Indigestible neutral detergent fibers: Relationship between forage fragility and neutral detergent fibers digestibility in total mixed ration and some feedstuffs in dairy cattle

Mostafa Soufizadeh, Rasoul Pirmohammadi*, Yunes Alijoo, Hamed Khalilvandi Behroozyar

Department of Animal Science, Faculty of Agriculture, Urmia University, Urmia, Iran.

Abstract

Indigestible neutral detergent fibers (iNDF) accurately predict forage digestibility when measured in situ. The objective of this study was to determine the effects of rumen incubation times on the estimated concentrations of iNDF for four forages (alfalfa hay, corn silage, wheat straw and orchard grass), four concentrates (barley grain, soybean meal, beet pulp and wheat bran) and two total mixed ration samples in dairy cows. The iNDF contents of the samples were evaluated in 10 feeds using three ruminally cannulated Holstein cows in a completely randomized design. Five grams of the samples were incubated up to 240 hr. The iNDF fraction was significantly affected by incubation time for all of the tested samples, but the potentially digestible NDF (pdNDF) was not affected for wheat straw, barley grain and wheat bran (32.32, 10.11 and 20.60 g per 100 g of dry matter, respectively). For most of concentrates feedstuffs, the iNDF fraction could be measured after 120 hr of incubation, while for forages ruminal incubation should be lasted up to 240 hr. Statistically significant differences (p < 0.01) were observed between forage samples regarding fragility and NDF digestibility (NDFD). Also, a positive correlation was observed between fragility and NDFD. In some of the cases, it appears that NDFD can be a more helpful index in adjusting pdNDF values than direct fragility measurements.

Key words: Cell wall Fibers digestibility Fragility Rumen fibers pools

© 2018 Urmia University. All rights reserved.
Introduction

A wide range of *in vitro* and *in situ* techniques are used as alternatives to *in vivo* measurement of ruminal fiber availability. Fiber digestibility and forage fragility are critical factors should to be considered in forage evaluation and diet formulation for ruminants.\(^1\) Digestive characters of dietary neutral detergent fibers (NDF) fraction have been reported to greatly affect feeding behavior, chewing activity, rate of particles breakdown, ruminal turnover rate, ruminal fill dry matter (DM) intake and overall efficiency of milk and milk components production. Traditionally, nutritionists have focused primarily on fiber digestibility parameters measures and kinetics of ruminal neutral detergent fiber (NDF) and acid detergent fiber (ADF) degradations. However, recent studies have suggested to include indigestible fiber estimates as important fiber characters with ability to influence turnover rate of ruminal fiber pools and eventually set the rate and final extent of rumen fiber digestion.\(^1\) Accurate estimation of the potentially digestible NDF (pdNDF) fraction and its rate(s) of digestion in nutritional modeling requires precise determination of indigestible NDF (iNDF) content.\(^1,2\) Digestibility of the fiber has been reported to be related to potentially digestible portion of NDF.\(^3\)\(^,\)\(^4\) The pdNDF are defined as the difference between the NDF and iNDF fractions. Potential digestibility is repeatedly defined as the NDF fraction disappearing after a long ruminal incubation period.\(^5\) The iNDF will not be available to microbial digestion in ruminants, even if the total tract residency of fibers extended to an infinite time.\(^6\) Indigestibility of the iNDF can be attributed to the cross-links between cell wall lignin and hemicellulose.\(^7\) According to Ellis *et al.*, iNDF determination should be considered as an inevitable test in forage evaluation for the estimation of pdNDF.\(^8\) Because of zero digestibility and potential effects on animal performance, it is recommended to define an upper limit for dietary iNDF in high producing of dairy cows. Lippke has suggested about 20 g kg\(^{-1}\) of metabolic weight a day as a maximum tolerable dietary iNDF consumption.\(^9\)

Fragility is defined as a relative rate of forage particle size reduction during chewing or laboratory simulation of chewing action. Fragility has been reported to be related to digestibility, lignin content and anatomical characters such as cell wall thickness.\(^7\)

Consequently, fragility measures of the forage cell wall can be used as predictive tools for forage digestibility. According to Minson,\(^10\) grass forages can be classified into high (81.00%), medium (72.00%) and low (56.00%) digestible feed for sheep, based on chemical composition and resistance to comminution and voluntary intake. Additionally, Minson has reported a negative correlation between NDF digestibility (NDFD) and chewing activity.\(^10\) However, correlation coefficient was reported to be positive between NDFD and samples type. Grant has found that the 24 hr *in vivo* NDFD explains about 60.00% of the variation in forage fragility.\(^11\) The potential exists to combine a fragility factor related to NDFD, with the physically effective factor (pef) derived by sieving to arrive at a superior value to predict cow chewing response.\(^11\)

Notwithstanding, there is a small, but developing data set regarding forage fragility and its relations with potential ruminal digestibility of plant cell wall. The objective of this study was to determine the effects of ruminal incubation time on iNDF concentration estimation in total mixed ration (TMR) and some of the forage and concentrate feeds in dairy cow nutrition programs. Additionally, relationships between fragility and NDFD of forage samples were addressed.

Materials and Methods

**Animals, samples and chemical analysis.** All of the experimental protocols were approved by the Animal Use Committee in Urmia University, Urmia, Iran (proposal No. 2606; 06.06.2016). The animals were cared according to Guide for the Care and Use of Agricultural Animals in Research and Teaching.\(^12\) Rumen cannulated mature Holstein steers (520.00 ± 15.00 kg) were fed a TMR containing 20.00% chopped alfalfa hay, 30.00% corn silage, 25.00% wheat straw and 25.00% barely grain plus mineral/vitamin supplement according to their requirements for two times daily at 8:00 and 18:00 hr. Four concentrate feeds (barley grain, soybean meal, beet pulp and wheat bran), four forages (alfalfa hay, wheat straw, orchard grass hay and corn silage) and two TMR (the concentrate and forage ratio in 45:55 on DM basis, Table 1) samples were used in this study. The effects of incubation time (96, 120 and 240 hr) on the concentrations of iNDF were evaluated using three ruminally cannulated Holstein cows in a completely randomized design. Feed samples were dried in a forced-air oven at 60 °C for 48 hr and ground to pass a 1,00-mm screen by a Wiley mill (Ankom\(^100\), Fiber Analyzer; Ankom Technology, Macedon, USA) before chemical analysis.\(^13\)

Feed samples were analyzed for DM (at 55 °C for 48 hr),\(^14\) NDF, ADF and lignin.\(^15\) Neutral detergent solution contained sodium sulphite and heat stable alpha amylase.\(^16\) Ash content was determined by ignition of the dried samples at 500°C for 4 hr.\(^13\) Ash was determined in the bag residues and NDF was expressed free of residual ash. The lignin content was determined by solubilizing of the ADF fraction in 12 M sulfuric acid.\(^17\)

**Forage fragility.** Fragility parameters were analyzed according to Miner Institute developed ball milling method.\(^18\) Briefly, forage samples were dried at 60 °C for
Table 1. Ingredient (% of dry matter) and chemical composition (g per 100 g of DM) of experimental rations and feed samples used in situ.

| Ingredient composition | TMR1 | TMR2 | Alfalfa hay | Wheat straw | Orchard grass hay | Corn silage | Barley grain | Soybean meal | Beet pulp | Wheat bran |
|------------------------|------|------|-------------|-------------|------------------|-------------|--------------|--------------|------------|-----------|
| DM                     | 72.31| 72.00| 91.50       | 93.30       | 92.55            | 32.50       | 91.90        | 90.90        | 92.20      | 89.10     |
| Ash                    | 5.20 | 5.70 | 8.20        | 7.80        | 9.80             | 3.33        | 2.50         | 6.75         | 6.30       | 6.90      |
| NDF                    | 35.29| 36.05| 44.43       | 73.12       | 56.13            | 45.30       | 19.77        | 13.68        | 41.49      | 43.33     |
| aNDFom¹                | 34.40| 34.90| 41.00       | 69.78       | 54.96            | 41.16       | 17.53        | 9.93         | 30.05      | 39.80     |
| ADF                    | 23.95| 25.51| 36.40       | 48.04       | 33.27            | 30.37       | 6.72         | 8.54         | 26.52      | 13.43     |
| Lignin                 | 6.75 | 7.10 | 14.13       | 14.23       | 10.30            | 10.88       | 1.57         | 2.37         | 4.31       | 5.65      |

¹NDF with sodium sulfite, amylase and ash correction; TMR: Total mixed ration.

48 hr and placed in a ball mill loaded with ceramic balls (1-2.60 mm, milling time: 15 min at 80 rpm). The forage samples were sieved for calculation of physically effective fiber index (particles greater than 1.18 mm) prior to (pef) and followed milling (pefsm).¹¹ Fragility was determined as a change in proportion of particles greater than 1.18 mm sieved by dry vertical sieving of the ball-milled forage from the original sample:

\[
\text{Fragility} = \left(\frac{\text{pef} - \text{pefsm}}{\text{pef}}\right) \times 100
\]

**In situ incubations.** The iNDF concentration of each feed sample (2.00 mm screen) was determined following in situ incubations for up to 96, 120 and 240 hr in the rumen. All samples (5.00 g) were weighed into polyester bags (7 cm × 8 cm) with a pore size of 15.00 ± 2.00 μm and a pore area equal to 6.00% of the total surface area and incubated in duplicate within each cow.¹³ Samples of the internal dimensions of the nylon bags and the sample size were adjusted to give a sample size to surface area ratio of 10 mg per cm². After removal, the bags were rinsed twice (for 12 min) in 25 °C water in a washing machine, boiled for 1 hr in neutral detergent solution including sodium sulfite (100 mL g⁻¹ of sample) and thoroughly rinsed twice with boiling water.¹⁶ The washed samples were rinsed twice with 30 mL of acetone, allowing for 2 min soak with each rinse, dried at 100 °C for 24 hr and weighed.¹⁹

The bag residues were analyzed for NDF and ash content. The iNDF content and iNDF 2.40 were calculated according to NDF content of the bag residues and 2.40 times of acid detergent lignin (ADL), respectively.²⁰

The pdNDF content was calculated based on difference of total NDF and iNDF and NDFD of forages was determined based on methods outlined by Grant as follows:¹¹²¹

\[
\text{NDFD}_i = 100 - \text{iNDF}_i
\]

\[
\text{pdNDF}_i = \text{NDF} - \text{iNDF}_i
\]

**Statistical analysis.** The complete data set was analyzed as a completely randomized design using PROC GLM of SAS (version 9.1; SAS Institute Inc., Cary, USA). Least square means were adjusted by Tukey and separated using PDIF option. Additionally, PROC REG was used to investigate the relationship between different measurements. Data were presented as least square means and corresponding standard errors.

**Results**

**Chemical analysis.** Chemical compositions of tested feed samples are represented in Table 2. Calculated iNDF and pdNDF values of the feed samples and TMRs are also reported in Tables 1 and 3. All of the feeds displayed a chemical composition within expected ranges. Concentrations of iNDF within each sample varied according to incubation time (p < 0.01). Generally, iNDF was higher in forage than concentrate samples. The TMR rations were contained different ingredient but formulated to have similar chemical composition (Table 2). The range for lignin concentration was very wide from 1.57 to 14.23% of DM for barley and wheat straw, respectively.
enhancement in NDFD. It does make sense that above about 55.00% of the variance in fragility, the R² value was 59.19% compared to 77.72% of DM for alfalfa hay and corn silage (Table 4). The fragility indices in alfalfa hay and wheat straw were higher and lower than other forage samples, respectively (49.49% of DM versus 13.98% of DM). Orchard grass hay had lower fragility than alfalfa hay and corn silage (p < 0.01). This result confirmed the reduction of fragility as a function of particle size. In the case of orchard grass, it had shown a greater impact than alfalfa hay and corn silage. The NDFD coefficient after 240 hr of ruminal digestion showed that for the orchard grass hay, barley grain and beet pulp measured values were greater than estimated values using lignin content.

The values for pdNDF are presented in Table 3. As shown, pdNDF in alfalfa hay and orchard grass hay had higher (p < 0.01) digestion rates than corn silage (at 96 hr incubation time). In the case of other feeds, pdNDF rates were slow. However, digestion rate for potentially digestible fraction of NDF can have a big impact on ruminal digestion extent. The pdNDF digestibility of alfalfa hay was the same as corn silage one (at 240 hr incubation) alongside with a quite different process. In corn silage, larger fractions of potentially digestible fiber digest slowly, but in the case of alfalfa hay, a smaller proportion of potentially digestible fiber accompanied with higher digestion was found compensating greater INDF pool.

### Frailty and NDF digestibility

Physical affectivity of fiber was shown to be affected by ball milling (Table 4). The fragility indices in alfalfa hay and wheat straw were higher and lower than other forage samples, respectively (49.49% of DM versus 13.98% of DM). Orchard grass hay had lower fragility than alfalfa hay and corn silage (p < 0.01). This result confirmed the reduction of fragility as a function of particle size. In the case of orchard grass, it had shown a greater impact than alfalfa hay and corn silage. The NDFD coefficient after 240 hr of ruminal incubation was greater for alfalfa hay than other forage samples. The in situ 240-hr NDFD for wheat straw was 59.19% compared to 77.72% of DM for alfalfa hay (p < 0.01) and averaged 74.94% of DM for orchard grass hay and corn silage (Table 4).

Figure 2 shows the regression line between fragility and NDFD after 96, 120 and 240 hr of incubation, respectively. There is a relationship between fragility and NDFD up to a certain point, perhaps ~55.00% of fragility. The R² indicates that the fragility explains about 55.00% of the variation in NDFD. However, beyond this point, there is no relationship between fragility and NDFD. It does make sense that above certain fragility, greater fragility results in no further enhancement in NDFD.
Discussion

Within tested samples, forages had higher NDF and slightly higher iNDF contents than concentrate sources (Tables 1 and 2). In wheat straw and corn silage, the NDF are mainly concentrated in vascular tissues of leaves and stems. Progressing with maturity, NDF are increasingly lignified and the digestibility is declined. In alfalfa hay and orchard grass, the NDF are likewise concentrated in vascular tissues of stems and only to small amounts in the leaves. Higher leaf to stem ratio and low NDF content of the leaves resulted in higher forage fragility (Table 4). The

Table 4. Fragility and NDF digestibility (NDFD as % of dry matter) of forages after 96, 120 and 240 hr in situ incubations.

| Item                          | Alfalfa hay | Wheat straw | Orchard grass hay | Corn silage | SEM  | p-value |
|-------------------------------|-------------|-------------|-------------------|-------------|------|---------|
| Fragility (%)                 |             |             |                   |             |      |         |
| Initial physical effective factor | 88.93<sup>b</sup> | 95.06<sup>a</sup> | 82.76<sup>c</sup> | 96.63<sup>a</sup> | 0.743 | < 0.0001 |
| After milling physical effective factor | 44.93<sup>d</sup> | 81.76<sup>a</sup> | 63.56<sup>c</sup> | 70.33<sup>b</sup> | 0.410 | < 0.0001 |
| Fragility index               | 49.46<sup>a</sup> | 13.98<sup>d</sup> | 23.19<sup>c</sup> | 27.21<sup>b</sup> | 0.990 | < 0.0001 |
| Un-fragility                  | 50.53<sup>d</sup> | 86.01<sup>a</sup> | 76.81<sup>b</sup> | 72.78<sup>c</sup> | 0.990 | < 0.0001 |
| NDFD (%)                      |             |             |                   |             |      |         |
| 96 hr                         | 71.22<sup>a</sup> | 54.98<sup>b</sup> | 65.84<sup>a</sup> | 65.71<sup>a</sup> | 15.063 | < 0.005 |
| 120 hr                        | 75.37<sup>b</sup> | 54.67<sup>c</sup> | 70.94<sup>b</sup> | 71.26<sup>b</sup> | 1.223 | < 0.0001 |
| 240 hr                        | 77.72<sup>b</sup> | 59.19<sup>c</sup> | 73.90<sup>b</sup> | 75.98<sup>c</sup> | 6.600 | < 0.0001 |

Means within a row with different superscripts differ (p < 0.05).

**Fig. 1.** Comparisons between different values of iNDF concentration of feed samples at 120 hr and 240 hr in situ incubation times and estimation of indigestible fiber by lignin × 2.40 (iNDF<sub>2.40</sub>). Mean values with different letters are significantly different at (p < 0.05).

**Fig. 2.** Relationship of the 96-hr (A), 120-hr (B) and 240-hr (C) in situ NDF digestibility of forages with fragility index of the forages as measured by change in physical effectiveness factor following ball milling.
NDFD was reported to be a function of various factors including forage species, maturity stage, number of harvest, latitude and climate.\textsuperscript{10} Van Soest has outlined that the nature and extent of lignification in forage cell walls control NDF.\textsuperscript{7} The results showed that lignin cannot be an accurate estimator for iNDF or pdNDF contents, because lignin is not an uniform chemical entity of the forage cell wall.\textsuperscript{12} This results were confirmed by other studies\textsuperscript{22,23} attempted to describe that forage iNDF from lignin contents generally have low accuracy and precision. Reaching a point where the sample residue weight does not change significantly with additional hr of fermentation is a measure of uNDF. As iNDF influence ruminal retention, digestion and passage dynamics and physical effectiveness of the fiber, they can be used for effective estimation of digestion rates and extent.\textsuperscript{24} In this study, for accurate estimation of the values of iNDF and pdNDF of feeds, the values obtained at different time in \textit{in situ} incubation (Tables 2 and 3), because a single time point assay is not a direct measure of iNDF and pdNDF or rate of fiber digestion. Lopes \textit{et al.} have suggested that the use of a single-time point incubation to predict NDFD is not adequate.\textsuperscript{19} A single-time point \textit{in vitro} NDFD assay simply indicates how much residual fiber remains after a specific period of exposure to rumen fluid. The measured residue includes not only the indigestible fiber fraction, but also potentially digestible fiber did not degrade. Lopes \textit{et al.} have showed that for each percentage unit increase in iNDF content, 0.96 percentage units reduction in total-tract NDFD can be expected.\textsuperscript{19} This relationship confirms the importance of an accurate iNDF measurement in forage evaluation and to NDFD prediction models development.\textsuperscript{13} Huhtanan et al. have noted that a moral model of NDFD estimation has to separate NDF into indigestible and potentially digestible fractions.\textsuperscript{23}

In the present study, all of the samples were analyzed for lignin by solubilization of cellulose in 12 M sulfuric acid after extraction with acid detergent according to the procedure described by Gomes \textit{et al.} and iNDF were estimated by long-term rumen incubation and 2.40 folds of the lignin content.\textsuperscript{17} However, the measured \textit{in situ} values at 120 and 240 hr were significantly different (Fig. 1, p < 0.05) compared to those predicted by iNDF 2.40. These differences between measured and calculated can potentially bias rate and extent of NDFD and dietary energy predictions.\textsuperscript{22} Lower accuracy of iNDF 2.40 values in the present study is in line with other studies tried to predict forage iNDF fraction via ADL content.\textsuperscript{7,24} Huhtanan \textit{et al.} have revealed that the fixed relationship between lignin and iNDF is not fitting and cannot accurately describe the digestible pool of NDF in all of the feed classes.\textsuperscript{23} Generally, lignin has been regarded as a primarily limiting factor in forage digestion. Van Soest \textit{et al.} have confirmed the linear relationship (R\textsuperscript{2} = 0.94, iNDF = 1.89) between lignin and iNDF for various forage sources.\textsuperscript{32} Despite the biological relevance of using lignin in iNDF predictions, it has been shown to be unsuccessful
when compared across the years and forage types. Even though, the 2.40× as a predictive coefficient was confirmed between permanganate lignin and iNDF by Huhtanen et al. This index for individual forage species (primary and regrown grasses, red clover and whole-crop cereals) varied between 2.80 and 5.50. Krämer et al. have observed an even greater range (from 0.30 to 4.70) for the relationship between iNDF and acid detergent lignin ADL when concentrates and byproducts were evaluated alongside different types of forages.

In addition to inconsistent inter- and intra-laboratorial lignin analysis results, lignin is not a uniform entity of the cell walls. Unpredictable relationships between lignin and indigestible NDF fraction, can be attributed to variances in cross-links of the lignin and cell wall carbohydrates among different forage species and maturity stages. Different factors such as adopted methods for ADL, iNDF and non-lignin characteristics of cell walls were reported to affect NDF indigestibility estimates. Furthermore, different environmental factors such as temperature and light intensity have been reported to affect relationship between lignin and cell wall carbohydrates. In a recent study using tropical forages, lignin analyzed by several methods was significantly correlated with iNDF concentration, but the resulting prediction errors were relatively high (58.70 to 87.30 g kg⁻¹ of NDF). This may reflect high errors in the determinations of iNDF concentration, but the resulting prediction errors were relatively high (58.70 to 87.30 g kg⁻¹ of NDF). This may reflect high errors in the determinations of iNDF and lignin, despite protein contamination correction of the latter. As a result, iNDF estimations based on lignin content of feed cannot be reasonably estimated.

In this experiment, the fragility indices of the forage samples via ball milling were measured and their relationships with NDFD were assessed. Fragility index as an important factor has been reported to influence chewing response of dairy cattle. A fragility index of 0, reveals very tough sample, with no reduction of particle size upon ball milling and a 100 percent index is parallel with complete particle size reduction to less than 1.18 mm, 

\[
\text{pen} = \text{pef}_{\text{BM}}\text{.11}
\]

As shown in Table 4, there were significant differences in forages fragility as measured by changing in particle size. This can be a hint to accounting forage fragility index in models to predict chewing activity, ruminal retention, passage rate and digestibility. Fragility may be related to lignin content and digestibility as well as some of the anatomical differences such as cell wall thickness among forages. According to Table 4 the fragility index was lower for wheat straw and orchard grass compared to alfalfa hay and corn silage, respectively. The greater NDFD of alfalfa hay versus wheat straw can be related to the greater susceptibility to particle size reduction. This likely reflects the lower NDF and ADL contents for alfalfa hay as opposed to wheat straw. Forage NDFD can be used successfully as a diagnostic tool to evaluate forage quality when NDF concentrations are similar, but it cannot be used directly in rations formulation. Figure 1 shows relationships between fragility and NDFD at 96, 120 and 240 hr, respectively. Grant and Zali et al. have observed that there is a positive relationship between NDFD and fragility, it appears that fragility may be more useful in adjusting peNDF values than digestibility. Grant has concluded that NDFD and fragility are related and this relationship can be used to improve predictions of chewing response to peNDF. Because measurements of NDFD and fragility can be highly variable, it is possible that a ball milling method would have much less variation associated with it. In line with that, the relationship between NDFD and fragility is needed to be tight. More samples are needed to be analyzed to know the true relationship between NDFD and fragility, although at this point, we are assuming that the general relationships shown in 1 are true.

In conclusion, although lignin plays a central role in cell wall degradation and iNDF of plant materials, its concentration cannot be used for estimation of iNDF or pdNDF digestibility across a wide range of feed samples. Determination of uNDF should be included in routine forage and feed analysis because iNDF are uniform feed fractions with a predictable digestibility (i.e. 0). In contrast, NDF are non-uniform feed fractions containing multiple pools that digest predictably as a primary function of lignification. Further development of mechanistic models will be required for proper disclosing of diet composition effects on iNDF concentrations. Thus, the in situ incubation method can be considered as an invaluable tool in forage evaluating techniques in ruminant nutrition. The relationship of NDFD and fragility can be used to improve prediction of chewing response to peNDF. Assessment of forage physical properties can be used to precisely predict chewing and productive responses of dairy cattle.

Acknowledgments

Authors would like to thank Urmia University for funding, laboratory and technical supports.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Cotanch KW, Grant RJ, Van Amburgh ME, et al. Applications of uNDF in ration modeling and formulation. New York, USA: Miner Agricultural Research Institute 2014;10-38.
2. Zali SM, Teimouri Yansari A, Jafari Sayyadi A. Effect of particle size and fragility of corn silage and alfalfa hay on intake, digestibility, performance, and chewing activity of fattening male lambs. Res Rev J Vet Sci 2015; 1:47-57.
3. Allen M.S, Mertens DR. Evaluating constraints on fiber digestion by rumen microbes. J Nutr 1988; 118: 261-270.
4. Poppi DP, France J, McLennan SR. Intake, passage and digestibility. In: Theodorou MK, France J (Eds). Feeding systems and feed evaluation models. Oxfordshire, UK: CAB International 2000; 35-52.
5. Wilkins RJ. Potential digestibility of cellulose in grasses and its relationship with chemical and anatomical parameters. J Agric Sci 1972; 78: 457-464.
6. Huhtanen P, Nousiainen J, Rinne M. Recent developments in forage evaluation with special reference to practical applications. Agr Food Sci 2006; 15: 293-323.
7. Van Soest PJ. Nutritional ecology of the ruminant. 2nd ed. Ithaca, USA: Comstock Pub. Associates 1994; 476.
8. Ellis WC, Poppi DP, Matis JH, et al. Dietary-digestive-metabolic interactions determining the nutritive potential of ruminant diets. Champaign, USA: American Society of Animal Science 1999; 423-481.
9. Lippke H. Regulation of voluntary intake of ryegrass and sorghum forages in cattle by indigestible neutral detergent fiber. J Anim Sci 1986; 63: 1459-1468.
10. Minson DJ. Forage in ruminant nutrition. San Diego, USA: Academic Press 1990.(483):9-59.
11. Grant R. Forage fragility, fiber digestibility and chewing response in dairy cattle. In proceedings: Tri-state dairy nutrition conference. Columbus, USA 2010; 27-40.
12. National Research Council. Nutrient requirements of dairy cattle. 7th ed. Washington DC, USA: National Academies Press 2001: 20-35.
13. Krizsan SJ, Huhtanen P. Effect of diet composition and incubation time on feed indigestible neutral detergent fiber concentration in dairy cows. J Dairy Sci 2013; 96: 1715-1726.
14. Feldsine P. AOAC International methods committee guidelines for validation of qualitative and quantitative food microbiological official methods of analysis. J AOAC Int 2002; 85: 1187-1200.
15. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci 1991; 74: 3583-3597.
16. Mertens DR, Allen M, Carmany J, et al. Gravimetric determination of amylose-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles. Collaborative study. J AOAC Int 2002; 85: 1217-1240.
17. Gomes DI, Detmann E, Filho SCV, et al. Evaluation of lignin contents in tropical forages using different analytical methods and their correlations with degradation of insoluble fiber. Anim Feed Sci Technol 2011; 168: 206-222.
18. Cotanch KW, Grant RJ, Darrah J, et al. Development of a method for measuring forage fragility. J Dairy Sci 2007; 90 (Suppl 1): 563.
19. Lopes F, Ruh K, Combs DK. Validation of an approach to predict total-tract fiber digestibility using a standardized in vitro technique for different diets fed to high-producing dairy cows. J Dairy Sci 2015; 98: 2596-2602.
20. Chandler JA. Predicting methane fermentation biodegradability. USA: Cornell University 1980; 93-107.
21. Raffrenato E, Van Amburgh ME. Development of a mathematical model to predict sizes and rates of digestion of a fast and slow degrading pool and the indigestible NDF fraction. In proceedings: Cornell nutrition conference for feed manufacturers. New York, USA; 2010; 52-65.
22. Mertens DR. Using uNDF to predict dairy cow performance and design rations. Belleville, USA: Mertens Innovation & Research LLC 2016; 12-19.
23. Lucas HL. Stochastic elements in biological models, their sources and significance. In: Gurland J (Ed). Stochastic models in medicine and biology. Madison, USA: Univ Wisconsin Press 1964; 355-384.
24. Mertens DR. Kinetics of cell wall digestion and passage in ruminants. In: Jung HG, Buxton DR, Hatfield RD, et al (Eds). Forage cell wall structure and digestibility. Madison, USA: American Society of Agronomy 1993; 535-570.
25. Mertens DR, Taysom D, Steinlicht B. Factors affecting in vitro undegested NDF as estimates of indigestible NDF. J Dairy Sci 2012; 93: 291.
26. Goering HK, Van Soest PJ. Forage fiber analysis (apparatus, reagents, procedures and some applications). Washington DC, USA: Agricultural Research Service 1970; 20.
27. Rinne M, Huhtanen P, Jaakkola S. Grass maturity effects on cattle fed silage-based diets. Cell wall digestibility, digestion and passage kinetics. Anim Feed Sci Technol 1997; 67: 19-35.
28. Rinne M, Huhtanen P, Jaakkola S. Digestive processes of dairy cows fed silages harvested at four stages of maturity. J Anim Sci 2002; 80: 1986-1998.
29. Koukolová V, Wisbjerg MR, Hvelplund T, et al. Prediction of NDF degradation characteristics of grass and grass/clover forages based on laboratory methods. J Anim Feed Sci 2004; 13: 691-708.
30. Mertens DR. Rate and extent of digestion. In: Dijkstra J, Forbes JM, France J (Eds). Quantitative aspects of ruminant digestion and metabolism. 2nd ed. Wallingford, UK: CAB International 2005; 13-47.
31. Robinson PJ, Fadel JG, Tamminga S. Evaluation of mathematical models to describe neutral detergent residue in terms of its susceptibility to degradation in the rumen. Anim Feed Sci Technol 1986; 15: 249-271.
32. Van Soest PJ, Van Amburgh ME, Robertson JB, et al. Validation of the 2.4 times lignin factor for ultimate extent of NDF digestion, and curve peeling rate of
fermentation curves into pools. In proceedings: Cornell
nutrition conference for feed manufacturers. New
York, USA 2005; 139-149.
33. Huhtanen P, Asikainen U, Arkkila M, et al. Cell wall
digestion and passage kinetics estimated by marker
and in situ methods or by rumen evacuations in cattle
fed hay 2 or 18 times daily. Anim Feed Sci Technol
2007; 133: 206-227.
34. Raffrenato E, Fievisohn R, Cotanch KW, et al. Effect of
lignin linkages with other plant cell wall components
on in vitro and in vivo NDF digestibility of forages and
potential energy yield. In proceedings: 3rd EAAP
international symposium on energy and protein
metabolism and nutrition. Parma, Italy 2010; 723-724.
35. Krämer M, Weisbjerg MR, Lund P. Estimation of
indigestible NDF in feedstuffs for ruminants. In
proceedings: 1st Nordic feed science conference.
Uppsala, Sweden 2010; 15-20.