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Modelling phytate degradation kinetics in soaked wheat and barley

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ABSTRACT

The objective of this study was to identify an appropriate mathematical function describing the *in vitro* phytate degradation profile of soaked wheat or barley (20 °C; 1 cereal: 2.75 water (w/w)) as affected by heat-treatment (stem pelleting at 90 °C) and phytase addition (Phytase 1: 0, 250, 500, 750, 1000, 1500, 2000 or 2500 FTU/kg; Phytase 2: 0, 375, 750, 1125, 1500, 2250, 3000 or 3750 FYT/kg). Samples were collected at 0, 2, 4, 8 and 24 h of soaking. The full dataset was split in two sub-sets as Phytase 1 and Phytase 2 are defined by different phytase activities. A segmented linear (SL) function, a first-order (FO) function and a Generalised Michaelis Menten (GMM) function were considered. The GMM fitted the data best. The GMM function was used to derive the relative instantaneously degradable fraction of phytate (F_0) and the half-life ($t1/2$) of phytate in hours (K). Addition of Phytase 1 or Phytase 2 had no effect on the degradation of phytate. The F_0 was greater in the heat-treated barley compared with the heat-treated wheat (0.19 *vs.* 0.14; P=0.02; Phytase 1). Heat-treatment of the cereals increased the F_0 from 0.05 to 0.15 (P=0.0007; Phytase 2). The K was lower in the non-heat-treated wheat compared with the non-heat-treated barley (averaging 23.3 *vs.* 43.5 h), whereas K was higher in the wheat compared with the barley (averaging 12.3 *vs.* 11.1 h; P=0.02 and P=0.006; Phytase 1 and Phytase 2) when the cereals were heat-treated. In conclusion, the GMM function is a suitable model for describing the phytate degradation profiles of the soaked wheat and barley. The *in vitro* results showed that heat-treatment of the cereals increased the F_0 and reduced the K although the plant phytase activity was reduced. This was possible due to induced structural changes of the grain that increased the contact between phytate and plant phytases. The lack of effect of additions of Phytase 1 or Phytase 2 on the phytate degradation in the cereals was possible due to a poor access of the microbial phytases to phytate. Therefore, more knowledge is needed about processing technologies that increase the access of plant and microbial phytases to phytate in the grains.

1. Introduction

Phytate, the main storage form of phosphorus (P) in plant feedstuffs, is difficult to digest for pigs unless plant or microbial phytase is present in the feed (Pointillart et al., 1984; Simons et al., 1990). Wheat and barley are rich in plant phytases

Abbreviations: AIC, akaike's information criteria; DM, dry matter; F_0, the relative instantaneously degradable fraction of phytate; FO, first-order function; FTU, FYT or PU, phytase activity expressed in units; GMM, Generalised Michaelis Menten; K, the $t1/2$ of phytate; LRT, Likelihood ratio tests; n, description of the shape of the phytate degradation curve; NLME, non-linear mixed effects model; P, phosphorus; RSD, residual standard deviation; SL, segmented linear function; $t1/2$, half-life.

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(Eeckhout and De Paepe, 1994; Viveros et al., 2000) and are the main constituents of pig feed in North European countries. The feed is prepared either directly without any heat-treatment (on-farm-mixed meal) or commercially as pellets requiring heat-treatment; often at temperatures above 81 °C. Consequently, a substantial part of the plant phytases is inactivated. Liquid feeding systems are widely used and provide possibilities of improvements of the digestibility of plant P, because mixing of feed and water initiates phytate degradation before feeding when phytase are present (Lyberg et al., 2005, 2006; Blaabjerg et al., 2010b). In practice, often only the cereal part of the feed is prepared as liquid feed. The time course of phytate degradation in soaked feedstuffs depends on different factors such as plant phytase present in the feedstuff, addition of microbial phytase and heat-treatment of the feedstuff (Carlson and Poulsen, 2003; Blaabjerg et al., 2010a). Mathematical functions can be used as tools to describe the time course of phytate degradation in soaked feedstuffs, providing information about the effect of these factors. Mathematical functions are commonly used in ruminant nutrition to describe the time course of nutrient degradation in *in vitro* systems, *e.g.* gas production (Lopez et al., 2000). These functions yield valuable information on fractional rates of degradation and half-life ($t1/2$) of feeds from their *in vitro* degradation profiles. Such approaches have not been undertaken in the study of *in vitro* phytate degradation in soaked cereals. Hence, the objective of this study was to identify an appropriate mathematical function describing the *in vitro* time course of phytate degradation at 20 °C in soaked wheat or barley as affected by heat-treatment and addition of two different microbial phytase products.

2. Materials and methods

2.1. Experimental design

Wheat and barley were ground by a roller mill (set to 3 mm between the rolls). Subsequently, half of each cereal was mixed in a cascade mixer with steam injection (about 70 °C) and pelleted through 3 mm dies at about 90 °C followed by cooling. Finally, the pellets were crumbled. The feedstuffs were supplemented with 0, 250, 500, 750, 1000, 1500, 2000 or 2500 FTU/kg (Phytase 1; *Aspergillus niger*; Natuphos; BASF) and 0, 375, 750, 1125, 1500, 2250, 3000 or 3750 FYT/kg (Phytase 2; *Peniophora lycii*; Ronozyme P; Novozymes) as shown in Table 1. The design resulted in 32 combinations of the 3 experimental factors (*i.e.*, 2(cereals) × 2(heat-treatment) × 8(phytase activity levels)) for each of the phytases. Hence, there were 8 replicates on the product factor 2(cereals) × 2(heat-treatment), which was the main interaction term of interest. These 32 combinations were assigned randomly to 32 containers where the individual container constitutes the experimental unit. Collections of samples during soaking are described in detail below.

Table 1
Analysed phytase activity (PU[a]/kg DM) in cereals (wheat or barley) subjected to heat-treatment or not and supplemented with 8 different levels of Phytase 1[b] (FTU/kg) or Phytase 2[c] (FYT/kg).

	Cereal			
	Wheat		Barley	
	Non-heat-treated	Heat-treated	Non-heat-treated	Heat-treated
Phytase 1, FTU/kg				
0	1010	270	696	254
250	1330	533	1020	468
500	1710	869	1280	703
750	1870	941	1690	1060
1000	2220	1280	1880	1470
1500	2730	1870	2790	1910
2000	3600	2390	3270	2290
2500	4130	2790	4110	2930
Phytase 2, FYT/kg				
0	1100	261	713	276
375	1690	867	1280	877
750	2360	1430	1710	1390
1125	2840	1990	2110	2040
1500	3490	2920	2580	2550
2250	4620	3400	3390	3410
3000	5160	4070	4060	4260
3750	6290	5130	4780	5120

[a] Phytase units as defined by Engelen et al. (1994).
[b] 32 combinations: 2(cereal) × 2(heat-treatment) × 8(activity levels of Phytase 1 [FTU/kg]). Declared activity of Phytase 1: 6500 FTU/kg; derived from *Aspergillus niger*; Natuphos; BASF.
[c] 32 combinations: 2(cereal) × 2(heat-treatment) × 8(activity levels of Phytase 2 [FYT/kg]). Declared activity of Phytase 2: 6390 FYT/kg; derived from *Peniophora lycii*; Ronozyme P; Novozymes.