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Review

The Role iNDF in the Regulation of Feed Intake and the Importance of Its Assessment in Subtropical Ruminant Systems (the Role of iNDF in the Regulation of Forage Intake)

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Abstract: The intake and digestibility of forages is largely influenced by the fibre content and specifically the neutral detergent fibre (NDF). Currently, the focus in commercial diet formulation and the modelling of animal performance is on the total NDF so as to achieve higher ruminant feed intakes, higher production performance and rumen health. Rations are often formulated for a specific level of NDF in the diet assuming that the digestibility of NDF operates over a narrow range. Forage NDF, particularly in C4 forages, varies greatly in potential digestibility within the rumen. This potential digestibility is defined as the NDF fraction which disappears after a long incubation period and the remaining indigestible component of NDF (iNDF) is unavailable for microbial digestion. It is hypothesized that this dietary iNDF has an important role in contributing to rumen digesta load and voluntary intake. Formulating a diet to a specific level of NDF without reference to the iNDF could markedly affect the resulting intake, digestibility and metabolisable energy (ME) content of the diet. It is concluded that nutritional models need to be modified to accept directly determined iNDF.

Keywords: iNDF; NDF; C4 forages; intake

1. Introduction

The digestibility of forage and the capacity of ruminants to consume it, are largely influenced by its content of neutral detergent fibre (NDF). Currently, the focus in commercial diet formulation and the modelling of animal performance is on the total NDF to optimise feed intakes and productivity without unduly compromising rumen health. Diets are often formulated to a specific level of NDF with an underlying assumption being that the digestibility of that NDF spans a narrow range. When feeding temperate forages or corn silage this assumption holds true, however, when considering a broader selection of tropical (C4) forages the NDF does vary in ruminal digestibility, to an important extent. This digestibility of the fibre is directly related to potentially digestible portion of NDF (pdNDF) [1,2]. Potential digestibility is defined as the NDF fraction which disappears after a long incubation period, leaving the indigestible component of NDF (iNDF) which is unavailable for microbial digestion [3,4]. Tropical forages can have the same NDF content but differ vastly in iNDF. Nutritional models predict dietary iNDF to be an important driver of rumen digesta load and therefore feed intake [5]. *In vivo*, strongly negative relationships between iNDF and feed intake have also been demonstrated when iNDF content exceeds 15% of total dietary dry matter [6]. *In vivo* data also implicate iNDF as a predictor of organic matter (OM) digestibility in forage-based diets [7]. Formulating a diet to a specific level of NDF without reference to iNDF could markedly affect energy supply through reduced feed intake, digestibility and metabolisable energy (ME) content of the diet. The digestible proportion of NDF has the greatest impact of all energy supplying nutrients on energy supply to the ruminant, in forage-based feeding systems. However, in Australia, despite the importance of iNDF, few nutritionists knowingly take iNDF into direct account when formulating diets. Moreover, iNDF values for a range of forages commonly used in diets are not readily available in feed analysis databases. Better assessment and awareness amongst nutritionists of the importance of iNDF for the range of tropical pasture species commonly used will improve the capacity of nutritionists to predict NDF digestibility and therefore to more effectively develop balanced diets.

2. Defining NDF

The NDF is important as a source of ME, a controller of rumen turnover rate, and is required to stimulate saliva production, which buffers the rumen thereby promoting rumen health. The NDF comprises the cell wall fraction of forages and includes a complex matrix of lignin, small amounts of protein, and various polysaccharides, particularly cellulose, hemicellulose and pectin [8]. Cell wall structure and composition vary greatly across species, plant tissues, and within a plant as it matures. The polysaccharides of plant cell walls are potentially digested by the rumen bacteria, protozoa and fungi. Classical reviews [9–12] identify the significance of the anatomical and chemical structure of leaf and stem, and the detrimental effect of lignin on the capacity of rumen microbes to digest NDF. In initial development, a plant cell wall consists of a primary wall principally made of cellulose. After the cell completes growth and elongation, the secondary thickening in the cell wall occurs with large proportions of cellulose and hemicellulose being laid down. In the rumen, cellulose and hemicellulose are slowly digested, but given enough time can be completely digested if these components are not protected by lignin [13]. Once the secondary wall deposition is completed the primary wall is lignified and the

lignification then progresses to deposition of the secondary wall which occurs on the inner surface of the cell lumen. The concentration of lignin is highest in the primary wall but because the secondary wall is greater in volume and mass it contains the greater amount of lignin [14]. Also during the maturation phase there is a greater accumulation of stem mass compared with leaf material with the stems containing more tissue with secondary thickening [15] and subsequently higher concentrations of cellulose, xylan and lignin [16]. In the rumen the lignin component is the primary factor responsible for limiting the digestion of the cell walls [16] and its presence further restricts the digestion of the polysaccharide fraction to which it is cross-linked.

Non-lignified cell walls parts can be accessed by rumen bacteria either on the external exposed surface of the cell wall, internally through the lumen and via adjoining cells walls [17,18]. A high concentration of lignin in the primary walls creates a barrier to microbes, thus preventing total digestion of the plant cell [15,19]. Rumen bacteria digest cells from the interior initially attacking the secondary wall and then progressively digesting the primary wall [19]. The low accessibility of the secondary wall surface to the rumen microorganisms highlights the importance of the mastication and rumination process that causes the physical disruption of the lignified plant cells [15], as well as increasing the surface area available for microbial colonization [16]. It is estimated that within an ingested piece of a typical grass forage approximately one third of the cells leave the rumen without being digested due to the inaccessibility or lack of exposure to rumen microbes [12].

Lignin is a non-carbohydrate polymer composed of phenolic units that are extensively cross-linked to form a lignin-carbohydrate complex. The chemistry of forage lignin and the biosynthesis of various forms have been reviewed by Jung (2011) [20]. Forage lignin is a mixture of guaiacyl and syringyl monolignols with the latter increasing in proportion with maturity. The nature of the lignin-carbohydrate complex varies with the type of cell wall and the species of plant [21]. In grasses, lignin is bound to the hemicellulose fraction through ferulate cross-links which result in lower cell wall digestibility, independent of lignin concentration [22,23]. Various corn mutants, e.g., brown midrib mutants, have been discovered with reduced ferulate ester deposition resulting in improved cell wall digestibility [24]. It is unknown whether C4 forages have a greater amount of ferulate cross-links and would be worthy of examination. In legumes, it is assumed that lignin is cross-linked to the cell wall polysaccharides but the chemical linkage has not been identified [25]. More needs to be understood about the structure and chemistry of lignin within forage tissues if the often presumed predictive relationship between lignin and dietary energy supply is to be improved. Alternatively and more practically, a direct determination of iNDF may circumvent the need to use lignin as a predictor.

3. Indigestible NDF and Its Importance to Voluntary Feed Intake

NDF has been proposed as a reliable predictor of voluntary dry matter (DM) intake under certain conditions [26]. The relationship between NDF content and food intake is complex and not a linear relationship [27]. The amount and quality of NDF in a diet can either enhance or limit intake. At lower NDF concentrations (7.5%–35.5%), DM intakes increased with increasing dietary NDF concentration, but DM intakes decreased sharply as NDF concentration increased over the range of 22.2%–45.8% in high-producing animals [28]. Low DM intakes at higher NDF contents is associated with rumen fill constraints [2]. The relationship between DM intake and NDF is more than just NDF content in the diet

but also dependent on the potential digestibility of NDF (pdNDF) [2,3,29]. The pdNDF fraction, is defined as the difference between the NDF and iNDF. It is the iNDF component that is the rate-limiting constituent of forages at higher NDF level [6].

The iNDF is unavailable to microbial digestion in ruminants even if the total tract residence time of fibre is extended to effectively an infinite time [30]. The lack of digestibility in the iNDF fraction of forage is attributable to the cross-linking between cell wall lignin and hemicellulose [31].

A higher iNDF intake limits a ruminant's ability to consume sufficient forage to meet nutrient requirements. The intake of forage-based diets by ruminants is often controlled by rumen fill and the rate of disappearance [32]. The rate of disappearance is largely influenced by the inherent rate of digestion and passage rate [1,2]. The indigestible portion is removed from the rumen by passage only [3] and will accumulate in the rumen relative to the potentially digestible portion [1], therefore having a longer rumen retention time [33]. A longer retention time in the rumen results in a lower intake [34]. According to Ellis *et al.*, (1999) [5] the determination of iNDF should be included in all basic feedstuff analysis because it is an ideal fraction which has zero digestibility and can be used for the estimation of pdNDF. It is therefore recommended that there should be a defined proportion of iNDF in the diet. Lippke (1986) [6] suggested that maximum iNDF consumption is about 20 g/kgBW$^{0.75}$.day, however more research is required to resolve if this value is relevant for different production systems and different forages.

4. The Use of NDF in Diet Formulation

Currently the commercial focus in diet formulation and modelling of higher performance ruminant animals is on total NDF so as to achieve higher intakes of diets containing a higher energy content. The National Research Council [35] recommends dairy cow diets should contain approximately 30%–35% NDF in the DM. This range represents a balance between increasing voluntary intake of DM and milk production without compromising milk fat [28]. This low level of NDF can be difficult to achieve in subtropical/tropical areas due to the predominance of high NDF forages and so 40% is considered a more achievable target in subtropical grazing systems. In terms of feedlot beef production, the NDF content in the diet is often much lower (16%) [28] due to the high starch diets. Lower levels of NDF in the diet could cause rumen dysfunction therefore a minimal level of NDF is required for animal health, whilst still meeting market specifications.

Balancing diets for high-producing ruminants based on NDF content is still problematic due to the high variability in forage NDF. In our laboratory 200 summer dairy forages (temperature and subtropical) were analysed for NDF where content ranged from 20%–80%. This is not dissimilar to the range (30%–80%) published by Jung and Allen (1995) [36]. The NDF content of a forage increases as the plant matures [16] and is also dependent on the growth environment [16] including temperature, light intensity, water availability and latitude. Even accounting for the large variation in NDF contents of forages, the relationship between NDF level and animal performance often does not hold. Even when diets are composed of similar NDF concentrations the NDF in the constituent forages may have variable degrees of potential digestibility. This variability is especially apparent in subtropical areas. In the analyses of the 200 summer dairy forages the pdNDF ranged from 26% to 90% of the NDF. These large differences in the pdNDF will have a large impact on DM intake. When ruminants are fed a forage diet,

increased fibre digestibility generally improves animal performance. Accurate estimation of pdNDF is a prerequisite for improving diet formulation, forage evaluation and productivity responses.

A model based on the concept of potential digestibility was developed by Waldo *et al*., (1972) [3] and is still useful for the examination of intake regulation and digestion [1,2]. Indigestible NDF is a prime determinate of the utilization of forages in many models [37]. Nutritional models such as Cornell Net Carbohydrate and Protein System (CNCPS) [38,39] and the Nordic model of dairy cow metabolism "Karoline" [40,41] also utilise an indirect calculation of iNDF to calculate OM and NDF digestibility, rumen NDF pool and microbial N flow which consequently has effects on the supply of energy and microbial protein.

The effect iNDF content has on food intake is especially apparent in grazing ruminant systems in subtropical and tropical regions. The challenge from the producer's perspective is to ensure higher intakes from forage. Increased production can be achieved by ensuring the iNDF content of the diet is low without necessarily decreasing the NDF content of the diet. This would involve identifying agronomic options to improve forage quality by lowering the iNDF content of both pasture and silages and requires a better assessment of the iNDF content rather than relying on just the NDF content.

However, despite the known importance of iNDF in both intensive and extensive ruminant production systems, particularly in subtropical and tropical areas, such data are still not present in readily available feed analysis data bases used in Australia. The actual values do not exist in well-known databases such as Feedipedia or in feed tables associated with popular nutritional models. The most likely explanation for this lack of information is due to the difficulty in assessing iNDF.

5. Assessment of iNDF

There are a number of methodologies including *in situ* or *in vitro* techniques, used in the determination of iNDF. The *in situ* techniques attain the degradability of forages by incubating samples in nylon bags which are placed in the rumen of fistulated animals. Bags are then extracted at 10–12 days. This technique is a useful predictor as it ferments feed in the actual rumen environment, and requires minimal effort to perform [42,43]. However, this technique often has poor repeatability due to a lack of standardized procedures [42,44,45].

The alternative long term ruminal *in vitro* methodology is more suitable for larger scale feed evaluations and is more cost effective. Commercial forage testing laboratories offer *in vitro* assays for NDF digestibility, however there is no standard analytical procedure, therefore results across laboratories are essentially not comparable. In addition these are generally short term fermentations (24 h or 48 h) and do not measure the actual iNDF component which require the 10 day fermentation. *In vitro* methodologies are commonly modifications of the Goering and Van Soest (1970) [46] *in vitro* procedure whereby forage is incubated in ruminal fluid, buffer and minerals, followed by a neutral detergent rinse and the residues dried and weighed. This technique is a modification of the two-stage *in vitro* DM digestion technique of Tilley and Terry (1963) [47], whereby feed is incubated in ruminal fluid and buffer, the remaining solid residue is then incubated in acid pepsin to mimic the digestion in the abomasum and the final residues are then dried and weighed. The use of rumen fluid provides the system with a large array of enzymes to effectively digest the feed samples, however, there is significant inter-assay error introduced by the rumen fluid inoculum due to the variable activity related to the host

animal from which it is collected and its dietary quality. To maximise the effectiveness of the rumen fluid it is advised that the donor animals should be fed a diet similar to what is being analysed [42,44], the rumen ammonia level must be above 50 mg/L [48], fluid needs to be collected at a fixed time, contamination with oxygen and saliva needs to be avoided, the collected rumen fluid needs to be kept at 39 °C, and there needs to be a rapid transfer to the *in vitro* system [49]. The development of the ANKOM Daisy system was based on a modification of the Goering and Van Soest (1970) [50] technique This technique uses the F57 Ankom filter bags which streamline NDF analysis following digestion, by eliminating the need to filter digesta samples. With all long term fermentations, media needs to be replenished after five days.

Any inter-laboratory variations in the determination of digestion rates and iNDF must be reduced if meaningful estimates of ruminal degradation can be generated for use in predictive models. Some sources of variation are inevitable due to rumen fluid variations, but a large amount of variation can be reduced with the adoption of a common methodology such as the standardisation of forage grinding length, bag porosity, open surface area, and sample weight to bag size [42,44].

Many laboratories find long term fermentations for determining the iNDF fraction laborious and expensive therefore many nutritional models attempt to predict iNDF from the acid detergent lignin (ADL) concentration [51–53]. Chandler (1980) [51] estimated the iNDF fraction as the measured ADL content multiplied by 2.4 and this relationship is used in a number of laboratories and nutritional models. This concept was validated using several forage species (corn, alfalfa, grasses and wheat straw) and obtained a satisfactory regression (R^2 = 0.94) between observed and predicted iNDF [54]. This (2.4) factor is presumed to be universal across forage species and growth environments. However, this simplistic mathematical relationship does not hold across C4 forages where large variation in the iNDF/ADL relationship is observed [55]. In this study a diverse set of forages were analysed for iNDF using long term fermentation and was also analysed for ADL. The iNDF/ADL ratios ranged from 1.5 to 6.2 and averaged 3.82 which were markedly higher than the 2.4 ratio used in forages in the CNCPS model used to predict iNDF. This work is supported by other studies [30,56] where attempts to describe forage iNDF from ADL contents generally had low accuracy and precision.

It has been suggested that specific equations for forage groups, based on morphological similarities, may improve the accuracy of iNDF prediction from ADL content [44,57]. In the study by Harper *et al.*, (2014) [55] the iNDF/ADL ratio variation within most subtropical forage groups, was very large. The iNDF/ADL ratio for kikuyu pasture ranged from 1.4–4.8. Variability within single forage species was also found by Huhtanen *et al.*, (2006) [30] whereby diets for individual forage species varied between 2.8 and 5.5. Therefore, iNDF estimations using a simplistic ADL model even within a forage species, would still be unacceptably inaccurate. This raises the issue of the relevance of the calculation of iNDF from ADL in models in many subtropical and tropical areas. Using this equation on C4 forages leads to inaccuracies with regard to predicting NDF digestibility of forages and would result in errors in predicting ME content.

Lignin is not a uniform entity of cell walls and the variability of the ADL relationship with iNDF is probably attributable to the differences in cross-linking between the lignin and hemicelluloses between C4 forage species and as these forages mature [31]. In addition, the ADL relationship may be prone to environmental conditions such as temperature and light intensity [30]. C4 forages are grown across variable and harsh growing conditions and have an extreme nutritive range with maturity. With this in

mind, it is not surprising that there were differences in the ADL/iNDF relationship when comparing temperate forages grown in cooler environments with C4 forages grown over a range of harsh environments.

6. Impact on the Prediction of Production

The inclusion of iNDF in nutritional models such as CNCPS, albeit indirectly, indicates its importance as a predictor of animal production. The way in which it is determined (ADL × 2.4) is likely to be unreliable in situations where C4 forages are fed. This is due to the poor relationship between iNDF and ADL found across a wide range of C4 forages. In general, an underestimation of iNDF results in the overestimation of the fractional rate of digestion (K_d) for many of the C4 forages. Modelling readily indicates how true iNDF values can change the predictions of ME supply and milk production in lactating dairy cattle [58] (Table 1). Generally the nutritional models underestimate iNDF content in most C4 forage groups and therefore over-predict potential milk production. The hypothesis that directly measured iNDF will increase model accuracy needs to be vigorously tested using C3 as well as C4 forages.

Table 1. Milk production (kg/day) and total metabolisable energy (ME) supply (MJ/day) estimates from the CNCPS, based on predicted iNDF2.4 or *in vitro* iNDF240 of forage groups relevant to dairy diets in Queensland, Australia.

Forage Group	iNDF2.4 (%DM)	iNDF240 (%DM)	K_d (%/h)	ME Supply iNDF2.4	Milk Yield iNDF2.4	ME Supply iNDF240	Milk Yield iNDF240	Δ Milk Yield
Barley silage	13.1	10.5	7	135	12.2	140	13.5	+1.3
Sorghum silage	12.7	22.4	5	117	6.9	99	0.6	−6.3
Tropical pasture	12.2	21.4	5	126	9.5	110	4.4	−5.1
Ryegrass fresh	7.9	10.3	9	146	12.5	141	11.2	−1.3

Inputs were: continuously grazed, 45 month old dairy cows, shrunk LW 576 kg, 120 days in milk, milk fat 4%, DMI 15 kg/day; iNDF240 was inputted by adjusting the ADL content as determined by iNDF240/2.4 [58].

An over-prediction of ME supply and estimated milk production by CNCPS, when iNDF is calculated from ADL in contrast to actual iNDF estimates, is due to the way in which the potential ME is estimated from the NDF fraction in this model. The potentially digested NDF, therefore the ME available, is estimated from the difference between NDF and iNDF content. In turn, the iNDF content is estimated from the ADL content multiplied by 2.4 [53]. The estimation of iNDF based on the ADL × 2.4 model has further implications on other calculations used to estimate key metabolic activities within the model. This includes the prediction of the available NDF fraction (CB3), the ruminally degraded CB3 (RDCB3), the ruminally escaped CB3 (RECB3) and ruminally escaped iNDF (RECC), which are all affected by the iNDF input [39]. These fractions comprise part of the carbohydrate intake variable which is applied in the equation for calculating ME supply. The implications are, that when the CNCPS model under-estimates the iNDF content in the forages, it could over-predict milk yield in dairy cows.

7. Alternative Assessment of iNDF

Indigestible NDF is a useful index for predicting OM digestibility and its inclusion in feed evaluation models increases accuracy [30]. Its use, however, is constrained by the difficulty in conducting long term fermentation procedures, and it is not accurately predicted from ADL content. It is therefore important to develop short-term techniques to predict iNDF. The application of near infrared reflectance spectroscopy (NIRS) is a rapid, cost effective and accurate forage analytical technique [59]. A close relationship between NIRS and iNDF of grass silage was reported by Nousiainen *et al.*, (2004) [56,60]. The NIR spectral data from a forage sample has a strong correlation with OM components such as NDF, acid detergent fibre, ADL and crude protein [61] which is directly related to organic carbon bonds. These relationships have been used as predictors of various OM components [62,63] with universal calibration equations developed from large (>100) sample sets. The prediction accuracy is dependent on a significant and strong correlation ($R^2 > 0.85$) of the spectral data and wet chemistry [64].

Robust NIRS calibrations have been developed using largely C4 forages for the prediction for iNDF [65]. The use of FT-NIR provides rapid and reproducible results. These calibrations have acceptable prediction for iNDF for use in practical feed evaluation for a broad range of forage samples. Using the iNDF values developed from NIRS calibrations would improve the accuracy and utility of feed and diet evaluation models for ruminants compared with the current technique that uses ADL to estimate iNDF values.

8. Model Recommendations

A frustration with many of the current nutrition models is that they do not allow direct input of iNDF values. The CNCPS for example, only allows direct input of ADL values and then calculates iNDF from ADL according to iNDF = ADL × 2.4. In such models, input of actual forage iNDF values can only be achieved by modifying ADL values. For example, if the intended iNDF is 12 units, then the input value for ADL needs to be 12/2.4 = 5 units. This adjustment is awkward and time consuming. Computer models need to be modified to allow easy direct input of iNDF values which are just as easily estimated as chemical determined ADL values.

9. Conclusions

The quantity of iNDF in a forage-based diet plays a significant role in the regulation of the digestibility of diet and the feed intake of ruminants to which it is offered. This is particularly apparent for C3 *versus* C4 forages, the latter having characteristics such as targeted lignification that allow the plant to cope with harsh environmental stress. These morphological and anatomical characteristics provide support and strength to C4 plants but also provide resistance to microbial degradation [15] and therefore energy availability. The C4 forages often have high NDF concentrations and, depending on stage of maturity and environmental growing conditions, have a generally higher but variable proportion of iNDF in the total NDF. A better assessment of iNDF rather than just NDF is beneficial as a model input to accurately estimate total energy intake of ruminants fed C4 forages. Long-term fermentation experiments on forages provide an accurate assessment of the iNDF content. Alternatively, the iNDF can be estimated with high accuracy and quickly using NIRS. The iNDF of individual forage samples

should be included in diet formulation models, especially for diets based on C4 forages. Nutritional models need to be modified to accept directly determined rather than mathematically derived iNDF values as inputs. This would improve the capacity of nutritionists to predict NDF digestibility and therefore balance diets more effectively, particularly in subtropical and tropical ruminant production systems.

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Author Contributions

Karen Harper conceived and wrote this review with the assistance of David McNeill.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Allen, M.S.; Mertens, D.R. Evaluating constraints on fiber digestion by rumen microbes. *J. Nutr.* **1988**, *118*, 261–270.
2. Poppi, D.P.; France, J.; McLennan, S.R. Intake, passage and digestibility. In *Feeding Systems and Feed Evaluation Models*; Theodorou, M.K., France, J., Eds.; CAB International: Oxfordshire, UK, 2000; pp. 35–52.
3. Waldo, D.R.; Smith, L.W.; Cox, E.L. Model of cellulose disappearance from the rumen. *J. Dairy Sci.* **1972**, *55*, 125–129.
4. Wilkins, R.J. Potential digestibility of cellulose in grasses and its relationship with chemical and anatomical parameters. *J. Agric. Sci.* **1972**, *78*, 457–464.
5. Ellis, W.C.; Poppi, D.P.; Matis, J.H.; Lippke, H.; Hill, T.M.; Rouquette, F.M. *Dietary-Digestive-Metabolic Interactions Determining the Nutritive Potential of Ruminant Diets*; American Society of Animal Science: Champaign, IL, USA, 1999; pp. 423–481.
6. 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.
7. Nousiainen, J.; Rinne, M.; Hellamaki, M.; Huhtanen, P. Prediction of the digestibility of the primary growth of grass silages harvested at different stages of maturity from chemical composition and pepsin-cellulase solubility. *Anim. Feed Sci. Technol.* **2003**, *103*, 97–111.
8. Moore, K.J.; Hatfield, R.D. *Carbohydrates and Forage Quality*; Alliance of crop, soul, and environmental science society: Madison, WI, USA, 1994; pp. 229–280.
9. Wilson, J.R. Influence of plant anatomy on digestion and fibre breakdown. In *Microbial and Plant Opportunities to Improve the Utilization of Lignocellulose by Ruminants*; Akin, D.E., Ljungdahl, J.R., Wilkins, R.J., Harris, P.J., Eds.; Elsevier Science Publishing Company: New York, NY, USA, 1990; pp. 99–117.

10. Wilson, J.R. Plant structures: Their digestive and physical breakdown. In *Recent Advances on the Nutrition of Herbivores*; Ho, Y.W., Wong, H.K., Abdullah, N., Tajudding, Z.A., Eds.; Malaysian Society of Animal Production: Kuala Lumpur, Malaysia, 1991; pp. 207–216.
11. Wilson, J.R. Organization of forage plant tissues. In *Forage Cell Wall Structure and Digestibility*; Jung, H.G., Buxton, D.R., Hatfield, R., Ralph, J., Eds.; ASA-CSSA-SSSA: Madison, WI, USA, 1993; pp. 1–32.
12. Wilson, J.R.; Mertens, D.R. Cell-wall accessibility and cell structure limitations to microbial digestion of forage. *Crop Sci.* **1995**, *35*, 251–259.
13. Weimer, P.J. Why don't ruminal bacteria digest cellulose faster? *J. Dairy Sci.* **1996**, *79*, 1496–1502.
14. Terashima, N.; Fukushima, K.; He, L.F.; Takabe, K. Comprehensive model of the lignified plant cell wall. In *Forage Cell Wall Structure and Digestibility*; Jung, H.G., Buxton, D.R., Hatfield, R., Ralph, J., Eds.; ASA-CSSA-SSSA: Madison, WI, USA, 1993; pp. 247–270.
15. Wilson, J.R.; Kennedy, P.M. Plant and animal constraints to voluntary feed intake associated with fibre characteristics and particle breakdown and passage in ruminants. *Aust. J. Agric. Res.* **1996**, *47*, 199–225.
16. Jung, H.G. Forage Digestibility: The Intersection of Cell Wall Lignification and Plant Tissue Anatomy. Available online: http://dairy.ifas.ufl.edu/rns/2012/12jungrns2012.pdf (accessed on 18 April 2015).
17. Jung, H.G.; Engels, F.M. Alfalfa stem tissues: Rate and extent of cell-wall thinning during ruminal degradation. *Neth. J. Agric. Sci.* **2001**, *49*, 3–13.
18. Engels, F.M.; Jung, H.J.G. Alfalfa stem tissues: Impact of lignification and cell length on ruminal degradation of large particles. *Anim. Feed Sci. Technol.* **2005**, *120*, 309–321.
19. Engels, F.M. Some properties of cell wall layers determining ruminant digestion. In *Physio-Chemical Characterization of Plant Residues for Industrial and Feed Use*; Chesson, A., Orskov, E.R., Eds.; Elsevier Applied Science: London, UK, 1989; pp. 80–87.
20. Jung, H.G. Forage lignins and their effects on fiber digestibility. *Agron. J.* **1989**, *81*, 33–38.
21. Chesson, A. Lignin polysaccharide complexes of the plant-cell wall and their effect on microbial-degradation in the rumen. *Anim. Feed Sci. Technol.* **1988**, *21*, 219–228.
22. Casler, M.D.; Jung, H.J.G. Selection and evaluation of smooth bromegrass clones with divergent lignin or etherified ferulic acid concentration. *Crop Sci.* **1999**, *39*, 1866–1873.
23. Grabber, J.H. How do lignin composition, structure, and cross-linking affect degradability? A review of cell wall model studies. *Crop Sci.* **2005**, *45*, 820–831.
24. Jung, H.G.; Phillips, R.L. Putative seedling ferulate ester (*sfe*) maize mutant: morphology, biomass yield, and stover cell wall composition and rumen degradability. *Crop Sci.* **2010**, *50*, 403–418.
25. Jung, H.G.; Raeth-Knight, M.; Linn, J.G. Forage fiber digestibility: Measurement, variability, and impact. In Proceedings of the 65th Minnesota Nutritional Conference, University of Minnesota: Minneapolis, MN, USA, 2004; Available online: http://google.sidney-aldebaran.me/url?sa=t&rct=j&q=&esrc=s&source=web&cd=2&ved=0CCUQFjABahUKEwju6K_2-ujHAhXHSo4KHWN5Boc&url=https%3A%2F%2Fbooks.google.com%2Fbooks%2Fabout%2F65th_Minnesota_Nutrition_Conference.html%3Fid%3Di2LXMgEACAAJ&usg=AFQjCNFrf8x1VOoIvKqeSh4UQSGLANmfFA (accessed on 9 September 2015).

26. Minson, D.J. *Forage in Ruminant Nutrition*; Academic Press: San Diego, CA, USA, 1990; p. 483.
27. Mertens, D.R. Regulation of forage intake. In *Forage Quality*, *Evaluation*, *and Utilization*; American Society of Agronomy, WI, USA, 1994, pp. 450–493.
28. Arelovich, H.M.; Abney, C.S.; Vizcarra, J.A.; Galyean, M.L. Effects of dietary neutral detergent fiber on intakes of dry matter and net energy by dairy and beef cattle: Analysis of published data. *Prof. Anim. Sci.* **2008**, *24*, 375–383.
29. Wilkins, R.J. Potential digestibility of cellulose in forage and faeces. *J. Agric. Sci.***1969**, *73*, 57–64.
30. Huhtanen, P.; Nousiainen, J.; Rinne, M. Recent developments in forage evaluation with special reference to practical applications. *Agr. Food Sci.* **2006**, *15*, 293–323.
31. Van Soest, P.J. *Nutritional Ecology of the Ruminant*, 2nd ed.; Comstock: Ithaca, NY, USA, 1994; p. 476.
32. Poppi, D.P. Nutritional constraints for grazing animals and the importance of selective grazing behaviour. In *Grassland Productivity and Ecosystem Services*; CABI: Wallingford, UK, 2011; pp. 19–26.
33. Poppi, D.P.; Minson, D.J.; Ternouth, J.H. Studies of cattle and sheep eating leaf and stem fractions of grasses. 2. Factors controlling the retention of feed in the reticulo-rumen. *Aust. J. Agric. Res.* **1981**, *32*, 109–121.
34. Poppi, D.P.; Minson, D.J.; Ternouth, J.H. Studies of cattle and sheep eating leaf and stem fractions of grasses. 1. The voluntary intake, digestibility and retention time in the reticulo-rumen. *Aust. J. Agric. Res.* **1981**, *32*, 99–108.
35. NRC. *Nutrient Requirements of dairy Cattle*, 7th ed.; National Academy Press: Washington, DC, USA, 2001.
36. Jung, H.G.; Allen, M.S. Characteristics of plant-cell walls affecting intake and digestibility of forages by ruminants. *J. Anim. Sci.* **1995**, *73*, 2774–2790.
37. Poppi, D.P. Predictions of food intake in ruminants from analyses of food composition. *Aust. J. Agric. Res.* **1996**, *47*, 489–504.
38. Fox, D.G.; Tedeschi, L.O.; Tylutki, T.P.; Russell, J.B.; van Amburgh, M.E.; Chase, L.E.; Pell, A.N.; Overton, T.R. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. *Anim. Feed Sci. Technol.* **2004**, *112*, 29–78.
39. Tylutki, T.P.; Fox, D.G.; Durbal, V.M.; Tedeschi, L.O.; Russell, J.B.; van Amburgh, M.E.; Overton, T.R.; Chase, L.E.; Pell, A.N. Cornell Net Carbohydrate and Protein System: A model for precision feeding of dairy cattle. *Anim. Feed Sci. Technol.* **2008**, *143*, 174–202.
40. Danfaer, A.; Huhtanen, P.; Uden, P.; Sveinbjornsson, J.; Volden, H. *The Nordic Dairy Cow Model*, *Karoline-Description*; CABI Publishing: Wallingford, UK, 2006; pp. 383–406.
41. Danfaer, A.; Huhtanen, P.; Uden, P.; Sveinbjornsson, J.; Volden, H. *The Nordic Dairy Cow Model*, *Karoline-Evaluation*; CABI Publishing: Wallingford, UK, 2006; pp. 407–415.
42. Nocek, J.E. *In situ* and other methods to estimate ruminal protein and energy digestibility—A review. *J. Dairy Sci.* **1988**, *71*, 2051–2069.
43. Damiran, D.; DelCurto, T.; Bohnert, D.W.; Pulsipher, G.D.; Findholt, S.L. Comparison of techniques and grinding size to estimate digestibility of forage base ruminant diets. *Proc. West. Sect. Am. Soc. Anim. Sci.* **2002**, *53*, 341–344.

44. Vanzant, E.S.; Cochran, R.C.; Titgemeyer, E.C. Standardization of *in situ* techniques for ruminant feedstuff evaluation. *J. Anim. Sci.* **1998**, *76*, 2717–2729.
45. Lund, P.; Weisbjerg, M.R.; Ahvenjarvi, S.; Huhtanen, P.; Uden, P.; Olafsson, B.; Volden, H. Nordic ringtest on INDF content and NDF degradation characteristics in three feeds. *J. Anim. Feed Sci.* **2004**, *13*, 139–142.
46. Goering, H.K.; Van Soest, P.J. Forage fiber analyses. (Apparatus, reagents, procedures, and some applications). In *Agriculture Handbook*, *United States Department of Agriculture*; U.S. Agricultural Research Service: Washington, DC, USA, 1970, p. 20.
47. Tilley, J.M.A.; Terry, R.A. A two-stage technique for the *in vitro* digestion of forage crops. *Grass and Forage Sci.* **1963**, *18*, 104–111.
48. Satter, L.D.; Slyter, L.L. Effect of ammonia concentration on rumen microbial protein production *in vitro*. *Br. J. Nutr.* **1974**, *32*, 199–208.
49. Rymer, C.; Huntington, J.A.; Williams, B.A.; Givens, D.I. *In vitro* cumulative gas production techniques: History, methodological considerations and challenges. *Anim. Feed Sci. Technol.* **2005**, *123*, 9–30.
50. Goering, H.K.; van Soest, P.J. *Forage Fiber Analyses* (*Apparatus*, *Reagents*, *Procedures*, *and Some Applications*); ARS-USDA: Washington, DC, USA, 1970.
51. Chandler, J.A.; Jewell, W.J.; Gossett, J.M.; Vansoest, P.J.; Robertson, J.B. Predicting methane fermentation biodegradability. *Biotechnol. Bioeng.* **1980**, *22*, 93–107.
52. Weiss, W.P.; Conrad, H.R.; Stpierre, N.R. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. *Anim. Feed Sci. Technol.* **1992**, *39*, 95–110.
53. Traxler, M.J.; Fox, D.G.; van Soest, P.J.; Pell, A.N.; Lascano, C.E.; Lanna, D.P.D.; Moore, J.E.; Lana, R.P.; Velez, M.; Flores, A. Predicting forage indigestible NDF from lignin concentration. *J. Anim. Sci.* **1998**, *76*, 1469–1480.
54. Van Soest, P.J.; Van Amburgh, M.E.; Robertson, J.B.; Knaus, W.F. 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 of the Cornell Nutrition Conference for Feed Manufactures, East Syracuse, New York, NY, USA, 18–20 October 2005; Cornell University: Ithaca, NY, USA, 2005; pp. 139–149.
55. Harper, K.J.; Barber, D.G.; Callow, M.; McNeill, D.M.; Poppi, D.P. Assessment of the iNDF of Subtropical Pastures. Available online: http://espace.library.uq.edu.au/view/UQ:354480 (accessed on 18 April 2015).
56. Nousiainen, J.; Ahvenjarvi, S.; Rinne, M.; Hellamaki, M.; Huhtanen, P. Prediction of indigestible cell wall fraction of grass silage by near infrared reflectance spectroscopy. *Anim. Feed Sci. Technol.* **2004**, *115*, 295–311.
57. Raffrenato, E.; Fievisohn, R.; Cotanch, K.W.; Grant, R.J.; Chase, L.E.; Amburgh, M.E.V. 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 of the Energy and Protein Metabolism and Nutrition. 3rd EAAP International Symposium on Energy and Protein Metabolism and Nutrition, Parma, Italy, 6–10 September 2010; Crovetto, G.M., Ed.; Wageningen Academic Publishers: Wageningen, The Netherlands, 2010; pp. 723–724.

58. Harper, K.J.; McNeill, D.M. Direct *in vitro* measurement of iNDF to improve the prediction of milk production from subtropical forages. In Proceedings of the 8th International Workshop on Modelling Nutrient Digestion and Utilization in Farm Animals, Cairns, Australia, 15–17 September 2014; McNeill, D.M., Bell, A., Eds.; CSIRO Publishing: Victoria, Australia, 2014; p. 21.
59. Deboever, J.L.; Cottyn, B.G.; Vanacker, J.M.; Boucque, C.V. The use of NIRS to predict the chemical-composition and the energy value of compound feeds for cattle. *Anim. Feed Sci. Technol.* **1995**, *51*, 243–253.
60. Kramer, M.; Weisbjerg, M.R.; Lund, P. Estimation of indigestible NDF in feedstuffs for ruminants. Available online: http://forskningsbasen.deff.dk/Share.external?sp=Sce3d98ad-7aaa-444b-b101-2c4e7b1490ab&sp=Sau (accessed on 18 April 2015).
61. Norris, K.H.; Barnes, R.F.; Moore, J.E.; Shenk, J.S. Predicting forage quality by infrared reflectance spectroscopy. *J. Anim. Sci.* **1976**, *43*, 889–897.
62. Shenk, J.S.; Westerhaus, M.O.; Hoover, M.R. Analysis of forages by infrared reflectance. *J. Dairy Sci.* **1979**, *62*, 807–812.
63. Abrams, S.M.; Shenk, J.S.; Westerhaus, M.O.; Barton, F.E. Determination of forage quality by near-infrared reflectance spectroscopy—Efficacy of broad-based calibration equations. *J. Dairy Sci.* **1987**, *70*, 806–813.
64. Mentink, R.L.; Hoffman, P.C.; Bauman, L.M. Utility of near-infrared reflectance spectroscopy to predict nutrient composition and *in vitro* digestibility of total mixed rations. *J. Dairy Sci.* **2006**, *89*, 2320–2326.
65. Harper, K.J.; Isherwood, P.; Barber, D.G.; Callow, M.; McNeill, D.M.; Poppi, D.P. Prediction of iNDF of subtropical forages by Near Infrared Reflectance Spectroscopy. Available online: http://espace.library.uq.edu.au/view/UQ:354481 (accessed on 18 April 2015).