The effects of nitrogen management and cultivar on the yield, total protein and amino acid composition of feed barley

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Abstract

Variation in the total protein content of barley grain can be attributed to environmental and genetic effects. Characterisation of protein quality in terms of amino acid composition, especially the essential or limiting amino acids, is important when formulating diets for growing pigs and chickens. Two experiments were conducted to determine the variation in grain yield and quality caused by location, cultivar and nitrogen (N) fertiliser application. In the first experiment, grain N did not differ among four cultivars, but variation between nine sites was large (1.37 to 1.94%). Cultivar ranking within sites was consistent. The cv. Nugget invariably had highest grain N content and cv. Dash had the lowest. Cultivars differed in their amino acid composition. In the second experiment, cv. Nugget had a better feed value than cv. Dash because of its higher grain N concentration and improved complement of amino acids. This was consistent over the range of N applied (0-160 kg/ha). Nitrogen fertiliser improved the protein content and generally increased the concentration of amino acids. It also significantly increased N uptake and grain yield as well as improving protein quality. Split N fertiliser applications at sowing and tillering had no effect on yield compared with a single application at sowing, although there was a small significant ($P<0.05$) improvement in the total grain protein content. Cultivar choice was an important factor in management for improved yield and quality.

Additional key words: Hordeum vulgare *L., grain quality, crop management, essential amino acids, grain nitrogen*

Introduction

The feed grain industry has requested more precise specifications for barley quality because of the increasing demand for high quality protein sources for feeding pigs and chickens. Protein composition is important because sub-optimal concentrations of essential amino acids such as threonine, histidine, lysine, methionine and cystine are common in barley grain.

Production of high quality grain invariably requires increased inputs such as fertiliser N. Characterisation of environmentally-induced variation, and that due to fertiliser responses, may lead to more efficient production and utilisation systems for specific cultivars. Precise specifications of grain quality are required, preferably before harvest, so that grain lines can be handled in bulk. This may require adjustments to traditional crop management to ensure best quality grain. Complete quality data including detailed compositional analysis of amino acid content are required for blending barley with alternative protein sources for optimum utilisation by animals.

There is little doubt that total protein can be improved by the application of N fertilisers (Birch and Long, 1990; Grant *et al.*, 1991; Boila *et al.*, 1996; Dalal *et al.*, 1997), but there is debate as to whether the protein quality is improved. There have been reports of changes in the amino acid composition in response to fertiliser N application in wheat (Abrol *et al.*, 1971) and barley (Bulman *et al.*, 1994). Improvements in quality (Linko *et al.*, 1989; Bulman and Smith, 1993) with increased fertiliser applications are often complicated by inconsistent effects from year to year and cultivar differences in composition (Bulman *et al.*, 1994).

There are also reports of strong environmental effects, such as available soil water, on total protein content in feed barley. Drought caused increased free proline content in grain during filling but this effect disappeared by maturity as the free proline was incorporated into protein (Pearson and Stewart, 1990). Dalal *et al.* (1997) showed that increased plant available water at sowing linearly reduced grain protein content in contrast to the effect of increased available N at sowing. These authors

have proposed a system using the available water:available N ratio to predict grain protein content.

The objectives of this study were to (1) determine the extent of variation in essential amino acid composition of feed barley by analysing the composition of a range of cultivars in regional trials in New Zealand and (2) determine the effects of N fertiliser (amount and timing) and cultivar on feed grain quality. This should assist in forecasting grain feed quality and suggest methods for adjusting essential amino acid concentrations.

Materials and Methods

Experiment 1

Four feed barley cultivars (Cask, Dash, Nugget and Regatta) were grown at nine locations in 1997/98 (two in the Lower North Island, five in Canterbury and two in Southland) as shown in Table 1. N fertiliser was applied according to normal farmer practice at each site.

The trials were planted as randomised complete block designs with four replicates at each site. Trial husbandry has been described in detail (FAR, 1998). For each cultivar, a 2 kg grain sample was saved from each plot and passed twice though a grain divider. A 50 g undried sample was then used to determine screenings by passing over a 2.34 mm screen for 30 seconds. Another subsample from each plot was retained for N content determination using a LECO 2000 analyser. For amino acid analysis, equivalent volumes of sub-samples from the four replicates of each cultivar were combined, then mixed thoroughly and screened before grinding with a Udy Mill (1 mm screen). Combined samples carried though amino acid analysis were also analysed by LECO for total nitrogen. The difference between amino acid nitrogen and total nitrogen constituted non-protein N.

Experiment 2

A field trial was conducted at Lincoln with two feed cultivars, Dash and Nugget. The trial was sown on 25 September 1997 on a free draining Templeton silt loam and had five N treatments and three replicates of the treatments in randomised blocks. Each 9-row plot was 26 m x 1.35 m. The trial was sown with two border plots, and spare plots at 13 m spacing were used for irrigation wheel tracks within the replicates. The crops were sown to achieve a plant population of 275 plants/m^2 assuming an 80% establishment rate for both cultivars and germination of 86 and 96% respectively for Dash and Nugget. Sowing rates were 197.8 and 159.3 kg/ha for cv. Dash (TGW = 49.4 g) and cv. Nugget (TGW = 44.3 g).

No base fertiliser was applied at sowing as soil test results indicated adequate levels of macro nutrients (P 27, K 10).

Nitrogen fertiliser treatments were applied as urea (46% N) in a single application at emergence (14 October) or split applications at emergence and late tillering (21 November, Feekes GS5), at the following rates: 0, 40, 40 + 40, 80 and 80 + 80 kg N/ha. Both N applications were washed into the soil with an 8 mm irrigation (combined with an 18 mm rainfall at the second N application). On the second occasion, the crops showed signs of N stress as the first and second leaves were partially senesced. This was possibly caused by the very dry conditions prior to the fertiliser application.

Herbicide (12 g/ha Glean (a.i. 750 g/kg chlorsulphuron) and 500 ml Cougar/ha (a.i. 100 g/l diflufenican and 500 g/l isoproturon)) was applied to the whole trial on 19 November. Folicure (a.i. 250 g/l terbuconazole) was applied at 600 ml/ha on 12 December

Table 1. Location, soil type, sowing date and rate and time of N fertiliser applications for regional trials.

Trial location	Soil type	Sowing date	N fertiliser (kg N/ha) and time of application
Marton	Marton silt loam	25 Oct 97	26 (sowing)
Wanganui	Egmont black loam	28 Oct 97	26 (sowing)
Aylesbury	Lismore stoney silt loam	1 Nov 97	138 (stem elongation)
Highbank	Gorge silt loam	2 Oct 97	30 (sowing) + 57 (tillering)
Rakaia	Paparua sandy loam	3 Oct 97	100 (tillering)
Lyndhurst	Lyndhurst silt loam	23 Sep 97	50 (sowing)
Waimate	Willowbridge silt loam	28 Aug 97	40 (sowing)
Balfour	Kaweku silt loam	15 Oct 97	60 (sowing)
Oreti	Pukemutu silt loam	22 Oct 97	90 (sowing)

as a precaution against fungal disease. No disease was observed in the trial. The first full irrigation (32 mm) was delayed until five days after the herbicide application. Thereafter, water was applied on five occasions (ending 5 January) with 40 mm at each application with the aim of maintaining a deficit less than 100 mm. A weekly update of soil water deficit was calculated from accumulated potential evapotranspiration and adjusted for rain and evaporation during the period.

Mineral N levels

Mineral N levels (nitrate and ammonium) in the soil were measured on 16 December. Five soil cores, each 4.5 cm in diameter, were taken from each plot to a depth of 20 cm. They were bulked, mixed thoroughly, passed through a 2 mm screen, mixed again and stored at -10°C until analysis. The samples were thawed and 4 g fresh weight sub-samples were extracted in 20 ml of 2 M KCl. Nitrate and ammonium levels were determined on an RFA3000 autoanalyser.

Biomass

Duplicate 0.3 m^2 (0.50 m x 4 rows) samples were taken from each plot at maturity. Five-plant sub-samples were taken from the bulk and the plant material separated into grain and rest (stem + leaves + chaff). Both the bulk and five-plant sub-samples were dried at 80°C for 24 hours. Tiller population and yield (total biomass and grain yield) were determined on the bulk sample. Yield components (harvest index, grain weight, grain number per unit area and grains per ear), N components for determination of total N uptake, and efficiency of nitrogen use (total N uptake/total N applied as fertiliser) were determined on the five-plant sub-samples. Grain and 'rest' samples were ground to pass a 1 mm screen.

Grain yield

Single 1 m^2 samples (1.33 m x 5 rows) were taken from each plot for determination of yield and grain quality. Samples were threshed using a stationary Kurt Pelz thresher and samples were saved for determination of grain yield and N content of the screened and unscreened fractions. Representative grain samples were obtained by repeated sub-sampling of grain for a total sample weight of 5 kg from each plot. The volume was reduced further by repeatedly passing the sample through a grain divider to end with a well mixed sample representative of the whole plot. Grain sub-samples were dried at 80°C for 2 days and weights adjusted to 14% moisture content. Another split sample was saved from each plot for nitrogen (LECO) and protein quality analysis. This sample was screened and then ground to pass a 1 mm screen in a Udy mill.

Quality analysis

Nitrogen content of ground grain and herbage samples was determined using a LECO 2000 analyser on 200 mg sub-samples weighed directly from an oven at 80°C and compared against known grass and sulphamethazine standards. Duplicate hydrolysates were prepared for amino acid analyses by weighing 10 mg of ground material in 1.0 ml of redistilled 6 M HCl with 0.1% phenol added. Samples were heated for 24 h at 110°C in glass tubes sealed under vacuum. Amino acid concentrations were measured using a Waters HPLC ion exchange chromatography system. Methionine and cystine were measured as methionine sulphone and cysteic acid respectively by separate duplicate hydrolyses following a preliminary oxidation of the samples in performic acid (Moore, 1963). Non-protein N was calculated by difference between total N (LECO) and amino N (hydrolysed protein). Total N content of screenings was also determined.

Statistical analysis

Analysis of variance was performed using Genstat 5 Release 4.1, making the usual checks for validity of assumptions. Data from all sites were examined with the same analysis. Because replicate samples at the Balfour site were inadvertently combined, these samples were omitted from the N component and amino acid analyses. Where individual replicate data were used, the cultivar and site x cultivar interactions were tested using the Site.Rep residual. In cases where the replicates were bulked, the site effects were compared using the standard error of the mean (SEM) and cultivars tested using the site x cultivar interaction mean square.

Results and Discussion

Site effects

Site was the main contributor to variation in N concentration in the unscreened grain (Table 2). Grain N concentration did not differ significantly among cultivars. Averaged over sites , cultivars differed more for screenings than for N content. However, there was considerable variation among sites in level of screenings (Table 2). The Balfour site, in particular, had a high screening level. Cultivar effects were significant ($P<0.001$) and the ranking of cultivars within sites was not consistent, as there was significant ($P<0.01$) cultivar x site interaction. Details of the within site differences were not considered to be important in the context of this study.

Table 2. Grain quality and yield characteristics for four barley cultivars grown at nine locations in New Zealand.

Treatments and Effects	N concentration (%) (unscreened grain)	Screenings (%)	TGW[1] (g/1000)	Yield[2] (t/ha)
Cultivar				
Cask	1.68	4.1	46.9	6.6
Dash	1.64	3.9	45.7	6.5
Nugget	1.71	3.7	44.5	6.4
Regatta	1.70	2.4	50.6	6.6
$LSD^{3}_{P<0.05}$; df	0.18; 24	1.00; 24	0.91;21	-
Sites				
Marton	1.37	5.9	47.0	6.2
Wanganui	1.62	5.0	48.2	6.5
Aylesbury	1.91	4.0	49.1	9.5
Highbank	1.83	2.6	44.8	4.4
Rakaia	1.78	1.9	49.4	6.8
Lyndhurst	1.94	4.5	39.8	3.8
Waimate	1.54	1.7	48.8	8.8
Balfour[4]	1.78	11.2	40.5	6.6
Oreti	1.49	2.5	48.2	6.1
$LSD_{P<0.05}$; df	0.056; 71	0.64; 72	1.23 (SEM)	-

[1] Data source, FAR (1998) Arable Update No 28.
[2] Site x cultivar interaction was used to test cultivar effect for TGW only as field reps were bulked.
[3] LSD; least significant difference between two means at the 5% level. df = degrees of freedom.
[4] ANOVA excluded samples from Balfour as field replicates were bulked.

The differences in N concentration among sites were caused by many environmental factors including fertiliser level, initial soil N fertility, general crop management or climatic pattern but these relationships could not be determined from the results of this study. Experiments in which these factors are individually controlled will be the only practical way of determining the appropriate causal relationships

Lincoln Trial

The experiment conducted at Lincoln provides a partial explanation for the scale of the variability induced by one factor alone, that of nitrogen fertiliser application level.

Establishment

Uniform establishment was achieved for both cultivars in the trial. Mean populations were 279 plants/m^2 (se = 70) for cv. Dash, and 246 plants/m^2 (se = 45) for cv. Nugget.

Weather

The season was extremely hot and dry with conditions typical of an El Nino weather pattern. Average monthly temperatures were up to 2.5°C higher than the long term means for October, November, January and February (Table 3). Wind run was slightly higher than average in November and lower in all other months. Rainfall in all months was less than average (Table 3). Ideally, soil moisture deficits should not have exceeded 90 mm. However, deficits up to 152 mm occurred because of a delay in irrigation. The calculated maximum deficits were overestimates as the crop transpiration rate was not adjusted for partial cover before canopy closure. Dry topsoil conditions and hot northwest conditions during the tillering and early stem elongation phase caused unusually rapid development, a shortened tillering phase and rapid development through to heading. Levels of Olsen P>20 indicated that P was unlikely to be limiting. Soil tests showed other nutrients were also non-limiting and therefore not likely to have contributed to accelerated crop growth and development in this period.

Table 3. Mean monthly temperature, wind run and rainfall during the Experiment 2 trial compared to the long term means for Lincoln.

	Lincoln (1997/98)			Long term averages (1975-91)		
Month	Temperature (°C)	Rain (mm)	Wind run (km/day)	Temperature (°C)	Rain (mm)	Wind run (km/day)
September	8.3	26	298	9.2	40	361
October	12.0	32	338	11.3	55	397
November	14.0	23	406	13.1	56	398
December	15.2	43	380	15.7	61	395
January	17.9	17	371	17.0	50	415
February	19.6	14	360	16.3	51	397

Soil fertility and crop N uptake

A mid-season soil analysis (MAF quick test) on 16 December confirmed a medium to high fertility status for the experimental site. Soil mineral N levels (KCl extractable nitrate and ammonium) were monitored at anthesis to determine residual soil N under the N fertiliser treatments. These were consistent with the levels of N fertiliser applied. In the control, very little N remained and any additional N uptake was assumed to be derived from mineralisation. At the high N rate (160 kg N/ha) there was about 10 ppm of total mineral N in the top 0-20 cm soil layer at anthesis. This was equivalent to 20 kg N/ha potentially available for uptake by the plants during grain filling excluding any additional mineralised N.

During tillering and stem elongation the demand for N by the crop was high. At maturity, the total N uptake averaged for all cultivars, ranged from 107 kg/ha in the control treatment to 211 kg/ha in the 80 + 80 kg N/ha treatments. N remaining in the plant (non-grain fraction) ranged from 24 to 46 kg N/ha. The level of N present in the crops under high N treatments was approximately double the level in the control. This response was highly significant ($P<0.001$) with respect to fertiliser N level but there were no significant difference between cultivars.

The proportion of N in the crop derived from fertiliser increased with increasing level of applied N, and approached a maximum of 75% at the high N rate (80 + 80 kg N/ha). There was no difference between cultivars but differences among fertiliser N treatments were significant when levels exceeded 40 kg N/ha.

Grain Yield

Cv. Dash yielded approximately 0.5 t/ha more grain than cv.Nugget (Fig.1). This difference was due to a significant increase in grain number (19 885/m^2 for cv. Dash compared with 17 944/ m^2 for cv. Nugget), and more grains/ear (18.6 versus14.1) (Table 4). Cv. Dash had a lower TGW (Table 4), although the difference was small at 1 g/1000 kernels. Cv. Dash produced fewer tillers/m^2 than cv. Nugget (1089 versus 1280). However, this was more than compensated for by higher grain number and grains per ear.

Grain yield was increased from an average of 5.4 to 8.3 t/ha over the range of N applications (Fig. 1). At the higher rates there were significantly more tillers/m^2 were

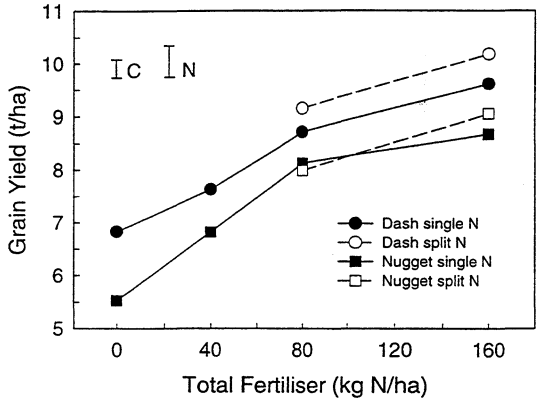

Figure 1. Response of grain yield (adjusted to 14% moisture) to nitrogen fertiliser in single or split applications for the cultivars Dash and Nugget at Lincoln. Vertical bars indicate least significant difference ($P<0.05$) between cultivar (C) and nitrogen (N) treatment means.

Table 4. Grain yield and quality characteristics of cultivars Dash and Nugget at Lincoln determined on 0.3 m^2 quadrats (tiller number, grain number and N content) and on 1.0 m^2 grain samples (screenings and thousand grain weight (TGW)).

Treatments	Tillers/m^2	Grain number/m^2	Grains/ear	TGW (g/1000)	Screenings (%)	%Nitrogen	
						Screenings	Screened grain
Cultivar							
Dash	1089	19885	18.6	39.3	5.4	1.81	1.72
Nugget	1280	17944	14.1	40.3	5.0	1.90	1.72
$LSD^1_{P<0.05}$	31	892	0.70	0.82	1.57	0.07	0.02
Fertiliser							
0	862	13591	17.4	41.4	2.7	1.72	1.53
40	1067	17031	16.8	40.2	4.0	1.72	1.61
40+40	1224	20003	15.6	39.9	5.2	1.85	1.71
80	1218	19177	16.2	39.5	4.6	1.79	1.73
80+80	1383	21896	15.9	39.2	6.7	2.00	1.87
160	1353	21808	16.3	38.5	8.1	2.05	1.86
$LSD_{P<0.05}$	54	1544	1.15	1.41	2.71	0.14	0.04

[1] LSD; least significant difference between means at the 5% level; degrees of freedom =22.

supported (1383 compared to 862) and therefore there were more kernels/m^2 (21 896 compared to13 591) (Table 4). The harvest index was also significantly lower in the control (0.54) compared with the high N treatment (0.58). This result was surprising, as the efficiency of conversion of dry matter to grain would normally increase with reduced N availability.

There were no significant interactions between cultivar and N treatments for yield component variables. Yield components which strongly correlated with grain yield were tiller number (r = -0.71), total dry matter yield (r = 0.98), harvest index (r = 0.71), TGW (r = -0.56) and grain number per unit area (r = 0.98).

Grain quality

N fertiliser had a large effect on grain quality variables, but differences between cultivar were small (Table 4). Grain N concentrations were consistently higher in cv. Nugget than in cv. Dash (Fig. 2) but these differences were not statistically significant.

Nitrogen fertiliser caused linear increases in both grain nitrogen concentration and yield (Table 4 and Figs 1 and 2), but these improvements occured at a potential cost to the grower in terms of quality. Screening level increased with increased grain N concentration (r^2 = 0.82)

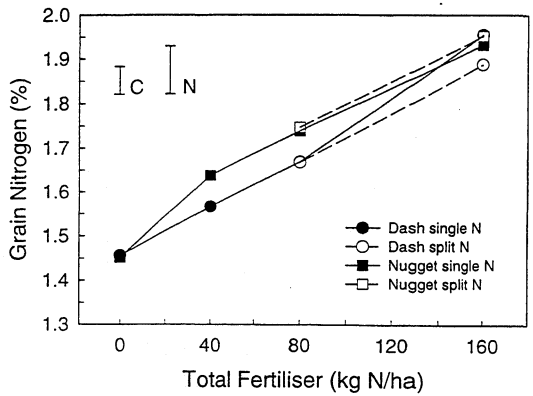

Figure 2. Grain nitrogen concentration from unscreened barley cultivars Dash and Nugget in response to the total fertiliser nitrogen applied as a single application at sowing or split application (sowing + tillering). Vertical bars indicate least significant difference (P<0.05) between cultivar (C) and nitrogen (N) treatment means.

and, similarly, grain size was decreased with increased grain yield ($r^2 = 0.79$).

Split applications of fertiliser were marginally more effective in raising the yield (Fig. 1) but there was little effect on the grain N concentration. Protein content was positively correlated with grain yield (r = 0.80) in agreement with other studies of feed barley responses to N (Garcia del Moral *et al.*, 1985; Birch and Long, 1990).

Amino acid composition

Expt. 1: Site effects

Site means could not be compared using standard analysis of variance because replicates were bulked. Instead, standard errors for site means were calculated by pooling the data for cultivars at each site. Amino acid composition differed considerably from site to site (Table 5). The mean total protein content varied from 85.3 mg/g at Marton (equivalent to 8.5% protein) to 133.2 mg/g (13.2%) at Rakaia.

Expt. 1: Cultivar effects

All amino acid concentrations except proline varied significantly among cultivars. With few exceptions the ranking of cultivars for their individual amino acid composition showed a consistent trend from low to high in the following order: Dash, Cask, Nugget, Regatta. The difference between cv. Dash and cv. Cask was always significant (Table 5). Cv. Nugget and cv. Regatta only differed in their contents of glycine, isoleucine, phenylalanine and histidine. In terms of total protein (amino acid) composition, cv. Nugget (119.5 mg/g) was the best of the cultivars, followed by Regatta and Cask (116 mg/g) and then Dash (114 mg/g).

Expt. 2: Cultivar effects

In the Lincoln trial the differences in amino acid composition between cv. Dash and cv. Nugget were small (Fig. 3). Background variability in the trial was low, so some of the differences were statistically significant. The concentrations of aspartic acid, leucine, tyrosine, phenylalanine, histidine, lysine, arginine and cystine were all significantly higher in cv. Nugget than in cv. Dash. However, the proline concentration was significantly lower in cv. Nugget. This single difference was sufficient for cv. Dash to have a higher total amino acid content (109 mg/g versus 105 mg/g). This contrasts with the site experiment where Nugget consistently had a higher total amino acid content.

Expt. 2: Fertiliser response

For all the amino acids except proline, the response to N fertiliser was highly significant ($P<0.001$) (Fig. 4). In most cases, the application of 80 kg N/ha (either single or split applications) significantly ($P<0.05$) increased amino acid concentration many cases the difference was significant when comparing the control and 40 kg N/ha treatments (data not shown). An additional application of 80 kg N/ha at tillering further improved the amino acid concentration compared with the single application at emergence ($P<0.05$). Generally, there was little difference between the amino acid composition of the 80 kg N/ha single and 40 + 40 kg N/ha split applications.

The improvement in amino acid concentration due to N fertiliser alone ranged from 14.3% (lysine) to 35.5% (glutamate). The appearance of these two amino acids at the extremes of the range of response to fertiliser is significant for the interpretation of feed value of barley flour. Lysine and threonine are essential amino acids in that no metabolic precursors can substitute for them in the monogastric diet (Butts *et al.*, 1991). Lysine is the most limiting of the essential amino acids in barley grain and appears to respond least to N fertiliser application. Conversely, glutamic acid, a non-essential amino acid, is one of the primary compounds synthesised from carbon and N (ammonium and glutamine) precursors. The positive response is possibly a direct result of higher concentrations of non-protein N in the grain during grain development in the high N fertiliser treatments.

Levels of non-protein N at maturity were minimal as the total amino acid content was nearly equivalent to the calculated crude protein content (%N x 6.25). Therefore, there appeared to be no residual effects of high rates of N fertiliser application on plant composition at maturity. The small amounts of residual mineral N in the soil for the high N treatments at anthesis had little significance for grain quality at maturity. No data were collected to analyse the accumulation of amino acids and non-protein N during grain filling. However, there is some indication from the literature that changes occur in the relative composition of glutamic acid, for example, during grain development. Bulman *et al.* (1994) showed that N fertiliser decreased the proportion of lysine in grain protein but did not affect other amino acids. In a second year, increases in N fertiliser raised the proportion of glutamic acid and proline in grain protein. Results for sulphur-containing amino acids were inconsistent (Bulman *et al.*, 1994). Higher N rates reduced the proportion of cystine in one cultivar but not in another.

Amino acid requirements for monogastrics

In the intensive livestock industry, the protein quality of ingredients for compounded feeds is generally

Table 5. Amino acid concentrations in screened grain of four barley cultivars grown at nine locations throughout New Zealand. Samples were taken from bulked field replicates.

Treatments	Arginine	Alanine	Aspartic	Cystine	Glutamic	Glycine	Histidine	Isoleucine	Leucine
Cultivar									
Cask	5.62	4.53	7.08	1.57	26.41	4.58	2.63	3.99	7.68
Dash	5.38	4.36	6.69	1.45	25.86	4.40	2.47	3.88	7.44
Nugget	5.88	4.66	7.23	1.61	26.89	4.67	2.73	4.10	7.86
Regatta	5.83	4.69	7.29	1.59	26.04	4.73	2.64	3.97	7.66
LSD[1] df=22	0.15	0.14	0.29	0.09	0.89	0.12	0.08	0.12	0.20
Sites									
Marton	4.46	3.80	6.36	1.35	18.83	3.95	2.08	3.08	6.02
Wanganui	5.42	4.12	7.03	1.38	24.91	4.33	2.50	3.64	7.09
Aylesbury	6.23	4.87	7.90	1.59	30.74	4.95	3.00	4.36	8.44
Highbank	6.23	5.00	7.56	1.72	30.34	5.00	2.97	4.51	8.60
Rakaia	6.25	5.05	7.49	1.66	30.72	4.99	2.90	4.53	8.61
Lyndhurst	6.18	4.96	7.44	1.77	30.55	4.97	2.83	4.57	8.72
Waimate	5.07	4.24	6.19	1.56	21.87	4.21	2.26	3.52	6.79
Balfour	5.96	4.65	7.08	1.59	27.59	4.63	2.66	3.97	7.87
Oreti	5.38	4.45	6.61	1.43	22.44	4.38	2.42	3.64	7.01
SEM[2]; df= 9	0.21	0.15	0.20	0.05	1.53	0.13	0.11	0.18	0.33

	Lysine	Meth-ionine	Proline	Phenyl alanine	Serine	Threonine	Tyrosine	Valine	Total
Cultivar									
Cask	3.97	1.86	22.68	5.55	4.62	3.99	3.68	5.62	116.1
Dash	3.84	1.76	21.09	5.60	4.46	3.87	3.54	5.32	111.4
Nugget	4.10	1.91	23.74	5.81	4.72	4.08	3.73	5.77	119.5
Regatta	4.11	1.97	22.04	5.49	4.65	4.14	3.69	5.66	116.2
LSD; df=22	0.08	0.09	3.03	0.16	0.21	0.18	0.09	0.15	4.6
Sites									
Marton	3.53	1.53	11.55	4.16	3.65	3.32	2.93	4.52	85.3
Wanganui	3.82	1.74	23.67	5.39	4.30	3.78	3.50	5.20	111.8
Aylesbury	4.22	2.01	27.91	6.39	5.33	4.59	4.03	6.02	132.6
Highbank	4.32	2.10	24.62	6.34	5.48	4.69	4.12	6.13	129.7
Rakaia	4.28	2.03	28.22	6.40	5.24	4.51	4.09	6.21	133.2
Lyndhurst	4.31	2.08	24.55	6.46	4.75	4.17	4.03	6.33	128.7
Waimate	3.71	1.79	18.29	4.83	4.06	3.50	3.24	5.02	100.1
Balfour	4.15	1.90	11.91	5.68	4.67	4.00	3.72	5.76	107.8
Oreti	3.83	1.73	20.29	4.94	4.11	3.59	3.34	5.28	104.9
SEM2; df=9	0.10	0.06	2.08	0.28	0.21	0.17	0.14	0.21	5.67

[1] LSD - least significant difference between means at the 5% level. Site x cultivar interaction was used to test cultivar effect.
[2] SEM - standard error of the mean.

evaluated on the basis of the total lysine content and the balance of the other essential amino acids relative to the total lysine content (Butts *et al.*, 1991). Lysine is used as the reference amino acid because it is the first limiting amino acid in the diets of pigs and poultry. The ideal balance of dietary essential amino acids to satisfy the nutrient requirements of protein accretion for the pig is: lysine 100 units, arginine 48, histidine 32, isoleucine 54, leucine 102, methionine 27, methionine + cystine 55, phenylalanine 60, phenylalanine + tyrosine 93, threonine 60, tryptophan 18, valine 68 (NRC, 1998). In practice, a proportion of the dietary amino acids is not biologically available to the animal. Therefore, evaluation of quality based on gross amino acid compositions will overestimate the nutritional value and this needs to be kept in mind when interpreting these results.

In order to evaluate the protein quality of all samples from both the cultivar/site and fertiliser trials, the total lysine contents were expressed on the basis of g total lysine/100 g crude protein (calculated on a dry weight basis as total N x 6.25) and compared with a reference value of 7 g lysine/100 g crude protein. The lysine requirement for young growing pigs is 6.88 g/100 g crude protein for 1.4 to 4.5 kg live weight and 5.77 g/100 g crude protein for 4.5 to 9.0 kg live weight (ARC, 1981). For the purpose of this report, the value of 7% is a reference value by which to compare the deficiencies of lysine and other essential amino acids in barley as the sole source of dietary protein to support growth of pigs.

To systematically assess the nutritional consequences of the amino acid contents of the various samples, the amino acid content of the dried ground sample was calculated on a per gram crude protein basis. Methionine and cystine contents were added together as the requirement for sulphur containing precursors can be met by both amino acids, but with the constraint that up to half the total requirement can be met by cystine, and the entire requirement can be met by methionine. Tyrosine and phenylalanine were also assumed to partially substitute for one another. Arginine was not considered a limiting amino acid for the growing pig.

It was apparent from both experiments that the lysine content of barley flour was insufficient to support growing pigs, providing only half of their requirement. For the pig, amino acid content was marginal for leucine, isoleucine, threonine and methionine + cystine (Table 6), whereas the concentrations of histidine, phenylalanine + tyrosine, leucine, isoleucine and valine were sufficient.

In this study, N fertiliser increased the protein content of the grain but its nutritional value was potentially

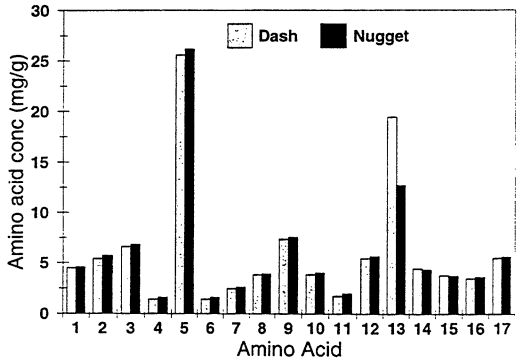

Figure 3. Differences in amino acid concentration for two cultivars grown at Lincoln. Values are means over all N treatments. Amino acids: 1 = Alanine, 2 = Arginine, 3 = Aspartic acid, 4 = Cystine, 5 = Glutamic acid, 6 = Glycine, 7 = Histidine, 8 = Isoleucine, 9 = Leucine, 10 = Lysine, 11 = Methionine, 12 = Phenylalanine, 13 = Proline, 14 = Serine, 15 = Threonine, 16 = Tyrosine, 17 = Valine.

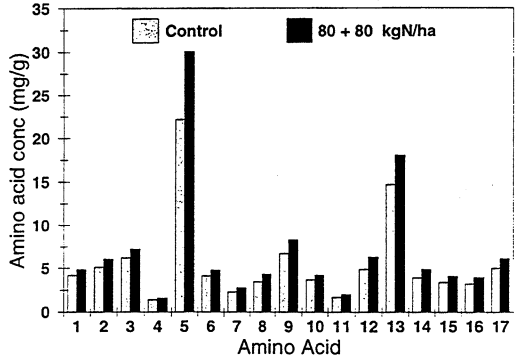

Figure 4. The effect of fertiliser N level on the composition of amino acids of barley grown at Lincoln. Data are means over two cultivars. Amino acids as for Fig. 3.

Table 6. Mean ratios[1] for amino acid composition of barley flour relative to the requirements for growing pigs.

Trial/Treatments		Proportion of the amino acid[2] requirement for growing pigs								
		Lys	Arg	His	Ile	Leu	Met +Cys	Phe +Tyr	Thr	Val
Trial 1: Multi - sites										
Site x cv.z(n=36)	Mean	0.53	1.57	1.08	0.97	0.99	0.83	1.31	0.88	1.09
	SD[3]	0.038	0.075	0.044	0.034	0.030	0.060	0.037	0.047	0.047
Trial 2: Lincoln										
Fert. x cv.z(n=30)	Mean	0.54	1.57	1.08	0.97	0.99	0.84	1.32	0.85	1.11
	SD	0.027	0.075	0.053	0.030	0.030	0.055	0.050	0.028	0.044

[1] Values were calculated as ratio of the actual level in the sample to the known requirement of individual amino acid (NRC, 1998). These values were corrected for the lysine requirement of 7 g/100 g crude protein for 1.4 - 9.0 kg pigs (ARC, 1981).

[2] Lys = lysine, Arg = arginine, His = histidine, Ile = Isoleucine, Met = methionine, Cys = cysteine, Phe = phenylalanine, Tyr = tyrosine, Thr = threonine, Val = valine.

[3] Standard deviation.

lowered because of a low response of lysine content to N fertiliser. The results of Bulman *et al.* (1994) support this finding. While the concentration of individual amino acids may be improved by selection of cultivar, source of grain (site effects) or application of N fertiliser, the real value to the animal must be evaluated either with reference to the most limiting amino acid (lysine in the case of barley) or a precise determination of utilisation (availability/digestibility). Amino acid requirements vary according to the energy density of the diet and it is also conceivable that fertiliser, cultivar or environmental effects on protein composition may be different if evaluated in an *in vivo* digestion. Our calculations (Table 6) point to limitations of specific amino acids such as lysine, methionine and threonine, but do not give any indication of the improvement in composition that may be attributed to site, cultivar or N fertiliser effects. The calculations relating to proportion of the requirements were standardised relative to the lysine content. Therefore, significant gains were only made if the relative content of an individual amino acid was significantly greater than the corresponding improvement in lysine content. In addition, expressing the proportions of amino acids relative to the total crude protein in the samples showed that most amino acids were increased by an amount comparable to the increase in total protein. Nevertheless, any improvements in the essential amino acid concentrations directly related to agronomic influence will have potential benefits in feed formulation.

The nutritional value of grain depends on the use of the amino acids in the digestive system of monogastric animals. In practice, a proportion of the dietary amino acids is not biologically available to the animal and, therefore, evaluation of quality based on gross amino acid compositions will overestimate the nutritional value. The key to formulating diets is to manage the concentration of limiting amino acids. Currently, diet formulation is based on tabled values for digestible lysine and total energy. Future work on barley feed quality should include *in vivo* evaluation to determine the true metabolic value in monogastric animals.

Conclusions

1. Site effects were the main contributor to variation in grain N concentration (crude protein) and screening level. In general, the protein levels were comparatively low, indicating a significant capacity for raising the N concentrations with additional fertiliser N. When analysed over multiple sites there were no consistent differences among cultivars.
2. N fertiliser strongly affected crop growth, N uptake, grain yield and quality (N concentration and amino acid composition). The cultivars cv. Dash and Nugget differed in the amount of N partitioned to the grain but not in total N uptake.
3. Grain N concentration was increased by around 0.50% (3.15% protein) over the range of fertiliser applied (0-160 kg N/ha) or 0.15% N /50 kg of N applied. Split applications also tended to improve grain N content, but the effect was not significant compared with the single application at emergence.

At most levels of N fertiliser application, cv. Nugget tended to have higher grain N content, but the cultivar effect was not statistically significant.

4. Although yields were increased by N fertiliser, the screening percentage was also increased, and mean grain size was reduced. Therefore, management to improve productivity has a potential downside with reduced payouts for undersized grain or excessive screenings.
5. The composition of amino acids varied significantly over sites. There were strong cultivar differences in the composition of all amino acids except proline. Cv. Nugget and Regatta were invariably better than cv. Dash or Cask.
6. While Dash was a higher yielding cultivar than Nugget, the grain quality characteristics of cv. Nugget were invariably better by virtue of the improved amino acid composition rather than the total crude protein level. Cv. Nugget had higher levels of all amino acids except proline, but only half of these differences were statistically significant. The total amino acid content was, however, similar for both cultivars.
7. Fertiliser effects were strong for all the component amino acids except proline. The improvement due to N fertiliser alone ranged from 14% to 36%. Nutritional gains in terms of feed formulation were significant, especially for amino acids such as lysine, methionine and threonine that are naturally limiting in barley.

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