Heat treatment of rapeseed meal increases phytate flow into the duodenum of sheep

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Abstract

The effect of heat treatment on duodenal flow of phytate, i.e., inositol hexaphosphate, from rapeseed meal was studied in four sheep fitted with duodenal cannula. The rapeseed meal was fed untreated, heated at 133°C (H133) or heated at 143°C (H143) for 3 h. Levels of phytate and its hydrolysis products, i.e., inositol tri-, tetra- and pentaphosphates were measured in duodenal digesta. Phytate was the major form of inositol phosphate (IP) in the duodenal digesta. Heat treatment increased daily flow of inositol hexaphosphate, inositol pentaphosphate, and inositol tetraphosphate into the duodenum. Approximately 22, 37 and 55% of dietary phosphorus in the form of IPs was recovered at the duodenum of sheep fed untreated, H133- and H143-treated rapeseed meals, respectively.

Results suggest that heat treatment of rapeseed meal increases flow of phytate into the duodenum, decreasing the digestibility of dietary phytate phosphorus for sheep. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Phytate; Duodenal flow; Rapeseed; Heat treatment; Sheep

1. Introduction

Rapeseed meal is a widely used protein source for ruminants (Fenwick, 1982), and treatment with heat generally decreases rumen degradability of its protein. This can increase the supply of dietary protein to the lower gut, thereby improving protein retention in ruminants (Dakowski et al., 1996).
Reddy et al. (1982) reported that rapeseed meal contains 2–4% phytate (inositol hexaphosphate). Monogastric animals poorly utilize phytate phosphorus because they lack phytase to release its inorganic phosphorus (Näsi et al., 1995; Weremko et al., 1997). Conversely, phytate phosphorus has been suggested to be available to ruminants due to the high phytase activity of ruminal microorganisms (Reid et al., 1947; Raun et al., 1956; Tillman and Brethour, 1958). However, our previous study, using the nylon bag technique, suggested that 32% of the phytate in a rapeseed meal was not degraded in the rumen of sheep and heat treatment suppressed ruminal degradation of phytate in the meals (Konishi et al., 1999).

The objective of this experiment was to determine the effect of heat treatment of rapeseed meal on duodenal flow of phytate and its partially hydrolyzed products in sheep.

2. Materials and methods

2.1. Heating procedure

Rapeseed meal was roasted as previously described (Konishi et al., 1999). Briefly, rapeseed meal was treated in a heated forced air oven at 133 or 143°C for 3 h. The heated oilseed meals were then allowed to cool at room temperature and stored in plastic bags. These treatments were replicated three times and the three batches were mixed well for the study.

2.2. Animals and feeding

Four crossbred ewes (Suffolk × Corriedale) weighing approximately 50 kg and fitted with T type duodenal cannula 50 mm posterior to the pylorus were used. The experimental design was a three-period change-over with three treatments (i.e., feeding with untreated rapeseed meal, or heat-treated rapeseed meals at 133 or 143°C). Sheep were randomly allocated to the three treatments, with none receiving the same diet twice. The sheep were individually housed in metabolism cages and cared for according to the Guide for the Care and Use of Laboratory Animals (Kyoto University Animal Care Committee). Untreated or treated rapeseed meals were labeled by ytterbium (Yb) according to the method of Vega and Poppi (1997).

Diet consisted of 70% beet pulp, 20% timothy hay and 10% untreated or treated rapeseed meals that included 0.5% of the corresponding Yb-labeled rapeseed meal. Almost all of the dietary phytate originated from the rapeseed meal. The animals were fed twice daily (10:00 and 22:00 h) at 0.85% of body weight and had free access to water and a mineral block that contained (%): NaCl, 97.1; Fe₂O₃·H₂O, 0.174; (FeO₂, FeO₃), 0.02; CuSO₄, 0.038; CoSO₄, 0.007; ZnSO₄, 0.124; MnCO₃, 0.105; Ca(IO₃)₂, 0.008; NaSeO₃, 0.003. The experimental diets contained 12.5% crude protein, 0.58% calcium and 0.22% phosphorus. The content of digestible energy was estimated to be 2.8 Mcal/kg fresh weight (NRC, 1985). Animals consumed all feed that was given. Daily intakes of crude protein, calcium, phosphorus and digestible energy were 106 g, 4.9 g, 1.9 g and 2.4 Mcal, respectively, sufficient for the requirements of maintenance ewes weighing 50 kg (NRC,
During the experiment, body weight did not change. All sheep were allowed a 7-day-preliminary feeding period before each collection.

2.3. Sampling procedures

Duodenal digesta were collected for 30 min at 1, 3, 5, 7, 9 or 11 h after the morning feeding for 2 days. Samples obtained at the same time on two successive days were pooled to a composite for each sheep. The duodenal digesta were freeze-dried and used for the analysis of phytate, its hydrolyzed products, and Yb.

2.4. Chemical analysis

The amounts of inositol hexa-, penta-, tetra- and triphosphates (IP6, IP5, IP4 and IP3) were determined in rapeseed meal and duodenal digesta according to ion-pair high performance liquid chromatography (HPLC) as described by Rounds and Nielsen (1993). The method included extraction of samples with HCl, partial purification of inositol phosphates (IPs) from the crude extract by an anion-exchange chromatography, separation by ion-pair HPLC, and UV detection after the post-column reaction.

Phosphorus concentration in the diets and Yb concentration in Yb-labeled rapeseed meal and duodenal digesta were measured using an inductively coupled plasma emission spectrometer (ICPS-1000, Shimadzu, Kyoto, Japan) after digestion with concentrated nitric acid and perchloric acid (5:1, v/v).

Calcium concentration in the diets was determined by an atomic absorption spectrophotometer (AA-6600F, Shimadzu, Japan). Crude protein in the diets was determined according to a Kjeldahl method of the AOAC (1990).

2.5. Estimation of the amount of IP reaching the duodenum

Daily flow of each IP into the duodenum was calculated as:

\[
\text{Daily flow of IP (\mu mol/day)} = \frac{\text{average IP concentration in duodenal digesta}}{\text{average Yb concentration in duodenal digesta} \times \text{daily intake of Yb}}
\]

Total phosphorus in the form of IPs was calculated as the sum of individual IPs multiplied by the number of phosphorus that each IP contained. Flow of phosphorus in the form of IPs at each sampling time was expressed as a ratio to Yb.

2.6. Statistical analysis

The effect of heat treatment on daily flow of each IP was analyzed by two-way ANOVA using the GLM procedure. Treatment differences were determined by the least-significant difference test. The effects of treatment and sampling time on the ratio of IPP to Yb were analyzed by the MIXED procedure. All statistical analyses were performed using the SAS (1985, 1996).
3. Results

3.1. IP concentrations in rapeseed meals

Heat treatments reduced the IP6 concentration and increased the IP5 and IP4 concentrations in rapeseed meal (Table 1). IP4 was not found in the untreated rapeseed meal and IP3 was not detected in any rapeseed meal. Phosphorus in the form of all IPs was slightly reduced by heat treatments.

3.2. Daily flow of IPs into the duodenum

To determine the flow of duodenal digesta the single marker Yb, which is known as a marker of the solid phase of digesta, was used since phytate was not detected in the soluble phase of duodenal digesta.

Table 1
IPs and phosphorus in the form of IPs in untreated and heat-treated rapeseed meal (μmol/g DM)

<table>
<thead>
<tr>
<th>Rapeseed meal</th>
<th>IP6 (^a)</th>
<th>IP5</th>
<th>IP4</th>
<th>IP3</th>
<th>IPP (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>38.05</td>
<td>3.40</td>
<td>ND</td>
<td>ND</td>
<td>245.3</td>
</tr>
<tr>
<td>H133(^c)</td>
<td>30.75</td>
<td>8.75</td>
<td>1.00</td>
<td>ND</td>
<td>232.3</td>
</tr>
<tr>
<td>H143(^c)</td>
<td>28.25</td>
<td>10.40</td>
<td>1.25</td>
<td>ND</td>
<td>226.5</td>
</tr>
</tbody>
</table>

\(^a\) IP3, IP4, IP5 and IP6 denote inositol tri-, tetra-, penta- and hexaphosphate, respectively.
\(^b\) Phosphorus in the form of IPs.
\(^c\) H133, heat treatment at 133°C; H143, heat treatment at 143°C.
\(^d\) Non-detectable levels.

Table 2
IPs and phosphorus in the form of IPs entering the duodenum of sheep fed untreated or heat-treated rapeseed meal (μmol/day kg live weight)\(^a\)

<table>
<thead>
<tr>
<th>IPP(^b) intake</th>
<th>IP flow to the duodenum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IP6(^c)</td>
</tr>
<tr>
<td>Untreated</td>
<td>382.4</td>
</tr>
<tr>
<td>H133(^d)</td>
<td>367.7</td>
</tr>
<tr>
<td>H143(^d)</td>
<td>360.0</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
</tr>
</tbody>
</table>

Statistical significance of effect (P)

<table>
<thead>
<tr>
<th>Diet</th>
<th>ND(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.011</td>
</tr>
<tr>
<td>Animal</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^a\) Mean values within a column not sharing a common letter (d, e, f) were significantly different, \(P < 0.05\). Means for four sheep.
\(^b\) Phosphorus in the form of IPs.
\(^c\) IP3, IP4, IP5 and IP6 denote inositol tri-, tetra-, penta- and hexaphosphate, respectively.
\(^d\) H133, heat treatment at 133°C; H143, heat treatment at 143°C.
\(^e\) Not determined because IPP intake per kg body weight was same among sheep in each treatment.
Heat treatment increased the flow of IP6 into the duodenum (Table 2). The increment was proportional to the heating temperature. Daily flows of IP5 and IP4 were also increased with heating. However, the flow of IP3 was not affected by the treatments. Since IP6 was the major form of IPs in the duodenal digesta, the flow of phosphorus in the form of all IPs showed a similar trend to that of IP6.

3.3. Daily changes of IPs flow into the duodenum

Heat treatment affected the ratio of phosphorus in the form of IPs to Yb in duodenal digesta and the effect of sampling time was also significant (Fig. 1). The ratio tended to increase by 3 h after feeding in all sheep. In sheep fed untreated and H133-treated rapeseed meal, the ratio gradually decreased through 11 h after feeding. Conversely, the ratio was relatively stable after 3 h post-feeding in sheep fed H143-treated rapeseed meal.

4. Discussion

Heat treatment slightly reduced IP6 but increased IP5 and IP4 concentrations in rapeseed meal, suggesting that heat treatment partially hydrolyzed IPs. Phosphorus in the form of IPs was slightly decreased by heat treatment, in agreement with previous reports of Mansour et al. (1993) and Konishi et al. (1999) on lower phytate-phosphorus concentration in heat-treated rapeseed meal, resulting in slightly higher intake of phosphorus in the form of IPs in sheep fed untreated rapeseed meal versus sheep fed heat-treated rapeseed meal.
Inositol hexaphosphate and other IPs were detected in duodenal digesta of sheep given untreated rapeseed meal, and 22% of dietary phosphorus in the form of IPs was recovered at the duodenum. Phytate is known to be firmly bound to protein in oilseed meals (Fontaine et al., 1946; Saio et al., 1967), and our previous results using the nylon bag technique suggested that approximately 37% of the protein in untreated rapeseed meal escaped ruminal degradation. In contrast, Nelson et al. (1976) reported no phytate in rumen, abomasum, and small intestine digesta of steers fed a ration based on sorghum grain and soybean meal after an 18 h fast. The present experiment shows that duodenal flows of IPs decreased postprandially, after a temporal increase, in sheep fed untreated rapeseed meal. As a result, duodenal IP flow was very low 11 h after feeding untreated rapeseed meal. Additionally, we previously suggested that phytate was more stable in untreated rapeseed meal than in untreated soybean meal during incubation in the rumen of sheep, and the effective rumen degradability of phytate was 50% less in rapeseed meal than in soybean meal (Konishi et al., 1999). These differences may explain why Nelson et al. (1976) did not detect phytate in the digesta of steers fed soybean meal, while we found phytate in the duodenal digesta of sheep fed rapeseed meal.

Heat treatment increased duodenal flow of all IPs other than IP3. As a result, phosphorus flow in the form of IPs was increased by heat treatment. Approximately 37 and 55% of dietary phosphorus in the form of IPs was recovered in the duodenum of sheep given H133- and H143-treated rapeseed meals, respectively, which agrees with previous results, 43 and 48% of phytate phosphorus using the nylon bag technique (Konishi et al., 1999) and suggests that duodenal flow of IPs may be increased by reduction of rumen protein degradation as a result of heat treatment.

A large part of the IP reaching the duodenum is considered unavailable to ruminants, analogous to phytate metabolism in monogastric animals. This may attain importance when dietary phosphorus is minimized to reduce fecal phosphorus excretion to reduce the environmental impact of ruminants.

The ratio of phosphorus, in the form of IPs, to Yb was temporally increased and then gradually decreased after the sheep were given untreated or H133-treated rapeseed meals. The reduction in this ratio suggests that phytate is gradually degraded in the rumen and that the flow of IPs into the duodenum is decreased with increased rumen retention times. Additionally, it seems that the ratio is low in digesta collected 1 h after feeding, perhaps because this digesta is mainly derived from diet ingested in the previous meal. However, the IP/Yb ratio was high in sheep fed the H143-treated rapeseed meal during this experiment, compared to sheep fed the untreated or H133 rapeseed meal, and it did not change substantially after feeding. We conclude that phytate in H143-treated rapeseed meal is not readily degraded in the rumen even if it is retained for a long time.

**Acknowledgements**

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References


