The Relationship Between Acid Detergent Insoluble Nitrogen and Nitrogen Digestibility in Lactating Dairy Cattle

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ABSTRACT

Five trials (19 treatments) conducted at the University of Nebraska-Lincoln (UNL) on lactating dairy cattle were analyzed to determine how the concentration of ADIN in the ration affects total tract N digestibility. Additionally, 6 published studies (13 treatments) were included to expand the data set. Results from the UNL trials showed that as the concentration of ADIN in the ration increased, the digestibility of ADIN also increased. However, the relationship was poor ($r^2 = 0.29$). To account for random effects among trials, a meta-analysis was conducted. In the UNL trials, as the ration concentration of ADIN increased, total tract N digestibility decreased; this relationship was moderate ($r^2 = 0.55$). A meta-analysis of the published studies illustrated similar results with a moderate correlation ($r^2 = 0.44$). All data were combined for a meta-analysis, and similar results illustrated a moderate relationship ($r^2 = 0.58$). There was positive relationship between ADF and the concentration of ADIN in the UNL trials; however, the relationship was poor ($r^2 = 0.19$). Additionally, a poor, negative relationship ($r^2 = 0.14$) was observed between ADF and N digestibility in the UNL trials. Milk yield ($31.9 \pm 3.1$ kg/d) in the UNL trials was unaffected ($r^2 = 0.01$) by the concentration of ADIN in the ration. These data suggest ADIN is partially digestible, N digestibility is moderately influenced by ADIN concentration in rations, there is a poor relationship between ADF and the concentration of ADIN, and milk yield is unaffected by the concentration of ADIN.

Key words: acid detergent insoluble nitrogen, dairy cattle, meta-analysis, nitrogen digestibility

INTRODUCTION

The concentration of ADIN in feedstuffs is commonly used to determine the degree of heat damage. Specifically, this is estimated by determining the amount of insoluble N in ADF residue (Firkins et al., 1984). Several sources have reported variable ADIN levels, ranging from 0.4% of CP for corn gluten feed and cottonseed meal to 31.1% of CP for dried distillers grains with solubles (DDGS) for forages and concentrates in wet and dried forms (Pena et al., 1986; Clark et al., 1987; Edionwe and Owen, 1989; Weiss et al., 1989; NRC, 2001; Kleinschmit et al., 2007; Vargas-Bello-Pérez et al., 2007).

In the Cornell Net Carbohydrate and Protein System, the assumption is made that ADIN represents the portion of protein in a feedstuff that is unavailable for use by the animal because it is completely indigestible (Sniffen et al., 1992). Additionally, Van Soest (1994) noted that as the concentration of ADIN increased, total tract N digestibility decreased. This negative association has been observed in several studies (Yu and Thomas, 1976; Thomas et al., 1982; Weiss et al., 1986; Van Soest, 1994). However, these observations were made with forages, and the relationship in other feedstuffs remains unclear. Additionally, the association in forages is generally a one-to-one reduction in N digestibility as ADIN increases, indicating that ADIN is indigestible. However, limited evidence in concentrates and diets fully supports the assumption that ADIN is indigestible (Weiss et al., 1986; Weiss et al., 1989; Nakamura et al., 1994). Nakamura et al. (1994) observed that ADIN was approximately 58% digestible in nonforage fiber sources, suggesting that ADIN is digestible and that it is inaccurate to assume ADIN is indigestible. Thus, it may be useful to evaluate diets that contain corn-milling coproducts.
Limited research has been conducted on how the concentration of ADIN in rations of lactating dairy cattle affects total tract N digestibility. The objective of this research is to use a statistical meta-analysis approach to determine the relationship between the concentration of ADIN on total tract N digestibility in rations fed to ruminants. It is hypothesized that as the concentration of ADIN increases in the ration, N digestibility will decrease, and that ADIN is partially digestible.

**MATERIALS AND METHODS**

**Animals and Dietary Treatments**

Data from 5 trials, with a total of 19 dietary treatments, were collected from the University of Nebraska-Lincoln (UNL) Dairy Research Unit (Mead, NE) and were used to evaluate the relationship between the concentration of ADIN in the ration and total tract N digestibility. In these trials, DMI was measured and fecal output was estimated on each animal within period for each experiment. The following is a brief description of the studies.

**Trial 1.** Four multiparous Holstein cows were fed 1 of 2 diets (Table 1) in each of 3 periods. The 2 dietary treatments included 1) a control diet with no inclusion of coproducts, and 2) 30% DDGS.

**Trial 2.** Twenty primiparous and 20 multiparous Holsteins were fed 1 of 5 diets (Table 1) in each of 5 periods. The 5 dietary treatments included 1) a control diet with no inclusion of coproducts, 2) 15% wet distillers grains with solubles (WDGS), 3) 15% wet corn gluten feed (WCGF), 4) 7.5% WDGS and 7.5% WCGF, and 5) 15% WDGS and 15% WCGF (Gehman and Kononoff, 2008).

**Trial 3.** Four lactating Holstein heifers were fed 1 of 4 diets (Table 1) in each of 4 periods. The 4 dietary treatments included 1) a control diet with no inclusion of coproducts, 2) 15% DDGS, and 3) 15% HP-DDG = high-protein dried distillers grains, 15% DDGS; Germ = corn germ, 15% DM; HP-DDG = high-protein dried distillers grains, 15% DM (no solubles included).

**Trial 4.** Twenty-eight lactating Holsteins were fed 1 of 4 diets (Table 2) in each of 4 periods. The 4 dietary treatments included 1) sorghum silage and 0% WCGF, 2) sorghum silage and 30% WCGF, 3) 25% WDGS and 18.3% corn silage, and 4) 25% WDGS and 15.8% alfalfa haylage.

**Trial 5.** Twenty lactating Holsteins were fed 1 of 4 diets (Table 2) in each of 4 periods. The 4 dietary treatments included 1) sorghum silage and 0% WCGF, 2) sorghum silage and 30% WCGF, 3) 25% WDGS and 18.3% corn silage, and 4) 25% WDGS and 15.8% alfalfa haylage.
To increase the size of the data set, additional observations were added from 6 published studies that reported the concentration of ADIN in the ration and total tract N digestibility (MacGregor et al., 1983; Edionwe and Owen, 1989; Weiss et al., 1989; Dann et al., 2006, 2007; Vargas-Bello-Pérez et al., 2008). Additionally, various feedstuffs such as barley, soybean silage, alfalfa silage, corn dried distillers grains, beet pulp, and a corn milling coproduct mix were included in the rations of the published studies.

Feed and Fecal Chemical Analysis

Samples of the TMR collected at the UNL Dairy Research Unit were composited by trial and treatment within each period. A 0.5-g sample of each dietary treatment within each period from every trial was weighed in triplicate into Ankom bags (Ankom Technology, Fairport, NY) and sealed. Each TMR sample was analyzed for DM and ADF. Acid detergent fiber was analyzed using an Ankom Fiber Analyzer (Ankom Technology). Coefficient of variation was determined after ADF analysis, and samples with a CV greater than 5% were eliminated. For samples that were eliminated, new samples were prepared, and DM and ADF were analyzed again. Residues with a CV of less than 5% remaining in the Ankom bags were combined into Whirl-Pak bags (VWR International, West Chester, PA) by dietary treatment within each period. Crude protein analysis was conducted on the ADF residue for each treatment within period to determine the amount of acid detergent insoluble CP in each TMR. Crude protein was determined using the Leco Tru-Spec N Analyzer (St. Joseph, MO). Each dietary treatment within period had only one measurement of acid detergent insoluble CP because of the lack of sample remaining after ADF analysis. Acid detergent insoluble N of TMR samples was determined from the protein analysis on the ADF residue by using the equation

$$\text{ADIN (\% DM)} = \frac{\text{ADICP (\% DM)}}{6.25}, \quad [1]$$

where ADIN (\% DM) is the ration ADIN concentration calculated after CP analysis, and ADICP (\% DM) is the acid detergent insoluble CP analyzed from ADF residue.

Fecal samples were composited by cow within each period for all trials and analyzed for ADIN by using the same procedures as the TMR samples. Fecal ADIN was determined from the protein analysis on the ADF residue by using equation [1].

ADIN Digestibility

Acid detergent insoluble N digestibility was calculated by treatment within period. Digestibility calculations for ADIN were as follows:

| Ingredient, % DM | Treatment2 | Trial 4 | | Treatment2 | Trial 5 | |
|-----------------|-----------|--------|--------|-----------|--------|
| C-CS | C-AH | W-CS | W-AH | C-C | C-WCGF | BMR-C | BMR-WCGF |
| WCGF | — | — | 25.2 | 25.2 | — | 29.8 | — | 29.8 |
| WDGS | — | — | — | — | 27.0 | 16.7 | — | — |
| Control sorghum silage | — | — | — | — | — | — | 27.0 | 16.7 |
| BMR sorghum silage | 31.5 | 17.4 | 18.3 | 7.9 | 19.1 | 16.7 | 19.1 | 16.7 |
| Corn silage | 15.8 | 34.7 | 9.2 | 15.8 | — | — | — | — |
| Alfalfa haylage | — | — | — | — | — | 6.2 | 4.2 | 6.2 |
| Alfalfa hay | 12.3 | 5.5 | 15.4 | 16.0 | 5.2 | 4.2 | 5.2 | 4.2 |
| Brome hay | 13.8 | 23.3 | 8.4 | 14.9 | 20.8 | 14.9 | 20.8 | 14.9 |
| Ground corn | 9.7 | 4.6 | 2.1 | 1.3 | 8.6 | 7.3 | 8.6 | 7.3 |
| Soybean meal | 9.0 | 4.4 | 15.6 | 13.8 | 3.2 | — | 3.2 | — |
| Soybean hulls | 5.7 | 8.2 | 3.5 | 3.1 | 5.3 | 2.9 | 5.3 | 2.9 |
| Urea | — | — | — | — | 1.0 | — | 1.0 | — |
| Vitamins and minerals | 2.4 | 2.9 | 2.5 | 2.1 | 3.5 | 3.5 | 3.5 | 3.5 |

1University of Nebraska-Lincoln.
2Trial 4: C-CS = 0% DM wet distillers grains with solubles (WDGS), 31.5% corn silage; C-AH = 0% WDGS, 34.7% alfalfa haylage; W-CS = 25% WDGS, 18.3% corn silage; W-AH = 25% WDGS, 15.8% alfalfa haylage. Trial 5: C-C = control sorghum silage, 0% wet corn gluten feed (WCGF); C-WCGF = control sorghum silage, 30% WCGF; BMR-C = brown mid-rib (BMR) sorghum silage, 0% WCGF; BMR-WCGF = BMR sorghum silage, 30% WCGF.
3LignoTech (Overland Park, KS).
[(TMR ADIN × DMI) − (FO × fecal ADIN)] /
(TMADIN × DMI),

where TMR ADIN is ADIN content
of the ration, DMI is feed intake, FO
is fecal output and was estimated by
indigestible ADF, and fecal ADIN is
ADIN content of the fecal matter. Af-
ter ADIN digestibility was determined
for each cow within each period, an
average ADIN digestibility was cal-
culated for each dietary treatment in
each trial.

Total Tract Nitrogen
Digestibility

Nitrogen digestibility was calcu-
lated by treatment within period. The
digestibility calculation for N was as
follows:

\[
\frac{(DMI \times \text{feed } N) - (FO \times \text{fecal } N)}{(DMI \times \text{feed } N)},
\]

where DMI is feed intake, feed N is
N content of the feed; FO is fecal
output, and fecal N is N content in
the fecal matter. After N digestibility
was determined for each cow within
period, an average N digestibility was
calculated for each dietary treatment
in each trial.

Statistical Analysis

Data from the UNL trials and the
published studies were analyzed sta-
tistically by using the mixed modeling
methodology of SAS (St-Pierre, 2001)
to account for random effects of trials
in the concentration of ADIN in the
ration and total tract N digestibility.
The linear model for this analysis was
as follows:

\[
Y_{ij} = \beta_0 + s_i + \beta_i X_{ij} + b_i X_{ij} + \varepsilon_{ij},
\]

where \(Y_{ij}\) is the experiment-adjusted
outcome for dependent variable, total
tract N digestibility, observed at level
j of the continuous variable, ADIN,
in experiment i; \(\beta_0\) is the overall
intercept across all experiments; \(s_i\)
is the random effect of experiment i;
\(\beta_i\) is the overall regressing coefficient
across all experiments; \(X_{ij}\) is the value
of j of the continuous variable in
experiment i; \(b_i\) is the random effect
of experiment i on the regression coef-
ficient in experiment i; and \(\varepsilon_{ij}\) is the
unexplained residual error of j of the
continuous variable on experiment i.

The power of the relationship between
2 variables was determined using \(r^2\)
values.

RESULTS AND DISCUSSION

Acid Detergent Insoluble
Nitrogen Digestibility

The concentration of ration ADIN
was determined for each dietary treat-
ment in the UNL trials and ranged
from 21.5 to 87.4% of N (Table 3).
The concentration of ADIN in the
published studies was variable as well.
However, the UNL trials had a con-
centration of ADIN that was higher
compared with the published studi-
es. The difference in concentration
of ADIN between the UNL trials and
the published studies may be due to
the difference in diets fed during the
trials, the amount of forage included
in the diets, or the method used for
analyzing ADIN.

Acid Detergent Insoluble
Nitrogen Digestibility

The mean ADIN digestibility from
UNL trials was approximately 58.0%.
The digestibility of ADIN in the UNL
trials was similar to that of Nakamura
et al. (1994), who observed ADIN to
be approximately 58% digestible when
nonforage protein sources were fed to
steers. In the UNL trials, ADIN di-
gestibility ranged from approximately
41.0 to 74.0%. Figure 1 illustrates the
relationship between the concentra-
tion of ADIN in the ration and ADIN
digestibility. As the concentration of
ADIN increased in the ration, ADIN
digestibility also increased \([y = 45.6 +
0.3 (ADIN)]\). However, this rela-
tionship is rather poor \((r^2 = 0.29)\),
indicating that other possible factors,
such as animal variation, level of
DMI, or level of the feedstuffs in-
cluded in the TMR may affect ADIN
digestibility.

Total Tract Nitrogen
Digestibility

Total tract N digestibility from the
UNL trials ranged from approximately
49.0 to 73.0% across treatments
before the meta-analysis. The mean
total tract N digestibility was ap-
proximately 63%.

The statistical meta-analysis sug-
gested there is a negative relationship
between the concentration of ADIN
in the ration and study-adjusted total
tract N digestibility from UNL trials
(Figure 2). As the concentration of
ADIN increased in the ration, total
tract N digestibility decreased, with
a moderate relationship \((r^2 = 0.55)\).
These results can be compared with
the UNL trials before a meta-analysis
was conducted (Figure 3). Although
there was a negative relationship
before the meta-analysis, the relation-
ship was poor \((r^2 = 0.11)\).

A meta-analysis was also con-
ducted on the observations from the
published studies. The relationship
between the concentration of ADIN
in the ration and study-adjusted total
tract N digestibility from the pub-
lished studies is illustrated in Figure
4. As the concentration of ADIN in
the ration increased, total tract N
digestibility also decreased; this rela-
tionship was moderate \((r^2 = 0.44)\).

An additional meta-analysis was
conducted to increase the number of
plotted observations. This data set
combined both the UNL trials and
published studies. Figure 5 illustrates
this relationship. As the ration con-
centration of ADIN increased, total
tract N digestibility decreased, as ob-
served in the previous meta-analyses,
and this relationship was moderate
\((r^2 = 0.58)\).

Van Soest (1994) illustrated the re-
lationship between the concentration
of ADIN in the ration and apparent
N digestibility from work conducted
on cattle and sheep, as observed by
Yu and Thomas (1976). Results sug-
gested that a negative relationship
existed between the concentration of
Acid detergent insoluble nitrogen and nitrogen digestibility

The relationship between unheated forages was moderate ($r^2 = 0.51$), whereas the relationship was stronger in heated forages ($r^2 = 0.91$). Additionally, the slope in both types of forages was close to or greater than $-1.0$. Acid detergent insoluble N is completely indigestible when a negative one-to-one relationship between the concentration of ADIN and N digestibility exists and the slope is close to $-1.0$ (Van Soest, 1994). The slope illustrated by Van Soest (1994) in unheated and heated forages was greater than the slope from the combined data ($-0.01$) from the UNL trials and published studies (Figure 5). Biologically, a greater slope means a smaller

### Table 3. Dry matter intake, CP, NDF, ADF, ADIN, and milk yield for each UNL trial

<table>
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<th>Diet</th>
<th>DMI, kg/d</th>
<th>CP, %</th>
<th>NDF, %</th>
<th>ADF, %</th>
<th>ADIN, % of N</th>
<th>Milk yield, kg/d</th>
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1UNL = University of Nebraska-Lincoln.

2Control = 0% DM coproducts. Trial 1: DDGS = dried distillers grains with solubles, 30% DM. Trial 2: WDGS = wet distillers grains with solubles, 15% DM; WCGF = wet corn gluten feed, 15% DM; 15% Mix = 7.5% DM WDGS plus 7.5% DM WCGF; 30% Mix = 15% DM WDGS plus 15% DM WCGF. Trial 3: DDGS = 15% DM DDGS; Germ = corn germ, 15% DM; HP-DDG = high-protein dried distillers grains, 15% DM (no solubles included). Trial 4: C-CS = 0% DM WDGS, high-corn silage; C-AH = 0% WDGS, high-alfalfa haylage; W-CS = 25% WDGS, high-corn silage; W-AH = 25% WDGS, high-alfalfa haylage. Trial 5: C-C = control sorghum silage, 0% WCGF; C-WCGF = control sorghum silage, 30% WCGF; BMR-C = brown mid-rib (BMR) sorghum silage, 0% WCGF; BMR-WCGF = BMR sorghum silage, 30% WCGF.

![Figure 1. Relationship between total tract ADIN (% of N) and ADIN digestibility from University of Nebraska-Lincoln trials.](image-url)
change in the concentration of ADIN in the ration will have a greater impact on total tract N digestibility. Therefore, a small increase in concentration of ADIN in the ration will decrease total tract N digestibility at a faster rate with a greater slope.

Another study conducted on sheep presented effects (Nakamura et al., 1994) similar to our findings. As the concentration of ADIN in the ration increased, true N digestibility decreased; this relationship was moderate ($r^2 = 0.66$). The slope was $-0.4$, indicating ADIN was digestible (Nakamura et al., 1994).

**Acid Detergent Fiber**

Acid detergent fiber residue is useful for measuring the amount of insoluble N in a feedstuff (Van Soest, 1994). Therefore, it may be possible that as the amount of ADF increases in feedstuffs, the concentration of ADIN increases as well. However, based on the UNL trials, this did not occur (Figure 6). As ADF increased, ADIN as a percentage of N increased as well; however, the relationship between these 2 variables was poor ($r^2 = 0.19$). This suggests that other factors may assist in determining the concentration of ADIN.

The use of ADF as a predictor of digestibility is based on statistical association, not on a theoretical basis (Van Soest, 1994). Therefore, ADF may not be used to predict digestibility accurately, such as N digestibility. The relationship between ADF and N digestibility (Figure 7) in the UNL trials was poor ($r^2 = 0.14$), indicating that ADF cannot be used to predict digestibility. Although as ADF increased, N digestibility decreased, other factors were hard to determine that might better explain N digestibility.

**Milk Yield**

In cases in which the intake of metabolizable protein is limiting, field nutritionists might suspect that feeding diets higher in ADIN might negatively affect protein availability.
and, in turn, milk production. Figure 8 illustrates the lack of relationship between the concentration of ADIN and milk yield in the UNL trials, where mean milk yield was 31.9 ± 3.1 kg/d. Although the slope was positive (0.02), the relationship was very poor \( r^2 = 0.01 \), indicating this relationship did not exist. This observation might suggest that metabolizable protein was adequate in all diets; it is equally likely that factors such as energy balance were more important than ADIN in affecting milk yield. Weiss et al. (1989) evaluated the performance of lactating dairy cattle fed barley distillers grains. Cattle consumed either a control diet containing soybean meal, a diet containing soybean meal plus barley distillers grains (5% of DM), or a barley distillers grains diet (18% of DM). Acid detergent insoluble N was also measured (1.3, 1.6, and 2.5% of CP for the soybean meal diet, the soybean meal plus barley distillers grains diet, and the barley distillers grains diet, respectively). The ADIN increased in the diet as barley distillers grains increased, and there was no significant difference in milk yield (20.4 ± 0.8 kg/d), milk protein (3.4 ± 0.1%), or milk fat (3.7 ± 0.1%). The results in the experiment by Weiss et al. (1989) were similar to the results in the UNL trials.

**IMPLICATIONS**

Acid detergent insoluble N may not be a good indicator of unavailable protein. Based on this research, total tract ADIN is approximately 58% digestible. There is a moderate, negative relationship between the concentration of ADIN and total tract N digestibility. Consequently, there is not a one-to-one negative relationship between the concentration of ADIN in the ration and total tract N digestibility in TMR of lactating dairy cattle. Additionally, ADF may not accurately estimate the concentration of ADIN in rations or N digestibility. As shown in previous experiments, milk yield is not affected by the concentration of ADIN. The assumption by
the Cornell Net Carbohydrate and Protein System that ADIN is unavailable may need to be reevaluated to account for the portion of protein that is received from ADIN. Although total tract ADIN concentration is only a small portion of protein, it is additional protein available for use by the animal.

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LITERATURE CITED


