Effects of Nitrogen Fertilizer on Protein Quantity and Gluten Strength Parameters in Durum Wheat (*Triticum turgidum* L. var. *durum*) Cultivars of Variable Gluten Strength

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ABSTRACT

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Field studies were conducted over three years at two locations in Saskatchewan, Canada, to determine the effect of nitrogen fertilizer on protein quantity and protein strength in 10 cultivars of durum wheat (*Triticum turgidum* L. var. *durum*) representing a range of gluten strength. Increasing nitrogen fertilizer resulted in increased protein content in all cultivars across environments. Cultivars were clearly differentiated on the basis of gluten strength using a gluten index (GI), SDS sedimentation (SDSS), alveograph indices of overpressure (*P*) and deformation energy (*W*), mixograph energy to peak (ETP), and mixograph bandwidth energy (BWE) at all fertilizer levels. Variable cultivar response to nitrogen fertilizer was observed only for protein content, GI, and alveograph *W*. The nature of the cultivar-by-fertilizer interaction for GI suggested that the conventional strength cultivars would benefit more from nitrogen fertilizer than the extra-strong types, which showed no change or slight decreases in GI with nitrogen fertilizer despite an increase in total gluten. SDSS increased with nitrogen fertilizer, following similar trends as protein. Gluten strength rankings of the cultivars by SDSS were maintained with increased fertilizer. Fertilizer had little effect on alveograph *P*, mixograph ETP, and mixograph BWE. Overall, GI values were more stable across increasing levels of nitrogen fertilizer and resultant increased protein content compared with SDSS, mixograph development time, and alveograph *W* and *L*, suggesting it is a good test for estimating intrinsic gluten strength for cultivars with a wide range of protein content.

Under nitrogen (N) limiting soil conditions, application of N fertilizer is known to increase protein content and alter flour functionality in bread wheat. Fewer studies are available on the effects of N fertilizer on quality traits in durum wheat, particularly regarding the effects on extra-strong gluten types.

Protein content and gluten strength are two of the major quality criteria used to predict pasta quality of durum wheat cultivars. Matsuo et al (1972) showed farinograph dough development time decreased and maximum consistency and tolerance index increased significantly with increased protein content. Increased protein content was also associated with firmer cooked pasta (Matsuo et al 1972). A review on protein in durum wheat concludes “a high protein content and a strong gluten are required to process semolina into a suitable pasta product” (Feillet 1988).

Increasing protein content is normally associated with increased dough strength and improved baking quality for bread wheat (Johansson et al 2001), although very high levels (>17%) have been linked to weakening of dough strength properties for some Canadian hard red spring wheat cultivars (Tipples et al 1977; Bushuk et al 1978; Kosmolak and Crowle 1980). Increased protein content in Canadian durum wheat of conventional gluten strength is also associated with increased dough strength (Dexter et al 1994). To our knowledge, there are no reports of a negative relationship between protein and gluten strength for durum wheat.

Protein increases due to N fertilizer have been associated with changes in the distribution patterns of high molecular weight (HMW) and low molecular weight (LMW) proteins (Doekes and Wennekes 1982; Lasztity et al 1984; Wieser and Seilmeier 1998). Changes in quantities of gluten proteins (gliadins and glutenins) and the ratio of HMW/LMW subunits affect technological quality of bread wheat (Hamada et al 1982; Uthayakumaran et al 1999). In durum wheat, protein quantity is believed to account for much of the variability in cooking quality of pasta (Matsuo et al 1972; Dexter and Matsuo 1977; Autran et al 1986; D’Egidio et al 1990). Still, gluten and dough strength parameters continue to be used to assess quality of durum wheat, and the increasing demand for extra-strong durum types suggests protein strength also plays a significant role in end-product quality. Most studies suggest that increased protein is accompanied by increased gluten strength (Abad et al 2000; Johansson et al 2001). However, Dexter et al (1982) concluded that for durum wheat of weak to moderate gluten strength, N fertilizer increased protein content but gluten strength was not affected.

In recent years, durum breeding programs in western Canada have been successful in developing cultivars with significantly stronger gluten, thus resulting in the creation of an extra-strong durum wheat classification, as well as cultivars with higher protein levels (Marchylo et al 1998, Dexter and Marchylo 2001). Although the effect of fertilizer N on durum quality has been investigated (Dexter et al 1982), there are several reasons to readdress this question. The first reason is related to the development of North American durum cultivars with significantly stronger gluten than those studied in the past (Ames et al 1999). Second, gluten index and alveograph measurements have become recognized durum wheat gluten strength specifications in many markets, and to our knowledge no published documentation exists on the effects of N fertilizer on these measurements. Finally, while it is generally accepted that higher protein or very strong gluten results in better quality durum, it is not known whether N affects the gluten properties of conventional strength and extra-strong gluten types similarly. Identification of cultivars that maintain high gluten quality and dough strength characteristics with minimal fertilizer input would be particularly useful in situations where reduced input is desired for environmental reasons or for “organic” production.

The objective of this study was to determine the effect of increased N fertilizer on protein quantity and gluten strength in cultivars of durum wheat with a range of gluten strength including novel extra-strong gluten strength types.

MATERIALS AND METHODS

Plant Material and Experimental Design

The 10 locally adapted durum cultivars selected for this study included registered Canadian cultivars Kyle (Townley-Smith et al 1987), AC Avonlea (Clarke et al 1998), AC Navigator (Clarke et

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TABLE I
Analysis of Variance (ANOVA) for Grain Protein Characteristics

Source of Variation	DF[a]	Grain Protein Content	Whole Meal SDSS[b]	Whole Meal GI[b]
Fixed effects				
Cultivar (C)				
F value	9	6.3**[c]	50.0**	20.3**
Den DF[d]		37.1	40.8	47.4
Fertilizer (F)				
F value	4	19.7**	23.5**	0.5
Den DF		20.6	20.8	6.1
C × F				
F Value	36	1.8**	1.4	1.9**
Den DF		360.3	361.2	304.2
Random effects (est. ratio)[e]				
Year (Y)		0.00	0.71	1.44
Location (L)		0.00	0.00	0.69
Y × L		0.15	3.06	0.00
Rep (Y × L)		0.25	0.16	0.01
Environment (E) × C		0.06	0.30	1.61
E × F		0.35	0.16	0.01
Residual		0.38–2.58	0.41–1.45	0.29–2.18

[a] Degrees of freedom.

[b] SDSS, sodium dodecyl sulfate sedimentation; GI, gluten index (%).

[c] *, ** = significant at $P \le 0.05$, $P \le 0.01$, respectively.

[d] Denominator degrees of freedom.

[e] Ratio of parameter estimate to average residual variance estimate. For residual, the range indicates the ratios of the variance estimates for the lowest and highest environment to the average.

al 2000a), AC Pathfinder (Clarke et al 2000b), and AC Morse, as well as breeding lines DT 674, DT 672, DT 675, DT 666, and a U.S. cultivar, Durex. These cultivars were selected to cover a range of gluten strength types, with the lower limit of the range typical of what is currently grown in Canada and utilized by industry (Kyle and AC Avonlea) and the higher end represented by the new extra-strong types (Durex and AC Navigator). The 10 cultivars were grown near Swift Current and Beverley, SK, in 1997, 1998, and 1999 on land fallowed the previous year. Available soil N at planting at the Swift Current site (0–60 cm depth) was 11, 59, and 36 kg/ha in 1997, 1998, and 1999, respectively. The available soil N was 13 kg/ha in 1997, 35 kg/ha in 1998, and 39 kg/ha in 1999 for the Beverley site. The experimental design was a randomized complete block with two replicates utilizing a factorial arrangement for five fertilizer treatments (0, 40, 80, and 120 kg/ha applied as urea [46-0-0] at seeding and 120 kg/ha at seeding plus 40 kg/ha top-dressed at head emergence). The trials near Swift Current received supplemental irrigation of $\approx$100 mm in two applications. The first application was applied at the time of fertilizer top-dressing to ensure N uptake, and the second was applied at midgrain fill. The trials at Beverley were grown under rainfed conditions. Protein content and gluten strength were determined on grain or whole meal for all replicated samples across locations, fertilizer treatments, and years, to evaluate the main effects of cultivar, fertilizer, and their interactions. Due to limited sample size and milling resources, semolina milling and testing were conducted on a subset including three years, but only three of the five fertilizer treatments (0, 80, 120+40 kg/ha) and grown at one location. The fertilizer treatments chosen represented the largest differences in protein and the Swift Current site had the highest yields, ensuring adequate material.

Milling Procedure

Grain test weight was determined using the Schopper Chondrometer equipped with a 1-L container. Grain was milled into whole meal with a Udy cyclone mill equipped with a 1-mm screen. The wheat samples were cleaned and conditioned as described by Dexter and Tipples (1987) and milled into semolina by a four-stand mill (Allis-Chalmers) according to the procedure of Dexter et al (1990) to an extraction rate of $\approx$68%.

Analytical Tests

Analytical results were expressed on a 13.5% moisture basis (mb) for whole meal, and 14.0% mb for semolina. Moisture was determined by Approved Method 44-15A (AACC 2000). Protein content of whole grain was determined by near-infrared scanning (NIR) on a NIR model 6500 equipped with infrasoft version 3.10 software. Protein content referred to hereafter is that of whole grain. SDS sedimentation (SDSS) values were determined using 3% SDS as described by Dexter et al (1980). Approved Method 38-12 (AACC 2000) was followed for the determination of a wet gluten and gluten index (GI) of whole meal samples. GI of semolina was determined using the two-stage method of gluten washing before centrifugation according to Standard No. 158 (ICC 2001).

Alveograph curves were obtained by Standard No. 121 (ICC 2001) using a constant pressure Alveograph model MA82. Extensibility values for overpressure (*P*), average abscissas at rupture (*L*), deformation energy (*W*), and configuration ratios (*P/L*) were computed automatically by the instrument. Semolina mixograph curves were obtained using a 10-g, computer-based mixograph system (Buckley et al 1990) at 56.5% water absorption. Mixing development time (MDT), bandwidth energy (BWE), peak bandwidth (PBW), energy to peak (ETP), and total energy (TEG) were recorded.

Statistical Analysis

The experiment was set up and analyzed to evaluate the effect of fertilizer using a sampling of environments typical of durum growing areas. A linear mixed model was fitted to the data (SAS Institute, Cary, NC). In the full model, fixed effects were cultivar, fertilizer, and their interaction. Random effects were environment (year-by-location), replicate within environment, and interactions between environment and the fixed effects. A separate error variance was fitted for each environment. This allowed pooling the data from all environments when the residual variances were heterogeneous. Satterthwaite's approximation formula was used for the degrees of freedom (DF) (SAS). Because inclusion of environment interactions, however small, sharply reduced DF for the *F* tests, Akaike's information criterion (SAS) was used to select which random effects to retain for the model and whether to use a common error variance. Variance components were estimated using restricted maximum likelihood (REML). Estimate ratios from

TABLE II
Analysis of Variance (ANOVA) for Semolina Quality Alveograph Characteristics

Source of Variation	DF[a]	GI[b]	Alveograph Indices[c]			
			P	*L*	*W*	*P/L*
Fixed effects						
Cultivar (C)						
F value	9	61.9**[d]	13.5**	8.9**	22.8**	6.7**
Den DF[e]		18.7	17.2	143.0	18.1	18.2
Fertilizer (F)						
F value	2	0.3	1.3	13.0**	7.5*	7.1**
Den DF		28.3	3.9	143.0	3.9	125.3
C × F						
F value	18	1.0	0.8	0.5	1.9*	0.8
Den DF		28.3	109.2	143.0	107.2	125.3
Random effects (est. ratio)[f]						
Year (Y)		3.98	0.79	1.86	1.75	1.93
Rep (Y)		0.05	0.56	0.33	0.43	0.32
Y × C		0.41	1.02	0.41	0.27	
Y × F		0.00	0.19	0.16		
Y × C × F		0.25				
Residual		0.6–1.8	0.8–1.5	1.00	0.7–1.4	1.00

[a] Degrees of freedom.

[b] Gluten index (%).

[c] *P*, overpressure (mm); *L*, abisicssa at rupture (mm); *W*, deformation energy (10^{-4} J).

[d] *, ** = significant at $P \leq 0.05$, $P \leq 0.01$, respectively.

[e] Denominator degrees of freedom.

[f] Ratio of parameter effect to residual or mean of residual variance estimates. Blanks indicate effect was not included in the model. For residual, the range indicates the ratios of the variance estimates for each year.

TABLE III
Analysis of Variance (ANOVA) for Semolina Quality Mixograph Characteristics

Source of Variation	DF[a]	GI[b]	Mixograph Values[c]			
			MDT	ETP	PBW	TEG
Fixed effects						
Cultivar (C)						
F value	9	61.9**[d]	18.0**	27.1**	6.9**	4.2**
Den DF[e]		18.7	18.2	18.2	18.2	18.1
Fertilizer (F)						
F value	2	0.3	14.5*	1.3	3.3	3.8
Den DF		28.3	4.0	125.4	4.0	4.0
C × F						
F value	18	1.0	1.0	1.5	0.8	0.9
Den DF		28.3	36.0	125.4	121.3	121.0
Random effects (est. ratio)[f]						
Year (Y)		3.98	2.16	1.56	0.09	0.27
Rep (Y)		0.05	0.28	0.14	0.35	0.04
Y × C		0.41	0.21	0.13	0.19	0.31
Y × F		0.00	0.08		0.41	0.61
Y × C × F		0.25	0.37			
Residual		0.6–1.8	1.00	1.00	1.00	1.00

[a] Degrees of freedom.

[b] Gluten index (%).

[c] MDT, mixing development time (min); ETP, energy to peak (J); PBW, peak bandwidth (N m); TEG, total energy (J).

[d] *, ** = significant at $P \leq 0.05$, $P \leq 0.01$, respectively.

[e] Denominator degrees of freedom.

[f] Ratio of parameter effect to residual or mean of residual variance estimates. Blanks indicate effect was not included in the model. For residual, the range indicates the ratios of the variance estimates for each year.

the analysis provide the relative contribution of each random effect to the total variance of a single observation. Estimates of the random effects are used in the *F* tests of fixed effects. Comparisons between and within two cultivar groups were made using contrasts. One group consisted of the extra-strong cultivars, while the other group included the remaining cultivars.

The REML method does not provide explicit denominators for the *F* tests. However, in the full model, each *F* test can be approximated by testing the fixed effect against the interaction between environment and itself. For environment interactions that are excluded or estimated as zero, any such two-way interaction should be replaced with the three-way interaction, and if the three-way interaction is missing, it should be replaced with the residual (mean of the error variances). A relatively large environment interaction makes the corresponding fixed effect more difficult to detect.

Because there was a relationship between the variance and the mean for some variables, transformation was used before analysis to stabilize the variances. The arcsine square root transformation was used for GI, square root for alveograph parameters, SDSS and ETP, and log for MDT and BWE. Where variables were transformed, mean data are presented as both transformed and back-transformed to provide both statistically and numerically relevant data.

TABLE IV
Fertilizer Least Square Means for Grain and Whole Meal Quality Characteristics[a,b]

Nitrogen Rate (kg/ha)	Grain Protein Content (%)	Whole Meal SDSS[c]	Whole Meal SDSS[d]	Whole Meal GI[c]	Whole Meal GI[d]
0	12.2	6.0	36.4	0.77	48.4
40	12.5	6.1	37.6	0.77	48.2
80	13.2	6.3	39.6	0.76	47.7
120	14.3	6.4	41.4	0.77	48.4
120+40	15.0	6.6	42.9	0.77	48.6
SED[e]	0.38	0.06		0.007	
DF[f]	20.6	20.8		6.1	

[a] Averaged over 10 cultivars, three years, and two locations.
[b] SDSS, sodium dodecyl sulfate sedimentation (mL); GI, gluten index (%).
[c] Indicates transformed data.
[d] Indicates back-transformed data.
[e] Standard error of the difference between two least square means.
[f] Degrees of freedom.

TABLE V
Cultivar Least Square Means for Grain and Whole Meal Quality Characteristics[a,b]

Cultivar	Grain Protein Content (%)	Whole Meal SDSS[c]	Whole Meal SDSS[d]	Whole Meal GI[c]	Whole Meal GI[d]
AC Avonlea	14.2	5.9	34.2	0.55	27.0
DT 674	13.7	5.7	32.5	0.58	30.5
Kyle	13.5	5.7	32.8	0.61	33.0
AC Morse	13.5	6.1	37.4	0.74	45.9
DT 666[e]	12.8	6.3	39.5	0.85	56.3
AC Pathfinder[e]	13.0	6.7	45.3	0.85	56.0
DT 672[e]	13.5	6.7	45.4	0.85	56.4
AC Navigator[e]	13.5	6.4	40.3	0.84	55.4
DT 675[e]	13.5	6.7	44.7	0.87	58.0
Durex[e]	13.2	6.7	45.2	0.94	65.6
SED[f]	0.22	0.09		0.04	
DF[g]	37.1	40.8		47.4	

[a] Averaged over five fertilizer treatments, three years, and two locations.
[b] SDSS, sodium dodecyl sulfate sedimentation (mL); GI, gluten index (%).
[c] Indicates transformed data.
[d] Indicates back-transformed data.
[e] Indicates extra-strong cultivars as described in text.
[f] Standard error of the difference between two least square means.
[g] Degrees of freedom.

RESULTS AND DISCUSSION

Analyses of variance (ANOVA) of all quality attributes showed significant main effects of cultivar (Tables I–III). Nitrogen fertilizer did not significantly affect all of the quality estimates of gluten strength. Significant fertilizer-by-cultivar interactions for some of the variables observed in this study suggest the effects of added N varies with cultivar.

Protein Content

Nitrogen fertilization increased grain protein content for all cultivars tested across years and locations. Significant ($P \leq 0.01$) effects of N fertilizer on protein content are shown in Table IV. With no added N, cultivar mean protein content was 11.2–12.8% and with the highest rate of N (120+40 kg/ha), cultivar mean protein content was 14.3–15.6%. While increased grain protein is a common response to applied N, these results provided confirmation that all 10 cultivars selected for the study, responded positively to increasing levels of N fertilizer in these environments. Cultivar effects were also significant ($P \leq 0.01$) but the differences did not reflect the gluten strength characteristics associated with the various strength types (Table V).

Significant cultivar-by-fertilizer interactions for protein content indicated that the level of response varied with cultivar, but this variation appeared unrelated to gluten strength characteristics (Tables IV and V). On average, the extra-strong cultivars showed similar rates of protein increase as cultivars in the lower gluten strength range. Increased protein with added N appeared relatively consistent over the years at Swift Current, although a wider range of protein was observed in 1998 (9–18%). Total gluten weight (data not shown) was highly correlated to the protein response to N fertilizer. Because protein data obtained from the analysis of whole meal samples across locations and five fertilizer rates indicated a consistent positive response, only the most extreme fertilizer treatments (0, 80, and 120+40 kg/ha) and the locations with the largest sample sizes (1997-99, Swift Current) were selected to mill into semolina for further evaluation.

Gluten Strength

Cultivar differences were observed for all estimates of gluten strength regardless of protein content (Tables I–III). The cultivars tended to cluster into two groups for most of the gluten strength measurements (Tables V–VII). Kyle, AC Avonlea, and DT 674 showed similar trends across N levels at the lower end of the strength range. For ease of discussion, this group of cultivars will be referred to as conventional gluten strength types. Durex, DT 675, AC Navigator, DT 672, AC Pathfinder, and DT 666 were considered to be extra-strong types as they consistently showed greater levels of gluten strength in all strength measurement tests. AC Morse was intermediate between the two groups depending on the gluten strength estimate. While AC Morse is not considered to be an extra-strong type, it clearly falls above or in the upper end of the strength range of the conventional group.

Gluten index and SDS sedimentation. The main effect of fertilizer on semolina and whole meal GI was not significant (Tables I–III), suggesting that, on average, increased protein content as a result of N fertilizer does not significantly influence gluten strength as measured by GI.

TABLE VI
Cultivar Least Square Means for Semolina Dough Strength Alveograph Characteristics^{a,b}

			Alveograph Indices							
Cultivar	GI^{c}	GI^{d}	*P*^{c}	*P*^{d}	*L*^{c}	*L*^{d}	*W*^{c}	*W*^{d}	*P/L*^{c}	*P/L*^{d}
AC Avonlea	0.29	8.2	8.2	67.2	6.4	40.8	9.8	96.2	1.32	1.74
DT 674	0.41	16.0	8.8	77.8	6.0	36.7	10.4	107.4	1.49	2.21
Kyle	0.35	12.0	8.2	67.4	6.3	40.0	9.8	95.5	1.33	1.78
AC Morse	0.68	39.2	9.6	91.2	6.3	39.5	11.7	137.2	1.56	2.43
DT 666^{e}	0.90	61.7	8.9	79.4	7.2	51.6	12.3	150.3	1.28	1.64
AC Pathfinder^{e}	0.95	65.7	9.9	97.4	7.0	48.5	13.4	180.8	1.45	2.10
DT 672^{e}	1.00	70.9	10.0	100.4	7.0	48.3	13.7	186.4	1.46	2.14
AC Navigator^{e}	0.94	65.1	10.8	116.4	6.2	39.0	13.7	186.4	1.75	3.08
DT 675^{e}	1.00	70.5	10.2	105.0	6.6	43.8	13.3	178.1	1.58	2.50
Durex^{e}	1.11	80.0	9.8	96.7	7.2	52.4	14.0	196.5	1.38	1.92
SED^{f}	0.055		0.34		0.20		0.50		0.078	
DF^{g}	18.7		17.2		143		18.2		18.2	

[a] Averaged over three fertilizer treatments, one location (Swift Current), three years, and two reps.
[b] GI, gluten index (%); *P*, overpressure (mm); *L*, abicissa at rupture (mm); *W*, deformation energy (10^{-4} J); *P/L*, configuration ratio.
[c] Indicates transformed data.
[d] Indicates back-transformed data.
[e] Indicates extra-strong cultivars as described in text.
[f] Standard error of the difference between two least square means.
[g] Degrees of freedom.

TABLE VII
Cultivar Least Square Means for Semolina Dough Strength Mixograph Characteristics^{a,b}

			Mixograph Values							
Cultivar	GI^{c}	GI^{d}	MDT^{c}	MDT^{d}	ETP^{c}	ETP^{d}	BWE^{c}	BWE^{d}	PBW	TEG
AC Avonlea	0.29	8.2	0.80	2.23	3.05	9.3	2.5	12.5	0.05	28.9
DT 674	0.41	16.0	0.91	2.47	3.38	11.4	2.7	14.1	0.06	32.0
Kyle	0.35	12.0	0.85	2.33	3.17	10.0	2.6	12.9	0.05	30.0
AC Morse	0.68	39.2	0.97	2.63	3.50	12.2	2.7	15.6	0.06	32.5
DT 666^{e}	0.90	61.7	1.15	3.17	3.75	14.1	2.8	15.9	0.06	30.6
AC Pathfinder^{e}	0.95	65.7	1.12	3.06	3.98	15.8	2.9	18.1	0.07	36.1
DT 672^{e}	1.00	70.9	1.12	3.07	3.90	15.2	2.9	17.9	0.07	34.2
AC Navigator^{e}	0.94	65.1	1.19	3.28	4.12	17.0	3.0	19.7	0.07	35.6
DT 675^{e}	1.00	70.5	1.17	3.21	4.00	16.0	3.0	19.0	0.07	34.7
Durex^{e}	1.11	80.0	1.19	3.28	4.04	16.3	3.0	19.2	0.07	34.4
SED^{f}	0.055		0.106		0.05		0.003	1.71		
DF^{g}	18.7		18.2		18.5		18.2	18.1		

[a] Averaged over three fertilizer treatments, one location (Swift Current), three years, and two reps.
[b] GI, gluten index (%); MDT, mixing development time (min); ETP, energy to peak (J); BWE, bandwidth energy (J); PBW, peak bandwidth (N m); TEG, total energy (J).
[c] Indicates transformed data.
[d] Indicates back-transformed data.
[e] Indicates extra-strong cultivars as described in text.
[f] Standard error of the difference between two least square means.
[g] Degrees of freedom.

A large significant cultivar effect was observed for whole meal and semolina GI (Tables I–III). Cultivar effects were expected because the samples selected for this study varied greatly in gluten strength characteristics. With the exception of AC Morse, both the extra-strong and conventional gluten strength groups were clearly distinguished by gluten index (Table V). A significant cultivar-by-fertilizer interaction for whole meal GI indicated that response to N fertilizer varied depending on cultivar. Contrasts between and within conventional and extra-strong gluten strength groups (data not shown) showed the interaction was due to differences between but not within the strength groups. Classification of cultivars into either conventional strength or extra-strong groups was not affected by year or by N fertilizer, but there were some minor changes in rank order within each gluten strength group from year-to-year, indicating that the presence of a significant cultivar-by-fertilizer interaction did not seriously compromise the ability of the GI to rank strength, particularly at lower levels of N. For the extra-strong gluten types, GI decreased or remained stable, while those cultivars with conventional gluten strength showed a slight increase with increased N (Fig. 1). The gluten strength of AC Morse is intermediate between the other cultivars and, while relatively consistent across N levels, it followed a slight downward trend with increasing levels of protein. A linear regression plot of whole meal GI versus protein content illustrates the potential differences in cultivar response due to inherent gluten strength. It is clear that the trend observed in Fig. 2 is not an artifact of the data transformation used because the untransformed data behaved the same way. There are significant differences in the slope between cultivar groups but not within cultivar groups.

Because total gluten weight increased with N fertilizer for all cultivars, increases or decreases in GI reflect changes in the relative proportions of gluten remaining on or passing through the screen after centrifugation. With added N, the firm, well-developed gluten portion remaining on the screen (probably HMW glutenin) (Rao et al 2000) increased to a greater extent (with concurrent reduction in material, probably gliadins, going through the screen) in cultivars with conventional gluten strength.

The relationship between composition of wheat protein and total protein content has been considered in a number of previous studies with bread wheat, and while results are somewhat conflicting, they do suggest that changes in gluten protein composition occur as a result of N fertilizer (Doekes and Wennekes 1982;

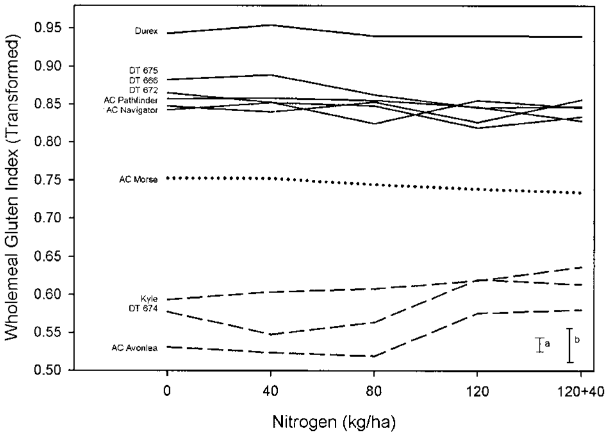

Fig. 1. Effect of nitrogen fertilizer (five levels) on whole meal gluten index (transformed data). Cultivar responses represent means of two replicates, two locations, and three years. Cultivars with extra-strong (——), conventional (– – – –), and intermediate (• • • • •) gluten strength. Standard error for comparisons (a) within the same cultivar and (b) among cultivars at the same fertilizer level.

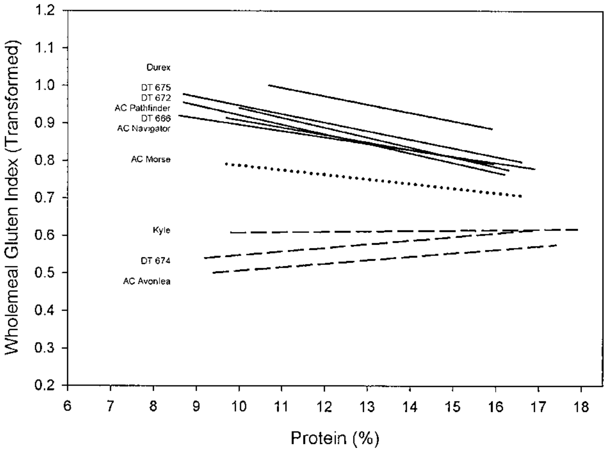

Fig. 2. Whole meal gluten index response (transformed data) for each cultivar at varying protein levels, expressed as regression lines. Cultivars with extra-strong (——), conventional (– – – –), and intermediate (• • • • •) gluten strength.

Wieser and Seilmeier 1998; Johansson et al 2001). Other results for bread wheat have reported no change in the proportion of gliadins or glutenins with increased protein (Tanaka and Bushuk 1972). While few studies of durum wheat have considered changes in protein composition with N fertilizer, in a study using conventional strength cultivars, Dexter and Matsuo (1977) showed that with successively higher protein contents the gliadin levels increased for one durum wheat cultivar but remained constant for another. Dexter et al (1982) showed that the proportion of lactic acid insoluble residue protein in durum wheat, which is comprised primarily of glutenin macropolymer protein, was not affected by N fertilizer.

Significant differences in whole meal SDSS were observed between cultivars and fertilizer treatments but the absence of a significant cultivar-by-fertilizer interaction indicates that fertilizer affected all cultivars in a similar manner (Table I). Whole meal SDSS increased with increased N for all cultivars tested (Table IV) following the trends of protein content. In contrast to the GI data, a linear regression plot of whole meal SDSS versus protein content illustrates the significant effect of fertilizer in the absence of a cultivar-by-fertilizer interaction (Fig. 3).

Increased SDSS levels with increased N were consistent across environments. The stronger gluten types tended to have higher than average sedimentation levels particularly at the higher levels of N

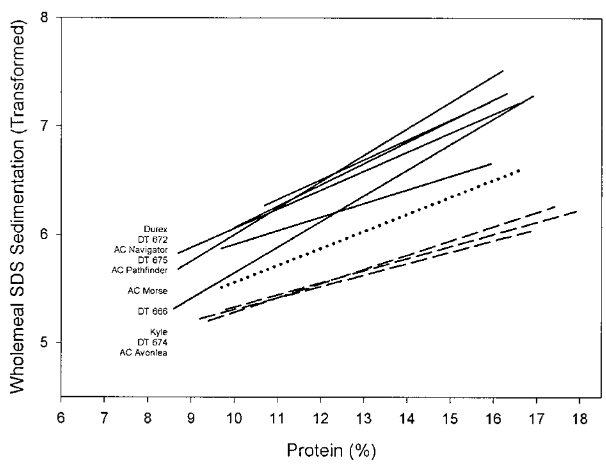

Fig. 3. Whole meal SDS sedimentation (transformed data) for each cultivar at varying protein levels, expressed as regression line. Cultivars with extra-strong (——), conventional (– – – –), and intermediate (• • • • •) gluten strength.

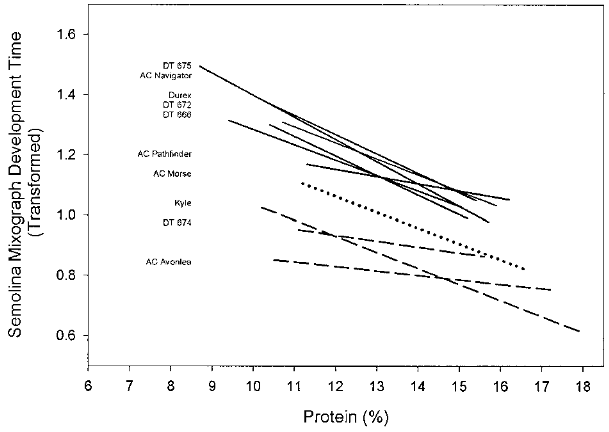

Fig. 4. Semolina mixograph mixing development time (transformed data) for each cultivar at varying protein levels, expressed as regression lines. Cultivars with extra-strong (——), conventional (– – – –), and intermediate (• • • • •) gluten strength.

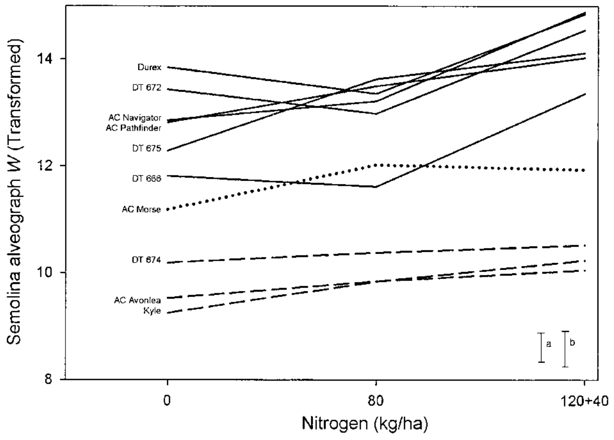

Fig. 5. Effect of nitrogen fertilizer (five levels) on semolina alveograph *W* values (transformed data). Cultivar responses represent the means of two replicates, two locations, and three years. Cultivars with extra-strong (——), conventional (– – – –), and intermediate (• • • • •) gluten strength. Standard error for comparisons (a) within the same cultivar and (b) among cultivars at the same fertilizer level.

(Table V). While DT 666 and AC Morse were similar to each other and to the conventional strength types (Kyle, AC Avonlea, and DT 674) for SDSS at low protein levels, these cultivars showed levels

TABLE VIII
Fertilizer Least Square Means for Semolina Dough Strength AlveographCharacteristics[a,b]

Nitrogen Rate (kg/ha)	GI[c]	GI[d]	Alveograph Indices							
			P[c]	*P*[d]	*L*[c]	*L*[d]	*W*[c]	*W*[d]	*P/L*[c]	*P/L*[d]
0	0.77	48.5	9.4	88.4	6.4	40.3	11.7	137.3	1.52	2.30
80	0.76	47.3	9.4	87.34	6.6	43.7	12.0	144.9	1.45	2.09
120+40	0.76	47.3	9.6	91.8	6.9	47.8	12.9	165.1	1.42	2.02
SED[e]	0.018		0.15		0.11		0.30		0.026	
DF[f]	28.3		3.9		143.0		3.9		125.3	

[a] Averaged over 10 cultivars, one location (Swift Current), three years, and two reps.
[b] GI, gluten index (%); *P*, overpressure (mm); *L*, abicissa at rupture (mm); *W*, deformation energy (10^{-4}); *P/L*, configuration ratio.
[c] Indicates transformed data.
[d] Indicates back-transformed data.
[e] Standard error of the difference between two least square means.
[f] Degrees of freedom.

TABLE IX
Fertilizer Least Square Means for Semolina Dough Strength Mixograph Characteristics[a,b]

Nitrogen Rate (kg/ha)	GI[c]	GI[d]	Mixograph Values							
			MDT[c]	MDT[d]	ETP[c]	ETP[d]	BWE[c]	BWE[d]	PBW	TEG
0	0.77	48.5	1.12	3.07	3.7	13.8	2.8	15.9	0.057	30.6
80	0.76	47.3	1.05	2.86	3.7	13.3	2.8	16.0	0.059	32.1
120+40	0.76	47.3	0.97	2.63	3.7	13.7	2.8	17.1	0.066	36.0
SED[e]	0.018		0.029		0.04		0.04		0.0039	2.00
DF[f]	28.3		4.0		125.4		3.8		4.0	4.0

[a] Averaged over 10 cultivars, one location (Swift Current), three years, and two reps.
[b] GI, gluten index (%); MDT, mixing development time (min); ETP, energy to peak (J); BWE, bandwidth energy (J); PBW, peak bandwidth (N m); TEG, total energy (J).
[c] Indicates transformed data.
[d] Indicates back-transformed data.
[e] Standard error of the difference between two least square means.
[f] Degrees of freedom.

similar to those of the extra-strong group at high protein (Fig. 3). The overall trend of increasing whole meal SDSS with protein was expected because the SDSS test reflects protein content. However, because gluten strength cultivar groups were still distinguishable at all N levels, SDSS is capable of differentiating cultivars of comparable protein content on the basis of gluten strength. Dexter et al (1982) reported similar increases in SDSS with N fertilizer for several conventional strength durum wheat cultivars, but concluded the increases were based on increased protein alone and not related to gluten strength.

Dough strength. Significant cultivar effects ($P \le 0.01$) were observed for all the semolina mixograph parameters measured, which in most cases reflect distinct gluten strength properties (Table III). Consistently higher MDT, ETP, BWE, PBW, and TEG were observed for the cultivar group with extra-strong gluten (Table VII). With the exception of MDT, fertilizer effects were not significant for mixograph parameters, which suggests that these parameters are predictive of gluten strength and relatively insensitive to variations in protein content (Tables VIII and IX). Wooding et al (2000a,b) reported increased flour protein resulted in increased mechanical dough development work but significant decreases in MDT, while the effect on dough strength (extensigraph resistance to extension) was small, suggesting these measurements depend on different aspects of protein composition. In the present study, a significant effect of fertilizer on MDT ($P \le 0.05$) showed an overall downward trend for MDT with N fertilizer (Table IX). Mixograph parameters indicated that MDT was significantly reduced and mixing energy TEG slightly increased with N fertilizer. These trends have been previously reported in hexaploid wheat (Lukow and Townley-Smith 1996) and in conventional strength durum wheat (Dexter and Matsuo 1982; Wooding et al 2000a,b). The extra-strong gluten cultivars in this study exhibited longer mixing times than the conventional strength gluten types at similar protein levels, although these differences were less at the highest protein, where conventional strength and extra-strong types overlapped for MDT, as illustrated in a linear regression plot of MDT versus protein content (Fig. 4). Long mixing times are generally associated with superior gluten quality in durum wheat (Irvine et al 1961; Matsuo et al 1972; Wasik and Bushuk 1975; Dexter and Matsuo 1980). In this study, mixographs were performed at constant absorption and reduction in mixograph MDT with increasing N may be partially due to increased water absorption requirements associated with higher protein content. The absence of cultivar-by-fertilizer interactions for any of the mixograph parameters suggests that all cultivars show a similar response to N fertilizer regardless of gluten strength type. This is in contrast to reports for hexaploid wheat where mixograph properties of extra-strong cultivars were less affected by protein content than conventional strength bread wheat cultivars (Khatkar et al 1996). One exception may be AC Pathfinder (Fig. 4) which showed lower rates of decrease in MDT with increased protein compared with the other extra-strong cultivars.

Significant cultivar effects ($P \le 0.01$) were observed for all semolina alveograph parameters (Table II). *P* and *W* values were higher for cultivars with extra-strong gluten strength (Table VI). However, *L* values appeared unrelated to gluten strength groupings, and increased with N fertilizer (Table VIII). This confirms that dough extensibility is highly dependent on protein content. An increase in alveograph *L* with increased protein is in agreement with previous reports for bread wheat (Shogren et al 1963a,b; Uthayakumaran et al 1999) and durum wheat (Dexter et al 1994; Edwards et al 1999).

Gluten strength differences among cultivars were clearly distinguishable by *P*, *W*, and *P/L* measurements. Significant increases in *W* and decreases in *P/L* with increased N (Tables II and VIII) were due to the significant increasing effect of N on *L* but not *P*. The extra-strong cultivars showed greater increases in *W* compared with conventional gluten strength types (Fig. 5). A significant cultivar-by-fertilizer interaction for *W* but not *L* (Table II) suggests variable cultivar response to N fertilizer is a reflection of both gluten strength and protein content. Contrasts between and within conventional and extra-strong gluten strength groups (data not shown) showed the interaction was due to differences between, but not within, the two strength groups. From alveograph data, it appears that N fertilizer influences dough extensibility as measured by *L* and the subsequent calculation of dough strength measured as *W* but not dough elasticity which is measured by *P*.

CONCLUSIONS

This study found that all cultivars responded to N by showing increased protein content, and where there were differences in response they were in intensity only and not related to gluten strength characteristics. For strength measurements such as SDSS, alveograph *W*, and MDT, all cultivars responded to N but inherent differences in strength were retained. Although protein composition was not characterized in the present study, the results of GI suggest changes in gluten composition with added N may affect the gluten strength of the stronger cultivars differently than the conventional strength types. Despite the presence of a significant cultivar-by-fertilizer interaction, GI remained quite stable across increasing levels of N fertilizer and resultant increased protein content. Therefore, GI is considered a good test for estimating gluten strength across cultivars with a wide range of protein contents compared with tests such as mixograph, SDSS, and alveograph *W* or *L*, which are highly affected by changes in protein content. Tests that are less influenced by fertilizer allow breeders to rank breeding lines consistently. The extra-strong gluten types appeared less dependent on protein content for maintaining inherently higher gluten strength compared with the conventional gluten strength types, which continued to show improvements in GI with increased protein content. Therefore, in situations where nitrogen availability is limited, such as in organic production systems, gluten strength quality will be better maintained using extra-strong gluten cultivars rather than conventional strength cultivars. Overall, the superior gluten strength of extra-strong cultivars compared with conventional cultivars was maintained with or without application of N fertilizer.

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