CHARACTERIZATION OF FIBER FRACTIONS IN PLANT BY-PRODUCTS AND MODELING FIBER DIGESTION WITH APPLICATION IN CNCPS 7.0

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CHARACTERIZATION OF FIBER FRACTIONS IN PLANT BY-PRODUCTS AND MODELING FIBER DIGESTION WITH APPLICATION IN CNCPS 7.0

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Studying fiber digestion in ruminants is important to quantify the effect it has on cattle performance. Cornell Net Carbohydrate and Protein System (CNCPS) demonstrates the importance of having accurate values of fiber digestion rate for the prediction of milk production. In-vitro fermentation studies demonstrated that the fiber (aNDFom) component of forages presented a residue that remained undigested over time (uNDF), and was recovered at 240 h. Further, the digestible fraction (dNDF = aNDFom – uNDF) was characterized as having a fast and a slow digesting pool, thus aNDFom digestion was modeled with a 3-pool dynamic exponential decay. The inputs for the models were residues at 30, 120 and 240 h; and the model's outputs were the pools size and respective rates, these being useful information when formulating diets for cattle. Plant by-products are fed to dairy cattle in discrete amounts and used to replace forages when of poor quality or not available. The objectives of this research were 1) to characterize aNDFom digestion in plant by-products; 2) formulate diets for lactating dairy cattle testing the effects that the multiple fractions of aNDFom had on cattle performance; 3) re-derive a model to describe aNDFom digestion with a sigmoidal decay as observed with the in-vitro fermentation; and 4) understand the implication of the "new" modeling approach (sigmoidal vs exponential) to the CNCPS predictions. Plant by-products contained an uNDF residue recovered at 120 h and only one dNDF fraction. Further, a two pool dynamic exponential decay was employed. The inputs for the by-products

model were residues at 12, 72 and 120 h. The experiment on farm was a pen study, where diets were formulated to be iso-aNDFom, 33% on dry matter basis, and consisted of two high forage diets (68%) with approximately 87% aNDFom from forages (corn silage and haycrop silage) and one low forage diet (34% forage) with 67% aNDFom from by-products sources (e.g. citrus pulp, cottonseed). The forage diets were formulated to be 32% uNDF (HUF) and 26% uNDF (LUF) and the by-products diet was 32% uNDF (HUNF). The design was a 3 x 3 Latin square with 21-d adjustment and 5-d sampling periods. No differences were observed for dry matter intake (DMI) between treatments HUF and LUF, however cattle fed HUNF consumed about 3.75 kg greater DMI ($P < 0.05$). Rumination per kg of aNDFom intake was greatest for the cattle consuming the LUF diet ($P < 0.05$), whereas total tract digestibility (TTD) was greatest for cattle fed the HUF diet ($P < 0.05$). Energy-corrected milk yields were 43.6, 44.9, and 46.4 kg/d for HUF, LUF and HUNF, respectively, and they were different ($P < 0.05$). The assessment of the two modeling approaches was made by first looking at goodness of fit, second testing the robustness of the model predictions in contrast to the variation associated to the analytical technique of aNDFom digestion, and third looking at the usefulness of models outputs for balancing diets for dairy cattle into the structure of CNCPS. The goodness of fit was evaluated with overall slope, intercept, R^2 and RMSE; and they were, respectively, for the exponential and sigmoidal decays 1.01 and 1.04; 0.01 and 0.02; 0.97 and 0.96; 0.02 and 0.04. Further, the robustness of the models was assessed by employing a Monte Carlo and looking at the CV of the predictions. In all the cases the sigmoidal decays were more robust. Finally, the usefulness of the model predictions were assessed by evaluating the RMSE of CNCPS predictions of ME-allowable milk and aNDFom TTD, using information from the lactating cattle study. The RMSE were 1.4 and 0.8 kg for ME-allowable milk and 3.8% and 4.2% for aNDFom TTD, respectively, for the exponential and sigmoidal curves.

BIOGRAPHICAL SKETCH

Alessandro Maria Zontini was born in Casalmaggiore (Italy) on September 22nd, 1986. He grew up in the countryside where he developed his interest in animal and plant kingdom. Alessandro obtained the Veterinary Medicine degree at Università degli Studi di Parma in 2011. During his time at the Vet school, Alessandro met his tutor, Dr. Federico Righi from the animal nutrition department, who seeded in him the interest in dairy nutrition. In the spring semester of 2011, Dr. Righi sent Alessandro to Cornell University, in an exchange program with Dr. Michael Van Amburgh. It was during his time at Cornell University, in the Dairy Cattle Nutrition course, taught at the Animal Science Department, that Alessandro realized that becoming an animal scientist with training in modeling was his calling. In July 2012, Dr. Van Amburgh invited Alessandro to enroll in a Ph.D program at Cornell University to pursue studies on fiber characterization and modeling fiber digestion with application to the CNCPS model.

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CHAPTER 1: Literature Review

1.1 THE PLANT CELL WALL

The plant cell wall forms the fibrous component of plants. Fiber is, by definition, the fraction not (or slowly) digestible by the digestive enzymes of mammals, thus having low nutritional value for them. However ruminants have the rumen, an organ of the digestive system that contains a large microbiome able to synthesize enzymes that attach and degrade fiber into simpler components, like volatile fatty acids, hence, making them available to the host. Therefore, fiber for ruminants can be nutritionally important because their diets are high in fiber content and they can obtain a consistent amount of energy from it. For this reason the feed industry invests resources to understand how ruminants utilize fiber in order to make this biological process more efficient and profitable. Forages and plant by-products are the major sources of fiber, and in some countries are almost the only components of ruminant diets. Increasing forage and plant by-products usage would have positive effects in several areas of animal production such as farm profitability and sustainability by allowing for more farm-raised feeds to be used and providing a broader area for spreading manure and recycling nutrients. In addition, the ability to dispose of more plant by-products of the human food system is more environmentally friendly since ruminants can convert the plant by-products into human food. Also, rumen health and microbial yield increases from increasing the amount of digestible fiber in the diet and maintaining adequate rumen fill of that fiber. Finally, increasing the intake of digestible fiber enhances the public perception of ruminants by consuming what is thought by consumers to be more appropriate for them

and not in competition with humans for food. Before describing how ruminants utilize fiber, it is important to know something about plant cell wall synthesis and structure.

1.1.1 Plant cell wall structure

The cell wall is an extra overlay around the cytoplasmic membrane (Figure 1.1). The cell wall is composed of different layers synthesized in different phases during cell maturation: the primary cell wall that makes up the exterior and envelopes the cell starting at the development of the cell while the cell is still expanding and dividing (Wilson, 1993). The primary cell wall has a fiberglass-like structure with a backbone of cellulose microfibrils and a matrix of structural proteins and polysaccharides such as pectin and hemicellulose that cross-link with the chains of cellulose (Figure 1.2). The secondary cell wall appears only after the cell reaches maturation and is composed of three different layers of cellulose microfibrils submerged into the matrix of polysaccharides, and is the part of the cell wall that highly lignified and together with its thickness makes the cell rigid, undegradable to enzymes, and impenetrable to pathogens (Harris, 1990). The most important difference that can be found among different cells in different tissues, but also in different regions of the cell wall itself, is the cross-linking network between matrix polysaccharides and cellulose microfibrils that a plant develops in response to the effects of the environment.

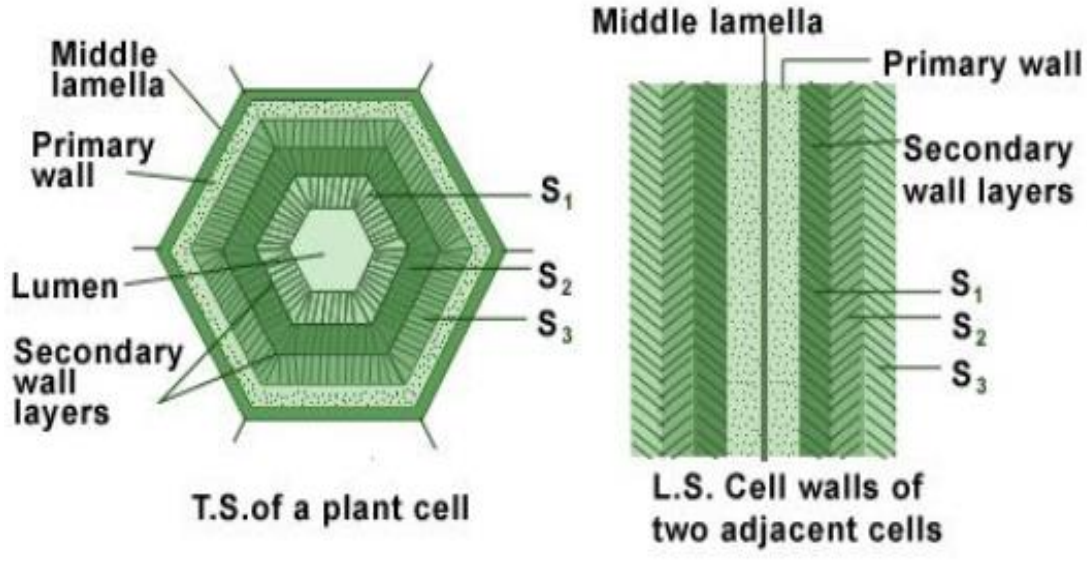

Figure 1.1. Cell wall structure: primary and secondary cell wall.

(http://www.tutorvista.com/content/biology/biology-iii/cell-organization/cell-wall.php)

Examining the cell wall, different types of bonds hold together the entire structure (Fry, 1988) and they include: 1) the weak hydrogen bonds that create strong linkages by forming in large numbers and interacting among the cellulose microfibrils stabilizing the structure; 2) the ionic bonds which are stronger and then required in lower frequency, such as Ca^{2+} ion bridges that build up the gel-network of pectins (Grant, et al., 1973), and those formed between structural proteins and uronic acid (Showalter, 2001); 3) the covalent bonds between structural proteins (Gorshkova and Morvan, 2006), and between cell wall polysaccharides through boron (Kobayashi et al., 1996), but more important between the lignins and tyrosine or the lignin sub-entities.

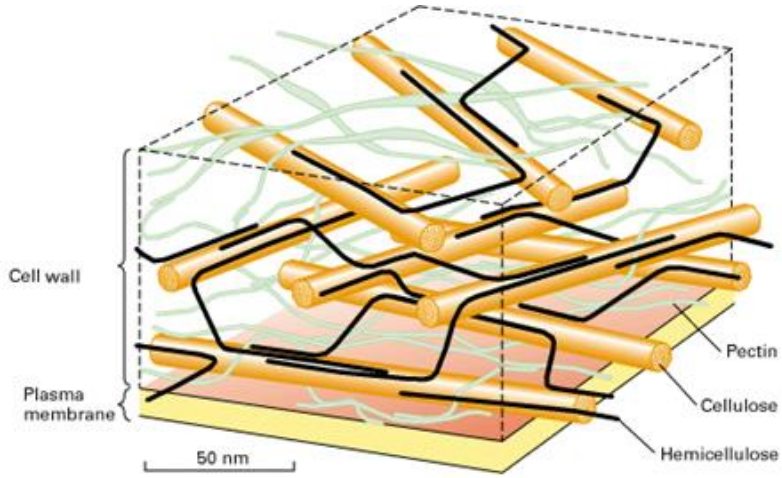

Figure 1.2. Network of cellulose, hemicellulose and pectin creating the fiber-glass like structure of the cell wall.

(http://plantcellbiology.masters.grkraj.org/html/Plant_Cellular_Structures2-Cell_Wall.htm)

1.1.2 Plant cell wall polysaccharides

The backbone of the cell wall is cellulose, a linear polymer consisting of about 10,000 glucose residues linked by β-(1-4) bonds. Individual polymers of cellulose are connected with each other to form microfibrils (Fry, 1988; Carpita and McCann, 2000; Gorshkova and Morvan, 2006). These microfibrils are clustered together and held by hydrogen bonds to form macrofibrils of cellulose, which bind with each other to form fibers of cellulose (Figure 1.3). The matrix of the cell wall is composed of both proteins and polysaccharides (Figure 1.2). The polysaccharides include hemicelluloses that are very similar to cellulose in that they can bind, but not form microfibrils due to branches and other modifications on their structure. Xyloglucan is the dominant and the most frequently cross-linked glycan, while other hemicelluloses such as arabynoxylan and

mannans are present in smaller amounts. Xyloglucan has a backbone that is similar to that of cellulose but is surrounded by xylose branches on 3 of 4 glucose residues. Arabinoxylans consists of a (1,4)-linked β-D-xylan backbone surrounded by arabinose branches and other residues such as glucuronic and ferulic acid esters. Mannans are also found in primary cell walls and have a backbone of (1,4)-β-D-Glycan that resemble cellulose. Other types of polysaccharides includes pectins like rhamnogalacturonan I and homogalacturonan, with smaller amounts of xylogalacturonan, arabinan, arabinogalactan I, and rhamnogalacturonan II. Covalent bonds bind pectin domains together which are in turn covalently and non-covalently linked to xyloglucan.

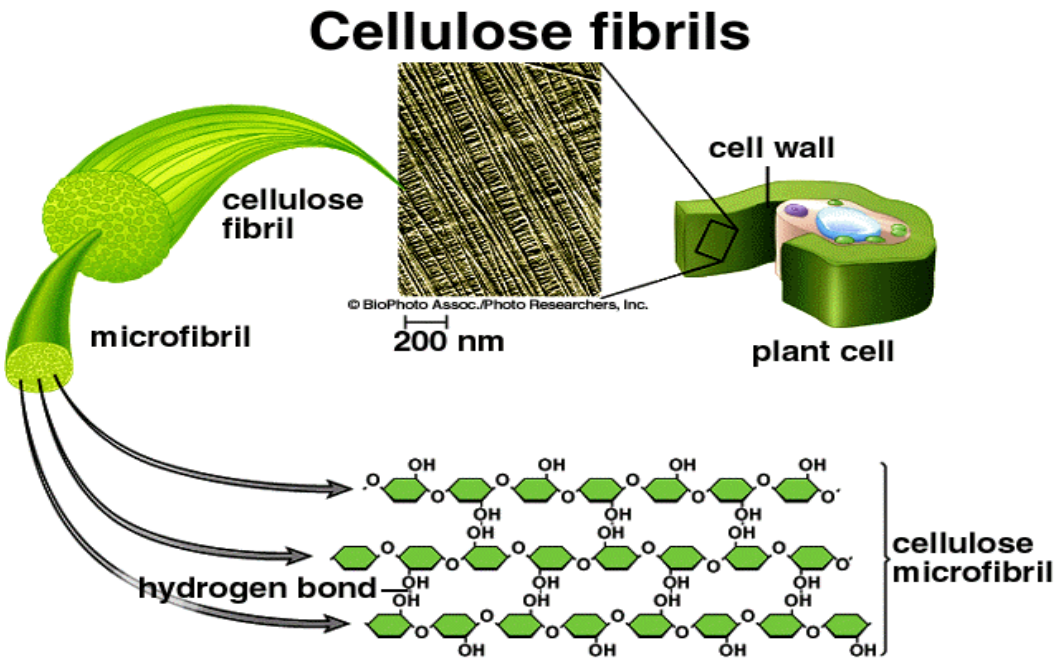

Figure 1.3. Chains of cellulose hold together by hydrogen bonds, likewise the microfibrils and fibrils, making up the fibrous component of the cell wall.

(http://www.desertbruchid.net/4_GB_LectureNotes_f/4_GB_03_Chem_J_Spr2003.html)

1.1.3 Lignin

From a chemical point of view lignin is a heterogeneous polymer and the building blocks are monolignols which are phenylpropanoids derived primarily from hydroxycinnamil alcohol monomers (Vanholme et al., 2010); (Figure 1.4). Lignin is the resource that plants have evolved in response to their condition of sessility. Lignin strengthens cell walls making them and consequently the whole plant rigid, hence able to stand against gravity, weather conditions or waves (for aquatic plant). Rigidity is also meaningful for transporting the water absorbed from the roots to the top of the plant. Through lignin deposition into the fibrous tissue, plants become less digestible to herbivores and insects; and with the incorporation into the polymer of other anti-nutritional factors plants avoid being eaten by herbivores and insects. Compounds such as tannins that give to the plant a bitter flavor, isoflavones with hormonal activity, terpenoids and essential oils known for their antimicrobial properties and toxicity for non-ruminants, along with cutins, alkaloids, cyanides, and silica (Van Soest, 1994; Van Soest, 2006), are all mechanisms for creating protection from predation.

Other than lignin deposition, another function plants developed in response to environmental factors like light and water is lignin cross-linking: the network of links that lignin can generate within the cell-walls (Figure 1.5) that makes them almost un-degradable by enzymes, thus limiting the conversion of fiber into animal or industrial products (Brown, 1985; Jung and Deetz, 1993), and even impenetrable by pathogens. Because of the complexity of the cell wall and this cross-linking, it is not correct to associate cell wall degradability and digestibility with lignin content solely. For instance, Chabannes et al. (2001) demonstrated that genetically modified plants for

lower lignin content responded by increasing the amount of cross-linking without changing the net digestibility. Furthermore, Sederoff et al. (1999) reported many cases in which lignin incorporates phenolic compounds (i.e. ferulic acid) not considered the traditional monolignols when their synthesis was restricted. From a nutritional standpoint lignin content and lignin cross-linking within the cell wall are the primary limiting factors of fiber fermentation (Besle et al., 1994).

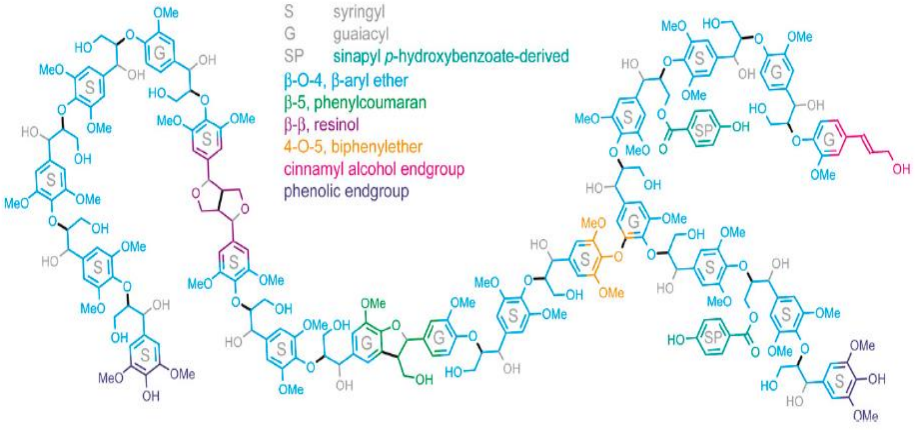

Figure 1.4. Representation of a lignin polymer (adapted from Stewart et al., 2009).

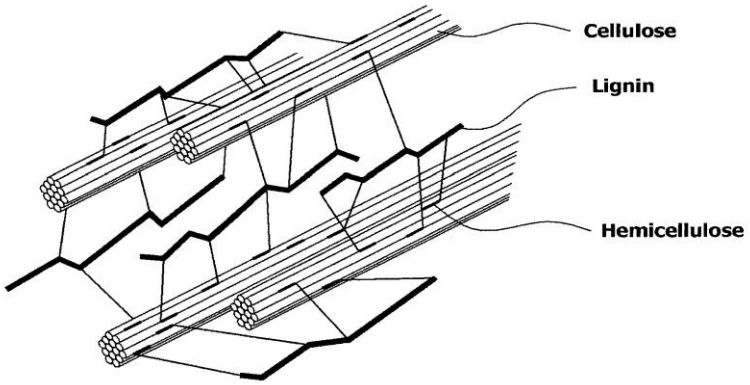

Figure 1.5. Lignin cross-linking between fibrils of cellulose and chains of hemicellulose (adapted from Cho et al., 2009).

1.1.4 Lignin and indigestibility

"Lignin is the most important single fiber component limiting nutrient availability; however, its effects are not uniform" (Van Soest, 1994). The study conducted by Chandler et al. (1980) provides one of the most important descriptions of the connections between lignin analysis and fiber indigestibility. After fermenting fiber sources for 90 and 120 days in methane digesters, Chandler et al. (1980) found that by back calculating the ratio of lignin to total NDF, the ratio of 2.4 (as % of fiber content) time lignin would provide an acceptable estimation of the indigestible fraction of fiber. Van Soest et al. (2005) found an R^2 of 0.94 between the undigested fraction analyzed in several forages through long in-vitro fermentation (240 h) and the value predicted by the 2.4 factor. Another study conducted by Conrad et al. (1984) reported that the indigestible portion of fiber could be calculated with the formula $ADL^{2/3}/NDF^{2/3}$. Nevertheless, these methods are not reliable for the estimation of the indigestible fiber as undigested aNDFom (uNDF) for the following reasons:

1) As previously mentioned in the quote by Van Soest, there are interactions between lignin structure and cross-linking, and the environment that would make this method difficult to validate.

2) The studies have been done only on forages, and it might be not applicable to plant by-products, not included in the analysis.

3) It might be that other forages do not hold the relationship with 2.4 x lignin:

Furthermore, several procedures for lignin quantification have been approved by AOAC (Hatfield and Fukushima, 2005), but inconsistencies among them (Huhtanen et al., 2006b; Raffrenato and Van Amburgh, 2011) lead to the conclusion that lignin

quantification cannot estimate the indigestible portion of the cell wall. Van Soest (1994) suggested that there is no chemical fractionation system that can separate available from unavailable cell wall and that the only real mechanism for that is through live rumen bacteria or a source of other cell-wall degrading enzymes. In-vitro or in-vivo fermentation techniques can help elucidate the availability of cell wall components, but it is easily done under principles where complete recovery of the indigestible fraction is recovered. In conclusion the undigested fraction of fiber recovered with long-term in-vitro fermentation is the proposed estimate of the indigestible fraction of fiber (Van Amburgh et al., 2015).

1.1.5 Ferulate and cross-linking.

Ferulate is a well-known component of grass cell walls that is implicated in cell wall cross-linking (Jung and Ralph, 1990; Hatfield and Ralph, 1999). The existence of bridges of aromatic components between two polysaccharides (diferulic bridges) has been proven biochemically (Grabber et al., 2004). Not only dimers but also tri- and tetramers of ferulic acid has been isolated from cell wall (Fry et al., 2000; Rouau et al., 2003; Bunzel et al., 2006). In a review paper by Ishii (1997) there was much discussion about the ability of ferulate to attach to different saccharide residues by treating grasses with mild acid or with enzymatic hydrolysis. The important isolation and structural elucidation of Xyl-Xyl-Ara-FA-(5-5)-FA-Ara-Xyl-Xyl (Xyl=xylose, Ara= arabinose, FA=ferulate) (Ishi,1991) and Ara-FA-(5-5)-FA-Ara (Saulnier et al., 1999), provided structural evidence that ferulate dehydromerization was a mechanism to cross-link saccharide units and therefore presumably to cross-link the cell wall. The extent of

cross-linking in grasses occurs with the acylation of the primary hydroxyl at the C5 position of L-arabinofuranosyl residues, therefore after a ferulate dimer is produced two polysaccharides can covalently bind. Finally Iiyama et al. (1994) demonstrated that ferulic acid can be etherified to lignin and esterified to arabinoxylans, thus demonstrating that ferulates are indeed cross-linking lignin and polysaccharides. From a nutritionist perspective, the important aspect of the cross-linking is how it affects the availability of cellulose and hemicellulose for microbial attachment and digestion. Cross-linking is thought to occur when a plant needs to create a more rigid structure, for example water stress is known to create conditions where the plant creates more ferulic acid ester and ether linkages between the lignin and hemicellulose to strengthen the cell wall and enhance rigidity to maintain structural integrity and stand ability of the plant (Van Soest, 1996).

1.2 METHODS TO DESCRIBE FIBER DIGESTION

1.2.1 Neutral Detergent Analysis System

The neutral detergent system is a laboratory procedure developed by Goering and Van Soest (1970) to separate the insoluble cell wall from the soluble cell content. There are several methods for the analysis of neutral detergent fiber (NDF) and the methodology used depends on the application of the analysis (Mertens, 2002). For example, if conducting sequential analysis (NDF, then acid detergent fiber, then acid detergent lignin), the best method to ensure full recovery of lignin is to use the neutral detergent residue method, which involves the use of neutral detergent solution and no amylase or sodium sulfite. However, for improved nutritional formulation, the

approach that utilizes both alpha-amylase and sodium sulfite, along with organic matter correction is preferred because it removes any components that will artificially inflate the residue causing an underestimation of true NDF in a diet. The procedure is the feed sample is combined with neutral detergent solution, α-amylase, and sodium sulfite, and boiled for 1 hour. The residue after filtration is defined as neutral detergent fiber corrected for amylase and sodium sulfite or aNDF, but not corrected for ash. Much of the insoluble ash stays into the filter and becomes perceived as aNDF. To overcome this issue, the glass filters holding the aNDF residues are placed into furnaces at 500° C for two hours. Such a practice is employed for burning the organic fraction (ash correction), thus by difference we obtain organic aNDF: [(filter + aNDF) – (filter + ash)] = aNDFom; which nutritionally represents the cell-wall components.

For the filtration of the residues, Raffrenato and Van Amburgh (2010) suggested using a glass microfiber filter "manufactured from 100% borosilicate glass that is binder free and chemically inert". This filters have been shown to increase the recovery of fine particles compared to the commonly used filtration system explained by Udén (2006).

1.2.2 aNDFom digestion using in-vitro and in-vivo techniques

Two techniques are employed to analyze fiber digestion: 1) the in-vitro technique, in which feed samples are dried at 55°C for 48 hours, ground to 1 mm screen, weighed into flasks and combined to 40 ml of Goering and Van Soest (1970) buffer, and incubated in a water bath at 39°C under continuous CO_2. After the flask environment becomes anaerobic and reaches 39°C, 10 ml of rumen fluid (from two lactating cows) containing the digesting bacteria (Siddons et al., 1985a; Broderick, 1987), are added to

each flask to start the fermentation. Continuous CO_2 is maintained throughout the analysis. 2) The other is the in-vivo technique, where polyester bags containing feed samples are simply placed into the rumen (Van Keuren and Heinemann, 1962; Schoeman et al., 1972). However, Mertens (1993) described the problems associated with the in-vivo technique when the purpose of the analysis is to study the rate of fiber digestion. To fully investigate the degradation behavior of aNDFom, multiple time-points are necessary to characterize the shape of the curve and to obtain this using the in-vitro technique the following time-points are utilized: 3, 6, 9, 12, 15, 18, 21, 24, 30, 48, 72, 96, 120, 240 hours. An example of decay obtained from an in-vitro analysis is in (Figure 1.6).

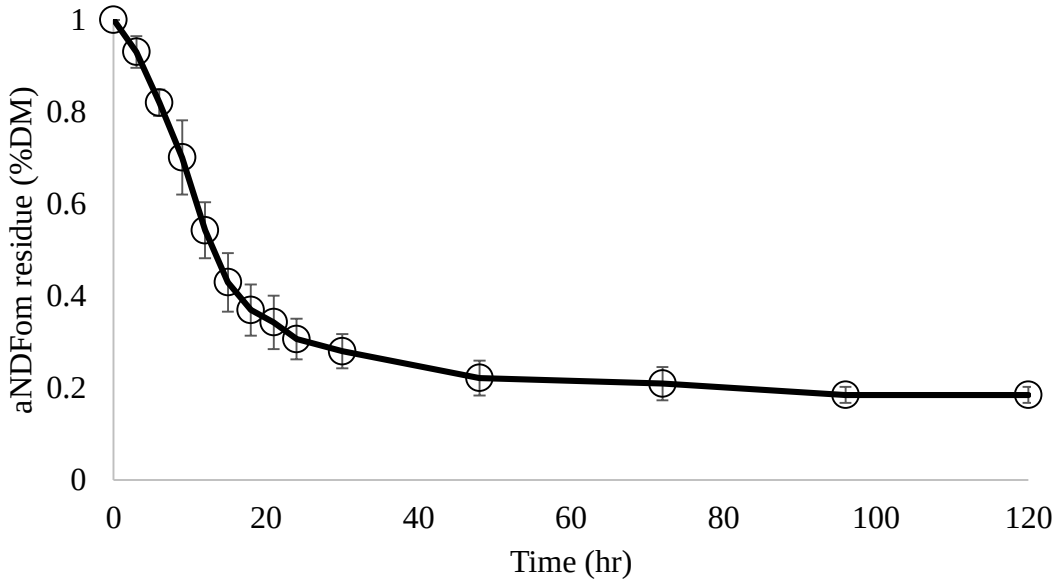

Figure 1.6. Fiber fermentation decay of beet pulp analyzed with the in-vitro technique at multiple time points up to 120 hr, which is the time when aNDFom digestion exhausts in plant by-products. The standard error bars represent the standard error of the measurement at each time point of the aNDFom residue.

The mathematical description of fiber digestion, as with many biological processes displays a sigmoidal behavior where the profile decays exponentially until it has reached an asymptote. Furthermore, fiber digestion follows a first-order behavior, hence its rate of digestion depends only on the amount of the substrate over time. When someone is interested in describing fiber digestion, the first step then, is to find a mathematical equation that represents the process. Fiber digestion can be mathematically expressed by a simple dynamic exponential decay equation. A simple exponential equation that can be used to describe fiber digestion is:

Eq.1: $\mathrm{aNDFom_t} = \mathrm{dNDF}^{-\mathrm{kd}*(\mathrm{t}-\mathrm{L})} + \mathrm{uNDF}$

Where $\mathrm{aNDFom_t}$ is the fiber residue at time t, dNDF and kd are the digestible fiber fraction and its rate of digestion, L is the lag time, and uNDF is the fraction of fiber that remains undigested over time.

The mathematical approach from which Equation 1 is the solution is:

Mathematical process 1:

a) $\frac{\mathrm{dY}}{\mathrm{d_{t-L}}} = -\mathrm{kY}$

The first-order differential equation in step a states that dY is the fiber substrate digesting over time (dt-L), adjusted for the lag phase, and -K the fractional rate of digestion of Y (KY), therefore solving step a for dY/Y gives:

b) $\frac{\mathrm{dY}}{\mathrm{Y}} = -\mathrm{kd_{t-L}}$

Integration of step b yields:

c) $\int_0^{\mathrm{t}} \frac{\mathrm{dY}}{\mathrm{Y}} \mathrm{dY}/_{\mathrm{Y}} = -\,\mathrm{k} \int_0^{\mathrm{t}} \mathrm{d}_{t-L} \quad \rightarrow \quad \int_0^{\mathrm{t}} \frac{1}{\mathrm{Y}} \mathrm{dY} = -\,\mathrm{k} \int_0^{\mathrm{t}} 1 \, \mathrm{d}_{t-L}$

d) $|\mathrm{Log}|\mathrm{Y}||_{\mathrm{o}}^{\mathrm{d}} = -\mathrm{k}_{(\mathrm{t}-\mathrm{L})}$

e) $\mathrm{Log}\mathrm{Y}_{\mathrm{t}} - \mathrm{Log}\,\mathrm{Y}_{0} = -\mathrm{k}_{\mathrm{t}-\mathrm{L}}$

f) $\mathrm{Log}\frac{\mathrm{Y}_{\mathrm{t}}}{\mathrm{Y}_{0}} = -\mathrm{k}_{\mathrm{t}-\mathrm{L}}$

g) $\frac{\mathrm{Y}_{\mathrm{t}}}{\mathrm{Y}_{0}} = \mathrm{e}^{-\mathrm{K}(\mathrm{t}-\mathrm{L})}$

h) $\mathrm{Y}_{\mathrm{t}} = \mathrm{Y}_{0}\mathrm{e}^{-\mathrm{k}(\mathrm{t}-\mathrm{L})}$

Where Yt has been adapted with aNDFom(t), Yo adapted with dNDF, (t-L)is the time t adjusted for the lag phase L, k adapted with kd and the uNDF fraction was then simply added to step h since it is constant.

The absolute amounts of aNDFom and uNDF can by directly analyzed in laboratory; dNDF is indirectly analyzed after subtracting uNDF from aNDFom at time zero; kd is the parameter to calculate; and the Lag time, which is the time required by the fermenting bacteria to attach to the food particles and start digesting fiber, can be either calculated in advance (Van Amburgh et al., 2003) or set as a parameter to be calculated by the model as for kd.

The lag-phase that occurs before the onset of fermentation might be bigger if the rumen fluid is not handled appropriately (i.e. without ensuring anaerobic and temperature conditions), hence shocking the bacteria. Once fermentation starts, rate of digestion depends solely by the amount of substrate. Finally, rate of fiber digestion can be associate to rumen's fiber mean retention time (MRT) or fiber passage rate kp (1/MRT) to estimate its extent of degradation in the rumen by using the formula kd/ kd +kp, as described by Waldo et al. (1972) (Figure 1.7).

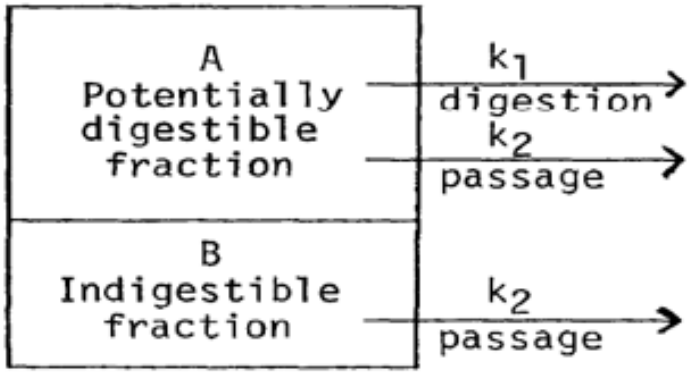

Figure 1.7. Characterization of cellulose disappearance from the rumen with two fractions: a digestible fraction that disappears by digestion and passage and an indigestible fraction that disappears only by passage, thus two rates describe the process: rate of digestion and rate of passage. Adapted from Waldo (1972).

1.3 MODELING FIBER DIGESTION

The rate of fiber digestion is an input in feed formulation systems and nutrition models (Van Amburgh et al., 2015; Higgs et al., 2015). The application of this information the CNCPS model (Van Amburgh et al., 2015) can demonstrate the importance of having accurate values of the digestion rate of fiber for the estimation of cattle performance in terms of feed intake and consequent milk production and nutrient excretion.

1.3.1 First order behavior and lag phase.

The analysis of fiber digestion has been of interest since the 1950's when researchers found difficulties in describing a process that was non-linear, as shown in the feed fermentation degradation in Figure 1.6. Waldo (1970) introduced the concept whereby if only the digestible part of fiber was taken into consideration, after subtraction of the indigestible fraction, then a first-order behavior would best explain the process in which,

for instance, the only limiting factor was the substrate. According to Mertens (1993) the implicit assumptions of a first-order model are:

1. The potentially digestible and indigestible pools act as distinct compartments with homogeneous kinetic characteristic.
2. The fractional rate of digestion is constant and is an intrinsic function of the digestive system and substrate.
3. Digestion begins instantly at time zero and continues indefinitely.
4. Enzyme or microbial concentrations are not limiting.
5. Flux or absolute rate is strictly a function of the amount of substrate present at any time.

Mertens (1993) stated that the classical test for appropriateness of the first-order mass-action model is to plot the natural logarithm of the digestible residue versus time (Figure 1.8).

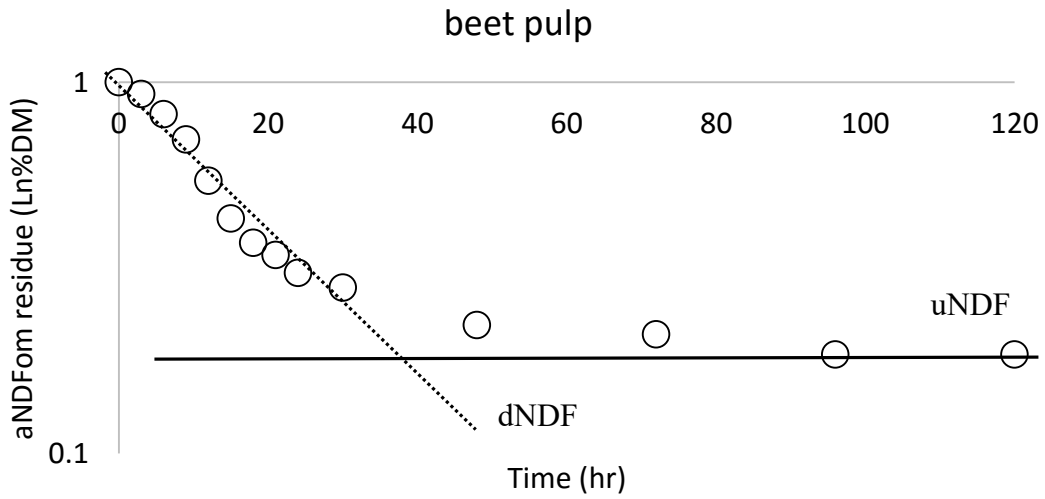

Figure 1.8. Degradation of aNDFom of beet pulp fermented with the in-vitro technique, plotted using a semi-log function. Dashed line indicates the first-order behavior process of fiber digestion, and solid line the undigested aNDFom (uNDF).

If the plot shows a linear relationship then the absolute rate of the digestion reaction is constant and proportional to the size of the digestible pool; therefore the first-order model is plausible and cannot be rejected. When fermentation decays are logarithmically transformed, it can be seen mathematically that linearity is not met in the first four hours, and hence, the prediction of the digestible aNDFom exceeds the concept of unity (slope equal to 1) (Figure 1.8). Mertens (1977) explained that phenomenon by introducing the concept of a lag phase. From a biological perspective, the lag phase can be considered as the time needed by the ruminal bacteria to attach to fiber and make it available for digestion (Allen and Mertens, 1988; Van Milgen et al., 1991). Mertens (1990), furthermore observed that a discrete lag term was a necessary addition to the dynamic equation to adequately describe digestion processes. Van Amburgh et al. (2003), using corn silages as an example demonstrated how the lag term can be calculated from the logarithmic transformation and its value added to the equation. It is now possible to understand each of the parameters of the equation (Eq.1) that we have adopted in our lab to express the fiber digestion profile:

Eq.1: $\mathrm{aNDFom_t} = \mathrm{dNDF}^{-\mathrm{kd(t-L)}} + \mathrm{uNDF}$

The fourth and fifth assumptions of the first-order model clearly state that rate and extent of digestion are not limited by anything else then the substrate itself, however France et al. (1982) suggested that microbial mass or enzymatic activity may be a limiting factor when the inoculum is not handled carefully or the rumen buffer is not prepared properly. Finally digestion of feed components follows a first-order behavior if no contamination from outside the fermentation vessel occurs during data collection (Mertens, 1993) and

if feeds are finely ground (Michalet-Doreau and Cerneau, 1991).

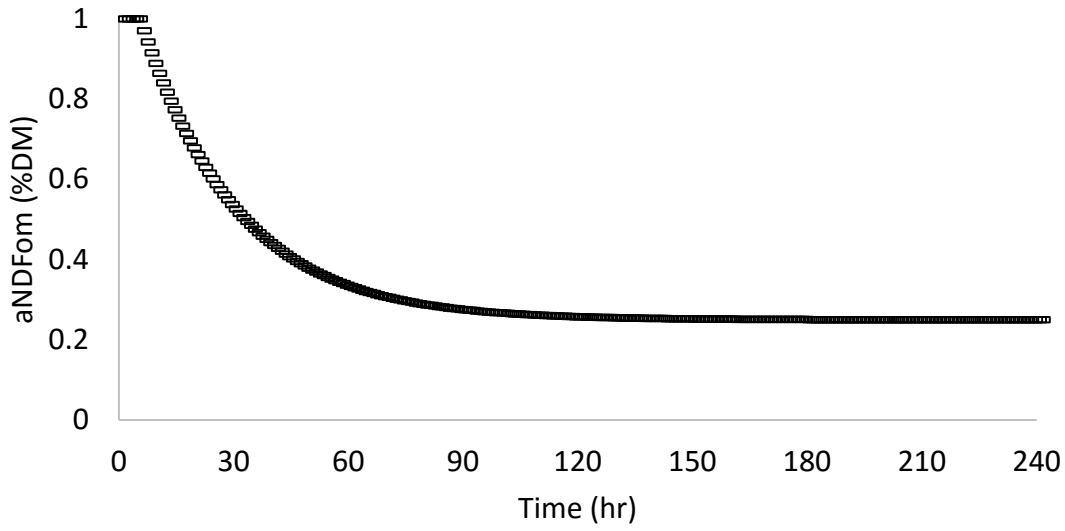

Figure 1.9. First-order, exponential decay expressed by equation 1.

1.3.2 Multiple pools of digestible aNDFom

Within forages, digestion is better described mathematically when the dNDF component is considered as split in two digestible fractions degrading with different rates and first order functions (Figure 1.10) (Mertens, 1973; Mertens, 1977; Mertens and Ely, 1979; Van Milgen et al., 1991). More recently Ellis et al. (2005) demonstrated that models of fiber digestion provided better predictions of degradation if two pools of dNDF were utilized . Also, Huhtanen et al. (2008) described gas production from in-vitro fermentations and reported a gain in model prediction of total NDF digestibility when dNDF was assumed to be composed of a two digestible pools. Therefore if the digestible fraction of fiber in forages is composed by more than one pool, a dynamic composite decay equation is needed to describe its digestion profile. To utilize that approach, Raffrenato and Van Amburgh (2010) described fiber digestion in forages with

the use of such equation, using a non-linear least-squares-fit method to solve it (Villuendas and Pelayo, 1987). Based on the available data that describes two digestible fractions of aNDFom equation 2 has been used to describe digestion curves and calculate the respective rates of digestion; along with the indigestible fraction (Raffrenato, 2011). Thus, the residual aNDFom at time t is described by:

Eq. 2 : $\mathrm{aNDFom_t} = \mathrm{dNDF_1^{-kd_1(t-L)}} + \mathrm{dNDF_2^{-kd_2(t-L)}} + \mathrm{uNDF}$

where $dNDF_1$, and kd_1 are the size at time (t) and fractional rate of the fast pool, respectively; $dNDF_2$, and kd_2 are the size and fractional rate of the slow pool, respectively; L is the lag and uNDF is the undigested aNDFom (Raffrenato, 2011).

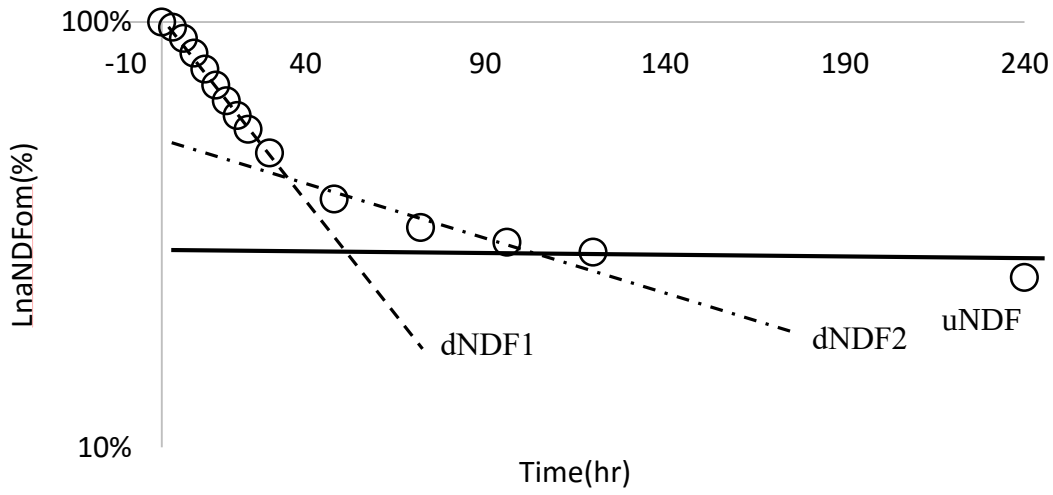

Figure 1.10. Degradation of aNDFom of a bmr corn silage fermented from 0 to 240 hr using the in-vitro technique and plotted on a semi-log basis. The dashed line indicates the fast pool, dashed-dot line indicates the slow pool, and solid line the uNDF.

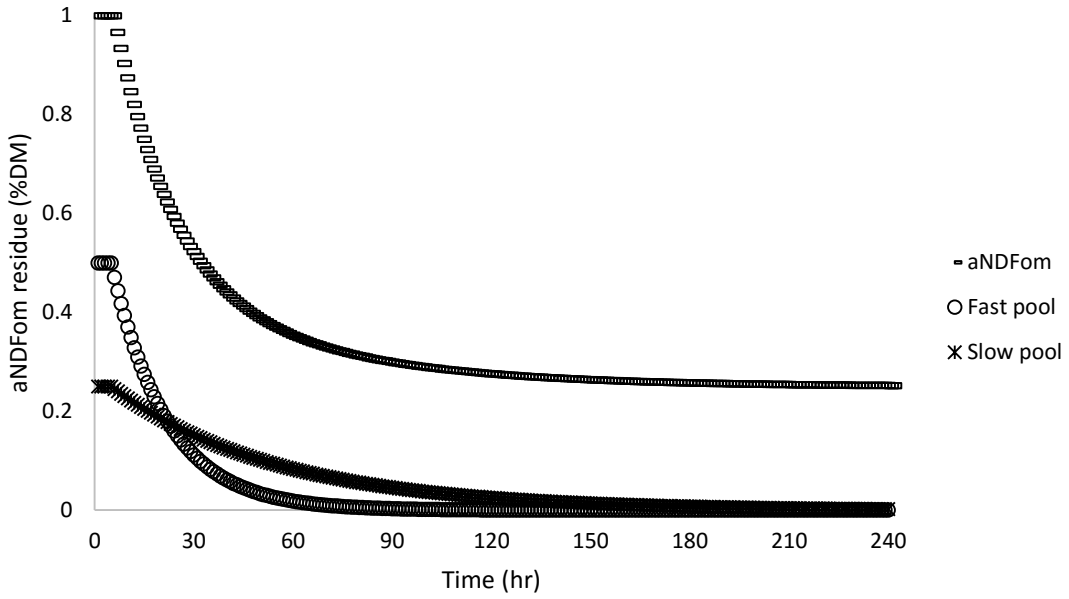

Figure 1.11. Decays of fast, slow and integrated curve of aNDFom digestion expressed by equation 2.

1.3.3 Dynamic models and modeling

There are several software programs available for model development that allow for dynamic approaches to solving biological calculations. One such program is Vensim® (Ventana Simulation Environment; Ventana System Inc., Belmont, MA, 2005), an interactive software environment that allows the development, exploration, analysis, simulation and optimization of dynamic models. The benefit of using Vensim® is that one can represent a dynamic system with a diagram made of boxes and flows, a concept that human mind easily understand. After the user has described the system with a visual tool (diagram), the researcher can associate to it the mathematical equations that explain the dynamic process. Therefore, Vensim® merges diagrammatic models to mathematical models, and this approach is very helpful for conducting research and developing a model. A diagram, along with the mathematical equations, describing

aNDFom in-vitro digestion of forages was built in Vensim® by Raffrenato and Van Amburgh (2010). Such a model (Figure 1.12) can calculate the aNDFom in-vitro digestion curve analyzed in the laboratory, and requires a minimum of three time-points from the fermentation to adequately estimate the rate of digestion and size of the aNDFom pools. By mathematically replicating the aNDFom digestion curve, Vensim® also estimates the rate of aNDFom digestion, the extent of the lag phase, and the dimensions of the digestible pools (Fast and Slow). For instance aNDFom in-vitro digestion of forages is modelled by using 0, 30, 120, and 240h fermentation time-points as model's input (Raffrenato, 2011). Size and rate of aNDFom fractions are used into the CNCPS for the prediction of structural carbohydrate fermenting bacterial growth, subsequent rumen nitrogen balance and nutrient supply (Russel et al., 1992). Therefore, having a mechanistic deterministic dynamic model as a tool that accurately quantifies the extent and rates of digestion of the fiber fractions, is very important for the CNCPS. The model has to be mechanistic because the objective of modeling fiber digestion is to quantify rate of fiber digestion, and that can only be done with an equation that describes the mechanism by which the process occurs, whereas that cannot be done with an empirical model that can only quantify the correlation between two variables. Deterministic because a stochastic output would not be of help for the CNCPS (balancing diets for cattle), and dynamic as a consequence of describing the mechanics of the process that occurs over time: a static equation cannot replicates the sigmoidal behavior of aNDFom digestion.

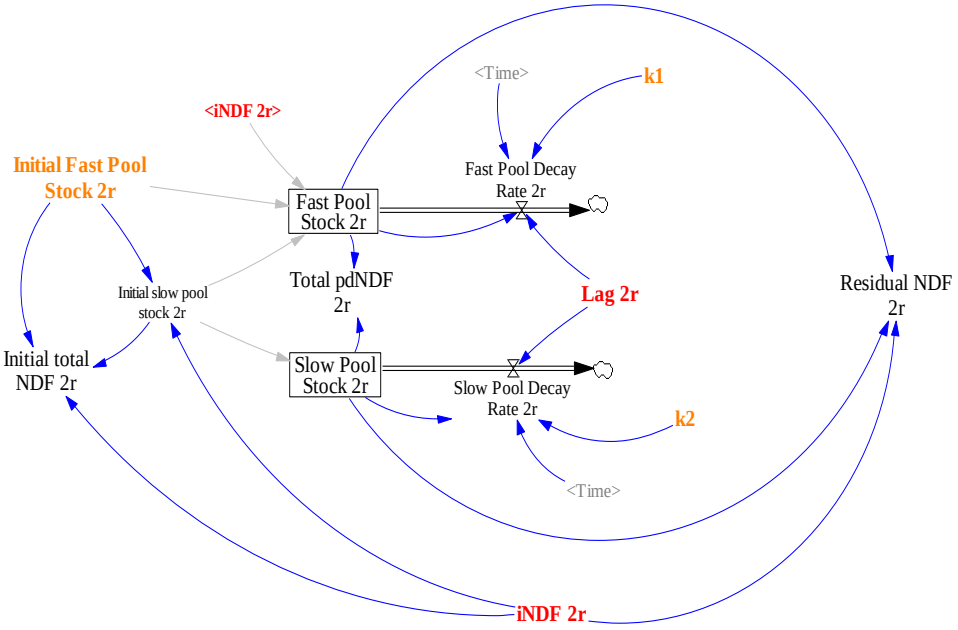

Figure 1.12. Diagram of fiber digestion model developed with Vensim® to estimate the rate of digestion of aNDFom and calculate three pools of aNDFom digestion by Raffrenato (2011).

1.4 APPROACHES TO DESCRIBE FIBER FLOW KINETICS IN THE RUMEN

1.4.1 Surgical preparation to measure fractional rate of passage

For studying the dynamics by which digesta pass out of the rumen, the reticulum, even though is proximal to the rumen, is not considered a correct compartment for sampling since there is no physical separation from the rumen. Thus, the reticulum still holds large particles not found after the reticulo-omasal sphincter (Hogan, 1964), thus the omasum is the preferred sampling site. The omasal cannulation technique is reported in different studies and it involves a device designed to sample digesta leaving the rumen (Ash, 1962; Hume et al., 1970; Engelhardt and Hauffe, 1975). Another procedure developed by Punia et al. (1992), requires the use of a tube linked to a vacuum pump,

entering via the ruminal cannula and collecting omasal samples by aspiration through the reticulo-omasal orifice. However, Wenham and Wyburn (1980) showed by radiological observation that cannulas disturb cows other than altering normal digesta. Furthermore, as reported by Poncet and Ivan (1984) electrical activity is altered in the gastro-duodenal tract due to cannulation, especially with re-entrant cannulas. Therefore the method that has been developed and most widely adopted relies on the collection of samples by aspiration of omasal fluid with a tube entering via the rumen fistula and into the omasum. Such a technique is well described by Hart and Leibholz (1983) and as reported by Huhtanen et al. (1997) control cows and cows in which devices were inserted had similar behavior. After animals are surgically prepared for digesta flow analysis, they need to be treated with infusion of indigestible external markers.

1.4.2 Markers for digesta flow

The kinetics of the passage of fiber into the rumen are the result of the combination of fiber digestion rate within the rumen and fiber rate of passage from the rumen. Rate of fiber digestion (kd) can be associate to rumen's fiber passage rate (kp = 1/MRT) to estimate its extent of degradation in the rumen. Generally, the passage of digesta into the gastrointestinal tract (GI) are studied with the use of markers, both internal and external. Markers are compounds utilized to estimate particulate flow through the rumen and GI tract and markers have particular criteria to be useful. In that regard, Faichney (1975) listed the criteria for the ideal marker:

1. It must not be absorbable;
2. It must not affect or be affected by the GI tract or its microbial population;

3. It must flow parallel with or be physically similar to or intimately associated with the material it is to mark;
4. Its method of estimation in digesta samples must be specific and sensitive and must not interfere with other analyses.

For studying digesta flow in ruminants, markers are usually recovered in the abomasum or in the feces after either a single dose of marker, or after continuous marker infusion (Owens and Hanson, 1992). Single dose is employed to assess average retention time (which is the time required by digesta before passing through a specific section of the GI tract) and average flow rate (France et al., 1985). Continuous infusion, on the other hand, is employed for the estimation of instantaneous flow at a specific section of the GI tract (France and Siddons, 1986). The term to express digesta flow is fractional rate of passage and it is calculated as the inverse of the mean retention time. After markers are selected, the animals have to be surgically prepared for sampling if sampling along the GI tract is to occur, otherwise markers can be applied to the feed and the appearance of them in the feces can be measured through multiple time-point sampling.

To study instantaneous digesta flow, markers are usually infused continuously, after a priming dose, to uniformly stain the digesta and creating a constant ratio of digesta to marker. Furthermore, with this technique, digesta sampling can only occur after steady-state conditions are achieved. Then, dividing the infusion rate of the marker by the marker concentration in the sample, digesta flow rate is calculated. Steady state condition can be difficult to obtain because cattle do not eat continuously during the day, and even so there is diurnal variability thus some deviation has to be expected. To partially overcome this issue a representative sample of the daily flow is obtained by

compositing samples taken every 1-2 hours, in a 24 hour cycle. Another consideration is that ruminal digesta are not uniform but constituted of different pools or phases; the liquid phase and the small and large particles (Owens and Hanson, 1992). Furthermore, the sampling techniques known to study digesta flow in ruminants do not guarantee collection of representative samples (representative sample = different phases of sample in same proportion as digesta), and if a representative sample of the digesta cannot be taken; the use of a single marker would lead to incorrect estimates of the flow. A commonly used strategy introduced by Weston and Hogan (1967) to overcome this last issue considers the utilization of a marker that associates exclusively to the liquid phase and another to the particle phase (double marker), allowing for a more uniform distribution between markers and digesta component, thus summing them to measure total digesta flow. Furthermore, France and Siddons (1986) have demonstrated that, assuming different phases flow independently and that samples are phase-representatives but not digesta-representative; and providing markers distribution is significantly different among phases, the double marker methods can be extended to the use of more markers (i.e. triple marker). Finally this technique requires complete separation of the different phases for the analysis. Thus after all these considerations, digesta flow (DF) can be calculated as (Faichney, 1993):

Eq. 3: $$DF = \frac{I_{liq}}{C_{liq}} + \frac{I_{small}}{C_{small}} + \frac{I_{big}}{C_{big}}$$

Where I_{liq}, I_{small}, and I_{big} are the infusion rate (mg/day) of the marker for the liquid phase, small and big particle phase respectively (i.e. Cobalt-EDTA complex (Udén et al., 1980), Ytterbium complex (Siddons et al., 1985b), and uNDF (Ahvenjärvi et al., 2003) respectively), and C_{liq}, C_{small}, and C_{big} are the concentrations of the markers in the sample

(mg marker / mg phase).

The flow rate of any digesta component (CF) is then calculated as:

Eq.4: $\mathrm{CF} = \mathrm{CC_{liq}}\left(\frac{\mathrm{I_{liq}}}{\mathrm{C_{liq}}}\right) + \mathrm{CC_{small}}\left(\frac{\mathrm{I_{small}}}{\mathrm{C_{small}}}\right) + \mathrm{CC_{big}}$

Where CC_{liq}, CC_{small}, and CC_{big} are the concentrations of the digesta component in the different digesta phases.

1.4.3 Multi-compartment passage models

After observing the profile that stained feed particles were following when exiting the GI tract of sheep (Figure 1.13) (assuming that the flow profile the labelled particles were showing in compartment n was resembling the flow profile in compartment n-1), Blaxter et al. (1956) described for the first time the digesta passage process with a multi compartments model (Figure 1.14) using differential equations to quantify the flow from one compartment to the other.

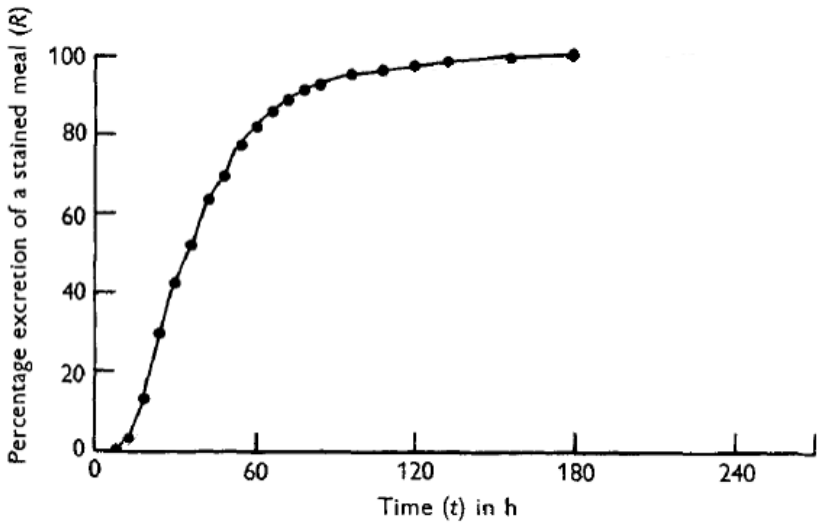

Figure 1.13. The curves represent the cumulative total of the stained particles excreted, expressed as a percentage over time. (Adapted from Blaxter et al., 1956).

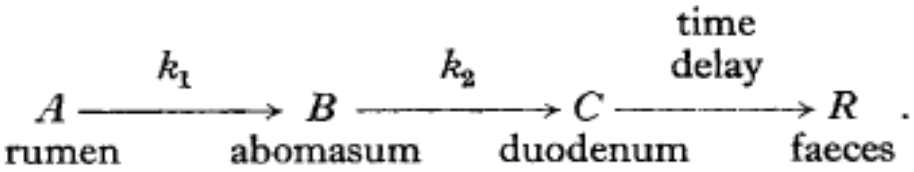

Figure 1.14. Multi-compartmental model describing the flow of digesta through the digestive tract of ruminants. (Adapted from Blaxter et al., 1956).

Much later, using the basic information developed by Blaxter, Thornley and France (2007) gave a very elegant explanation of how to mathematically explain the digesta flows in a multiple compartment model (up to 4 compartments), using differential equation:

Eq.5: Compartment 1 (Rumen): $^{dX1}/_{dt} = -K_1X_1$

Eq.6: Compartment 2 (Abomasum): $^{dX2}/_{dt} = K_1X_1 - K_2X_2$

Eq.7: Compartment 3 (Duodenum): $^{dX3}/_{dt} = K_2X_2 - K_3X_3$

Eq.8: Compartment 4 (Feces): $^{dX4}/_{dt} = K_3X_3$

Therefore solving such equations for X1 ≠ X2 ≠ X3 ≠ X4 yields (look mathematical process 1):

Eq. 9 (Ingesta remaining in the rumen over time): $X_1 = De^{-K_1t}$

Eq.10 (Ingesta remaining in the abomasum over time): $X2 = K_1D(\frac{e^{-K_1t}}{K_2-K_1} + \frac{e^{-K_2t}}{K_1-K_2})$

Eq.11 (Ingesta remaining in the duodenum over time):

$X3 = K_1K_2D[\frac{e^{-K_1t}}{(K_2-K_1)(K_3-K_1)} + \frac{e^{-K_2t}}{(K_1-K_2)(K_3-K_2)} + \frac{e^{-K_3t}}{(K_1-K_3)(K_2-K_3)}]$

Eq.12 (Ingesta exiting the duodenum over time):

$$X4 = D[1 - \frac{K_2K_3e^{-K_1t}}{(K_2-K_1)(K_3-K_1)} - \frac{K_1K_3e^{-K_2t}}{(K_1-K_2)(K_3-K_2)} - \frac{K_1K_2e^{-K_3t}}{(K_1-K_3)(K_2-K_3)}$$

And the fecal marker outflow rate becomes:

Eq.13: $$dX4/dt = K_1K_2K_3D[\frac{e^{-K_1t}}{(K_2-K_1)(K_3-K_1)} + \frac{e^{-K_2t}}{(K_1-K_2)(K_3-K_2)} + \frac{e^{-K_3t}}{(K_1-K_3)(K_2-K_3)}$$

And the analytical solution to the N-pool model, given $K_1 \neq K_2 \neq \ldots \neq K_{N-1}$ is:

Eq. 14: $$dXN/dt = (\prod_{i=1}^{N-1} Ki)D \sum_{i=1}^{N-1}[\frac{e^{-K_1t}}{\prod_{\substack{j=1 \\ J\neq i}}^{N-1}(Kj-Ki)}]$$

Using another approach, Matis (1972) described the lifetime of the feed particles in the rumen using a gamma distribution, which is a statistical probability approach that makes passage from rumen to abomasum time dependent. For example during a meal a cow swallows feed particles that enter the rumen (compartment 1) and the new particles start to mix with other feed particles (digesta) that have been consumed in previous meals, thus the mix of particles are of different "ages". If the newly ingested feed particles have same probability of exiting the rumen than older feed particles, the process is defined as age-independent, and an exponential decay equation (first-order) can describe the residence time of the digesta in the rumen. However, if the process is time dependent, considering instead that the digesta particles are following an age-dependent process, the probability of a particle to pass out of the rumen increases with increased residency time. To generate an age-dependent distribution the rumen compartment needs to be sub-compartmentalized with *n* independent exponential sub-compartments having same distribution (Figure 1.15). Pond et al. (1988), reported how the gamma distribution varies according to time-dependency; in particular higher orders of gamma

function are used to model higher orders of time dependency (i.e. ingesta that take longer to escape the rumen) (Figure 1.16).

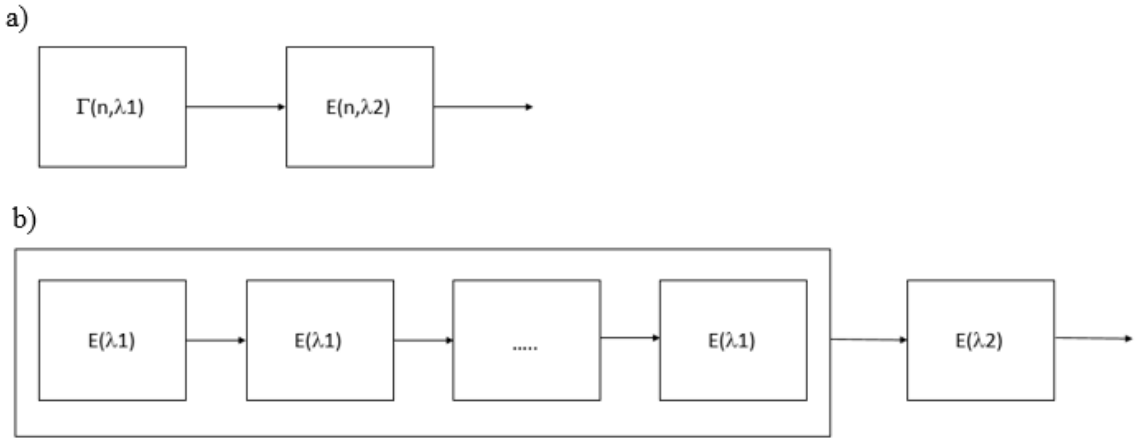

Figure 1.15. In figure 15a: A two compartment model with gamma distribution in the first and exponential distribution in second compartment. In Figure 15b is the schematic of the first compartment showing *n* independent exponential sub-compartments, depending on the order of the gamma distribution. Higher orders of gamma distribution express higher orders of time dependency (i.e. second order = two sub-compartment, third order = three sub-compartments). (Adapted from Matis,1972).

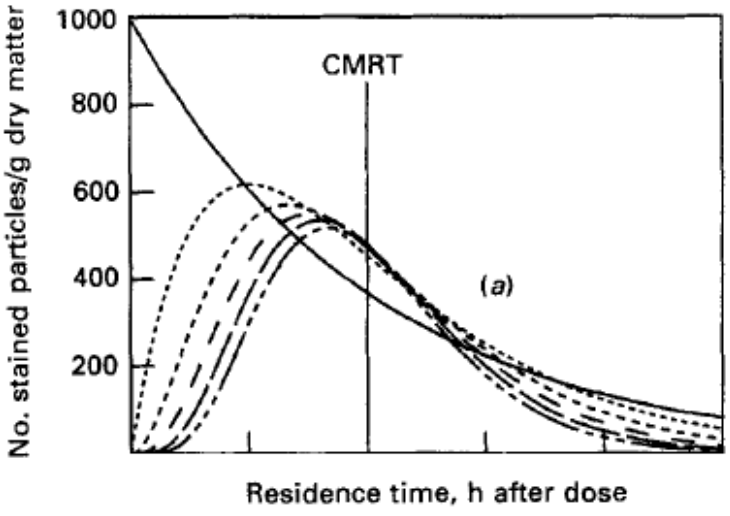

Figure 1.16. Increasing orders of gamma distribution in one-compartment model, as the order increases so does the time-dependency. (Adapted from Pond et al., 1988).

Pond et al. (1988) described how to incorporate time-dependency into one or two compartment models, and how the flow parameters would vary according to the different degrees of the gamma distribution. The mathematical functions adapted from Pond et al. (1988) can be used for expressing higher order of time-dependency in one and two compartment models and this is described in Table 1.1,
Table 1.2,

Table 1.3 and Table 1.4. As reported in different studies (Pond et al., 1988; Matis et al., 1989; Poppi et al., 2001), despite the apparent mechanistic approach the gamma functions bring to modeling approach, the application is very empirical, since researchers only analyzed and fitted the model or models that best fit the dataset, and, from that the flow rates (or compartment mean retention time) were obtained. Therefore, the gamma distribution approach does not provide any insight into the mechanism that explains the nature of digesta flow in ruminants, except that a multi-compartment model it is likely to be the right approach for modeling this biological process. It is likely that the need to apply different gamma distributions to different studies involves the differences in digestibility in aNDFom among and within studies. The digestibility of the aNDFom is rarely considered during the evaluation of the studies involving passage rate where particle size is normally the factor of interest.

Table 1.1. Increasing orders of gamma function expressing total marker remaining in the compartment over time, in one-compartment model. Adapted from Pond et al. (1988).

Model	Distribution	Total marker remaining in the compartment
G1	Gamma-1 (exponential)	De^{-Kt}
G2	Gamma-2	$De^{-\lambda t}(1 + \lambda t)$
G3	Gamma-3	$De^{-\lambda t}[1 + \lambda t + (\lambda t)^2/2]$
Gn	Gamma-n	$De^{-\lambda t} \sum_{(n-1,i=0)} (\lambda t)^i/i!$

Table 1.2. Increasing orders of gamma function expressing total marker exiting from the system over time, in a one-compartment model. Adapted from Pond et al. (1988).

Model	Distribution	Marker concentration
G1	Exponential	Ce^{-kt}
G2	Gamma-2	$C\lambda te^{-\lambda t}/0.59635$
G3	Gamma-3	$C\lambda^2t^2e^{-\lambda t}/(2 \times 0.47454)$
G4	Gamma-4	$C\lambda^3t^3e^{-\lambda t}/(6 \times 0.40857)$
G5	Gamma-5	$C\lambda^4t^4e^{-\lambda t}/(24 \times 0.36528)$
G6	Gamma-6	$C\lambda^5t^5e^{-\lambda t}/(120 \times 0.33929)$

Table 1.3. Increasing orders of gamma functions expressing total marker remaining in the system over time, in a two-compartment model. Adapted from Pond et al. (1988).

	Distribution		
Model	Compartment1	Compartment2	Total marker remaining in the system
G1G1	Exponential	Exponential	$D(K_1 e^{-K2t} - K_2 e^{-K1t})/(K_1 - K_2)$
G2G1	Gamma-2	Exponential	$D\{\delta^2 e^{-K2t} + e^{-\lambda 1t}[1-\delta^2 + (1-\delta)\lambda_1 t]\}$
G3G1	Gamma-3	Exponential	$D\{\delta^3 e^{-K2t} + e^{-\lambda 1t}[1-\delta^3 + (1-\delta^2)\lambda_1 t + (1-\delta)(\lambda_1 t)^2/2]\}$

Table 1.4. Increasing orders of gamma function expressing total marker exiting from the system over time, in a two-compartment model. Adapted from Pond et al. (1988).

	Residence-time distribution		
Model	Compartment1	Compartment2	Marker Concentration
G1G1	Exponential	Exponential	$C_2K_1(e^{-K2t} - e^{-K1t})/(K_1 - K_2)$
G2G1	Gamma-2	Exponential	$C_2[\delta^2 e^{-K2t} - e^{-\lambda 1t}(\delta^2 + \delta\lambda_1 t)]$
G3G1	Gamma-3	Exponential	$C_2[\delta^3 e^{-K2t} - e^{-\lambda 1t}(\delta^3 + \delta^2\lambda_1 t + \delta\lambda_1^2 t^2/2)]$

1.4.4 Mechanistic sub-models of rumen fiber digestion kinetics

Mertens (1997) postulated that rumen kinetics were biologically best represented by a multi-compartment model. Different theories have been developed to describe the kinetics of fiber digestion with a sub-compartmentalization of the rumen. Mertens and Ely (1979) proposed a theoretical mass-action model (sub-compartments with exponential distribution) of particle size reduction. Using a similar concept, Poppi and Norton (1980) postulated that the likelihood of a particle to exit the rumen was inversely related to its size. Sutherland (1988), however, concluded that a better criteria to compartmentalize the rumen was according to buoyant properties of particles, even if he acknowledged that size was correlated to buoyant properties, and hence, subdivided the rumen in buoyant particles and particles that sediment in the rumen. Ahvenjärvi et al. (2001) found that particles were similar in fiber digestibility from dorsal to ventral sac and reticulum, pointing out that buoyancy may not be the correct approach either. Nevertheless, Hristov et al. (2003) observed that particles that sediment contained more indigestible fiber than buoyant particles. Furthermore, Huhtanen et al. (2006a), in support to that, reported different experiments in which the mean retention time estimated by rumen empting was longer for digestible compared to indigestible fiber, in spite of the fact that they are both in the same particles. Allen and Mertens (1988) proposed a three compartment model incorporating a lag-phase for non-digestible fiber and selective retention into the rumen for not escapable digestible fiber (Figure 1.17).

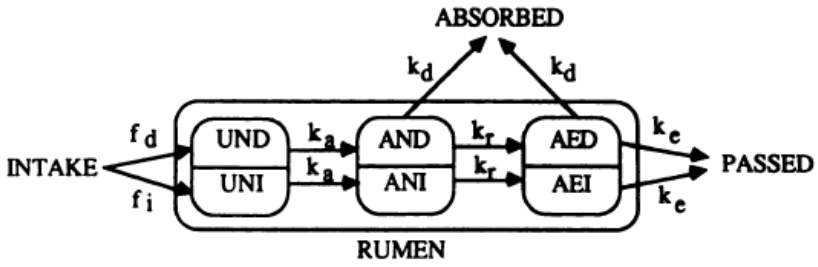

Figure 1.17. Rumen model of fiber disappearance. (Adapted from Allen and Mertens, 1988).

Poppi et al. (2001) described the non-escapable pool using the raft theory, where particles into the raft were modelled with an age-dependent function and the particles available in the escapable pool were modelled with a first-order behavior (Figure 1.18). In summary, whatever is the mechanism underlying selective retention of feed particles, the process of selective retention should be understood and incorporated into mechanistic dynamic rumen models. Again, in a similar discussion concerning gamma functions, defining the true aNDFom digestion or potentially digestible pools would most likely add some mechanism to all of these models and would help provide a quantitative platform for framing some of these multi-compartment models.

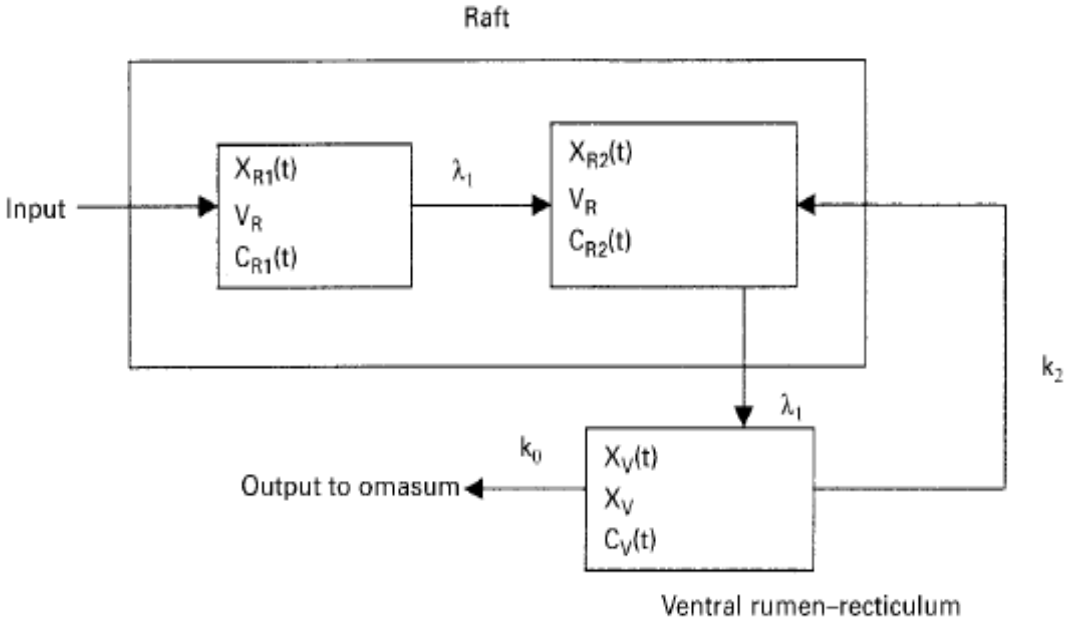

Figure 1.18. Model of fiber kinetics into the rumen. The raft is considered as a pool of two sequential compartments, R_1 and R_2, representing conceptual dosing and sampling sites respectively which are embedded in the same volume, V_R. The rate parameter, λ_1 describes age-dependent turnover flow from the raft pool to the ventral-reticulum compartment, v. Mass action, age independent turnover from v is via exit to the omasum or by recycling back into the sampling compartment of the raft pool, R_2, and the return via rate λ_1. The quantities, X, and concentration, C, of marker in each compartment's volume, V, is indicated as a function of time (Adapted from Poppi et al., 2001).

1.5 VOLUNTARY DRY MATTER INTAKE

Feed intake has always been a topic of interest because of the impact it has on the performance of the livestock. It is also central to the discussion of modeling aNDFom digestibility since aNDFom plays such an important role in the concept of physical fill limitations in ruminants. Baile and Forbes (1974) presented the large number of physiological, psychological, and environmental factors that contribute to explain feed intake. Voluntary dry matter intake is stimulated by hunger and inhibited either by a physical limitation, if the flow of the digesta in the digestive apparatus is slow, therefore creating a ballast that stretches the wall of the gastro intestinal tract (GIT) inhibiting hunger in the animal (Campling, 1970; Forbes, 2007); or by physiological limitation when the animal eating a high energy diet reaches its energy requirements sooner (Gherardi and Black, 1989; Mertens, 2010). In support of that, Blaxter et al. (1961) reported a decreased response in voluntary dry matter intake (VDMI) as digestibility of forages increased. Furthermore, Conrad et al. (1964) demonstrated that voluntary food intake was positively related to the digestibility of the diet when using medium-poor quality feeds (up to 67% in digestibility), and negatively correlated for high quality feeds (above 67% in digestibility) (Figure 1.19). They also stated that the higher is the animal's production the higher has to be the digestibility of the diet to limit feed intake (further above 67%). In other words voluntary feed intake of high producing cows is more likely to be controlled by physical limitation. Therefore since fiber is not soluble and is slowly digested in the rumen compared to other nutritional factors, it has a greater filling effect over time, and hence considered the main driver of feed intake in ruminants (Mertens and Ely, 1979; Mertens, 1997). Due to their relatively high fiber content,

forages have a greater capacity to cause physical distention and thus are one of the best predictors of voluntary dry matter intake (Van Soest, 1965; Waldo, 1986). However, other factors associated and not to aNDFom affect fill: aNDFom fractionation, particle size, particle fragility, chewing frequency and effectiveness (Allen, 1996).

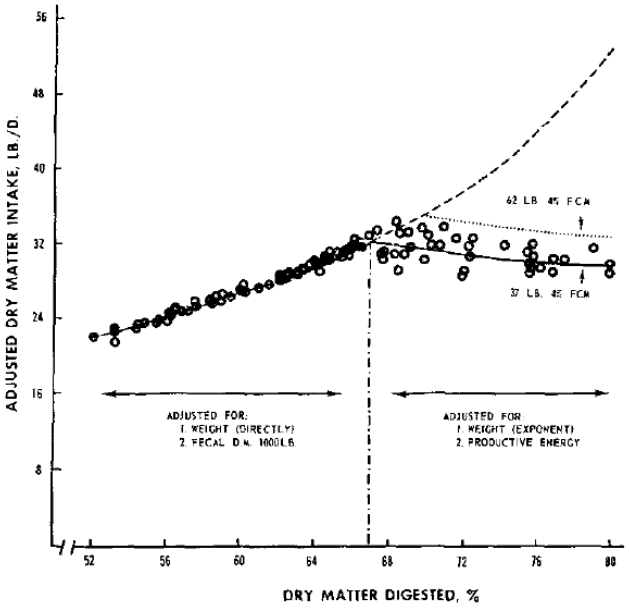

Figure 1.19. This figure describes the effect of digestibility of the diet on the effect of dry matter intake with a breakpoint at approximately 67% digestibility. Below 67% dry matter digestibility, intake is limited by physical fill while above 67% intake is more limited by chemical fill or metabolites of digestion, like volatile fatty acids and glucose (from Conrad et al., 1964)).

1.5.1 *Models to predict dry matter intake*

Feed intake prediction is important when formulating diets for dairy cattle. An accurate prediction limits under- or over-feeding of nutrients that can have a negative impact on an animal's health and production, environment, and also on feed costs. Many models have been designed to predict feed intake (Ingvartsen, 1994; Poppi, 2008). Models can be classified as empirical or mechanistic, static or dynamic, deterministic

or stochastic. To generate an empirical model the researcher has to fit an equation, with one or multiple independent variables that he considers good in explaining the system, to a dataset (Fuentes-Pila et al., 2003). The selection of the variables of an empirical model is made with a step-wise procedure that can be either a forward selection or backward elimination (Brown et al., 1977; Halachmi et al., 2004), or bidirectional elimination. Most of the models in use for predicting dry matter intake are empirical; the limitation of an empirical model, though, is that it doesn't give any explanation of the correlation between dependent and independent variables, and it must be applied in systems where the conditions are the same as the ones from where the model has been developed. Mechanistic models are derived from theoretical equations (Sauvant et al., 1996; Sauvant et al., 2014), therefore to develop such models the researcher needs to make an important step, i.e. understanding the "mechanism" of the system in order to build some theory and hypothesis to test. Therefore a good mechanistic model does not depend on data-sets, thus more applicable if accurate; it improves the knowledge of the researcher and can provide insights to where to direct future studies. The distinction between static and dynamic models is that the latter present dynamic parameters, and therefore dynamic models include differential equations and have a time function. In the prediction of dry matter intake, the "dynamic parameters" usually considered are the fractional rate of digestion and passage (Mertens, 1987). Static models, on the other hand are purely algebraic and among the ones found in the literature they mostly predict dry matter intake from factors related to the animal only, as for instance, body weight, milk production and stage of lactation (Rayburn and Fox, 1993). Others model have among their independent variables factors associated with components and digestibility

of the diet such as digestible organic matter in the dry matter, total nitrogen, and ammonia nitrogen (Rook et al., 1991). Finally, Brown et al. (1977) considered factors associated to season. The last distinction is between deterministic and stochastic models. Deterministic models are mainly developed to explain how the system behave on "average", and they give precise solutions; whereas stochastic models present the error term to account for the unexplainable.

An important aspect to consider when building a model is to understand at which hierarchical level the model will belong. Thornley and France (1984) introduced the organizational hierarchy of biological systems.

Level	Description of the Level
i + 1	Herd of animals
i	Animal
i – 1	Organs
i – 2	Tissues
i – 3	Cells

The equations generated by empirical modelers can only describe systems at the same level where data were collected, i.e. information taken at the animal level can be used to describe systems at the animal level. Mechanical models, on the other hand, are built by gathering data at lower levels than the systems they want to describe. Finally from a different perspective, there are models developed on the principles of the fill- unit system (Jarrige et al., 1986; Zom et al., 2012), exclusively used in French and Denmark.

Notwithstanding the availability of many models, cow's feed intake is still not completely understood, and therefore there is not an "absolute" model for its prediction yet.

1.6 CONCLUSION

Characterizing the aNDFom fractions (fast, slow digesting pool, and uNDF) along with the rates by which these fiber components are digested, can help the CNCPS with the description of rumen digestion dynamics. Describing accurately rumen digestion dynamics would allow for an improved prediction of microbial yield, rumen nitrogen balance, and nutrient supply, and hence, final better characterization of animal performance, in terms of milk production and excretions. The aNDFom digestion displays a sigmoidal behavior, similar to the one seen for feed particles moving through the different compartments of the GI tract of ruminants, and thus it is likely that similar equations can be employed to describe these processes. Researchers, while using different approaches in describing the multiple steps process of digesta flow in the GI tract, arrived to the conclusion that a multiple compartment model was the best solution to replicate the sigmoidal behavior. However, the mathematical equations that they have adopted (gamma function) were probabilistic, and thus, they could not be used for describing aNDFom digestion where deterministic solutions of aNDFom rate of digestion are needed, if they want to be applied into the structure of the CNCPS. Therefore, describing the two step process taking place during aNDFom digestion (the lag phase where the rumen bacteria prepare the substrate for digestion, and the digestion phase where the substrate is actually digested) with a multi compartmental model using

differential equations, could help modeling aNDFom digestion more realistically then Equation 1 and 2 that express an exponential decay. The differential equations would still provide deterministic values of the size and digestion rates of the aNDFom fractions, to be used as CNCPS inputs.

1.7 OBJECTIVES

At the start of this project most of the work on aNDFom digestion analysis had been done on forages, very few if not data were available on plan by-products. Therefore the first objective of this project was to analyze aNDFom digestion in plan by-products. Specifically to understand where aNDFom digestion exhausted and how aNDFom was fractionated in these feeds; and which fermentation time points to analyze to use as inputs for the calculation of fractions size and rates of aNDFom digestion. The second objective of this project was to perform an experiment to test and quantify the effects of balancing diets for dairy cattle, considering the three fractions of aNDFom, on productivity and diet digestibility. The third objective of this project was to develop a mechanistic model that describes the sigmoidal behavior of in-vitro aNDFom digestion, using differential equations to provide deterministic values of digestion rates.

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CHARACTERIZING FIBER DIGESTION IN PLANT BY-PRODUCTS

Interpretive summary:

Fiber quality and digestibility is nutritionally very important in dairy cattle diets, especially for rumen health and is also important for maximizing income over feed costs. Fiber digestion has been characterized in forages but not in plant by-products. Characterizing fiber digestion in plant by-products can provide useful information for nutritionists and provide information about different fiber sources when forages are of poor quality or not available. The objective of this study was to characterize fiber digestion in plant by-products for ruminants and the paper provides a protocol for fiber analysis in by-products to provide useful information to dairy nutritionist.

CHAPTER 2: Characterizing and modeling fiber digestion in plant by-products

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2.1 ABSTRACT

Previous work demonstrated that the extent of aNDFom digestion in forages was achieved by 240 h and resulted in what is termed undigested aNDFom (uNDF). Further, it was demonstrated that the digestible aNDFom (dNDF = aNDFom – uNDF) of forages can be fractionated into two digestible pools (fast and slow), and that forage aNDFom digestion kinetics can be characterized with a composite dynamic deterministic model utilizing four data points (0, 30, 120 and 240 h). The objectives of this study were to understand when fermentable carbohydrates were exhausted in plant by-products and

determine the point at which the uNDF is realized in non-forage fiber feeds; compare the uNDF analyzed through long term in-vitro fermentation with the undigestible fiber fraction calculated using the formula [(2.4 x ADL)/NDF] and employed in the CNCPS; and the formula ($ADL^{2/3}/NDF^{2/3}$), used in the TDN equation; study plant by-products aNDFom digestion to develop an equation to describe the rate and extent of digestion; and obtain the combination of time-points that allows for the highest goodness of fit of the model when predicting aNDFom digestion. Samples of 15 plant by-products were collected, each from two suppliers, and analyzed in duplicate in three separate batches for aNDFom digestion using the in-vitro technique. To determine uNDF the samples were analyzed for 96, 120, and 240 h to evaluate the residues and the overall aNDFom digestion curve was described using the following time points: 3, 6, 9, 12, 15, 18, 21, 24, 30, 48, 72, 120 h. Decays were plotted on a semi-log scale to study the inflection points on the curves that reveal the aNDFom fractions. Data were analyzed using a mixed effect model and treatment effects were analyzed using Tukey's test. Compared to forages, the uNDF of by-products was achieved at 120 h and the time-point combination of 0, 12, 72 and 120 h yielded the highest goodness of fit with an average slope and intercept of 0.95, and 0.04, and overall slope, intercept, and RMSE of 1.00, 0.002, and 0.002, respectively. The dNDF of plant by-products was determined to be one digestible pool and the uNDF and the dNDF decayed exponentially following first-order behavior, thus a simple exponential decay model was proposed.

Keywords: aNDFom, uNDF, modeling, plant by-products

2.2 INTRODUCTION

The rate and extent of aNDFom digestion is an input in feed formulation systems and nutrition models and application of this information in the CNCPS model (Van Amburgh et al., 2015) can demonstrate the importance of having accurate values of aNDFom digestion to better estimate the most limiting nutrient when evaluating milk yield and overall productivity of lactating or growing cattle. For most cellulosic feeds, the in-vitro digestion of aNDFom displays an exponential profile which decays until it has reached an asymptote. The asymptote represents the fraction of aNDFom that is not digestible (Waldo et al., 1972). It is important to have an accurate measure of the undigested aNDFom (uNDF) to accurately calculate size and rate of digestion of the digestible aNDFom (dNDF); (Raffrenato, 2011; Van Amburgh et al., 2013). Previous work to determine the unavailable NDF was conducted by fermenting fiber sources for 90 and 120 days in methane digesters and the residue could be calculated as (lignin x 2.4)/NDF and this equation was used in the CNCPS to calculate the indigestible fraction for all feeds Chandler et al. (1980). In a similar approach Conrad et al. (1984) published that the indigestible fiber could be calculated as $lignin^{2/3}/NDF^{2/3}$, and this equation is used to predict TDN (Weiss et al., 1992). Recent work has described the uNDF of various forages analyzed using the in-vitro technique (Palmonari et al., 2014) and a summary of various studies indicated that the uNDF obtained with long-term in-vitro fermentation was an accurate representative of the indigestible fiber fraction (Cotanch et al., 2014).

Once the uNDF is subtracted from the amount of aNDFom digesting over time, the

digestible fraction of aNDFom (dNDF) is obtained. Digestion of dNDF follows a first-order behavior, thus the rate of digestion depends only on the amount of the substrate over time. Further in forages the dNDF fraction has been characterized as composed of a fast digesting and a slow digesting pool. Therefore, fiber digestion in forages can be modelled by a dynamic exponential decay (Equation 1; adapted from Raffrenato, 2011).

Eq.1: $\mathrm{aNDFom_t} = \mathrm{dNDF_1^{-kd_1*(t-L)}} + \mathrm{dNDF_2^{-kd_2*(t-L)}} + \mathrm{uNDF}$

where $\mathrm{aNDFom_t}$ is the fiber residue at time t, $\mathrm{dNDF_1}$ and $\mathrm{kd_1}$ are the fast pool and fast rate of digestion, $\mathrm{dNDF_2}$ and $\mathrm{kd_2}$ are the slow pool and slow rate of digestion, L is the lag time, and uNDF is the undigested aNDFom at the determined endpoint of digestion that describes the full extent under anaerobic conditions.

The absolute amounts of aNDFom and uNDF can be directly analyzed in a laboratory and dNDF is indirectly analyzed after subtracting uNDF from the aNDFom that changes over time. The resulting pools size and rates of digestion (kd) are calculated with optimization methods by the model and the lag time, which is the time required by the digesting bacteria to attach to the feed particles and initiate digestion can be either calculated in advance (Van Amburgh et al., 2003) or set as a constant parameter if data exist to describe the consistency of the lag within laboratory and feed type.

Non-forage fiber sources or by-products have been used as replacements for forages in formulation of diets for lactating cattle and with mixed success depending on the level of substitution and the consistency of the byproduct (Grant, 1997). Concerns exist about the inclusion level of non-forage fiber sources and associative effects related to particle size and rate of digestion that can negatively affect passage rate, extent of digestion and potentially set up acidosis conditions (Armentano and Pereira, 1997;

Pereira and Armentano, 2000). Data were generated to describe digestion kinetics and extent of digestion as summarized by Firkins (1997) however to our knowledge, a comprehensive analysis of digestibility from a more mechanistic perspective has not been conducted. A better description of aNDFom digestion in plant by-products, consistent with the approach for forages, might help nutritionists employ these feeds when forages are of poor quality or not available and more critically understand how to use them in diet formulation.

The objectives of this study were: 1) to understand when dNDF is exhausted in plant by-products to determine the uNDF fraction, 2) to compare the uNDF analyzed through long term in-vitro fermentation with the indigestible fiber fraction calculated using the formulas presented by Chandler et al. (1980) and Conrad et al. (1984), 3) to develop a dataset of plant by-product aNDFom digestion to analyze digestion curves and develop equations to predict the rate and extent of digestion, and 4) to obtain the combination time-points that allow for the highest goodness of fit of the model when predicting aNDFom digestion decays for use in calculating rates of digestion for use in the CNCPS. The hypothesis is that aNDFom digestion of plant by-products differs from forages because of the difference in cell wall structure among the byproducts and between forages along with the effects of processing, thus the amount of time necessary to identify the uNDF is most likely less than what is needed in forages.

2.3 MATERIALS AND METHODS

Samples of fifteen plant by-products (beet pulp, canola meal, citrus pulp, corn gluten feed, corn distillers, corn germ, flaked corn, rice hulls, soybean meal, soy hulls,

Soyplus™, sunflower hulls, wheat distillers, wheat middlings and whole cottonseed) were collected, each from two suppliers, and analyzed in a three-step process to determine the time required to achieve the uNDF, the number of time points of fermentation required to describe the curve with the least amount of time points, and the actual time points that best describe the digestion of the non-forage byproducts for estimating digestible NDF (dNDF).

For the first step, samples were dried at 55° C for 48 h and ground to 1 mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA). Half-gram samples were weighed into Erlenmeyer flasks with 40 ml of Goering and Van Soest (1970) buffer. Flasks were then held under continuous CO_2 in a water bath at 39°C to hydrate and reduce oxygen concentration. After 2 h, 10 ml of mixed rumen fluid blended from two high producing lactating cattle, (fed a TMR that consisted of corn silage, alfalfa silage with a starch content of approximately 27%), were added to each flask and 39°C and continuous CO_2 was maintained throughout the fermentation. Fermentations were conducted using duplicates samples, and in three different batches for 96, 120, and 240 h consistent with previous data from Raffrenato (2011). Fermentation was stopped by placing flasks on ice and analysis of aNDFom residues was conducted immediately after using the procedure explained by Mertens (2012). Residues were filtered on a glass microfiber filter (934-AH, Whatman) with a 1.5 µm pore size to ensure residue recovery (Raffrenato and Van Amburgh, 2010).

Statistical analysis

To analyze changes in uNDF the residues were compared with a Tukey's test. Significance was declared at *P*-values < 0.05. Furthermore, the uNDF analyzed with

long-term fermentation was compared to that calculated from the lignin (ADL) content using the equations from Chandler et al. (1980), and Conrad et al. (1984) equation. Data were analyzed using the following mixed effects model in JMP (JMPv.11 SAS Institute, Inc., Cary, NC):

$Y_{ijk} = H_i + F_j + S_k + R_m + e_{ijkm}$

where,

Y_{ijkm} is the dependent, continuous variable

H_i is the fixed effect of the i^{th} treatment (i= 1, 2, 3)

F_j is the fixed effect of the j^{th} feed (j= 1,…, 15)

S_k is the fixed effect of the k^{th} supplier (k = 1, 2)

R_m is the random effect of the m^{th} run (m = 1, 2, 3)

e_{ijkm} is the residual error

To determine the digestion behavior and kinetics the same feeds were analyzed in duplicate and in three separate batches for aNDFom digestion using the in-vitro technique. Digestion of aNDFom was obtained by analyzing feed samples through in-vitro fermentation for 3, 6, 9, 12, 15, 18, 21, 24, 30, 48, 72, 96, and 120 h using the procedure described above. The residues from the time points were plotted on semi-log scale and visually analyzed to determine if inflection points that fractionate the dNDF into more than one digestible pool were present (Van Soest et al., 2005). Further, Eq. 1 that described fiber digestion in forages was adapted to model fiber digestion in plant by-products and the ability of the equation to predict the fermentation residues was evaluated.

To determine the time points required to model aNDFom in-vitro digestion and

subsequently calculate aNDFom digestion rates, 8 combinations of four time points were selected and used as inputs to a model (Eq.1) following the discussion of Mertens (1993). Inputs were values of aNDFom residues at 0 h, two intermediate time-points, and the uNDF. The corresponding average slope and intercept, and overall slope, intercept and RMSE of observed versus predicted decays were analyzed for the goodness of fit of the model.

2.4 RESULTS AND DISCUSSION

Having a robust estimation of the uNDF value is important for nutritional uniformity to more accurately determine the amount of dNDF for use in calculating rates of digestion and predict the metabolizable energy content of individual feeds. In many cases, information on the overall digestibility, the rate of digestion of the aNDFom and the uNDF for non-forage feeds has been found only in feed libraries (NRC, 2001, Higgs et al., 2015), whereas forages are routinely analyzed for such characteristics. Thus to improve diet formulation and the prediction of nutrition models, having these data should reduce some of the bias in predicting ME and MP allowable milk if applied properly in a model (Higgs et al., 2015).

For several of the plant by-products evaluated, the extent of digestion of aNDFom was achieved by 96 h of in-vitro fermentation. However, to achieve complete extent of in-vitro digestion, the time point where all but one of the feeds had stopped digesting was 120 h (Table 2.1). This is in contrast to forages that required 240 h of digestion to achieve the same outcome (Raffrenato, 2011; Palmonari et al., 2014). Of the feeds evaluated, only citrus pulp aNDFom continued to digest out to 240 h ($P = 0.002$); (Table

2.1). Although significantly different, the amount of citrus pulp aNDFom digested between 120 and 240 h represents only 4.8% of the total aNDFom digested and nutritionally, the difference would be inconsequential given the variability associated with the measurement and small amount of digested aNDFom that would affect the prediction of ME and MP yield. Also, given that a method like this should be repeatable and if needed, commercially available, having an endpoint that represents the uNDF for the majority of the typical feeds is important to ensure consistent results, thus the 120 h endpoint meets that criteria.

Table 2.1. The aNDFom content at time 0 h (% of DM), and the undigested NDF (%aNDFom) residues of 14 by-product feeds and one commercial feed after in-vitro fermentation for 96, 120, and 240 h.

	Time (h)				
Feed	0	96	120	240	SEM
Beet pulp	46.6	22.4[a]	19.2[b]	17.4[b]	0.8
Canola meal	28.9	40.2 [a]	41.3 [a]	40.7 [a]	1.0
Citrus pulp	22.6	20.7[a]	20.3[a]	16.3[b]	1.2
Corn gluten feed	39.1	15.8[a]	14.4[a,b]	12.9[b]	0.7
Corn distillers	41.6	16.8 [a]	15.6 [a]	14.5 [a]	1.5
Corn germ	63.2	33.6 [a]	29.2 [a]	27.0 [a]	5.1
Flaked corn	13.2	14.1 [a]	14.0 [a]	11.5 [a]	4.1
Rice hulls	71.1	94.1 [a]	93.6 [a]	93.2 [a]	0.6
Soybean meal	9.4	8.2 [a]	7.9 [a]	7.9 [a]	0.8
Soy hulls	71.6	10.3[a]	9.1[a,b]	7.5[b]	0.7
Soyplus™	22.8	9.0[a]	6.1[a,b]	5.8[b]	1.5
Sunflower hulls	74.3	76.6 [a]	76.6 [a]	74.4 [a]	1.3
Wheat distillers	37.7	28.6 [a]	25.6 [a]	25.3 [a]	2.2
Wheat middlings	45.3	36.1[a]	31.3[b]	30.1[b]	1.5
Whole cottonseed	54.2	59.7 [a]	57.7 [a]	54.4 [a]	1.9

[a,b,c] Values in rows with different superscripts differ $P < 0.05$ analyzed using a Tukey's test.

The residues of uNDF observed after 120 h of in-vitro fermentation were in most

comparisons different from those calculated with the equations of Chandler et al. (1980) and Conrad et al. (1984); (Table 2.2). This is consistent with the data from forages where the uNDF is almost always different and usually lower than the values calculated from either Chandler et al. (1980) or Conrad et al. (1984) equations (Cotanch et al., 2014). Given the application of those equations in predicting the size of the digestible aNDFom pool and subsequent energy and protein supplies, some of the error that has been observed in prediction of net energy or similar calculations might be related to the difference between observed versus predicted uNDF. Application of using the uNDF approach to calculate more appropriate dNDF pool sizes in an updated version of the CNCPS demonstrated enhancements when accounting for most limiting nutrients through improved predictions of ME and MP from microbial sources (Higgs, 2014).

Table 2.2. A comparison of three methods of estimating the undigested NDF using the values obtained after 120 h of in-vitro fermentation compared to the calculations of Chandler et al. (1980) and Conrad et al. (1984), respectively.

Feed	Method		
	uNDF (%aNDFom)	2.4xADL/NDF (Chandler et al., 1980)	$(ADL/NDF)^{2/3}$ (Conrad et al., 1984))
Beet pulp	19	28	24
Canola meal	41	73	45
Citrus pulp	20	19	53
Corn gluten feed	14	15	4
Corn distillers	16	26	23
Corn germ	29	23	21
Flaked corn	14	26	23
Rice hulls	93	20	21
Soybean meal	9	23	21
Soy hulls	9	10	7
Soyplus™	6	9	35
Sunflower hulls	77	59	47
Wheat distillers	26	29	22
Wheat middlings	31	17	23
Whole cottonseed	57	45	49

The digestibility of rice hulls was very low and the uNDF was 93% of aNDFom and that was consistent with previous data. Van Soest (2006) described the low digestibility as an effect of the silica content that acted as an anti-microbial factor, thus limiting fiber digestion. Application of the Chandler et al. (1980) or Conrad et al. (1984) equations to calculate the uNDF from lignin content for the rice hulls was misleading due to the effect of the silica and thus would not predict the dNDF fraction compared to the measured value. Using either approach related to constants and surface area is not adequate if inhibitory complexes are present in the feed.

To mathematically describe the digestion data, the natural log transformation of the data demonstrated that aNDFom of these plant byproducts is composed of one digestible fraction and the uNDF. Using soy hulls as an example, this can be seen through the detection of only one inflection point along the digestion data of the samples analyzed (Figure 2.1 and Figure 2.2). Therefore, to describe aNDFom digestion in plant by-products, the Eq.1 (Raffrenato, 2011) was adapted into Eq 2. which considers only one digestible pool (dNDF) and the uNDF fraction.

Eq.2: $\mathrm{aNDFom_t} = \mathrm{dNDF}^{-\mathrm{kd(t-L)}} + \mathrm{uNDF}$

where $\mathrm{aNDFom_t}$ is the fiber residue at time t, dNDF and kd are the digestible pool and relative rate of digestion, L is the lag time, and uNDF is the undigested aNDFom.

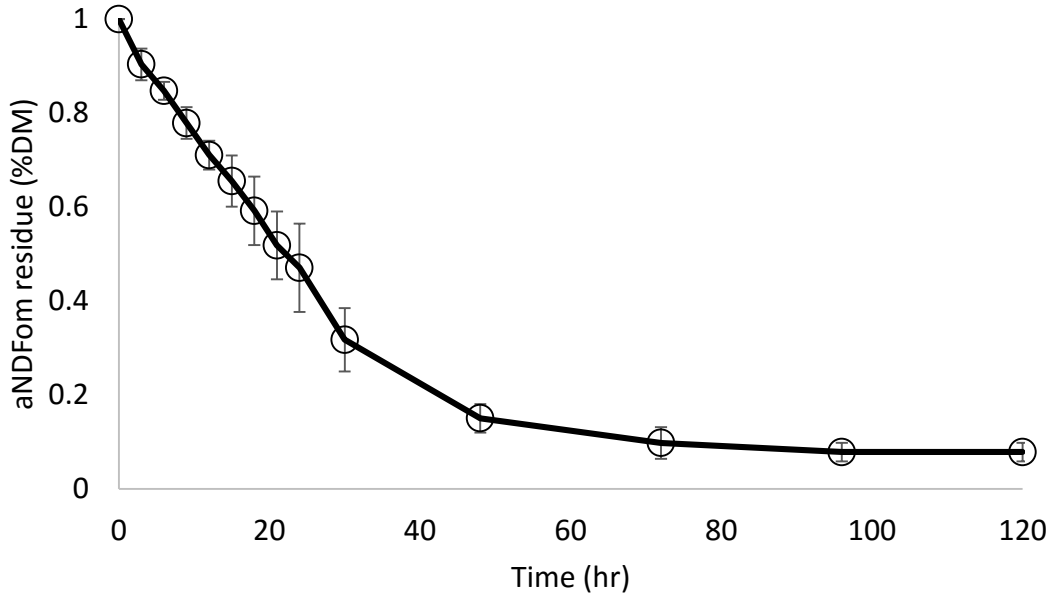

Figure 2.1. The residues remaining after in-vitro aNDFom digestion of soy hulls at multiple time points from zero to 120 h.

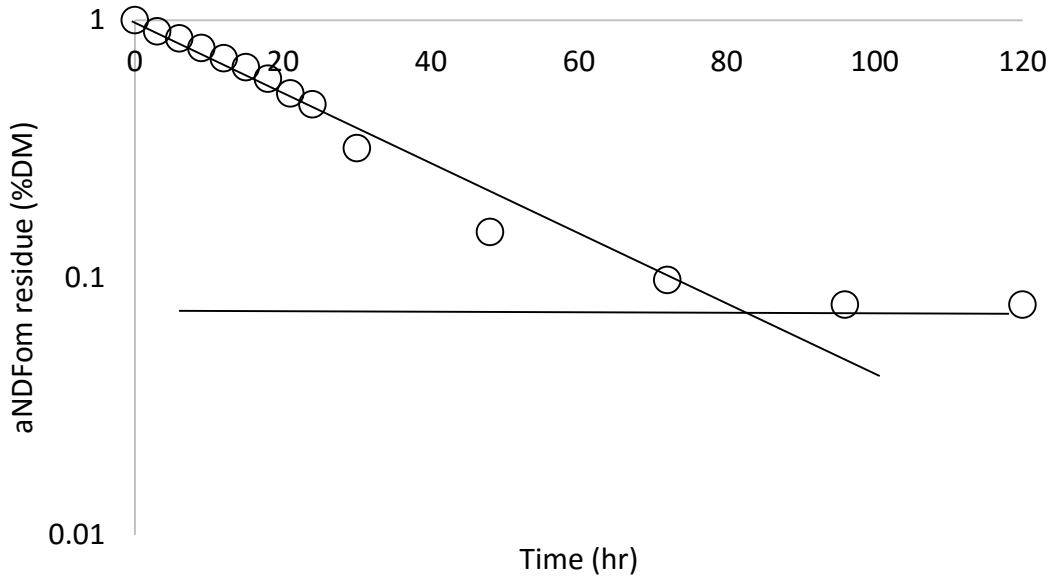

Figure 2.2. The residual aNDFom after in-vitro digestibility of soy hulls plotted on semi-logarithm basis to observe the inflection points that reveal aNDFom fractionation, into digestible NDF and undigested NDF.

The time-point combinations selected were not all possible combinations of time

points because as discussed by Mertens (1993), for modeling purposes it is necessary to know the beginning and exhaustion of fermentation, along with one time-point before and one after the inflection of the decay. Furthermore, only combinations of time points that represented values that were achievable in a commercial laboratory setting were considered. The accuracy of the statistical model did not allow for detecting significant differences in goodness of fit among time point combinations and that is likely because with only two pools of aNDFom, any time point in the linear phase of digestion would adequately represent the pool. However, 0, 12, 72, and 120 h were the time points that provided model predictions with an average slope = 0.95 and intercept = 0.04, which were the closest to unity and zero respectively; and overall slope, intercept, and RMSE of 1.00, 0.002, and 0.002 respectively (Figure 2.3).

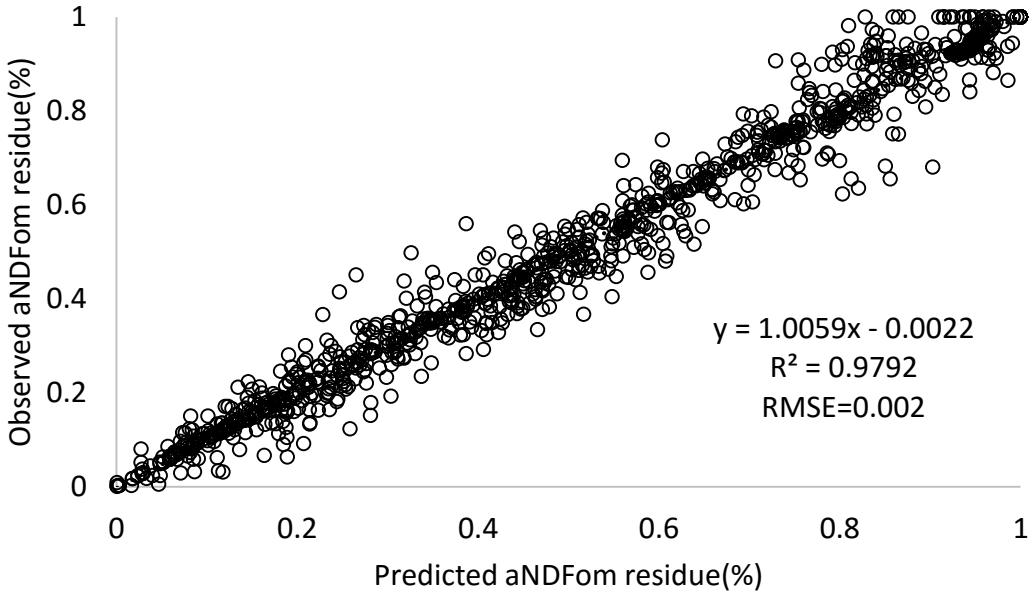

Figure 2.3. Regression of overall observed versus predicted aNDFom values, using residues at 0, 12, 72, and 120 h as inputs for the model ($aNDFom_t = dNDF^{-kd*(t-L)} + uNDF$) and individual data time point residues from all of the byproduct digestibility analysis.

To explain how the model works using corn gluten feed as example, it can be seen that after providing the aNDFom residues at 0, 12, 72, and 120 h as inputs, Equation 1 can recalculate the value of the residues at the same time points analyzed in the laboratory (Figure 2.4) and reports which value of kd has been used to fit the dataset. Table 2.3 shows the calculated rates of digestion of the by-products employed in this experiment. If the predicted residues are plotted against the observed residues the goodness of fit yields slope = 1.00, intercept = 0.02 and R^2 = 0.99 (Figure 2.5). This demonstrates that based on residues there is one digestible fraction of aNDFom in the majority of the feeds analyzed in this study. This is consistent with data on digestibility developed using gas production, but it is easier to visualize the outcome using residues since gas production asymptotes are not fully descriptive of when the digestible fraction is exhausted and also, usually only one time point for residues is identified (Getachew et al., 2004).

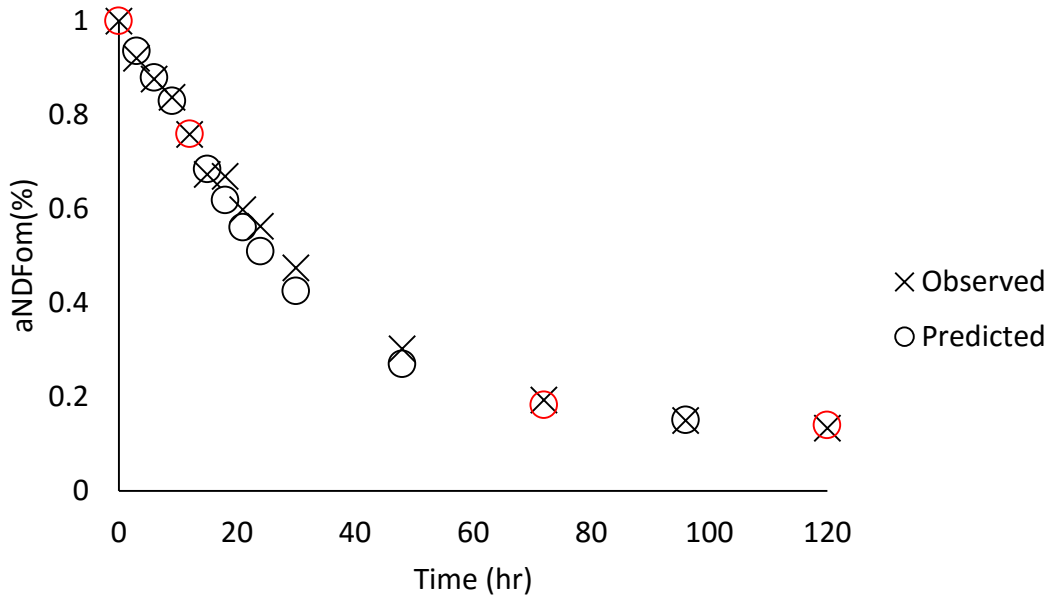

Figure 2.4. Observed and predicted aNDFom residues of corn gluten feed, using residues at 0, 12, 72, and 120 h (in red) as inputs for the model ($\mathrm{aNDFom_t} = \mathrm{dNDF}^{-\mathrm{kd}*(\mathrm{t}-\mathrm{L})} + \mathrm{uNDF}$).

Table 2.3. The digestibility and rate of aNDFom digestion (kd) of 14 by-product feeds and one commercial feed. The rate of kd was determined using the following equation: $\mathbf{aNDFom_t = dNDF^{-kd(t-L)} + uNDF}$.

Feed	dNDF (%)	kd (%/hr)
Beet pulp	81	7.0
Canola meal	59	5.8
Citrus pulp	80	8.1
Corn gluten feed	86	4.0
Corn distillers	84	5.2
Corn germ	71	7.5
Flaked corn	86	5.1
Rice hulls	7	40
Soybean meal	91	9.4
Soy hulls	91	4.0
Soyplus™	94	2.7
Sunflower hulls	23	8.7
Wheat distillers	74	5.5
Wheat middlings	69	9.8
Whole cottonseed	43	4.0

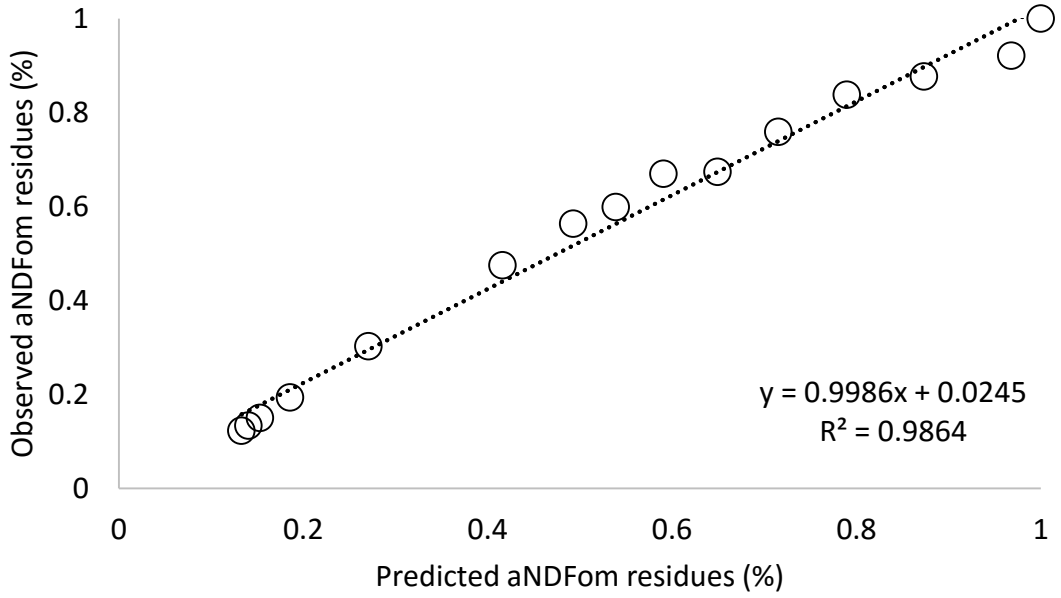

Figure 2.5. Regression of observed versus predicted aNDFom values of corn gluten feed, using residues at 0, 12, 72, and 120 h as inputs for the model ($aNDFom_t = dNDF^{-kd*(t-L)} + uNDF$).

2.5 CONCLUSIONS

Plant by-products present a fraction of aNDFom that remains undigested over time (uNDF). The amount of time necessary to achieve the undigested fraction is 120 h of in-vitro fermentation. This study provides a protocol for analysis of or plant by-products that can be used to accurately determine the amount of digestible or undigestible aNDFom along with a method to generate rates of digestion for single digestible pool feeds when applying a simple exponential decay equation as long as the uNDF is known or measured. The outputs of the model such as size of dNDF and relative digestion rate are useful information for balancing diets for dairy cattle, having them quantified allow nutritionists to critically understand how to include them in a cattle diet, and replace forages when of poor quality or not available. As data become available it is recommended to not use the reported rates of aNDFom digestion as reference for formulating diets or the uNDF calculated by either Chandler et al. (1980) or Conrad et al. (1984) equations since those will generally under predict the size of the dNDF pool.

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THREE POOL NEUTRAL DETERGENT FIBER DIGESTIBILITY, FEED INTAKE AND MILK YIELD

Interpretive summary:

Dairy cattle nutritionists prefer to maximize forage fiber intake over other sources of carbohydrates, because forage is an inexpensive source of digestible energy that maintains rumen health and can be produced on farm. Thus, the feed industry is interested in characterizing fiber digestion in dairy cattle and developing methods that allow nutritionists and dairy producers to make better utilization of forages and non-forage fiber sources. The study evaluated diets of lactating cattle formulated using a new approach for neutral detergent fiber digestion and analysis and this approach described multiple digestible fractions of fiber and these fractions improved the ability to better characterize cattle performance and fiber digestibility.

CHAPTER 3: Application of a three pool approach to describe organic matter corrected neutral detergent fiber digestibility for formulating diets for lactating dairy cattle: effects on feed intake, milk yield and diet digestibility and model evaluation.

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3.1 ABSTRACT

The first objective of this study was to quantify the effect of partitioning aNDFom into two digestible and an undigestible fraction (uNDF) on DMI, milk and milk component yield, rumination and total tract-digestibility (TTD). The second objective was to assess the sensitivity of CNCPS 7.0 in predicting ME allowable milk and aNDFom total tract digestibility. Holstein cattle (n=144) were stratified by milk yield and allocated randomly into 9 pens each containing 4 primiparous and 12 multiparous cows and each pen was assigned in a 3 X 3 Latin square design with 21-d adaptation periods and 5-d sampling periods. Cattle averaged 127 ± 54 DIM and 727 ± 66 kg of BW at trial start. Treatments ingredients were analyzed using the in-vitro fermentation,

with 30, 120, and 240 h as time points for forages, and 12, 72, and 120 h for plant by-products. Fermentation residues were used as model inputs for the calculation of the three aNDFom pools size and rates of digestion. Treatment diets were formulated to be iso-aNDFom (33% of DM), iso-metabolizable energy and protein, and consisted of two high forage diets (68%) with approximately 87% aNDFom from forages and one low forage diet (34% forage) with 67% aNDFom from high-fiber by-product feeds. The forage diets were formulated to be 32% uNDF (high uNDF, HUF) and 26% uNDF (low uNDF, LUF) and the by-product diet was formulated at 32% uNDF (HUNF). The uNDF was used as the internal marker for calculating TTD. Dry matter intake was not different between the cattle fed the HUF and LUF diets; however, cattle fed the HUNF diet consumed approximately 3.6 kg/d greater DM. The uNDF intake was different among all treatments and accordingly, energy-corrected milk yield was different among treatments and rumination per kg of aNDFom intake was greatest for the cattle consuming the LUF diet whereas TTD was highest for cattle fed the HUF diet. The diet, cow and environment characteristics were loaded into CNCPS 7.0 to evaluate the predictions of TTD and ME allowable milk. The ME allowable predictions were within 1 kg of the actual milk yield with an RMSE of 1.4 kg, and the predicted TTD ranged from 0.6% to 7% of observed with a RMSE of 3.1% aNDFom. In conclusion the use of the multiple pool approach to describing rates of digestion and passage of aNDFom within the structure of the CNCPS appears to be reasonable. However, further evaluation is necessary to understand cattle response and determine the robustness of the approach.

Keywords: aNDFom, uNDF, dNDF, CNCPS

3.2 INTRODUCTION

Fiber, compared to the other nutritional components, is slowly digested and bulky, thus, it creates a ballast in the digestive tract of ruminants. For this reason it is thought to be a primary regulator of feed intake (Mertens and Ely, 1979). Mertens (1987) provided a mathematical framework for modeling feed intake using NDF content of the diet and within the structure of the model presented, NDF was treated as a homogenous pool. Further, Mertens (1977) suggested that when digested, NDF was not a homogenous fraction and that more than one digestible pool might exist and this was also discussed by Allen and Mertens (1988). For instance, NDF contains a fraction that is not available to microbial digestion even if fiber fermentation could be extended to infinite time (Allen and Mertens, 1988; Huhtanen et al., 2006). This undigestible fraction can be analyzed in laboratories using lon-term in-vitro fermentation and is defined as uNDF (Raffrenato, 2011; Cotanch et al., 2014). The component available to microbial digestion is defined as digestible NDF (dNDF) and these pools are dynamic in size, primarily driven by forage type and the agronomic conditions the plant is grown under given light, heat, and water conditions (Raffrenato, 2011; Krämer et al., 2012).

Calculations of NDF digestibility by Van Soest et al. (2005) demonstrated that the dNDF of forages can be decomposed into two digestible fractions, and both fractions followed first order behavior with different digestion rates and were defined as fast and slow digesting pools (Raffrenato and Van Amburgh, 2010). For plant by-products, semi-logarithmic plots of undigested NDF residues demonstrated that the dNDF within those feeds can be described as one digestible pool that disappears with first order

behavior and the uNDF (Chapter 2; Cotanch et al., 2014). When integrated, the size of the various pools of dNDF and the associated digestion rates, when combined to create differences in total NDF pool size of fast, slow and uNDF within a total mixed ration, might affect feed intake and rumination behavior, milk production, and total tract digestibility.

In addition, data from forage studies conducted at Miner Institute were re-analyzed using some of the components of a multi-pool NDF approach, primarily the uNDF, and among several studies, the data strongly suggested that cattle filled to a particular uNDF content in the rumen (Cotanch et al., 2014). Among several studies and treatments, the ratio of uNDF in the rumen to uNDF intake from forages was approximately 1.6, suggesting a consistent relationship with rumen fill albeit with different diets, forages and treatments (Cotanch et al., 2014). From this observation a question arises about how the uNDF might behave to cause rumen fill and also how the shift in the size and rate of digestion of the digestible pools might interact with the uNDF as part of the regulation of physical fill. This follows on the work of (Huhtanen et al., 2007) that demonstrated that the uNDF passed at a faster rate than the dNDF, demonstrating there was an interaction among the digestible and undigestible fractions when describing rumen retention and thus fill. The implication from Huhtanen et al. (2007) is that dNDF is preferentially retained and since uNDF is integral to dNDF, uNDF would accumulate in the rumen until the fermentable fraction is exhausted, thus it implies some relationship between intake of uNDF and rumen pool size that is directly related to the size of the digestible pool of NDF.

The first objective of this study was to balance diets for high producing dairy cattle

using these concepts, and evaluate the effect that diets with different proportions of the aNDFom pools might have on feeding behavior, rumination, milk production and total tract digestibility. Based on the previous data (Cotanch et al., 2014) our hypothesis was that cattle fed diets varying in uNDF content and related aNDFom pools would demonstrate differences in feed intake, rumination, and feed efficiency; and that diets similar in aNDFom and uNDF content would have similar intake behavior independently from source e.g. source of uNDF from forage or plant by-products would behave similarly. The second objective was to assess the sensitivity of CNCPS 7.0 in predicting metabolizable energy (ME) allowable milk and aNDFom total tract digestibility (TTD).

3.3 MATERIALS AND METHODS

3.3.1 Animals, Treatments and Experimental Design

This experiment was approved by Cornell University Animal Care and Use Committee and was conducted between April 8 and July 21, 2015. One-hundred and eight multiparous cows (727 ± 66 kg BW; 127 ± 54 DIM) and thirty-six primiparous cows (615 ± 53 kg BW; 106 ± 46 DIM) were enrolled in this study and housed at the Cornell University Research Center (CURC). Cattle were stratified by DIM, BW, and milk yield and distributed into 9 pens of 16 (12 multiparous and 4 primiparous) cattle. Pens were assigned randomly to the three treatments (Table 3.4) in a 3 x 3 Latin square design with 21-d adaptation periods and 5-d sampling periods. Cows had free access to water and were fed a TMR once a day at 0900 h, allowing 5% refusals and feed was pushed up three times daily corresponding to milking times. Cows received rbST (Posilac, Elanco Animal Health, Indianapolis, IN) every 14 days according to label and

Rumensin® (Elanco Animal Health, Indianapolis, IN) was formulated in the diets at 400 mg/cow/d.

Diet ingredients were analyzed by wet chemical methods as needed for use in the CNCPS 6.5 (Higgs et al., 2015). Treatment diets were developed using the fractionation of aNDFom into fast and slow digestible pools, and uNDF for forages. Digestibility of the aNDFom was conducted using in-vitro fermentation and forages were analyzed for residues of aNDFom after 0, 30, 120 and 240 h fermentation whereas non-forage byproduct residues were analyzed after 0, 12, 72 and 120 h as described in Raffrenato (2011) and Chapter 2 (Table 3.1 and Table 3.2) to measure the fractionation of aNDFom into pools for each ingredient (Table 3.3). Three treatment diets were developed, two diets with approximately 86% aNDFom from forages and the remaining diet with 34% aNDFom from forages (Figure 3.1). The formulated high forage diets were 32% uNDF (High uNDF, HUF) and 26% uNDF (low uNDF, LUF) and the low forage diet was formulated to match the uNDF of the High uNDF high forage diet (32% uNDF, HUNF) (Table 3.4, Figure 3.2). Diets were formulated using the Cornell Net Carbohydrate and Protein System (CNCPS v6.5 via AMTS.Cattle.Pro v4.0) to support 45 kg of ME and MP allowable milk production at approximately 27 kg DMI using library values adjusted with the measured chemical composition of the actual ingredients.

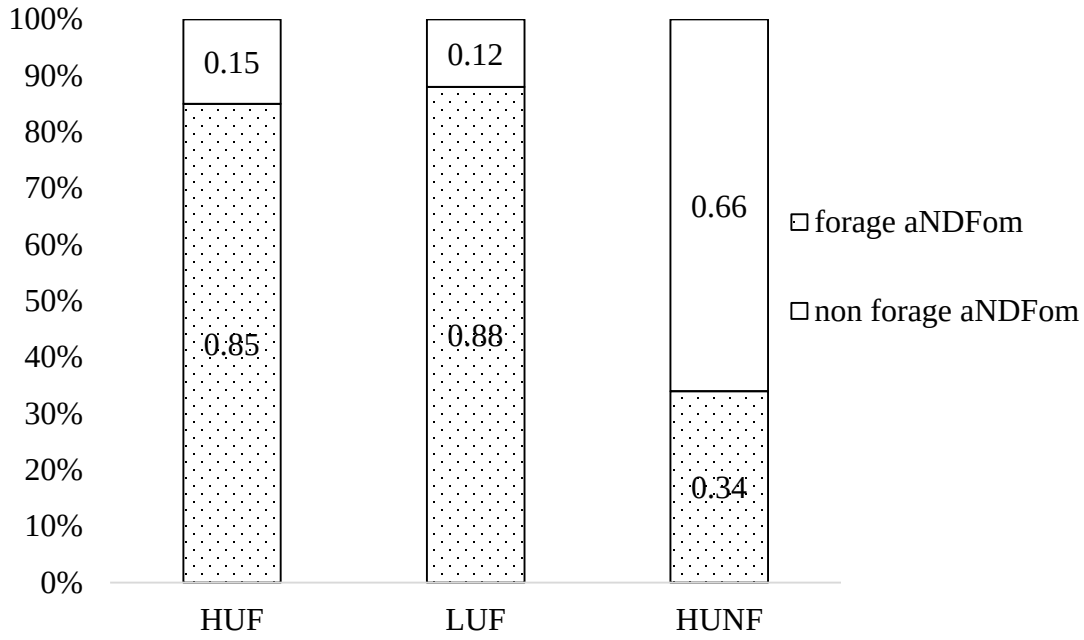

Figure 3.1. The aNDFom inclusion rate from forages versus the plant by-products among the three treatments.

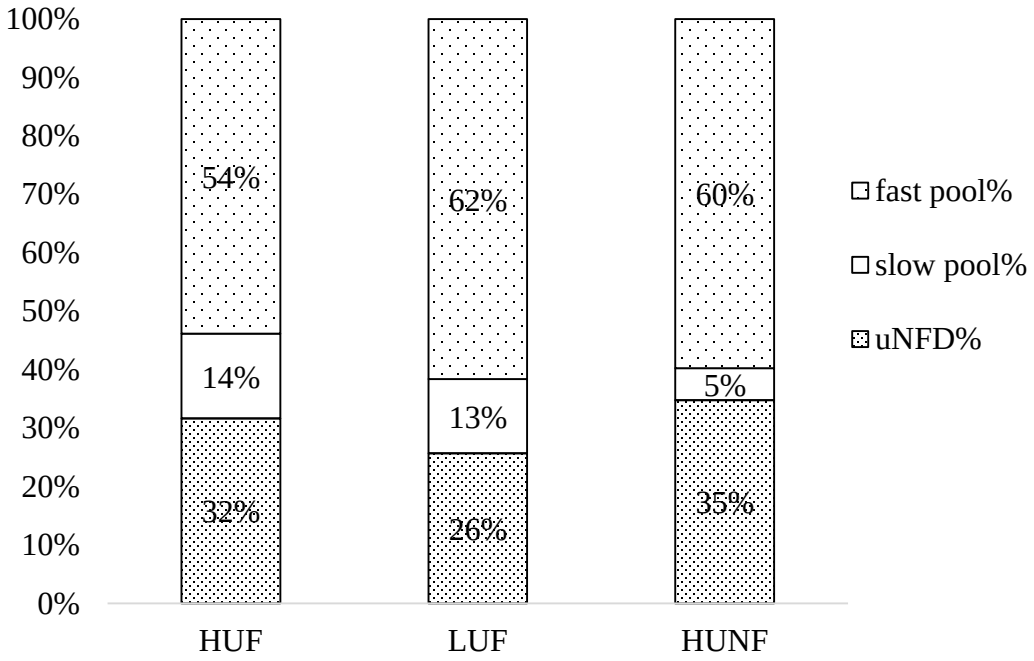

Figure 3.2. The formulated proportion of the fast and slow digestible pool, and uNDF in the three treatment diets. The experimental diets were high forage, high uNDF (HUF) (~70% forage, 32% uNDF), high forage, low uNDF (LUF) (~70% forage, 26% uNDF) and low forage, high uNDF (HUNF) (30% forage, 32% uNDF).

3.3.2 Sampling Procedure and Analysis

Representative samples of feed ingredients, TMR and individual pen orts were taken each day of the sampling period and analyzed in triplicate for DM. Dry matter was determined by drying samples in a forced air oven at 55°C for 48 h. Feed samples were then ground using a Wiley mill (Arthur H. Thomas, Philadelphia, PA) with a 1-mm screen and collected as composites on a period basis for forages, TMR and orts, whereas on an experiment basis for plant by-products. Composites were analyzed for determination of nutrient content and aNDFom digestibility using 30, 120, and 240 h as time points of in-vitro fermentation for forages, TMR and orts, and 12, 72, and 120 h as time points for plant by-products. Dry matter intake was measured daily by pen and recorded using FeedWatch (Valley Agricultural Software, Tulare, CA). Minutes of rumination were recorded using SCR monitors (SCR Global, Netanya, Israel) and this occurred during the first 3 days of the 5-d sampling periods. Cows were milked three times daily at 0830, 1630, and 2430 h and milk production from all milking's was recorded using Alpro™ herd management software (DeLaval International AB, SG). Milk samples were collected at each milking for the first 3 days of the sampling period and preserved with 2-bromo-2-nitropane-1, 3-diol at 4°C until analyzed. Milk samples were analyzed by mid-infrared methods (DairyOne Ithaca, NY) for fat, true protein, lactose, MUN (Foss Milkoscan FT+, Foss In., Eden Praire, MN) and somatic cell count (SCC) (Fossomatic FC, Foss Inc., Eden Praire, MN). Furthermore, feces were collected on 72 randomly selected cows (8 per pen) at 0530, 1330, and 2130 on day 4, and at 0930, 1730, and 0130 on day 5 of the sampling period. At any fecal sampling 500ml of sample was obtained from each cow and frozen immediately at -20°C until further

analysis. Fecal samples were then composited per cow per period, dried, ground, and analyzed for aNDFom digestibility as for feed forages. Samples were analyzed at Cumberland Valley Analytical Services Inc. (Maugansville, MD). The total tract aNDFom digestibility was calculated using uNDF as an internal marker using the formulas: fecal aNDFom (kg) = fecal uNDF (kg) * 100 % / fecal uNDF (% of fecal aNDFom); and aNDFom TTD = aNDFom intake – fecal aNDFom.

Body weights were recorded at the beginning of the experiment and at the end of each experimental period, one time after the first milking using scale located at the exit of the parlor. Body condition scores were recorded from the same two people right after the body weight measurements.

3.3.3 Statistical Analysis

Data were analyzed with the following mixed effects model using the fit model procedure of JMPv.11 (SAS Institute, Inc., Cary, NC):

$Yijkl = T_i + PR_j + D_k + PN_l + PN(PR)_{l(j)} + \varepsilon_{ijklm}$

where,

Y_{ijkl} is the dependent, continuous variable,

T_i is the fixed effect of the ith treatment (i=1, 2, 3);

PR_j is the fixed effect of the jth period (j=1, 2, 3);

D_k is the fixed effect of the kth day (j=1, 2, 3);

PN_l is the random effect of the lth pen (j=1,…, 9);

$PN(PR)_{l(j)}$ is the random effect of the jth (j=1, 2, 3) period nested within lth (j=1,…, 9) pen,

ε_{ijlkm} is the residual error.

Overall treatment effects were analyzed using Tukey's test. Significance was declared at $P < 0.05$. A reduced model without the day effect was used for the analysis of composite samples.

3.3.4 CNCPS 7.0 Evaluation

The latest beta version of the CNCPS 7.0 was developed using the multiple pool approach for aNDFom (Higgs et al., 2015). This approach was adopted because it improved the prediction of amino acid flows from the rumen and because it is a mechanistic model through the entire gastrointestinal tract, the multiple pool approach for aNDFom also improved the prediction of TTD of fiber. To evaluate CNCPS 7.0 predictions of ME allowable milk and TTD with an independent dataset, the necessary inputs to describe the cattle and the treatment diets were inputted into the model. Cattle descriptions included days in milk, days pregnant, actual DMI, milk yield, milk composition, BW and BCS were averaged per treatment per period, whereas inputs for mature body weight was the average weight of the mature cattle of the farm. Total tract digestibility of aNDFom predicted by the model per treatment per period was compared to the observed TTD averaged per treatment per period. Accuracy of the prediction was evaluated with RMSE.

Table 3.1. The measured chemical composition and aNDFom residues at 30, 120, and 240 hr of in-vitro fermentation of the forages used in the diets

	Forages				
Item[1]	CCS[2]	BMR[2]	AS-LU[2]	AS -HU[2]	AH-HU[2]
DM, % as fed	29.8	28.3	41.4	46.9	92.3
CP, % DM	7.2	7.6	21.8	21.0	16.8
Soluble CP, % of CP	59.8	57.7	58.8	53.1	40.3
ADICP, % of CP	10.9	8.1	7.9	9.0	8.8
NDICP, % of CP	12	10.9	8.8	12.8	10.4
aNDFom, % of DM	43.3	39.8	34.0	36.8	43.1
ADF, % of DM	27.4	24.6	30.5	33.8	37.2
Lignin, % of aNDFom	7.0	4.5	16.6	19.1	18.2
uNDF 30 h, % of aNDFom	39.1	29.0	45.7	54.6	58.6
uNDF 120 h, % of aNDFom	26.3	19.4	37.5	41.4	49.5
uNDF 240 h, % of aNDFom	25.1	18.1	38.4	43.6	51.3
[3]Chandler et al., 1980	16.8	10.8	39.8	45.8	43.7
[3]Conrad et al., 1984	29.7	23.4	62.0	64.6	56.3
Ether extract, % DM	2.1	2.8	3.0	2.7	1.1
Ethanol soluble carbohydrates, % DM	0.5	0.9	1.5	2.3	6.7
Starch, % DM	29	31.7	0.9	0.6	1.8
Ash, % DM	3.7	3.3	9.8	10.7	10.1

[1]DM: dry matter; CP: crude protein; SP: soluble protein; ADICP: acid detergent insoluble protein; NDICP: neutral detergent insoluble protein; ADF: acid detergent fiber; EE: ether extracts; ESC: ethanol soluble carbohydrates.
[2]CCS: conventional Corn Silage; BMR: corn silage brown midrib; AS-LU: alfalfa silage low uNDF; AS-HU: alfalfa silage high uNDF; AH-HU: alfalfa hay high uNDF.

[3]The indigestible aNDFom calculated using the equation published by Chandler et al. (1980) and Conrad et al. (1984)

Table 3.2. Nutrient composition and aNDFom residues at 12, 72, and 120 hr of in-vitro fermentation, of the plant by-product ingredients used in the diets.

	Experimental Ingredients[2]							
Item[1]	CM	CP	WM	CSD	SP	SH	CGF	SF
DM, % as fed	88.0	88.6	89.0	92.0	89.0	91.0	89.7	91.3
CP, % DM	8.6	7.1	21.7	25.4	47.4	13.4	22..9	6.0
SP, % DM	18.9	31.1	30.6	20.2	4.4	16.3	40.1	22.0
ADICP, % CP	7.4	14.8	4.0	6.7	2.2	8.4	7.3	37.5
NDICP, %CP	8.0	28.5	11.7	9.4	10.1	27.9	11.8	45.6
aNDFom, % DM	9.5	22.7	35.3	38.5	15.6	63.6	32.0	70.9
ADF, % DM	2.6	16.8	12.2	29.9	8.6	47.2	14.1	62.6
Lignin, % aNDFom	7.3	10.5	11.3	27.7	7.5	3.9	6.7	30.5
uNDF 12h, % aNDFom	62.5	40.4	64.9	90.7	60.0	59.0	62.5	87.0
uNDF 72h, % aNDFom	15.6	27.1	37.0	57.2	20.6	8.5	15.6	76.8
uNDF 120 h, % aNDFom	14.6	24.4	32.1	51.1	17.9	7.4	14.6	72.1
[3]Chandler et al., 1980	17.5	25.2	27.1	66.5	18.0	9.4	16.1	73.2
[3]Conrad et al., 1984	83.9	59.8	46.8	80.3	61.4	15.6	35.3	57.0
EE, % DM	2.8	2.7	3.3	19.4	5.8	1.5	4.8	0.0
ESC, % DM	2.0	18.5	4.0	2.8	10	1.3	5.1	0.0
Starch, % DM	70	1.1	19.4	0.9	1.7	0.5	9.0	0.0
Ash, % DM	2.0	7.8	7.6	4.8	6.3	5.3	7.8	5.0

[1]Same as Table 1.
[2]CM: corn meal; CP: citrus pulp; WM: wheat middlings; CSD: cottonseed delinted; SP: Soyplus®; SH: soybean hulls; CGF: corn gluten feed; SF: sunflower hulls.
[3]The indigestible aNDFom calculated using the equation published by Chandler et al. (1980) and Conrad et al. (1984)

Table 3.3. Fractionation of aNDFom into pools with respective rates of digestion, of the feed ingredients used in the experimental diets

	aNDFom fractionation and rates of digestion				
Item	Fast pool, %aNDFom	Slow pool, %aNDFom	uNDF, %aNDFom	kd1, %/hr	kd2, %/hr
Corn silage conventional	55.9	19.1	25.1	9.0	2.1
bmr corn silage	65.9	16.0	18.1	11.2	2.2
Alfalfa silage low uNDF	49.5	12.0	38.4	24.7	1.8
Alfalfa silage high uNDF	39.1	17.3	43.6	9.1	2.0
Alfalfa hay high uNDF	40.6	9.0	50.4	20.5	1.3
Corn meal	77.1	-	22.9	4.4	-
Citrus pulp	75.6	-	24.4	15.4	-
Wheat middlings	67.9	-	32.1	5.9	-
Cottonseed delinted	48.9	-	51.1	3.3	-
Soyplus™	82.1	-	17.9	5.5	-
Soybean hulls	92.6	-	7.4	5.9	-
Corn gluten feed	85.4	-	14.6	5.9	-
Sunflower hulls	27.9	-	72.1	4.4	-

3.4 RESULTS AND DISCUSSION

The forage and by-product ingredients used for formulation were from the same silage bunker and mill used for pre-trial formulation, nevertheless aNDFom content (% of DM) shifted from when diets were formulated before the start of the experiment to when diets were fed, and went from 33 (iso-aNDFom) to 29.1, and 30.8 for LUF and HUNF respectively; and uNDF (% aNDFom) content of HUNF shifted from 32 to 34.7. Approximately three weeks into the study, the bmr corn silage shifted in aNDFom content and that altered the iso-aNDFom formulation of the LUF diet and subsequently altered some of the preplanned comparisons among diets. The primary differences between the high forage diets (HUF and LUF) was in uNDF content obtained by using conventional corn silage and a blend of two low digestibility alfalfa forages (silage and hay high uNDF) for diet HUF, and a bmr corn silage and a higher digestibility alfalfa silage (low uNDF) for diet LUF (Table 3.1 and Table 3.3). For the low forage, high by-product diet (HUNF) the objective was to develop a diet that contained the same aNDFom and uNDF content as HUF to determine if the fiber fractions could provide regulation of DMI independently of source. For the HUNF diet, the sunflower seed hulls provided a significant amount of uNDF to help maintain the expected levels.

The uNDF content of the diets remained similar and allowed for a comparison of uNDF intake as a percent of BW and on a mass basis. Based on the preliminary data from the studies at Miner Institute (Cotanch et al., 2014) a lower uNDF diet was expected to allow for greater DMI between the two high forage diets, HUF and LUF. Cattle fed the low forage, high by-product diet (HUNF) consumed approximately 3.75 kg more dry matter than cattle fed the two high forage diets (Table 3.4). Contrary to

our hypothesis the cattle fed the two high forage diets (HUF and LUF), despite the differences in uNDF content and aNDFom digestion rates, did not demonstrate differences in DMI. The lack of difference in DMI, but higher milk production, for cattle fed bmr corn silage is similar to Oba and Allen (2000) for the inclusion level of aNDFom (Table 3.5). However, for the HUNF treatment, the cattle consumed significantly more DMI compared to the high forage containing diets, despite having the highest uNDF content and intake (Table 3.5). There was a significant difference in uNDF intake among the three treatments and the range in uNDF intake was from 2.1 to 3.4 kg per day and that corresponded to an uNDF intake of 0.3 to 0.5% BW, which was consistent to the range described in Cotanch et al. (2014). Again, the hypothesis was that the high content of uNDF in the HUNF diet might limit feed intake, thus the DMI of the HUNF diet was expected to be similar to that observed in cattle fed the HUF diet, but cattle fed the HUNF diet consumed up to 3.9 kg more DMI than the high forage diets and this most likely was partially related to the particle size of the byproducts. Although the byproducts used in this study were primarily high fiber containing feeds, the particle size and structural integrity were different from the forages and that most likely contributed to a moderately increased passage rate, which allowed for greater DMI (Kennedy and Murphy, 1988; Seo et al., 2006; Krizsan et al., 2010).

Table 3.4. Ingredients, chemical composition and digestibility of experimental diets

	Treatment[1]		
Ingredient, kg DM	HUF	LUF	HUNF
Conventional corn silage	11.1	-	4.1
Bmr corn silage	-	10.6	-
Alfalfa silage low uNDF	-	8.4	3.1
Alfalfa silage high uNDF	4.5	-	-
Alfalfa hay high uNDF	3.5	-	-
Corn meal	4.0	4.4	6.6
Citrus pulp	1.1	0.7	2.3
Wheat middlings	-	-	2.1
Cottonseed delinted	-	-	2.0
Soyplus™	1.8	2.3	1.6
Soybean hulls	0.5	-	1.5
Corn gluten feed	-	-	1.1
Sunflower hulls	-	-	2.0
Mineral and vitamins	1.2	1.3	1.1
Total	27.7	27.7	27.7
Chemical composition[2]			
DM, % as fed	46.0	41.0	63.0
CP, % DM	15.8	15.8	16.8
SP, % DM	42.8	44.6	31.7
ADIP, % DM	8.9	7.3	10.7
NDIP, % DM	11.6	9.8	14.7
ESC, % DM	3.1	2.4	3.7
Starch, % DM	22.0	24.3	23.5
aNDFom, % DM	33.1	29.1	30.8
uNDF 30, % aNDFom	40.0	35.3	43.8
uNDF 120, % aNDFom	33.4	27.7	36.2
uNDF 240, % aNDFom	31.8	25.7	34.7
ADF, % DM	23.5	20.3	20.9
Lignin, % aNDFom	10.2	8.9	11.6
EE, % DM	4.0	3.4	3.9
Ash, % DM	7.0	7.9	6.8
Lys:Met	3.0	2.9	2.8
Forage, % DM	68.7	68.8	33.6

Forage aNDFom, % DM	86.6	88.1	33.4
Digestibility			
Weighted average forage kd of TMR, %aNDFom/hr	4.2	5.6	4.9
Weighted average by-product kd of TMR, %aNDFom/hr	4.4	4.1	3.9

[1]HUF: high forage, high uNDF; LUF: high forage, low uNDF; HUNF: high uNDF non-forage

[2]DM: dry matter; CP: crude protein; SP: soluble protein; ADICP: acid detergent insoluble protein; NDICP: neutral detergent insoluble protein; ADF: acid detergent fiber; EE: ether extracts; ESC: ethanol soluble carbohydrates.

Table 3.5. Dry matter intake, rumination, fecal excretion, body weight and body condition score

	Treatments			
Item[1]	HUF	LUF	HUNF	SEM
Intake				
DMI, kg/cow/d	27.7[a]	28.0[a]	31.6[b]	0.3
aNDFom intake, kg/cow/d	9.2[b]	8.2[a]	9.8[c]	0.1
aNDFom intake, % BW	1.3[b]	1.2[a]	1.4[c]	<0.01
dNDF intake, kg/cow/d	6.2[b]	6.1[a]	6.4[b]	0.09
dNDF intake, % BW	0.89[b]	0.86[a]	0.89[b]	<0.01
uNDF30, kg/cow/d	3.7[b]	2.9[a]	4.3[c]	0.06
uNDF30, % BW	0.52[b]	0.41[a]	0.60[c]	0.01
uNDF120, kg/cow/d	3.1[b]	2.3[a]	3.5[c]	0.04
uNDF120, % BW	0.44[b]	0.32[a]	0.50[c]	0.01
uNDF 240 intake, kg/cow/d	2.9[b]	2.1[a]	3.4[c]	<0.01
uNDF intake, % BW	0.42[b]	0.30[a]	0.48[c]	<0.01
Rumination				
min/cow/d	596[b]	607[c]	530[a]	2.7
min/kg aNDFom intake	65[b]	75[c]	54[a]	0.5
min/kg dNDF intake	95[b]	100[c]	83[a]	1.3
min/kg uNDF intake	205[b]	290[c]	156[a]	1.6
Feces				
Fecal aNDFom, % DM	52.0[b]	47.1[a]	55.5	0.1
Fecal uNDF, % of aNDFom	65.9[c]	54.0[a]	57.8[b]	0.2
Fecal lignin, % of aNDFom	23.1[a]	23.6[a]	23.4[a]	0.3
Fecal aNDFom, kg/cow/d	4.4[b]	3.9[a]	5.9[c]	0.1
aNDFom TTD, % aNDFom	52.7[b]	53.2[c]	39.8[a]	<0.01
dNDF TTD, % aNDFom	77.2[c]	71.7[b]	60.9[a]	<0.01
Body weight and BCS				
BW initial, kg	702[a]	705[a]	713[b]	1.7
BW change, kg/trt/period	3.2[a]	9.3[a]	17.8[b]	4.2
BCS change	0.01[a]	0.01[a]	0.1[a]	<0.1

[1]HUF: high forage, high uNDF; LUF: high forage, low uNDF; HUNF: high uNDF non-forage

[abc]Values in rows with different asterisk differ $P < 0.05$

Given the high level of non-forage fiber sources in the HUNF diet, the aNDFom digestion was basically composed of only one digestible pool with a faster rate of digestion compared to an integrated rate of digestion of the higher forage diets. The implication of this effect suggests that after the single digestible aNDFom pool was

exhausted the feed particles lost structural integrity and were available to escape the rumen and this would help explain why passage rate appears to be faster for non-forage fiber sources and this would partially explain the higher DMI for cattle fed the HUNF diet. The concept follows on the data of Huhtanen et al. (2006a) where uNDF appears to pass at a faster rate than the digestible aNDFom fraction because the digestible pool is preferentially retained due to the fermentation, allowing for more complete digestion. The HUF and LUF diet were composed of two digesting pools and once the fast pool was exhausted the slow pool was still slowly fermenting preventing the feed particles from escaping the rumen at the same rate as HUNF diet, and making them more likely to be ruminated.

In high producing lactating cattle, chewing and rumination are strong indicators of rumen pH and health. In this study cattle fed the HUF and LUF diets had the highest rumination per day per cow of approximately 600 minutes, whereas the cattle fed the HUNF diet ruminated about 70 minutes less per day for a total of 530 minutes (Table 3.5; $P < 0.05$). The minutes of rumination observed in this study were greater than what was observed in several other studies (Maekawa et al., 2002; Kononoff et al., 2003) among all treatments, thus cattle on this study demonstrated adequate rumen fill and time budgets suggest acceptable rumen health. The rumination time for the HUNF treatment was in an acceptable range (>500 min) despite the low forage inclusion level and demonstrates that the non-forage fiber from the byproducts were stimulatory for rumination which is consistent with data from Penner et al. (2009) where they replaced forage with distillers grains and observed similar rumination rates as observed in this study, and this again implies that a considerable part of that diet was actually processed

and digested instead of passing out to a faster rate. It is likely that cattle time budgets or at least lying time should be accounted for when evaluating rumination (Grant, 2007). A recent evaluation of the lying time at the CURC dairy indicated that the cattle were averaging approximately 13 h of lying time and rumination time is likely influenced by the hours of lying time available to cattle (Grant, 2007). A recent evaluation of lying times in North America indicated the average herd had about 11 h of lying time (Von Keyserlingk et al., 2012) and if the average cow had 11 h of lying time, subtracting off the equivalent of 2 h of rumination time would equal 480 minutes of rumination, which is similar to the data available from other studies (Maekawa et al., 2002; Kononoff et al., 2003).

Further, rumination per unit of aNDFom and uNDF intake was greatest for the cattle fed the LUF diet ($P < 0.05$) and this coincides to the relationship between the dNDF and uNDF intake of the LUF treatment compared to the other two treatments. The significant difference in rumination per unit of uNDF intake appears to be related to the ratio between the dNDF intake and the uNDF among treatments. Cattle fed the LUF diet consumed 2.9 times more dNDF than uNDF, whereas the other two treatments consumed about twice as much dNDF. This suggests that the amount of dNDF associated with the uNDF will positively impact the rumination time, most likely because it indirectly causes retention of the pool in the rumen due to the buoyancy from fermentation which maintains the dNDF in the rumen mat until the fermentable substrate is exhausted to allow for movement into the escapable pool (Lund et al., 2007). And given the dNDF intake of the HUF and HUNF treatments were similar, the expected rumination time should be similar; however, the particle size of the byproducts

in the HUNF diet most likely contributed to an increased passage rate, which decreased the potential to be ruminated due to a reduced rumen residency time. However, overall the rumination time for the cattle fed the HUNF diet was more than adequate to maintain positive rumen health, thus diets comprised of by-products can be developed to maintain rumen health, provided the ingredients are formulated at an adequate aNDFom level to maintain adequate rumen fill.

The formulation of these diets created a problem with the terms "fast" and "slow" digesting pool of aNDFom, because they are relative to each other and differ among ingredients. For example, the kd's of fast pool in forages varied from 0.09 hr^{-1} for conventional corn silage to 0.247 hr^{-1} for alfalfa silage low uNDF, and kd's of slow pool range between 0.013 hr^{-1} for alfalfa hay high uNDF and 0.022 hr^{-1} for BMR corn silage (Table 3.3). Furthermore the digestible aNDFom fraction of plant by-product have kd's that range from 0.033 hr^{-1} for delinted cottonseed to 0.154 hr^{-1} for citrus pulp. The byproducts have only one digestible pool and little cross-linking between the hemicellulose and lignin, thus their ruminal behavior is going to be different than forages and this most likely contributes to the difference in rumen retention time and passage rate. Also of interest are the differences in measured uNDF compared to the calculated values from the Chandler et al. (1980) calculations or those from Conrad et al. (1984) that use fixed coefficients or surface area calculations to predict the unavailable NDF (Table 3.1 and Table 3.2). The observed uNDF values are significantly different from the predicted values using the fixed coefficients. This suggests that digestible aNDFom estimated from the calculations would be either over or under-estimated, thus ME and MP allowable milk predictions and microbial yield would be

similarly influenced.

Fecal excretion of uNDF was different among treatments but fecal excretion of lignin was not (Table 3.5) demonstrating that lignin cannot be used to calculate the indigestible fiber as reported in Chandler et al. (1980). Generally what makes fiber indigestible is the extension of cross-linking of lignin within the cell wall and that really depends and the agronomic and atmospheric condition the plant is grown in, and thus not constant to guarantee a relationship between lignin content solely and indigestible fiber (Hatfield et al., 1999; Grabber et al., 2009). Using uNDF as internal marker, aNDFom total tract digestibility was estimated for the treatment diets. For the HUF and LUF diets, TTD was approximately 53% on an aNDFom basis, whereas cattle fed the HUNF diet demonstrated a 24% reduction in TTD (Table 3.5). This observation is consistent with the higher DMI and expected faster passage rate of the by-products, however using the aNDFom obscures the digestibility of all treatment diets, and especially the HUNF treatment since it contained the largest uNDF pool. Subtracting out the uNDF from the aNDFom and re-calculating the TTD, the digestibility of dNDF was 76% (HUF), 71% (LUF) and 61% (HUNF) demonstrating that a significant amount aNDFom was digested in these diets, suggesting that there was a modest increase in the passage rate of the smaller feed particles in the HUNF diet, but the extent of ruminal or total tract digestion was not overly compromised due to faster escape from the rumen. The overall dNDF pool size was 6.3 kg, 6.1 kg and 6.4 kg per day for the HUF, LUF and HUNF diets respectively. Given the TTD of the dNDF, the digested aNDFom was 4.8 kg, 4.3 kg and 3.9 kg for the HUF, LUF and HUNF diets, respectively. Thus the range in digestibility of aNDFom among the diets (HUF to HUNF) was 18%, still significant,

but less so than when calculating aNDFom as an integrated pool.

Milk yield and ECM were different ($P < 0.05$) among treatments (Table 3.6) and followed the DMI, although milk yield among all treatments was high. This demonstrates that although digestibility among aNDFom pools was being altered by treatments, the overall diet digestibility was good. That in itself is problematic when trying to design studies of this nature because treatment differences can only be as significant as your differences in forage digestibility for the year and season the work is being conducted in. The cattle fed the HUNF diet had significantly lower milk fat percentage than the higher forage diets and the cause for this was evaluated. Based on the level of DMI along with the decreased aNDFom digestibility, passage rate was most likely higher in cattle fed this diet compared to the higher forage diets. The rumination time exceeded 500 min/d, so it is unlikely that acidosis was a significant problem, and upon re-evaluation of the diet formulations, due to the ingredients used in the diet, the unsaturated fatty acid intake in cattle fed the HUNF diet was approximately 877 g/d, whereas for the HUF and LUF diets were approximately 488 and 550 g/d, respectively. In combination with the faster passage rate in the HUNF treatment and the inclusion of Rumensin, the combination of risk factors were quite high for milk fat depression, and that all fits with the observed milk fat percent (AlZahal et al., 2008). Milk protein percentage and yield was highest for the cattle fed the HUNF diet ($P < 0.05$), further suggesting there was no concern with ruminal acidosis on that treatment.

Table 3.6. Milk yield and milk composition from cattle fed three diets differing in source and pool size of aNDFom.

	Treatment[1]			
Item	HUF	LUF	HUNF	SEM
Milk production				
Milk, kg/d	41.9 [a]	44.2 [b]	47.8 [c]	0.6
Energy corrected milk, kg/d	43.6 [a]	44.9 [b]	46.4 [c]	0.6
Fat yield, kg/d	1.6[a]	1.6[a]	1.5[b]	<0.1
True protein yield, kg/d	1.2 [a]	1.3 [b]	1.5 [c]	<0.1
Lactose yield, kg/d	2.0 [a]	2.1 [b]	2.3 [c]	<0.1
Milk composition				
Fat, %	3.79 [a]	3.58 [b]	3.18 [c]	0.04
True protein, %	2.91 [a]	2.95 [b]	3.05 [c]	0.01
Lactose, %	4.77 [a]	4.84 [b]	4.89 [c]	0.01
MUN, mg/dl	11.1 [b]	8.8 [a]	12.1 [c]	0.21
SCC (log1000/ml)	85 [a]	81 [a]	88 [a]	11.88

[1]HUF: high forage, high uNDF; LUF: high forage, low uNDF; HUNF: high uNDF non-forage

[abc]Values in rows with different asterisk differ P<0.05

The diet, chemical composition, cow and environment characteristics were loaded into CNCPS 7.0 to evaluate the predictions of TTD and ME allowable milk (Table 3.7). The model predicted the ME allowable milk yield with reasonable accuracy and for all three treatments the ME allowable predictions were within 1 kg of the actual milk yield with an RMSE of 1.4 kg. Of greater interest for this exercise were the predictions of aNDFom TTD by the model and in this evaluation, the predicted TTD ranged from 0.5% to 8% of observed with a RMSE of 3.1 % aNDFom. Thus, overall the use of the multiple pool approach to describing rates of digestion and passage within the structure of the CNCPS appears to be reasonable, especially given the range in aNDFom sources used among the treatments. However, further evaluation is necessary to determine the robustness of the approach.

An important input in this evaluation of ME allowable milk prediction was the BW change observed during the study for the cattle fed the HUNF diet. Although not significant, BW tended to increase with increasing DMI for the cattle fed the HUNF diet and if not accounted for, the predicted ME allowable milk would not align with the observed milk yield. This suggests that the BW change should be accounted for when evaluating the feed efficiency of the HUNF treatment since that results in a substantial increase in overall energy balance despite the apparent reduced feed efficiency.

Table 3.7. The evaluation of ME allowable milk and aNDFom TTD predictions from the Cornell Net Carbohydrate and Protein System v7 using the multi-pool approach

	Treatment			
	HUF	LUF	HUNF	RMSE
Observed				
Milk yield, kg/d	41.9 (±1.0)	44.2 (±1.6)	47.8 (±2.8)	-
aNDFom TTD, % aNDFom	52.7 (±1.2)	53.2 (±0.6)	39.8 (±1.7)	-
Model predicted				
ME allowable milk, kg/d	39.9 (±1.3)	43.1 (±1.6)	48.1 (±2.8)	1.4
aNDFom TTD, % aNDFom	53.3 (±0.6)	57.7 (±6.7)	41.1 (±0.6)	3.1

[1]HUF: high forage, high uNDF; LUF: high forage, low uNDF; HUNF: high uNDF non-forage

3.5 CONCLUSIONS

In conclusion, the cattle fed the diets did not behave as expected, however, formulating diets using the multiple aNDFom pool approach appeared to influence intake behavior. In this study, digestible aNDFom was positively related to rumination behavior. There appears to be differences in passage rate and digestion of feeds when considering one or two digestible pools, possibly related to structural integrity of the particle. Fractionating the aNDFom into fast and slow digestible pools and uNDF when

balancing diets for dairy cattle seems to be a reasonable approach in the structure of CNCPSv7. However more studies, where feed ingredients are analyzed with the same methodology followed in this study are necessary to test the sensitivity of this approach.

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CREATING A MECHANISTIC MODEL TO DESCRIBE ORGANIC MATTER NEUTRAL DETERGENT FIBER DIGESTION

Interpretive summary:

Fiber is one of the main chemical fraction of dairy cattle diets. Being able to accurately quantify rate of fiber digestion would help in the prediction of energy supply and make dairy cattle more efficient in their performances. The objective of this study was to give a tool to dairy nutritionist to use when balancing diets for dairy cattle. A mechanistic model for fiber digestion along with guidelines of fiber digestion analysis are proposed in this study.

CHAPTER 4: In-vitro digestion of organic matter corrected neutral detergent fiber and development of a multi-compartment model and evaluation of predictions in the CNCPS 7.0 model

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4.1 ABSTRACT

Models such as the Cornell Net Carbohydrate and Protein System rely on having accurate values for feed ingredients including digestion rate to aid in predicting metabolizable energy and protein yield and subsequent milk yield. To estimate rates of digestion, the use of in-vitro digestion where residues are analyzed at specific time points is employed to characterize microbial degradation of the feeds of interest. In-vitro aNDFom digestion displays a sigmoidal shape which is the result of a two-step

process: the lag phase, where bacteria attach to the substrate and establish digestion and the digestion phase where substrate is being degraded. In many mechanistic models, the lag phase was calculated as a discrete function, meaning that aNDFom digestion could occur only after the lag phase was complete and this does not follow normal biological behavior. In other statistical models aNDFom digestion has been described with a multi-compartment system using gamma functions. Such models can describe the sigmoidal shape of aNDFom digestion but the estimated rate of digestion were probabilistic and not deterministic. The objective of this study was to develop a mechanistic model that describes the sigmoidal behavior of in-vitro aNDFom digestion, using differential equations to provide deterministic values of digestion rates while describing lag as a continuous function. The proposed are multi-compartments models, one describing aNDFom in plant by-products, where the digestible aNDFom fraction (dNDF) is unique; and the other describing aNDFom digestion in forages, where dNDF is further fractionated into fast and slow digesting pools. In both feed types a fraction remains un-digested over time (uNDF). In these models, the lag phase is a rate and the life-time of the dNDF fractions are consistent with gamma distributions and the behavior of the system is sigmoidal. The model was fitted to 36 conserved forages, 32 fresh forages; and 15 plant by-products analyzed using in-vitro methods. The quality of fit was evaluated with an overall slope (1.03), intercept (0.01), R^2 (0.98), and a RMSE (0.02) of the regressions of observed versus predicted. Further, the relevance of the model predictions were assessed by evaluating the RMSE of CNCPS predictions of ME allowable milk and aNDFom total tract digestibility, using information from a lactating cattle study where treatment diets were formulated to quantify the effects of aNDFom

source and digestibility. Parameters of aNDFom digestion were calculated for each ingredient of the diets, and used as inputs for CNCPSv7.0. The RMSE of predictions among the three treatments were 0.8 kg for ME allowable milk and 4.2 % of aNDFom for TTD. Overall, the multi compartmental modeling approach improved the robustness of predicting pool sizes and rates of digestion of aNDFom and when model solutions were utilize within the structure of a dynamic nutrition, the predictions of ME allowable milk and TTD were within acceptable ranges and variation.

Keywords: aNDFom, modeling, multi-compartment

4.2 INTRODUCTION

The fiber component (aNDFom) of the diets fed to cattle is not uniformly digestible. For example, aNDFom contains a fraction that is not available to microbial digestion even if fiber fermentation could be extended for infinite time (Allen and Mertens, 1988, Huhtanen et al., 2006). This indigestible fraction can be analyzed by commercial laboratories with long term in-vitro fermentation and is defined as uNDF (Cotanch et al., 2014). Furthermore the component available to microbial digestion is defined digestible aNDFom (dNDF) and can be obtained by subtracting the uNDF from total aNDFom (dNDF = aNDFom - uNDF). Previous work demonstrated that the dNDF of forages can be composed of two digestible fractions (Van Soest et al., 2005), both fractions following first order behavior but with different digestion rates and defined as fast and slow digesting pools (Raffrenato and Van Amburgh, 2010); whereas in plant by-products the dNDF is identifiable as one fraction and disappearing with a first order behavior as discussed in Chapter 2. Digestion of aNDFom can be modeled to obtain

rate of fiber digestion. Rate of aNDFom digestion is an input in feed formulation system and nutrition models (Van Amburgh et al. 2015). The application of the CNCPS model can demonstrate the importance of having accurate values of the aNDFom rate of digestion, for the estimation of microbial yield, metabolizable energy and protein supply, and subsequent milk production and nutrient excretion (Higgs et al., 2015; Van Amburgh et al. 2015). The equations used in the CNCPS to model aNDFom digestion have the form of Equation 1 for plant by-products (Chapter 2), and of Equation 2 for forages (Raffrenato, 2011); which describe exponential decays:

Eq.1: $\mathrm{aNDFom_t} = \mathrm{dNDF}^{-\mathrm{kd}*(\mathrm{t}-\mathrm{L})} + \mathrm{uNDF_{120}}$

where $aNDFom_t$ is the fiber residue at time t, dNDF and kd are the digestible fiber fraction residue over time and its fractional rate of digestion, L is the lag phase and uNDF is the undigested fraction of fiber analyzed at 120 h.

Eq.2: $\mathrm{aNDFom_t} = \mathrm{dNDF_1}^{-\mathrm{kd_1}*(\mathrm{t}-\mathrm{L})} + \mathrm{dNDF_2}^{-\mathrm{kd_2}*(\mathrm{t}-\mathrm{L})} + \mathrm{uNDF_{240}}$

where $dNDF_1$ and kd_1 are magnitude and rate of digestion of the fast pool and $dNDF_2$ and kd_2 are magnitude and rate of digestion of the slow pool, and uNDF is the undigested fraction of fiber analyzed at 240 h.

From a biological perspective the lag phase is the time needed by the ruminal bacteria to attach to the fiber substrate and make it available for digestion through rehydration, microbial attachment, and initiating digestion after enzymes are produced (Russell, 2002). Using corn silage as example, Van Amburgh et al. (2003) demonstrated how

the lag phase can be calculated from a logarithmic transformation and its value added to the equation. In this form, equations 1 and 2 consider the lag phase as a discrete process, meaning that aNDFom digestion does not start as long as the lag phase has not finished, and therefore the curves that Eq.1 and Eq.2 create are exponential decays moved to the right on the X axis for the amount of the lag phase (Figure 4.1). Evaluation of the digestion curve from a typical in-vitro aNDFom fermentation shows that aNDFom digestion as many biological processes is more sigmoidal in shape and that is because the lag phase is not discrete (Figure 4.2). Part of aNDFom substrate starts to be digested even though microbes are not attached on the all substrate and aNDFom hasn't been fully hydrated and colonized (the lag phase has not completed), and according to Russell (2002) the substrate is prepared to digestion exponentially, parallel to the substrate's colonization of the rumen bacteria cells and this is consistent with the process described by McAllister et al. (1994). Therefore, the lag parameter could be better modeled not as a discrete term, but as a continuous exponential process, and hence, not as amount of time needed for microbial colonization and substrate preparation but as a rate: amount of substrate available over time. One approach to translate this concept into a mechanistic model is through the use of a multi-compartmental system. A multi-compartmental system is a diagram of box and flows that describes how matter (in boxes) move or changes (in flows) continuously through different processes (Blaxter et al., 1956). An example of this is aNDFom digestion that must undergo a "lag phase" where substrate is prepared for digestion and a "digestion process" where substrate is actually digested. Multi-compartment systems, describing digesta flow in the gastrointestinal tract of ruminants have been employed previously,

and data sets were fit with the gamma functions (Matis, 1972; Pond et al., 1988; Poppi et al., 2001). Such models were reproducing the sigmoidal shape similar to the one observed with in-vitro aNDFom digestion, and thus, they could be used to model such process (Vieira et al., 2008). However, their estimates and outcomes were probabilistic values for passage, which cannot be used as inputs for nutrition models that require deterministic values as inputs.

The objective of this study was to develop a mechanistic model that describes the sigmoidal behavior of in-vitro aNDFom digestion, using differential equations, to provide deterministic values of digestion rates. The proposed models are multi-compartment models where the flows are expressed by differential equations, and rates are fractional. In this model, the lag phase is quantified as the rate by which the substrate is made available to digestion, and the life-time of aNDFom is consistently sigmoidal. A subsequent objective was to evaluate if the new approach of describing aNDFom digestion could reduce the variability associate to the predictions of ME allowable milk and aNDFom total tract digestibility into the CNCPS 7.0.

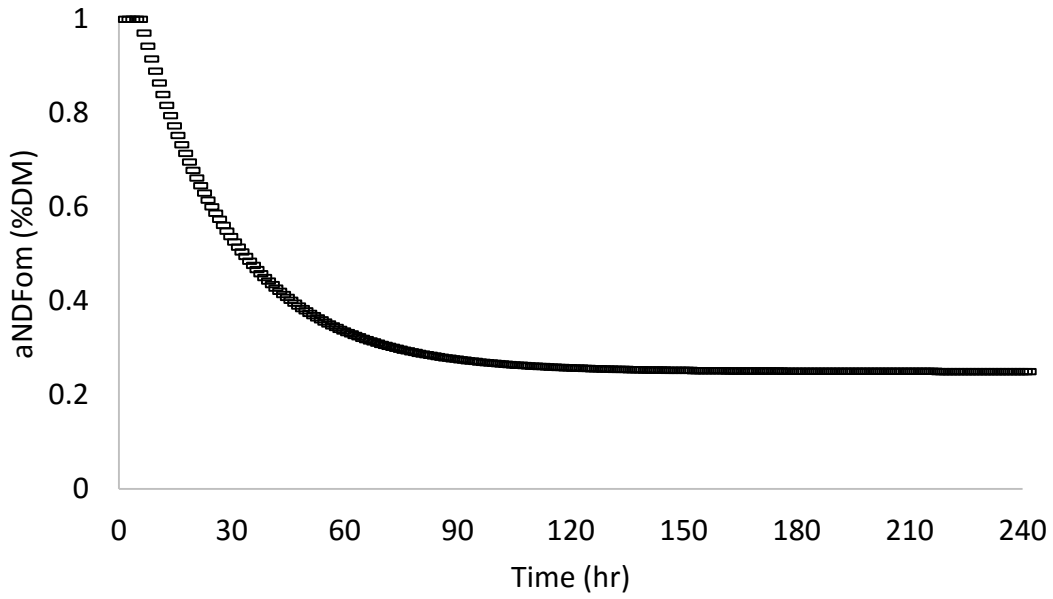

Figure 4.1. Exponential decay representing fiber digestion using Equation 1 as example, accounting for the lag-phase as a discrete term.

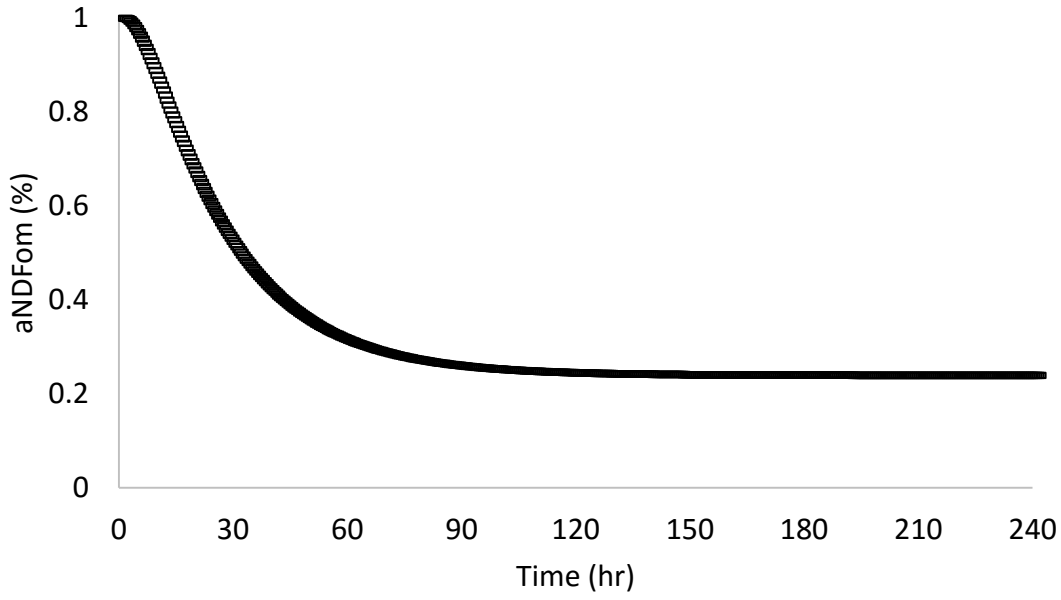

Figure 4.2. Sigmoidal behavior representing aNDFom digestion as analyzed with in-vitro fermentation.

4.3 MATERIALS AND METHODS

4.3.1 Describing aNDFom Digestion in Plant By-Products with a Two Compartment System.

A two compartment system describing in-vitro aNDFom digestion in plant by-products is presented in Figure 4.3Error! Reference source not found.. The first compartment (lag-compartment) depicts the amount of aNDFom present in a feed sample, divided into $dNDF_{LAG}$: the potentially digestible aNDFom still not available to digestion; and the uNDF: the aNDFom fraction that remains undigested over time. The first reaction is an exponential decay ($-k_{LAG}(dNDF_{LAG})$) and expresses the gradual progression by which the $dNDF_{LAG}$ fraction becomes available for digestion, and thus leaves the first compartment. The second compartment depicts the amount of dNDF that is available to digestion over time, and the second reaction ($-k_{dig}(dNDF)$) is still an exponential decay that describe the first order behavior of aNDFom digestion.

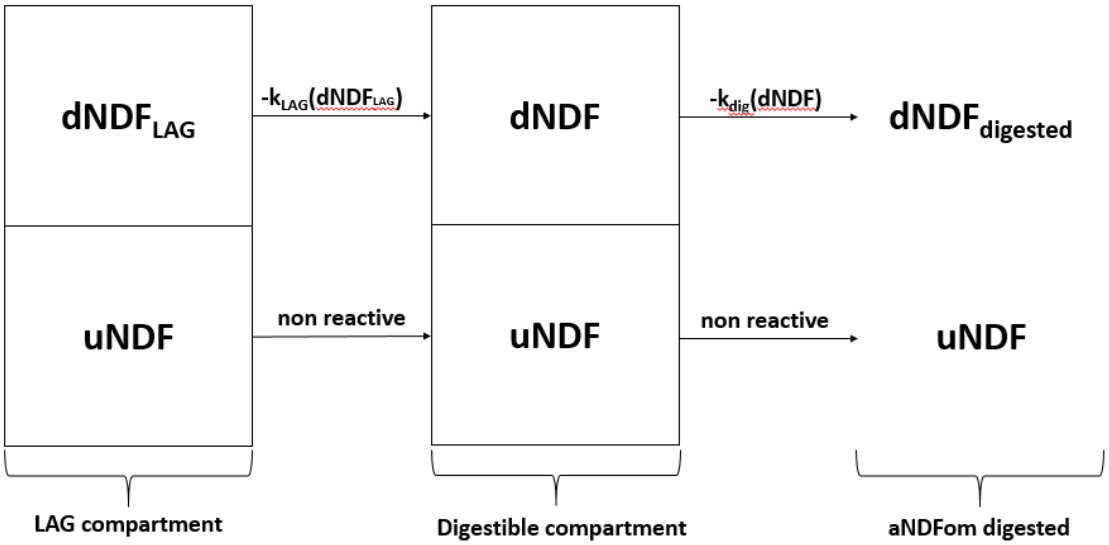

Figure 4.3. Two compartment system describing in-vitro aNDFom digestion of plant by-products: $dNDF_{LAG}$ is the substrate in the lag phase, k_{LAG} is the rate by which substrate is prepared to be digested; dNDF is the digestible fraction, k_{dig} is the rate by which is digested; and uNDF is the undigested fraction of aNDFom.

The lifetime of the substrate (aNDFom digestion) has a sigmoidal shape similar to the one observed during in-vitro aNDFom digestion. The differential equations that describe the processes of Figure 4.3 are:

Eq.3: Lag-compartment: $\frac{\mathrm{ddNDF_{LAG}}}{\mathrm{dt}} = -(\mathrm{k_{LAG}})(\mathrm{dNDF_{LAG}})$

where $dNDF_{LAG}$ is the substrate not available to digestion, and K_{LAG} is the rate by which substrate becomes available to digestion.

Eq.4: Digestible-Compartment: $\frac{\mathrm{ddNDF}}{\mathrm{dt}} = \left[(\mathrm{k_{LAG}})(\mathrm{dNDF_{LAG}}) - (\mathrm{k_{dig}})(\mathrm{dNDF})\right]$

where $dNDF_{LAG}$ and dNDF are the substrate not available for digestion and available for digestion respectively; and k_{LAG} and kdig are the rate by which substrate becomes

available to digestion and the rate of substrate digestion.

Eq.5a: aNDFom digested: $\frac{\mathrm{ddNDF_{digested}}}{\mathrm{dt}} = (\mathrm{k_{dig}})(\mathrm{dNDF})$

Eq.5b: aNDFom residue: $\frac{\mathrm{daNDFom_{residue}}}{\mathrm{dt}} = 1 - (\mathrm{k_{dig}})(\mathrm{dNDF})$

where in Eq.5a $\mathrm{aNDFom_{digested}}$ is the dNDF being digested and hence exiting the system, kdig is the rate by which the dNDF is digested (i.e. exit out of the system). In Eq 5b $\mathrm{aNDFom_{residue}}$ is the digestible substrate remaining in the system over time, which is what is analyzed with the aNDFom in-vitro digestion technique.

The solutions of the differential equations (the integral functions) that describe the digestion behavior as a function over the degradation period as depicted in Figure 4.4 are:

Equation 6 is the solution of Equation 3 describing the substrate remaining into the Lag-compartment:

Eq.6 : $\mathrm{dNDF_{LAG(t)}} = \mathrm{dNDF_{LAG(0)}}\ \mathrm{e^{-K_{LAG}t}}$

Equation 7 is the solution of Equation 4 describing the period of time to move substrate from the undigested into the digestible compartment:

Eq.7 : $\mathrm{dNDF_t} = (\mathrm{dNDF_0})(\mathrm{k_{LAG}})\left[\left(\frac{\mathrm{e^{-k_{LAG}t}}}{k_{\mathrm{dig}}-\mathrm{k_{LAG}}}\right) + \left(\frac{\mathrm{e^{-k_{dig}t}}}{\mathrm{k_{LAG}}-\mathrm{k_{dig}}}\right)\right]$

Equation 8a is the solution of Equation 5a describing the amount of aNDFom being

digested (i.e. leaving the compartment) over time; whereas the inverse function is found in Equation 8b that describes the amount of aNDFom remaining in the feed compartment over time:

Eq.8a: $$\mathrm{aNDFom}_{\mathrm{digested(t)}} = \mathrm{dNDF}_{(0)}\left[1-\left(\frac{k_{dig}e^{-k_{LAG}t}}{k_{dig}-k_{LAG}}\right)-\left(\frac{k_{LAG}e^{-k_{dig}t}}{k_{LAG}-k_{dig}}\right)\right]$$

Eq.8b: $$\mathrm{aNDFom}_{\mathrm{residue(t)}} = 1-\left\{\mathrm{dNDF}_{(0)}\left[1-\left(\frac{k_{dig}e^{-k_{LAG}t}}{k_{dig}-k_{LAG}}\right)-\left(\frac{k_{LAG}e^{-k_{dig}t}}{k_{LAG}-k_{dig}}\right)\right]\right\}$$

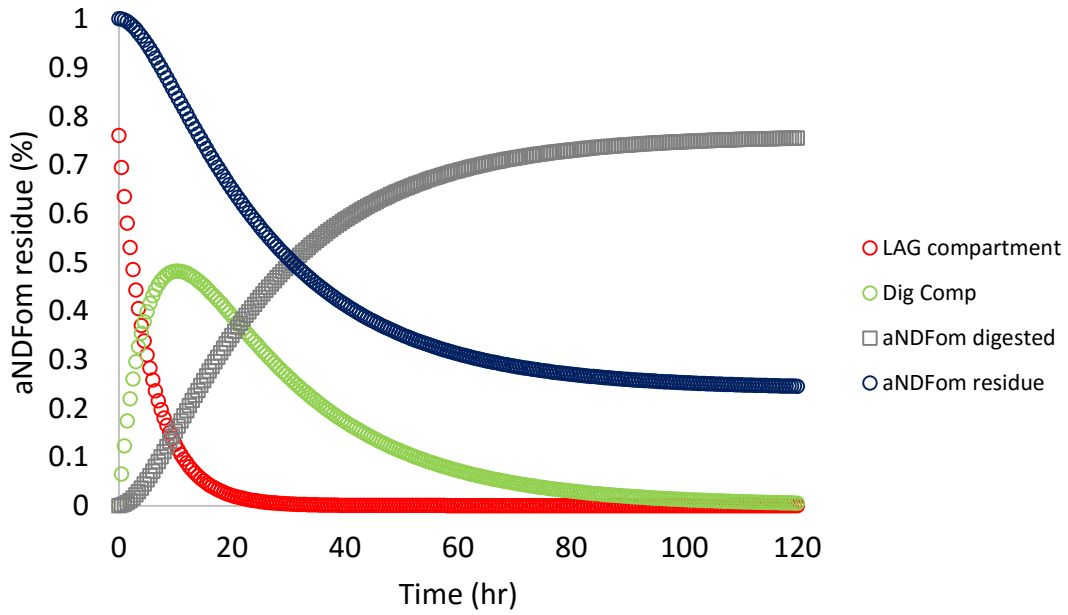

Figure 4.4. Profile expressed by Equations 6, 7, 8a, and 8b representing aNDFom in-vitro digestion of plant by-products modeled with a two compartment system, when k_{LAG} is 18 % of dNDF/hr, k_{dig} is 4.4 % of dNDF/hr, and uNDF is 24 % of aNDFom.

4.3.2 Describing aNDFom Digestion in Forages with a Two Compartment System.

A two compartment system describing in-vitro aNDFom digestion in forages is depicted in Figure 4.5. In this case the dNDF of forages is composed of two digesting pools (on relative basis, a Fast and Slow pool). For this description it is assumed that

the lag phase is biologically not differentiated for the two pools hence no differentiation is made for the lag compartment. Once the substrate is made available to digestion, the dNDF is split into the two digesting fractions. The fraction that belongs to the Fast pool will digest at a faster rate than the fraction that belongs to the Slow pool. Therefore the shape of the digestion curves are similar to those depicted in Figure 4.6**Error! Reference source not found.**.

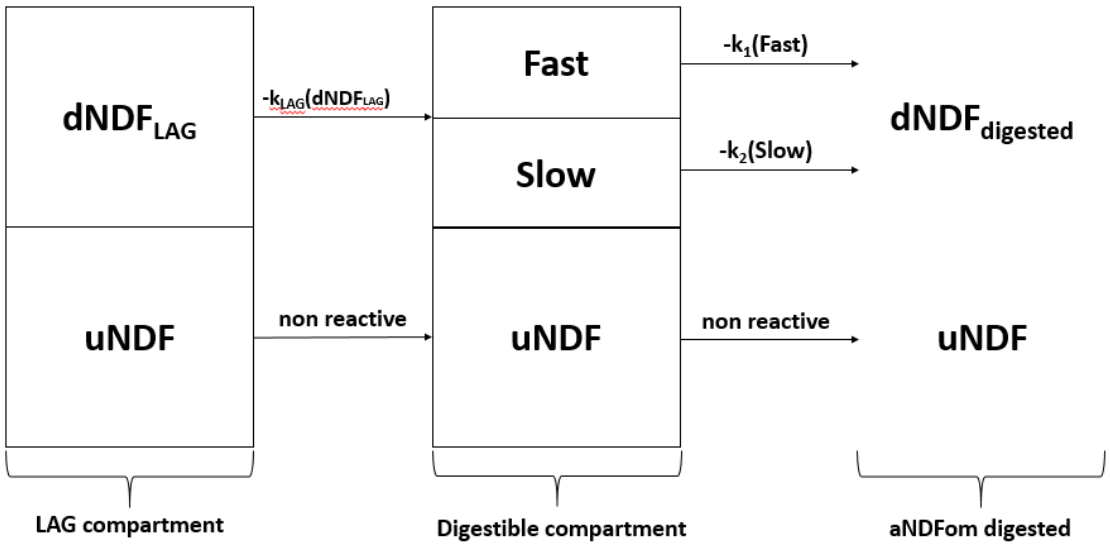

Figure 4.5. Two compartment system describing in-vitro fiber digestion of forages: $dNDF_{LAG}$ is the substrate in the lag phase, k_{LAG} is the rate by which substrate is prepared to be digested; Fast is the fast pool, k_1 is the rate by which the fast pool is digested, Slow is the slow pool, k_2 is the rate by which the slow pool is digested; and uNDF is the undigested fraction of aNDFom

The differential equations that describe the processes of this two pool digestion behavior**Error! Reference source not found.** are:

Eq.3: Lag compartment: $\frac{\mathrm{ddNDF_{LAG}}}{\mathrm{dt}} = -(\mathrm{k_{LAG}})(\mathrm{dNDF_{LAG}})$

Eq.9: Digestible compartment: $\frac{\mathrm{dFast}}{\mathrm{dt}} = [(\mathrm{k_{LAG}})(\mathrm{dNDF_{LAG}} * \mathrm{F})] - (\mathrm{F} * \mathrm{k_1})$

where dFast is the fast pool becoming available to digestion over time, $\mathrm{dNDF_{LAG}}$ is the substrate not available to digestion yet; F is the amount of fast pool (as % of aNDFom), and $\mathrm{k_{LAG}}$ and k1 are the rate by which substrate becomes available to digestion and the digestion rate of the fast pool.

Eq.10: Digestible compartment: $\frac{\mathrm{dSlow}}{\mathrm{dt}} = [(\mathrm{k_{LAG}})(\mathrm{dNDF_{LAG}} * \mathrm{S})] - (\mathrm{S} * \mathrm{k_2})$

where $\mathrm{dNDF_{LAG}}$ and dSlow are the substrate not available to digestion and the slow pool available to digestion respectively; S is the amount of slow pool, and $\mathrm{k_{LAG}}$ and k2 are the rate by which substrate becomes available to digestion and the digestion rate of the slow pool.

Eq.11a: aNFDom digested: $\frac{\mathrm{ddNDF_{digested}}}{\mathrm{dt}} = (\mathrm{k_1} * \mathrm{F}) + (\mathrm{k_2} * \mathrm{S})$

Eq.11b: aNDFom residue: $\frac{\mathrm{daNDFom_{residue}}}{\mathrm{dt}} = 1 - [(\mathrm{k_1} * \mathrm{F}) + (\mathrm{k_2} * \mathrm{S})]$

where in Eq.11a $\mathrm{NDF_{exit}}$ is the dNDF being digested and hence exiting the system, k1 and k2 are the rates by which the Fast and Slow pool are digested respectively (i.e. exit out of the system). In Eq.11b $\mathrm{aNDFom_{residue}}$ is the digestible substrate remained in the system over time.

The solutions to the equations that describe the digestion behavior as depicted in Figure 4.6 are:

Equation 12 is the solution of Equation 9 describing how the Fast pool moves into the digestible compartment:

Eq.12: $\mathrm{dNDF_{(t)}} = \left(k_{LAG} * F_{(0)}\right)\left(\frac{e^{-k_{LAG}t}}{k_1 - k_{LAG}} + \frac{e^{-k_1 t}}{k_{LAG} - k_1}\right)$

Equation 13 is the solution of Equation 10 describing how the Slow pool moves into the digestible compartment:

Eq.13: $\mathrm{dNDF_{(t)}} = \left(\mathrm{k_{LAG}} * \mathrm{S_0}\right)\left(\frac{\mathrm{e^{-k_{LAG}t}}}{\mathrm{k_2 - k_{LAG}}} + \frac{\mathrm{e^{-k_2 t}}}{\mathrm{k_{LAG} - k_2}}\right)$

Equation 14a is the solution of Equation 11 representing the amount of aNDFom residue remaining in the feed compartment over time:

Eq.14a: $\mathrm{aNDFom_{digested(t)}} = 1 - \left\{1 - \left[\mathrm{F}\left(1 - \frac{\mathrm{k_1 e^{-k_{LAG}t}}}{\mathrm{k_1 - k_{LAG}}} - \frac{\mathrm{k_{LAG} e^{-k_1}}}{\mathrm{k_{LAG} - k_1}}\right)\right] - \left[\mathrm{S}\left(1 - \frac{\mathrm{k_2 e^{-k_{LAG}t}}}{\mathrm{k_2 - k_{LAG}}} - \frac{\mathrm{k_{LAG} e^{-k_2}}}{\mathrm{k_{LAG} - k_2}}\right)\right]\right\}$

Eq.14b : $\mathrm{aNDF_{residue(t)}} = 1 - \left[\mathrm{F}\left(1 - \frac{\mathrm{k_1 e^{-k_{LAG}t}}}{\mathrm{k_1 - k_{LAG}}} - \frac{\mathrm{k_{LAG} e^{-k_1}}}{\mathrm{k_{LAG} - k_1}}\right)\right] - \left[\mathrm{S}\left(1 - \frac{\mathrm{k_2 e^{-k_{LAG}t}}}{\mathrm{k_2 - k_{LAG}}} - \frac{\mathrm{k_{LAG} e^{-k_2}}}{\mathrm{k_{LAG} - k_2}}\right)\right]$

where equation 14a is the solution of Equation 11a describing the amount of aNDFom being digested (i.e. escaping the feed compartment) over time; whereas the inverse Equation 14b describes the amount of aNDFom remaining in the feed compartment over

time.

It is important to recognize that the lag in the multi-compartment model was developed as a continuous function, thus it is described as a percent per hr, not as a discrete variable, and the approach negates any direct comparison to a discrete lag, but does allow for comparisons of goodness of fit, rates of digestion and application to nutrition models.

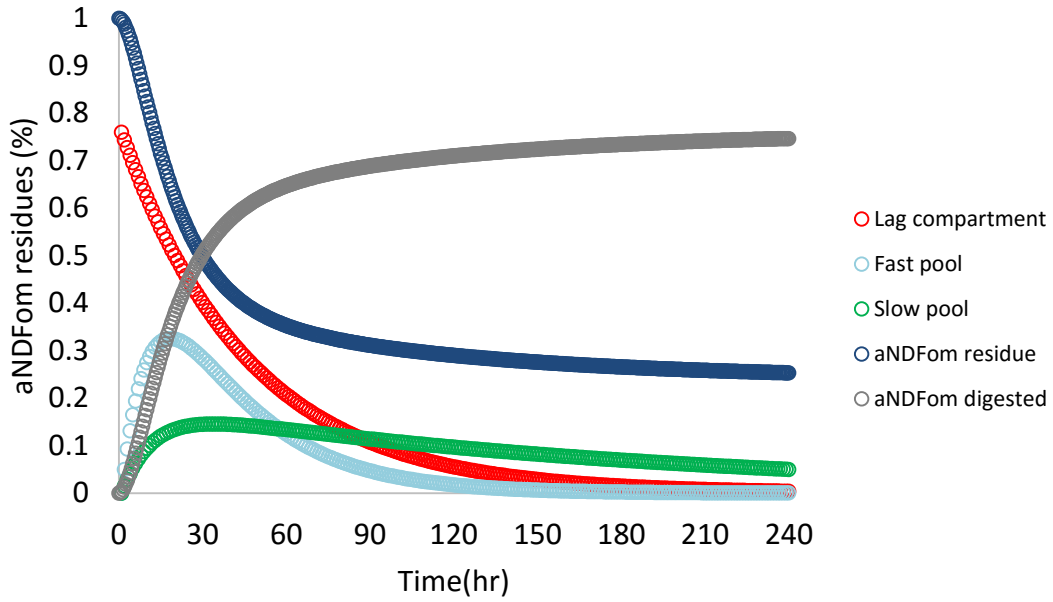

Figure 4.6. Profile expressed by Equations 6, 12, 13, 14a and 14b representing fast and slow pool, aNDFom residue and the lag-phase with a two-compartment system, when k_{LAG} is 18 %/hr, k_1 is 6.7 %/hr, k_2 is 1.1 %/hr, F is 59 % , S is 17 %, and uNDF is 24 % (all as % of aNDFom).

4.3.3 Model Evaluation

4.3.3.1 Generating the dataset to test the quality of model fit

Samples of 36 conserved forages (23 corn silages, 8 grass silages, 2 wheat straw, 3

grass hay), 32 fresh grasses (8 lower cut spring season, 8 higher cut spring season, 8 lower cut summer season, 8 higher cut summer season) and 15 plant by-product feeds (beet pulp, canola meal, citrus pulp, corn germ, corn gluten feed, corn distillers, flaked corn, rice hulls, soybean meal, soy hulls, Soyplus™, sunflower seed hulls, wheat distillers, wheat middlings, and whole cotton seed), were analyzed for aNDFom digestion using the in-vitro technique. Fermentations were following the methods of Goering and Van Soest (1970). Analysis of aNDFom residues were performed as described by Mertens (2002). Residues were filtered on a glass microfiber filter (934-AH, Whatman) with a 1.5 µm pore size to enhance residue recovery (Raffrenato and Van Amburgh, 2010). Two set of inputs for each type of feed were used for the models, and they were the aNDFom residues at 30, 120 and 240 h (set 1), and 6, 30, 120 and 240 (set 2) for the conserved forages; the aNDFom residues at 24, 120, and 240 h (set 1), and 8, 24, 120, and 240 (set 2) h for fresh forages; and the aNDFom residues at 12, 72, and 120 h (set 1), and 6, 12, 72, and 120 h (set 2) for plant by-product. The time points for forages were different due to previous observations of very rapid digestion of the fresh forage in the early period of fermentation, thus having an earlier time point was deemed necessary to ensure the Fast pool was identified before it was exhausted. Further, the data on fresh forages was determined from New Zealand pasture samples and the early time point of 8 hr was selected by the investigators because they expected a fast digestion rate and wanted an early time point to reflect expectation (Ryan Higgs, personal communication). This time point differs from our decision to use a 6 h time point but the modeling exercise is robust and flexible enough to accommodate both time points.

To evaluate the models, Vensim® (Ventana Systems, Harvard, MA) was utilized since it allows the user to develop dynamic, non-linear models and link to datasets for quick evaluation of model function and output. The diagrams build in Vensim® that represent the multi compartments model discussed above are depicted in Figure 4.7 and Figure 4.8. The optimization method employed in Vensim® for the evaluation of the digestion decays was a Powell hill-climbing algorithm Powell (1964). The models provided estimates of goodness of fit and to evaluate the performance of the mathematical approach (discrete versus continuous lag) the goodness of fit was compared for each regression (observed on predicted) using the variance accounted for R^2, along with the slope and intercept of prediction, and the root mean squared error of the prediction MSPE (Tedeschi, 2006).

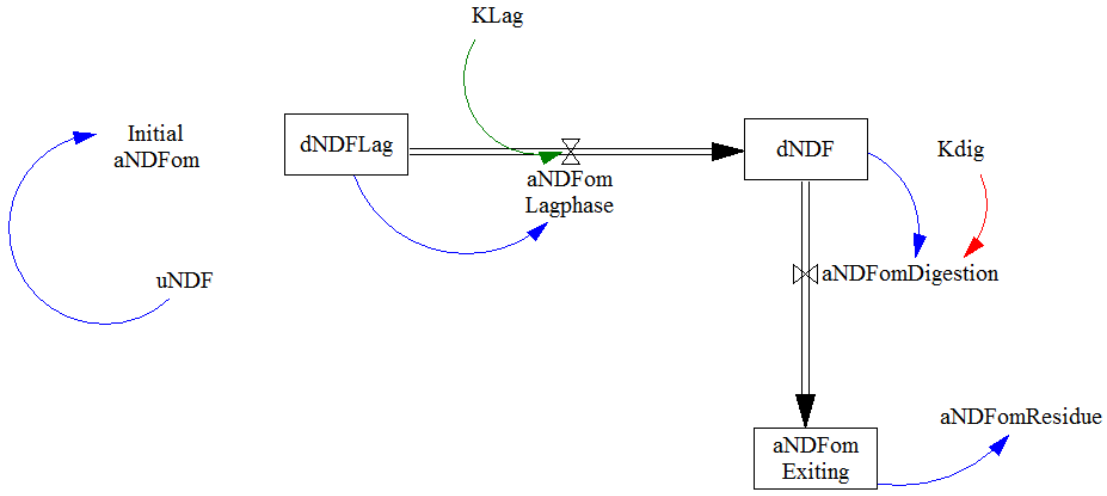

Figure 4.7. Diagrammatic representation of plant by-products aNDFom digestion of a multi-compartment model in Vensim®.

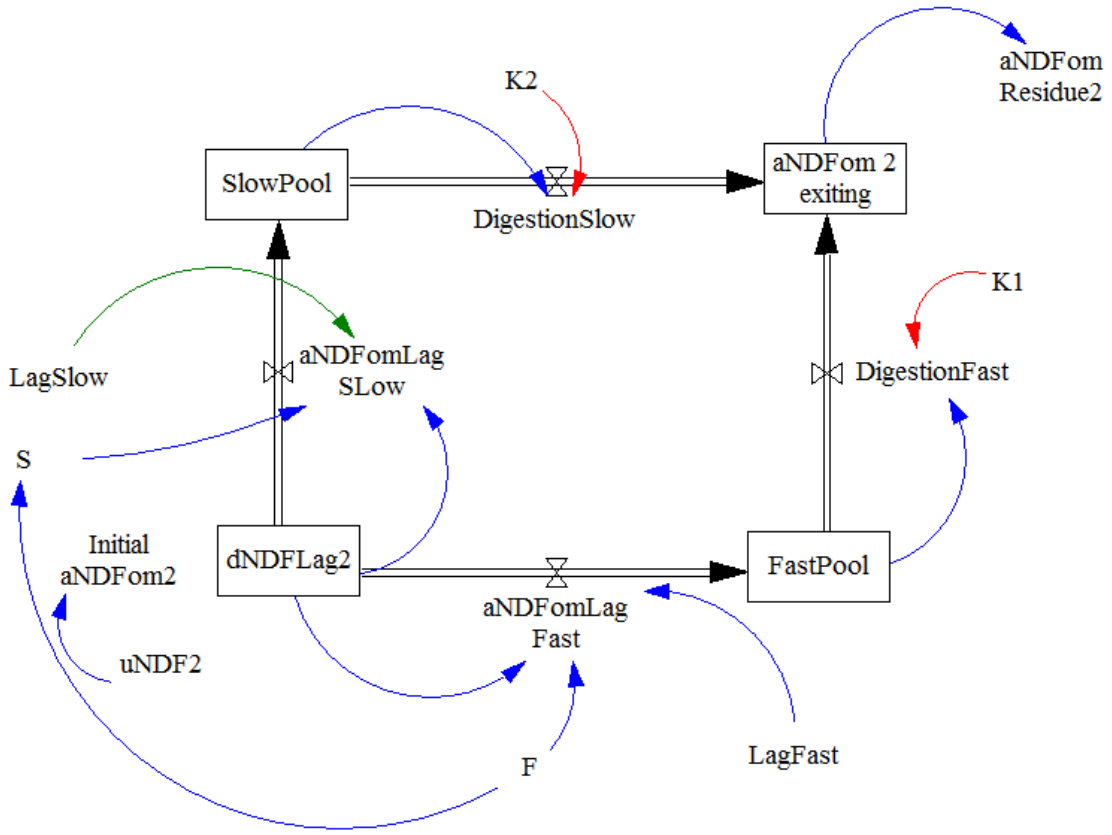

Figure 4.8. Diagrammatic representation of forages aNDFom digestion of a multi-compartment model in Vensim®.

4.3.3.2 Assessing the robustness of the model

To assess the robustness of the models, 10 conserved forages (2 corn silage conventional, 2 corn silage BMR, 2 grass silage, 2 grass hay, 2 wheat straw), 4 fresh forages (1 lower cut spring season, 1 higher cut spring season, 1 lower cut summer season, and 1 higher cut summer season) and 10 plant by-products (beet pulp, citrus pulp, canola meal, corn distillers, corn gluten feed, soybean meal, soy hulls, wheat distillers, wheat middling, and whole cotton seed) were selected for the analysis. A Monte Carlo simulation was performed by allowing a standard deviation of 2.5% around the aNDFom residue at each fermentation time point. The Monte Carlo simulation's

inputs were aNDFom residues at 6, 30, 120, and 240 h for the conserved forages, aNDFom residues at 8, 24, 120, and 240 h for the fresh forages, and aNDFom residues at 6, 12, 72, and 120 h for the plant by-products. Therefore, for each feed sample, 100 digestion curves were generated, and for each curve the digestion parameters were predicted (Fast pool, Slow pool, kd1, kd2, kd-lag). The robustness of the models was assessed by looking at the coefficient of variation of each predicted parameter for forages and plant by-products, respectively. To assess statistical differences of the coefficient of variation of the predicted parameters, the fit model function was performed in JMP considering the treatment effect the only variable of the statistical model, overall treatment effects were analyzed using Tukey's test. Significance was declared at *P*-values < 0.05.

4.3.3.3 Evaluating the usefulness of model predictions

The usefulness of the model predictions were assessed by evaluating the RMSE of CNCPS predictions of ME allowable milk and aNDFom TTD, using information from a lactating cattle pen study where treatment diets were formulated to quantify the effects of aNDFom source and digestibility. The experimental design was a 3 x 3 Latin square with 21 d adjustment and 5 d sampling periods, this was used to develop the RMSE calculations. Parameters of aNDFom digestion (Fast pool, Slow pool, uNDF, and rates of digestion) were calculated for each treatment's feed ingredients, following the procedures proposed by Raffrenato (2011) for forages and described in Chapter 2 for plant by-products. Parameters of aNDFom digestion, along with cattle and farm information were therefore used as inputs for CNCPS 7.0.

4.3.3.4 Comparing the multi compartments model of aNDFom digestion with the exponential decay model

Both type of models: the multi compartments models (Eq. 8b and Eq. 14b); and the exponential decay model (Eq.1 and Eq.2); were compared along with the evaluation of the study. In the development of the exponential decay model (Raffrenato, 2011) the model as applied was implemented in Vensim® and to fit the observed digestion behavior and provide accurate rates of digestion and pool sizes for the Fast and Slow pool of aNDFom, the model required constraints. These constraints were developed to ensure the prediction of the digestion of the Fast pool remained within observed values using the discrete lag and a minimum of time points needed to implement the discrete lag, and describe the inflection between the Fast and Slow pools and the uNDF. The constraints remained in place through this evaluation, however the new equations describing the multi-compartment models were not constrained when implemented in Vensim®.

4.4 RESULTS AND DISCUSSION

4.4.1 Development and application of multi-compartment models to describe digesta kinetics in the rumen

The approach of describing aNDFom digestion with a multi-compartment model could be extended to describe other biological processes that similarly show an overall sigmoidal behavior. An example could be modeling the kinetics of the aNDFom in the rumen to better characterize energy supply and possibly dry matter intake. Similarly to

the gamma functions proposed by (Matis, 1972; Pond et al., 1988; Poppi et al., 2001), to model the rumen residency time of digesta within a specific compartment of the gastrointestinal tract (GIT) of a ruminant, a multi-compartment model proposed in this study can replicate the behavior observed in Figure 4.4 and Figure 4.6. However the gamma functions proposed are of little applicability to predict aNDFom dynamics in the rumen because they were fit a posteriori to the particle flow observed, and especially because researchers used different gamma functions with different degrees (one for each compartment of the GIT) until they find a combination that fit best the data set, and gamma functions are not consistent among studies (Pond et al., 1988; Poppi et al., 2001). The need for different gamma functions to describe particle flow and digestion behavior might be due to the differences in aNDFom digestibility, uNDF and other factors not considered in those studies that might influence the rate and extent of digestion that is not described by the gamma distribution function.

Furthermore the rates of digesta flow from one compartment to the other of the GIT were stochastic and having a deterministic model that can be used a priori to model the kinetics of the digesta in the rumen could help in the prediction of energy supply and dry matter intake. According to Allen and Mertens (1988) digesta that enter the rumen are initially in the lag compartment, then move to a digestible but non-escapable compartment, and finally move to a digestible and escapable compartment, before escaping the rumen and this is consistent with the approach described by Huhtanen and Kukkonen (1995). The lag compartment proposed by those papers is not the same lag compartment described in this study. In this study the lag phase is associated to in-vitro fermentation and the initiation of digestion and is used as the basis for modeling the

rates of aNDFom digestion, thus it would be incorrect to consider the lag present in the in-vitro analysis as the lag occurring in the rumen. Alternatively, the aNDFom fractions and corresponding digestion rates calculated by the aNDFom digestion model could be similar to those in the rumen. Therefore, if the rates of lag and digestion can be used to help model the passage rates that describe when digesta moves from the non-escapable to escapable compartment, the ability to better predict the dynamics of rumen turnover could possibly be simplified and other aspects could be modeled around this, such as DMI.

4.4.2 Goodness of fit and evaluation of the effect of models inputs to models outputs

This analysis was different to any approach found in the literature (Van Milgen et al., 1991; Huhtanen et al., 2008; Vieira et al., 2008), and aimed to evaluate the time-point combinations currently in use for aNDFom analysis (30, 120, and 240 h for forages; and 12, 72, and 120 h for plant by-products; Van Amburgh et. al., 2015) to determine if three time points were providing information for an accurate estimation of the aNDFom digestion parameters or if an early time point (6 or 8 h) was necessary. In particular the objective was to understand if providing the 6 or 8 h residue that gives information about the first inflection of the aNDFom digestion curve, yielded similar model output than not providing it. Overall, the goodness of fit was acceptable and similar for both approaches (Table 4.1). There was a small reduction in R^2 and RMSE when the 6 or 8 h time point was not used, therefore, when simply evaluating the ability of either set of equations to describe the digestion curve, both approaches are adequate.

Table 4.1. Overall goodness of fit of the models, and comparison of models performance of fit using 6h or 8h time point as input versus without using it.

Item	Slope	Intercept	R^2	RMSE
Eq. 1 and 2, 6h	1.01	0.01	0.99	0.02
Eq. 1 and 2,	1.01	0.01	0.97	0.02
Eq. 8b and 14b, 6h	1.04	0.02	0.98	0.03
Eq. 8b and 14b,	1.06	0.02	0.96	0.04

However, validating a biological model cannot be accomplished only through a mathematical process (i.e. by fitting the model to dataset), the user needs to ascertain that the model also provides accurate biological outputs that are evaluated and applicable in the field. For these reason it was necessary to evaluate the model's output with and without the 6 or 8 h residue provided. The overall ranges in the digestibility of the aNDFom by pools, the associated rates of digestion along with the estimated lags are found in

Table 4.2. The data are described by model and whether the early time points were useful in adding information to the early part of the digestion curve to better predict the rate and pool size of the aNDFom. Providing the early time point fermentation residue provided the model with information about the early inflection of the curve and yielded different model results compare to not including it. For example, the ranges of the fast pool kd1 calculated with and without the early time points did not overlap for the fresh forages when comparing the two modeling approaches. Furthermore, for some conserved forages the exponential decay approached the model constrains in the calculations of the fast pool kd without the early time point provided. Whereas, when the information for the early time point was given, the rates of digestion were all calculated within the expected biological range. Likewise the multi-compartment

models for the fresh forages, without the information provided by early time points, were not able to predict any slow pool, and that doesn't fit the digestion curve described in Figure 4.5. The opposite was true when the 8 h residue was given. In feeds such as the fresh forages where the rates of aNDFom digestion were higher and much of the Fast pool substrate is exhausted by or before 30 h the early time point information becomes more important for parameterization, specifically the 6 or 8 and 24 h values.

According to Mertens (1993) mathematical models require information about the beginning, end and any inflection point of the curve; and this seems to be confirmed in Table 4.2. Indeed, to fully describe the pool behavior of aNDFom digestion, it is important to have time points that correspond or fall within the linear portion of the specific curve, whether it is the Fast or Slow pool. Furthermore for byproducts, the ranges in the digestion parameters were similar within model structures, and the inclusion of the early time point didn't seem to affect much the outputs. The explanation of this can lie in the fact that 12 h can be sufficiently close to 6 or 8 h to provide adequate information of the early inflection of the curve, and also aNDFom digestion models of plant by-product have only two parameters to calculate (kd and Lag) and hence more stable compare to aNDFom digestion models for forages that have to calculate five parameters. Finally this study demonstrated that exponential decays and multi-compartment models that describe aNDFom digestion kinetics with two different approaches can fit the data set very well, while providing different solutions. Those application and evaluation of those solutions are relevant to use in extant nutrition models and require evaluation for applicability. Mathematically, the multi-compartment models require no constraints, suggesting they are more mechanistic and robust and

might be adaptable to further development of integrated models.

Table 4.2. Ranges of the estimated parameters of in-vitro aNDFom digestion calculated by Eq. 1 and 2, and Eq. 8b and 14b with and without the 6 or 8 hour time point.

Conserved	Fast, %aNDFom	Slow, %aNDFom	kd1, % /hr	kd2, % /hr	Lag[3]
Eq. 2, 6h	31.0 – 70.6	15.5 – 44.3	6.0 – 13.3	1.0 – 5.1	2.6 – 4.0
Eq. 2, 30h	33.3 – 57.5	6.4 – 48.1	6.0 – 30.0	1.1 – 5.8	0.3 – 4.0
Eq. 14b, 6h	41.5 – 86.1	0.0 – 40.3	6.0 – 11.1	0.0 – 1.2	9.9 – 24.5
Eq. 14b, 30h	42.4 – 86.1	0.0 – 39.4	5.8 – 11.0	0.0 – 1.1	9.6 – 24.8
Fresh forages	Fast, %aNDFom	Slow, %aNDFom	kd1, %/hr	kd2, %/hr	Lag*
Eq. 2, 8h	67.9 – 78.3	11.5 – 18.2	20.9 – 29.0	2.6 – 4.3	3.9 – 4.0
Eq. 2, 30h	73.0 – 81.1	8.6 – 13.1	14.5 – 18.8	2.2 – 2.5	2.1 – 3.6
Eq. 14b, 8h	79.3 – 87.4	0.4 – 6.8	18.1 – 22.8	0.6 – 1.7	18.5 – 22.2
Eq. 14b, 30h	86.1 – 89.9	0.0 – 0.0	13.4 – 15.0	0.0 – 0.0	13.4 – 15.0
By-products	Fast, %aNDFom	Slow, %aNDFom	kd1, %/hr	kd2, %/hr	Lag*
Eq. 1, 6h	–	–	3.8 – 13.5	–	0 – 4
Eq. 1, 12h	–	–	4 – 11.8	–	0 – 4
Eq. 8b, 6h	–	–	5.6 – 17.8	–	7.7 – 17.9
Eq. 8b, 12h	–	–	5.1 – 20.3	–	7.7 – 28.7

[3]Lag units for the Eq. 1 and 2 are hours, units for the Eq. 8b and 14b are %aNDFom/hour.

4.4.3 Model's Robustness

This part of the study was important because a certain degree of variation in the analysis of aNDFom digestibility is present, even from run to run of the same feed sample (for example in our lab we reported a standard deviation of 2.5%). Variation associated to the in-vitro analytical technique might affect the model's outputs and consequently reduce the applicability for diet formulation. For instance it is unsatisfactory that the same feed sample analyzed at two different times or laboratories would lead two different digestion rate predictions. Therefore it was important to employ a Monte Carlo simulation that by producing 100 virtual in-vitro aNDFom

digestion curves per sample, considering a standard deviation of 2.5% around the fermentation time points, to determine if the models were consistent in estimating aNDFom digestion parameters, despite the variation present in the analytical procedure. The coefficient of variation from the Monte Carlo simulations along with the mean values of the parameters estimated by the two types of models are in Table 4.3. It is important to consider that the coefficient of variation needs to be compared to the mean value to which it is related, indeed a CV is less meaningful when the mean value is smaller. Given that, the most direct examples are among the kd1 of the conserved forages where the means of the predicted kd1 values are very similar for the two types of models (10.5 and 10.2 %/hr) but the coefficient of variation are statistically different and about 4 times higher for the exponential decay model compared to the multi-compartment models; and among the slow pools of the conserved forages the coefficient of variation of the predicted values are not statistically different (9.1 and 9.3), but the means of the estimated values are about 3.4 time lower for the multi-compartment models versus the exponential decays models. Further evaluation of the data presented in Table 4.3 shows that the multi-compartment models were more robust in estimating parameters, despite the variation of the Monte Carlo simulation. The CVs of the lag parameters cannot be compared because of the different approach between the two models in describing the lag phase. The robustness of the model is very important for the standardization of a protocol can be used in commercial laboratories, where consistent results are needed for application in formulating diets.

Table 4.3. Coefficient of variation calculated from a Monte Carlo simulation of the parameters estimated with Eq. 1, 2 and Eq. 8b, 14b using residues at 6, 30,120, and 240 h for conserved forages; 6, 24, 120, and 240 h for the fresh forages; and 6, 12, 72, 120 h for plant by-products.

	Models			
Item[1]	Eq. 8b and 14b	Eq. 1 and 2	SEM	P-val
Conserved Forages				
Fast, %	1.5 (68.9)	5.1 (51.9)	0.1	<0.05
Slow, %	9.1 (7.1)	9.3 (24.1)	0.3	0.71
kd1, %	3.1 (10.5)	12.3 (10.2)	0.4	<0.05
kd2, %	10.2 (3.6)	8.2 (2.0)	1.2	0.25
Lag[2]	3.6 (11.5)	1.0 (0.6)	NA	NA
Fresh forages				
Fast, %	0.1 (87.7)	2.1 (75.2)	<0.1	<0.05
Slow, %	0.0 (0.0)	12.8 (12.4)	0.5	<0.05
kd1, %	0.6 (15.8)	6.9 (14.1)	0.2	<0.05
kd2, %	0.0 (0.0)	12.1 (2.9)	0.4	<0.05
Lag[2]	0.5 (14)	0.7 (0.6)	NA	NA
By-products				
Kd, %	3.0 (12.6)	5.3 (7.6)	0.2	<0.05
Lag[2]	2.5 (13.4)	0.3 (2.7)	NA	NA

[1] In brackets are reported the mean values of the parameters estimated.
[2] The lag parameter cannot be compared because the units are different among the two models. In the exponential decays model units are hours, in multi compartments models units are % of aNDFom/hour.

4.4.4 Application of the parameter estimates

This evaluation was conducted to understand if the aNDFom digestion models were providing outputs that were biologically realistic, and thus, could be used for formulating diets for dairy cattle. The outputs of the aNDFom digestion models were the size of the aNDFom fractions (fast pool, slow pool and dNDF) along with the digestion rates of the selected feeds, these were used as inputs for the CNCPS 7.0 (Table 4.4). The lag parameters, either in terms of time extent (for Eq. 1 and 2) or rate (for Eq. 8b and 14b), were not useful information for balancing diets, but were important to help predict the aNDFom pools and rates. To make the model comparisons the data from Chapter 3 for the animal, environmental and diet descriptions from the lactation experiment was used and the rates of digestion generated from both models were inputted. All aNDFom containing feeds were evaluated using both models and then the predicted rates of digestion were used in the CNCPS 7.0 to evaluate the model predicted ME allowable milk and aNDFom TTD (Table 4.5). Use of the more mechanistic modeling approach for estimation of rates of digestion improved the prediction of ME allowable milk for all three treatments and provided the lowest RMSE for ME allowable milk. For the exponential model, the RMSE was 1.26 kg whereas for the multi-compartmental model the RMSE was 0.8 kg suggesting that the multi-compartment model provided better information for rates of digestion for the dietary ingredients used in this study. For the prediction of aNDFom TTD, the RMSE were 3.1 and 4.2 as % of aNDFom for the exponential decays and multi compartment models respectively. However the model inputs from exponential decays models were not consistent in the estimation of aNDFom TTD when related to the estimation of ME allowable milk. For

instance the rates from exponential decays were predicting lower values of ME allowable milk, compared to observed, but similar or higher amounts of aNDFom digested, among treatments.

Table 4.4. Fractions of aNDFom and respective digestion rate, as calculated by the digestion decay and multi-compartment models, of the feeds used in the analysis of CNCPS v.7.0

	aNDFom fractionation and rates of digestion				
Item	Fast pool, %aNDFom	Slow pool, %aNDFom	uNDF, %aNDFom	kd1, %/hr	kd2, %/hr
Exponential decay models					
Corn silage conventional	55.9	19.1	25.1	9.0	2.1
Bmr corn silage	65.9	16.0	18.1	11.2	2.2
Alfalfa silage low uNDF	49.5	12.0	38.4	24.7	1.8
Alfalfa silage high uNDF	39.1	17.3	43.6	9.1	2.0
Alfalfa hay high uNDF	40.6	9.0	51.3	20.5	1.3
Corn meal	77.1	-	22.9	4.4	-
Citrus pulp	75.6	-	24.4	15.4	-
Wheat middlings	67.9	-	32.1	5.9	-
Cottonseed delinted	48.9	-	51.1	3.3	-
Soyplus™	82.1	-	17.9	5.5	-
Soybean hulls	92.6	-	7.4	5.9	-
Corn gluten feed	85.4	-	14.6	5.9	-
Sunflower hulls	27.9	-	72.1	4.4	-
Multi compartments models					
Corn silage conventional	49.3	25.6	25.1	11.9	2.2
Bmr corn silage	58.9	23.0	18.1	25.5	2.7
Alfalfa silage low uNDF	46.6	15.0	38.4	28.2	2.6
Alfalfa silage high uNDF	39.5	16.8	43.6	12.0	4.3

Alfalfa hay high uNDF	32.6	16.1	51.3	20.6	3.6
Corn meal	77.1	-	22.9	4.8	-
Citrus pulp	75.6	-	24.4	14.7	-
Wheat middlings	67.9	-	32.1	6.5	-
Cottonseed delinted	48.9	-	51.1	3.3	-
Soyplus™	82.1	-	17.9	6.0	-
Soybean hulls	92.6	-	7.4	6.4	-
Corn gluten feed	85.4	-	14.6	6.6	-
Sunflower hulls	27.9	-	72.1	5.0	-

Table 4.5. CNCPS predictions of ME allowable milk and aNDFom total tract digestibility using inputs generated from Eq 1 and 2, and Eq 8b and 14b.

	Treatments			
	HUF	LUF	HUNF	RMSE
ME allowable milk, Kg				
Observed	41.85	44.21	49.38	
Predicted Eq. 1 and 2	39.90	43.13	48.14	1.26
Predicted Eq. 8b and 14b	41.28	44.40	51.1	0.80
aNDFom TTD, %aNDFom				
Observed	52.7	53.2	39.8	
Predicted Eq 1 and 2	53.3	57.7	41.1	3.1
Predicted Eq 8b and 14b	45.3	50.0	43.7	4.2

[1]HUF = high uNDF (32% of aNDFom) forage base (80% of DM); LUF = low uNDF (25% of aNDFom) forage base (80% of DM); HUNF = high uNDF (32% of aNDFom) non-forage base (30% of DM).

4.5 CONCLUSIONS

Describing aNDFom digestion with a multi-compartment model improved the ability to fit the observed digestion data, specifically the sigmoidal behavior observed in the in-vitro aNDFom fermentation analysis. Furthermore, the multi-compartment model showed it was capable of adequately quantifying aNDFom digestion parameters in different ways. First it does not require the use of constraints for the predictions compared to the exponential decay model. Accordingly, it is seemingly more robust in dealing with the variation associated with the aNDFom in vitro procedure. Further, when the multi-compartment model was used to calculate rates of digestion, the predictions of a field usable model were improved suggesting the approach was more robust. Finally, as rates of aNDFom digestion increase, especially the fast pool, the amount of information needed to best describe the rates of digestion from in-vitro fermentation becomes more important for an accurate calculation of aNDFom digestion parameters.

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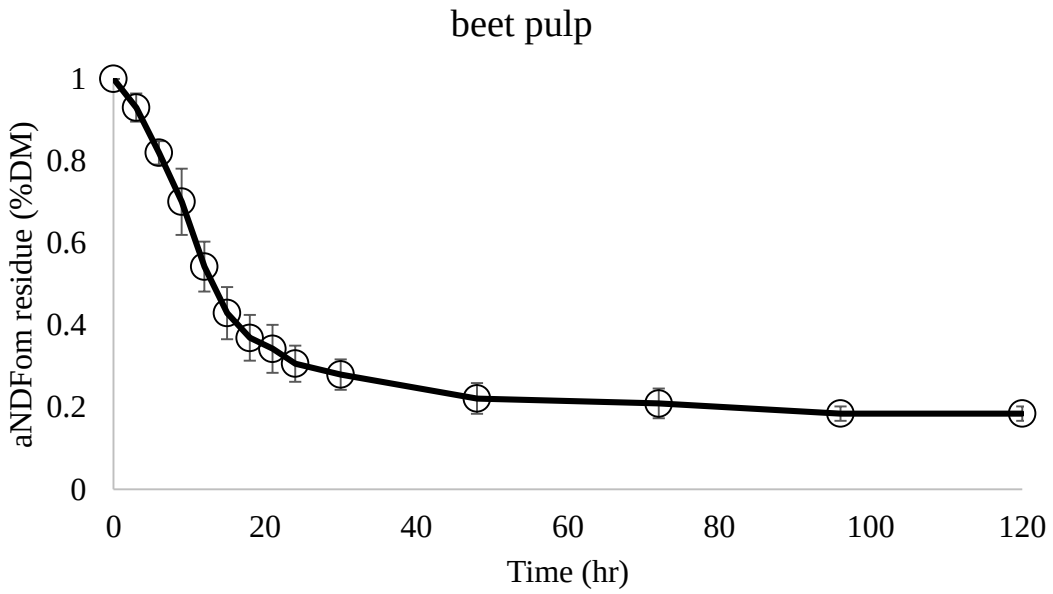
5 SUMMARY

Describing the multiple fractions of aNDFom seems to be a reasonable approach within the structure of CNCPSv7. This project, after the analysis of aNDFom in-vitro digestion in plant by-products (Chapter 2), completed the work that had been ongoing for the characterization of the aNDFom fractions of the feeds used in formulation of diets for lactating dairy cattle and residing in the library of the CNCPS. The development of a laboratory protocol for the analysis of aNDFom and aNDFom digestion from byproducts provided the necessary information, such as pool size and rate of digestion, to use as inputs for the CNCPSv7 when balancing diets for lactating dairy cattle. This enabled us to design an experimental study controlling for these factors and thus quantify their effect on feed intake, milk production and composition, rumination time and aNDFom total tract digestibility (Chapter 3).

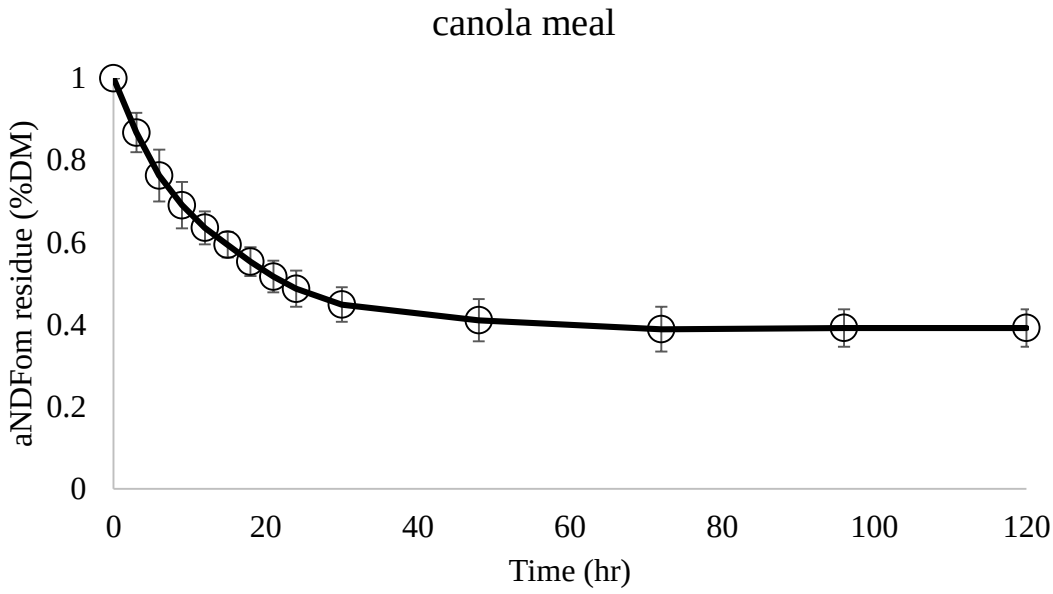
As the CNCPS is improved to more mechanistically describe the biology of the rumen and cow, it becomes more sensitive to inputs and for this reason we wanted to revisit the equations currently employed to model aNDFom digestion. The equations presented in Chapter 4 describe aNDFom digestion with a more mechanistic approach, shifting the description of aNDFom digestion from an exponential decay to a multi-compartment approach to more mechanistically describe the sigmoidal behavior associated with digestion. This resulted in reduced variation associated to CNCPSv7 prediction of ME allowable milk, for the experiment in Chapter 3. Finally this dissertation makes an effort by providing tools (aNDFom digestion model) and guidelines (fermentation time points) to generate accurate data in the laboratory, and how to use them for field application.

APPENDICES

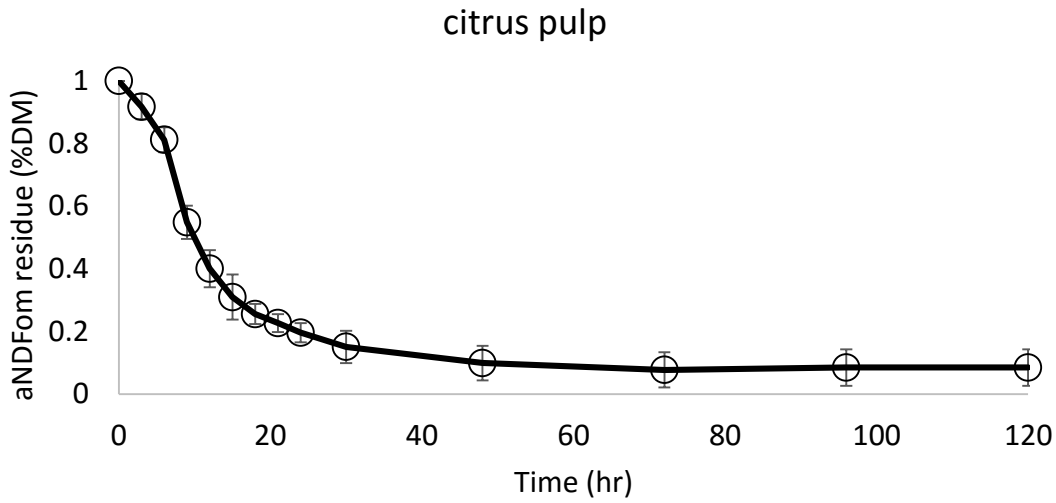
DIGESTION DECAYS OF THE PLANT BY-PRODUCTS ANALYZED FOR THE EXPERIMENT IN CHAPTER 2



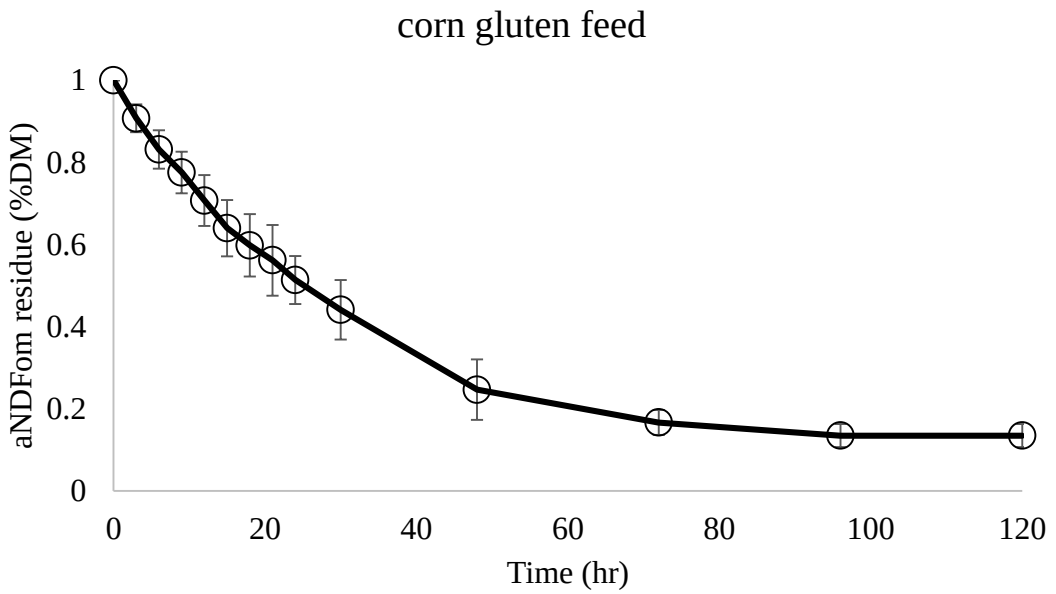
The in-vitro aNDFom decay of beet pulp, averaged on 2 samples and 3 batches.



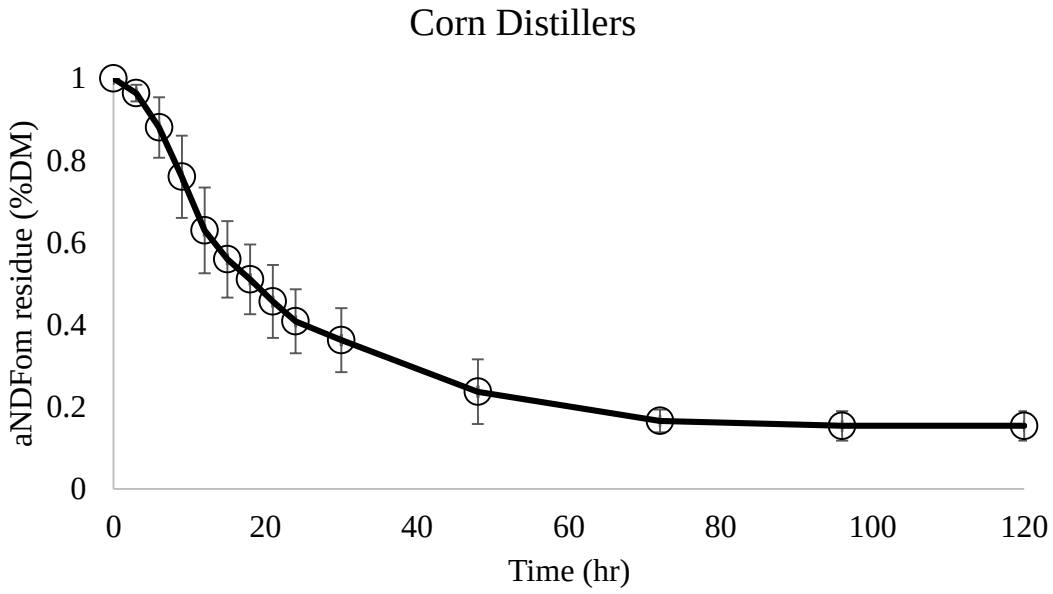
The in-vitro aNDFom decay of canola meal, averaged on 2 samples and 3 batches



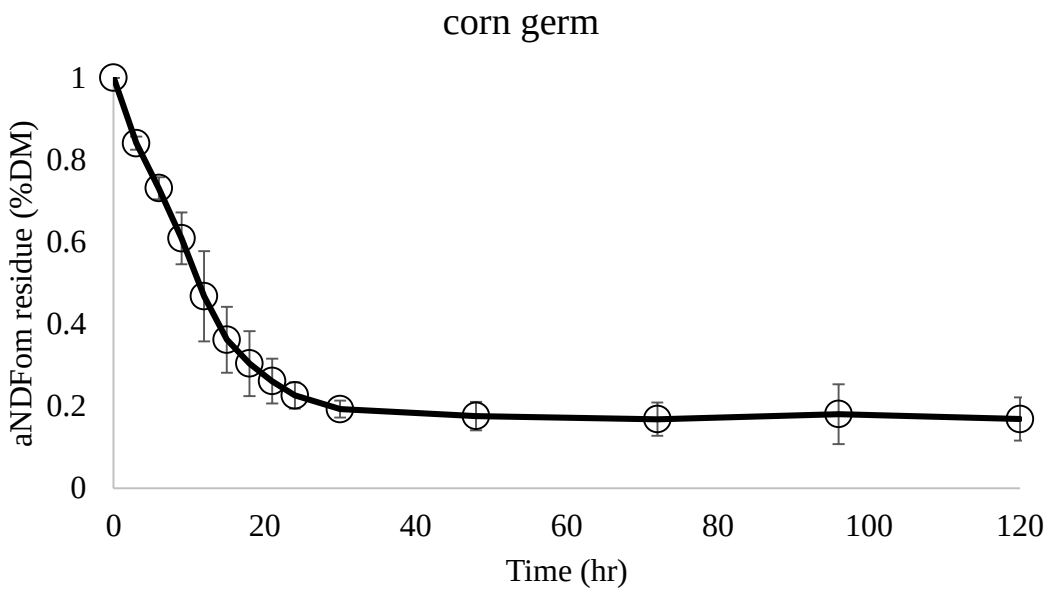
The in-vitro aNDFom decay of citrus pulp, averaged on 2 samples and 3 batches



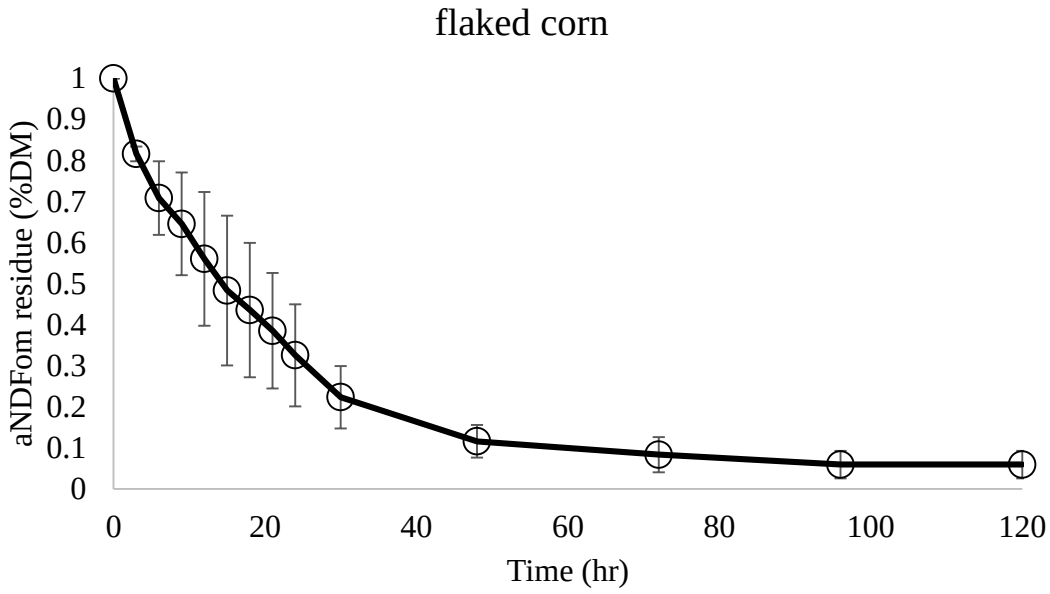
The in-vitro aNDFom decay of corn gluten feed, averaged on 2 samples and 3 batches



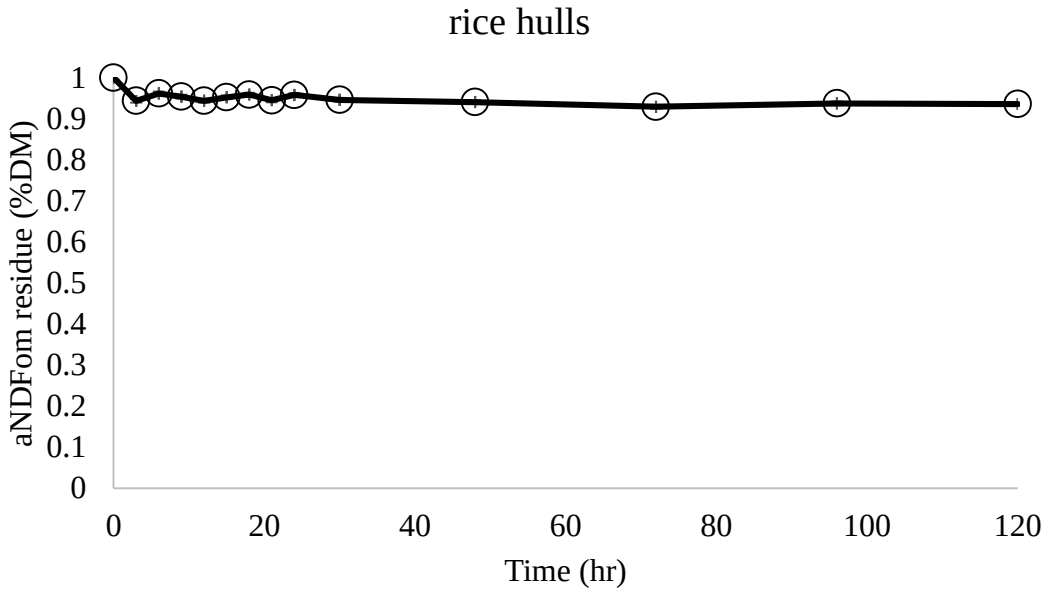
The in-vitro aNDFom decay of corn distillers, averaged on 2 samples and 3 batches



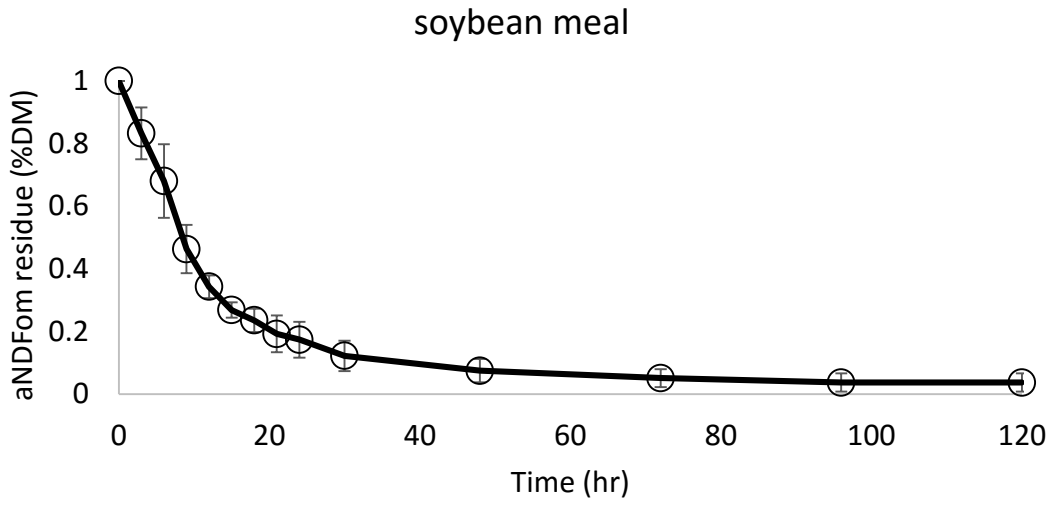
The in-vitro aNDFom decay of corn germ, averaged on 2 samples and 3 batches



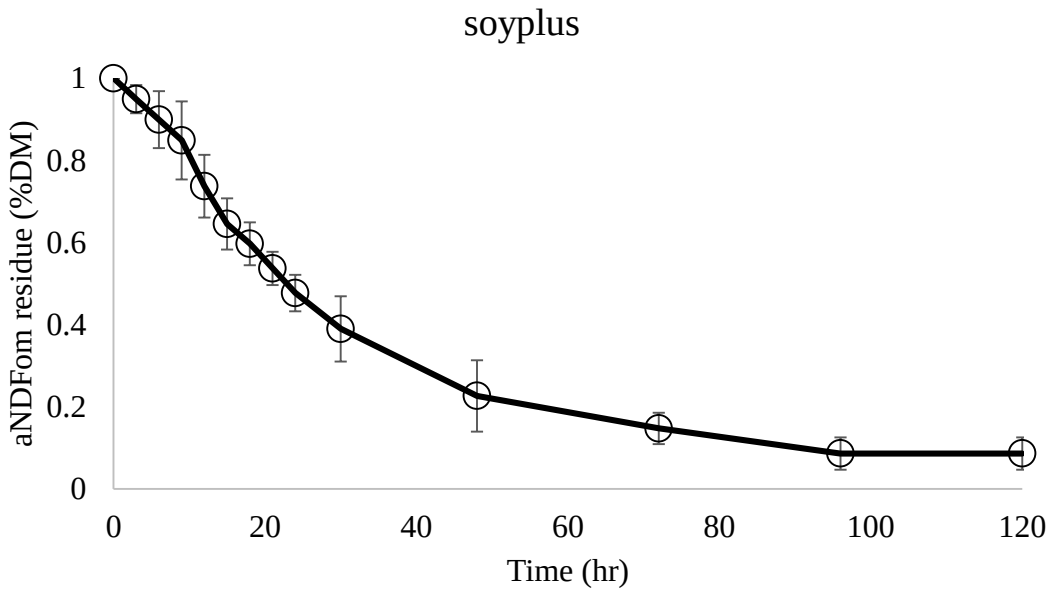
The in-vitro aNDFom decay of flaked corn, averaged on 2 samples and 3 batches



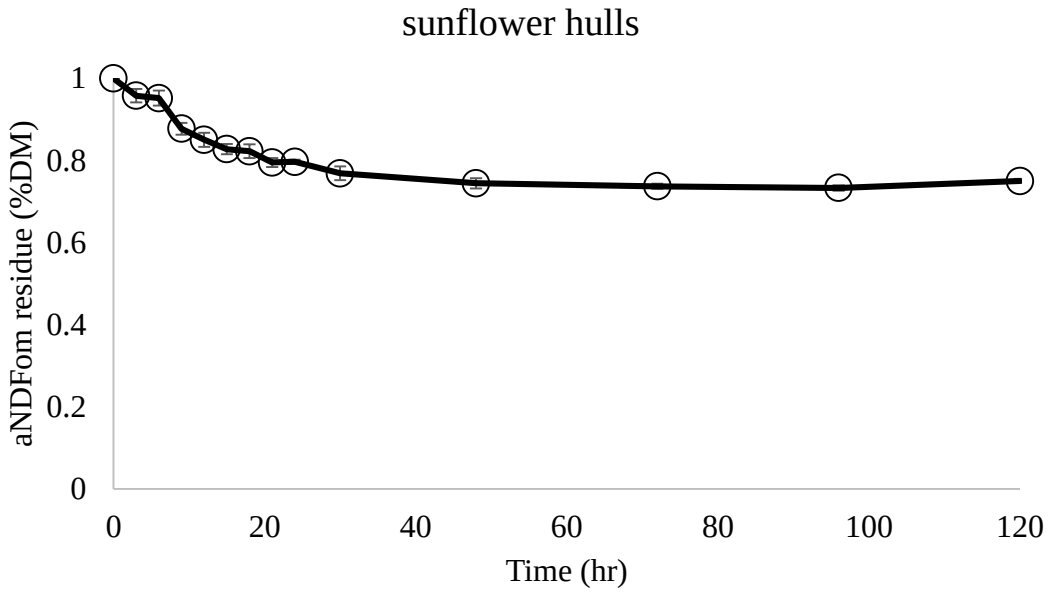
The in-vitro aNDFom decay of rice hulls averaged on 2 samples and 3 batches



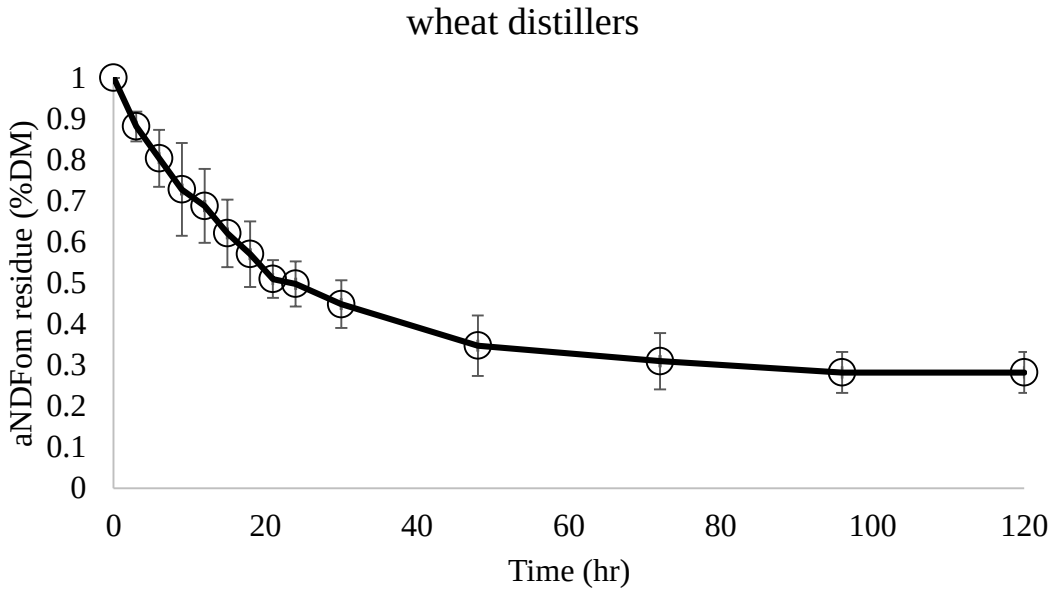
The in-vitro aNDFom decay of soybean meal averaged on 2 samples and 3 batches



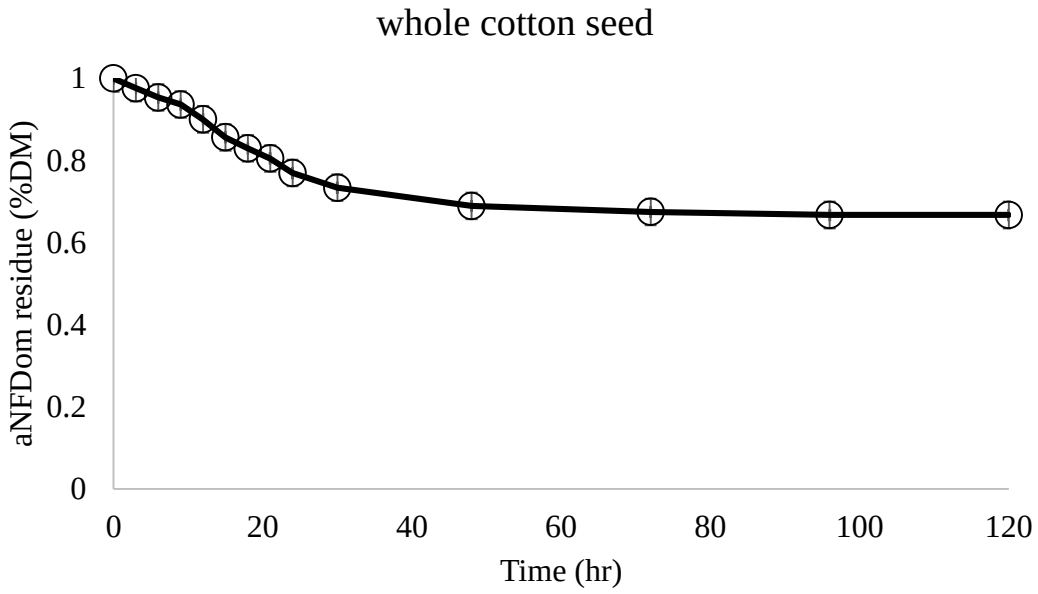
The in-vitro aNDFom decay of soyplus averaged on 2 samples and 3 batches



The in-vitro aNDFom decay of sunflower hulls averaged on 2 samples and 3 batches



The in-vitro aNDFom decay of wheat distillers averaged on 2 samples and 3 batches



The in-vitro aNDFom decay of whole cottonseed averaged on 2 samples and 3 batches