H_0 : \beta_1 = 0$, in which β_0 and β_1 are the intercept and slope, respectively. The model was assumed to have a good fitting when H_0 was not rejected.

The models were evaluated comparing with different dependent variables. The coefficient of determination (R^2) and relative standard deviation (RSD) were also taken into account to assess the models. The statistical procedures were performed using the MIXED, CORR, and REG procedures of SAS 9.2 (SAS Institute, Cary, NC, EUA), at 5% probability.

KL and KLp contents were greater in legumes (Table 1). Conversely, the protein contamination (PK) on KL was significant ($p<0.01$) in both grasses and legumes, but it was greater in legume samples. This pattern can be explained by the positive correlation between protein contamination and the contents of KL (r=0.872), CP (r=0.718), and NDIP (r=0.452), which were all greater in legume samples. These values reinforce that protein correction is mandatory to accurately estimate KL contents in tropical forages. The pattern obtained here agrees with previous assessment of protein contamination on KL in tropical forages (Gomes et al., 2011), but disagrees with Hatfield et al. (1994), who found that CP content in samples is not correlated with the degree of protein contamination in lignin residues. At least part of the greater protein contamination in legumes can be traced back to the greater tannin content of this group, which favors the formation of insoluble complexes with the protein components of forages (Van Soest, 1994).

Table 1. Contents of Klason lignin (KL), KL corrected for protein (KLp), protein contamination in KL (PK), crude protein (CP), and neutral detergent insoluble protein (NDIP), as well as significance of evaluation models using different dependent variables.

Variable	Grasses	Legumes	p value	Parameters of the models		
				R^2	RSD[(1)]	Lack of fit (p value)
KL (g kg^{-1} DM)	151±11.8	225±11.7	<0.001	-	-	-
KLp (g kg^{-1} DM)	129±8.5	161±8.4	0.009	0.983	8.7	0.983
PK (g kg^{-1} DM)	22.2±4.36	64.2±4.29	<0.001	-	-	-
PK (g kg^{-1} KL)	146±12.6	285±12.5	<0.001	0.843	29.1	0.994
PK (g kg^{-1} CP)	276±23.1	311±22.8	0.283	-	-	-
CP (g kg^{-1} DM)	84.8±7.08	208.0±6.96	<0.001	-	-	-
NDIP (g kg^{-1} DM)	30.1±6.62	109.7±6.52	<0.001	-	-	-
KLp/KL (g g^{-1})	0.854	0.716	-	0.171	11.5	<0.001

[(1)]RSD, relative standard deviation.

Considering that protein contamination is relevant for KL estimates, a procedure for protein correction must be recommended to correctly obtain lignin content and to estimate more reliable relationships between rate and extent of cell degradation in the rumen. However, the procedures for protein correction are time and labor intensive, and correction using a model could be helpful. Three dependent variables were evaluated to propose such a model: KLp, PK, and the KLp/KL ratio (Table 1).

The model based on the ratio of KLp to KL did not present a significant fit and was discarded. The models based on KLp and PK fitted well to data (Table 1), with greater R^2 and smaller RSD for the model used to predict KLp. The fitted model was: KLp = 0.8807KL - 0.0938KL x D - 0.00338CP, in which D is a "dummy" variable assigned as 0 for grasses and as 1 for legumes.

The fitted model is quite simple and soundly expresses the relationships between chemical characteristics of forage samples and the extent of protein contamination on KL contents. The model was adjusted without an intercept, since it is expected that there could not be contamination without lignin in the sample. Furthermore, the extent of contamination was proportional to KL content, which makes sense because the unit used to adjust the model was based on the fraction of total DM. In the present study, different protein contamination between grasses and legumes can be a reflection of the greater tannin content in the latter, as discussed previously. Finally, the regression coefficient associated with CP content in the sample reinforces that protein contamination is proportional to the total nitrogen content.

Klason lignin isolated from tropical forages is significantly contaminated with protein, and this contamination is proportional to lignin and nitrogen contents of the sample.

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References

DETMANN, E.; SOUZA, M.A. de; VALADARES FILHO, S. de C.; QUEIROZ, A.C. de; BERCHIELLI, T.T.; SALIBA, E. de O.S.; CABRAL, L. da S.; PINA, D. dos S.; LADEIRA, M.M.; AZEVEDO, J.A.G. (Ed.). **Métodos para análise de alimentos**. Visconde do Rio Branco: Suprema, 2012. 214p.

DRAPER, N.R.; SMITH, H.A. **Applied regression analysis**. New York: John Wiley, 1966. 427p.

GOMES, D.I.; DETMANN, E.; VALADARES FILHO, S. de C.; FUKUSHIMA, R.S.; SOUZA, M.A. de; VALENTE, T.N.P.; PAULINO, M.F.; QUEIROZ, A. de C. 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. DOI: 10.1016/j.anifeedsci.2011.05.001.

HATFIELD, R.D.; JUNG, H.-J.G.; RAPLH, J.; BUXTON, D.R.; WEIMER, P.J. A comparison of the insoluble residues produced by the Klason lignin and acid detergent lignin and acid detergent lignin procedures. **Journal of the Science of Food and Agriculture**, v.65, p.51-58, 1994. DOI: 10.1002/jsfa.2740650109.

JUNG, H.G.; MERTENS, D.R.; PAYNE, A.J. Correlation of acid detergent lignin and Klason lignin with digestibility of forage dry

matter and neutral detergent fiber. **Journal of Dairy Science**, v.80, p.1622-1628, 1997. DOI: 10.3168/jds.S0022-0302(97)76093-4.

THEANDER, O.; WESTERLUND, E.A. Studies on dietary fiber. 3. Improved procedures for analysis of dietary fiber. **Journal of Agricultural and Food Chemistry**, v.34, p.330-336, 1986. DOI: 10.1021/jf00068a045.

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

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