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The use of acid detergent insoluble nitrogen to predict digestibility of rumen undegradable protein of heat processed plant proteins[1]

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The usefulness of acid detergent insoluble nitrogen (ADIN) to predict digestibility of rumen undegradable protein (UDP-D) of heat processed and unprocessed plant proteins was investigated. A series of drum roasted protein sources, sunflower, cottonseed and soybean oilcake meals, in addition to whole soybeans, whole sunflowerseed, whole cottonseed and whole lupinseed, were evaluated in this study to test the relationship between ADIN (% of total N) and UDP-D. Using ADIN as a screening parameter, five samples on average of each protein source together with the unprocessed control were selected for an *in situ* study using the mobile bag technique (MBT) to determine UDP-D. Digestibility of undegradable protein was not significantly decreased as a result of heat treatment, when ADIN values were below 12–15%, but progressively decreased when ADIN was higher, suggesting substantial heat damage. Ruminal nitrogen digestibility also decreased (p <0.05) with heat treatment. Digestibility of undegradable protein was predicted with a r^2 = 0.72 and error of estimate (Sy.x) of 4.78%. The prediction equation (p <0.001) of UDP-D (%) = 91.9 – 0.025 (ADIN, % in DM)^2, could therefore be a satisfactory guideline for predicting UDP-D and heat damage of the above-mentioned heat processed and unprocessed plant protein sources.

Die doel van die studie was om die suurseep-onoplosbare-stikstof- (ADIN) analise te gebruik om die verteerbaarheid van die nie-degradeerbare proteïen (UDP-V) van 'n wye reeks hitte-geprosesseerde en ongeprosesseerde plantaardige proteïenbronne te voorspel. Om die verwantskap tussen ADIN (% van totale N) en UDP-V te evalueer, is sonneblom-oliekoekmeel, katoensaad-oliekoekmeel, en soja-oliekoek meel sowel as heel sojabone, heel sonneblomsaad, heel katoensaad en heel lupiensaad dromgerooster. Resultate van ADIN-analise is gebruik om gemiddeld vyf hitte-geprosesseerde asook die ongeprosesseerde kontrole te selekteer vir 'n *in situ* evaluasie deur die mobielesakkie-tegniek (MBT) te gebruik om UDP-V te bepaal. Verteerbaarheid van nie-degradeerbare proteïen het nie betekenisvol verlaag as gevolg van hittebehandeling wanneer ADIN onder 12–15% was nie, maar het progressief verlaag wanneer ADIN hoër was, wat aansienlike hittebeskadiging voorstel. Ruminale-stikstofverteerbaarheid het ook verlaag (p <0.05) met hittebehandeling. Verteerbaarheid van nie-degra-deerbare proteïen is voorspel met 'n r^2 = 0.72 en beramingsfout (Sy.x) van 4.78%. Die voorspellingsvergelyk-ing (p <0.001) van UDP-V (%) = 91.9 – 0.025 (ADIN, % in DM)^2, behoort 'n nuttige riglyn te wees om UDP-V en hitte-beskadiging van hitte-geprosesseerde en ongeprosesseerde plantproteïenbronne te voorspel.

Keywords: roasting, plant protein, undegradable protein, ADIN, dairy cows

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There has been much interest in using indices of heat damage to protein sources, such as laboratory tests or even visual appraisal, to determine the value of a protein source (Merchen, 1990). Moderate heating may enhance the value of many protein sources by decreasing degradability in the reticulo-rumen, while not affecting digestion in the small intestine (SI), whereas overheating may be detrimental. Acid detergent insoluble nitrogen (ADIN) has been known to indicate heat damaged proteins in forage (Yu, 1976; 1977; Van Soest & Mason, 1991; Broderick *et al.*, 1993; Yang *et al.*, 1993; Boila & Ingalls, 1994) and in plant proteins (Drackley & Schingoethe, 1986; Pena *et al.*, 1986; Faldet *et al.*, 1991; 1992; Reddy *et al.*, 1993). Nakamura *et al.*, (1994) furthermore obtained a linear relationship (r^2 = 0.66) between ADIN and true nitrogen digestibility, but non-forage sources such as corn dried distillers grain and milo dried distillers grain were included, and he concluded that ADIN was a weak indicator of heat damage. Owing to its correlation with heat damage, % ADIN may prove useful to predict digestibility of undegradable protein (UDP-D) in the SI and this study further investigates this possibility.

Seven plant protein sources, sunflower oilcake meal (SFOC), cottonseed oilcake meal (CSOC), soybean oilcake meal (SOC), whole soybeans (WSB), whole sunflowerseed (WSS), whole cottonseed (WCS) and whole lupinseed (WLS), were drum roasted using a 6 temperature × 5 time interval factorial design as described by Schroeder *et al.* (1995). An electrically driven drum roaster (45 l) using a gas flame as energy source and rotating at 23 rpm was used. The protein sources were processed from 110°C to 210°C with 20°C intervals and the time intervals were 10, 30, 60, 90 and 120 min per setting. The temperature of the product was measured inside the drum using an electronic thermometer. After roasting, the product was cooled by an electrically powered fan situated underneath the drum. All processed protein sources as well as the corresponding unprocessed controls were analysed for ADIN as described by Goering & Van Soest (1970). Demjanec *et al.* (1995) indicated that a maximum amount of amino acids (AA) ultimately disappeared in the SI of sheep when SOC was heated to the point where 12 to 15% of total N was in the form of ADIN. Using this as a norm, between four and seven samples of each protein source varying in ADIN from below 12% ADIN to above 15% ADIN, together with the unprocessed control, were selected for the *in situ* study.

Three rumen and duodenally cannulated lactating dairy cows were used in this study. The cows received a 17% CP total mixed diet *ad lib*, maintaining an intake of about 23 kg DM/d. The mobile bag technique (MBT) as described by Kirkpatrick & Kennelly (1985) was used to determine UDP-D. Six mobile bags per cow per sample were filled (1 g; 2 mm) using two additional blanks to correct for microbial contamination. All bags were firstly incubated in the rumen (16 h) inside a large net bag (Nalsen *et al.*, 1987). Following incubation, three bags and one blank were washed (10 min; cold water, washing machine) and dried (55°C/48 h). The remaining three bags and one blank were incubated in a pepsin-HCl solution (39°C/3 h). After digestion, these bags were kept on ice at 4°C and inserted randomly into the duodenum via the duodenal cannula at a rate of one bag per 20 min. Upon recovery from the faeces the bags were kept on ice at 4°C until all bags were recovered whereafter they were washed and dried as described. Digestibility values obtained using the MBT can be considered as an estimate of true digestibility (de Boer, *et al.*, 1987; Hvelplund *et al.*, 1994). For N-analysis, Macro-Kjeldahl (A.O.A.C., 1984) was used. Linear and non-linear regression equations of ADIN on UDP-D were tested for best fit (SAS, 1990).

The acid detergent insoluble nitrogen and digestibility of undegradable protein values of drum roasted and unprocessed plant protein sources are shown in Table 1. Digestibility of undegradable protein was non-significantly decreased up to 12–15% ADIN, but was significantly and progressively decreased above this level, suggesting substantial heat damage. The ruminal nitrogen digestibility values are also depicted in Table 1 showing a significant decrease with temperature and time, clearly indicating the effect of heat processing. Digestibility of undegradable protein was predicted best using a non-linear function with $r^2 = 0.72$ and error of estimate (Sy.x) of 4.78%. The prediction equation ($p < 0.001$) of UDP-D(%) = 91.9 – 0.025 (% ADIN of total N, % in DM)^2, appears to be a satisfactory guideline to predict UDP-D across plant proteins (Figure 1.). If however, a specific protein source is investigated the prediction equation for that protein source as shown in Table 2, may be used.

The low r^2 value of 0.19 for WSB is inexplicible but could possibly be because only three processed samples were selected with no large variation in % ADIN. Secondly, it has been suggested that ADIN is not considered to be a useful indicator of heat damage with whole soybeans (Satter *et al.*, 1994). Recent digestibility studies in sheep using SOC, a by-product of WSB, in a total mixed diet have shown that ADIN is not an adequate measure of indigestible N for high-protein supplements (Hussein *et al.*, 1995). Their conclusion was that ADIN was highly digestible in the digestible tract. Similar studies would be needed for other high-protein supplements.

Figure 1 depicts both the UDP-D and ADIN values of the plant protein sources and the non-linear equation. Increases in ADIN resulted in non-significant decreases in UDP-D up to 12–15% ADIN, but above 12–15% ADIN, small increases

Table 1 ADIN and UDP-D[1] of drum processed and unprocessed plant protein sources

		Control	Temp (°C)	110	130	130	130	130	130	150	150	150	150	170
Protein source			time (min)	90	30	45	60	90	120	10	30	40	60	10
Sunflower	ADIN	2.4					6.3	9.3	12.0		14.4		18.2	10.3
oilcake meal	UDP-D[2]	96.2[a]					93.0[b]	91.2[bd]	89.9[d]		85.6[c]		78.3[e]	85.8[c]
	N-D[4]	71.6[a]					38.2[b]	33.8[d]	29.6[c]		29.1[c]		29.8[c]	39.6[b]
Cottonseed	ADIN	2.7					4.2	6.2			9.5	11.7	34.9	
oilcake meal	UDP-D	91.5[a]					92.1[a]	92.7[a]			91.9[a]	90.8[a]	53.0[b]	
	N-D	63.9[a]					45.0[b]	44.9[b]			39.5[c]	37.4[d]	31.7[e]	
Soybean	ADIN	1.4			2.1	6.8	15.9	33.3			24.0			
oilcake meal	UDP-D	94.1[ac]			96.8[a]	96.3[a]	90.5[c]	68.1[b]			68.2[b]			
	N-D	57.8[a]			31.0[b]	26.9[bd]	25.7[bd]	23.1[cd]			23.2[cd]			
Whole soybeans	ADIN	1.7					9.5	16.0			20.3			
	UDP-D	89.5[a]					92.4[a]	83.4[a]			88.6[a]			
	N-D	87.6[a]					55.7[b]	55.0[b]			55.9[b]			
Whole	ADIN	2.1			7.6		14.8			12.4	27.8			19.9
sunflowerseed	UDP-D	59.3[3]			89.5[b]		89.0[bd]			91.8[b]	83.7[c]			86.0[cd]
	N-D	93.4[a]			53.3[b]		37.3[c]			50.0[bd]	43.8[cd]			41.8[c]
Whole cottonseed	ADIN	4.9					6.6	9.2			12.6		26.0	12.1
	UDP-D	85.3[ab]					85.3[a]	85.3[a]			83.0[ab]		68.6[c]	81.4[b]
	N-D	70.7[a]					61.6[b]	43.8[c]			52.0[d]		42.0[c]	53.1[d]
Whole lupinseed	ADIN	1.8		2.9			7.5				9.4			8.3
	UDP-D	89.4[ab]		95.0[a]			90.0[ab]				83.9[b]			84.8[b]
	N-D	94.1[a]		62.1[b]			63.7[b]				67.7[c]			64.4[b]

[1] Least square means, alphabetic superscripts within rows differ (a#b#c#d#e, $p < 0.05$). [2] Digestibility of undegradable protein. [3] Not included in regression owing to exceptionally low value, possibly caused by the blocking of the pores in the mobile nylon bags. [4] Nitrogen digestibility in the rumen.

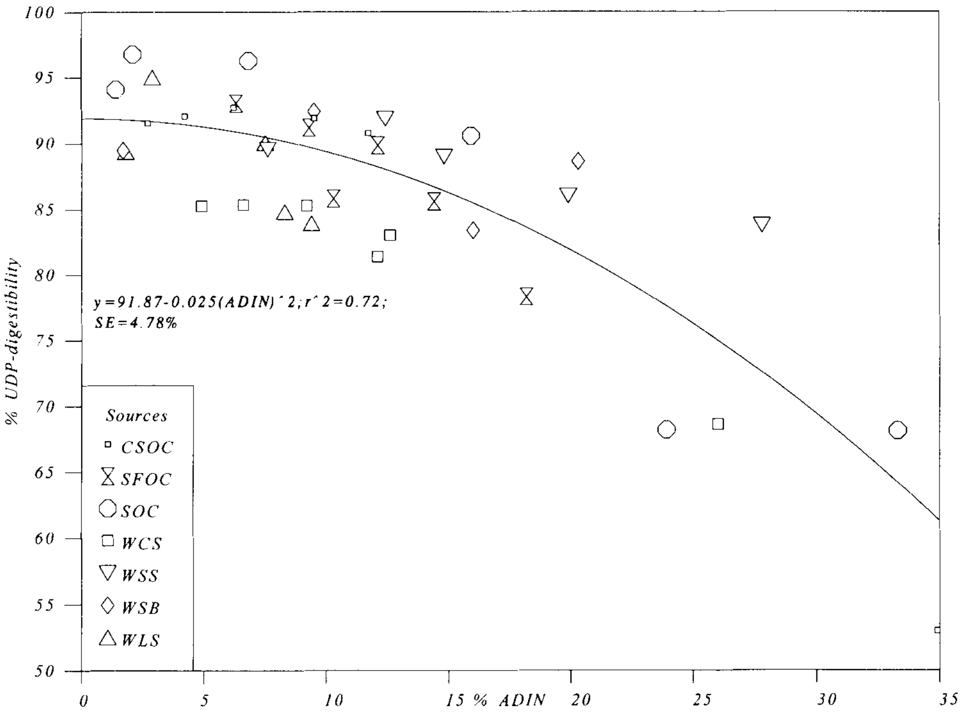

Figure 1 Relationship between acid detergent insoluble nitrogen (ADIN) as a percentage of total N and UDP-digestibility of various heat processed plant protein sources.

Table 2 UDP-D prediction equations for the individual protein sources

Source	n	Prediction equation	r^2	p
SFOC	7	UDP-D (%) = 95.2 – 0.05 (ADIN)^2	0.89	p <0.01
CSOC	6	UDP-D (%) = 90.4 + 0.61 (ADIN) –0.05 (ADIN)^2	0.99	p <0.01
SOC	6	UDP-D (%) = 95.2 – 0.029 (ADIN)^2	0.84	p <0.01
WSB	4	UDP-D (%) = 90.9 – 0.20 (ADIN)^2	0.19	p <0.57
WSS	5	UDP-D (%) = 48.8 + 5.02 (ADIN) – 0.14 (ADIN)^2	0.78	p <0.10
WCS	6	UDP-D (%) = 86.5 – 0.026 (ADIN)^2	0.98	p <0.01
WLS	5	UDP-D (%) = 92.9 – 0.09 (ADIN)^2	0.66	p <0.10

SFOC = sunflower oilcake meal; CSOC = cottonseed oilcake meal; SOC = soybean oilcake meal; WSB = whole soybeans; SS = whole sunflowerseed; WCS = whole cottonseed; WLS = whole lupinseed; ADIN = % acid detergent insoluble nitrogen as a % of total N, in DM

in ADIN resulted in large and significant decreases in UDP-D, clearly indicating the non-linear relationship.

This is in contrast to the results of Nakamura *et al.* (1994), who found a linear relationship (r^2 = 0.66) between ADIN and true nitrogen digestibility, concluding that ADIN was a weak indicator of heat damage in non-forage protein sources which included corn dried distillers grain (CDG) and milo dried distillers grain (MDG). A possible explanation for the conclusion of Nakamura *et al.* (1994), is that ADIN in distillers by-products may be as high as 50% digestible, and ADIN therefore is a poor predictor of N-digestibility in dried distillers grain. Nakamura *et al.* (1994), however, reported a linear relationship (r^2 = 0.84) between UDP-D and true nitrogen digestibility of the heat processed soybean oilcake, sunflower oilcake and cottonseed oilcake samples, which is in agreement with our study (r^2 = 0.74) when using these three processed sources. In both our study and in that of Nakamura *et al.* (1994), a poor relationship (r^2 = 0.53) existed when only the unprocessed protein sources were taken into account. In their study only one heat treatment (150°C for 90 min) was used with added xylose to induce severe heat damage. This resulted in abnormally high ADIN values, whereas in our study a broader range of ADIN values resulted from the factorial temperature × time interval arrangement. It can be concluded that ADIN is a useful indicator of UDP-D for selected heat processed and unprocessed plant proteins namely sunflower, cottonseed and soybean oilcakes as well as whole soybean, whole sunflowerseed, whole cottonseed and whole lupinseed. Dairy producers can help assure quality products by insisting that such test information be supplied with the purchase of heat processed products.

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