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Review

Allometries in Plants as Drivers of Forage Nutritive Value: A Review

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Abstract: The nutritive value of forage for herbivores has been for a long time determined by the concentration in protein and, hence in nitrogen (N), the concentration in different minerals (P, K, Ca, Mg, and oligo-elements), and the in vivo dry matter (DM) digestibility. Forage DM digestibility, the proportion of ingested DM being metabolized by ruminant animals has been related to different components of plant tissue composition such as Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF); the NDF concentration represents an estimate of cell wall content while the ADF concentration is an estimate of the more lignified cell wall content. Forage nutritive value is generally analyzed by relating the attributes of nutritive value to plant phenology, in order to predict the decline of these attributes with plant age. A more functional approach, initially developed for the analysis of N concentration dynamic analysis (Lemaire et al. 2008 and Lemaire et al. 2019), and extended for digestibility for this review, is based on the assumption that above-ground plant mass (W) is composed of two compartments: (i) the metabolic compartment (Wm), associated with plant growth process scaling with leaf area, having a high N concentration (%N), and a high Digestibility (%D); (ii) the structural compartment (Ws) associated with architectural plant development, scaling with plant height and thickness and having low %N and %D. With the postulate that Wm is allometrically related to W ($Wm = c \times W^{\alpha}$ with $\alpha < 1$), the ontogenetic decline of both %N and %D as the plant gets bigger and forage mass increases can be explained, and the purely empirical statistical approach of forage quality based on plant phenology can be replaced by a more mechanistic and comprehensive analysis linking forage production and forage quality dynamics within the same functional approach for a better understanding of genotype-environment-management interactions.

Keywords: forage quality; forage digestibility; protein content; neutral detergent fibre; acid detergent fibre; leaf/stem ratio

1. Introduction

The nutritive value of forage harvested for feeding domestic herbivores is generally estimated by two characteristics: (i) the concentration in Crude Protein (%CP) that corresponds roughly to N concentration (%N) with %CP = 6.25 × %N, and (ii) the concentration in metabolizable energy, i.e., the fraction of the total energy content of the forage dry mass being potentially digested and metabolized by animals. The fraction of forage mass being digested by animals can be expressed by its digestibility (%D). Digestibility can be measured directly on animals through the dry mass balance between ingestion and feces excretion or more simply estimated by in vitro digestion of forage samples in an artificial rumen (%ivD).

The pioneering works of Van Soest and Wine in 1967 [1] and Chesson in 1993 [2] provide the possibility to relate %D of forages to some chemical characteristics of plant tissues representing their

degree of degradation within the rumen. These chemical characteristics are: Neutral Detergent Fiber (%NDF), Acid Detergent Fiber (%ADF), and Lignin (%L) concentrations. The whole forage mass (W) can be separated in a first fraction: W-ADF with highly digestible plant tissues (%D = 100%) corresponding roughly to cytoplasmic components while ADF corresponds to cell wall content; a second fraction, NDF-ADF, corresponds to the cell wall fraction, having a moderate level of degradability, and the third fraction ADF would represent the non-degradable cell wall. So the digestibility of the whole plant is, in the first approximation, determined by the proportion between these three plant tissue fractions. But as the digestibility of the ADF fraction is very variable, the use of these three biochemical fractions for predicting the energy value of forages through chemical analysis is very unprecise.

It is very important for grassland management and livestock farming to develop a fine-tuned model representing the trade-offs between the dynamic of forage accumulation and the correlative decline in forage nutritive value for adapting the best strategy in the timing of grazing or mowing. Coupling forage growth models with nutritive forage models are of great importance for providing farmers and stockbreeders to optimize their production systems. The objective of this review paper is to show the limits and insufficiency of the normative approach relating forage nutritive value to plant phenology for predicting forage nutritive value and to establish the basis for a more functional approach allowing the expression of the causal link between plant biomass accumulation processes and the ontogenetic decline in the nutritive value of forages.

2. The Classical Approach of Forage Nutritive Value Based on Plant Phenology

Animal nutritionists and agronomists were always interested in predicting the in-field values of crude protein concentration and energy of forages along with their evolution with time within seasons in order to harvest forages for optimal animal nutrition and performance. The strong evidence of the decline of forage crude protein concentration (%CP) and digestibility (%D) as forage plants develop and grow leads to a strong trade-off between harvested forage mass and its nutritive value. For solving this trade-off and for optimizing the quantity-nutritive value compromise, agronomists and farmers have long related the decrease of forage nutritive value, which is the decline in %CP and in %D, to plant phenology progression. Each plant phenology stage could be related to a range of value in both %CP and %D, giving farmers the possibility to anticipate the decline in forage nutritive value and to decide the best harvest date by simply monitoring plant phenology.

Because of the main period for forage production in both grass-based or legume-based or mixture-based meadows in the spring, the plant phenology used for monitoring the best harvest date for optimizing forage yield and the nutritive value was linked to the flowering of the main forage species. Quantified phenology scales have been elaborated in grasses [3] and legumes, such as alfalfa [4] and sulla [5], so that quantified relationships could be established between the nutritive value and phenological stages, as illustrated in a recent study [6]. This study reported that the decline in nutritive value with advancing phenological development of alfalfa and timothy during spring growth varied across three contrasting agro-climatic regions. The relationship between the stage of development of a forage species and its neutral detergent fiber (NDF) concentration was previously shown to not always be reliable [7]. Predictive equations of pre-harvest forage nutritive values of alfalfa [8,9] and alfalfa-grass mixtures [10] were more successful when plant height was also taken into account.

So tables of forage quality values have been established for different forage species (see [11]). But as observed rapidly, the variability in %CP or %D observed within each phenology stage was of the same amplitude that the variation across different successive stages that led to a too fuzzy a prediction. So obviously, even if the general trend for the decline in forage nutritive as plants progress in terms of phenology remains in evidence, it is clear that this relationship is not strong enough as a causal determination of the forage nutritive value decline. It appears that both forage nutritive value decline and plant phenology progression are only correlated with time. Some authors tried to replace the phenology scale with degree-days, but the problem of the pure correlation and the absence of a causal process remained the same as phenology progression of a plant is directly driven by temperature.

Moreover, this large uncertainty in the determination of both forage production and forage nutritive value obtained at different times within a season or at the same date between different years prevents farmers from optimizing their forage harvest management and also making difficult for agronomist and plant breeders the development of a relevant genotype-environment-management interaction interpretation. For doing such an analysis, it is absolutely necessary to overpass the purely empirical approach of static correlations between forage nutritive value and plant phenology and to develop a more dynamic and functional approach of processes linking plant growth and plant architecture development with components of forage nutritive value.

3. A Theoretical Framework for Coupling Crude Protein Concentration and Forage Mass

As forage crude protein concentration is simply estimated as a function of N concentration (%CP = 6.25%N), the dynamic of N uptake by plants and its consequence in terms of plant N concentration during plant growth should provide a useful approach for analyzing variations in forage crude protein concentration. As well demonstrated by Lemaire and Salette [12,13] for forage grass species, by Lemaire et al. [14] for alfalfa, and by a great number of authors for several crop species [15–18], plant %N declines monotonically as crop mass increases. This decline in %N is more pronounced when the N supply is limiting plant growth (Figure 1). Above a critical N supply level, plant %N increases without any corresponding increase in crop mass (W), indicating a "luxury N consumption" by plants. Below this critical N supply, both plant %N and crop mass (W) decrease when the level of N supply is decreasing, indicating that the level of N supply is limiting plant growth.

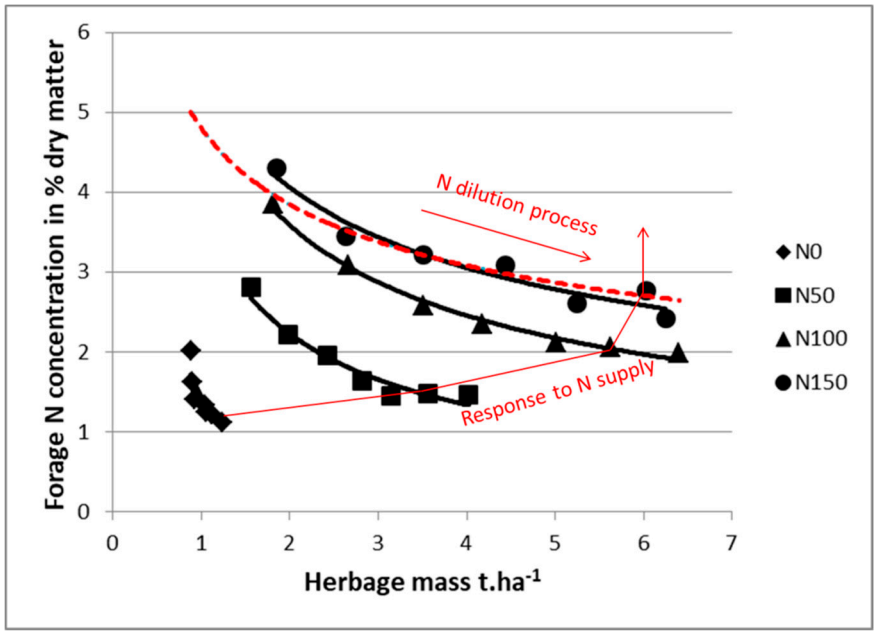


Figure 1. Variation of forage N concentration (%N) in relation to forage mass (W) during a spring growth of tall fescue under different levels of N fertilizer applications. The dotted line in red represents the critical N dilution curve: $\%N = 4.8W^{-0.32}$ [12], corresponding to the minimum plant %N for achieving maximum forage mass. Above this critical curve, plant %N can increase as luxurious N accumulation, and below this curve, both %N and W decrease with the decreasing N supply. Data obtained at Lusignan (46.46° N; 0.07° E; France) and graph adapted from Lemaire and Denoix [19].

Consequently, forage N concentration is at each moment during the growth cycle is determined by two factors: (i) the level of soil N availability that determines plant N uptake and (ii) the rate of forage Dry Matter accumulation (dW/dt) that drives the N dilution process. An allometry equation has been

proposed by Lemaire and Salette [12] for relating crop N uptake (N) and crop mass (W) accumulation during the time course of crop growth

$$N = a \times W^b \quad (1)$$

where N is expressed in kg ha^{-1}, W is expressed in t DM ha^{-1}, a is the N uptake for W = 1 t ha^{-1}, and b is the allometry coefficient. By dividing the two terms of Equation (1) by W, it is then possible to express plant %N in relation to W, referred to as a N dilution curve:

$$\%N = a/10 \times W^{b-1} \quad (2)$$

The factor 1/10 is introduced for expressing forage N concentration as a percent of dry matter.

The critical N dilution curve that is the minimum %N for achieving the maximum W was shown to be very similar for a large range of grass species and for alfalfa, and very stable across seasons [13,14]. This stability of the critical N dilution curve strongly suggests that the N dilution process is not mechanistically associated with plant phenology stages because the relationship between %N and plant phenology stages depends highly on genotype and seasons.

A theoretical framework allowing the explanation of N dilution processes has been developed by Lemaire and Gastal [20], and more recently generalized [21]. Lemaire et al. [16] have shown, following the initial assumption of Caloin and Yu [22], that N dilution curves result from the relative size of two major plant compartments:

1. W_m, the "metabolic compartment" of plant tissues directly associated with photosynthesis and growth processes, with a high N concentration: $\%N_m$;
2. W_s, the "structural compartment" of plant tissues associated with plant architecture and hydraulic conductivity, with a low N concentration: $\%N_s$. With W being total forage mass, it is possible to write:

$$W = W_m + W_s \quad (3)$$

It has been postulated that the absolute growth rate (dW/dt) of a crop should be, by definition, proportional to the size of its metabolic compartment (W_m):

$$dW/dt = k \times W_m \quad (4)$$

Rearranging Equation (4) and dividing both sides by W gives a relationship between the proportion of the metabolic component in plant mass and the relative rate of biomass accumulation:

$$W_m/W = 1/k \times (dW/dt)/W \quad (5)$$

Plant N concentration (%N) can be calculated from the relative contribution of the two compartments, W_m and W_s, to the plant shoot mass (W):

$$\%N = 1/W \times ((\%N_m\ W_m + \%N_s\ W_s)) \quad (6)$$

Using Equations (5) and (6) gives:

$$\%N = (1/k \times 1/W \times (\%N_m - \%N_s) \times dW/dt) + \%N_s \quad (7)$$

Caloin and Yu [21] proposed that the metabolic component W_m is linked to the whole plant shoot mass (W) by an allometric relationship:

$$W_m = c \times W^{\alpha} \quad (8)$$

Equation (8) with $\alpha < 1$ indicates that the proportion of metabolic tissues within whole-plant mass declines allometrically with any increase in plant mass. Substituting W_m from Equation (8) into Equation (4) gives:

$$dW/dt = kc \times (W)^{\alpha} \quad (9)$$

Including Equation (9) within Equation (7) gives:

$$\%N = (c \times (\%N_m - \%N_s) \times W^{\alpha-1}) + \%Ns \quad (10)$$

This theoretical relationship is different from the empirical one (Equation (2)). However, Lemaire and Gastal [20] showed that the difference due to the existence of an asymptote different of 0 on Equation (10) representing the minimum N concentration in structural tissues was only sensible for W > 20 t ha^{-1}, and that under this value, Equation (2) could be considered as an acceptable approximation of the N dilution process, which is the case for most forage harvests.

The crude protein concentration of forages can be directly related to the quantity of forage harvested, and the daily decline in %CP due to a delayed harvest is directly linked to the daily rate of forage mass accumulation. This decline in %CP with forage mass accumulation is the consequence of the ontogenetic plant shape and plant architecture changes associated with plant growth process as determined by allometry (Equation (8)). Lemaire et al. [23] have demonstrated that the metabolic compartment of a plant (W_m) scales with plant leaf area or the crop Leaf Area Index (LAI), while the structural compartment (W_s) scales with canopy height and leaf thickness. It has been shown that the decline in %N in a large range of crops, including C3 and C4, or monocot- or dicot-species strictly parallel the decline in Leaf Area Ratio (LAR). So this decline in both %N and LAR is a consequence of the competition for the light within dense canopies and the adaptive response of plants for positioning their leaf area within the illuminated layers of the canopy [21]. As a consequence, an increasing proportion of structural tissues having a low N concentration is necessary for supporting the leaf area.

4. Generalization of the Approach for Forage Digestibility

The theoretical framework used for analyzing the decline in plant %N as forage mass increases can be generalized for studying the decline in forage digestibility with increasing forage mass as suggested by Lemaire and Allirand [24] for alfalfa (*Medicago sativa* L.) and Bélanger et al. [25] for timothy (*Phleum pratensis* L.). If we suppose that the two compartments W_m and W_s of Equation (3) have respectively a high and a low digestibility ($\%D_m \approx 1$ and $\%D_s < 1$), then Equation (10) can be adapted for relating the digestibility of the whole plant, %D, to the forage mass W:

$$\%D = (c \times (\%D_m - \%D_s) \times W^{\alpha-1}) + \%D_s \quad (11)$$

However, an additional difficulty arises as compared with the %N estimation. The $\%D_m$ can be expected to be close to 100% and independent of time, which is a reasonable assumption because, by definition, the compartment W_m is composed only of very degradable tissues. However, the $\%D_s$ is not constant during crop growth. The $\%D_s$ can be estimated by measuring the digestibility of the neutral detergent fiber (D_{NDF}), which corresponds to the digestibility of the plant cell walls. An example of this decrease of $\%D_s$ can be seen from a study conducted with timothy where the D_{NDF} of leaves and stems decreased with forage mass accumulation during a spring growth cycle (Figure 2).

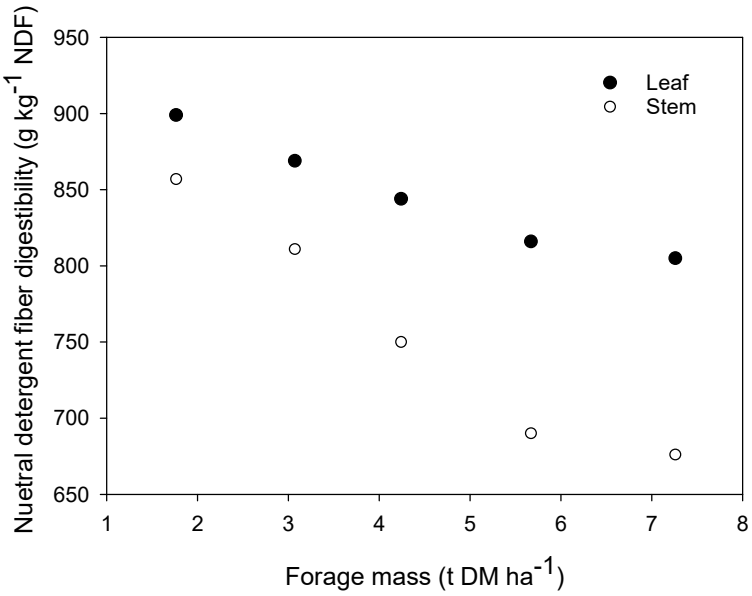


Figure 2. Changes in neutral detergent fiber digestibility of leaves and stems of timothy during spring growth under non-limiting N conditions. Drawn from data obtained at Fredericton (45.55° N; 66.36° W; Canada) and presented in Bélanger and McQueen [26].

A function relating $\%D_s$ with time is therefore necessary for taking into account the "maturation" of structural tissues during plant ontogeny through lignin deposition within cell walls [27]. So Equation (10) could be transformed:

$$\%D = (c \times (1 - \%D_s(t)) \times W^{\alpha-1}) + \%D_s(t) \quad (12)$$

The term $\%D_s(t)$ corresponds to the function relating to the digestibility of the structural compartment with time or with any other plant or canopy dynamic parameter evolving with time. This decrease in $\%D_s$ of the leaves and stems is taken into account in existing process-based forage grass models in which the rate of decrease is primarily driven by temperature [28,29].

Empirical relationships between forage in vitro digestibility (%Div) and forage mass have been established for different species. For alfalfa (*Medicago sativa* L.), as shown in Figure 3, a negative linear regression accounts for the decline in forage digestibility with increasing forage mass. This decline is more pronounced for the second regrowth in summer (July–August) than for the first regrowth at the end of spring (May–June), and also for non-irrigated conditions when water deficit is important (2nd regrowth). Water deficit, therefore, seems to have two opposite effects on in vitro digestibility:

(i) A positive effect associated with the reduction in forage mass; this affects the lower decline in the Leaf/Stem ratio with forage mass accumulation [30].

(ii) A negative effect corresponding to a decline in digestibility at similar forage mass; this effect is associated with a lower digestibility of the ADF fraction of stems [30].

So, variations in the rate of decline of the forage digestibility of alfalfa with forage mass d(%D)/d(W) can be obtained across environmental conditions such as higher air temperatures during summer than in spring and a water deficit during summer. As it is well known that high temperatures favor lignin deposition within cell wall structure [31], we can suppose that water-stressed plants should have a higher temperature in their shoot tissues that could have led to this more rapid decline of digestibility per unit of herbage mass. The difference in the relationship between in vitro digestibility (%Div) and crop mass of timothy between Spring and Summer shown in Figure 4 can be interpreted by a difference in temperature between the two seasons.

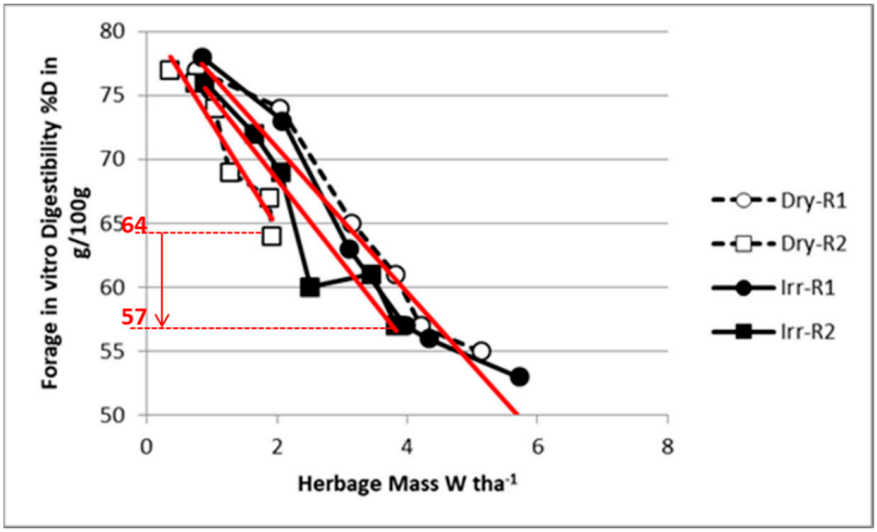


Figure 3. Relationship between in vitro forage digestibility (%D) and forage mass (W) of alfalfa during two successive regrowths (R1: 15th May–1st July and R2: 1st July–15 August), under irrigated (Irr) and non-irrigated (Dry) conditions. Linear regressions are: %D = 82–5.67W, R^2 = 0.94 for both irrigated and non-irrigated R1; %D = 81–6.44W, R^2 = 0.89 for irrigated R2; and %D = 81–8.17W, R^2 = 0.92 for non-irrigated R2. (Data obtained at Lusignan (46.46° N; 0.07° E; France) and re-calculated from Lemaire et al. [30]).

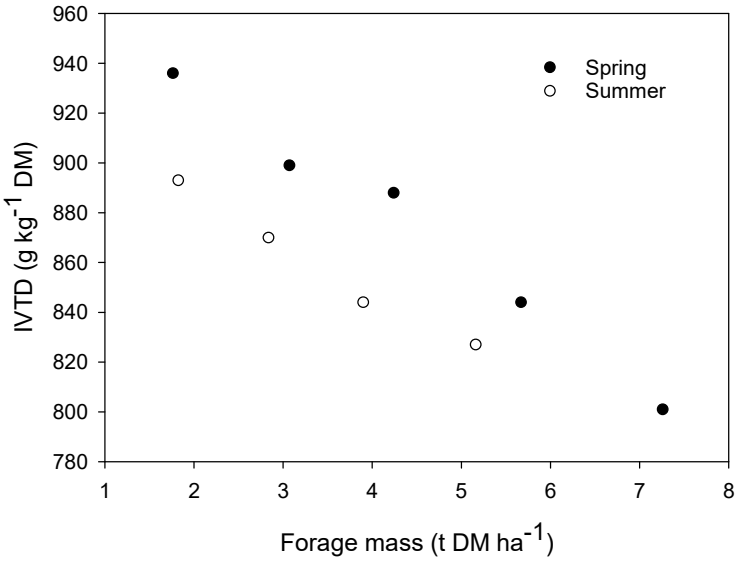


Figure 4. Relationship between in vitro forage digestibili (IVTD) and forage mass (W) of timothy during spring and summer regrowth under non-limiting N conditions. Redrawn from Bélanger and McQueen [32].

For grass forages such as timothy, as shown in Figure 5, a two-stick negative linear regression allows for accounting the decline in %Div as herbage mass increases. There are no differences across years or cultivars, the breakpoint indicating the start of the high decline in %ivD being then independent of the phenology stages, knowing the difference in date of ear emergence or flowering between the early and the late cultivar was more than one week, that would correspond to about 2 t in term of herbage mass. So the acceleration in the decline rate of %ivD from about 2% per ton of herbage mass accumulated down to about 4% cannot be related directly to any phenology stage. This breakpoint is probably an artefact due to the choice of a two-stick regression, and the acceleration of the decline in %Div with increasing W is probably more progressive. This acceleration would correspond to the

progressive ontogenetic decrease in %Ds with time or with W, as described by the function $\%D_S(t)$ in Equation (12).

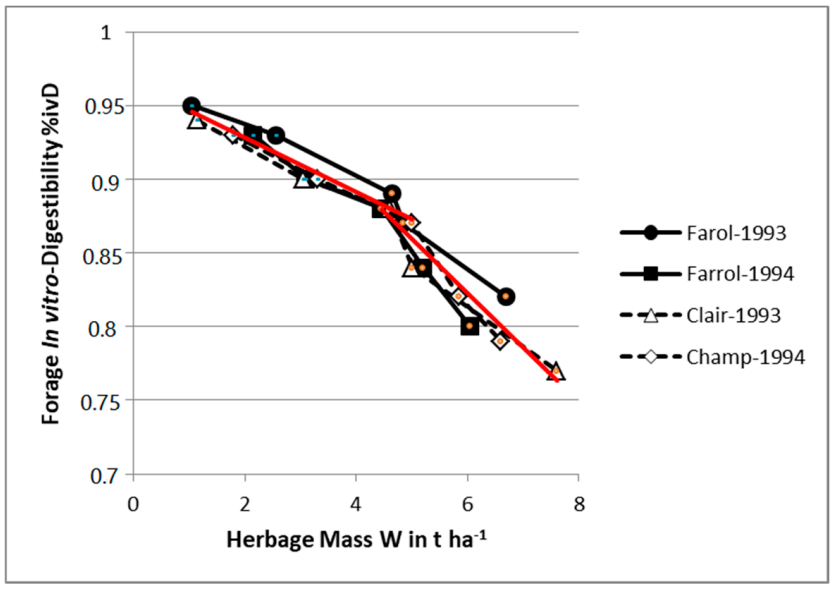


Figure 5. Decrease in forage digestibility during the herbage mass accumulation process in spring for meadows of timothy (*Phleum pratense* L.) in Eastern Canada for two consecutive years, according to different cultivars having either early or late phenology (flowering stage). The initial regression until herbage mass W ≈ 5 t ha^{-1} is %Div = −0.019W + 0.97 (R^2 = 0.937), and the final regression for W > 5 t ha^{-1} is: %Div = 0.037W + 1.04 (R^2 = 0.873). Data obtained at Fredericton (45.55° N; 66.36° W; Canada) and redrawn from Bélanger and McQueen [33].

The fact that we obtain the same pattern of decline of %Div for the different years and the different cultivars would indicate that $\%D_s$ should decline in relation to whole herbage mass itself. So Equation (12) could be re-written as follows

$$\%D = c \times (1 - \%D_s(w))W^{\alpha-1} + \%D_s(w) \quad (13)$$

where $\%D_s(w)$ would represent a "maturation" function of the structural tissue compartment as the size of the plant, and then the herbage mass increases. So the decline in %D of herbage with increasing herbage mass would be the consequence of two embedded ontogenetic processes linked with plant growth in size:

(i) An allometric increase of the proportion of structural compartment (W_s/W_m) of plant tissue as plant size increases;
(ii) a "maturation" of cell walls in structural tissues leading to a decline in their digestibility and then represented by the term $\%D_S(w)$ in Equation (13).

The distortion between empirical linear regressions and the theory is due to the fact that Equation (11) supposes a fixed value for %Ds, while the more relevant Equation (13) postulates a decreasing function linking $\%D_s$ and W. So even if an allometric equation should hold at the beginning of the herbage growth period, as the value of $\%D_s$ progressively declines as W increases, the value of %D drops more rapidly than the decrease is only due to the decline in W_m/W_s ratio as herbage mass increases. This decline in %Ds is due to a "maturation" of cell walls as a plant grows due to secondary deposition of lignin and polysaccharides [34] because the cross-links between polysaccharides and lignin reduce the degradability of cell walls [35].

5. Are the Leaf/Stem Ratio or the Leaf Proportion Relevant Indicators of Forage Digestibility?

Several attempts were made to use the Leaf/Stem ratio or the fractional leaf mass within the forage mass (W_L/W; W_L being the leaf mass) for explaining the variability in forage digestibility [25] or as an indicator of forage digestibility. If we accept that the W_m/W_s ratio or the W_m/W are the main drivers of forage digestibility, the question arises whether or not the Leaf/Stem ratio and the leaf proportion are relevant estimates of the W_m/W_s ratio and the W_m/W ratio, respectively. This assumption is relatively well verified for forage species such as alfalfa and timothy, for which leaf tissues contain very few structural components such as petioles or midribs (Figures 6 and 7). Kühbauch and Voigtländer, [36] found that the nutritive value of alfalfa leaves remained high and declined only little during the growing period in contrast to stems whose nutritive value declined sharply.

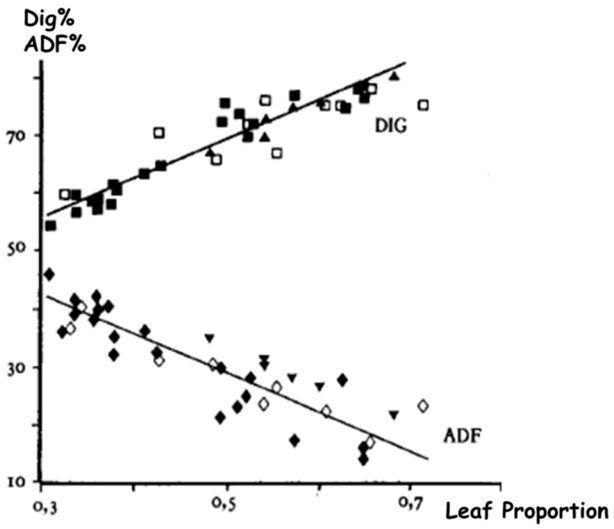


Figure 6. Relationship between forage digestibility (%D) or ADF concentration (ADF%) with the proportion of leaves in the forage mass (W_L/W) for alfalfa (same conditions as for Figure 2). Data obtained at Lusignan (46.46° N; 0.07° E), France, and redrawn from Lemaire et al. [30].

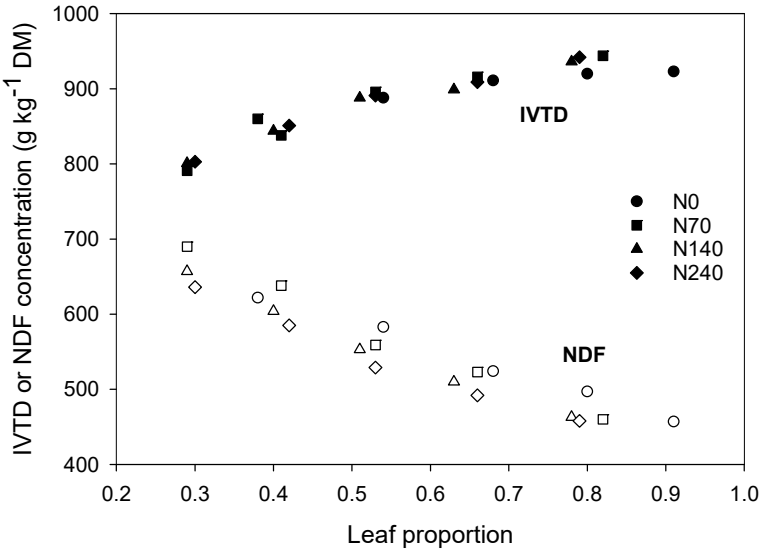


Figure 7. Relationship between forage digestibility (IVTD) or neutral detergent fiber (NDF) concentration with the proportion of leaves in the forage mass (W_L/W) of timothy during spring growth under four N fertilization rates. Data obtained at Fredericton (45.55° N; 66.36° W; Canada) and redrawn from Bélanger and McQueen [32]. N0, 0 kg N ha^{-1}; N70, 70 kg N ha^{-1}; N140, 140 kg N ha^{-1}; N240, kg N ha^{-1}.

However, for some grass species, this assumption might not be valid because the leaves are composed of a variable proportion of "metabolic" and "structural" components.

As shown in Figure 8 for sorghum forage, successive leaves with increasing size have an increasing proportion of midrib tissues. As a result, the W_m/W_s ratio at whole-plant level decreases long before stem elongation occurs and W_s becomes an important component of W. Moreover, when stem internodes elongate rapidly later in the season, as a result of plant phenology changes, the size of the new leaves starts to decline as their leaf primordia are pushed up by stem internode elongation. The proportion of midrib tissues within the leaves then decline, which contributes to a re-increase of the leaf digestibility.

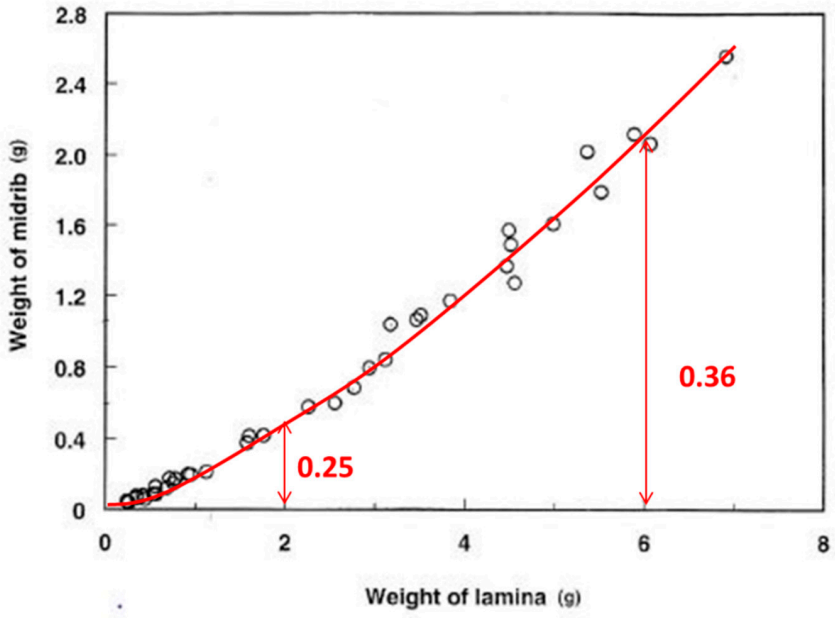


Figure 8. Relationship between the weight of midrib and the weight of lamina for successive leaves in a sweet sorghum plant. Redrawn from Fig 1.7 in Lemaire and Gastal [20]. Values in red are the midrib/lamina ratio that shows the increasing proportion of structural tissues as lamina size increases.

As well demonstrated by Ratjen et al. [37], the Leaf/Stem ratio is not always a relevant indicator for the estimation of W_m and W_s proportions within plant mass because of the strong trade-off between Leaf/Stem ratio and the Specific Leaf Area. The relevant parameter for estimating the W_m/W_s ratio in plants is the Leaf Area Ratio (LAR), which is the plant mass per unit of leaf area or W/LAI at the level of canopy. As the metabolic forage mass (W_m) scale with canopy LAI, LAR represents the W_m/W ratio while the Leaf/Stem ratio and the Specific Leaf Area (SLA) are only two components of LAR:

$$LAR = SLA/(W_{leaf}/W_{stem} + 1) \quad (14)$$

When stem internode elongation remains very low or inhibited because of plant phenology, successive leaves produced by plants are of increasing size for reaching the well-illuminated layers at the top of the canopy. Therefore, for being maintained erected, these leaves must contain an increasing proportion of structural tissues with strong nervures and vascular bundles that leads to a declining SLA and then also a declining LAR according to Equation (14). When stem internodes elongate, as determined by plant phenology, i.e., the onset of reproductive development, then primordia of young growing leaves are pushed up to the well-illuminated layer of the canopy, and leaf size is decreasing and SLA re-increases compensating then the Leaf/Stem ratio declines while maintaining the overall allometric relationship between LAI and crop mass W

$$LAI = k \times W^{\alpha} \quad (15)$$

and then the allometric decline of LAR as forage mass increases:

$$LAR = k \times W^{\alpha-1} \quad (16)$$

Lemaire et al. [16] have shown that the allometry coefficient α = 2/3 matches very well with observed data for a large range of crop species. This value of 2/3 corresponds to the fact that crop LAI scales with an area while W scales with a volume. So the LAR declines with increasing crop mass seem to result from a very general feature for plant growing in dense canopy being forced to grow isometrically, i.e., with the same relative rate in the three dimensions [38,39]. Such a constraint obliges plants to invest progressively a higher proportion of their mass in the structural dimension (height or thickness) for capturing light through leaf area expansion. It is important to note here that for a large range of species coefficient α of Equation (13) and coefficient b of Equation (1) have both close to the value of 2/3 demonstrating then that this fundamental allometry between LAI and crop mass is driving both plant N uptake and plant architecture dynamics at the level of canopy, determining then the ontogenetic decline of forage Crude Protein concentration and Digestibility as forage mass accumulates.

Even if a general correlative trend shows that low forage digestibility is frequently associated with low leaf/stem ratio of harvested forages, these correlations are too fuzzy for providing an accurate estimate of forage nutritive value and are not supported by any functional process. The determinism of the decline in forage nutritive value with increasing forage mass is due to the allometry between leaf area and plant mass resulting from two fundamental adaptive responses of plants growing in dense stands:

(i) The competition for access to light that constraints plants to orient their architecture towards the vertical dimension to colonize the well-illuminated layers of the canopy through their photo-morphogenetic responses to light micro-climate signals [40,41];
(ii) the mechanical constraint leading plants to invest in structural tissue in the thickness dimension as their expansion in height increases [42].

Neither of these two mechanisms are driven directly by plant phenology, but they are linked to forage growth dynamics through the general allometry governing plant size expansion. As plant size and forage mass increase, plant tissues are composed of an increasing proportion of structural components, and whole plant digestibility declines. As a result, a big plant always has lower digestibility than a small one. Consequently, the analysis of differences in forage digestibility between species and genotypes must be carried at similar forage mass for detecting any possible difference in their intrinsic nutritive value. Otherwise, the conclusion of differences in nutritive value, due only to differences in forage mass, would be trivial. In this sense, the different coefficients K, c, and a in Equations (1), (8) and (13) representing the inherent plant architecture parameters should help in characterizing inherent aptitude of different species and genotypes to provide high quality forages whatever their forage production capacity:

"K" is the LAI when W = 1 t ha^{-1}. It has been named the "leafiness coefficient" by Lemaire et al. [20], who have shown a large variation across values of 1.94 for alfalfa, 1.59 for canola, 1.13 for wheat, 1.41 for sorghum, and 1.06 for maize.

"c" is, in theory, the analogous of K and then should follow the same variation across genotypes.

"a" is the quantity of N uptake necessary to produce the first ton of dry forage matter. It represents the reciprocal of N efficiency in the plant. It has been demonstrated by Greenwood et al. [14] and by Lemaire et al. [16] that "a" remains very constant across species of the same metabolic group with C4 species having a lower value (≈34 kg N ha^{-1}) than C3 species (48 kg N ha^{-1}). This difference between C3 and C4 species reflects then the well-known difference in N efficiency between the two metabolic groups of species.

The effect of the N supply on forage digestibility in grass-based meadows cannot be analyzed without taking into account the effect of N on forage mass accumulation. As shown in Figure 9, the N supply has two opposite effects on forage digestibility:

(1) An increase in %D at similar forage mass (W) as a consequence of an increase in Wm/Ws of the leaves or stems;

(2) a decrease in %D as a consequence of the increased forage mass and the "dilution" effect mediated through a decrease in Wm/Ws.

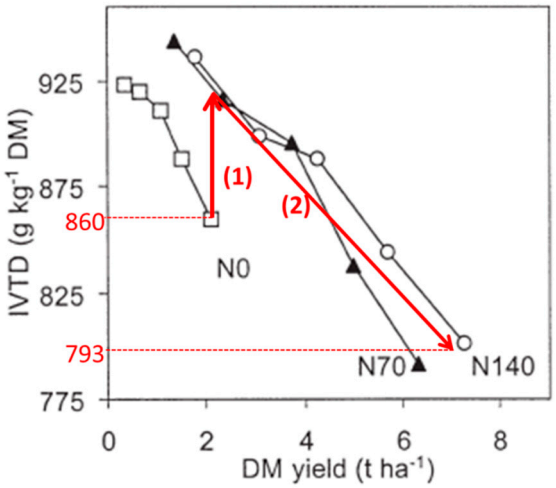


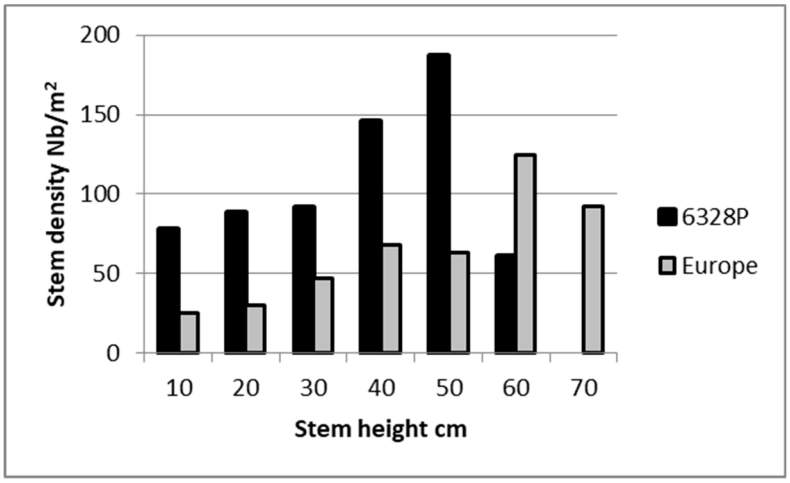
Figure 9. Changes in forage in vitro true digestibility (IVTD) of timothy with increasing forage mass (DM yield) in spring under three different N fertilizer application: N0, 0 kg N ha^{-1}; N70, 70 kg N ha^{-1}; N140, 140 kg N ha^{-1}. Data obtained at Fredericton (45.55° N; 66.36° W; Canada) and redrawn from Bélanger and McQueen [32]. Arrows express the two opposite effects of the N supply on %D: (1) the increase in %D at similar herbage mass (W) as a consequence of an increase in Wm/W and (2) the decrease in %D as a consequence of the increased forage mass, and the "dilution" effect.

As a result of those two opposite effects of the N supply on %D, the effect of the N supply on %D is the subject of conflicting reports [25]. Because the second effect due to the increased forage mass is generally greater than the first effect, the %D generally decreased as a consequence of N fertilization. However, in other circumstances, primarily in studies where the levels of N nutrition due to N fertilization rates are not very contrasted, the compensation between the two opposite effects can be more complete, leading then to an absence of any apparent effect of the N supply on forage digestibility in grass-based meadows.

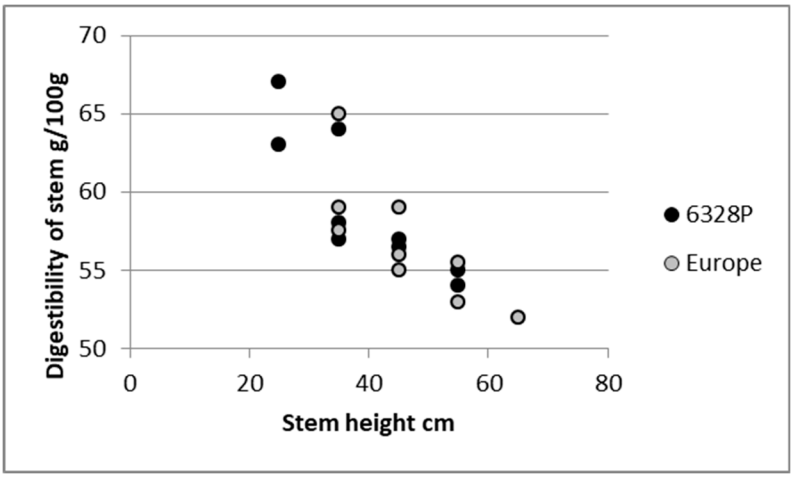
6. Towards an Integrated Approach of Forage Nutritive Value at the Canopy Level

The different results presented and discussed above demonstrate that plant growth processes, i.e., the increase in plant size through its architectural development, is the main driver of the decline in forage nutritive value. As plants increase in size, their investment in structural tissues proportionally increases, and in parallel, the maturation of these tissues necessary for supporting an increasing plant weight leads to a decline of the digestibility of the whole plant associated with the dilution of its N concentration. Consequently, as well shown above, plant phenology, even if correlatively linked with forage mass accumulation through time, is not directly the driver of forage nutritive value. Drivers of forage nutritive value have to be determined at the plant morphogenesis and architecture level: phytomer (lamina, petiole or sheath, and stem internode) appearance and growth. Moreover, these drivers have also to be analyzed at the canopy level: a collection of individual plants interacting with each other for competition for light.

As shown by Figure 10, the less productive alfalfa cultivar 6328P has a lower stem height population than Europe but has a higher digestibility because the digestibility of stems is mainly determined by stem height. So the stem internode elongation as a determinant of the stem height seems to be the main driver of the decline of forage digestibility with forage mass accumulation.



(**A**)



(**B**)

Figure 10. Distribution of stem height within an alfalfa stand for two cultivars (**A**) and the relationship between stem height and digestibility of stems (**B**). Data obtained at Lusignan (46.46° N; 0.07° E; France) and the graph was redrawn from Lemaire and Allirand [25].

As well demonstrated by Vallet et al. [43], stem internode diameter continues to increase after internodes have reached their final length, and this stem diameter enlargement is accompanied by an increasing cell wall deposition in secondary xylem and phloem with an increasing lignin deposition (Figure 11).

During the stem growth process, any new stem internode appearing at the top of the stem, along with its corresponding leaf area, corresponds to highly digestible plant tissues. This production of digestible plant tissues is counterbalanced by the "maturation" of basal stem internodes having less and less digestibility because of an accumulation of more and more lignin components within cell walls during stem diameter enlargement through cambial activity.

As shown in Figure 12, the digestibility of each internode should be determined by its distance to the top of the canopy. So as stem height increases during the growth period, the upper internodes having high digestibility are progressively diluted within an increasing mass of maturing internodes leading to a control of whole stem digestibility by stem height. As demonstrated clearly by Moulia et al. [42], the perception by plants of mechanical constraints leads to the control of both stem internode length and diameter and also cell wall and lignin deposition. As stem height increases, mechanical constraint increases, and the internode cell wall "maturation" is accelerated, leading to the decrease in stem

digestibility with stem height. In a similar way, Lemaire et al. [44] have shown that N distribution among leaves within alfalfa canopies follows the light extinction profile. So both digestibility and N concentration decline from the top layers to the bottom layers of alfalfa stands as forage mass increases.

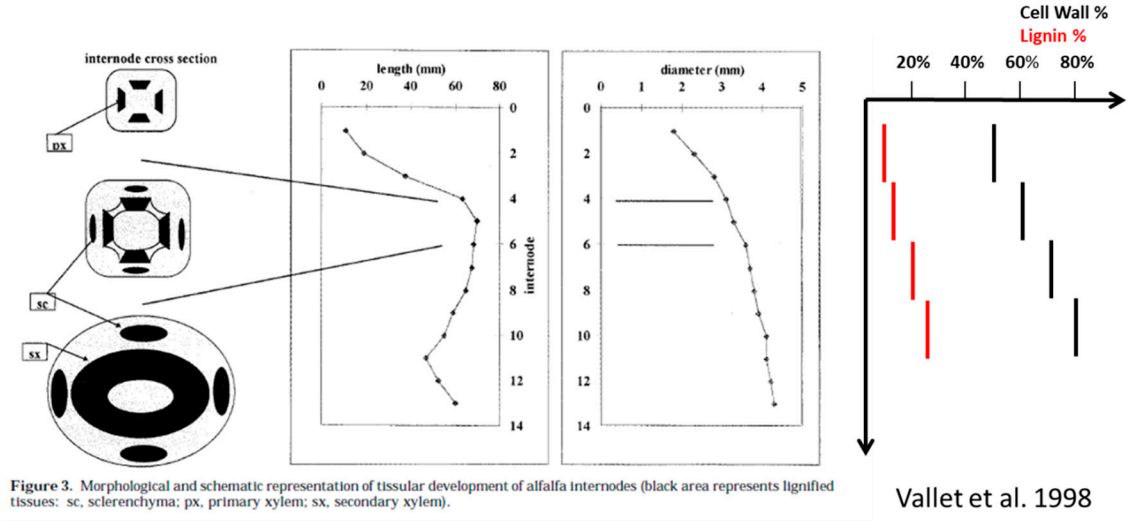


Figure 3. Morphological and schematic representation of tissular development of alfalfa internodes (black area represents lignified tissues: sc, sclerenchyma; px, primary xylem; sx, secondary xylem).

Figure 11. Progression of stem internode length and diameter from top to bottom stems of alfalfa associated with the secondary cell wall and lignin deposition. Data obtained at Lusignan (46.46° N; 0.07° E), France, and graphs were redrawn from Vallet et al. [43].

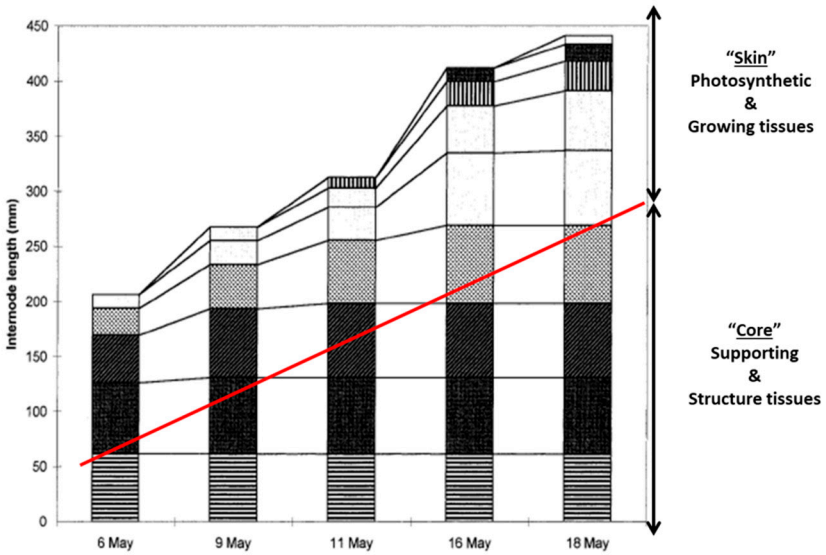


Figure 12. Evolution of the structure of an alfalfa stand during a spring growth period showing the length of the successive internodes on the dominant stems. As the digestibility of each internode and the N concentration in its associated leaf area is determined by its relative position within canopy layers, it is then possible to represent the whole canopy as being consisting of a more or less constant "Skin", an upper envelope of young growing internodes and their well-illuminated leaves having high digestibility and a high N concentration, and an increasing "Core", consisting of maturing stem internodes having a decreasing digestibility associated with shaded and senescing leaves with a low N concentration. Adapted from Vallet et al. [45].

7. Conclusions

This review provides several convergent pieces of evidence demonstrating that the general links established between digestibility and crude protein of forage crop and the plant phenology were due to correlations with time, but were not a causal relationship. As a result, the prediction of forage quality

through observation of plant phenology stages is highly uncertain in the context of contrasting growing conditions related to cultivars, seasons, climate, and crop N nutrition. The theoretical framework developed in this review allows the expression of a mechanistic link between an increase in plant size and a decrease of both forage crude protein concentration and digestibility. As the plant gets bigger, its structural tissues that have a low N concentration and low digestibility increase proportionally more rapidly than its metabolic tissues that have a high N concentration and high digestibility, which leads then to a parallel decrease of crude protein concentration and forage digestibility as crop mass increases. The Leaf/Stem ratio provides a relevant approximation of the proportion of structural and metabolic tissues for species with a very low proportion of structural tissues in their leaves such as alfalfa or timothy, but not for species with leaves that have more abundant vascular bundles and midrib tissues. Stem height also appears to be an important parameter for explaining the decrease in forage quality as forage mass increases. As stem height increases, the lignification of cell walls at the base of the stem increases, which leads then to a decline in the digestibility of stem tissues.

The general decline in both crude protein concentration and digestibility of forage with crop mass accumulation is, therefore, the consequence of plant adaptive capacity to compete for the light within a dense canopy. This adaptive capacity results in leaves being positioned at the top of the canopy for reaching light with the mechanical constraint for developing relevant supporting tissues. All these adaptive architectural characteristics of plants converge for providing a strong trade-off between forage mass and forage quality. The possibility to formulate this trade-off within a crop model by using the different algorithms presented in this review should allow (i) farmers to determine the best compromise between forage production and forage quality in their management decisions, and (ii) plant breeders to identify the relevant plant morphological characteristics being able to optimize this trade-off.

The theoretical framework presented in this review has been based on general allometry relationships in plant architecture observed mainly for perennial grasses and legumes, and illustrated by results obtained in a few numbers of species, mainly timothy and alfalfa. The degree of genericity of this framework to a larger range of species, including annual forage, can be questioned. However, the wide and successful use of these allometry relationships across a large range of annual crop species for analyzing crop N nutrition and crop N concentration dynamics in relation to crop mass accumulation suggests high genericity. The use of this framework as a tool for analyzing these genetic or environmental variations should then help in a better understanding of Genotype-Environment-Management interactions on the trade-off between forage production and forage quality.

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