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Effect of nitrogen and sulphur fertilization on the quality of barley protein

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ABSTRACT

A small-plot field experiment was set up during 2011, 2012 and 2013 to test the effect of nitrogen (N) fertilizers and nitrogen fertilizers combined with sulphur (S) in the nutrition of spring barley cv. Bojos. The following parameters were studied, the effect of fertilization on grain yield, specific weight, percentage of the grain retained on a 2.8 mm and 2.5 mm sieve, the content of starch and proteins, and the percentage of protein fractions. Also the influence of weather conditions on the studied parameters was evaluated. Starch content and specific weight of grain were not influenced by the fertilizer applied. Grain yield was rising until the overall dosage reached 50 kg N/ha and 21 kg S/ha while any higher rate of fertilizer reduced the yield. The percentage of A- and B-hordeins increased and the content of C- and D-hordeins reduced as the fertilizer rate increased. A significant effect of weather on the monitored parameters as well as on the percentage of protein fractions was demonstrated. The results enabled a conclusion that there is no improvement in technological quality of barley grain once the optimal rate of fertilizer has been reached. The optimal rate is influenced by weather conditions during the growing season.

Keywords: *Hordeum vulgare* L.; malt quality; sulphur deficiency; amino acids

Sulphur (S) plays an essential role in plant metabolism, its deficiency adversely affecting crop quality (Zhao et al. 1999). In the barley grain, the element is present in the form of sulphur amino acids (Hřivna et al. 2011). Essentially, its biochemical role is the formation of disulphide bonds between peptide chains and the stabilization of protein structures (Hawkesford and De Kok 2006), which greatly influences the quality of malting barley as the factors determining the same include protein content and fractional composition. Hulín et al. (2008) reports that an average barley grain contains 12.1% albumins, 8.4% globulins, 25% prolamins and 54.5% glutelins. The content of each fraction has a significant influence on the technological, nutritional and, indirectly, the hygienic quality of grain (Kuktaite 2004).

Sulphur deficiency was demonstrated to affect the composition of proteins; there is a decrease in sulphur-rich fractions of B-hordeins and high-molecular D-hordeins and increase in sulphur-poor C-hordeins (Shewry et al. 1983). This means that the availability of sulphur to the plant may influence the malting quality since S-rich B- and D-hordeins are the main components of the gel protein fraction, one that may limit the endosperm modification during malting (Shewry et al. 2001). Under certain conditions, compounds may form from sulphur amino acids during malting, adversely affecting the sensory quality of beer. They include substances such as dimethylsulphide (DMS) and its precursor, S-methyl-L-methionine (SMM), which are formed as early as the germination stage. When exposed to higher temperatures during kilning and the brewing process, SMM decomposes to form DMS, a substance that may be sensorially unfavourable for beer starting at concentrations around 35–40 μg/L (Hřivna et al. 2011).

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Table 1. Weather conditions during the growing periods

Month	2010–2011		2011–2012		2012–2013		Normal	
	T (°C)	P (mm)	T (°C)	P (mm)	T (°C)	P (mm)	T (°C)	P (mm)
January	–1.0	–2.0	0.1	49.4	–2.1	26.5	–2.5	21.9
February	–1.4	–3.0	–4.4	26.1	0.0	43.4	–0.7	18.1
March	5.1	3.9	6.3	8.00	0.8	55.5	3.5	27.8
April	11.8	8.9	10.5	31.0	9.7	41.8	9.5	29.8
May	15.0	14.3	16.5	38.0	13.8	112.7	14.6	63.8
June	19.0	17.1	19.0	100	17.3	117	17.3	68.3
July	18.4	18.9	21.2	84.2	21.2	1.2	19.4	71.4
August	20.3	18.7	20.7	71.2	19.6	87.7	19.1	62.7

T – temperature; P – precipitation

The aim of the study was focused on the explanation of the effect of different fertilizer combinations on the changes of the content of protein fraction. The effect of fertilizer combinations on the technological characteristics of barley grain was investigated as well.

MATERIAL AND METHODS

Barley cv. Bojos was cultivated in 2011–2013 on a plot situated in Agrospol Velká Bystřice (agricultural cooperative) as a small-plot area 20.6 m^2. The land is located in a region of slightly warm and moderately humid climate. There is a moderately heavy soil, one of the brown earth soil types. Sugar beet was the preceding crop. The weather conditions per growing season are shown in Table 1.

Nitrogenous fertilizers and nitrogen (N) fertilizer mixture with sulphur were applied as indicated in Table 2. Each of the treatments was set up in four runs. LAV 27 is a nitrogen fertilizer containing 27% N. It is a mixture of the ammonium nitrate and finely ground limestone. DASA 26/13 is a nitrogen fertilizer, formed by a mixture of ammonium sulphate and ammonium nitrate. It contains 26% N (7.5% N-NO_3 and 18.5% N-NH_4), and 13% S. It is in a form of 2–5 mm large whitish till light

Table 2. The total applied dose of nitrogen (N) fertilizer and nitrogen fertilizer containing sulphur (S; kg/ha)

Treatment	Application period				Total		Code
	after emerging (DC 13)		stem elongation start (DC 31)				
	fertilizer	N	fertilizer	N	N	S	
1	–	0	0	–	0	0	K0
2	LAV 27	30	–	–	30	0	N1
3	LAV 27	30	DAM 390	20	50	0	N2
4	SA	30	–	–	30	36	N1S1
5	SA	30	SAM 19N-5S	20	50	42	N2S2
6	DASA 26/13	30	–	–	30	15	N1S1
7	DASA 26/13	30	SAM 19N-5S	20	50	21	N2S2
8	SAM 19N-5S	30	–	–	30	10	N1S1
9	SAM 19N-5S	30	SAM 19N-5S	20	50	16	N2S2
10	LAV 27 + S1	30	–	–	30	30	N1S1
11	LAV 27 + S1	30	DAM 390	20	50	30	N2S1
12	LAV 27 + S2	30	–	–	30	50	N1S2
13	LAV 27 + S2	30	DAM 390	20	50	50	N2S2

LAV 27 – ammonium nitrate with limestone (27% N); SA – ammonium sulphate (20.3% N, 24% S); DASA 26/13 (26% N, 13% S); SAM 19N-5S (19% N, 5% S); DAM 390 (30% N); S1, S2 – elementary sulphur (1/2 indicates the size of dosage)

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yellow granules. Liquid fertilizer SAM 19N-5S contains 19% N (12.4% N-NH_2 and 6.6% N-NH_4) and 5% S. Nitrogen liquid fertilizer DAM 390 contains 30% N (15.0% N-NH_2, 7.5% N-NH_4 and 7.5% N-NO_3).

The barley crop was harvested when fully ripe using a small scale thresher of Wintersteiger type. Grain yield was determined for each of the treatments along with the grain quality. This involved determining specific weight as per (ISO 7971-2, 1995), the percentage of the grain retained on the sieve for sieve mesh sizes of 2.5 mm and 2.8 mm (ČSN 461011-7, 1988), protein content (ISO 1871, 2009) and starch (ISO 10520, 1997).

The content of protein fraction was measured by RP-HPLC with UV detection (214 nm). Barley meal (500 mg) was suspended in 4.5 mL of buffer 25% acetonitrile + 0.15% TFA before centrifugation for 20 min. The supernatant was analysed using the Vydac column 218TP C18, which was eluted using acetonitrile + TFA (0.1%). The absolute area of the peaks and relative areas of the peaks were recorded by the Agilent Chemstation software for LC nad LC/MS systems (Santa Clara, USA). The protein fractions were identified according to eluting time with the respect to the characteristics of barley protein fraction published by Celus et al. (2006). The content of fraction was obtained by dividing area of peak by absolute area of peaks.

The results were statistically analyzed using the analysis of variance (ANOVA). The differences were tested on $\alpha = 0.05$ significance level by using the Tukey's test. Statistical analysis was performed using Statistica CZ 12 (StatSoft, Prague, Czech Republic).

RESULTS AND DISCUSSION

Nitrogen fertilization and the fertilization using nitrogen mixed with sulphur impacted grain yield (Table 3). Even if the grain yield was significantly increased in fertilized treatments, the differences among fertilized treatments were not significant. However, grain yield was slightly increased in treatments fertilized by DASA 26/13 (treatments 6, 7). It can be assumed that this parameter was affected mostly by the characteristics of the growing region.

Differences between treatments in starch content were not significant. Similarly to the starch content, the fluctuation of specific weight values was not statistically significant between the treatments. The highest specific weight was shown for grain grown as part of treatment 7, while the lowest such value was identified in grains grown as part of the control treatment.

The higher the ratio of rather larger grains, the higher the estimated extractivity of raw materials, particularly starch. The values obtained for the mesh size of 2.8 mm suggest a raw material of good

Table 3. Average values of grain technological parameters

Treatment	Yield (t/ha)	Starch (% dry matter)	Specific weight (kg/hL)	2.8 mm	2.5 mm	Protein (% dry matter)
				(%)		
1	7.032^{a}	65.59^{a}	64.88^{a}	77.57^{b}	17.20^{ab}	10.81^{a}
2	7.734^{bc}	65.29^{a}	64.84^{a}	72.2^{ab}	20.56^{abc}	11.36^{b}
3	7.723^{bc}	66.00^{a}	64.65^{a}	70.4^{ab}	21.89^{c}	11.19^{ab}
4	8.086^{c}	65.52^{a}	65.29^{a}	77.87^{b}	16.61^{a}	11.26^{abc}
5	7.947^{bc}	66.30^{a}	65.32^{a}	70.09^{ab}	22.28^{c}	11.34^{bc}
6	8.161^{c}	66.04^{a}	64.99^{a}	75.53^{b}	18.46^{abc}	11.23^{ab}
7	8.175^{c}	65.56^{a}	66.05^{a}	71.88^{ab}	21.55^{c}	11.45^{bc}
8	7.580^{b}	66.15^{a}	65.89^{a}	75.02^{b}	19.00^{abc}	11.36^{bc}
9	7.741^{bc}	65.52^{a}	64.84^{a}	71.09^{ab}	21.37^{c}	11.54^{bc}
10	7.697^{bc}	65.44^{a}	65.68^{a}	72.96^{ab}	19.30^{abc}	11.32^{bc}
11	7.919^{bc}	65.47^{a}	65.10^{a}	70.03^{ab}	22.31^{c}	11.34^{bc}
12	7.916^{bc}	65.89^{a}	64.93^{a}	72.14^{ab}	20.71^{bc}	11.41^{bc}
13	7.708^{bc}	65.59^{a}	65.07^{a}	66.09^{a}	20.94^{bc}	11.70^{c}

Values with different letters in the column differ significantly ($P < 0.05$). 2.8 mm and 2.5 mm – percentage retained on the 2.8 mm or 2.5 mm sieve

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Table 4. The effect of the crop year on barley grain technological parameters

Parameter	Crop year		
	2011	2012	2013
Yield (t/ha)	7.26[a]	8.48[b]	7.66[c]
Starch (% dry matter)	64.83[a]	66.02[b]	66.31[b]
Specific weight (kg/hL)	59.3[a]	67.6[b]	68.6[c]
2.8 mm (%)	64.42[a]	72.21[b]	80.95[c]
2.5 mm (%)	26.14[a]	19.73[b]	14.63[c]
Protein (% dry matter)	11.09[a]	10.36[b]	12.55[c]

Values with different letters in the row differ significantly ($P < 0.05$). 2.8 mm or 2.5 mm – percentage retained on the 2.8 mm or 2.5 mm sieve

malting quality. Larger grains were harvested as part of treatments with lower nitrogen fertilization intensity. It is expected that lower nitrogen fertilization reduced number of spikelets per spike, thus affecting the distribution of assimilates into a lesser number of storage organs. Application of sulphur did not show any significant effect on the grain size. High dosage of nitrogen and sulphur significantly reduced the grain size, which is evident from the parameters of the grain (treatment 13) with a total of 50 kg N/ha and 50 kg S/ha.

The optimal protein content in barley grain is 10.0–11.5% (Kosař and Procházka 2000). The protein content in the dry matter is moderately above the upper limit of malting optimum. Sulphur is expected to encourage nitrogen utilization and increase protein content in the grain, which was reflected in samples as well. The highest protein content was obtained in treatment 13 fertilized with 50 kg N/ha and 50 kg S/ha; in addition, high values were seen for treatment 9 (50 kg N/ha and 16 kg S/ha) and treatment 7 (50 kg N/ha and 21 kg S/ha).

The technological parameters were also influenced by weather (Table 4). Crop year 2013 appears to be the best one; it was above average as regards total precipitation volume. It was the period of harvesting grain of the largest size, the highest starch content and the highest specific weight. The protein content ranged well above the optimum level. Lower values of all the technological parameters were recorded in 2011, probably due to low total precipitation and the average temperatures.

The application of fertilization significantly decreased the content of A- and B-hordeins in barley grain (Figure 1a). Significantly lowest was the content of these fractions for treatment 2. Conversely, treatments 12 and 13 were found to have the significantly highest content of A- and B-hordeins within the fertilized treatments. For the content of S-poor C-hordeins, the applied fertilization was found to have a significant effect as well (Figure 1b). Compared with control, the increased content of the protein fraction was found only for treatment 4. For other treatments, the C-hordein content was significantly reduced. The grain grown as part of treatments 2, 11 and 13 was identified to have the lowest content of C-hordeins. Conversely, the content of D-hordeins, albumins and globulins significantly increased after the application of nitrogen fertilization and fertilization with nitrogen and sulphur, with the largest increase in the content of the fractions in treatment 2 (Figure 1c). The content of high-molecular weight glutelin subunits was increased by fertilization only for treatment 4, while for the other treatments the content of the fractions reduced compared with control (Figure 1d). The content of low-molecular weight glutelin subunits was significantly increased for all the treatments (Figure 1e).

The content of protein fractions in the barley grain was significantly influenced by the crop year (Table 5). In 2011, the grain contained significantly lowest percentage of A- and B-hordeins, C-hordeins and high-molecular weight glutelin subunits. Conversely, the highest ratio of the fractions was found in the grain harvested in 2013. The content of D-hordeins and low-molecular weight glutelin subunits was the lowest in 2012. Nearly twice the amount was achieved in 2011. Shewry et al. (2001) reports four different fractions of hordeins (A to D), classified per size and com-

Table 5. The effect of the crop year on the content of protein fractions (%)

Crop year	A- and B-hordeins	C-hordeins (S-poor)	D-hordeins Alb, Glo	LMW GS	HMW GS
2011	30.05[a]	8.97[a]	31.40[c]	25.25[c]	4.28[a]
2012	53.97[b]	8.70[b]	16.48[a]	15.21[a]	5.55[b]
2013	36.27[c]	10.07[c]	23.17[b]	23.28[b]	7.09[c]

Values with different indices in the column differ significantly ($P < 0.05$). Alb – albumins; Glo – globulins; LMW – low molecular weight; HMW – high molecular weight; GS – glutelin subunits

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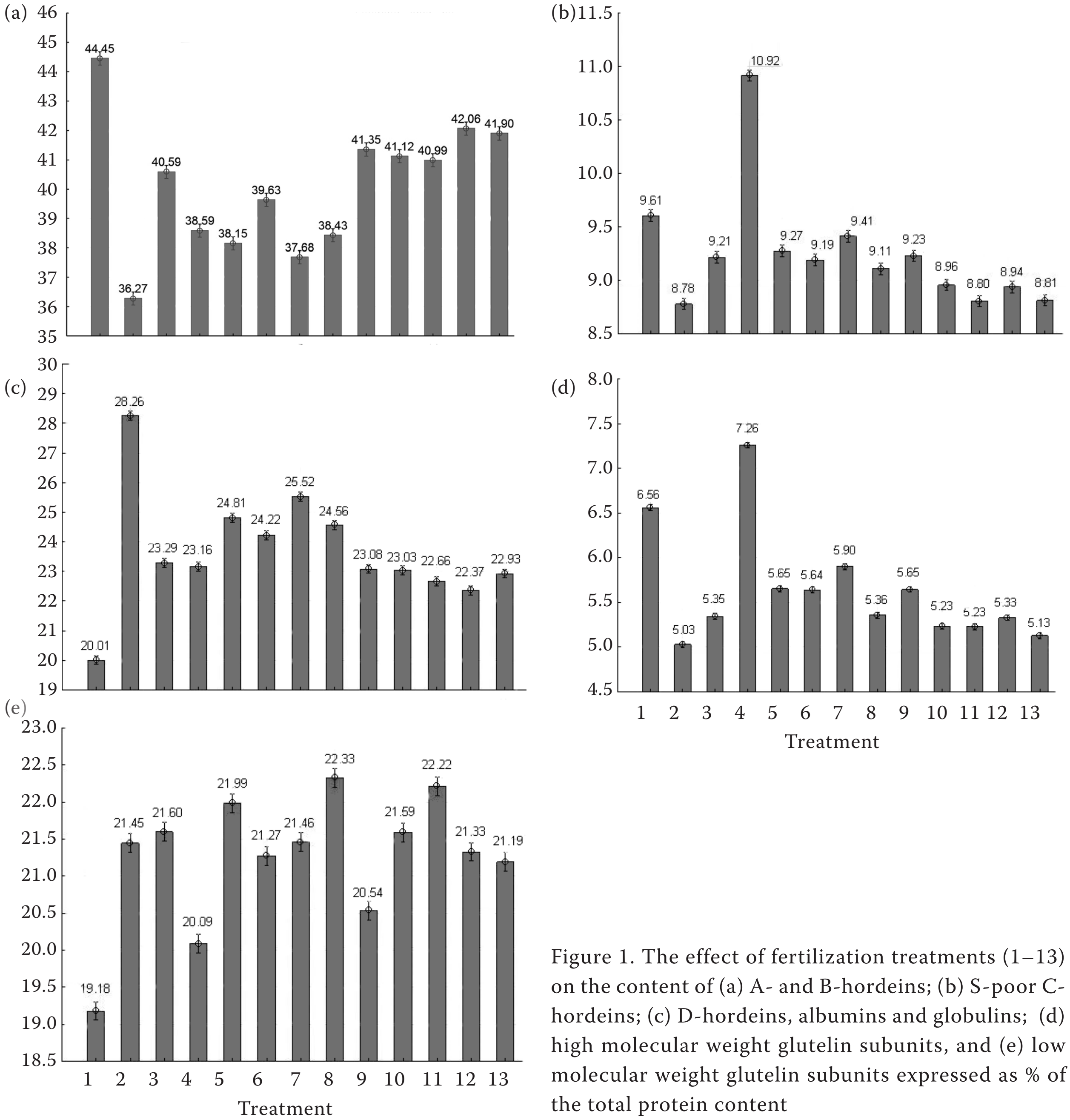

Figure 1. The effect of fertilization treatments (1–13) on the content of (a) A- and B-hordeins; (b) S-poor C-hordeins; (c) D-hordeins, albumins and globulins; (d) high molecular weight glutelin subunits, and (e) low molecular weight glutelin subunits expressed as % of the total protein content

position of amino acids. A-hordeins (15–25 kDa) are storage proteins that contain protease and alpha-amylase inhibitors. B-hordeins (32–45 kDa) are rich in sulphur and constitute 80% of all hordeins. This type consists of peptides composed of 19 amino acid residues and a central repeating domain that is rich in proline and glutamine residues (Steiner et al. 2011). The rate of the sulphur fertilization applied can be assumed to reflect on the composition of proteins. There is a decrease in sulphur-rich fractions of B-hordeins and HMW (high molecular weight)-D hordeins (> 100 kDa), while an increase in the content of sulphur-poor fraction of C-hordeins. Such changes may subsequently affect proper grain hulling (Shewry et al. 2001) and malting quality (Zhao et al. 2006). The maximum amount of A- and B-hordeins was found in the control treatment (44.45%). A higher nitrogen rate (treatments 3) had almost a similar influence on the content of A- and B-hordeins in the grain as seen in the treatments within which a higher rate of sulphur was applied. However, the higher content of sulphur dispensed increased the content of A- and B-hordeins in grain.

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Sulphur-poor C-hordeins cover about 10–30% of total hordeins (Qi et al. 2006). Samples with higher rate of applied sulphur and nitrogen contained a higher ratio of C-hordeins (8.78–9.61%). As for treatment 4, there was an increase in this fraction up to 10.92% after applying 30 kg N/ha and 36 kg S/ha. Globulins form about 15% of barley proteins. Albumin accounts for about 11% of the total protein barley. The content of D-hordeins, albumins and globulins in the barley grain ranged from 22.37–28.26%. The largest amount of this fraction was measured for treatment 2 after applying 30 kg N/ha. Conversely, the control treatment had the lowest value of all the treatments studied.

The HMW glutelin content was relatively balanced (5.03–7.26%). The application of higher sulphur rates did not influence the increase in the amount of HMW GS (glutelin subunits); in contrast, a positive result was obtained in this regard after applying lower rates of sulphur and nitrogen, 36 kg N/ha and 30 kg S/ha, respectively, as part of treatment 4 (Figure 1d). For the values ranging from 20.09–22.33%, indicating clearly what affected the content of these substances is not possible. The highest values were reached as part of treatments 8, 11 and 5, while the control treatment was the worst.

In conclusion, the differentiated application of sulphur in interaction with nitrogen plays an important role in barley nutrition. Compared to the control treatment, nitrogen fertilization and fertilization with nitrogen and sulphur increased the content of S-rich hordeins, albumins and globulins. Contrarily, the content of A- and B-hordeins was decreased in nearly the same extent. The average content of S-poor C-hordeins was significantly decreased in fertilized treatments as well. In addition to the fertilization, the percentage of the fractions was influenced by weather conditions during the growing season. The analysis of the results indicated that the optimum fertilization rate that still improves the technological quality and the content of protein fractions relates to the weather in the respective year.

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