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Relevance of Phytase Activity for the P-Utilisation in Pigs

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Aim of the project

Little is known about the activity of alkaline phosphatases and phytases in the different parts of the gut of pigs and about their effects on the phytate degradation in the digestive tract. Phytate, the salt of *myo*-inositol 1,2,3,4,5,6 hexakis dihydrogen phosphate (IP_6) is widely distributed in plants. As a storage form of phosphorus and minerals, phytate is essential for germination processes in seeds and hence it is present in all sorts of plant seeds. The concentration of phytate in cereals, legumes and nuts ranges from ~1 to 3 % (Ingelmann *et al.,* 1993). Predominately, phytate is present in unprocessed feed and food, but can be strongly degraded during processing including fermentation (Phillippy *et al.,* 1988) while heat treatment up to boiling temperature causes only minor degradation (Schlemmer *et al.,* 1995). Under the acidic conditions of the stomach, phytic acid binds bi- and trivalent cations and precipitates at neutral pH of the small intestine. Thereby, it inhibits the intestinal absorption of trace elements and minerals and has, therefore, been regarded as an antinutrient for decades. In the last few years, however, beneficial properties of phytic acid such as antioxidative (Graf *et al.,* 1984, Rimbach and Pallauf, 1998) and anticancerogenic activities (Shamsuddin *et al.,* 1997) as well as 'second messenger' function of certain phytate hydrolysis products (DL-Ins(1,4,5)P_3 and DL-Ins(1,3,4,5)P_4 ; Berridge and Irvine, 1989) have been reported. These findings have revived discussions of the significance of phytic acid and other inositol phosphates in nutrition. Information as to the part of the gut in which phytate degradation takes place and which enzymes are involved, however, is scarce. The present study, therefore, was designed to gain more clarity about the whole mechanism of phytate degradation in the gut.

Methods

Finishing pigs (99-102 kg, bw) were fed two different diets (17 d), one high (control diet A) and one low (experimental diet B) in activity of feed enzymes. The composition of both diets was the same, however, the cereals of the one diet were extruded to inactivate feed enzymes Pigs were slaughtered 5 hours after the last feeding and chyme from the stomach, small intestine and colon was removed. Chyme from stomach, small intestine and colon was collected, homogenised, pH measured, the inositol phosphate isomers analysed and the activity of phytases and alkaline phosphatases determined.

In feed and faeces, total phosphorus (P) and phytate-phosphorus were determined according to the Official Method of Analysis (1986).

Results

Phytase activity for the control diet in feed, stomach, small intestine and colon was 43.1 ± 2.1, 3.7 ± 0.6, 0.9 ± 0.3 and 2.2 ± 0.1 and for the extruded diet 0.2 ± 0.1, 0.3 ± 0.2, 0.7 ± 0.2 and 1.8 ± 0.1 mU/mg protein, respectively. Activity of alkaline phosphatases in the small intestine and in the colon for the control diet was 146 ± 37 and 29.9 ± 6.9 and for the extruded diet 153 ± 43 and 39.3 ± 5.8 mU/mg protein, respectively.

For the control diet, high content of phytate hydrolysis products (IP_2-IP_5)(58.1 ± 13.8 %) of total inositol phosphates was found in the gastric chyme of the stomach, indicating strong phytate degradation in the stomach of pigs fed the control diet rich in phytases. For the extruded diet 16.8±4.1 % of total inositol phosphates were IP_2-IP_5, indicating only low phytate degradation in the stomach of pigs fed the extruded, phytase-deactivated diet. The distribution of total inositol phosphates in the liquid and solid phase of the stomach chyme at the mean pH ~ 4.3-4.6 was similar for both diets, showing 56.5 ± 13.1 % (diet A) and 66.7 ± 8.6 % (diet B) of total inositol phosphates as soluble. The content of soluble phytate, however, was higher for the extruded diet (51.8 ± 6.9 %) than for the control diet (10.7 ± 1.5 %) due to the missing degradation by inactivated phytases in the extruded diet; the content of insoluble phytate, however, was the same for both diets (31.2 ± 3.5 % and 31.4 ± 6.4 % for diet A and diet B, respectively), based on the insusceptibility of insoluble phytate to enzymatic hydrolysis.

In the small intestinal chyme, higher concentrations of inositol phosphates than in the stomach were determined. Compared with the stomach, the same inositol phosphate isomers were found except for DL-Ins(1,3,4,5)P_4 and DL-Ins(1,4,5)P_3. The distribution of total inositol phosphates in the liquid and solid phase, however, varied depending on the increased pH (~ pH 6.6) and the content of soluble inositol phosphates was much lower (13.1 ± 1.7 % and 4.3 ± 0.9 % for diet A and diet B, respectively) than in the stomach. High phosphorylated inositol phos-

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phates (IP_6, IP_5) showed low solubility (<14%) and lower phosphorylated inositol phosphates (IP_3, IP_2) higher solubility (35 to 75 %) in the small intestinal chyme, similar for both diets. Phytase activity in the chyme of the small intestine for the control diet (~ 1 mU/mg protein) was only about 1/4 of that found in the stomach.

For the control diet, the composition of inositol phosphate isomers in the colon chyme was completely different from that in the small intestine. High concentration of IP_6, along with low concentration of IP_2 - IP_5 was observed. For the control diet, decreasing concentration of total inositol phosphates from small intestine to colon (2,714 ± 484 to 1,419 ± 394 nmol/g chyme, ww), parallel to an almost constant IP_6 concentration (1,224 ± 340 and 1,119 ± 311 nmol/g chyme, ww), showed predominant degradation of the lower phosphorylated inositol phosphates (IP_2 - IP_5). The decrease in total inositol phosphates from small intestine to colon was similar for both diets (diet A: 47.7 %, diet B: 57.1 %), while the decrease in IP_6 was significantly higher (P<0.05) for the phytase-inactivated than for the control diet (2,213 ± 648 to 877 ± 305 and 1,224 ± 340 to 1,119 ± 311 nmol /g chyme, ww). Total phosphate concentration of P_i and IP_1 as an indicator for intensive hydrolysis of inositol phosphates was also significantly higher (P<0.05) for the extruded than for the control diet. Phytase activity for the control diet increased about two fold from small intestine to colon, reaching a common level with the extruded diet at ~2 mU/mg protein, while alkaline phosphatase activity from small intestine to colon decreased strongly (~150 to ~35 mU/mg protein for both diets. The main inositol pentaphosphates were DL-Ins(1,2,4,5,6)P_5, typical of microbial phytases (Cosgrove, 1970), and DL-Ins(1,2,3,4,5)P_5, typical of *Escherichia coli* phytases (Greiner *et al.,* 1993). From colon to faeces a continuing decrease in inositol phosphates occurred and a high concentration of IP_6 along with a low concentration of IP_2 - IP_5 were determined. Distribution of inositol phosphates in the liquid and solid phase was not evaluated due to the very low quantity of soluble inositol phosphates (< 3 % of total inositol phosphates in the faeces). On the basis of the mean daily intake and excretion of phytic acid (5 d) per animal, the mean degradation of phytic during the passage throughout the digestive tract was calculated. The results show very similar degradation of phytic acid for both diets (diet A: 97.4 ± 2.3 %; diet B: 97.7 ± 2.2 %). The mean apparent digestibility of total phosphorus per day and animal, however, was significantly different (P<0.05) for the control and the extruded diet (45.7 ± 3.3 % and 29.5 ± 5.1 %, respectively).

Cooperation

The present study was a cooperation between the Institute of Animal Nutrition, Federal Agricultural Research Centre, Braunschweig, (FAL), experienced in all kind of animal studies and the Institute of Nutritional Physiology of the Federal Research Centre of Nutrition, Karlsruhe, (BFE), involved in various projects concerning the nutritional activity of phytate and other inositol phosphates, using pigs as model for humans and also as target species.

Conclusions and further research demand

For monogastric animals such as pigs, total phytate degradation in the digestive tract for both diets is almost complete. To utilise phytate-phosphorus, however, phytate hydrolysis has to take place in the stomach as phosphate is absorbed in the small intestine. To improve phytate degradation in the stomach, high phytate release from the feed matrix along with sufficient phytase activity in the gastric chyme has to be achieved. As the contribution of endogenous phytases in the stomach and small intestine to the phytate hydrolysis is neglectable the addition of microbial phytases to the feed is essential to increase phytate phosphorus digestibility. The mechanism of phytic acid degradation found in pigs can be transferred to humans. As cereals and legumes are normally heat treated during preparation, intrinsic food phytases are non active in the consumed food. The degradation of phytate from the phytase-inactivated diet, therefore, represents the phytate hydrolysis in the human gut. This means that in humans no gastric phytate hydrolysis occurs, but strong degradation in the large intestine. To use the antioxidative and anticancerogenic activity of phytic acid for colon cancer protection, however, a high content of phytate in the colon is required.

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