Effect of microwave treatment of wheat on the appearance of inositol phosphates in different segments of the digestive tract in broilers

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Abstract
The effect of microwave treatment of wheat on inositol phosphates (InsP) in the digestive tract was studied in broilers. Two wheat-soybean meal-based diets with high (Dh) or low (Dl, microwave treated wheat) intrinsic phytase activity were used (total P: 4.8 g/kg DM). Eight pens of 15 birds were allocated to each diet on d 16. On d 23, the content of crop, duodenum/jejunum together, terminal ileum and caeca was obtained. Samples were analysed for InsP isomers and the marker TiO2. In the crop, InsP6 degradation was higher for Dh (59%) than for Dl (26%), reflecting differences in intrinsic phytase activity. In the duodenum/jejunum, this difference disappeared (Dh: 65%; Dl: 67%). Microwave treatment had a significant effect in the ileum (Dh: 69%; Dl: 78%), probably due to enhanced accessibility of InsP6 to phosphatases present in the intestine, but not in the caeca (Dh and Dl: 97%). In the crop, Ins(1,2,3,4,5)P5 and Ins(1,2,4,5,6)P5 were the main InsP5 isomers, confirming results from in vitro studies with wheat phytase. In the duodenum/jejenum, Ins(1,2,3,4,5)P5 and Ins(1,2,3,4,6)P5 dominated, suggesting participation of phosphatases of different origin.

Key Words: inositol phosphates, microwave treatment, intrinsic phytase, broilers

Introduction and objective
In plant seeds, phytate (any salt of phytic acid (InsP6)) represents the major storage form of phosphorus (P). As the absorption of P primarily occurs as orthophosphate, InsP6 needs to be hydrolyzed by phytase via different inositol phosphate (InsP) isomers. Cereals like wheat contain high phytase activities. The potential of intrinsic plant phytases to increase phytate hydrolysis has already been shown in poultry. The pathway of intrinsic phytases has been specified in vitro, whereas in poultry, information about the formation of different InsP isomers by intrinsic phytases is lacking. In vitro, the simulation of the different conditions along the digestive tract is only possible to a limited extend. Additionally, phosphatases of different origin might be involved in InsP degradation in the digestive tract. Thus, the InsP pattern in different segments of the digestive tract might differ from the in vitro pattern of intrinsic phytase. Moreover, heat-treatment of cereals, reducing intrinsic phytase activity, might modify InsP degradation. The objective therefore was to specify the InsP degradation in different segments of the digestive tract in broilers fed wheat-based diets differing in intrinsic phytase activity due to microwave treatment of wheat.

Material and methods
Two wheat-soybean meal-based diets with high (Dh, 623 U/kg) or low (Dl, microwave treated wheat, 121 U/kg) intrinsic phytase activity were used (total P: 4.8 g/kg DM). Wheat inclusion in the pelleted (70°C) diets was 65 %. Eight pens of 15 unsexed broilers were allocated to each diet on d 16. On d 23, the content of the crop, duodenum/jejunum together, terminal ileum and caeca was obtained and pooled on a pen-basis. Freeze-dried samples were analysed for InsP isomers and the marker TiO2. InsP isomers were detected by HPIC and UV detection at 290 nm following postcolumn derivatization using an ICS-3000 system (Dionex, Idstein, Germany). InsP6 degradation was calculated based on TiO2 concentration. Statistical
significance was evaluated by ANOVA and treatment means were compared using a multiple t-test ($P \leq 0.05$).

**Results and discussion**

In the crop, InsP$_6$ degradation was significantly lower for the microwave treated D$_l$ (26%) than for the untreated D$_h$ (59%), which most likely was the consequence of lower intrinsic phytase activity (Table 1). In the duodenum/jejunum, this difference disappeared (D$_h$: 65%; D$_l$: 67%). Soaking in the crop and passage through the acid condition of the proventriculus/gizzard might have enhanced the accessibility of phytate. Microwave treatment might have further increased InsP$_6$ accessibility and extractability of naturally non-extractable intrinsic phytases. This can explain the compensation of InsP$_6$ degradation in D$_l$ measured in the duodenum/jejunum. Another explanation might be a higher substrate induction of microfloral or endogenous mucosa phytases in the duodenum/jejunum for D$_l$ compared to D$_h$. Microorganisms producing phosphatases might also be induced by more accessible nutrients in D$_l$. In the ileum, InsP$_6$ degradation was significantly higher for the D$_l$ treatment (78%) compared to the D$_h$ treatment (69%). This can be seen as a result of structural changes to substrate, induced by the microwave treatment, which enhanced the contact between InsP$_6$ and the microfloral or mucosal phytase as suggested by BLAABJERG et al. (2010). In the caeca, an InsP$_6$ degradation of 97% was found in both treatments.

In the crop, Ins(1,2,3,4,5)P$_5$ and Ins(1,2,4,5,6)P$_5$ were the predominating InsP$_5$ isomers in both treatments (Figure 1a). Percentage of Ins(1,2,4,5,6)P$_5$ in $\Sigma$InsP$_5$ isomers was significantly higher for D$_l$ compared to D$_h$. The predominating InsP$_5$ isomer in the crop confirms the *in vitro* degradation pathway of wheat phytase, starting hydrolysis at the position D/L-4 (equivalent with L/D-6) of the inositol ring (NAKANO et al., 2000). BOHN et al. (2007) showed wheat phytases initiating InsP$_6$ degradation at both the D/L-6 and D/L-3 position. The occurrence of InsP$_5$ isomers could be partially due to non-degraded InsP$_5$ isomers of the diet or may have been caused by microfloral or feed-associated fungal phytase. Higher percentage of Ins(1,2,4,5,6)P$_5$ in $\Sigma$InsP$_5$ isomers for the D$_l$ treatment could also have been caused by residual intrinsic soybean phytase, a 3-phytase (PHILLIPPY and BLAND, 1988). Soybean meal was not microwave treated and only intrinsic wheat phytase activity was lower in D$_l$ compared to D$_h$. This might have resulted in higher appearance of Ins(1,2,4,5,6)P$_5$, formed by soybean phytase, in D$_l$ compared to D$_h$. In regard to InsP$_4$ isomers, in both treatments Ins(1,2,5,6)P$_4$ and Ins(1,2,3,4)P$_4$ were predominantly formed (data not shown). This again confirms the *in vitro* described degradation pathways of wheat phytases, where the major pathway proceeds via Ins(1,2,5,6)P$_4$ (equivalent with Ins(2,3,4,5)P$_4$) and the minor pathway proceeds via Ins(1,2,3,6)P$_4$ (equivalent with Ins(1,2,3,4)P$_4$) (NAKANO et al., 2000).

As shown for the duodenum/jejunum (Figure 1b), Ins(1,2,3,4,5)P$_5$ and Ins(1,2,3,4,6)P$_5$ were the main InsP$_5$ isomers for both treatments in the duodenum/jejunum and ileum. The difference in Ins(1,2,4,5,6)P$_5$, which was found in the crop, no longer existed in the duodenum/jejunum and ileum. The InsP$_5$ pattern changed in the intestinal segments compared to the crop as shown for the duodenum/jejunum (Figure 1b). This suggests the participation of phytases and other phosphatases of different origin in different segments, with 6- and 5-phytases dominating in the intestinal segments. Ins(1,2,3,4)P$_4$ (equivalent with Ins(1,2,3,6)P$_4$), the only detected InsP$_4$ isomer in the duodenum/jejunum (data not shown), might be a degradation product of a 5-phytase.

**Conclusions**

We conclude that the crop was the main site of InsP$_6$ degradation when the untreated wheat was fed, while it was the proventriculus/gizzard or duodenum/jejunum when the microwave treated wheat was fed. In the crop, Ins(1,2,3,4,5)P$_5$ and Ins(1,2,4,5,6)P$_5$ as well as
Ins(1,2,5,6)P₄ and Ins(1,2,3,4)P₄ seem to be the main degradation products for wheat phytases. This confirms results from in vitro studies with wheat phytase. Likewise, the changing InsP₃ pattern in the duodenum/jejunum shows the participation of phosphatases of different origin in different segments.

Table 1. InsP₅ degradation (%) in different segments of the digestive tract of broiler chickens (Means and SD, n = 8 pens per treatment)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>D₉</th>
<th>D₁</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Segment</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Crop</td>
<td>59ᵃ</td>
<td>10.7</td>
<td>26ᵇ</td>
</tr>
<tr>
<td>Duodenum/jejunum</td>
<td>65</td>
<td>5.8</td>
<td>67</td>
</tr>
<tr>
<td>Lower ileum</td>
<td>69ᵇ</td>
<td>4.8</td>
<td>78ᵃ</td>
</tr>
<tr>
<td>Caeca</td>
<td>97</td>
<td>1.8</td>
<td>97</td>
</tr>
</tbody>
</table>

ᵃᵇ Means in the same row with different superscripts are significantly different according to the t-test (P ≤ 0.05).

D₉: diet contained untreated wheat, D₁: diet contained microwave treated wheat.

Figure 1: InsP₅ isomers in the digesta of the crop (a) and duodenum/jejunum (b), expressed as percentage of ∑InsP₅ isomers (means and SD)

References

