Effects of spontaneous heating on estimates of total digestible nutrients for alfalfa-orchardgrass hays packaged in large round bales

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ABSTRACT

Large round or large square hay packages are more likely to heat spontaneously during storage than hay packaged in conventional (45 kg) bales, and the effects of this phenomenon on the associated energy estimates for these hays can be severe. Our objectives for this project were to assess the relationship between estimates of total digestible nutrients (TDN) and spontaneous heating and to describe any important differences in energy estimates that may result specifically from 2 methods of estimating truly digestible fiber (TD-Fiber). Using the summative approach to estimate TDN, TD-Fiber can be estimated from inputs of protein-corrected neutral detergent fiber (NDFn) and acid detergent lignin (TD-FiberLIG) or from NDFn and 48-h neutral detergent fiber digestibility (TD-FiberNDFD). Throughout 2006 and 2007, mixed alfalfa (Medicago sativa L.)-orchardgrass (Dactylis glomerata L.) hays from 3 individual harvests were obtained from the same 8.2-ha research site near Stratford, Wisconsin. Both options for estimating TD-Fiber (TD-FiberLIG or TD-FiberNDFD) were then used independently via the summative approach to estimate the total TDN concentrations (TDN-LIG or TDN-NDFD, respectively) within these hays. Estimates of both TDN-LIG and TDN-NDFD then were related to heating degree days >30°C accumulated during storage by various regression techniques. Changes (poststorage – prestorage) in TDN-LIG that occurred during storage (ΔTDN-LIG) were best fitted with a nonlinear decay model in which the independent variable was squared [Y = (11.7 × e^{-0.0000033x^2}) − 11.6; R^2 = 0.928]. For changes in TDN-NDFD (ΔTDN-NDFD), a quadratic regression model provided the best fit (Y = 0.000023x^2 − 0.010x + 0.4; R^2 = 0.861). Generally, ΔTDN-LIG estimates were 2.0 to 4.0 percentage units lower than ΔTDN-NDFD estimates when heating exceeded 500 HDD. For regressions on maximum internal bale temperature, both ΔTDN-LIG (Y = −0.38x + 16.3; R^2 = 0.954) and ΔTDN-NDFD (Y = −0.25x + 10.2; R^2 = 0.848) were best fitted by linear models with heterogeneous (P < 0.001) slopes and intercepts. In both cases, coefficients of determination were high, suggesting that simple measures of spontaneous heating are excellent predictors of energy losses in heated forages. Regardless of method, reductions in TDN were associated primarily with losses of nonfiber carbohydrate, which is known to occur via oxidation of sugars during spontaneous heating. For heated forages, some discrepancy between TDN-LIG and TDN-NDFD existed because the relationship between NDFD and spontaneous heating was shown previously to be very poor, resulting in minimal changes for estimates of TDN-LIG as a consequence of heating. In contrast, TD-FiberLIG declined in close association with heating, largely because TD-FiberLIG was sensitive to changes in concentrations of both NDFn and acid detergent lignin. Discrepancies between TDN-LIG and TDN-NDFD were exacerbated further when neutral detergent fiber rather than NDFn was used to estimate TDN-LIG. Estimates of TDN declined by as much as 13.0 percentage units within severely heated hays, and this is a serious consequence of spontaneous heating.

Key words: energy, spontaneous heating, total digestible nutrient

INTRODUCTION

The harvest of alfalfa (Medicago sativa L.) and other hays can be complicated by poor drying conditions or the threat of unexpected rainfall events, each of which potentially places valuable hay crops at risk. As a result, it is not uncommon for hay producers to choose between 2 undesirable management options: bale their hay before it is desiccated adequately, or subject their wilting hay crop to rain damage. When hay is packaged before it is desiccated adequately, spontaneous heating is commonly observed during storage and the potential for combustion exists. Recently, we have reported on a series of experiments (Coblentz and Hoffman, 2009a,b;
Coblentz et al., 2010) describing spontaneous heating within large round bales of alfalfa-orchardgrass (Dactylis glomerata L.) and the subsequent effects of heating on recoveries of DM following storage, as well as on in vitro true digestibility, fiber and protein composition, and ruminal in situ disappearance kinetics of DM, NDF, CP, and neutral detergent insoluble CP (NDICP). In those reports, estimates of 48-h NDF digestibility (NDFD) and effective ruminal degradability of NDF exhibited limited and undetectable responses, respectively, to spontaneous heating (Coblentz and Hoffman, 2009b), thereby raising questions about estimates of total digestible nutrients (TDN) derived from the summative method (Weiss et al., 1992; NRC, 2001) when truly digestible fiber (TD-Fiber) is determined using 48-h NDFD as the digestion coefficient for protein-corrected NDF (NDFn). If TD-Fiber is determined within heated hays by this option (TD-FiberNDFD), it would be sensitive only to fluctuating concentrations of NDFn, primarily because NDFD remained largely static across a fairly wide range of heating (Coblentz and Hoffman, 2009b).

In contrast, TD-Fiber also can be estimated via the acid detergent lignin option (TD-FiberLIG; Weiss et al., 1992; NRC, 2001) that uses inputs of NDFn and acid detergent lignin. Unlike the static nature of NDFD in our heated hays, concentrations of acid detergent lignin increased in curvilinear relationships with heating degree days (HDD) >30°C or maximum internal bale temperature (MAX), exhibiting very high coefficients of determination (R² ≥ 0.885; Coblentz and Hoffman, 2009b). The practical consequence of increased concentrations of acid detergent lignin is that TD-FiberLIG is sensitive to concentrations of both NDFn and acid detergent lignin, thereby creating a potential discrepancy between the TD-FiberLIG and TD-FiberNDFD options for estimating TD-Fiber in heated hays. To the best of our knowledge, this potential discrepancy has not been evaluated. Furthermore, changes in the other nutrients that compose summative estimates of TDN, such as truly digestible CP (TD-CP) and truly digestible NFC (TD-NFC), have not been described in detail for heated forages. Our objectives for this project were to assess the relationship between estimates of TDN and spontaneous heating for large round bales of alfalfa-orchardgrass hay and to describe any important differences in energy estimates that may result from the 2 methods of estimating TD-Fiber.

**MATERIALS AND METHODS**

**Field Procedures**

This project comprised 3 separate hay harvests conducted on the same 8.2-ha field site over a 2-yr period (2006 and 2007). All details relating to the establishment of forages, soil fertility, harvest management, storage procedures, temperature measurements, and pre- and poststorage sampling procedures have been described previously in detail (Coblentz and Hoffman, 2009a,b; Coblentz et al., 2010). Therefore, these methods are outlined only briefly.

**Source of Hays.** The forage base at the 8.2-ha field site consisted of an alfalfa-orchardgrass and Extend orchardgrass that was established on April 14, 2004 near Stratford, Wisconsin (44°7’ N, 90°1’ W). The overall project consisted of 3 individual harvests, based largely on moisture ranges at baling. The moisture ranges for these harvests were 9.3 to 17.3%, 16.8 to 24.2%, and 26.7 to 46.6%, hereafter designated as low (LM), intermediate (IM), and high (HM) moisture. The LM and HM hay harvests were taken from the second and third cuttings, respectively, during 2006, whereas the IM harvest used forage obtained from the second cutting of 2007. Dry-weight percentages of alfalfa in the LM, IM, and HM harvests were 91, 76, and 68%, respectively, whereas orchardgrass composed 9, 22, and 31% of each sward, respectively. Because orchardgrass remains strictly vegetative following an initial harvest as hay or silage each spring, only second and third harvests were used for this project, thereby avoiding any potential confounding created by stem elongation within the orchardgrass portion of the sward. Alfalfa was harvested at first flower for the LM harvest and as plants approached full bloom for the IM and HM harvests.

It was necessary to use this multiple-harvest approach to provide adequate quantities of forage for an in-depth evaluation ultimately requiring 96 large round bales, and this objective could not be accomplished with the forage available from a single cutting at the 8.2-ha field site. The treatment structure within each hay harvest was similar; generally, bales were packaged in factorial arrangements of bale diameter (1.5, 1.2, or 0.9 m) and various concentrations of moisture. In each harvest, forage was mowed and conditioned (Model 8830; J. I. Case Co., Racine, WI), adjacent rows were gathered with a bifold rake, and hay was then baled with a Ford-New Holland round baler (Model BR 740A; CNH America LLC, Racine, WI). All bales were tied with 2 revolutions of net wrap, and each bale was then placed on its rounded side on top of wooden pallets located outdoors over a dense grass sod.

**Temperature Measurements.** After packaging, each bale was fitted with a thermocouple positioned near its geometric center and bales were monitored daily for internal bale temperature with a hand-held thermocouple thermometer (Omega 450 AKT Type K; Omega Engineering, Stamford, CN). Storage periods lasted...
Laboratory Analyses

To estimate TDN for our heated forages, all pre- and poststorage hay samples were analyzed for fiber composition (NDF, acid detergent lignin), 48-h NDFD, and whole-plant ash, as well as CP, NDICP, and acid detergent insoluble CP (ADICP). Initial evaluation of fiber composition was conducted sequentially using batch procedures outlined by Ankom Technology Corp. (Fairport, NY) for an Ankom200 Fiber Analyzer and was reported previously (Coblentz and Hoffman, 2009b). Neither sodium sulfite nor heat-stable α-amylase was included in the NDF solution. Concentrations of whole-plant ash were determined independently for each sample following combustion of 1.0-g samples in a muffle furnace at 500°C for 2 h (Coblentz and Hoffman, 2009a).

Concentrations of CP, NDICP, and ADICP were quantified by a macro-Kjeldahl technique (AOAC, 1998; method 988.05). Prior to analyzing NDF and ADF residues for CP, hay samples were digested independently in neutral and acid detergent, respectively, using the batch procedures outlined by Ankom Technology Corp. The neutral detergent solution did not contain sodium sulfite or heat-stable α-amylase; previously, Van Soest et al. (1991) recommended that sodium sulfite be omitted from digestions in neutral detergent that precede quantification of NDICP because sodium sulfite cleaves disulfide bonds and dissolves cross-linked proteins, which reduces protein recovery from NDF residues. In addition, before quantification of ADICP, digestions in acid detergent were conducted nonsequentially, without preliminary digestion in neutral detergent (Van Soest et al., 1991).

Procedures and apparatus for determining NDFD (University of Wisconsin Soil and Forage Analysis Laboratory, Marshfield, WI) consisted of incubating 0.5-g hay samples in 125-mL Erlenmeyer flasks containing rumen fluid, buffer media, and macro- and micromineral solutions (Goering and Van Soest, 1970). Flasks were purged continuously with CO2, maintained in a water bath at 39°C for 48 h, and then terminated by digestion in neutral detergent solution that included both heat-stable α-amylase and sodium sulfite (Goering and Van Soest, 1970; Mertens, 1992). Prior to ruminal incubation, rumen fluid was harvested from a nonlactating dairy cow fitted with a ruminal cannula and offered a diet containing 59% alfalfa-grass silage and 40% corn silage, with the balance consisting of vitamin and mineral supplements.

Calculation of TDN

The energy concentration of each hay sample was calculated from appropriate laboratory inputs using the equations of Weiss et al. (1992), as adapted by NRC (2001). No processing adjustment factor was applied in this work. Within this context, TDN was defined as

\[
\text{TDN} (\%) = \text{TD-NFC} + \text{TD-CP} + (\text{TD-FA} \times 2.25) + \text{TD-Fiber} - \text{metabolic fecal TDN},
\]

where TD-NFC, TD-CP, and TD-Fiber have been defined previously, TD-FA = truly digestible fatty acids, and the metabolic fecal TDN = 7 percentage units.
The concentration of TD-FA for each forage was estimated as ether extract - 1. This concept is based on the assumption that pigments and waxes, both of which are recovered with FA in ether extract, contain little or no digestible energy and make up approximately 1% of forage DM (Weiss et al., 1992). For purposes of this evaluation, tabular values (NRC, 2001) were used to approximate concentrations of ether extract. These estimates were based on those provided for (predominantly legume) mixed legume-grass forages subclassified on the basis of concentrations of NDF. The associated multiplier of 2.25 is based on the imperfect premise that lipids and fats contain 2.25 times the energy of carbohydrates (Van Soest, 1982). Concentrations of TD-FiberLIG were calculated as described within NRC (2001) guidelines, based on concentrations of acid detergent lignin and NDFn using the equation

\[
\text{TD-FiberLIG} = 0.75 \times (\text{NDFn} - \text{lignin}) \times [1 - (\text{lignin}/\text{NDFn})^{0.667}].
\]

In this equation, NDFn is expressed as a percentage of DM and calculated as NDF - NDICP. Alternatively, TD-FiberNDFD also was calculated as NDFn \times (NDFD/100), where NDFn was expressed as a percentage of DM and NDFD was expressed as a percentage of NDF. Following quantification of TD-FiberLIG or TD-FiberNDFD, TDN was summed over the various component parts, thereby yielding 2 respective estimates of energy for further comparison (TDN-LIG and TDN-NDFD). In practice, these estimates differed only on the basis of methodology for calculating TD-Fiber.

**Statistics**

**Regression of TDN-NDFD on TDN-LIG.**

Initially, TDN-LIG and TDN-NDFD were related directly using linear regression analysis, with TDN-LIG identified as the independent variable (PROC REG; SAS Institute, 1990). An additional test statement was included to compare the slope to unity, which would be an essential criterion for ideal agreement between methods. Subsequently, this approach illustrated that there was bias between methods and justified a more in-depth evaluation of the relationship between various truly digestible energy subunits that compose TDN and indices of heating (HDD or MAX).

**Regressions of TDN and Truly Digestible Subunits on HDD and MAX.**

Mean concentrations of TD-NFC, TD-CP, TD-FiberLIG, TD-FiberNDFD, TDN-LIG, and TDN-NDFD from 32 combinations of bale moisture and diameter taken from the core of large round bales were pooled from the LM, IM, and HM harvests and then regressed against HDD or MAX. Each data point represented the mean of 3 bales; for each treatment combination of bale moisture and diameter, 1 bale was obtained from each of 3 field blocks that were established on the basis of field topography (slope). Although similar across harvests, prestorage concentrations of these total or partial energy components were not identical; therefore, data were normalized before regression analysis as the simple mathematical difference resulting from storage (poststorage – prestorage; i.e., ΔTD-NFC, ΔTD-CP, ΔTD-FiberLIG, ΔTD-FiberNDFD, ΔTDN-LIG, and ΔTDN-NDFD), where positive and negative values indicate increased and decreased concentrations, respectively. These variables were then regressed against HDD and MAX using nonlinear regression models (PROC NLIN; SAS Institute, 1990) of the general form

\[
Y = b \times (e^{-kx}) - a \quad \text{if these } \Delta \text{ response variables became negative with spontaneous heating, or } Y = a - (b \times e^{-kx}) \quad \text{if they became positive.}
\]

For these model assessments, \( k \) was the rate constant, \( x \) was the independent variable (HDD or MAX), and \( a \) and \( b \) were parameters determined directly by the regression model. For nonlinear regression models, the independent variables (HDD or MAX) also were squared in an attempt to improve fit. Linear, quadratic, cubic, and quartic responses to HDD or MAX also were evaluated by PROC REG of SAS (SAS Institute, 1990). Generally, selection of the most appropriate model was based on the greatest coefficient of determination (R²); however, polynomial regression models were not selected if the coefficient or slope for the highest ordered term did not differ from zero.

**RESULTS AND DISCUSSION**

**Regressions of TDN-LIG on TDN-NDFD**

Concentrations of summative energy equation subunits, as well as TDN-LIG and TDN-NDFD, which were derived for hays from the LM, IM, and HM harvests, are summarized in Table 1. For estimated hay energies, the bias between estimates of TDN determined by TDN-NDFD or TDN-LIG is illustrated by simple linear regression (Figure 1). When TDN-LIG was designated as the independent variable, the resulting slope (0.73) differed from unity \((P < 0.001)\) and the intercept (15.4) differed \((P < 0.001)\) from zero; however, the coefficient of determination was very high \((R^2 = 0.953)\), indicating the 2 methods were associated closely. Generally, these findings suggest that the bias between the 2 methods of calculating TDN was greatest for low-TDN hays, which had previously incurred the most severe spontaneous heating. Under these circumstances, estimates of TDN-NDFD were consistently greater than those derived from TDN-LIG; however, this bias was largely undetectable when spontaneous heating was minimal and TDN exceeded about 55%.
Regressions of ΔTD-NFC on both HDD (Figure 2a) and MAX (Figure 2b) were best fitted to nonlinear decay models in which the independent variable was squared. In both cases, ΔTD-NFC declined rapidly with low or modest heating before becoming asymptotic at approximately 660 HDD or 60°C MAX when ΔTD-NFC = −6.2 or −6.4 percentage units of TDN, respectively. Both measures of spontaneous heating (HDD and MAX) explained approximately 70% of the variation within ΔTD-NFC (R² = 0.686 and 0.712, respectively). In the summative model (NRC, 2001), NFC is estimated as residual DM, which is not included within pools of NDFn, CP, ether extract, or ash; therefore, NFC represents primarily plant sugars that are known to be oxidized when hays heat spontaneously. In practice, the responses exhibited by ΔTD-NFC in regressions on HDD and MAX are essentially mirror-opposite responses from those exhibited by concentrations of NDF for these same hays (Coblentz and Hoffman, 2009b). Concentrations of NDF within heated hays increase primarily by losses of nonfiber constituents, whereas true fiber components remain largely inert (Rotz and Muck, 1994). In a previous study (Coblentz et al., 1997), alfalfa hay packaged at 29.7 and 20.2% moisture within small rectangular bales exhibited poststorage concentrations of total nonstructural carbohydrates of 2.1 and 4.2% of DM, which were only 35 and 58%, respectively, of the concentrations reported for corresponding prestorage controls (Coblentz et al., 1997). These data suggested that oxidation (losses) of nonstructural carbohydrates can be substantial as a consequence of spontaneous heating, or storage, or both, even when heating is relatively limited in scope. More importantly, the oxidative losses observed in the current study, as well as past studies, have serious consequences with respect to the final energy estimates of the hay because the true digestibility coefficient for NFC is 0.98 (Weiss et al., 1992; NRC, 2001), which is based on Lucas-test analysis of neutral detergent solubles from cattle and sheep fed at maintenance (Van

**Table 1.** Concentrations of truly digestible NFC (TD-NFC), truly digestible CP (TD-CP), truly digestible fiber (TD-Fiber) determined by 48-h NDF digestibility (TD-FiberNDFD), TD-Fiber determined by lignin approximation (TD-FiberLIG), and total digestible nutrients (TDN) determined by the 2 methods of calculating TD-Fiber (TDN-LIG and TDN-NDFD) summarized from round bales of alfalfa-orchardgrass hay made from 3 harvests during 2006 and 2007 at Stratford, Wisconsin

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<th>Number</th>
<th>Bales</th>
<th>Mean</th>
<th>SE</th>
<th>Mean</th>
<th>Maximum</th>
<th>Minimum</th>
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<td>60.9</td>
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1Harvest; HM = high moisture (26.7 to 46.6%); IM = intermediate moisture (16.8 to 24.2%); LM = low moisture (9.3 to 17.3%).
2Number of interactive treatments during each harvest. Harvest HM contained 1 baling treatment made at 26.7% moisture at the 0.9-m bale diameter only, whereas harvest LM contained 1 dry control made at 9.3% moisture at the 1.2-m bale diameter only. These additional treatments were made at only 1 diameter because insufficient forage was available to complete the entire factorial arrangement of treatments (bale diameters) at these moisture concentrations. Each interactive treatment represents the mean of 3 bales.
3Total numbers of bales made per harvest.
4Overall mean of all interactive bale moisture × bale diameter treatments.
5Standard error of the overall mean of all interactive bale moisture × bale diameter treatments.
6Maximum values across all interactive treatments.
7Minimum values across all interactive treatments.
8Standard error of the interactive (bale moisture × bale diameter) mean.
Soest, 1982). Therefore, NFC approaches ideality as a feed fraction, implying near complete bioavailability, and any loss of NFC as a consequence of spontaneous heating results in a near identical and direct loss of TDN units from the final energy estimate for the hay.

**TD-CP.** Regressions of \( \Delta TD-CP \) on HDD (Figure 3a) and MAX (Figure 3b) indicate that \( \Delta TD-CP \) was positive with minimal to modest spontaneous heating that ranged generally up to 500 HDD or 60°C MAX, but became negative as spontaneous heating became more extreme. For HDD, data were best fitted \((R^2 = 0.812)\) to the nonlinear decay model with the independent variable squared. For this regression, the curve became asymptotic at approximately 1,450 HDD when \( \Delta TD-CP = -2.6 \) percentage units of TDN. Although \( \Delta TD-CP \) generally exhibited a similar response pattern when regressed on MAX, a cubic polynomial model provided the best fit \((R^2 = 0.864)\).

Generally, the concentration of CP increases slightly in heated hays, particularly in the short term \(<60\) d; Rotz and Muck, 1994). Mostly, this occurs because forage carbohydrates, particularly sugars, are preferentially oxidized during storage, thereby concentrating CP indirectly. In our study, averaged over all treatments and harvests, the average change in CP concentration was \(1.0 \pm 0.67\) percentage units during storage (Coblentz et al., 2010), which supports the premise established by Rotz and Muck (1994). A second factor affecting estimates of TD-CP derived from summative energy equations is ADICP (Weiss et al., 1992; NRC, 2001). When expressed as a proportion of CP, ADICP is highly correlated with digestibility of forage CP and this relationship is used to estimate TD-CP (Thomas et al., 1982; Weiss et al., 1983). In the summative model, TD-CP in forages is calculated as \( CP \times e^{-1.2 \times (ADICP/CP)} \), where ADICP is expressed as a percentage of DM (Weiss et al., 1992; NRC, 2001). In practice, the expression \( e^{-1.2 \times (ADICP/CP)} \) approaches 1.0 when ADICP is in its native form within unheated alfalfa hays and composes a relatively small proportion of the total CP pool. Over several studies (Hoffman et al., 1993; Coblentz et al., 1996; Coblentz et al., 1998; Ogden et al., 2006), concentrations of ADICP have ranged between 0.7 and 1.6% of DM for oven-dried alfalfa forages and unheated hays. Within these forages, the corresponding coefficient generated by \( e^{-1.2 \times (ADICP/CP)} \) ranged narrowly from 0.90 to 0.96, thereby indicating that TD-CP is driven primarily by the concentration of CP within any specific unheated alfalfa forage. In our present study with heated hays, actual concentrations of ADICP ranged broadly from 1.1 to 4.5% of DM (Coblentz et al., 2010), yielding values for the \( e^{-1.2 \times (ADICP/CP)} \) term that varied from 0.94 for unheated hays to a minimum of 0.76 for those hays incurring the most extreme spontaneous heating. As a result, estimates of \( \Delta TD-CP \) were positive with low or limited heating, largely in response to slight increases in concentrations of CP coupled with relatively stable estimates of \( e^{-1.2 \times (ADICP/CP)} \). At more
extreme levels of heating, the coefficient generated by $e^{-1.2 \times (ADICP/CP)}$ decreased by as much as 19%, thereby resulting in negative estimates of $\Delta$TD-CP.

$TD_{-Fiber}$. Regressions of $\Delta$TD-FiberLIG or $\Delta$TD-FiberNDFD on HDD (Figure 4a) depict somewhat contrasting responses to spontaneous heating. The relationship between $\Delta$TD-FiberLIG and HDD was best fitted to the nonlinear decay model with the independent variable squared, which explained approximately two-thirds of the variation in the data ($R^2 = 0.676$).

Figure 2. Nonlinear regressions of the change in truly digestible NFC (poststorage − prestorage; $\Delta$TD-NFC) on a) heating degree days >30°C and b) maximum internal bale temperature. The mean prestorage concentration of TD-NFC (weighted on the basis of the number of treatments from the low, intermediate, and high moisture harvests) was 25.6%, which corresponds generally to $\Delta$TD-NFC = 0 on the y-axis. TDN = total digestible nutrients.
In this relationship, ΔTD-FiberLIG remained positive, indicating that TD-FiberLIG had increased relative to prestorage estimates until approximately 400 HDD were accumulated. After that point, ΔTD-FiberLIG was negative over the remainder of the heating continuum and became asymptotic at approximately 1,500 HDD when ΔTD-FiberLIG = −3.2 percentage units of TDN. In contrast, the regression of ΔTD-FiberNDFD...
1. NDFD is a relatively imprecise and variable measurement typically exhibiting relatively high standard errors; 2. NDFD is 1 of only 2 factors multiplied directly to determine TD-FiberNDFD; and 3) there was little evidence to suggest that NDFD was affected by spontaneous heating, particularly within bales incurring low to modest levels of HDD or MAX (Coblentz and Hoffman, 2009b).

**TDN**

Estimates of ΔTDN-LIG and ΔTDN-NDFD were related to HDD (Figure 5a) with a nonlinear decay model in which HDD was squared and with a quadratic regression model, respectively. Both relationships were characterized by high coefficients of determination ($R^2 \geq 0.861$), indicating that the poor relationship between ΔTD-FiberNDFD subunit and HDD that was discussed previously had only limited effects on the overall estimation of TDN. For ΔTDN-LIG, the nonlinear regression curve became asymptotic at approximately 1,300 HDD when ΔTDN-LIG = −11.6 percentage units of TDN. These responses were about 2.0 to 4.0 percentage units more negative than the corresponding regression for ΔTDN-NDFD whenever heating exceeded about 500 HDD. For regressions on MAX (Figure 5b), both ΔTDN-LIG and ΔTDN-NDFD were best fitted by linear models; in both cases, coefficients of determination were high ($R^2 = 0.954$ and 0.848, respectively), again suggesting that simple measures of spontaneous heating are excellent predictors of energy depressions for heated forages. Although both regressions on MAX were linear, the general relationship between ΔTDN-LIG and ΔTDN-NDFD was consistent with that observed for relationships with HDD. The slope associated with ΔTDN-LIG was more negative than that observed for ΔTDN-NDFD ($-0.38$ vs. $-0.25$; $P < 0.001$), and respective intercepts for these regressions differed ($16.3$ vs. $10.2$; $P < 0.001$). As a result, ΔTDN-LIG was consistently more negative than ΔTDN-NDFD when MAX exceeded $45^\circ C$, reaching a maximum differential of $-3.9$ percentage units at the greatest MAX ($77.2^\circ C$).

**Importance of Using NDFn**

Although our calculations of TD-FiberNDFD (and subsequently TDN-NDFD) have been based strictly on NDFn, NRC (2001) guidelines and associated programming codes specify only that TD-FiberNDFD can be estimated using NDFD as a digestibility coefficient for NDF. Intuitively, use of NDFn is logical for calculating TD-FiberNDFD because contributions made by NDICP to TDN already have been considered during estimation of the TD-CP energy subunit. In practice,
the concentration of NDICP is relatively low in most unheated dairy-quality forages and it is evaluated inconsistently throughout commercial forage evaluation packages. Therefore, actual values for NDICP are commonly unavailable or approximated, or the protein correction of NDF is ignored entirely when calculating TD-FiberNDFD and, subsequently, TDN-NDFD. Across the LM, IM, and HM harvests, prestorage means...
for NDICP ranged from 3.5 to 3.9% of DM (18.4 to 21.9% of CP; Coblentz et al., 2010); therefore, protein correction of NDF likely has only limited relevance for estimates of TD-FiberNDFD from unheated forages. However, within severely heated hays, concentrations of NDICP ranged as high as 9.3% of DM (47.6% of CP; Coblentz et al., 2010), thereby greatly increasing the potential for discrepancy between methods. This

Figure 5. Nonlinear regressions of the changes (poststorage − prostorage) in total digestible nutrients (TDN) with truly digestible fiber estimated by lignin equation (ΔTDN-LIG; ●, thick line) and by 48-h NDF digestibility (ΔTDN-NDFD; ○, thin line) on a) heating degree days >30°C and b) maximum internal bale temperature. The mean prestorage concentrations of TDN-LIG and TDN-NDFD weighted on the basis of the number of treatments from the low, intermediate, and high moisture harvests were 57.9 and 57.7%, respectively, which correspond generally to ΔTDN-LIG and ΔTDN-NDFD = 0 on the y-axis.
Figure 6. Regressions of the changes (poststorage − prestorage) in concentrations of total digestible nutrients (TDN) on spontaneous heating when truly digestible fiber was estimated from NDF (rather than protein-corrected NDF) with 48-h NDF digestibility as the corresponding digestibility coefficient (ΔTDN-NDFD_{uncorr}; gray dot, gray line). Responses are regressed on a) heating degree days >30°C and b) maximum internal bale temperature. The mean prestorage concentration of TDN-NDFD_{uncorr}, weighted on the basis of the number of treatments from the low, intermediate, and high moisture harvests, was 59.5% and corresponds generally to ΔTDN-NDFD_{uncorr} = 0 on the y-axis. Regression lines for change in TDN with truly digestible fiber estimated by lignin equation (ΔTDN-LIG; thick black line) and by 48-h NDF digestibility (ΔTDN-NDFD; thin black line) are described in Figure 5 and are provided here for reference.
concept is illustrated in Figure 6, where NDF, rather than NDFn, was used to calculate TDN-NDFD (TDN-NDFD\textsubscript{uncorr}). Changes (poststorage – prestorage) in TDN-NDFD\textsubscript{uncorr} during storage (ΔTDN-NDFD\textsubscript{uncorr}) were regressed on HDD yielding a simple linear relationship that explained approximately three-fourths of the variation within the data (Y = −0.0044x + 0.3; R\textsuperscript{2} = 0.748; Figure 6a). For the regression on MAX, a quadratic response, without the linear term, was the most appropriate response model (Y = −0.0017x\textsuperscript{2} + 3.5; R\textsuperscript{2} = 0.747; Figure 6b). Generally, these regressions illustrate that deviations by ΔTDN-NDFD\textsubscript{uncorr} from ΔTDN-LIG were approximately twice those observed for ΔTDN-NDFD for any hay incurring at least modest spontaneous heating. As a result, the regression curve established for ΔTDN-NDFD\textsubscript{uncorr} ranged up to 7 percentage units of TDN higher than that observed for ΔTDN-LIG. As such, any use of the NDFD option for determining TD-Fiber and, subsequently, TDN, should use NDFn if there is any corroborating evidence suggesting that hays heated during storage.

**IMPLICATIONS**

Estimates of TDN from hays are reduced as a consequence of spontaneous heating during storage. Most of the reduced concentrations of energy within these heated forages are associated with losses of TD-NFC, which occurs primarily via oxidation of sugars. For heated forages, some discrepancy exists between estimates of TD-FiberLIG compared with those estimated by the alternative TD-FiberNDFD approach. The poor association between NDFD and spontaneous heating caused only minimal changes in TD-FiberNDFD over an extensive range of heating. In contrast, TD-FiberLIG declined in close association with heating, largely because this approximation is sensitive to changes in both NDFn and acid detergent lignin. Discrepancies between TDN-LIG and TDN-NDFD were exacerbated further when NDF rather than NDFn was used to estimate TD-FiberNDFD. As such, any use of the NDFD option for determining TD-Fiber, and subsequently TDN, should use NDFn if there is any other evidence suggesting that hays were heated during storage. In severely heated hays from large round bales, estimates of TDN declined by as much as 13.0 percentage units during storage, and this is a serious consequence of spontaneous heating.

**REFERENCES**