New Developments in Analytical Evaluation of Total Mixed Rations

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INTRODUCTION

There are many new advancements in the analytical evaluation of forages and total mixed rations (**TMRs**). Despite new advancements, the world of "forage testing", as we commonly refer to it, is still difficult to understand. A plethora of concerns and myths exist. This paper will attempt to address new advancements in total mixed ration evaluation as well as address concerns and myths with analytical procedures and utility of forage evaluation systems.

SUMMATIVE ENERGY EQUATIONS

The amount of energy in a ruminant diet is arguably the single most important factor in predicting animal performance. It is the author's impression that nutrition consultants and dairy producers have lost confidence in the ability of feed testing systems to predict energy content of a forage or ration. In the past this perspective was somewhat valid. Empirical equations (Rohweder et al., 1978) were used for many years to predict forage energy content from a single analyte such as acid detergent fiber (ADF). Empirical equations to predict forage energy content by and large were accurate but imprecise. The aforementioned statement simply means that when examining a large data base of forage energy contents predicted by an empirical equation, the empirical equation accurately predicts the average of the data base but cannot precisely predict the energy content of any single forage in the data base. To be of real value, feed testing systems should be able to precisely predict the energy content of any single forage, feed, or diet.

Weiss (1996) proposed using a summative approach to predict energy content of feeds. The concept of a summative approach is simple: measure the principal components in the feed that contribute energy, give each component a digestion coefficient, multiply each component by its respective digestion coefficient, and add the products together. The greater utility of a summative energy system is that it can be used on any forage, grain, commodity, or even total mixed rations. The major drawback of summative equations is extensive laboratory measurements are needed. Seven principal nutrients need to be accurately and precisely measured in the laboratory: crude protein (CP), neutral detergent fiber (NDF), fat, ash, acid detergent fiber crude protein (ADF CP), and neutral detergent fiber crude protein (NDF CP) to facilitate the final determination of NFC. The digestion coefficients assigned to CP, fat, and NFC are well defined by

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research (Weiss, 1993); however, the digestion coefficient for NDF (NDFD, % of NDF) is not well defined by research and thus requires measurement in the laboratory.

A complete discussion of summative energy equations is available (Weiss, 1996; NRC, 2001) and is beyond the scope of this paper. An example of a summative energy equation adopted by the NRC (2001) to predict the energy content of a legume grass silage is presented in Table 1. The reader should be aware the summative equation concept presented in Table 1 has been modified for corn silage (Schwab and Shaver, 2001).

MEASURING NDF DIGESTIBILITY

Accurately and precisely predicting the NDFD content of the feed or forage NDF is extremely important in generating a quantitative summative forage energy prediction. Unfortunately NDFD is one of the more difficult assays to conduct in the laboratory. Most laboratories cannot conduct the assay because an in vitro NDFD laboratory procedure requires rumen fluid from a live cannulated cow.

Forage NDFD can be measured in one of two ways. First, forages can be placed in small dacron bags and inserted into the rumen of a cow via a ruminal cannula. The amount of NDF prior to ruminal incubation is compared to the amount of NDF remaining after ruminal incubation and NDFD is calculated. This is called an in situ method. The in situ method is a very viable method to estimate NDFD of forage NDF and is often used in research and other forage evaluation programs. Because of the lack of a large uniform database, the 2001 NRC, however, does not recommend the in situ method as its basis for NDFD of feeds and forages.

The 2001 NRC uses lignin as a base to predict potential NDF digestibility or advises the use of a 48 h in vitro NDFD as the basis for direct determination of the NDF digestibility coefficient. Again, advised use of a 48 h in vitro NDFD was not made based on analytical superiority over the in situ system, rather the in vitro NDF digestibility data base was larger and more uniform, making interpretation easier. An in vitro NDFD determination (Goering and Van Soest, 1970) is conducted as follows: 1) feed is weighed into a glass flask, 2) buffers, macro- and micro-minerals are added along with rumen fluid extracted from a cow fit with a ruminal cannula, 3) the forage, buffers, and rumen fluid are incubated in a water bath in an anaerobic environment (carbon dioxide) at a cow's body temperature (102° F) for 48 hours, 4) the flask containing the forage, buffers, and rumen fluid is removed from water bath and the remaining solution is refluxed in NDF solution for 1 hour, 5) after refluxing in NDF solution for 1 hour the remaining solution is filtered and the NDF that resisted digestion by rumen bacteria is retained on the filter, and 6) digestible NDF is calculated by difference.

Few changes have been made to the in vitro NDFD assay over the years, but some researchers and laboratories have reduced the incubation times from 48 hr to 30 or 24 hr, opting that shorter incubation times better describe the digestion potential of NDF in high producing lactating dairy cows. Reducing the incubation time of the in vitro NDFD assay

to 30 or 24 hr is logical because feed is not retained in the rumen of a high producing dairy cow for 48 hr. In the larger sense, however, this issue is somewhat clouded because changing the incubation time of the assay reduces the amount of NDF digested; therefore, NDF digestibility values obtained from 30 or 24 hr digestions cannot easily be compared to available NDF digestibility data bases (NRC, 2001). The recommendation of a 48 hr in vitro NDFD by the NRC (2001) is also designed to facilitate calculating TDN content of forages at maintenance intakes (which is TDN). The most important issue with NDF digestibility at this time is for laboratories to report forage NDF digestibilities that have a common scale and reference. Because the NRC, 2001 advises the use of a 48 hr in vitro NDF digestibility procedure to calculate TDN contents of forages at maintenance intakes, it is most logical to identify with the 48 h NDFD reference and scale.

Listed in Table 2 are 30 and 48 h NDFD (% of NDF) of many common feeds and forages. The NDFD values from 30 h in vitro evaluation systems typically yield lower NDFD values. With caution, these values can be substituted into summative energy equations (NRC, 2001) to calculate TDN at maintenance, but the user should be aware that low TDN predictions can occur if 30 h NDFD procedures are compromised. Substituting wet chemistry in vitro 48 h NDFD values into summative energy equations can increase the accuracy and precision of forage energy estimates if done correctly, but may slightly over-estimate the TDN content of the feed.

The NDFD content of a forage can be predicted using NIRS, but generally there is some loss of precision. Combs (1998) has used NIRS to predict in vitro 48 h NDFD contents of legume grass forages with success. The NIRS NDFD equations developed by Combs (1998) are commercially available and are currently being used in some commercial forage testing laboratories. Development of accurate and precise NIRS equations for the NDFD content of corn silage has proven more problematic because of the narrow range of NDFD in corn silage and the heterogeneous nature of corn silage (Lundberg, et al., 2003).

Ultimately, prediction of NDFD in forages by NIRS would be preferred because laboratories using NIRS prediction systems can be easily standardized. It is likely that large data bases of forage NDFD contents will be required to facilitate accurate and precise measures of forage NDFD by NIRS. Such projects are in progress and therefore it is likely that prediction of NFDF in forages using NIRS will improve in the future.

SUMMATIVE EQUATIONS: PRACTICAL PROBLEMS

The utility of summative equations to predict feed and forage energy content is well documented, but there are practical problems associated with the interface between laboratories and nutrition consultants in their use. First, summative equations require numerous and often difficult-to-conduct laboratory assays. For example, a simple lactation diet containing alfalfa silage, corn silage, grass hay, high moisture corn, soybean meal, and distillers grains would require 42 different wet chemistry assays to fully utilize a summative energy prediction for the diet. The cost of wet chemistry laboratory procedures could easily exceed $200.00 for the ration. Second, determination of 48 h IV

NDFD which is required of the summative systems requires a minimum of 3 to 5 days to properly conduct. Thus utilizing the full benefit of summative systems is relatively slow and expensive as compared to rapid NIRS procedures to test feeds and forages.

One method to fully exploit the utility of summative systems to predict feed or diet energy content is to conduct a summative laboratory analysis on the total diet. The advantages include the ability to do a re-check of the formulated diet on one sample for one nominal fee.

EVALUATION OF TOTAL MIXED RATIONS

In the author's opinion, summative energy prediction systems have great utility in the evaluation of total mixed rations. The author realizes that sampling and laboratory analysis of TMRs is unconventional. One of the greatest concerns with laboratory evaluation of TMRs is sampling error. Recently, Hutjens (2002) warned against TMR sampling error and suggested evaluating TMRs via wet chemistry for DM, CP, and ADF to determine accuracy of mixing. The recommendation of Hutjens (2002) is logically conservative, but overlooks the potential to use a precision summative technology to estimate of TMR energy content as compared to relying on commonly empirical generated ration energy contents. Evaluation of energy contents of TMRs is relatively simple with CP, NDF, ash, fat, NDF CP and 48 h in vitro NDF digestibility of the TMR evaluated in duplicate via wet chemistry procedures, thus minimizing potential lab error. The energy content of the TMR is then estimated using NRC, 2001 summative models and precision estimates are achieved. Laboratory error, however, must be controlled.

As previously stated, criticism of laboratory evaluation of TMRs is speculation that sampling error is high, although few data are available to substantiate this speculation. To assess TMR sample error, the author extracted random raw data of laboratory analyses conducted on a static research trial TMR over a 7 day period (Hoffman and Esser, 2001). The author selected these data because the diet was static (no feed changes), was sampled by the sample technician, and laboratory analysis was likewise conducted by the same technician. Thus the variation observed in Table 3 is mostly sampling error. While empirical, the standard deviation (**SD**) of nutrients in the TMR is relatively small and appears to be of limited concern (Table 3). The data in Table 3 can be compared to data in Figures 1-7 which contain nutrient profiles of 377 high group lactating cow TMRs from individual dairy herds evaluated using the TMR-Quality Control procedures at the Marshfield Soil and Forage Analysis Laboratory. The TMRs in Figures 1 - 7 were extracted from the TMR data base at the Marshfield Soil and Forage Analysis Laboratory. The reader is reminded that laboratory error was minimized due to the use of duplicate precision wet chemistry laboratory methods. The variation of TMR nutrients in Figures 1 - 7 far exceeds the TMR sampling variation observed in Tables 3; therefore, numerous TMR diets in Figures 1 - 7 are likely incorrectly formulated or fed. In addition, it should be noted that high group lactating cow diets containing a common 27.0 to 28.0% NDF can vary dramatically in dietary energy content. More research is needed on the normal relative sampling errors associated with TMRs. For the first time, however, the dietary energy content of a TMR can be systematically evaluated if proper laboratory

procedures are used. The precision summative TMR evaluations are, however, slow (1 week minimum) and expensive to conduct (≅$50.00). Dairy producers and nutrition consultants should not confuse precision summative TMR analysis systems with other common TMR testing systems. Evaluation of TMRs using NIRS or with TMR energy estimates made using empirical equations or book values are of limited value.

Finally, laboratory evaluation of TMRs for energy density using precision summative technology appears to be an excellent tool to re-check energy estimates developed from ration balancing techniques.

OTHER NEW DEVELOPMENTS IN FORAGE EVALUATION

Bypass Protein

Recent work from our laboratory (Dorshorst et al., 2000; Hoffman et al., 1999a, b, c) has demonstrated that NIRS can predict (R^2 = .87) bypass protein (3X maintenance) content of legume grass silages (Hoffman et al., 1999c) and legume grass hays (Dorshorst et al., 2000). The NIRS system to predict bypass protein of these forages was developed using a calibrated cow in situ technique and was then converted to NIRS techniques. The NIRS evaluation system is commercially available, but has limited use in field applications because the sample cannot be microwave dried because of protein matrix alteration due to overheating. Very good bypass protein numbers can be generated for legume/grass hays or silages if samples are dried at 55° C, then evaluated using bypass protein using NIR systems.

pH

Some laboratories now routinely offer the prediction of pH in ensiled forages using NIRS. Reeves et al. (1989) observed that NIRS could predict silage pH, but prediction was somewhat imprecise. The actual utility of silage pH is somewhat vague, but could be used as a screening tool to conduct further silage fermentation analyses.

Silage Fermentation Analysis

Similar to silage pH, silages can be evaluated for fermentation profiles which generally include pH, acetic, lactic, butyric, propionic (acids) and ammonia (NH_3). Silage fermentation analyses are generally done using high pressure or gas chromatography although some labs use NIRS on undried, unground samples which has been demonstrated to be feasible (Reeves et al., 1989). Silage fermentation analysis can be used to trouble shoot silage fermentation problems, assess potential dry matter intake problems, or evaluate silage inoculant performance.

CONCLUSIONS

There have been a number of new advancements in analytical evaluation of forages. Nutrition consultants and dairy producers need to be aware that these analytical advancements often exceed the program capabilities of commercial forage testing laboratories. To take advantage of these new analytical advancements, nutrition consultants and dairy producers should work closely with their laboratory to eliminate false expectations. Listed below are some general guidelines and concepts to keep in mind when working with any forage testing laboratory.

1) The old NIR vs wet chemistry argument is a moot point in modern forage evaluation. NIRS is an excellent tool for many nutrients, but not all nutrients.

2) Expect to pay more and wait longer for quantitative (precise) forage energy predictions.

3) Rapid, low cost forage evaluation systems are routine screening tools. It is difficult for any laboratory to provide accuracy and precision of every nutrient under these conditions.

4) Explain to your laboratory exactly what you are looking for and design a forage evaluation system to meet your needs. Be willing to pay more and wait bnger for custom or high precision forage evaluation systems.

5) Do not underestimate the importance of providing a good forage or TMR sample to your laboratory for analysis.

6) Because a laboratory can run an assay does not guarantee the results of the assay have a utility.

7) Evaluate TMRs using precision summative technology.

8) Be aware of forage and TMR testing gimmicks. Ask for research data to support a particular forage testing system.

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Table 1. Example of summative calculations made to estimate the energy content of legume grass silage.

Item	*Abreviation*	*Unit*	*Value*	Formula		*TDN Units*
Protein Fractions						
Crude Protein	***CP***	***% of DM***	**21.9**	**CP * .93**	**Ecp=**	**20.37**
Neutral Detergent Fiber Crude Protein	***NDFCP***	***% of DM***	**4.2**			
Fiber Fractions						
Neutral Detergent Fiber	***aNDF***	***% of DM***	**40.0**	**((NDF)*(NDFD/100))*.75**	**Endf=**	**14.40**
Neutral Detergent Fiber Digestibility, 48 h	***NDFD***	***% of NDF***	**48.0**			
Carbohydrates and Fats						
Non Fiber Carbohydrate [-]	***NFC***	***% of DM***	**29.1**	**(NFC*.98)**	**Enfc=**	**28.50**
Fat		***% of DM***	**3.2**	**((.97*(Fat-1))*2.25**	**Efat=**	**4.80**
Macro Minerals						
Ash		**%of DM**	**10.0**			
Energy Calculations:2001 NRC						
Total Digestible Nutrients,1X	***TDN***	***% of DM***		**Ecp+Endf+Enfc+Efat-7**		**61.06**
Net Energy , Lactation, 3X	***Nel***	***Mcals/lb***		**((.0245*TDN)-.012)/2.2046))**		**0.62**

[-] **NFC = 100-(CP+NDF+Ash +Fat-NDFCP)**

****** Note. Not for use with corn silage.**

Table 2. Typical NDF digestibility values for forages, total mixed rations and byproduct feeds.

Feed	In Vitro NDF Digestibility, % of NDF[1,2]					
	High	Medium	Low	High	Medium	Low
	48 h NDF Digestibility			30 h NDF Digestibility		
Alfalfa Hay	55.4	49.8	44.2	53.5	46.2	38.9
Alfalfa Silage	58.2	53.1	48.0	55.9	51.3	46.7
Grass Hay	64.8	54.2	43.6	na	na	na
Grass Silage	62.9	53.7	44.5	na	na	na
Legume/Grass Hay	59.4	48.0	36.6	na	na	na
Legume/Grass Silage	59.5	54.3	49.1	na	na	na
Ryegrass Silage	na	63.1	na	na	55.6	na
Red Clover Silage	50.3	47.1	43.9	na	na	na
Sorghum/Sudan Silage	na	57.2	na	na	49.2	na
Straw	na	32.5	na	30.5	26.6	22.7
Corn Silage	63.8	58.9	54.0	52.3	48.0	43.7
Brown Mid-Rib Corn Silage	72.8	68.6	64.4	na	na	na
Small Grain Silage	66.8	56.4	46.0	na	47.9	na
Total Mixed Rations, High Group	63.0	57.1	51.2	na	na	na
Total Mixed Rations, Prefresh	63.5	54.6	45.7	na	na	na
Total Mixed Rations, Postfresh	61.4	55.9	50.4	na	na	na
Total Mixed Rations, Dry Cows	64.9	59.4	53.9	na	na	na
Total Mixed Rations, Heifer Diets	61.5	54.4	47.3	na	na	na
Corn Gluten Feed	na	na	na	na	79.8	na
Distillers Dried Grains	na	na	na	81.2	76.2	71.2
Brewers Grains	na	na	na	na	49.9	na
Wheat Midds	na	na	na	53.0	51.2	49.4
Beet Pulp	na	na	na	89.6	83.6	77.6
Citrus Pulp	na	na	na	na	85.0	na
Soy Hull	na	92.0	na	na	91.6	na
Whole Cottonseed	na	na	na	61.9	53.3	44.7
Soybean Meal	na	na	na	90.8	87.3	83.8
Barley	na	na	na	na	52.0	na
Corn	na	85.0	na	na	na	na
Steam Flaked Corn	na	na	na	81.5	73.6	65.7

Adapted from data bases of the Marshfield Soil and Forage Analysis Laboratory and Peter Robinson, University of California.

High NDFD values represent the average plus 1 standard deviation. Low NDFD values represent the average minus one standard deviation. Feeds without high and low values do not contain enough samples to calculate a reliable standard deviation.

Table 3. Sampling induced variation of nutrients in a static TMR sampled over a 7 day period, (unpublished lab data; Hoffman and Esser, 2001).

	TMR Sampling Day							
Item	Mon	Tue	Wed	Thurs	Fri	Sat	Sun	SD
DM, % as fed	55.4	57.6	55.8	53.6	53.6	54.6	55.0	1.42
CP, % DM	17.4	17.5	17.4	17.6	17.9	17.9	17.5	0.24
ADF, % DM	18.5	18.0	17.9	18.6	18.6	18.1	18.2	0.29
NDF, % DM	28.0	27.4	28.1	27.2	27.0	27.3	28.4	0.53
NDFD[1], % NDF	56.0	57.5	58.7	60.2	58.7	58.1	59.8	1.42
Ash, % DM	8.3	8.4	8.5	8.5	8.0	8.0	8.8	0.28
P, % DM	0.50	0.52	0.48	0.51	0.53	0.54	0.53	0.02
Ca, % DM	0.77	0.83	0.78	0.84	0.79	0.82	0.79	0.03

[1] NDFD = NDF digestibility expressed as a percent of NDF

Figure 1. Distribution of CP Content in High Group TMRs

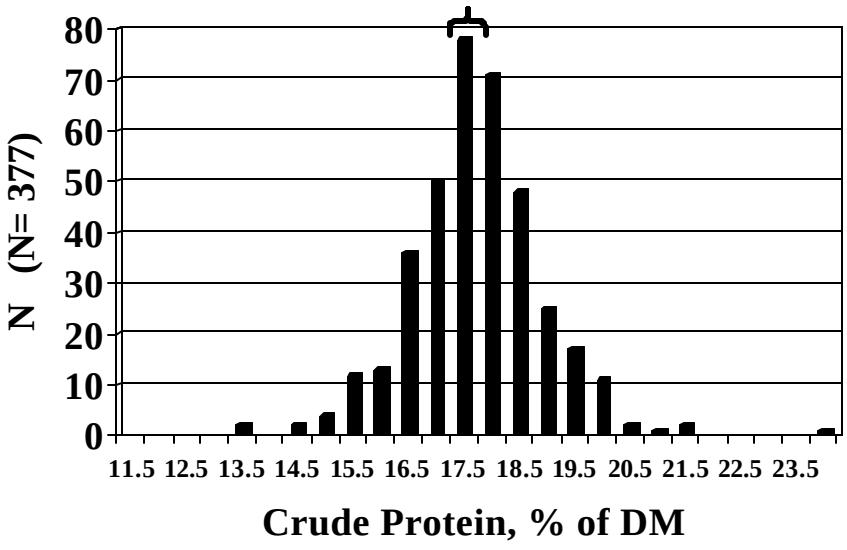

Figure2. Distribution of NDF Content in High Group TMRs

Sampling Error = 2.0

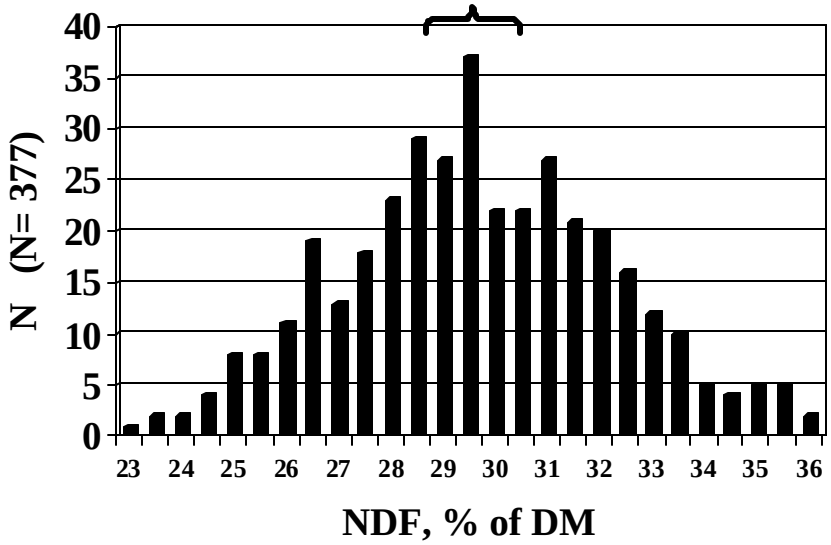

Figure 3. Distribution of NDFD Content in High Group TMRs

Sampling Error = 4.1

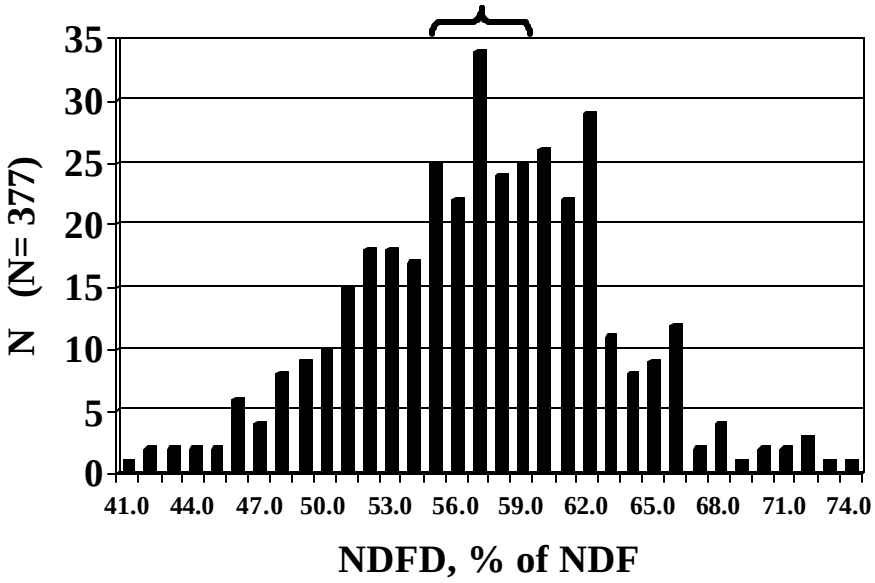

Figure 4. Distribution of Ash Content in High Group TMRs

Sampling Error = 0.4

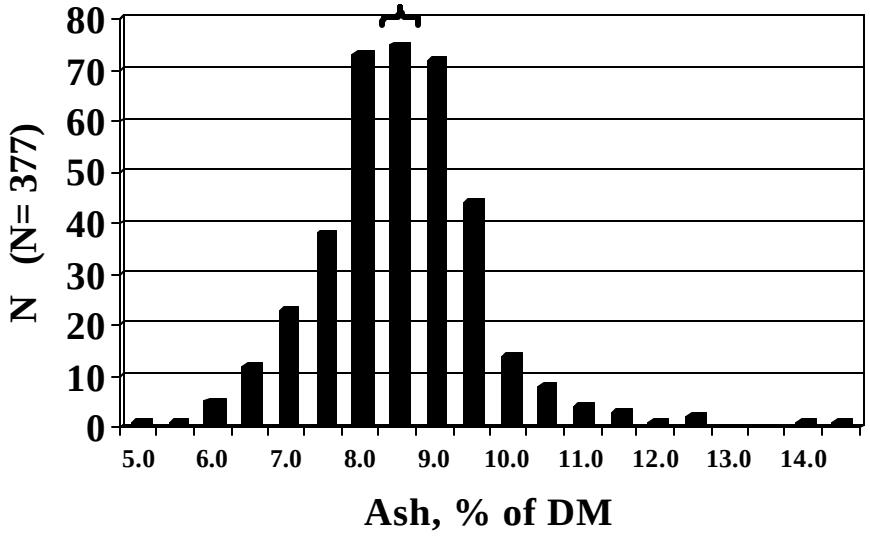

Figure 5. Distribution of NFC Content in High Group TMRs

Sampling Error = 2.9

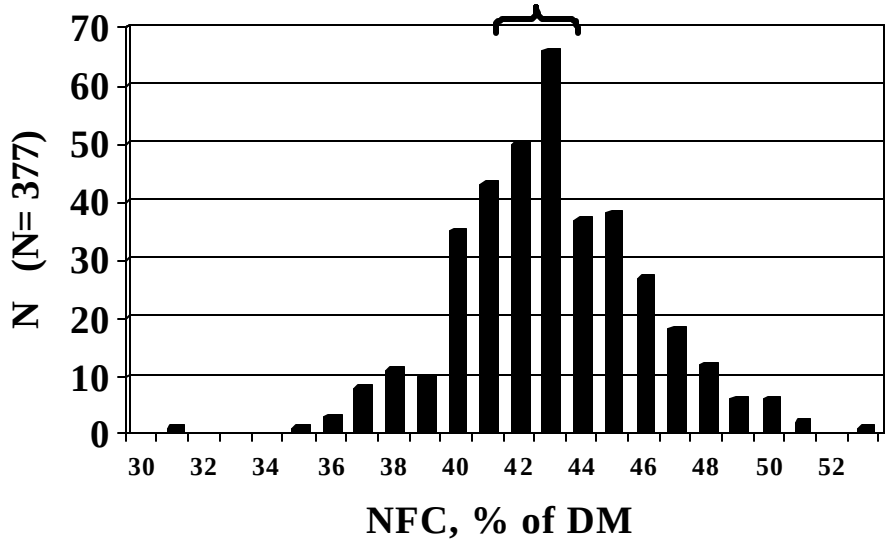

Figure 6. Distribution of Fat Content in High Group TMRs

Sampling Error = 1.0

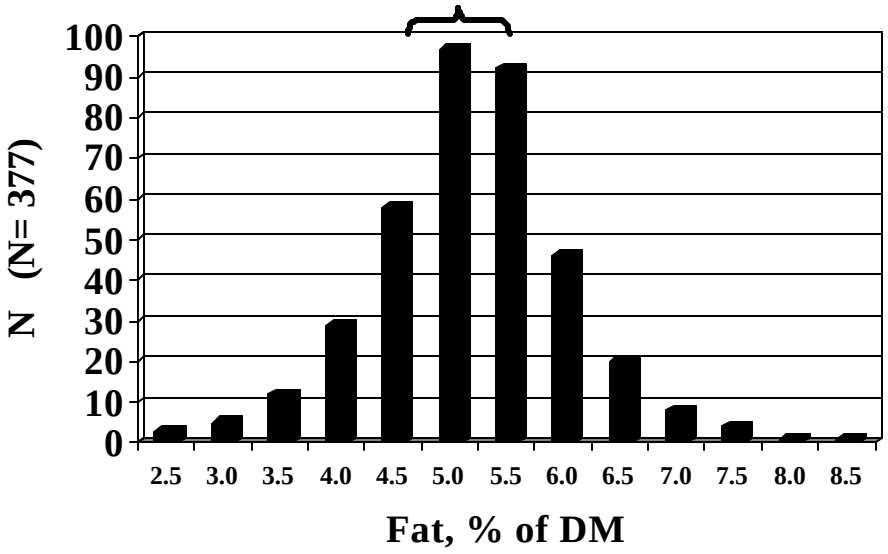

Figure 7. Distribution of NE_L Content in High Group TMRs

Sampling Error = .026