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by

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ABSTRACT

Seventy-four samples of wheat grain (c.v. Mercia) grown under a variety of different fertiliser nitrogen (N) regimes were milled through a 3mm screen. The rumen degradation characteristics of N were estimated using the nylon bag technique and N solubility in water was estimated in the laboratory. The values determined from the nylon bag technique for the immediately rumen soluble ('a') fraction (16.9 ± 3.81%) and the rate ('c') of N degradation (0.128 ± 0.0178 h^{-1}) were both considerably lower than in previous reports in the literature. The N content of the grain exhibited a strong curvilinear relationship with the total N applied as fertiliser. Increasing fertiliser N and the resulting increase in grain N contents reduced and increased ($P<0.01$) the immediately rumen soluble ('a') and insoluble but degradable N ('b') fractions respectively, although the relationships only accounted for about 10% of the variance. For both statistical and theoretical reasons these relationships may not be applicable to other samples of wheat. The work also showed that the water soluble N fraction as measured in the laboratory (8.3 ± 1.84%) was on average only about 0.4 of the nylon bag zero hour loss (21.2 ± 4.49%) and about 0.5 of the fitted 'a' value (16.9 ± 3.81%). This suggests that the nylon bag technique can seriously overestimate the immediately soluble N fraction of wheat and probably other cereal grains.

INTRODUCTION

It has been recognised for some time (Agricultural Research Council, 1984) that the assessment of the value of dietary proteins for ruminant animals requires a knowledge of the proportions of the dietary protein or nitrogen (N) that are immediately soluble in the rumen, subsequently degraded in the rumen and that which reaches the small intestine intact. More recently, this concept has been developed into a practical system for use in the UK (AFRC, 1992).

There are few reported measurements of the protein degradability of cereal grains in general and wheat in particular. Limited data for wheat, barley and oats have been reported by MAFF (1990) and for wheat by Fahmy *et al.* (1991) although there are no data related to specific varieties. Also, whilst there are now several reports (see for example Dampney, 1992) which conclude that additional fertiliser nitrogen results in additional grain protein concentrations, there appears to be little information relating fertiliser N or protein content of grain to its rumen degradation characteristics.

The rumen degradation characteristics of ruminant feedstuffs are commonly assessed by incubating small samples in nylon bags in the rumen for varying time periods. Material lost from the bag is assumed to be soluble or to have been microbially degraded. In the case of cereals, recent work (Michalet-Doreau and Cerneau, 1991) has indicated that different procedures for estimating degradability can influence the values obtained. This study and that of Lindberg and Varvikko (1982) have also raised doubts about the use of the nylon bag for estimating the immediately soluble nitrogen fraction in cereal grains.

Accordingly, the objectives of the present study were three-fold. Firstly, to obtain rumen N degradation characteristics of a range of wheat grains, secondly to examine any relationships between grain N content, fertiliser treatment and rumen N degradation characteristics and thirdly, to examine the applicability of the nylon bag method for estimating the proportion of cereal protein immediately soluble in the rumen.

MATERIALS AND METHODS

Wheat grains

A total of 74 samples of wheat grain (c.v. Mercia) were studied. These grains were obtained from an HGCA funded study (0018/5/88A) which examined the effects of rate and timing of fertiliser N on protein quality for bread-making. Total N fertiliser application rates ranged from 50 to 360 kg ha^{-1}. Full details of the sites of origin and the fertiliser N treatments are given in Appendix 1. Further details regarding the production of the grain can be obtained from Dampney (1992).

Rumen degradability studies

All 74 samples were thoroughly mixed and a representative 500g working sub-sample of each obtained. The working samples were milled in their entirety using a hammer mill fitted with a 3mm screen. The 3mm screen size was chosen since the work of Michalet-Doreau and Cerneau (1991) showed that smaller screen sizes led to the loss of large amounts of fine particles through the pores of the nylon bags.

Each milled sample was thoroughly mixed and about 5.5g of each accurately weighed into each nylon bag (pore size 42μm). Nylon bags were incubated in the rumens of three rumen cannulated non-lactating dairy cows for 0, 2, 5, 8, 12, 24 and 48h. The order of sample incubation was randomised across all samples. Details of the order and batching of samples are given in Appendix 2. Each time period was incubated in duplicate in each cow. All bags for incubation were placed in the rumen just before the morning feed. During the course of the work the cows were fed a basal diet of grass silage (4.3kg dry matter (DM)) and rolled mineralised barley (1.8kg DM) in two equal meals at 0830 and 1700h. Zero hour bags, which estimate the immediately soluble fraction, were not incubated but were mechanically washed in cold water along with the bags containing the incubation residues.

Following washing, the bags and residues were dried at 60°C for 48h.

Laboratory studies

The dried residues from the two bags from each cow at each incubation period were combined. The combined sample, along with samples of original grain, were milled through a Cyclotec mill (Perstop Analytical, Bristol) fitted with a 1mm screen. The milled material was then analysed for total N content using the Kjeldahl method (MAFF, 1986).

The solubility of N was also measured in the laboratory by saturating approximately 1g of milled grain in 40ml of de-ionised water for 1h with regular agitation. The samples were then filtered under vacuum onto a Whatman grade 541 filter paper, washed with 3 portions of 40ml of de-ionised water and the filter paper plus residue dried at 100^{o} C for 18h. The filter paper plus dried residue was then weighed to estimate DM solubility and then the insoluble N present was measured by the Kjeldahl method.

Statistical analysis

The rumen degradation characteristics of the N fraction was estimated by fitting the nylon bag disappearance values to the exponential model of Ørskov and McDonald (1979) viz:

$$P = a + b\,(1 - e^{-ct}) \text{ where,}$$

a = the immediately soluble fraction, b = the insoluble but degradable fraction, c = the rate of degradation of the 'b' fraction and P = the disappearance value at time 't'.

The zero hour losses and 'a' values for N measured by the nylon bag method were compared with the respective water solubility values measured in the laboratory. The N associated with any fine particles (NAFP) lost through the pores of the bags was estimated as ('a' - N solubility).

The effect of N fertiliser treatments on the degradability data were assessed by a stepwise linear regression technique using GENSTAT. This involved introducing into the regression model in order the effects of year of harvest, harvest site, total amount of N fertiliser applied and the timing of the nitrogen applied. This order was chosen to remove the effects of year and site from the effect of N fertiliser. However, since all sites were not represented in both years, the effect of site could not be fully included in the model.

RESULTS

Nitrogen content and degradation characteristics

Table 1 presents the mean results for N contents, N solubilities, zero hour N losses, NAFP and N degradation characteristics for the 74 samples studied. The results show a wide range of N contents (18.3-29.9 g kg^{-1} DM) and that the N in the wheats was extensively degraded with the potential degradability (a + b) nearing 100% in all cases. Whilst potentially degradable N varied little, there was considerable variability in the immediately soluble N ('a') and rate of N degradation ('c') values.

Table 1. Nitrogen (N) content, N solubility, N zero hour losses, N associated with fine particles (NAFP) and the fitted N degradation characteristics of 74 wheat grain samples.

Determination	Mean	SD[1]	CV[2]	Minimum	Maximum
N content (g kg^{-1} DM)	24.0	2.01	8.4	18.3	29.9
N solubility (%)	8.3	1.84	22.2	4.2	12.8
Zero hour N loss (%)	21.2	4.49	21.2	12.8	33.4
NAFP (%)	8.6	3.47	40.3	-1.0	17.8
Fitted N degradation characteristics:					
a (%)	16.9	3.81	22.5	9.4	26.0
b (%)	82.0	3.86	4.7	73.4	89.7
a + b (%)	98.9	1.06	1.1	95.2	100.2
c (h^{-1})	0.128	0.0178	13.9	0.092	0.168

1, Standard deviation of population. 2, Coefficient of variation (%).

The N solubility values measured in the laboratory were considerably lower than the dacron bag derived immediately soluble N expressed as either the zero hour N loss or the fitted 'a' value. The differences between N solubility and the immediately soluble N values were assumed to be NAFP lost through bag pores. The NAFP contents (Table 1) also showed a large variability, and on average represented about 0.5 of the fitted immediately soluble N ('a' value).

Effect of nitrogen fertiliser or nitrogen content and degradation characteristics

Table 2 indicates the probability of an effect of rate and timing of N fertiliser, year and site on the N content of the grain and on the N characteristics listed in Table 1. Year and site had significant ($P<0.05$) effects on all factors except N solubility and the rate of N degradation (c) and zero hour N loss for site only.

Table 2. The probability of an effect of rate and timing of nitrogen fertiliser, year and site on the nitrogen content and nitrogen degradation characteristics of wheat.

Determination	Modifications to regression model			
	+ Year	+ Site	+ Nitrogen rate	+ Nitrogen timing
N content	***	***	***	NS
N solubility	NS	NS	**	NS
Zero hour N loss	**	NS	***	NS
NAFP	***	*	***	NS
Fitted N degradation characteristics:				
a	***	**	***	NS
b	*	***	***	*
a + b	***	***	NS	NS
c	NS	NS	***	*

*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; NS, Not significant.

The rate of N fertiliser had a significant ($P<0.01$) effect on all variables except a + b, the potentially degradable N, although there appeared to be little further influence of the timing of fertiliser application. The amount of N fertiliser applied had a strong positive relationship with the N content of the grain. The relationship which is shown in Figure 1 was significantly ($P<0.05$) non-linear and described by a second order polynomial:

$$\text{N content (g kg}^{-1}\text{ DM)} = 16.5 + 0.0509\ NA - 0.00007\ NA^2, \qquad R^2 = 58.4\%.\ \text{r.s.d} = 0.130$$

Where NA is total nitrogen fertiliser applied (kg ha^{-1}).

Increasing amounts of fertiliser N tended to reduce the fitted 'a' value whereas it tended to increase the value of 'b'. The simple linear regressions relating to these variables were:

'a' (%) = 20.8 - 0.0187 NA, R^2 = 10.0%, r.s.d. = 3.62, P<0.01

'b' (%) = 78.0 + 0.0189 NA, R^2 = 10.0%, r.s.d. = 3.66, P<0.01

Where NA is total nitrogen fertiliser applied (kg ha^{-1}).

Similar relationships (Figure 2) existed between the N content of the grains and the fitted 'a' and 'b' values viz:

'a' (%) = 32.7 - 0.658N, R^2 = 10.9%, r.s.d. = 3.60, P<0.01

'b' (%) = 67.0 + 0.624 N, R^2 = 9.4%, r.s.d. = 3.67, P<0.01

Where N is the nitrogen content of the grain (gkg^{-1}DM).

All of these relationships whilst being highly significant (P<0.01) showed a low variance accounted for, although it should be noted that any influence of factors such as year and site of harvest are included in these relationships.

DISCUSSION

The N contents of the wheat grains (Table 1) are broadly in agreement with the values (range 15.5 - 25.8 g N kg^{-1}DM) reported by MAFF (1990) for 45 samples of wheat obtained from a variety of sources. Thus the range in N contents obtained in the present work appears to be representative of that found in practice despite being obtained from a fertiliser N experiment. However, the N degradation characteristics obtained in the present study (Table 1) differ considerably from those reported by MAFF (1990) for seven wheats. In particular, the fitted 'a' values from the present work (mean 16.9%) were much lower than obtained by MAFF (1990) which reported a range from 39 to 53%. Also of note is the much lower rate of N degradation obtained (mean 0.128h^{-1}, Table 1) than reported by MAFF (1990) (range 0.22 - 0.51h^{-1}).

Fahmy *et al.* (1991) reported a N solubility in water for wheat (c.v. Camp Remy) of 26.2% and Herrera-Saldana *et al.* (1990) a zero hour loss for N in wheat of 72.5%, both of which are much higher than the current values (N solubility, 8.3 ± 1.84%). One reason for the lower 'a' and solubility value in the present work compared with the findings of MAFF (1990), Fahmy *et al.* (1991) and Herrera-Saldana *et al.* (1990) may relate to the small screen sizes used by these workers (1mm, MAFF, 1990; 0.8mm, Fahmy *et al.*, 1991; 1mm, Herrera-Saldana *et al.*, 1990) during sample milling, whereas a 3mm screen was used in the present study. Screen size has been shown (Michalet-Doreau and Cerneau, 1991) to be an important factor in determining the 'a' value for N in a range of feeds, although wheat was not included in this work. Another reason for the lower 'a' values and solubility of N in the present work may relate to the fact that the wheat studied was a breadmaking type, the total protein of which may have been richer in gliadin and glutenin proteins than in non-breadmaking varieties. Gliadin and glutenins are known to be less soluble and less readily degraded than the cytoplasmic albumin and globulin proteins (Fahmy *et al.*, 1991). More work is needed to measure the N degradation characteristics across a range of wheat types.

An important finding is the fact that under the present experimental conditions at least, the nylon bag technique overestimated the true water solubility of N. On average, water solubility of N measured in the laboratory was only about 0.4 and 0.5 of the nylon bag zero hour value and the fitted 'a' value respectively. These differences have been assumed to relate to the loss of N associated with fine particles lost through the pores of the nylon bags. This assumption seems to be reasonable since Michalet-Doreau and Cerneau (1991) showed by direct measurement that barley milled through a 3mm screen lost 8.7% of its N through the nylon bag pores. This compares with a mean NAFP value calculated by difference of 8.6% in the present work (Table 1), although there was considerable variability around this value. The results therefore question the use of the nylon bag method for estimating the N degradation of grain, especially if used in conjunction with fine grinding. Although not measured in the present work, it seems likely that the loss of starch particles through bag pores would be very much greater than N. Thus the use of this technique for estimating the rate and extent of starch fermentation in the rumen is very questionable. More research is clearly needed in this area to develop more suitable techniques.

In agreement with the current work (Figure 1), other studies (e.g. Dampney, 1992) have shown a strong relationship between the amount of fertiliser N applied and the N content of the grain, yet there appear to be no reports which relate fertiliser N application to the N degradation characteristics. The small but significant ($P<0.01$) reduction observed in the 'a' value as a result of extra fertiliser N is however in accordance with the findings of Salmon (1992) who showed that additional fertiliser N either as ammonium nitrate or urea increased the N content of breadmaking wheats by increasing the amount of the gliadin and especially glutenin proteins. The concentrations of albumin and globulin proteins remained unchanged. This tends to question whether the relationships between the N content of the grain and the 'a' and 'b' values (Figure 2) are causal or merely indications of the fertiliser treatment and whether the observed effects of fertiliser N on 'a' and 'b' values would also apply to non-breadmaking wheats. Further work is needed to examine this area.

The present work has shown that the N content, N solubility and N degradation characteristics of wheat can vary considerably. The values obtained from the immediately rumen soluble N ('a') and the rate of N degradation ('c') were both considerably lower than in earlier reports. The N content of the grain was strongly related to the amount of fertiliser N applied during growth. Whilst increasing fertiliser N and hence increasing grain N contents reduced and increased the immediately rumen soluble N ('a') and insoluble but degradable N ('b') respectively, the relationships were not strong enough to be of use for predicting unknown samples. In addition, the use of grain N content for predicting 'a' and 'b' values across different types of wheat is questionable. The experiment also showed that the nylon bag technique can severely overestimate the immediately soluble N content of wheat and probably other cereal grains.

ACKNOWLEDGEMENTS

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Figure 1. Relationship between total nitrogen fertiliser applied and nitrogen concentration of the wheat grain.

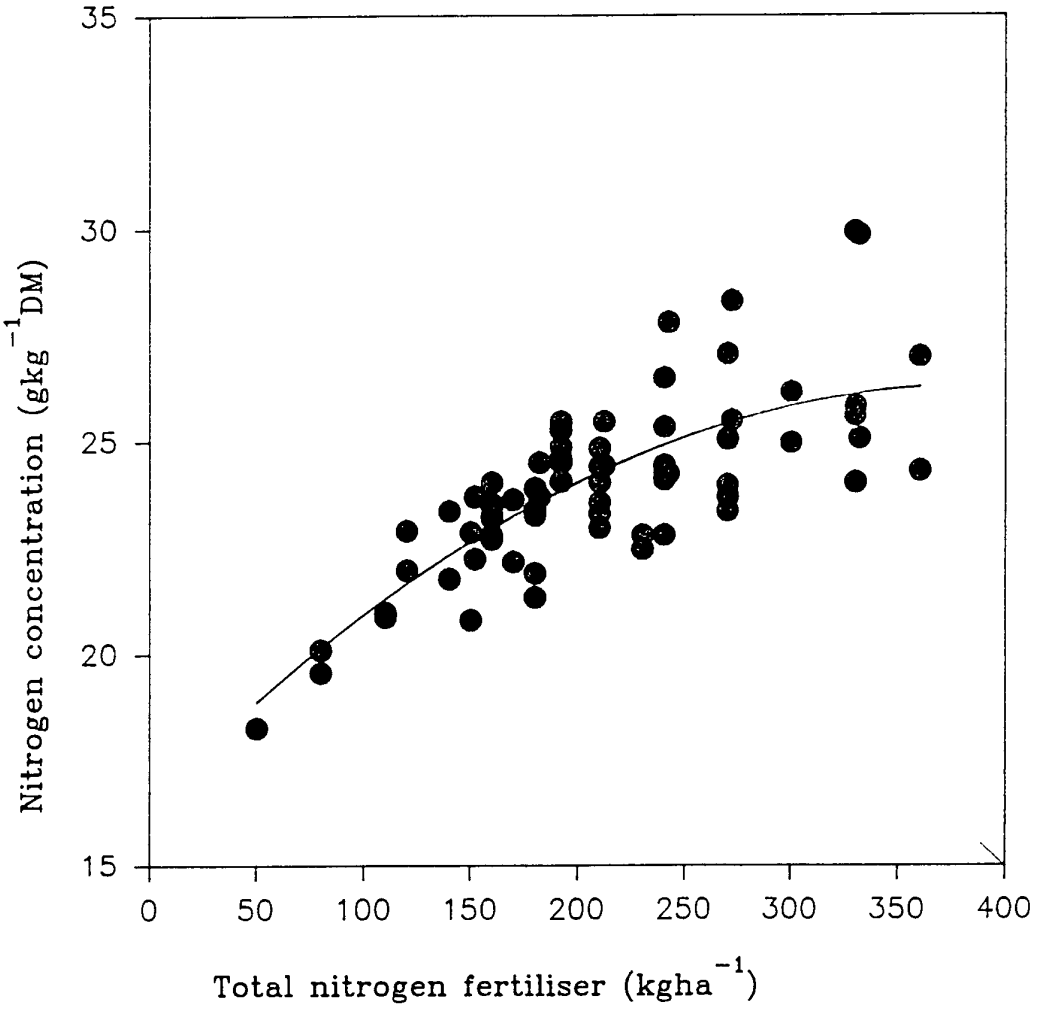
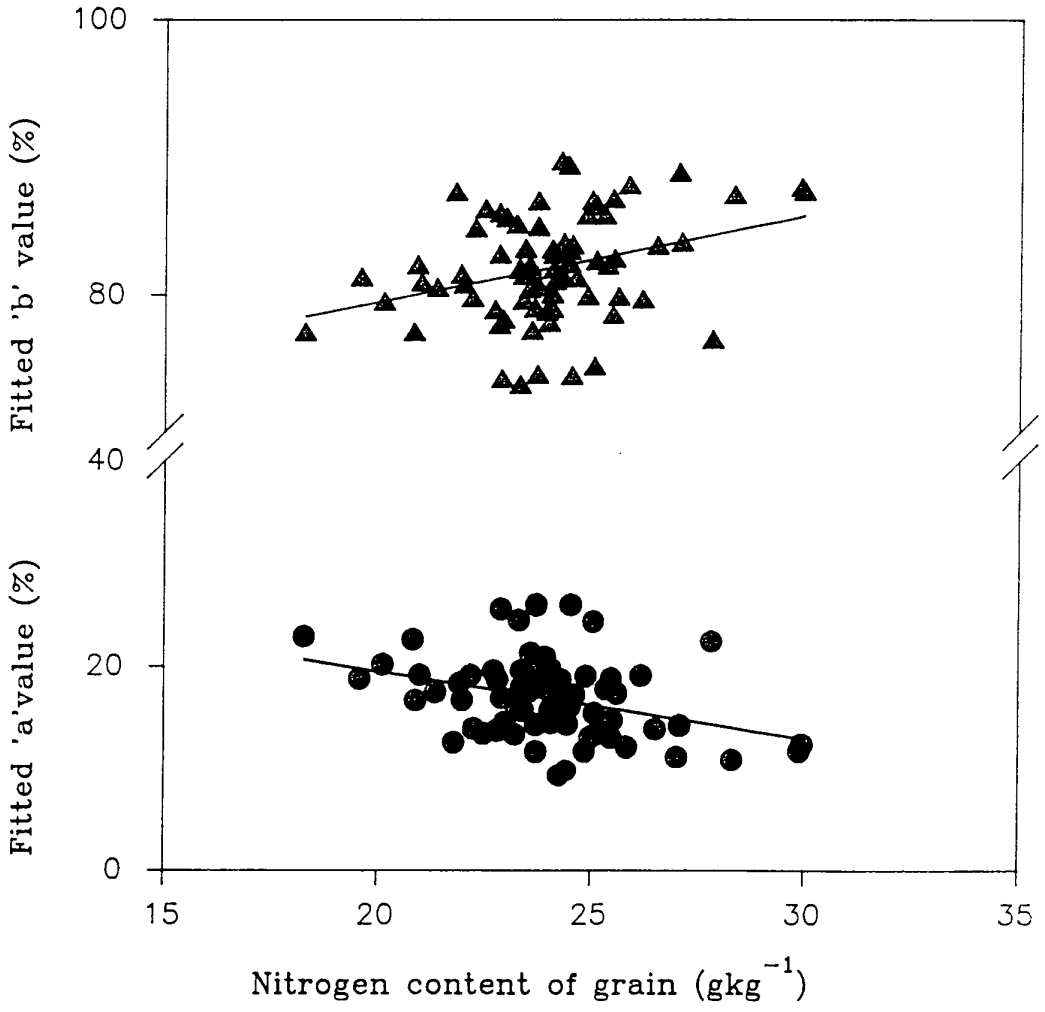


Figure 2. Relationship between nitrogen content of grain and fitted degradation constants 'a' and 'b'.



APPENDIX 1 DETAILS OF SITES AND FERTILISER TREATMENTS

FRAMPTON N RATES TRIAL 1990 HARVEST

Prelim. No.	M73	M74	M75	M76	M77	M78	M79	M80	M81	M82	M83
Treatment	1	2	3	4	5	6	7	8	9	10	11
No. of samples	2	2	3	3	3	4	3	4	3	2	4
Ammonium nitrate (kgN/ha)											
GS32	0	0	0	0	0	0	30	60	90	120	180
Foliar urea (kgN/ha)											
GS75-4 days	0	0	0	0	30	30	0	0	0	0	0
GS75-2 days	0	0	30	30	30	60	0	0	0	0	0
GS75	0	30	30	30	30	60	0	0	0	0	0
GS75+2 days	0	0	0	30	30	30	0	0	0	0	0
Total N applied (kg/ha)	50	80	110	140	170	230	80	110	140	170	230

All received 0 and 50 kg N/ha at GS 23 and 31 respectively as ammonium nitrate.

ROUNDWAY N RATES TRIAL 1990 HARVEST

Prelim. No.	M85	M86	M87	M88	M89	M90	M91	M92	M93	M94	M95
Treatment	1	2	3	4	5	6	7	8	9	10	11
No. of samples	4	4	3	4	4	4	4	4	4	4	4
Ammonium nitrate (kgN/ha)											
GS32	0	0	0	0	0	0	30	60	90	120	180
Foliar urea (kgN/ha)											
GS75-4 days	0	0	0	0	30	30	0	0	0	0	0
GS75-2 days	0	0	30	30	30	60	0	0	0	0	0
GS75	0	30	30	30	30	60	0	0	0	0	0
GS75+2 days	0	0	0	30	30	30	0	0	0	0	0
Total N applied (kg/ha)	150	180	210	240	270	330	180	210	240	270	330

All received 40 and 110kg N/ha at GS23 and 31 respectively as ammonium nitrate.

BLADON N RATES TRIAL 1991 HARVEST

Prelim. No.	M97	M98	M99	M100	M101	M102	M103	M104	M105	M106	M107
Treatment	1	2	3	4	5	6	7	8	9	10	11
No. of samples	4	3	4	4	3	4	4	4	3	4	4
Ammonium nitrate (kgN/ha)											
GS32	0	0	0	0	0	0	30	60	90	120	180
Foliar urea (kgN/ha)											
GS75-4 days	0	0	0	0	30	30	0	0	0	0	0
GS75-2 days	0	0	30	30	30	60	0	0	0	0	0
GS75	0	30	30	30	30	60	0	0	0	0	0
GS75+2 days	0	0	0	30	30	30	0	0	0	0	0
Total N applied (kg/ha)	180	210	240	270	300	360	210	240	270	300	360

All received 40 and 140 kgN/ha at GS 23 and 31 respectively as ammoniumn nitrate.

BISHOPS CANNING N RATES TRIAL 1991 HARVEST

Prelim. No.	M150	M151	M152	M153	M154	M155	M156	M157	M158	M159	M160
Treatment	1	2	3	4	5	6	7	8	9	10	11
No. of samples	4	3	3	3	4	4	4	4	4	4	3
Ammonium nitrate (kgN/ha)											
GS32	0	0	0	0	0	0	30	60	90	120	180
Foliar urea (kgN/ha)											
GS75-4 days	0	0	0	0	30	30	0	0	0	0	0
GS75-2 days	0	0	30	30	30	60	0	0	0	0	0
GS75	0	30	30	30	30	60	0	0	0	0	0
GS75+2 days	0	0	0	30	30	30	0	0	0	0	0
Total N applied (kg/ha)	150	180	210	240	270	330	180	210	240	270	330

All received 40 and 110 kg N/ha at GS 23 and 31 respectively as ammonium nitrate.

ADAS BOXWORTH RESEARCH CENTRE N TIMING TRIAL 1991 HARVEST

Prelim No.	M116	M117	M118	M119	M120	M121	M122	M123	M124	M125
Treatment	1	2	3	4	5	6	7	8	9	10
No. of sample	4	3	3	2	4	3	3	4	3	3
Nil	√	√								
Foliar urea (40kgN/ha)										
Flag leaf emerged (GS39)			√							
" + 10 days (GS46)				√						
" + 20 days (GS59)					√					
" + 30 days (GS67)						√				
" + 40 days (GS71)							√			
" + 50 days (GS75)								√		
" + 60 days (GS85)									√	
" + 70 days (GS91)										√
Total N applied (kg/ha)	120	120	160	160	160	160	160	160	160	160

All received 40 and 80 kg N/ha as ammonium nitrate at GS 23 + 31 respectively.

MILTON ERNEST N TIMING TRIAL 1991 HARVEST

Prelim No.	M140	M141	M142	M143	M144	M145	M146	M147	M148
Treatment	1	2	3	4	5	6	7	8	9
No. of samples	4	4	4	4	3	4	4	3	2
Nil	√	√							
Foliar urea (40kgN/ha)									
Flag leaf fully emerged (GS39)			√						
" + 10 days (GS45)				√					
" + 20 days (GS59)					√				
" + 30 days (GS69)						√			
" + 40 days (GS73)							√		
" + 50 days (GS75)								√	
" + 60 days (GS83)									√
" + 70 days*									
Total N applied (kg/ha)	152	152	192	192	192	192	192	192	192

*No sample. All received 42 and 110kg N/ha as ammonium nitrate at GS 23+ 31 respectively.

MILTON ERNEST N RATES TRIAL 1991 HARVEST

Prelim No.	M129	M130	M131	M132	M133	M134	M135	M136	M137	M138	M139
Treatment	1	2	3	4	5	6	7	8	9	10	11
No. of samples	4	4	4	3	4	4	4	3	4	4	2
Ammonium nitrate (kgN/ha)											
GS32	0	0	0	0	0	0	30	60	90	120	180
Foliar urea (kgN/ha)											
GS75-4 days	0	0	0	0	30	30	0	0	0	0	0
GS75-2 days	0	0	30	30	30	60	0	0	0	0	0
GS75	0	30	30	30	30	60	0	0	0	0	0
GS75+2 days	0	0	0	30	30	30	0	0	0	0	0
Total N applied (kg/ha)											
	152	182	212	242	272	332	182	212	242	272	332

All received 42 and 110 kg N/ha as ammonium nitrate at GS 23 and 31 respectively.

APPENDIX 2. ORDER OF INCUBATION OF WHEAT SAMPLES.

Batch No.	Preliminary No.	Batch No.	Preliminary No.
1	M83	12	M153
	M106		M19
	M132		M118
2	M102	13	M147
	M99		M123
	M133		M140
3	M152	14	M119
	M98		M100
	M137		M97
4	M75	15	M139
	M120		M131
	M77		M93
5	M134	16	M107
	M129		M103
	M80		M141
6	M92	17	M87
	M94		M89
	M144		M76
7	M122	18	M125
	M159		M154
	M138		M146
8	M116	19	M73
	M145		M121
	M142		M157
9	M78	20	M117
	M88		M104
	M156		M135
10	M82	21	M150
	M81		M101
	M160		M90
11	M148	22	M158
	M86		M85
	M143		M74
		23	M79
			M155
			M151
		24	M130
			M124
			M105
		25	M136
			M95
			Extra sample*

* Extra sample chosen at random and used to ensure that the same number of bags were incubated as in the other batches.