Scientific Research Publishing

American Journal of Analytical Chemistry, 2024, 15, 357-361
https://www.scirp.org/journal/ajac
ISSN Online: 2156-8278
ISSN Print: 2156-8251

Evaluation of the Technique of Acid Detergent Insoluble Protein Determination

Eloisa de Oliveira Simões Saliba*, Jaqueline Simões Saliba, Yara da Costa Guedes, Gabriela Fernanda da Silva Custodio, Ana Luiza Costa Cruz Borges, Diogo Gonzaga Jayme, Hemilly Cristina Menezes de Sà, Ricardo Reis e Silva

Department of Animal Science, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
Email: *saliba@ufmg.br

How to cite this paper: Saliba, E.O.S., Saliba, J.S., da Costa Guedes, Y., da Silva Custodio, G.F., Borges, A.L.C.C., Jayme, D.G., de Sà, H.C.M. and e Silva, R.R. (2024) Evaluation of the Technique of Acid Detergent Insoluble Protein Determination. *American Journal of Analytical Chemistry*, **15**, 357-361.
https://doi.org/10.4236/ajac.2024.1512023

Received: October 17, 2024
Accepted: December 2, 2024
Published: December 5, 2024

Abstract

Vegetable cell wall components are commonly present in animal feeds, and are able to be used by ruminant animals. However, some of these have little digestibility or may not be digestible, taking up a big space in their gastrointestinal tract, which can affect their nutrition and performance. The cell wall is chemically composed of cellulose, hemicelluloses, pectin, lignin, and minor parts of proteins and tannins. Thus, several studies have been performed aiming at practical techniques for measuring the concentration of such structural substances. The aim of the present study was to test whether the method of separation of cell wall components using detergents [1] in a sequential way could interfere with the value of acid detergent insoluble nitrogen (ADIN). The analysis was conducted for neutral detergent fiber (NDF), acid detergent fiber (ADF) and sequentially and non-sequentially, according to USDA Agriculture (method 379) [2]. Eight feeds were tested: Brachiaria hay (*Brachiaria* sp.), barley hay (*Hordeum vulgare* L.), Cratylia hay (*Cratylia argentea*), sunflower silage (*Helianthus annuus*), millet silage (*Pennisetum typhoides*), maize silage (*Zea mays* L.), ground and rehydrated, pequi fruit peels (*Caryocar brasiliense* Camb), and Tifton 85 hay (*Cynodon* sp.). Samples were ground in a Wiliey-type mill and went through a 1-mm sieve; then, they were analyzed through the ADF techniques sequentially and non-sequentially from NDF. The product of these steps was studied for the acid detergent insoluble protein (ADIP). The significant difference was seen in the determination of ADIP between the two methods for five feeds, while three feeds did not show any difference ($P < 0.05$). Due to our findings, we conclude that it is reasonable to determine ADIP for ADF non-sequentially from NDF.

Keywords

Protein, Cell Wall, Extraction through Detergents

1. Introduction

Plant cells have some peculiar features, such as the presence of plastids, vacuoles and a wall. The wall is the outermost layer in plant cells, and it is the one that remains after the cell's death. Its composition is made mainly of cellulose, hemicelluloses, lignin and other minor components, such as proteins and tannins.

The plant cell wall constituents can be used in ruminant animals' feed. However, some of these molecules have components of little or no digestibility, which take up space in the gastrointestinal tract of the animals and may restrain their consumption and performance [3].

The actual characterization of the cell wall components becomes truly important for predicting feed nutritional availability. However, although many methods can be chosen for this analysis, they usually focus on determining crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF) [4] [5].

Some proteins in the cell wall are interconnected through intermolecular interactions with amino acids such as tyrosine, which are resistant to most chemical extraction methods. Not all sources of crude protein (CP) are nutritionally equal. Some are more quickly used in the rumen (e.g. soluble peptides, ammonia), some take more time to be used (e.g. nitrogen (N) associated with NDF), and some can even be indigestible (N associated with lignin) [6].

Protein fractions have been separated based on their solubility [7]; however, solubility or certain fractions may not precisely describe nitrogen (N) digestibility. There are certain soluble fractions of N in heat-damaged feeds that may be indigestible and ADF fractions of N in thermally-treated feeds that may be digestible. Acid detergent insoluble nitrogen is understood as coming from feeds submitted to extraction using boiling acid detergent, and whose residue is assessed for crude protein, following N × 6.25. Such nitrogen comes from lignin or tannin-associated protein, which were damaged by heat. This protein is understood as indigestible in forage [6].

All the gravimetric analyses of fiber residues are subject to interference through contaminants. Fiber residue-associated contaminants may occur due to two distinguished causes: mistakes during the procedures or method-inherent contamination. In the former, contamination happens owing to either wrong conducting or omission of procedure steps; in the latter, contaminants are intrinsic to the method. Among these contaminants are, especially, fractions of neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) [8].

The assessment of fiber components is of great significance in animal nutrition, given the importance of dietary protein and protein/energy ratio.

Due to the significance of vegetables in animal nutrition, and taking into consideration that detergents can wash away the cell membranes, therefore, destroying their proteins, the present study was carried out aiming at checking whether the method of separation of cell wall components through sequential use of detergents [1], could affect the determination of cell wall proteins extracted. We have not yet checked the current publication date of this work, and we have not checked any articles on the topic.

2. Material and Methods

Following the USDA Agriculture guidelines (method 379) [2], the analysis was carried out after the procedure of acid detergent fiber (ADF), sequentially and non-sequentially from neutral detergent fiber (NDF), and were studied for acid detergent insoluble nitrogen (ADIN), for eight different feeds: Brachiaria hay (*Brachiaria* sp.), barley hay (*Hordeum vulgare* L.), Cratylia hay (*Cratylia argentea*), sunflower silage (*Helianthus annuus*), millet silage (*Pennisetum typhoides*), ground and rehydrated maize silage (*Zea mays* L.), pequi fruit peels (*Caryocar brasiliense* Camb.), and Tifton 85 hay (*Cynodon* sp.).

Samples were ground in a Willey-type mill and went through a 1-mm sieve. Samples from ADF, sequentially and non-sequentially from NDF, were studied for acid detergent insoluble nitrogen (ADIN) and N × 6.25 acid detergent insoluble Protein (ADIP). All feeds and analyses were carried out with 5 replicates, using the Ankom® device and FS67 bags from this same manufacturer.

3. Statistical Design

All the analyses were conducted using the R [9] software, using generalized linear models. The variables were evaluated in a completely randomized one-way design, as in the following equation:

$$Y_{ij} = \alpha + \beta_j + \varepsilon_{ij}$$

where:

Y_{ij} is the response variable assessed for the specimen that underwent the treatment j;

α is the trial's general mean;

β_j is the effect of the treatment j;

ε_{ij} is the trial error associated with Y_{ij}.

All variables were assessed for the presupposition of normality and homoscedasticity through the tests of [10] and [11], respectively. When the presuppositions were not met, the response variable was transformed and a matrix of variance and covariance was designed. The means of the treatments were compared using Tukey's test ($P < 0.05$) [12].

4. Results and Discussion

The results of the analytical determinations and statistical analyses are in Table 1 and Table 2.

Table 1. Studied values for ADIP (%) for ADF sequentially and non-sequentially from NDF.

Feed	Sequential ADIP %	Non-sequential ADIP %	SE[1]	P-value[2]
Brachiaria	2.42	1.29	0.112	<0.001
Barley	6.69	6.92	0.162	0.27

Continued

Cratylia	3.78	4.71	0.24	0.036
Sunflower	1.62	3.71	0.088	<0.001
Millet	2.35	2.22	0.04	0.197
Ground maize	2.56	1.29	0.27	0.011
Pequi fruit	1.15	1.62	0.032	<0.01
Tifton	1.43	1.44	0.05	0.84

[1]Mean's standard error; [2]F < 0.05.

Table 2. Studied values for ADIP (%) for ADF sequentially and non-sequentially from NDF.

Feed	Sequential ADIP %[2]	Non-sequential ADIP %[2]	SE[1]
Brachiaria	2.42 A	1.29 B	0.112
Barley	6.69 A	6.92 A	0.162
Cratylia	3.78 B	4.71 A	0.24
Sunflower	1.62 B	3.71 A	0.088
Millet	2.35 A	2.22 A	0.04
Ground maize	2.56 A	1.29 B	0.27
Pequi fruit	1.15 B	1.62 A	0.032
Tifton	1.43 A	1.44 A	0.05

[1]Mean's standard error; [2]different letters show significant difference F < 0.05.

Amongst the eight feeds studied through the different methods, five showed significant differences in the values of sequential and non-sequential ADIP. The value was bigger for ADIP for Brachiaria (*Brachiaria* sp.) when analyzed sequentially, rather than non-sequentially (2.42% vs. 1.29%, respectively), as well as for ground and rehydrated maize (*Zea mays* L.) (2.56% vs. 1.29%, respectively). On the other hand, the value was smaller when analyzed sequentially, rather than non-sequentially, for ADIP in Cratylia hay (*Cratylia argentea*) (3.78% vs. 4.71%, respectively), sunflower silage (*Helianthus annuus*) (1.62% vs. 3.71%, respectively), and pequi fruit peels (*Caryocar brasiliense* Camb.) (1.15% vs. 1.62%, respectively).

Three of the studied feeds, however, did not show a significant difference (P < 0.05) between sequential and non-sequential analyses: barley hay (*Hordeum vulgare* L.) (6.69% vs. 6.92%, respectively), Tifton 85 hay (*Cynodon* sp.) (1.43% vs. 1.44%, respectively) and millet silage (*Pennisetum typhoides*) (2.35% vs. 2.22%, respectively). Therefore, due to this variation in results using the different methods, we cannot predict whether a certain result would be greater or smaller for a certain method and a certain food.

5. Conclusion

From the results in the present work, we conclude that it is sensible to determine

ADIP through ADF, non-sequentially from NDF.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Van Soest, P.J., Robertson, J.B. and Lewis, B.A. (1991) Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *Journal of Dairy Science*, **74**, 3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

[2] AOAC (2005) Method 958.06. In: Horwitz, W., Ed., *Bar Official Methods of Analysis*, 18th Edition, AOAC International.

[3] Van Soest, P.J. (1994) Nutritional Ecology of the Ruminant. Cornell University Press. https://doi.org/10.7591/9781501732355

[4] Saliba, E.O.S., Rodriguez, N.M., Gonçalves, L.C., *et al*. (1999) Effect of Corn and Soybean Lignin Residues Submitted to Ruminal Fermentation on Structural Carbohydrates Digestibility. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *Belo Horizonte*, **51**, 85-88.

[5] Saliba, E.O.S., Rodriguez, N.M., Piló-Veloso, D. and Morais, S.A.L. (2002) Chemical Characterization of the Lignins of Corn and Soybean Agricultural Residues. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, **54**, 42-51. https://doi.org/10.1590/s0102-09352002000100007

[6] Hall, M.B. (2014) Feed Analyses and Their Interpretation. *Veterinary Clinics of North America*: *Food Animal Practice*, **30**, 487-505. https://doi.org/10.1016/j.cvfa.2014.07.001

[7] Nelson, D.L. and Cox, M.M (2022) Biological Membranes and Transport. In: Nelson, D.L. and Cox, M.M., Eds., *Lehninger—Principles of Biochemistry*, 8th Edition, Savier.

[8] Dettman, E. (2012) Métodos de análises de alimentos. Visconde do Rio Branco.

[9] R Core Team (2022) The R Project for Statistical Computing. https://www.r-project.org

[10] Shapiro, S.S. and Wilk, M.B. (1965) An Analysis of Variance Test for Normality (Complete Samples). *Biometrika*, **52**, 591-611. https://doi.org/10.1093/biomet/52.3-4.591

[11] Bartlett, M.S. (1937) Properties of Sufficiency and Statistical Tests. *Proceedings of the Royal Society of London*, *Series A—Mathematical and Physical Sciences*, **160**, 268-282. https://doi.org/10.1098/rspa.1937.0109

[12] Tukey, J.W. (1949) Comparing Individual Means in the Analysis of Variance. *Biometrics*, **5**, 99-114. https://doi.org/10.2307/3001913