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The effects of temperature, moisture, duration of incubation time, calcium level, and soaking with water or citric acid on *in vitro* phytate degradation in a wheat–barley–rye–soybean meal-based diet

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ABSTRACT

Three *in vitro* experiments were carried out to determine the effect of temperature, moisture content, duration of incubation, Ca level, soaking with water or citric acid onphytate degradation in a wheat–barley–rye–soybean meal-based broiler diet. In experiment 1, phytase activity of individual feed ingredients and 4 low-P broiler diets, containing 2, 4, 8, and 12 g Ca per kg diet, respectively, were measured in the presence or absence of sodium phytate or soybean meal. By using sodium phytate as substrate, phytase activity of rye, wheat, barley and soybean meal was 3350, 1170, 580 and 30 FTU/kg, respectively (P<0.001). Calcium level had no effect on the activity of intrinsic phytase of diets (P>0.05). In experiment 2, the effect of 2 moisture levels (0.25 and 50%), 3 temperatures (70, 75 and 80 °C) and 3 durations of incubation (2, 4, and 8 min) on the residual phytase activity of diet 1 (basal diet) were evaluated as a 2 × 3 × 3 factorial arrangement with 3 replicates per treatment. The loss of activity of intrinsic phytase increased from 0.25 at 70 °C to 0.61 at 80 °C (P<0.001). Increasing duration of incubation from 2 min to 8 min increased the loss of activity from 0.27 to 0.52 (P<0.001). By increasing the moisture content, loss of activity of intrinsic phytase also significantly increased from 0.25 at 25% moisture to 0.53 at 50% moisture. In experiment 3, the effects of 4 dietary Ca levels (2, 4, 8, and 12 g/kg), 2 types of soaking (with water or citric acid solution) and 4 duration times of soaking (2, 4, 8 and 24 h) on the amount of P released from the complete diet were determined as a 4 × 2 × 4 factorial arrangement with 3 replicates per treatment. Increasing Ca level of the diet from 2 to 12 g/kg decreased the amount of released P from 1.19 to 0.97 g/kg of diet (P<0.001). As time of soaking increased, the difference in released P due to soaking with citric acid comparison to soaking with deionized water became more prominent (interaction soaking × time; P<0.05).

In conclusion, soaking of a broiler diet, especially in a citric acid solution one day before feeding, may increase available P and decrease the need of supplemental inorganic P to these diets, thus improving the sustainable use of P resources.

Abbreviations: h, hour; min, minute; P, phosphorus; SPM, strokes per minute.

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1. Introduction

The majority of total P in vegetal feed ingredients is present in the structure of phytate, which has a low bioavailability in non-ruminant animals (Eeckhout and De Paepe, 1994). To meet P requirement for optimum performance and bone strength, inorganic P is usually added to non-ruminant diets. Phosphorus excretion and pollution is a serious concern in intensive animal systems, particularly in areas close to surface waters (De Boer et al., 1997). Microbial phytase is generally added to non-ruminant diets to render phytate P more available and reduce supplementation of inorganic P. Phytase, however, is present in some plant feed ingredients, *i.e.* in rye, wheat and barley, as well (Eeckhout and De Paepe, 1994) and this intrinsic phytase can be helpful in degrading phytate P (Esmaeilipour et al., 2012).

Time is a limiting factor for complete degradation of phytate in the gastrointestinal tract (Newkirk and Classen, 1998). Moreover, gastrointestinal conditions including pH and proteolytic digestion are possibly destructive for the intrinsic plant phytases (Esmaeilipour et al., 2012). Therefore, pre-treatments of feed may have a beneficial effect on phytate P hydrolysis before and during feeding (Newkirk and Classen, 1998). Phytate degradation and P utilization can be improved by either intrinsic plant phytase or supplementary microbial phytase (Kemme and Jongbloed, 1993). Combining both plant phytase and microbial phytase even was shown to have synergistic effects on P utilization (Afsharmanesh et al., 2008). These authors observed in a broiler experiment a limited increase in P digestibility (from 56.9 to 61.9%) if microbial phytase was added to diet in which plant phytases were inactivated by heat treating, whereas P digestibility largely increased (from 51.2 to 68.4%) if microbial phytase was added to a non-heat treated diet in which plant phytases were active. Näsi et al. (1995) reported that soaking of the feed increased the apparent absorption of P in pigs. Phosphorus absorption, however, might be negatively affected by the presence of Ca, due to the formation of Ca-phytate complexes, which are highly insoluble and not readily degradable (Cheryan, 1980; Reddy et al., 1982; Fisher, 1992; Angel et al., 2002). Also precipitation of Ca and released ortho-phosphate may limit P absorption (Angel et al., 2002). On the other hand, it has been reported that citric acid can improve availability of P *via* chelating effects on divalent cations (especially Ca), resulting in increased solubility and susceptibility of phytate to hydrolysis (Centeno et al., 2007). Moisture or wet conditions are necessary for enzyme activation. Stability of enzymes can be affected by several factors such as high temperature (Esmaeilipour et al., 2012), moisture at high temperature (Denstadli et al., 2006) and duration of incubation (Esmaeilipour et al., 2012). Denstadli et al. (2006) reported that a combination of low temperature and high moisture resulted in the highest phytate degradation during incubation. However, a high moisture level is destructive during high temperature because water unfolds and denatures enzymes and proteins (Denstadli et al., 2006). It has been reported that cowpea phytase had a lower activity after incubation at higher moisture levels than at lower moisture levels (Affrifah et al., 2005). Therefore, it can be hypothesized that intrinsic plant phytases are more stable at lower moisture levels especially at high temperatures.

The aim of this study was to investigate the effects of type of processing including soaking in water or citric acid solution, duration of soaking and Ca levels on the availability of phytate P by intrinsic plant phytases in an *in vitro* experiment. The effects of temperature, moisture, and time of incubation on the loss of activity of intrinsic phytase were also investigated.

2. Materials and methods

2.1. Feed ingredients and diets

Samples of feed ingredients wheat, barley, rye, and defatted soybean meal were obtained from a feed company in The Netherlands. The feed ingredients were ground with a rotor mill over a sieve of 1 mm (Retsch ZM100, Haan, Germany). In experiment 1, phytase activity of individual feed ingredients was measured with either an abundance of sodium phytate or soybean meal in a 1:1 ratio with each of the three cereals as source of phytate. After having demonstrated the activity of intrinsic phytase of the feed ingredients, the diets were formulated. A wheat–barley–rye–soybean meal based basal broiler diet, containing 20 g/kg Ca and 3.7 g/kg total P (without supplemental inorganic P), was formulated (diet 1, Table 1). In exp. 2, additional levels of limestone were added to the basal diet in order to investigate the effect of Ca content. There were 4 mash diets, only differing in Ca content: diet 1 (D1), diet 2 (D2), diet 3 (D3), and diet 4 (D4) containing 2, 4, 8, and 12 g/kg Ca, respectively.

2.2. Experimental procedures

In exp. 2, the effects of high temperature (relevant for pelleting conditions), moisture, and time of incubation on the efficacy of intrinsic phytase were investigated. Therefore, 0.5 gram samples of diet 1 were either mixed with 125–250 μl deionized-water (25 and 50%, respectively), incubated at high temperature (70, 75, and 80 °C) for different times of incubation (2, 4, and 8 min). After incubation, phytase activity of the samples was measured and the loss of activity of intrinsic phytase of incubated samples in comparison with non-incubated samples was calculated. Sodium phytate was used as phytate source in the exp. 2.

In exp. 3, the effects of Ca level, type of soaking (deionized-water or citric acid solution), and soaking times (2, 4, 8, and 24 h) on the amount of released P (g/kg diet) by intrinsic phytase were evaluated. Therefore, 0.5 g samples of each diet were soaked either in deionized-water or citric acid solution in the room temperature for the different times of soaking.