Relationship of NDF Digestibility to Animal Performance

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Summary

In order to optimize the utilization of forages, an accurate laboratory measure of fiber digestibility is essential. The measure should mimic in vivo digestion and should be consistent across forage types. A new in vitro lab assay has been developed that predicts total tract NDF digestion **(TTNDFD)** in ruminants. The test is based on a patented and licensed in vitro assay and model of fiber digestion. The in vitro TTNDFD assay is available through commercial labs and has been calibrated to NIR analysis. The TTNDFD model predicts fiber digestion of alfalfa, corn silage, and grass forages in cattle and has been validated against directly measured NDF digestibility in lactating dairy cattle.

Introduction

The digestibility of NDF is more variable than the digestibility of any other feed component and can profoundly affect intake and milk production. In high producing dairy cows, the variation in total tract fiber digestion can account for enough energy to support as much as 8 to 10 lb of potential milk yield. Fiber digestion is affected both by characteristics of the plant material and by the animal consuming the fiber. To accurately predict how fiber will be utilized, laboratory measures that predict the rate of fiber digestion and the proportion of total fiber that is potentially digestible are needed. The rate and potential extent of NDF digestion are heavily influenced by the genetics and growing environment of the plant. Fiber digestion is also affected by the rate of passage of the potentially digestible fiber through the animal's rumen and hindgut, and therefore, prediction of fiber utilization must also account for animal factors.

Predicting Fiber Digestion with Laboratory Tests and Modeling

There are at least 4 critical factors that affect fiber digestion and performance in ruminants:

1. *The proportion of feed fiber that is potentially digestible.* Forage NDF consists of 2 components, potentially digestible **(pdNDF)** and indigestible NDF **(iNDF)**. The proportion of NDF in the pdNDF fraction varies due to feed type and the growing environment. On average, the pdNDF fraction of alfalfa is about 60 to 65% of total NDF. The proportion of potentially digestible fiber in corn silage is typically greater than in alfalfa NDF; 75 to 85% of corn silage NDF is potentially digestible. The proportion of NDF that is indigestible is typically estimated from long term incubations of fiber in the rumens of cattle or long term in vitro digestions.

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The NDF residue remaining after 240 h of incubation ($uNDF_{240}$), for example, is often used as an estimate of iNDF. The pdNDF is typically determined by subtracting the uNDF fraction from total NDF. The iNDF proportion can only be cleared from the digestive tract by passage while the pdNDF fraction disappears by passage and microbial digestion. Since fiber is bulky and one of the slowest digesting components of the diet, clearance of fiber from the rumen is an important factor limiting feed intake.

2. *The rate of digestion of potentially digestible fiber* ***(kd)***. The rate of fiber digestion also differs due to forage type and the growing environment. The potentially digestible fiber in alfalfa digests nearly twice as fast (4 to 6%/hour) as the potentially digestible NDF in corn silage (2 to 3%/hour). Even though fiber digestion rates for forages are slow, differences in rate of fiber digestion have a big impact on how much of the potentially digestible fiber will digest. The total-tract NDF digestibility of alfalfa and corn silage are similar, but the process of NDF digestion is quite different. In corn silage, there is a larger fraction of digestible fiber that digests slowly. In alfalfa, there is a smaller proportion of digestible fiber, but the faster rate of digestion of the potentially digestible fraction compensates for the bigger pool of iNDF.

3. *The rate of passage of potentially digestible NDF through the cow* ***(kp)***. Both cow size and feed intake affect the passage rates of pdNDF and iNDF. Passage of fiber is much slower than the passage of forage DM. The passage rates of iNDF and pdNDF are not the same. Passage of the pdNDF fraction is slower than passage of the iNDF fraction (Lund et al., 2007). As intake goes up, the rate of passage of both fractions also increases, and as a result, NDF digestibility declines.

4. *Ruminal and hindgut fiber digestion.* Approximately 90 to 95% of fiber digestion occurs in the rumen (Huhtanen et al., 2010), but digestion beyond the rumen must be accounted for if one is to accurately predict the amount of energy derived from NDF. When both ruminal and hindgut digestion are accounted for, a total-tract NDF digestion (TTNDFD) measurement can be calculated and this digestion coefficient can be directly validated with dairy cattle.

An accurate assessment of fiber digestion requires that the 4 factors be integrated into a single measurement. The rumen fiber digestion process can be described mathematically as:

Proportion of fiber digested = pdNDF x (kd/(kd + kp)) ;

where, pdNDF is the fraction or amount of potentially digestible NDF, kd is the rate of digestion of potentially digestible fiber, and kp is the rate of passage of potentially digestible NDF (Mertens, 1993). One way of accounting for hindgut digestion is to divide the proportion of NDF digested in the rumen by the proportion of total fiber digested in the hindgut.

Challenges with Assessing Forage Quality with $uNDF_{240}$, kd, NDFD30, or NDFD48

Nutritionists currently use many different tests to assess fiber digestibility or to compare forages. Using assays that predict iNDF (such as $uNDF_{240}$), or in vitro digestion of fiber after a fixed time (NDFD30 or NDFD48), as stand-alone measures of forage quality have limitations. Using only the pdNDF (or inversely the $uNDF_{240}$) value or kd value is not an accurate assessment of fiber quality because forages can differ in both pdNDF content and kd. A simple analogy demonstrates this point. Fiber quality is an estimate of the

amount of digestible energy generated from a given quantity of forage NDF and is somewhat analogous to predicting how far you can drive a car before it runs out of gas. You need to know how much gas is in the tank and the fuel efficiency of the car to predict the distance that the car will travel. Forage quality is conceptually similar. The amount of digestible energy from fiber (i.e., how far you can drive a car) depends on the amount of fiber that is digestible (i.e., the amount of gas) and the efficiency of fiber digestion (i.e., the fuel efficiency). Knowing you have 10 gallons of fuel may be somewhat useful, but you can't accurately determine how far you can go unless you also know the fuel efficiency. The driver would also have some bearing on the distance traveled. If the driver has a 'lead foot', the distance traveled will be less than for someone who is a more conservative driver. This is a bit like the effect of rate of passage on fiber digestion. Integrating pdNDF, kd, and kp into a single term is a more comprehensive measure of fiber quality than any of the individual terms that are used to determine fiber utilization.

In vitro NDF digestibility measured after 30 h (NDFD30) or 48 h (NDFD48) is widely used to index forage fiber digestibility. Oba and Allen (1999) reviewed several feeding studies with dairy cattle and concluded that a 1% change in vitro or in situ NDF digestibility (NDFD30 or NDFD48) was correlated with a 0.37 lb increase in voluntary DMI, and 0.55 lb increase in 4% fat corrected milk yield. The change in situ or in vitro fiber digestibility within a study was correlated with intake and milk production, but there was no significant correlation between the absolute measures of fiber digestion and intake or milk yield across studies. For field nutritionists, this suggests that in vitro methods differ enough from lab to lab to make it impractical to compare results between labs.

There is also another challenge with using values like NDFD30 to assess forage quality. The NDF residue remaining after a given time in a flask of rumen fluid is simply *undigested* NDF. That residue consists of truly *indigestible* NDF and the portion of the *potentially digestible* NDF that has not yet been digested. There is no way of knowing or estimating the rate of fiber digestion or the fraction of indigestible NDF from this measurement alone. If we go back to the car and gas analogy, the NDFD30 value is like reading the gas gauge of the car. The gauge may indicate a half tank of fuel, but this doesn't tell you how big the tank is or the fuel efficiency of the car and so you can't accurately determine how far you can drive. The iNDF fractions and rates of fiber degradation can vary considerably within forage type. In forages measured in our lab, the iNDF fractions in alfalfa and grasses vary from less than 5% to over 55% of NDF, while corn silage iNDF values range from less than 10% to over 40% of NDF (unpublished data). Krizsan et al. (2010) reported that iNDF values in a database of 172 feeds ranged from 2.4 to 17.4% of feed DM. In addition, the estimated rates of degradation of pdNDF vary from about 1 to over 10%/hour when measured by using multiple incubation time points and fitting the disappearance of pdNDF to first order kinetics.

In vivo Measurement of Fiber Digestion

Total tract apparent NDF digestibility values for diets fed to dairy cows are readily available and are a valuable tool for field nutritionists. Goeser (2008) summarized total tract NDF digestibility measurements that were reported from 25 corn silage feeding trials (81 treatment comparisons) and in 20 trials in which legumes and grasses (64 treatment comparisons) were the primary forages fed to high producing ruminants. Summary statistics suggest that in vivo NDF digestibility coefficients can vary by 30 to 35% units among legumes, grasses, and

corn silages. The TTNDFD of corn silage based diets, for example, average about 42% of NDF but range from 20 to nearly 60% of NDF. A more recent survey of corn silage based feeding trials (Ferraretto and Shaver, 2012) reported that the treatment means for TTNDFD averaged 44.3 ± 2.5 % in 106 treatment observations from 24 dairy feeding trials that were published in peer-reviewed journals between 2001 and 2011. Diets for high producing dairy cows are typically formulated to contain between 28 and 35% total NDF. For cows that are expected to produce over 90 lb/day, a 30-unit change in TTNDFD is equivalent to the digestible energy needed to support more than 10 lb of milk production.

Measuring the Fiber Digestion Process *in vivo* with the Rumen Evacuation Method

Measuring the process of ruminal and hindgut fiber digestion *in vivo* is laborious and expensive, but it is the 'gold standard' to which other estimates of fiber digestion should be compared. Comprehensive evaluations of *in vivo* fiber digestion are most commonly measured by the 'rumen evacuation' technique (Huhtanen et al., 1997; Ivan et al., 2005; Taylor and Allen, 2005). With this method, the critical dynamic components that contribute to the digestion of fiber are directly measured in rumen-cannulated animals. Rumen pools of digestible and indigestible fiber are measured by total rumen evacuation. Rates of digestion of potentially digestible NDF and rates of passage of pdNDF and indigestible NDF are also measured as well as total tract NDF digestion.

Despite the cost and labor, a large number of rumen evacuation studies have been published from studies done in the US and Northern Europe with dairy cattle. Krizsan et al. (2010) compared ruminal passage rates of iNDF as measured by the rumen evacuation technique to empirical estimates of particulate passage rate in cattle. Their database included 49 studies in which 172 treatment means were measured. From this database, they published predictive equations for passage of iNDF in lactating cow fed diets based on corn silage, grass silage, alfalfa, and pasture-based grass diets. Huhtanen et al., (2010) also published a meta-analysis of the NDF digestion process using the rumen evacuation method. Thirty-two studies and 122 diets were included in this analysis. Most of the published studies are with lactating dairy cattle fed grass, alfalfa, or corn silage based diets. The fiber digestion module of the recently published Nordic Feed Evaluation system (NorFor) is based on fiber kinetic parameters estimated by the rumen evacuation technique (NorFor, 2011).

The rates of pdNDF degradation of diets when measured by the rumen evacuation method typically range from approximately 2 to 6%/hour (Greenfield et al., 2001; Ivan et al., 2005; Taylor and Allen, 2005; and Volker Linton and Allen, 2008). Corn silage based diets typically have slower rates of pdNDF degradation than alfalfa. The NDF in diets based on temperate grasses tends to have a similar proportion of pdNDF as corn silage, but grass fiber degrades faster than corn silage fiber, and slower than alfalfa fiber.

The University of Wisconsin in vitro TTNDFD Assay

University of Wisconsin researchers have recently developed an in vitro lab assay and model for predicting NDF digestion in dairy cattle that can be used by field nutritionists. The outcome is TTNDFD. The TTNDFD value is benchmarked to fiber digestibility values that have been obtained from feeding studies where NDF digestion has been directly measured. Total tract fiber digestibility is reported because

this value can be used not only to predict in vivo fiber utilization but also to predict forage digestible energy **(DE)**, net energy **(NE)**, or total digestible nutrient **(TDN)** values.

The TTNDFD assay accounts for pdNDF, kd, kp, and hindgut digestion of NDF (Figure 1). Measurement of the pdNDF fraction and the kd of pdNDF are based on a modified Goering and Van Soest (1970) in vitro procedure (Goeser and Combs, 2009). The pdNDF fraction is estimated from long term (120 or 240 h) in vitro incubations. Multiple measurements of in vitro NDF digestibility are used to calculate a rate of ruminal pdNDF digestion. The approach accounts for ruminal and post-ruminal fiber digestion and can be adjusted for changes in fiber passage as size or intake of the animal changes. Rates of fiber passage are estimated from regressions that have been derived from in vivo studies (Lund et al., 2007; Krizsan et al., 2010). In this model, the diet TTNDFD can be calculated by summing the amount of digestible fiber provided from each feed. The *in vitro* method has been calibrated to near infrared spectroscopy **(NIR)** so that kd and iNDF fractions in a feed can be predicted quickly and with little additional cost.

Several feeding studies have been conducted with various forages to test the model and to validate that the estimates of digestion and passage that are used in the model are consistent with what is measured in cattle fed diets containing the test forages (Verbeten et al., 2011; Lopes et al., 2013; Lopes et al., 2015a; Lopes et al., 2015b). In addition, our lab group has been monitoring commercial lab derived TTNDFD for corn silages, alfalfa, and grass forages and comparing these values to the digestibility coefficients for the respective forages that have been published in peer-reviewed feeding studies.

Field Observations with TTNDFD

We have been monitoring the TTNDFD values of corn silages, alfalfa, and grasses that have been submitted to a commercial forage-testing lab for routine analysis. The TTNDFD values for corn silage, alfalfa, and grasses are summarized in Table 1. The average values represent over 7000 samples each of corn silage or alfalfa and over 1200 grass forage samples.

The means, standard deviations **(SD)**, and ranges in TTNDFD values coincide with *in vivo* measures of TTNDFD that have been reported in dozens of controlled feeding studies published in peer reviewed journals. For consultants, we recommend that tested forages be compared these mean TTNDFD values. When comparing 2 forages with similar total NDF, a forage that is more than one SD below the mean TTNDFD value would be among the lowest 15% of forages sampled and a 6 to 7 unit difference from the mean TTNDFD value would indicate that their forage fiber would reduce the DE value of the forage by enough to reduce potential milk yield by 2 to 3 lb. A forage which is one SD above the mean TTNDFD value would be higher in fiber digestibility than 85% of the forages tested and would contain enough additional DE to potentially support 2 to 3 lb more milk production. Experiences with this test in the field suggests that diets that incorporate large amounts of low TTNDFD forage support less milk and cows consume less feed DM than expected. Cows fed these types of diets respond well to additions of extra starch, or addition of sources of more highly digestible fiber, such as soy hulls.

Validation with Controlled Feeding Studies

The laboratory prediction of TTNDFD of forages and diets has been validated to fiber digestibility values that have been directly

measured in feeding studies. One study (Lopes et al., 2015a) was designed to compare estimates of ruminal fiber digestion predicted from *in vitro* NDFD analysis of feeds to the ruminal fiber digestion measured in cattle fed the same feeds. The feeding study was conducted with lactating dairy cows fed either low fiber digestibility corn silage or to higher fiber digestibility corn silage as the main source of dietary NDF (Table 2). The fiber characteristics of the low fiber digestibility corn silage (34.4% NDF, pdNDF 58.6% of NDF, and kd 3.2%/h) and the higher fiber digestibility corn silage (38.4% NDF, pdNDF 74.3% of NDF, and kd 3.3%/h) were determined by our *in vitro* TTNDFD method prior to the feeding experiment. The fiber characteristics of the 2 silages and the other feeds used in the diets were then used to predict TTNDFD digestibility of the treatment rations. The predictions for each diet were then compared to the observed measures of fiber digestion in daiary cows fed the same feeds. The *in vitro* method predicted that the higher fiber digestibility corn silage was higher in TTNDFD than the low fiber digestibility corn silage because it contained a larger proportion of potentially digestible NDF. Rates of pdNDF digestion and passage and the measured pool of pdNDF in the rumens of cows fed the experimental diets were directly measured in cows and compared to the fiber digestion parameters from the TTNDFD assay and model. It is important to note that the fiber digestion parameters measured directly in the cows are independent of the *in vitro* measurements. Results of the study indicate that the *in vitro* TTNDFD were similar to the directly measured *in vivo* total tract NDF digestibility values and provide evidence that supports the concept that *in vivo* fiber digestion can be predicted from *in vitro* fiber kinetics.

The objective of another *in vivo* experiment (Lopes et al., 2013) was to compare estimates of total tract fiber digestion as predicted by the *in vitro* TTNDFD model to in vivo measurements in lactating dairy cows. Cows were fed diets that varied in proportions of corn silage and alfalfa. The *in vitro* fiber digestion parameters for corn silage (NDF = 34.4%, pdNDF kd = 3.2%/h, and pdNDF = 58.6% of NDF) and alfalfa silage (NDF = 34.7%, pdNDF kd = 6.1%/h, and pdNDF = 51.3% of NDF) indicate that fiber in the corn silage contains more pdNDF than alfalfa, but the rate of digestion of alfalfa fiber is nearly twice as fast as corn silage fiber. The feeding experiment measured how cows utilize forages that differ in pdNDF and kd (Table 4). The diets contained approximately 55% forage and the dietary NDF concentration was similar across the 4 treatments.

Feed intake was lower when cows consumed the diets that contained 100% of forage as alfalfa silage than it was when cows were fed diets containing corn silage. The observed (*in vivo*) total tract NDF digestion values were calculated from feed and fecal samples. Cows consuming the diet with alfalfa as the only forage had higher NDF digestibility than cows on the diets that contained corn silage. Milk and FCM yields did not differ due to treatment. The NDF digestibility coefficients predicted by the *in vitro* TTNDFD method were similar to the *in vivo* values. The fiber digestibility coefficients suggest that the faster rate of fiber digestion of alfalfa fiber compensates for content of pdNDF, but as higher proportions of alfalfa forage are fed, the amount of indigestible fiber in the rumen increases and rumen fill becomes a more predominant factor limiting feed intake.

These feeding experiments demonstrate that the *in vitro* TTNDFD analysis can provide important insights into fiber utilization by dairy cattle. The rates of fiber degradation determined from the *in vitro* NDFD assays are consistent with values measured in *in vivo* feeding

studies. The kd, kp, and pdNDF parameters predicted by the TTNDFD model appear to be consistent with in vivo measures, and the total tract digestion of NDF as predicted by the TTNDFD model is consistent with observed *in vivo* digestion. A third study (Lopes et al., 2015b) compared 21 diets from seven feeding experiments and showed that TTNDFD of total mixed rations analyzed by the *in vitro* TTNDFD method were highly correlated to the directly measured *in vivo* total tract NDF digestibilities of the same diets in lactating dairy cows.

Conclusions: How to Use the TTNDFD Test

The key to getting the most out of forages is understanding how energy values are affected by NDF and NDF digestibility. This test is intended to be an additional tool to provide a clearer understanding of how forage fiber is utilized in dairy cattle. It is not intended to be the only tool to use to evaluate forage quality or fiber utilization by dairy cattle. Table 5 summarizes important limitations to this assay. In top quality forages, NDF accounts for 35 to 45% of the total DM, and this fiber is the source of 30 to 40% of the digestible energy. A 30% NDF diet with a TTNDFD of 33% would support 7 to 10 lb less milk than a 30% NDF diet with a TTNDFD of 45%, assuming no reduction in feed intake. The average TTNDFD value for most diets formulated with alfalfa and corn silages will be about 42 to 44%, and this should be a target for ration formulations.

The TTNDFD value can also be used as a stand-alone value to index forages. A consultant could compare values from their forage test to the values in Table 1. For example, note in the Table 1 that an average alfalfa will have a TTNDFD value of 43%. An alfalfa with a TTNDFD value one SD below average (less than 36%), would be among the bottom 15% of the alfalfa samples tested. A sample with low TTNDFD likely will not be utilized as well as 'typical' alfalfa containing similar amounts of total NDF. Our validation studies with corn silages, alfalfa, and temperate grasses indicate that TTNDFD values of feeds can be used in ration formulation and evaluation to 'fine-tune' the amount and overall digestibility of NDF in rations for high producing dairy cattle. The ability to predict fiber digestibility and incorporate this information into rations could improve our ability to optimize forage utilization and milk production.

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Table 1. Typical total tract NDF digestibility (TTNDFD) values of corn silage, alfalfa, or grass.[1]

	TTNDFD, % of NDF	SD[2]	Range
Corn Silage	42	± 6	20-60
Alfalfa	43	± 7	25-80
Grass	47	± 8	6-80

[1]Samples submitted to Rock River Laboratories, Watertown, WI.
[2]SD = Standard deviation.

Table 2. Effects of source of corn silage on total tract NDF digestion (Lopes et al., 2015a).[1]

	LFDCS	HFDCS	SE
Feed, % of TMR DM			
Low fiber digestibility corn silage	47	0	
High fiber digestibility corn silage	0	47	
Alfalfa silage	17	13	
Concentrate mix	36	40	
Diet composition			
NDF, % of DM	27.5	28.3	
pdNDF, % of NDF	68.9	75.9	
Results			
DMI, lb/day	55	56	1.3
4% FCM, lb/day	76	77	1
Observed TTNDFD, in vivo	47	43	2.5
Predicted TTNDFD, in vitro	43	50	0.9

[1]LFDCS = Low fiber digestibility corn silage, HFDCS = high fiber digestibility corn silage, pdNDF = potentially digestible NDF, and TTNDFD = total tract NDF digestibility.

Table 3. Comparison of rumen and total tract NDF digestion of diets predicted from TTNDFD model and observed in vivo (Lopes et al. 2015a).[1]

Item	Predicted *in vitro*	Observed *in vivo*	SEM	*P* value
Input				
pdNDF kd, %/h	4.1	4.3	0.5	0.72
pdNDF kp, %/h	2.7	2.8	0.3	0.56
Output				
NDF digested in rumen, lb	6.01	5.79	0.48	0.64
NDF digested in hindgut, lb	0.79	1.41	0.42	0.05
NDF digested in total tract, lb	6.80	7.19	0.48	0.42
Total tract NDF digestibility, % of NDF	46.4	49.5	0.07	0.13

[1]pdNDF = Potentially digestible NDF, Kd = rate of digestion, and Kp = rate of passage.

Table 4. Effect of changing ratios of corn silage to alfalfa on intake, production, and fiber digestion in dairy cows (Lopes et al., 2013).[1]

Corn silage (CS):alfalfa (AS) ratio	100CS 0AS	67CS 33AS	33CS 67AS	0CS 100AS	
Corn silage, % of TMR	56	37	18	0	
Alfalfa silage, % of TMR	0	19	37	55	
Concentrate mix, % of TMR	44	44	45	45	
Diet composition					
NDF, % of DM	24.9	25.5	24.6	25.5	
iNDF, % of NDF	31.1	31.6	31.8	32.3	
Results					SE
DMI, lb/day	55.4[ab]	55.7[a]	53.5[b]	48.2[c]	1.8
4% FCM, lb/day	79.6	77.9	77.8	78.3	2.0
Observed TTNDFD, in vivo	38.3[a]	40.9[ab]	39.4[ab]	43.8[c]	1.9
Predicted TTNDFD, in vitro	38.0	41.0	41.0	45.0	2.1

[1]iNDF = Indigestible NDF and TTNDFD = total tract NDF digestibility.

Table 5. Guidelines for using total tract NDF digestibility (TTNDFD).

1. The TTNDFD assay is intended to evaluate the digestibility of NDF of feeds and rations in animals fed an otherwise balanced diet. Inadequacies of other nutrients (protein, amino acids, and minerals) or excesses of dietary components other than fiber (i.e., mycotoxins) are not accounted for in this assay.

2. The TTNDFD can be used to compare fiber utilization across forage or fiber sources. For example, fiber digestibility of corn silage can be compared to fiber digestibility of alfalfa, grass, or co-product feeds.

3. TNDFD does not account for differences in physical form (effective fiber) of forages.

4. TTNDFD estimates total tract digestibility of fiber for a dairy cow consuming about 54 lb/day of DM.

5. In vitro NDFD values (NDFD24, NDFD30, or NDFD48) should not be used as a single indicator to compare fiber digestibility of forages. These values do not factor in indigestible fiber or NDF concentration of forages. Single time NDFD values are poorly correlated to total tract fiber digestibility.

6. Total NDF and TTNDFD must be considered when comparing forages for quality.

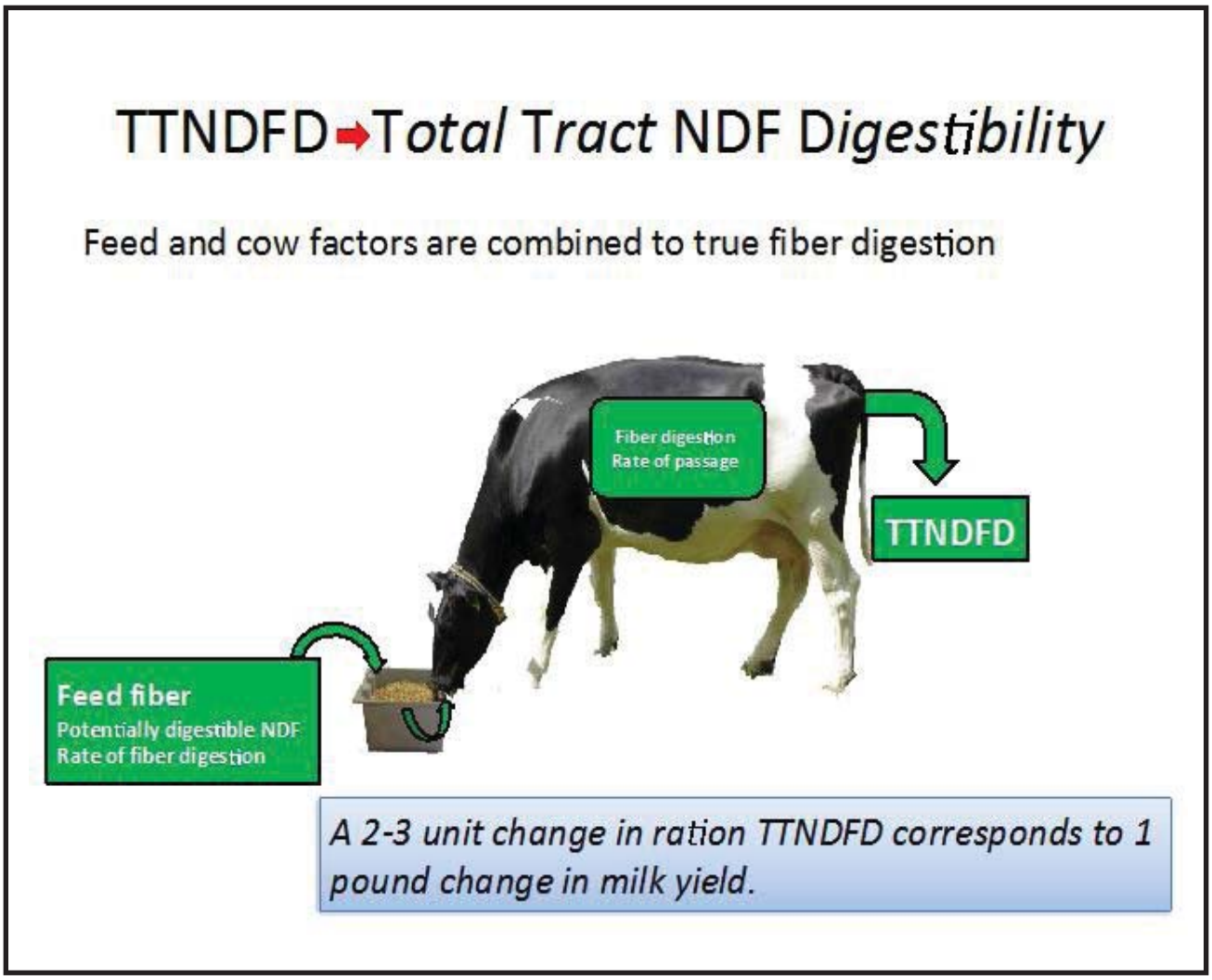

Figure 1. The TTNDFD model.