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# Predicting Forage Indigestible NDF from Lignin Concentration

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**ABSTRACT:** We used chemical composition and in vitro digestibility data from temperate and tropical forages to develop relationships between indices of lignification and forage indigestible NDF. Neutral detergent fiber indigestibility increased nonlinearly as the lignin concentration of the NDF increased. Differences in estimated indigestible NDF using equations developed for a specific forage class (C<sub>3</sub> and C<sub>4</sub> grasses and legumes) were small and are probably not biologically significant when compared to those estimated from a common equation. Selected equations were compared with the Cornell Net Carbohydrate and Protein System (CNCPS) for the prediction of ADG. The linear equation (2.4 times NDF lignin content) used by the CNCPS and the Beef NRC had some of the largest errors due to mean bias. A log-log model [ $4.37 \times (\text{lignin/NDF})^{.84}$ ] provided the best combination of low total prediction error, low mean

bias, and minimal error due to regression bias when permanganate lignin was used. A similar equation based on sulfuric acid lignin [ $6.17 \times (\text{lignin/NDF})^{.77}$ ] also met the above criteria. These equations then were evaluated with the CNCPS model against animal growth data from diets ranging in forage quality. Regardless of the equation used for predicting unavailable fiber, the CNCPS underpredicted daily gain, with mean biases ranging from  $-.10$  to  $-.22$  kg/d. Regression bias ranged from  $.13$  to  $.14$  kg/d and the coefficients differed from unity ( $P = .0001$ ). The new equations gave numerically lower energy allowable ADG by steers compared to the linear equation currently used by the CNCPS model. The estimates were lower due to a higher predicted indigestible NDF, which resulted in a lower estimated forage energy value.

Key Words: Lignin, Forage, Models

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## Introduction

Many factors such as temperature, light intensity, water availability, latitude, maturity, and harvest and storage methods affect forage composition, particularly lignin content (Van Soest, 1994, 1996). Lignin is generally accepted as the primary entity responsible for limiting the digestion of forages (Besle et al., 1994; Van Soest, 1994). Various equations have been proposed to estimate the indigestible NDF based on lignin and NDF content (Mertens, 1973; Chandler, 1980; Conrad et al., 1984; Weiss et al., 1992; Van Soest, unpublished data).

An analysis of the data of Mertens (1973) by Van Soest (1993, 1994) indicated that the limit imposed on extent of NDF digestion by lignin as measured by acid detergent lignin was similar, irrespective of lignin type or plant species. This contradicts the views of some authors (Buxton and Russell, 1988) but supports the surface-limiting concept introduced by Conrad et al. (1984).

Many advanced nutrition models (i.e., CNCPS, Sniffen et al., 1992; Weiss et al., 1992; NRC, 1996) calculate forage energy values from the digestible carbohydrate pools. Lignin is indigestible and acts to reduce the proportion of potentially digestible fiber fraction in forages. The indigestible NDF (**INDF**) does not contribute energy to the animal and therefore should not be included in the estimation of forage energy content. Thus, the primary objective of this study was to derive an equation for the prediction of INDF based on forage chemical composition data for use in models such as the CNCPS and NRC.

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Table 1. Collaborators and description of the samples used in the development and testing of equations to predict the indigestible NDF fraction

| Contact                               | Location  | Genus and species                             | Plant material       | Comments   |
|---------------------------------------|---|---|----------------------|--|
| — Development data set —              |   |   |                      |  |
| Dante Lanna and Max Bose              | Universidade de São Paulo, Piracicaba, SP, Brazil         | <i>Pennisetum purpureum</i>                   | Leaf and stem        | 45- to 105-d regrowth material collected at 10-d intervals                                   |
| Carlos Lascano                        | Centro Internacional Agricultura Tropical, Cali, Colombia | <i>Andropogon gayanus</i>                     | Leaf and stem        | Samples were collected over a period of 2 yr   |
|                                       |   | <i>Arachis pintoi</i>                         | Leaf and stem        |  |
|                                       |   | <i>Brachiaria decumbens</i>                   | Leaf and stem        |  |
|                                       |   | <i>Brachiaria dictyoneura</i>                 | Leaf and stem        |  |
|                                       |   | <i>Brachiaria humidicola</i>                  | Leaf and stem        |  |
|                                       |   | <i>Centrosema acutifolium</i>                 | Leaf and stem        |  |
|                                       |   | <i>Calliandra calothyrsus</i>                 | Leaf fraction        |  |
|                                       |   | <i>Dioclea guianensis</i>                     | Leaf fraction        |  |
|                                       |   | <i>Desmodium ovalifolium</i>                  | Leaf and stem        |  |
|                                       |   | <i>Flemingia macrophylla</i>                  | Leaf fraction        |  |
|                                       |   | <i>Panicum maximum</i>                        | Leaf and stem        |  |
|                                       |   | <i>Pueraria phaseoloides</i>                  | Leaf and stem        |  |
|                                       |   | <i>Tadehagi rodgeri</i>                       | Leaf fraction        |  |
| John E. Moore                         | University of Florida, USA                                | <i>Cynodon dactylon</i>                       | Whole plant          | Samples represent two growing seasons  |
|                                       |   | <i>Digitaria decumbens</i>                    | Whole plant          |  |
|                                       |   | <i>Paspalum notatum</i>                       | Whole plant          |  |
| — Test data set —                     |   |   |                      |  |
| Antonio Flores and Miguel Vélez       | Escuela Agrícola Panamericana, El Zamorano, Honduras      | <i>Andropogon gayanus</i>                     | Hay                  | The <i>Zea mays</i> and sorghum samples were from untreated and samples treated with ammonia |
|                                       |   | <i>Digitaria decumbens</i>                    | Pasture and hay      |  |
|                                       |   | <i>Enterolobium cyclocarpum</i>               | Fruit                |  |
|                                       |   | <i>Gliricidia sepium</i>                      | Leaf                 |  |
|                                       |   | <i>Panicum maximum</i>                        | Pasture, silage, hay |  |
|                                       |   | Sorghum                                       | Residue and silage   |  |
| Rogério P. Lana and Odilon G. Pereira | Universidade Federal de Viçosa, Viçosa, MG, Brazil        | <i>Zea mays</i>                               | Residue and silage   |  |
|                                       |   | <i>Brachiaria ruziziensis</i>                 | Whole plant          |  |
|                                       |   | <i>Brachiaria brizantha</i> cv. marandu       | Whole plant          |  |
|                                       |   | <i>Brachiaria decumbens</i>                   | Whole plant          |  |
|                                       |   | <i>Calopogonium mucunoides</i>                | Whole plant          |  |
|                                       |   | <i>Cenchrus ciliaris</i>                      | Whole plant          |  |
|                                       |   | <i>Cynodon dactylon</i>                       | Whole plant          |  |
|                                       |   | <i>Digitaria decumbens</i>                    | Whole plant          |  |
|                                       |   | <i>Hyparrhenia rufa</i>                       | Leaf and sheath      |  |
|                                       |   | <i>Macroptilium atropurpureum</i> cv. siratro | Whole plant          |  |
|                                       |   | <i>Neonotonia wightii</i> cv. clarence        | Whole plant          |  |
|                                       |   | <i>Neonotonia wightii</i> cv. tinaroo         | Whole plant          |  |
|                                       |   | <i>Pueraria phaseoloides</i>                  | Whole plant          |  |
| <i>Pennisetum purpureum</i> cv. Roxo  | Whole plant   |   |                      |  |
| <i>Pennisetum purpureum</i> cv. Mott  | Whole plant   |   |                      |  |
| <i>Setaria</i>                        | Leaf and sheath   |   |                      |  |

## Materials and Methods

We obtained samples of 145 forages from collaborators in tropical Latin America and Florida representing forages available to producers in those areas. Most of the samples were received as stem or leaf fractions from whole plant collections. A list of the collaborators and forages contributed to this project is presented in Table 1.

Forage samples were analyzed for crude protein (N × 6.25) by Kjeldahl (Pierce and Haenisch, 1947). Fiber (ADF and NDF) fractions were determined nonsequentially using the procedures of Van Soest

(1973) and Van Soest et al. (1991). Buffer insoluble protein and the N contents of NDF and ADF were determined using the procedures described by Licitra et al. (1996). Sulfuric acid lignin (SL; Van Soest and Robertson, 1980) and permanganate lignin (PL; Van Soest and Wine, 1968) determinations were performed on separate ADF preparations.

Long-term in vitro fermentations (> 96 h) were conducted to reach the maximum extent of digestion and then relate that to the forage lignin content. Mertens (1973) observed continued digestion after 96 h in some forages, provided the fermentation medium was renewed after an initial fermentation period

(either 48 or 72 h). A small preliminary study indicated that when fermentations were continued to 192 h, the extent of digestion was not significantly different from that obtained after 144 h of fermentation. Although 96 h was used by Mertens (1973), 144 h was used as the fermentation end point for all new samples.

A new in vitro apparatus (DAISY<sup>II</sup>; ANKOM Technology Corp., Fairport, NY) was used to perform the extended fermentations because of the ease of re-inoculating the samples after an initial incubation period with this equipment (Traxler, 1997). Handling of samples and preparation of ruminal fluid were described by Traxler (1997).

### Data Sets

The chemical composition and indigestibility data were divided to provide data sets for equation development and equation evaluation. The equation development data set consisted of the samples from CIAT, University of Florida, the *P. purpureum* samples from Brazil, and 75% of the sample composition data randomly selected from the data of Mertens (1973). The data collected by Mertens (1973) contained forage samples from 12 research stations in the United States, the Philippines, and Puerto Rico, representing 21 forage species or types. The evaluation data set consisted of the remaining samples, those from Escuela Agrícola Panamericana, Honduras, the University of Viçosa, Brazil, and the remaining samples of Mertens (1973). Because sample composition data used in the development data set were not used in the evaluation data set, the data sets were considered to be independent. The chemical composition data from Mertens (1973) included DM, CP, NDF (the 0-h in vitro NDF residues were used because all samples were treated equally for the determination of NDF), lignin (PL and SL), ash, and protein content of the ADF and NDF residues.

### Equation Development

There are two regularly used methods for the determination of lignin in forages: hydrolysis with sulfuric acid and oxidation with permanganate. The lignin values obtained with sulfuric acid are usually slightly lower than those obtained with permanganate (Van Soest and Wine, 1968; Van Soest, 1994). Neither method is dominant, and either method will provide a reasonable estimate of forage lignin content. Thus, the choice of lignin method to use is at the discretion of the scientist performing the forage analyses. The advantages of the permanganate method are that the reagents are not as corrosive and do not require standardization. However, permanganate oxidizes phenolic and unsaturated substances (e.g., tannins, pigments, or proteins) that are not completely removed during the acid detergent

preparatory step and that appear as lignin (Van Soest and Wine, 1968). However, 72% sulfuric acid allows non-phenolic matter (e.g., cutins and waxes) to remain in the crude lignin residue (Van Soest and Wine, 1968).

Several models relating various indices of lignification (i.e., percentage of DM and NDF, and grams of lignin per kilogram of NDF) to INDF were developed and evaluated for prediction accuracy. Separate models were developed to predict the INDF for different forage classes ( $C_3$  and  $C_4$  grasses and legumes). In addition, a common equation was developed. The models were developed from a database for which the indigestible residue was determined following a 96-h (Mertens, 1973) or a 144-h in vitro fermentation.

An exponential relationship (*exp*) was derived using PROC NLIN (SAS, 1990) without specifying a derivative. The  $b_1$  parameter was initialized at .01 and allowed to increase by increments of .01 up to 1.0. The general model was

$$U = (\text{Lig/NDF})^{b_1},$$

where U is the proportion of NDF remaining after a 96- or 144-h in vitro fermentation, and (Lig/NDF) is the lignin content of the NDF (grams per kilogram).

A log-log (*log*) equation was derived using the PROC REG procedure of SAS (1990). The general form of the model was

$$\log_{10}(U) = a + b_1 \times \log_{10}(\text{Lig/NDF}),$$

where (Lig/NDF) is the proportion of lignin contained in the NDF. The predicted indigestible NDF is determined by calculating the antilog of U.

Two models assumed that lignin had a constant effect on the maximum extent of digestion. The first model related the lignin content (% of NDF) to indigestible NDF and the other used lignin content (% of DM). The PROC REG procedure (SAS, 1990) was used to derive both of these relationships using models with the following form

$$U = a + b_1 \times X$$

where X is lignin expressed either as a percentage of the DM or of the NDF.

The equations derived from this study and three additional equations were compared for their prediction accuracy on the test data set. The first equation is from Chandler (1980) and is used to calculate the INDF in the CNCPS model (Sniffen et al., 1992) and in the level 2 solution of the beef NRC (NRC, 1996). The form of this equation, denoted as *Chandler*, is

$$U = 2.4 \times (\text{Lig/NDF})$$

Table 2. Average and range in chemical composition (% of DM) of the forages from the development and evaluation data sets

| Item                 | CP   | NDF   | PL <sup>a</sup> | SL <sup>b</sup> | Ash  | NDIP <sup>c</sup> | ADIP <sup>d</sup> | INDF <sup>e</sup> |
|----------------------|------|-------|-----------------|-----------------|------|-------------------|-------------------|-------------------|
| Development data set |      |       |                 |                 |      |                   |                   |                   |
| n                    | 312  | 312   | 312             | 270             | 237  | 257               | 253               | 304               |
| Mean                 | 12.7 | 62.8  | 7.1             | 5.8             | 8.1  | 4.1               | 1.2               | 34.1              |
| Median               | 12.1 | 64.3  | 6.6             | 5.4             | 7.7  | 3.7               | 1.1               | 30.6              |
| SD                   | 5.91 | 11.75 | 3.10            | 2.77            | 1.78 | 2.48              | .76               | 16.00             |
| Min.                 | 1.2  | 28.5  | 2.6             | .9              | 2.5  | .4                | .0                | 11.4              |
| Max.                 | 32.1 | 86.0  | 19.3            | 19.3            | 22.5 | 14.4              | 4.8               | 96.7              |
| Evaluation data set  |      |       |                 |                 |      |                   |                   |                   |
| n                    | 84   | 84    | 84              | 84              | 61   | 84                | 84                | 84                |
| Mean                 | 13.2 | 63.0  | 6.7             | 5.3             | 8.9  | 4.4               | 1.1               | 31.7              |
| Median               | 13.4 | 64.9  | 5.9             | 4.5             | 8.4  | 4.1               | 1.0               | 27.8              |
| SD                   | 5.23 | 10.89 | 2.83            | 2.31            | 3.24 | 2.08              | .48               | 14.11             |
| Min.                 | 3.2  | 28.9  | 2.7             | 1.8             | 4.2  | 1.0               | .5                | 11.4              |
| Max.                 | 24.4 | 81.6  | 16.2            | 11.6            | 17.4 | 9.0               | 2.9               | 68.4              |

<sup>a</sup>PL = permanganate lignin.

<sup>b</sup>SL = sulfuric acid lignin.

<sup>c</sup>NDIP = neutral detergent insoluble protein.

<sup>d</sup>ADIP = acid detergent insoluble protein.

<sup>e</sup>INDF = indigestible NDF.

where U is defined as above and Lig/NDF is the proportion of lignin contained in the NDF. The second equation (Weiss) is from Weiss et al. (1992) and is based on the surface limitation theory as applied to NDF availability by Conrad et al. (1984). In the Weiss equation, NDF is adjusted for its digestible nitrogen content. This adjustment is accomplished by subtracting the protein insoluble in neutral detergent (NDIP) and adding back the lignin protein ( $N \times 6.25$ ) content. The nitrogen content of lignin is difficult to measure, and the acid detergent insoluble protein (ADIP) can be used as an estimate (Mertens, 1973). Weiss et al. (1992), however, further adjusted the ADIP (70 and 40% for forages and concentrates, respectively) for its partial digestibility (IADIP). Thus,

$$\text{NDF}_N = \text{NDF} - \text{NDIP} + \text{IADIP}$$

where NDF, NDIP, and IADIP are expressed as grams per kilogram. The equation to predict NDF indigestibility is

$$U = \text{NDF} - [(\text{NDF} - \text{Lig}) \times (1 - (\text{Lig}/\text{NDF}_N)^{.677})]$$

where U is the indigestible NDF fraction (g/kg DM), Lig = sulfuric acid lignin, and  $\text{NDF}_N$  and Lig are expressed as grams per kilogram. The third equation (VanSoest) is a semi-log function from Van Soest (1994). Separate equations are presented for lignin determined by sulfuric acid and permanganate, which are for sulfuric acid lignin

$$U = 100 - [147.3 - 78.9 \log_{10} \times (\text{Lig}/\text{ADF})]$$

and for permanganate lignin

$$U = 100 - [180.8 - 96.6 \log_{10} \times (\text{Lig}/\text{ADF})]$$

where Lig/ADF is the proportion of lignin contained in the ADF.

The prediction accuracy of the equations was tested and compared with the evaluation data set using the mean square prediction error (MSPE) analysis of Theil (1966) and Bibby and Toutenburg (1977):

$$\text{MSPE} = \frac{1}{n} \sum_{i=1}^n (P_i - A_i)^2$$

where  $n$  is the number of pairs of  $A$  (actual) and  $P$  (predicted) values being compared. The MSPE can be decomposed into three components, yielding:

$$\text{MSPE} = (\bar{A} - \bar{P})^2 + s_P^2(1 - b)^2 + (1 - r^2) s_A^2$$

where  $s_A^2$  and  $s_P^2$  are the variances of the actual and predicted values, respectively. The slope of the regression of  $A$  on  $P$  is denoted as  $b$ , and  $r$  is the correlation coefficient of actual and predicted values. The component values represent the error due to mean bias, regression bias, and unexplained variation, respectively.

The first term, or mean bias  $(\bar{A} - \bar{P})^2$ , would be zero if the means of the predicted and actual values were equal. The second term, or regression bias, quantifies the regression of observed on predicted values as a deviation from a regression slope of unity, which occurs if the predictions were perfectly accurate. If the slope of the regression of  $A$  on  $P$  is less than

Table 3. Correlation coefficients associated with linear and quadratic relationships of the indigestible residue (DM and NDF) with NDF, ADF, and various indices of permanganate lignin for the equation development data set

| Item   | NDF    |        | ADF    |        | Lignin |        | Lignin/NDF |        | Lignin/ADF |        |
|--|--------|--------|--------|--------|--------|--------|------------|--------|------------|--------|
|  | r      | R      | r      | R      | r      | R      | r          | R      | r          | R      |
| C <sub>3</sub> grasses, n = 79                                 |        |        |        |        |        |        |            |        |            |        |
| Indigestible DM  | .73*** | —      | .79*** | .82*** | .83*** | .84*   | .71***     | .72*   | .62***     | —      |
| Indigestible NDF   | .62*** | —      | .71*** | .74*   | .81*** | .81*   | .73***     | .74*   | .64***     | —      |
| C <sub>4</sub> grasses, n = 137                                |        |        |        |        |        |        |            |        |            |        |
| Indigestible DM  | .74*** | —      | .78*** | —      | .86*** | —      | .75***     | —      | .79***     | —      |
| Indigestible NDF   | .62*** | —      | .73*** | —      | .82*** | —      | .77***     | —      | .77***     | —      |
| Legumes, n = 79  |        |        |        |        |        |        |            |        |            |        |
| Indigestible DM  | .81*** | —      | .93*** | .93*** | .91*** | —      | .41***     | —      | .37***     | —      |
| Indigestible NDF   | .46*** | —      | .71*** | —      | .82*** | .84**  | .63***     | .68**  | .57***     | —      |
| C <sub>3</sub> and C <sub>4</sub> grasses and legumes combined |        |        |        |        |        |        |            |        |            |        |
| Indigestible DM  | .30*** | .36*** | .77*** | .64*** | .84*** | .84*   | .56***     | .59*** | .57***     | .60*** |
| Indigestible NDF   | .20*** | .28*** | .47*** | .53*** | .91*** | .91*** | .86***     | .87*** | .85***     | .85*   |

\*  $P < .05$ .

\*\*  $P < .01$ .

\*\*\*  $P < .001$ .

unity, the equation tends to overpredict at high actual values and underpredict at low actual values. A large regression bias is indicative of underlying inadequacies in the structure of the equation to describe the data being analyzed. The last term,  $(1 - r^2) s_A^2$ , represents the variation in  $A$  that remains unaccounted for after the mean bias and the covariance between  $A$  on  $P$  have been removed. Results of the MSPE decomposition are given in units of the mean by taking the square root of each component.

## Results

### Chemical Composition

The chemical composition of the forages varied considerably in both data sets (Table 2). The mean composition values were similar between the two data sets, but the range of compositional values was greater in the development data set. Lignin measured as the weight loss upon oxidation with permanganate was greater than that determined with sulfuric acid, agreeing with Van Soest and Wine (1968). Linear relationships in the development data set existed among NDF, ADF, and various indices of lignification, determined by the permanganate method (i.e., % of DM, NDF, and ADF) and the indigestible DM and NDF when the forage classes were considered separately, or when they were combined (Table 3). Several quadratic relationships were evident for C<sub>3</sub> grasses and legumes, but not for the C<sub>4</sub> grasses. The three indices of lignification had curvilinear relationships with indigestible DM and NDF, except for that between lignin content (% of DM) and dry matter

indigestibility. A curvilinear relationship between lignin content of the NDF (Lig/NDF) and NDF digestibility has been reported previously (Van Soest, 1967; Jung and Vogel, 1986). Our results agree, except when the C<sub>4</sub> grasses were analyzed as a separate forage class.

Table 4 summarizes the equations derived with the development data set for the two lignin methods and three forage classes (C<sub>3</sub> and C<sub>4</sub> grasses and legumes) as well as a common equation for all forage classes. From this point on, discussion of the equations will follow the following nomenclature: equation type (lignin constant [% of DM] = *DM*, lignin constant [% of NDF] = *NDF*, log-log = *Log* and exponential = *Exp*) immediately followed by lignin method (permanganate lignin = *PL* and sulfuric acid lignin = *SL*) and forage class (separate classes = *-S* and common or single classification = *-C*).

### Mean Square Prediction Error

The equations were evaluated for their prediction accuracy and biological relevance, with the preferred model having a small regression bias and minimal unexplained variation. The regression bias for each equation was tested for its effect on prediction accuracy by regressing the actual values on predicted INDF and testing the linear regression coefficient ( $b_1$ ) for its equality to 1 or unity. The INDF values were converted to a dry matter basis (g NDF/100 g DM) prior to comparison of the equations.

Coefficients of determination, MSPE (g NDF/100 g DM)<sup>2</sup>, and component values in units of the mean (g NDF/100 g DM) were calculated for the prediction equations on the test data set for lignin determined

Table 4. Equations generated from the development data set to predict indigestible NDF (% of NDF) from various indices of lignification for the separate forage classes ( $C_3$  and  $C_4$  grasses, and legumes) and for the forage classes combined. The standard errors of the regression ( $SE_{y,x}$ ) are in units of g/100 g

| Forage class   | Permanganate lignin |            |       |               |            | Sulfuric acid lignin |            |       |               |            |
|--|---------------------|------------|-------|---------------|------------|----------------------|------------|-------|---------------|------------|
|  | $a$                 | $b_1$      | $r^2$ | SE of $b_1^a$ | $SE_{y,x}$ | $a$                  | $b_1$      | $r^2$ | SE of $b_1^a$ | $SE_{y,x}$ |
| Linear equations based on lignin as a % of DM                    |                     |            |       |               |            |                      |            |       |               |            |
| $C_3$ grasses  | 1.34                | 4.46X      | .62   | .356          | 5.06       | 3.30                 | 5.62X      | .63   | .539          | 5.36       |
| $C_4$ grasses  | 3.22                | 4.20X      | .69   | .257          | 5.39       | 3.26                 | 5.19X      | .69   | .330          | 5.50       |
| Legumes  | 19.56               | 2.90X      | .69   | .228          | 6.46       | 23.43                | 3.27X      | .66   | .272          | 6.70       |
| Combined   | 4.21                | 4.12X      | .81   | .113          | 6.08       | 6.36                 | 4.93X      | .79   | .158          | 6.67       |
| Linear equations based on lignin as a % of NDF                   |                     |            |       |               |            |                      |            |       |               |            |
| $C_3$ grasses  | -1.75               | 3.23X      | .51   | .333          | 5.81       | -1.10                | 4.22X      | .52   | .511          | 6.13       |
| $C_4$ grasses  | -1.40               | 3.56X      | .61   | .261          | 6.06       | -4.16                | 4.88X      | .68   | .321          | 5.62       |
| Legumes  | -11.41              | 3.16X      | .71   | .230          | 8.10       | 11.74                | 2.42X      | .61   | .221          | 8.83       |
| Combined   | 6.32                | 2.39X      | .80   | .068          | 7.09       | 10.35                | 2.56X      | .78   | .082          | 7.35       |
| Log-log equations based on lignin as a % of NDF <sup>b</sup>     |                     |            |       |               |            |                      |            |       |               |            |
| $C_3$ grasses  | 2.82                | $X^{1.03}$ | .48   | .109          | 5.81       | 4.17                 | $X^{.99}$  | .51   | .123          | 6.13       |
| $C_4$ grasses  | 3.09                | $X^{1.03}$ | .59   | .078          | 6.06       | 3.63                 | $X^{1.08}$ | .63   | .078          | 5.60       |
| Legumes  | 1.86                | $X^{1.10}$ | .72   | .079          | 8.02       | 6.17                 | $X^{.76}$  | .56   | .075          | 8.90       |
| Combined   | 4.37                | $X^{.84}$  | .77   | .026          | 7.11       | 6.17                 | $X^{.77}$  | .77   | .026          | 7.22       |
| Exponential equations based on lignin in NDF <sup>b</sup> , g/kg |                     |            |       |               |            |                      |            |       |               |            |
| $C_3$ grasses  | 1                   | $X^{.74}$  | .63   | .005          | 5.84       | 1                    | $X^{.80}$  | .64   | .007          | 6.14       |
| $C_4$ grasses  | 1                   | $X^{.76}$  | .69   | .004          | 6.08       | 1                    | $X^{.80}$  | .68   | .005          | 5.69       |
| Legumes  | 1                   | $X^{.75}$  | .30   | .004          | 8.30       | 1                    | $X^{.77}$  | .54   | .003          | 8.80       |
| Combined   | 1                   | $X^{.75}$  | .65   | .002          | 7.12       | 1                    | $X^{.78}$  | .73   | .002          | 7.21       |

<sup>a</sup>SE of  $b_1$  = standard error of the  $b_1$  regression coefficient.

<sup>b</sup>The form of the log-log and exponent equations is  $a \cdot X^{b_1}$  when calculating the indigestible NDF (% of NDF).

with the permanganate and sulfuric acid methods (Table 5). The predictions of indigestible NDF from PL were generally more accurate than predictions from SL.

The *Chandler* and *VanSoest* equations had the largest MSPE values, which ranged from 28 to 32 (g NDF/100 g DM)<sup>2</sup> when PL was used to predict INDF. The remaining equations had MSPE values less than 21 (g NDF/100 g DM)<sup>2</sup>. The *DMPL-S* equation had the smallest MSPE value. The *Chandler* equation had a mean bias of -3.41 g NDF/100 g DM. The *VanSoest* equation had a positive mean bias (3.25 g NDF/100 g DM). The *LogPL-S* equation had the smallest regression bias (-0.02 g NDF/100 g DM), followed by the *LogPL-C* and *VanSoest* equations with regression biases of -.24 and .86 g NDF/100 g DM, respectively. The relationship between the actual INDF with predicted values from PL for the *Chandler*, *Weiss*, *VanSoest*, and *LogPL-C* equations are displayed in Figure 1. The *Chandler* equation tended to underpredict INDF along the entire range of actual INDF values. The *Weiss* equation overpredicted INDF at low observed values and underpredicted at greater observed values. Indigestible NDF predictions by *VanSoest* were generally greater than the actual values. The values predicted by the *LogPL-C* equation seemed to be equally distributed along the unity line, agreeing with the small mean bias of .26 g NDF/100 g DM (Table 5). Coefficients from the linear regression of actual on predicted values for the *Chandler* and

*LogPL-C* equations ( $1.02 \pm .074$  and  $1.04 \pm .068$ , respectively) did not differ from unity ( $P > .6$ ). The *Weiss* equation had the greatest regression bias (-1.86 g NDF/100 g DM) and the linear regression coefficient ( $1.34 \pm .082$ ) significantly differed from unity ( $P = .0001$ ). The regression coefficient for *VanSoest* was  $.89 \pm .057$  and was not different from unity ( $P = .052$ ). The error due to unexplained variation ranged from 3.88 to 4.55 g NDF/100 g DM.

Indigestible NDF was less accurately predicted from SL than from PL. The mean bias ranged from -7.15 to 5.88 g NDF/100 g DM. The *Chandler*, *Weiss*, and *VanSoest* equations had the largest MSPE values, attributable primarily to the mean bias. The *Weiss* equation had the greatest regression bias (-2.34 g NDF/100 g DM). The *NDFSL-C*, *ExpSL-S*, and *Chandler* equations also had large regression biases (-1.08, -.89, and -.88 g NDF/100 g DM, respectively). The *VanSoest* equation had the smallest regression bias (.10 g NDF/100 g DM). Several equations had regression biases less than .50 g NDF/100 g DM in magnitude. The accuracy of predicting INDF from SL by the *Chandler*, *Weiss*, *VanSoest*, and *LogSL-C* equations is depicted in Figure 2. Indigestible NDF was underpredicted by the *Chandler* and *Weiss* equations across the range of actual values. The linear regression coefficients for these two equations were  $1.16 \pm .104$  and  $1.50 \pm .104$  for *Chandler* and *Weiss*, respectively. The *Weiss* coefficient differed from unity ( $P = .0001$ ). The *VanSoest* equation predicted a

Table 5. Mean square prediction errors (MSPE), components of bias, and unexplained variation for equations to predict the indigestible NDF fraction from indices of lignification (% of DM, % of NDF, and g/kg)

| Equation             | r <sup>2</sup> | MSPE,<br>(g/100 g DM) <sup>2</sup> | Mean bias,<br>g/100 g DM | Regression bias,<br>g/100 g DM | Unexplained<br>variation,<br>g/100 g DM |
|----------------------|----------------|------------------------------------|--------------------------|--------------------------------|---|
| Permanganate lignin  |                |                                    |                          |                                |   |
| <i>Chandler</i>      | .69            | 32.34                              | -3.41                    | -.11                           | 4.55                                    |
| <i>Weiss</i>         | .76            | 19.42                              | -.25                     | -1.86 <sup>a</sup>             | 3.99                                    |
| <i>VanSoest</i>      | .75            | 28.63                              | 3.25                     | .90                            | 4.16                                    |
| <i>DMPL-C</i>        | .75            | 16.95                              | .33                      | .43                            | 4.08                                    |
| <i>DMPL-S</i>        | .78            | 15.25                              | .32                      | .35                            | 3.88                                    |
| <i>NDFPL-C</i>       | .74            | 18.34                              | .51                      | -.41                           | 4.23                                    |
| <i>NDFPL-S</i>       | .71            | 20.88                              | .77                      | .54                            | 4.47                                    |
| <i>LogPL-C</i>       | .74            | 17.76                              | .26                      | -.24                           | 4.20                                    |
| <i>LogPL-S</i>       | .73            | 18.50                              | .28                      | -.02                           | 4.29                                    |
| <i>ExpPL-C</i>       | .76            | 17.80                              | .93                      | -.91 <sup>a</sup>              | 4.01                                    |
| <i>ExpPL-S</i>       | .73            | 20.20                              | 1.15                     | -.77                           | 4.28                                    |
| Sulfuric acid lignin |                |                                    |                          |                                |   |
| <i>Chandler</i>      | .60            | 71.19                              | -6.60                    | -.88                           | 5.18                                    |
| <i>Weiss</i>         | .71            | 33.82                              | -3.05                    | -2.34 <sup>b</sup>             | 4.36                                    |
| <i>VanSoest</i>      | .70            | 55.24                              | 5.88                     | .10                            | 4.55                                    |
| <i>DMSL-C</i>        | .73            | 19.11                              | .88                      | .14                            | 4.28                                    |
| <i>DMSL-S</i>        | .78            | 15.09                              | .62                      | .31                            | 3.82                                    |
| <i>NDFSL-C</i>       | .68            | 23.25                              | .77                      | -1.08 <sup>b</sup>             | 4.64                                    |
| <i>NDFSL-S</i>       | .69            | 22.45                              | .99                      | .51                            | 4.61                                    |
| <i>LogSL-C</i>       | .70            | 20.53                              | .29                      | -.58                           | 4.53                                    |
| <i>LogSL-S</i>       | .70            | 21.39                              | .84                      | .24                            | 4.54                                    |
| <i>ExpSL-C</i>       | .69            | 21.08                              | .26                      | -.48                           | 4.56                                    |
| <i>ExpSL-S</i>       | .71            | 21.62                              | 1.11                     | -.89                           | 4.43                                    |

<sup>a</sup>Linear regression coefficients differed from unity,  $1.34 \pm .082$  ( $P = .0001$ ) for *Weiss* and  $1.14 \pm .070$  ( $P = .042$ ) for *ExpPL-C*.

<sup>b</sup>Linear regression coefficients differed from unity,  $1.50 \pm .104$  ( $P = .0001$ ) for *Weiss* and  $1.19 \pm .089$  ( $P = .039$ ) for *NDFSL-C*.

greater quantity of INDF than was observed and had a linear regression coefficient of  $.98 \pm .072$ , which did not differ from unity ( $P = .85$ ). The predictions of INDF by the *LogSL-C* equation were distributed along the unity line, agreeing with the small mean bias (Table 5) with a regression coefficient of  $1.09 \pm .079$ , which did not differ from unity ( $P = .25$ ).

## Discussion

### Evaluation of Prediction Equations

Most research groups have concentrated on a single forage class when investigating the relationship between lignification and NDF digestibility (Jung and Vogel, 1986; Buxton and Russell, 1988). Others have only considered a common relationship (Conrad et al., 1984; Weiss et al., 1992). Van Soest (1993, 1994) concluded from an analysis of a subset of the data from Mertens (1973) that forage class is unimportant when estimating the INDF from lignin content of the NDF. Our findings support that conclusion. The coefficients derived from this study for C<sub>3</sub> and C<sub>4</sub> grasses were similar (Table 4). Although the coefficients for legumes were somewhat different from those of the grasses, the largest difference in the prediction

accuracy between the pairs of equations was 4 (g NDF/100 g DM)<sup>2</sup> (Table 5). The net effect of using separate equations for each forage class to predict INDF cannot be considered biologically different from those predicted by the common forage equations.

Lignin has no effect on the digestibility of cell solubles, and relating the digestibility of NDF based on lignin content of the dry matter should be avoided (Van Soest, 1967, 1994). The apparent relationship between forage lignin content (% of DM) and indigestible NDF results as a consequence of maturity; NDF increases while CP and cell solubles decline. Therefore, the high correlation and low MSPE values obtained in this study with the linear prediction equations based on the dry matter lignin content may be related to the data analyzed.

The equations resulting from the *log* and *exp* analyses provide some interesting mathematical comparisons. Both sets of equations have been written in the exponential form for ease of comparison (Table 4). During the generation of the exponential equations, the values were logarithmically transformed to derive the coefficient for the exponent. Because of the manner in which the lignin to NDF ratios were expressed (g/kg) and because no intercept was specified, the antilog of zero is one. The application of this equation is easy to use and the accuracy does not

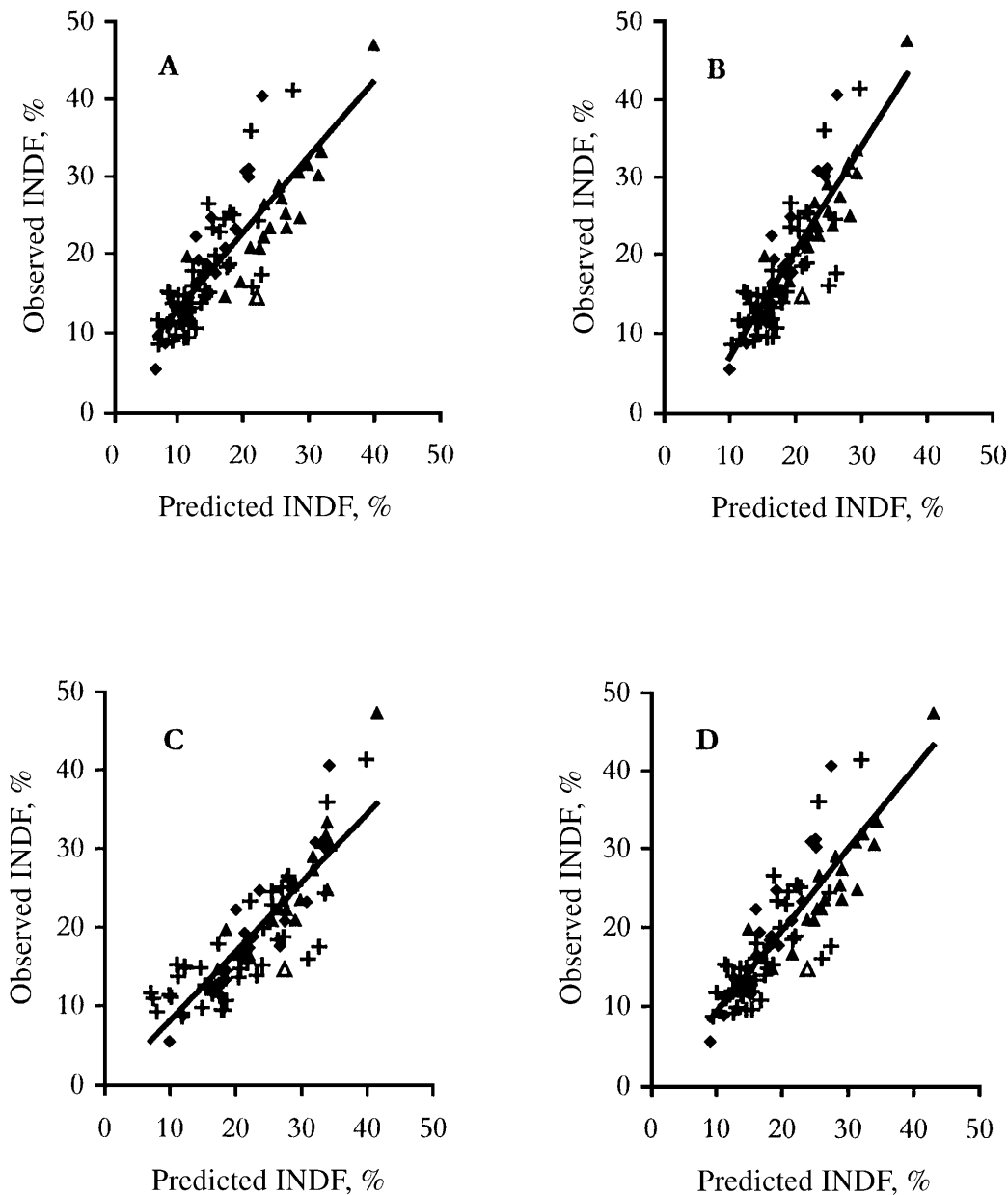


Figure 1. Relationships of predicted to observed indigestible NDF ( $C_3$  grasses [ $\blacklozenge$ ];  $C_4$  grasses [ $+$ ]; legumes [ $\blacktriangle$ ]) estimated from permanganate lignin by the *Chandler* (A), *Weiss* (B), *VanSoest* (C), and *LogPL-C* (D) equations. The overall linear regression (—) coefficients for *Chandler*, *VanSoest*, and *LogPL-C* did not differ from unity. However, the coefficient for *Weiss* ( $1.34 \pm .082$ ) differed from unity ( $P = .0001$ ). *Gliricidia sepium*, a tropical, tannin-containing legume, is identified with an open triangle.

differ greatly from that of the other equations. When lignin is expressed as a percentage of NDF and an intercept value is calculated, the exponential coefficients associated with the *log* equations result.

The *Chandler* equation was developed from 120-d anaerobic fermentations and represents the average effect of SL on the indigestibility of NDF from a small number of samples (Chandler, 1980). In vitro (96 and 144 h) NDF residues were 2.5 times the weight of lignin. The variability was greater with the in vitro data than with the 120-d fermenter data (1.6

and .4 [mass/mass], respectively). Despite the small size and origin of the database, the *Chandler* equation performed well in the prediction of the INDF in our test forages. However, it tended to underpredict the actual indigestibility values, with a greater underprediction when SL was used compared to PL.

The summative equation of Conrad et al. (1984) assumes that NDF digestion is limited by the surface limiting effect of lignin. The original equation was later modified by Weiss et al. (1992) to remove the available NDF protein from the calculation of energy

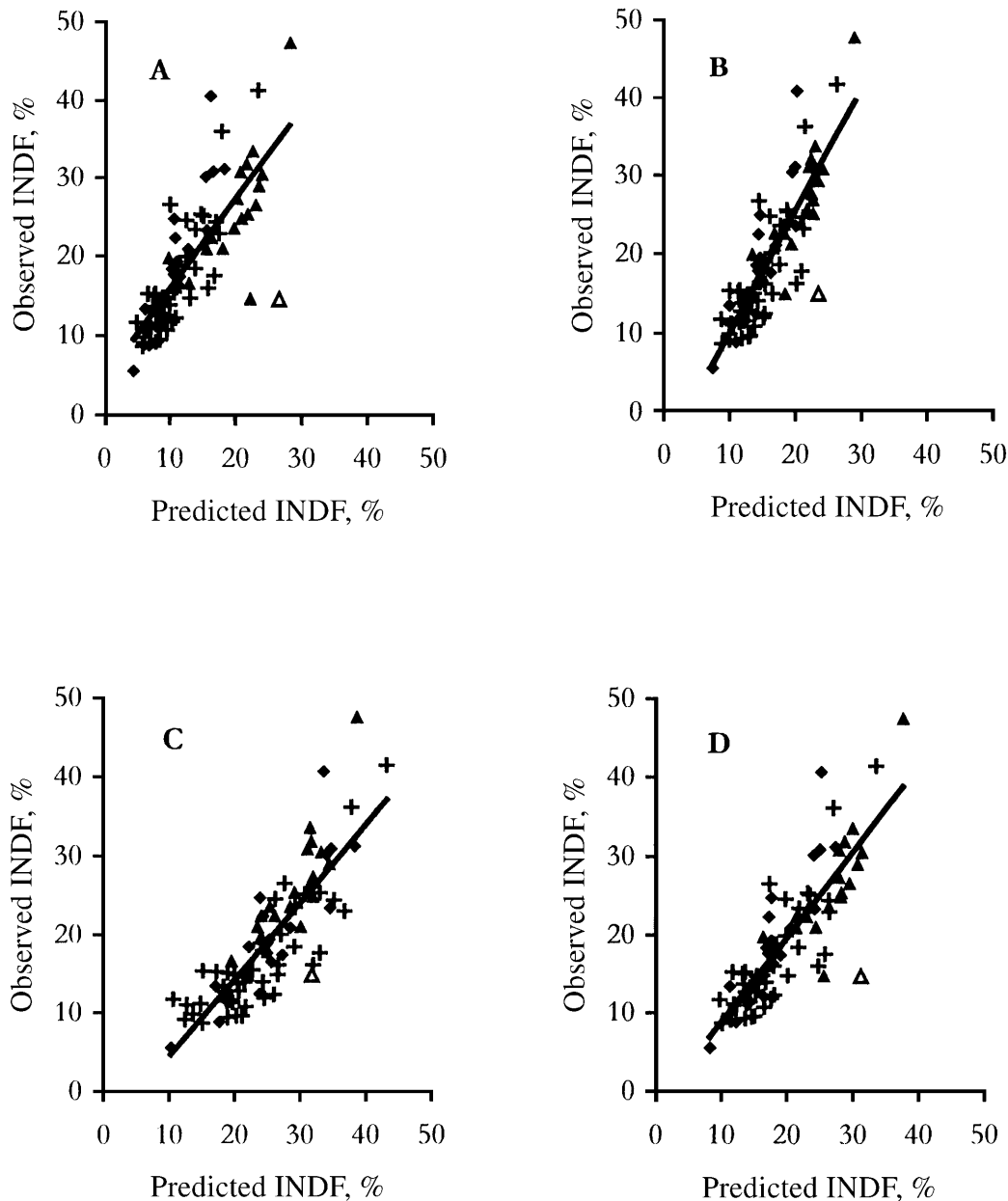


Figure 2. Relationships of predicted to observed indigestible NDF ( $C_3$  grasses [ $\blacklozenge$ ];  $C_4$  grasses [ $+$ ]; legumes [ $\blacktriangle$ ]) estimated from sulfuric acid lignin by the *Chandler* (A), *Weiss* (B), *VanSoest* (C), and *LogSL-C* (D) equations. The overall linear regression (—) coefficients for *Chandler*, *VanSoest*, and *LogSL-C* did not differ from unity. The regression coefficient for *Weiss* ( $1.50 \pm .104$ ) differed from unity ( $P = .0001$ ). *Gliricidia sepium*, a tropical, tannin-containing legume, is identified with an open triangle.

derived from the NDF fraction of the feedstuff. The full summative equation performed well in the prediction of feedstuff energy values for lactating cows (Weiss et al., 1992). However, results from this study indicated that the two-thirds power function for estimating the INDF results in an underprediction of the INDF (Figures 1b and 2b).

Figure 3 depicts the estimation of INDF as the ratio of lignin to NDF increases. As the surface limiting law suggests, the influence of lignin on the indigestibility of NDF is greater at lower than at higher lignin

concentrations. The two curvilinear functions in Figure 3 predict a greater INDF than the linear *Chandler* function at low lignin to NDF ratios. However, the *Weiss* equation deviated from the *LogPL-C* equation as the lignin content of NDF increased. The few feeds in the library that had high lignin to NDF ratios (grape pomace, sunflower seed meal, and two cottonseed products) were predicted to have 70 to 100% of the NDF unavailable for digestion by the *Chandler* and *LogPL-C* equations. Predictions of INDF by the *Weiss* equation on the same feeds were between 60 and 80% of the NDF.

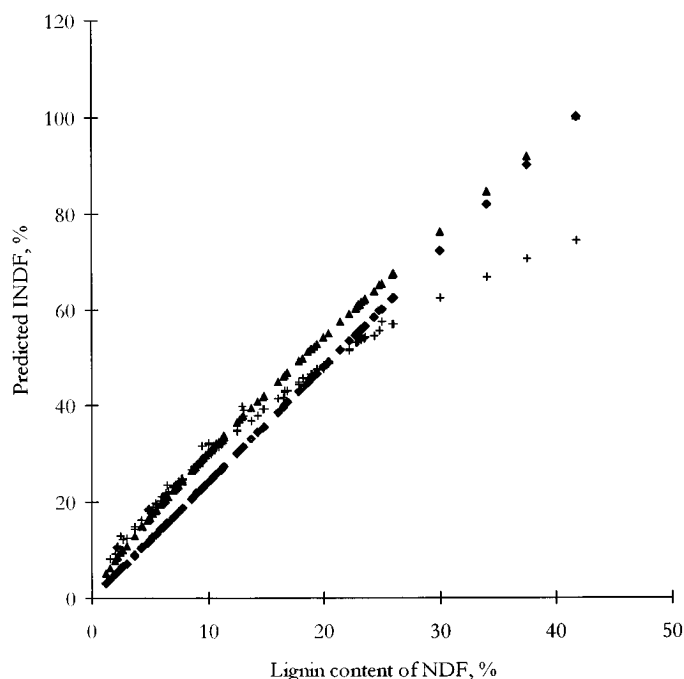


Figure 3. Relationships between lignin content (% of NDF) and indigestible NDF (% of NDF) as predicted by the *Chandler* ( $\blacklozenge$ ), *Weiss* (+), and *LogPL-C* ( $\blacktriangle$ ) equations. Data are from the CNCPS feed library (Sniffen et al., 1992).

The summative equations of Conrad et al. (1984) and Weiss et al. (1992) overcame this apparent underestimation of indigestible NDF by multiplying the potentially digestible NDF with a digestibility factor. This factor ranges from .82 at maintenance to .75 at three times maintenance intake. Adjusting for the undigested potentially digestible NDF would account for some of the discrepancy seen in Figure 3. However, this approach does not adequately consider differences in rates of digestion among forages. A more appropriate approach would be to integrate digestion and passage rates for the forage to derive an energy value for the desired animal class.

The lower predictions of INDF by *Chandler* and *Weiss* from SL were not unexpected. Lignin values determined from sulfuric acid are usually lower than the values determined by the permanganate method (Van Soest and Wine, 1968). This was evident in our test data set, because the SL values were approximately 76% of those determined by the permanganate method. This agrees with Van Soest and Wine (1968), in which SL values were 81% of the PL values.

After consideration of the problems associated with the equations based on a constant lignin factor (*DMPL-C*, *DMPL-S*, *DMSL-C*, and *DMSL-S*), the equations derived from the *log* analysis (*LogPL-C*, *LogPL-S*, *LogSL-C* and *LogSL-S*) provided the best estimates of the actual indigestible NDF. The difference in the estimates of INDF by these equations within a lignin method probably do not have any

biological significance; therefore, the common forage equations (*LogPL-C* and *LogSL-C*) were selected for comparison with the *Chandler* equation using the CNCPS model on an animal validation set.

#### Validation with the CNCPS Model

The *Chandler* equation is used in the prediction of feedstuff energy content in the CNCPS model (Sniffen et al., 1992), as well as the level 2 solution of the Beef NRC (NRC, 1996). Therefore, the predicted performance resulting from estimating the INDF with the *LogPL-C*, and *LogSL-C* equations was tested against that derived from the *Chandler* equation. The animal data set was selected to represent a wide range in forage quality. The NRC (1996) growth data validation set consisted of the data reported by Ainslie et al. (1993) and a subset of the data reported by Wilkerson et al. (1993). The data from Ainslie et al. (1993) consisted of 56-d growth periods with implanted Holstein steer calves (113 to 208 kg BW) fed diets based on corn silage or high-moisture ear corn, with supplements of either urea, raw soybeans, roasted soybeans (110 and 135°C), or corn gluten feed. Diet ME content ranged from 2.52 to 3.04 Mcal/kg, supporting average daily gains of .75 to 1.44 kg/d. The Wilkerson et al. (1993) data set consisted of 11 feeding trials with primarily Hereford  $\times$  Angus crossbred steers (mean BW ranged from 200 to 316 kg) in feeding periods lasting between 85 and 120 d. A variety of protein sources were evaluated in metabolizable protein-deficient roughage-based diets. Roughage sources included corncobs, corn stalks, corn silage, sorghum silage, and alfalfa hay. Protein sources included blood meal, corn gluten meal, dried distillers grains, soybean meal, and wet distillers grains. The roughage fraction ranged from 73 to 95% of the diet dry matter. In this analysis, the estimates of INDF by the *LogPL-C* and the *LogSL-C* equations were for the roughage sources only. The INDF of grains and supplements were estimated by the default *Chandler* equation. The proportion of the potentially digestible NDF (1 - INDF) degraded was calculated by integration of passage (based on level of intake and forage effective fiber content of the diet) and NDF digestion rates (4 to 6 %/h). The equations were compared using the MSPE analysis described earlier and with a *t*-test.

Although the *Chandler* and *LogPL-C* equations gave quite similar predictions of energy allowable daily gain ( $P = .12$ ), daily gain on the low-energy diets was more accurately predicted with *Chandler*. The predictions of gain by *LogSL-C* were different from those of *Chandler* ( $P = .036$ ) and similar to those of *LogPL-C* ( $P = .215$ ). The relationships between the actual and predicted daily gains are depicted in Figure 4 for the *Chandler* (a) and *LogPL-C* (b) equations (*LogSL-C* is similar to *LogPL-C*). The mean bias for the three equations (-.10, -.17, and -.22 kg/d for

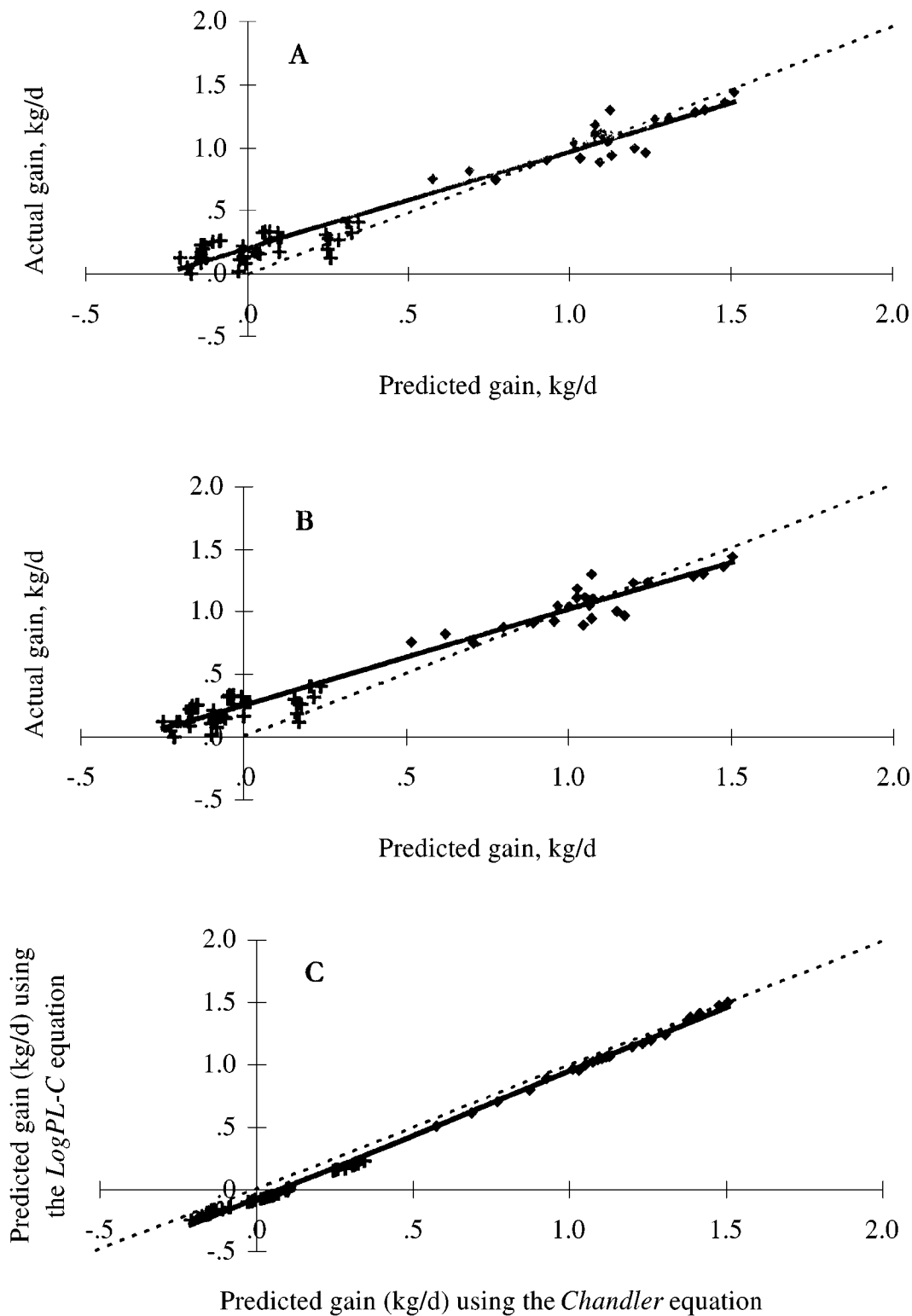


Figure 4. Comparisons of actual daily gain to those predicted by the CNCPS using either the *Chandler* (A) or *LogPL-C* (B) equation to predict the forage indigestible NDF. Regression line is solid and unity line is dashed. Linear regression coefficients were .77 and .76 for *Chandler* and *LogPL-C*, respectively. Both coefficients differed from unity ( $P = .0001$ ). Relationship between predicted daily gains ( $R^2 = .99$ ) resulting from the estimates of indigestible NDF by the *Chandler* and *LogPL-C* equations (C). Animal performance data are from Ainslie et al. (1993;  $\blacklozenge$ ) and Wilkerson et al. (1993; +).

*Chandler*, *LogPL-C*, and *LogSL-C*, respectively) indicated that daily gain was underpredicted. Each equation had numerous negative gain predictions for diets based on low-quality roughages, which were responsible for much of the underprediction of gain and the negative mean biases. The linear regression coefficients resulting from the regression of actual on predicted gains by the three equations were .77, .76, and .76 for *Chandler*, *LogPL-C*, and *LogSL-C*, respectively. Each of the regression coefficients differed from unity ( $P = .0001$ ). The corresponding regression biases were .13, .14, and .14 for *Chandler*, *LogPL-C*, and *LogSL-C*, respectively. The predicted values from *Chandler* and *LogPL-C* are plotted against each other in Figure 4c. The two estimates agree well with each other ( $R^2 = .99$ ). The *Chandler* and *LogPL-C* equations agreed closely with the data of Ainslie et al. (1993), largely because they were mostly corn silage-based (low lignin content, % of NDF), resulting in relatively small differences in estimates of INDF. However, with the data of Wilkerson et al. (1993), the *LogPL-C* equation predicted lower gains than *Chandler* because it estimated a greater INDF fraction leading to a lower forage energy value. Many of the diets fed in the studies summarized by Wilkerson et al. (1993) were based on low-quality roughages (corn cobs, corn stalks, corn and sorghum silage, and alfalfa hay) supporting gains ranging from  $-.02$  to  $.40$  kg/d that were not represented in the data sets used to generate the *log* equations and may have overestimated the INDF fraction for these feeds. The *log* equations need further evaluation with animal production data in a variety of feeding situations.

### Implications

Several equations were developed for the prediction of indigestible neutral detergent fiber (INDF), which is useful for the estimation of forage energy values in computer models. A common equation provides similar estimates among forage classes. However, separate equations are needed for the two lignin procedures commonly used. The equation used by the Cornell Net Carbohydrate and Protein System and Beef NRC resulted in better estimates of daily gain on low-quality roughages with the Beef NRC growth validation data set. However, the *log* equations are more biologically sound (based on biologically sound principles), had better estimates of the indigestible INDF fraction, and should provide better estimates of animal performance under widely varying conditions.

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