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Effect of variety and environment on the amount of thiamine and riboflavin in cereals and grain legumes

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ABSTRACT

Comprehensive information on B vitamin contents in cereals and grain legumes used for animal feeding is scarce. Thus, the objective of this study was to determine the contents of thiamine and riboflavin uniformly in a selection of cereals and grain legumes. Additionally, the evaluation of varietal and environmental effects on the amounts of both B vitamins was targeted. We analysed contents of thiamine and riboflavin with HPLC in 855 samples of different organically cultivated cereal and grain legume cultivars. Since the sample set was unbalanced, it had to be adapted for further statistical analyses. Data of 541 samples of ten cereal and grain legume cultivars was used to assess the influence of variety and environment with generalized linear models. Cereal grains contained 1.27–3.53 mg thiamine and 0.62–1.58 mg riboflavin/kg DM, which was less than expected from table values. Thiamine and riboflavin contents of grain legumes were mostly comparable with table values. Their thiamine contents ranged from 2.55 to 8.97 mg and their riboflavin contents from 1.00 to 3.84 mg/kg DM. Furthermore, variety, harvest site, and/or year affected B vitamin contents in all cultivars of our sample set. Due to wide variations of the contents of thiamine and riboflavin, we recommend to express values in food- and feed tables as ranges and to mention the number of underlying analysed samples. It must be considered that thiamine contents of cereal grains might be lower than expected from food- and feed tables.

1. Introduction

Providing water-soluble vitamins with cereal-based diets for monogastric animals is challenging. Thiamine and riboflavin are important coenzymes in energy metabolism (Depeint et al., 2006; Fattal-Valevski, 2011). Thus, deficiencies lead to decreased enzyme activity and therefore to health issues with specific symptoms, including decreased performance and even death (Blair and Newsome, 1985). Since the capacity of monogastric animals to store B vitamins in the organism and to microbially synthesise them in the digestive tract is low, B vitamins must be fed continuously to prevent deficiencies (McDowell, 2000).

However, comprehensive information on contents of thiamine and riboflavin in cereals and grain legumes used for animal feeding is scarce. Since B vitamin analyses are relatively expensive, native amounts in the feed are usually not determined. Most values used even in food and feed tables (Sauvant et al., 2004; USDA, 2016; Souci et al., 2008) originate from early studies. There have not been representative analyses of B-vitamins in cereal and legume grains for a long time. Furthermore, underlying laboratory methods are often unknown. Various methods used to determine B vitamins can lead to different results (Hollman et al., 1993). Besides differences arising from analytical methods, environmental and genetic factors might also affect the actual contents of B vitamins in plant material (Bognar and Kellermann, 1993; Shewry et al., 2011). Moreover, storage conditions (Bayfield and O'Donnell, 1945; Finglas,

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2003) or further processing (Gołda et al., 2004; Lebiedzińska and Szefer, 2006) can alter the amounts of B vitamins.

The objective of this study was to determine the contents of thiamine and riboflavin uniformly in a selection of cereals and grain legumes. We also wanted to evaluate varietal and environmental (harvest year and harvest site) effects on the contents of B vitamins in some cereals and grain legumes.

2. Material and methods

2.1. Sample set

Organically managed variety field trials of cereals and grain legumes are undertaken throughout Germany by independent institutions. Those institutions collected a selection of 855 available harvest samples of cereals and grain legumes from trials in the years 2011, 2012, and 2013.

2.2. Laboratory analyses

Immediately after harvesting samples were sent to the laboratory of the Institute of Organic Farming. Impure samples were cleaned using an air separator. We did not further process them before they were dried at 40 °C, ground to pass a 0.5 mm sieve, and stored in the dark at 8 °C. Contents of thiamine and riboflavin were analysed using high-performance liquid chromatography methods (HPLC) with fluorescence detection (FLD) according to EN 14122 (2014) and EN 14152 (2014). Oxidation of thiamine to thiochrome was necessary for FLD. We used the pre-column derivatisation for thiamine.

An Agilent 1260 Infinity HPLC system equipped with an FLD (Waldbronn, Germany) was used for analytical HPLC separations. Reversed-phase chromatography was performed using a SecurityGuard™Standard Gemini-NX C18 pre-column (Phenomenex, Aschaffenburg, Germany) and a Kinetex 5.0 µm C18 column (150 × 4.6 mm, Phenomenex, Aschaffenburg, Germany). A volume of 20 µl was injected.

Thiochrome separation was performed at a column temperature of 25 °C and a flow rate of 0.8 ml/min. The mobile phase consisted of methanol and 0.5 M sodium acetate (30/70, v/v, isocratic conditions, pH 5.2). The FLD operated at an excitation wavelength of 366 nm and an emission wavelength of 435 nm. The results were expressed as the total thiamine using the factor 0.787 for conversion from thiamine hydrochloride to thiamine.

Riboflavin was analysed at a flow rate of 1 ml/min at 35 °C. The mobile phase consisted of a phosphate buffer (5 mM potassium dihydrogen phosphate, 10 mM sodium heptanesulfonate, 36 mM trimethylamine, pH 3.0) and 60% methanol. We ran the system with a binary gradient as shown in Table 2. The FLD operated at an excitation wavelength of 468 nm and an emission wavelength of 520 nm.

2.3. Statistics

All statistical analyses were conducted using R 3.4.0 (R Core Team, 2017). For each cultivar, mean, standard deviation, minimum and maximum (range) were determined using the complete dataset (n total in Table 1). The relationships between B vitamins were tested using Pearson correlation analyses (package PerformanceAnalytics) (Peterson and Carl, 2014).

We used Wilcoxon rank sum tests to compare B vitamin contents in cereals with contents in grain legumes. Residuals of a combined data set containing all grain legume samples for a comparison between cultivars were not normally distributed. Thus, Wilcoxon rank sum tests were also used to compare the B vitamin contents of grain legume cultivars. Generalized linear models (GLM) were used to identify the effects of cultivar on thiamine and riboflavin contents in cereals.

An uneven availability of samples resulted in an unbalanced sample set for each cultivar. For our analysis, we used variety, site, and harvest year as factors. The following adjustments of the dataset were necessary to enable further analyses regarding the influence of variety, year, and site on the contents of B vitamins:

Table 1
Chromatographic gradient conditions for the analysis of riboflavin.

Time (min)	Eluent A[a]	Eluent B[b]
0	95	5
3	95	5
12	53	47
12.1	2	98
17	2	98
17.1	95	5
25	95	5

[a] 10 mM sodium heptanesulfonate; 5 mM potassium dihydrogenphosphate; 36 mM triethylamine pH 3.0.
[b] 60% methanol.