Evaluation of Acid Detergent Insoluble Nitrogen as an Indicator of Protein Quality in Nonforage Proteins[1]

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ABSTRACT: Two trials were conducted to determine the adequacy of ADIN as an indicator of N digestibility. In Trial 1, eight nonforage plant protein sources were heated at 150°C for 90 min in the presence of xylose (12.8% of CP, DM basis) to produce heat damage. Thirty-four wethers (average BW 40 kg ± 9.9) were used to determine the effect of heat on N digestibility. Differences in ADIN were evident among the protein sources, and no significant heat × protein source interaction was observed for N digestibility. Apparent N digestibilities were reduced ($P < .001$) for all protein sources by heat treatment. Acid detergent insoluble N and N digestibility were correlated ($r^2 = .66$). However, the assumption that ADIN was completely indigestible led to underestimation of N digestibility: approximately 58% of the ADIN was digestible in these feeds. In Trial 2, seven dried distillers grains from different distilling plants were tested for N digestibility using 24 wethers (average BW 35 kg ± 3.6). Visual differences in color indicated possible differences in degree of heating in these feeds. The ADIN contents were quite variable among these feeds; however, there were no differences in N digestibility. The correlation between ADIN and N digestibility was weak ($r^2 = .24$). These results indicate that ADIN values in nonforage protein sources predicted more protein damage than that estimated by in vivo N digestibility values.

Key Words: Sheep, Protein Concentrates, Nitrogen, Digestibility, Acid Detergent Insoluble Nitrogen

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Introduction

In recent years, more attention has been focused on meeting the AA needs of animals at the small intestine. More emphasis has been placed on using protein sources with higher ruminal escape characteristics, making accurate estimation of total tract digestibility of that protein important.

In forages, excellent relationships of ADIN to N indigestibility exist (Van Soest, 1965; Goering et al., 1972; Yu and Thomas, 1976). This relationship was essentially a one-to-one reduction in N digestibility as ADIN increased. Acid detergent insoluble N was also assumed to be a measure of heat damage in nonforage plant protein sources (Van Soest and Sniffen, 1984; Van Soest et al., 1984; Poos-Floyd et al., 1985).

However, Britton et al. (1986) presented evidence that the relationship of ADIN and heat damage in several nonforage protein sources was very poor and also that recovery of ADIN in feces was poor. Others (Rogers et al., 1986; Cleale et al., 1987b; Weiss et al., 1989) reported similar results. With this in mind, two studies were conducted to determine whether ADIN is an adequate indicator of protein digestibility in a cross-section of nonforage plant proteins fed to ruminants.

Materials and Methods

Trial 1

Thirty-four Finnsheep-Suffolk crossbred wethers (40 ± 9.9 kg BW) were fitted with canvas fecal collection bags and assigned to one of 17 dietary treatments in a completely randomized design. Two wethers were assigned per dietary treatment in each period and re-randomized at the beginning of the second and third periods. Weights of wethers were taken on two consecutive days at the beginning of each period. Wethers were individually housed in metabolism crates (51 × 104 cm^2) and fed at an equal percentage (2%) of BW under constant lighting and temperature (20°C). The wethers were prefed in individual pens (.84 m^2).

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Table 1. Dry matter composition of diets fed to lambs in Trial 1

Item	Supplemental nitrogen source in diet[a]								
	Urea	SBM	CGM	CDG	MDG	LSM	CM	SFM	CSM
Base mix, %[b]	87.2	87.2	87.2	87.2	87.2	87.2	87.2	87.2	87.2
Corn, %	11.7	4.9	7.4	—	—	1.7	3.6	3.4	3.8
Urea, %	1.1	—	—	—	—	—	—	—	—
Protein source, %	—	7.9	5.4	12.8	12.8	11.1	9.2	9.4	9.0
SN/TN[c]	—	.385	.385	.365	.345	.375	.370	.370	.370
PSN/SN[c]	—	.870	.810	1.00	1.00	.955	.900	.910	.900

[a]SBM = soybean meal, CGM = corn gluten meal, CDG = corn dried distillers grain, MDG = milo dried distillers grain, LSM = linseed meal, CM = canola meal, SFM = sunflower meal, and CSM = cottonseed meal.
[b]Base mix contained 78.1% ensiled ground corncobs, 18.9% ground alfalfa hay, 1.06% urea, and 1.94% vitamin-mineral premix. Vitamin-mineral premix contained 70.14% dicalcium phosphate, 15.45% salt, 10.30% ammonium sulfate, 2.57% trace mineral premix, and 1.54% vitamin A, D, and E premix. Trace mineral premix contained 10% Mg, 6% Zn, 4.5% Fe, 2% Mn, .5% Cu, and .3% I. Vitamin A, D, and E premix contained 15,000 IU of A, 3,000 IU of D, and 3.75 IU of E per gram of premix.
[c]SN = supplemental N, TN = total N, and PSN = protein source N.

Eight plant proteins plus urea were used as supplemental protein sources. The eight plant protein sources used were soybean meal (**SBM**), corn gluten meal (**CGM**), corn dried distillers grain (**CDG**), milo dried distillers grain (**MDG**), linseed meal (**LSM**), canola meal (**CM**), sunflower meal (**SFM**), and cottonseed meal (**CSM**). One-half of each plant protein source was mixed with xylose (12.8% of plant CP, DM basis) and reconstituted to 80% DM. Xylose as a reducing sugar and 80% DM were chosen to aid the Maillard reaction based on studies conducted by Cleale et al. (1987a). The protein-sugar mixtures were placed in aluminum pans, covered with aluminum foil, and heated at 150°C for 90 min in a forced-air oven. At the end of the sugar-heat treatment, mixtures were removed from the oven, aluminum foil was removed, and contents were equilibrated to room temperature. Diets (Table 1) consisted of 87.2% base mix (ensiled ground corncobs and ground alfalfa hay mixture) and 12.8% protein supplement on a DM basis. The majority of the N in the protein supplement was supplied by urea or eight different protein sources with or without heat treatment. Diets were formulated to be isonitrogenous (11.5% CP, DM basis).

The experiment consisted of three periods of a 14-d adaptation phase followed by a 7-d collection phase. During the collection phase, feed intake, feed refusal, and total fecal weight were recorded daily. Fecal samples and refused feed were composited by lamb in each period and frozen along with weekly feed samples for later analysis. Feed refusals were less than 1% of DMI in all classes.

At the completion of the trial, feed and fecal samples were dried at 60°C in a forced-air oven for 48 h and ground through a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA). Dry matter and apparent N digestibility (**APND**) coefficients were determined from feed and fecal samples. Feed and fecal sample N were determined by the Kjeldahl procedure (AOAC, 1984) and supplemental N digestibility of each protein source was calculated by difference in APND relative to the urea control. Urea was assumed to be 100% degradable in the rumen, so any decrease in the total tract N digestibility coefficient of the treatment group was assumed to be due to the protein source in the supplement. Therefore, true N digestibility (**TND**) of each supplemental protein source was calculated by the following equation:

$$\text{TND} = 100 - \frac{(\text{APND of urea control}) - (\text{APND of treatment group})}{(\text{Supplemental N/Total N}) \times (\text{Protein source N/Supplemental N})}$$

Approximately 1 g of each protein source (particle size = as fed) was placed in a Dacron bag (7.5 cm × 12.5 cm; 50 μm average pore size [Ankom, Fairport, NY]) and incubated in situ for 12 h to estimate ruminally degradable protein, using a steer fed a ground corncob-SBM diet according to procedures of Wilkerson (1992). Ruminally degradable protein was determined by 100 – protein content remaining after a 12-h in situ incubation and expressed as a percentage of total CP. Acid detergent insoluble N values of the protein sources were determined according to Goering et al. (1970).

Apparent N digestibility values were analyzed as a completely randomized design using the GLM procedures of SAS (1985) with heat treatment, source, and period in the model. Because there was no heat treatment × protein source interaction, it was removed from the model. Treatment means were compared using LSD. Regression of ADIN on TND values were performed using SAS (1985).

Trial 2

Seven batches of dried distillers grains from different distilleries were tested for N digestibility and their ADIN values. Because all distillers grains were subjected to heat during the production at distilleries, no further heat treatment was applied. Twenty-four Finnsheep-Suffolk crossbred wethers (35 ± 3.5 kg

Table 2. Dry matter composition of diets fed to lambs in Trial 2

Item	Supplemental nitrogen source in diet		
	% in Base	Urea	Distillers grains
Base mix, %	—	87.2	87.2
Ensiled ground corncobs, %	79.5	—	—
Ground alfalfa hay, %	17.4	—	—
Urea, %	1.1	—	—
Vitamin-mineral mix, %[a]	2.0	—	—
Corn, %	—	11.7	—
Urea, %	—	1.1	—
Protein source, %	—	—	12.8

[a]Mix contained 70.14% dicalcium phosphate, 15.45% salt, 10.30% ammonium sulfate, 2.57% trace mineral premix, and 1.54 vitamin A, D, and E premix. Trace mineral premix contained 10% Mg, 6% Zn, 4.5% Fe, 2% Mn, .5% Cu, and .3% I. Vitamin A, D, and E premix contained 15,000 IU of A, 3,000 IU of D, and 3.75 IU of D per gram of premix.

BW) were fitted with canvas fecal collection bags and randomly assigned to one of eight dietary treatments (Table 2), three lambs per treatment for two periods. The experimental protocol, response variables, and analytical and calculation procedures were the same as those for Trial 1. Apparent N digestibility values were analyzed as a completely randomized design using the GLM procedures of SAS (1985) with distillers grain source, period, and source × period interaction in the model. Mean comparison as well as regression of ADIN on TND was performed as in Trial 1.

Results and Discussion

Trial 1

There was no source × heat interaction, indicating that the heat treatment reduced ($P < .001$) apparent N digestibility (APND) of all nonforage protein sources studied (Table 3). Differences were evident in APND among the protein sources. Both dried distillers grains (CDG and MDG) were among the lowest APND, whereas CGM was the highest for untreated protein sources. Corn gluten meal seemed to suffer the most (10% reduction in APND) from the sugar-heat treatment applied.

Calculated TND of untreated protein sources were essentially 100%, except for both distillers grains (88.5 and 86.8% for CDG and MDG, respectively; Table 3). These values reflect the APND values observed. These lower values for the CDG and MDG may be due to the heat applied during the total alcohol production process, thus making protein in the distillers grains damaged to some degree by the Maillard reaction before additional sugar-heat treatment was applied. True N digestibility of treated protein sources ranged from 74 to 94%. The sugar-heat treatment reduced TND of all protein sources, but to different degrees.

Acid detergent insoluble N increased considerably in each source of protein as a result of sugar-heat treatment (Table 3). The increase in ADIN content was the highest in SBM (16.2 times that of untreated), whereas both dried distillers grains were the lowest (1.3 and 1.4 times that of untreated CDG and MDG, respectively). The rest of the protein sources ranged from four to seven times that of their untreated values. There seems to be some difference in the increase of ADIN among feeds with response to the sugar-heat treatment.

Table 3. Apparent nitrogen digestibilities of diets, true nitrogen digestibilities of supplemental protein sources, and acid detergent insoluble nitrogen (ADIN) contents of protein sources used in Trial 1

Item	Protein source[a]									SEM
	UREA	SBM	CGM	CDG	MDG	LSM	CM	SFM	CSM	
N digestibility										
Apparent, %										
C[b]	65.1^{cd}	65.3^{cd}	68.0^{c}	60.9^{e}	60.4^{e}	65.4^{d}	65.7^{cd}	64.3^{d}	62.7^{de}	1.2
H	—	63.2^{c}	61.6^{c}	55.7^{d}	57.5^{de}	62.3^{c}	59.7^{ce}	63.0^{c}	60.0^{ce}	1.2
True, %										
C	—	100.0	109.0	88.5	86.8	100.6	101.7	97.6	92.7	—
H	—	94.1	88.3	73.6	77.5	91.8	83.4	93.5	84.6	—
ADIN, % of total N										
C[b]	—	1.2^{c}	7.3^{d}	36.6^{e}	41.4^{f}	3.8^{g}	4.3^{g}	4.3^{g}	2.6^{cg}	.77
H	—	19.4^{cd}	30.4^{e}	46.6^{f}	59.5^{g}	14.7^{dh}	31.4^{e}	24.4^{c}	11.5^{h}	1.7

[a]SBM = soybean meal, CGM = corn gluten meal, CDG = corn dried distillers grain, MDG = milo dried distillers grain, LSM = linseed meal, CM = canola meal, SFM = sunflower meal, and CSM = cottonseed meal.
[b]C = untreated control, H = sugar-heat treated. Heat-treatment effect ($P < .001$).
[c,d,e,f,g,h]Numbers in the same row with different superscripts differ ($P < .05$).

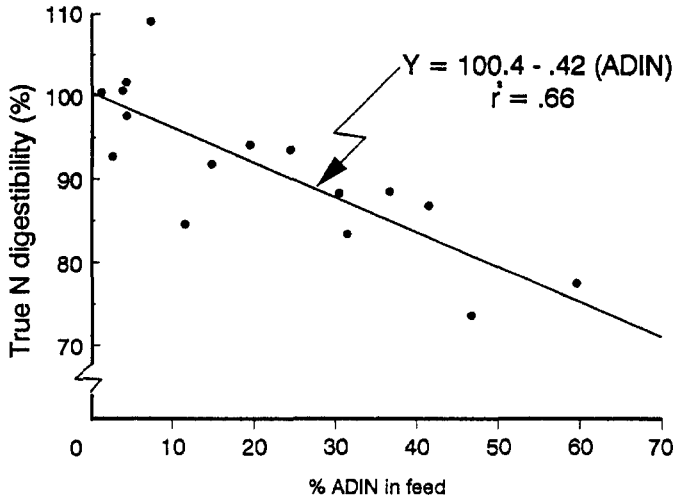

Figure 1. Correlation between acid detergent insoluble nitrogen (ADIN) as a percentage of total N and true N digestibility of various plant protein sources fed to lambs in Trial 1. Each data point represents treatment mean. Standard error of estimate = .079%.

When measured ADIN was correlated to TND of the protein sources, there was a relatively strong relationship (r^2 = .66; Figure 1). The equation (Figure 1) indicates that the ADIN was approximately 58% digestible in these feeds. Rogers et al. (1986) reported that the ADIN in diets supplemented with wet or dried brewers grains was 21 to 49% digestible. Values obtained from this current trial were slightly higher than the values reported in their paper. However, in either case, if ADIN is digestible then it would not serve as an adequate indicator of indigestible proteins. Therefore, if ADIN is used as an indicator of heat damage, protein digestibility will be underestimated.

The 12-h in situ incubation indicated that ruminally degradable protein (**RDP**) content of untreated proteins was the highest in CM and the lowest in CGM (Table 4). These values were in relatively good agreement with the mean in vivo values in NRC (1985), except for SBM and CGM. In the present trial, RDP for SBM was 60.1%, whereas the NRC value was 72%. However, the summary of literature values reported by Nocek and Russell (1988) indicated that RDP content for SBM ranged from 51.0 to 85.0%. Considering this range, RDP of SBM in the present trial is in reasonable agreement with literature values. The ruminally degradable protein content of CGM in the present trial was 6.5%, whereas that of the NRC was 45%, considerably higher than the value obtained in our trial. Also, values reported by Nocek and Russell (1988) for CGM were higher and ranged from 11.6 to 54.0%. When the same set of protein sources was treated, RDP was reduced and ADIN was increased across the protein sources. The increase in ADIN was related to the reduction in RDP of these treated feeds [RDP = 48.34 – .76 (% ADIN)]; however, only 31% (r^2 = .31) of the reduction in RDP in treated feeds was explained by the ADIN content of these feeds.

Trial 2

There were some differences in color of distillers grain samples, indicating possible differences in degree of heating. When ADIN of these distillers grains was measured, there were considerable differences (ranging from 8 to 28% of original N) among the samples. However, APND were not different ($P > .05$) among these distillers grains (Table 5).

When we applied the Cornell feed evaluation system (Van Soest et al., 1984) and calculated APND of these distillers grains according to the equation by Yu and Thomas (1976), it predicted 45.2 and 63.4% for distillers grains A and F, respectively. Differences in APND among distillers grains were far less than what would be predicted from ADIN values by the Cornell feed evaluation system. Similar results were

Table 4. Crude protein (CP) and ruminally degradable protein (RDP) contents of protein sources used in Trial 1

	Protein source[a]								
Item	SBM	CGM	CDG	MDG	LSM	CM	SFM	CSM	SEM
CP, % of DM									
C[b]	49.4[e]	67.8[f]	32.8[g]	29.3[h]	37.6[i]	40.9[j]	39.8[k]	40.9[jk]	.40
H	48.1[e]	64.7[f]	30.6[g]	29.0[h]	35.6[i]	40.4[j]	40.1[j]	40.9[j]	.36
RDP, %[c]									
C[d]	60.1[e]	6.5[f]	31.4[g]	20.2[h]	63.1[i]	81.2[j]	73.9[j]	46.5[k]	2.9
H	8.5[e]	5.0[f]	28.1[g]	12.3[h]	23.1[i]	20.6[i]	22.4[i]	11.2[eh]	1.0

[a]SBM = soybean meal, CGM = corn gluten meal, CDG = corn dried distillers grain, MDG = milo dried distillers grain, LSM = linseed meal, CM = canola meal, SFM = sunflower meal and CSM = cottonseed meal.
[b]C = untreated control, H = sugar-heat treated.
[c]Ruminally degradable protein expressed as percentage of CP.
[d]Heat-treatment effect ($P < .001$).
[e,f,g,h,i,j,k]Numbers in the same row with different superscripts differ ($P < .05$).

Table 5. Apparent nitrogen digestibilities of diets, true nitrogen digestibilities of distillers grains, and acid detergent insoluble nitrogen (ADIN) contents of distillers grains used in Trial 2

		Distillers grain source[a]							
Item	UREA	A	B	C	D	E	F	G	SEM
N digestibility									
Apparent, %	67.0[b]	62.7[c]	61.3[c]	65.1[bc]	64.1[bc]	62.0[c]	63.4[c]	63.1[c]	1.3
True, %	—	92.0	89.3	100.0	97.3	94.5	91.4	96.2	—
ADIN, % of total N	—	27.9[b]	23.8[bc]	7.8[d]	11.8[d]	23.0[e]	9.0[d]	23.7[c]	1.4

[a]Letters designate different distilleries.
[b,c,d,e]Numbers in the same row with different superscripts differ ($P < .05$).

reported by Plegge et al. (1985). When SBM was roasted at 145°C, ADIN content was increased from 4.1% in control SBM to 15.8% in roasted SBM. However, total tract digestibility of N was unaffected, 69.0 and 69.1% for control and roasted SBM, respectively.

True N digestibility calculated from APND relative to the urea control ranged from 89 to 100%. Considering the variation in ADIN of these feeds, TND was not highly variable.

Ruminally degradable protein values of these distillers grains after 12 h of in situ incubation were quite variable (Table 6). Ruminal escape protein values (100 - RDP) tended to follow the same direction as ADIN contents. Regression analysis indicated that ADIN contents explained 55% of RDP reduction ($r^2 = .55$) in these distillers grains. However, the differences in RDP were almost canceled out in the lower digestive tract, as indicated by similar APND. This suggests that rate or extent of digestibility of escape protein may be different among these distillers grains. Weiss et al. (1989) reported that digestion of ADIN increased as the amount of barley distillers grains in the diet increased. The differences among treatments for ADIN digestibility were much greater than for the digestibility of acid detergent soluble N, suggesting that the composition of ADIN varies more among feeds than does the composition of acid detergent soluble N.

When ADIN was correlated with TND (Figure 2), the relationship was weak ($r^2 = .24$). This correlation was much lower than that obtained in Trial 1. Furthermore, a considerable portion (approximately

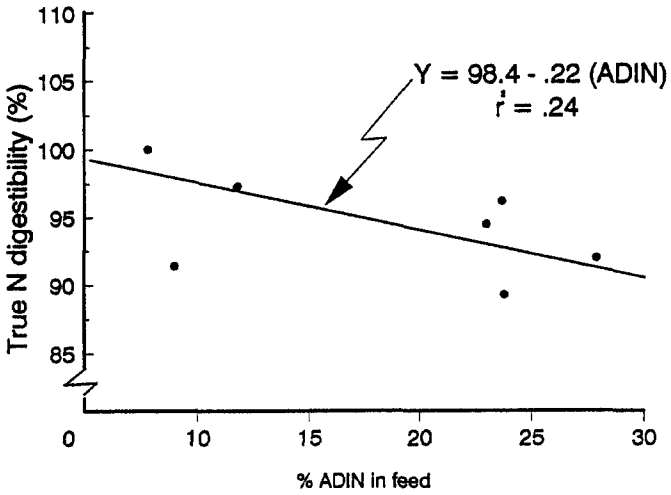

Figure 2. Correlation between acid detergent insoluble nitrogen (ADIN) as a percentage of total N and true N digestibility of distillers grains from different distilleries fed to lambs in Trial 2. Each data point represents treatment mean. Standard error of estimate = .176%.

78%) of ADIN in distillers grain was digested, according to the equation. This value was higher than the values obtained in Trial 1 or by Rogers et al. (1986). However, this emphasizes the point that ADIN is digestible and is not a measure of protein indigestibility. Therefore, underestimation of protein digestibility would occur if ADIN was used as the indicator of protein damage in dried distillers grains.

Table 6. Crude protein (CP) and ruminally degradable protein (RDP) contents of protein sources used in Trial 2

	Distillers grain source[a]							
Item	A	B	C	D	E	F	G	SEM
CP, % of DM	30.1[c]	28.5[d]	27.6[e]	29.1[f]	26.0[g]	29.9[c]	27.4[e]	.11
RDP, %[b]	38.5[d]	26.4[eg]	56.2[f]	45.1[df]	20.3[g]	84.2[c]	42.0[def]	4.8

[a]Letters designate different distilleries.
[b]Ruminally degradable protein expressed as percentage of CP.
[c,d,e,f,g]Numbers in the same row with different superscripts differ ($P < .05$).

Implications

These studies indicated that acid detergent insoluble nitrogen is not an accurate estimate of heat damage in nonforage proteins. In heat-damaged nonforage protein sources, acid detergent insoluble nitrogen increased; however, this increase was not a one-to-one relationship with indigestible nitrogen. Therefore, acid detergent insoluble nitrogen should not be subtracted as unavailable nitrogen on a one-to-one basis in these protein sources.

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