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# High moisture airtight storage of barley and triticale: Effect of moisture level and grain processing on nitrogen and phosphorus solubility

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ABSTRACT

The aim of this study was to evaluate the effect of storage time, grain processing (whole vs. rolled) and the combination of phytase, xylanase, β-glucanase and protease on nitrogen (N) and phosphorus (P) solubility during high moisture airtight (HMA) storage of barley and triticale at various moisture levels (20, 23, 26 and 29% moisture) and to compare HMA storage of cereals with dry storage for 49 days. Dry stored barley and triticale (10 and 13% moisture, respectively) were kept in 10 L plastic buckets for 0 and 49 days. HMA stored cereals were kept in airtight bags (400 g per bag) at 15 °C for 0, 14, 29 and 49 days. The cereals were dry stored or HMA stored in rolled or whole form without or with an enzyme combination. Samples in triplicate were measured for dry matter (DM), pH, N and P solubility, phytate P and total P. HMA storage of rolled barley and rolled triticale at 26 and 29% moisture increased N and P solubility and decreased ratio of phytate P to total P (Phytate P:Total P as a measure of phytate degradation) compared with grains before storage ($P < 0.05$) and dry storage at d 49 ($P < 0.05$). The added enzyme combination increased P solubility in all barley groups by 8–16% points ($P < 0.001$) but only increased N solubility by 4% points in HMA stored rolled barley at 29% moisture at d 49 ($P < 0.001$) compared with no enzyme addition. The enzyme combination also increased N (3 and 5% points on average) and P solubility (8 and 15% points) in HMA stored rolled triticale at 26 and 29% moisture, respectively ($P < 0.05$). The inclusion of the enzyme combination during storage of rolled barley and rolled triticale for 49 days increased phytate degradation (23 and 39% points, respectively) and N (16 and 24% points, respectively) and P solubility (25 and 52% points, respectively) in HMA storage at 29% moisture to a greater extent compared with dry storage ($P < 0.05$). At d 49, increasing moisture levels increased P solubility (rolled barley, whole and rolled triticale) and N solubility (whole and rolled triticale) linearly and decreased Phytate P:Total P (rolled barley) linearly. There was a positive linear correlation between P and N solubility of HMA stored rolled barley and triticale. Overall, HMA storage of rolled barley and rolled triticale at 29% moisture with the enzyme combination is the suitable condition for increasing N and P solubility.



*Abbreviations:* N, nitrogen; P, phosphorus; HMA, high moisture airtight; DM, dry matter; CP, crude protein; Phytate P:Total P, ratio between phytate P and total P; d, day; + E, with the enzyme combination; NSP, non-starch polysaccharides; ATTD, apparent total tract digestibility.

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## 1. Introduction

Cereals such as wheat, barley and triticale constitute up to 70% of a typical North European pig diet. However, cereal protein and phosphorus (P) are often embedded tightly in the grain matrix and are poorly digested by pigs due to the presence of anti-nutritional factors, for example non-starch polysaccharides (NSP) and phytate. In fact, NSP create a barrier preventing enzymes from accessing substrates bound within the cell wall (Baik and Ullrich, 2008), while phytate forms insoluble complexes with protein and P (Weremko et al., 1997; Selle et al., 2000). Therefore, unlocking the nutrient potential of cereal by different feed processes may improve protein and P digestibility and reduce nitrogen (N) and P emissions to the environment.

Processing of cereals by means of grinding or rolling is used to rupture grains and render nutrients more accessible to digestive, plant and/or exogenous enzymes (Rowe et al., 1999). Moreover, using an enzyme combination (e.g. phytase, xylanase, β-glucanase and protease) may bring a potential synergistic effect on N and P digestibility. The supplementation of NSP degrading enzymes (xylanase and β-glucanase) together with phytase increases the accessibility of phytase to phytate (Adeola and Cowieson, 2011). Also, the addition of protease may support protein digestion. However, the effect on pigs is still unclear (Adeola and Cowieson, 2011). The activation of plant and exogenous enzymes requires moist conditions and is initiated in the stomach. Moreover, the time allowing enzymes' active sites to access substrate is short, because N and P are mainly absorbed at the proximal part of the small intestine (Partridge, 1978; Low, 1979; Cross et al., 1990). Based on this perspective, high moisture airtight (HMA) storage can be a potential approach to improving nutrient availability as it creates ideal conditions for activating enzymes (high moisture) and for providing more time to degrade substrate (Choct and Hughes, 1997; Kim et al., 2005). In fact, HMA storage improved soluble protein in barley (Åman et al., 1990) and maize (Baron et al., 1986) along with soluble P in maize (Niven et al., 2007). Nutrient solubility seems to be a sensitive marker of biological changes during HMA storage (Åman et al., 1990). Phosphorus solubility is also used to estimate P availability (Niven et al., 2007; Columbus et al., 2010). Moreover, Christensen (2013) found the correlation between the increase of apparent total tract digestibility (ATTD) of barley protein and the ingested amount of soluble protein in pigs. Therefore, in the current study, N and P solubility was used as an indicator of the effect of HMA storage on improving nutrient availability of cereals. However, as mentioned above, the increase of nutrient solubility during HMA storage may depend on different factors such as moisture levels, storage time and grain processing. The reported effects varied (Prigge et al., 1976; Baron et al., 1986; Åman et al., 1990) and are not yet well known. To the best of our knowledge, no reports on exogenous enzyme addition during storage of cereal have been published.

The hypothesis of the current study is that HMA storage of barley and triticale activates endogenous enzymes prior to feeding, resulting in increased solubility of N and P compared with dry storage. Moreover, we hypothesize that together the addition of microbial phytase, xylanase, β-glucanase and protease to barley during storage will increase the solubility of N and P compared with no enzyme addition. Therefore, the study aimed to evaluate the effect of storage time, grain processing (whole vs. rolled), enzyme combination and storage (dry storage vs. HMA storage at different moisture levels) on N and P solubility of barley and triticale.

## 2. Materials and methods

### 2.1. Cereals and experimental procedure

Barley and triticale (13 and 10% moisture, respectively) were cleaned separately using a sample cleaner MLN (Wintersteiger AG, Ried/I., Austria) to remove dust and unwanted particles. Half of the barley and triticale remained as whole grain (whole), whereas the other half was coarsely ground (rolled) by a roller mill (Skiold crushers KB 200, SKIOLD A/S, Sæby, Denmark). The average particle size of rolled barley and rolled triticale were $2468 \pm 2$ μm and $1844 \pm 2$ μm, respectively. Before storage, whole and rolled cereals were supplemented without or with an enzyme combination of phytase (Natuphos 5000G, BASF SE, Ludwigshafen, Germany) at 1000 FTU/kg of feed; xylanase (Danisco Xylanase 8000 G, Danisco Animal Nutrition, Marlborough, United Kingdom) at 4000 U/kg of feed; β-glucanase (ECONASE Barley P700, AB Vista, Marlborough Wiltshire, United Kingdom) at 17,500 BU/kg of feed and protease (RONOZYME ProAct CT, DSM Nutritional Products Ltd., Basel, Switzerland) at 15,000 PROT/kg of feed.

Whole or rolled barley and triticale supplemented without or with the enzyme combination were either stored in 10 L covered plastic buckets (dry storage) at ambient temperatures (23 °C on average) or in vacuum packed plastic bags providing airtight conditions for the cereals at moisture levels of 20, 23, 26 and 29% (HMA storage) (Table 1). Before HMA storage, the dry matter (DM) content of barley and triticale was determined. Based on the DM content, the required amounts of water were added to the barley and triticale to obtain the planned moisture levels. The procedure for HMA storage was as follows: each sample (400 g) of whole or rolled barley or triticale was weighed in a plastic bag. After mixing thoroughly with demineralised water, the bag was sealed by a vacuum packaging machine (Webomatic I 22-D, Bochum, Germany). The combination of phytase, xylanase, β-glucanase and protease was added to the enzyme supplemented groups at the same time as water. Samples in triplicate were randomly placed in an incubator (New Brunswick Scientific Model G25 Controlled Environment Incubator Shaker, GST Technical Sales, Edmonton, Alberta, Canada) at 15 °C. The positions of samples in the incubator were changed randomly every week. The temperature of the incubator was measured every day at different