Rye Bread in the Diet of Pigs Enhances the Formation of Enterolactone and Increases Its Levels in Plasma, Urine and Feces

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ABSTRACT To obtain new insight into the quantitative and qualitative metabolism of rye and wheat lignans, we performed three series of experiments with catheterized pigs. Two diets with similar levels of dietary fiber and macronutrients but with contrasting levels of plant lignans (isolariresinol, lariciresinol, matairesinol, pinoresinol, secoisolariciresinol and syringaresinol) were prepared from rye (high in lignans) and wheat (low in lignans) soft and crisp breads. In two series of experiments we quantified the uptake from the gut of enterolactone in four pigs fitted with catheters in the portal vein and mesenteric artery and with an ultrasonic flow probe attached to the portal vein to monitor the blood flow. In a third study with six pigs, we quantified the bioavailability of the plant lignans that can be converted to enterolactone (lariciresinol, matairesinol, pinoresinol, secoisolariciresinol and syringaresinol) and the concentration in the peripheral blood. Plant and mammalian lignans in diets and stool were analyzed by isotopic dilution gas chromatography–mass spectrometry and enterolactone in plasma and urine determined by time-resolved fluoroimmunoassay. There was a significantly higher formation of enterolactone in pigs fed the rye diet, and higher fecal and urinary excretion and circulating levels of mammalian lignans than in pigs fed the wheat diet. The conversion of mammalian lignan precursors to enterolactone was 48% with the wheat diet and 60% with the rye diet. Mammalian lignans are absorbed by passive diffusion from the large intestine and a substantial fraction of the absorbed mammalian lignans undergoes enterohepatic circulation, resulting in low diurnal variation in plasma levels of enterolactone.


KEY WORDS: • catheterized pigs • enterolactone • lignans • metabolism

Lignans and isoflavonoids are two groups of diphenolic compounds with estrogen-like biological activity that are widely distributed in plants (1,2). Plant lignans occur as glycosides in whole grain, seeds, nuts, vegetables, berries and beverages such as tea and coffee (2,3). The plant lignans are converted to the mammalian lignans enterolactone (ENL) and enterodiol (END) by dehydroxylation and demethylation by the intestinal microflora (4–6). This process occurs primarily in the large intestine, as suggested by a significant change in the proportion of plant to mammalian lignans from ileal digesta to cecum and colon (7) and the very low urinary excretion of mammalian lignans in ileostomy patients who consume rye bread (8). Until recently, it was believed that secoisolariciresinol (SECO) and matairesinol (MAT) were the only plant lignans to be converted to ENL and END but recent studies have identified a number of plant lignans, pinoresinol (PINO), syringaresinol (SRY), isolariresinol (I-LAR) and lariciresinol (LAR), as potential new precursors for the formation of ENL and END (9). Among the newly identified mammalian lignan precursors, in vitro studies with human fecal microflora have shown that I-LAR was stable during the incubation, whereas the conversion of PINO, SRY and LAR after 24 h was 55, 4 and 101%, respectively. In rats the plant lignans undergo an enterohepatic circulation (10), and it is also probably the case in other mammals, as shown in humans for phenolic estrogens (11). Lignans are excreted in both the urine and the feces. From human data (12,13) it has been calculated that 0.09 and 0.17 μmol lignans were excreted per g dietary fiber ingested by omnivorous and vegetarian subjects, whereas in pigs, Glitsø et al. (7) calculated the excretion of lignans to be 0.01–0.12 μmol lignans per g dietary fiber when the pigs were fed diets based on whole grain rye, pericarp-testa, aleurone and endosperm. The circulation levels of lignans depend on the diet consumed (14,15). In a recent study it was...
found that the plasma ENL concentration was 12.5 nmol/L in men and 14.8 nmol/L in women who consumed wheat bread, which increased to 25.6 and 39.7 nmol/L for the two sexes, respectively, when the bread was switched to rye (15).

It has been shown that the mammalian lignans and soy isoflavonoids have relatively low estrogenic activity and that they may have antiestrogenic effects in certain situations (16,17). Lignans and isoflavonoids may stimulate production of sex-hormone-binding globulin in the liver (16) and may in this way alter the biological effects of sex hormones. It has also been hypothesized that by inhibiting growth and proliferation of hormone-dependent cancer cells, they may prevent breast and prostate cancer and perhaps also colon cancer (16,18,19).

Rye soft and crisp breads are traditional components of the diet in Denmark, Finland and other Northern European countries. Rye is an important source of dietary fiber (DF) in Scandinavia, accounting for ~30% of the DF intake in Denmark and Finland (20,21). The lignans in rye, like those in other cereals, are localized in the aleurone and pericarp-testa cell walls, giving a much higher concentration of plant lignans in the bran than in the extracted flour (7,22). Because lignans are probably metabolized by facultative anaerobe bacteria (23), it has been assumed that dietary components that alter the colonic environment may influence lignan metabolism. However, in a recent study with pigs fed diets that introduced huge differences in the metabolism of carbohydrates in the large intestine, it was not possible to demonstrate that degradation of plant cell walls was a necessity for converting plant to mammalian lignans (7).

There is a great need to know more about the conversion from plant to mammalian lignans, about the quantitative absorption and excretion of lignans in feces and urine and about the levels of circulating mammalian lignans, to understand the physiological effects of lignans. In the present investigation we studied the metabolism of dietary plant lignans in three series of experiments with catheterized pigs chosen as a model for human subjects. Rye- and wheat-based diets were chosen to provide the dietary plant lignans.

MATERIALS AND METHODS

Breads and diets. Wheat and rye soft and crisp breads were produced in pilot plant bakeries at Nordmills (Nordmills, Cerealia AB, Malmså, Sweden) and at Wasa Bread (Wasa Bread AB, Filipstad, Sweden), respectively. The ingredients for the wheat breads were white wheat flour, a purified wheat fiber (Vitacell), soy oil, sugar, salt, malt and yeast; and for the rye breads, whole grain rye, rye bran, soy oil, sugar, salt and yeast. The soft breads were frozen immediately after production and stored at ~20°C until consumption, whereas the crisp breads were stored dry. Soft and crisp breads were mixed with vitamins and minerals and provided ~19, 15 and 66% of the energy from fat, protein and carbohydrates, respectively (see Table 1 for diet ingredients and composition). Hereafter, the wheat diet is referred to as diet WD and the rye diet as diet RD. A plant lignan–free purified diet [semisynthetic diet (diet SSD)] was prepared from wheat starch (707.8 g/kg), cellulose (80.0 g/kg), casein (182.2 g/kg) and vitamins and minerals (28 g/kg).

Experimental design and pigs. The main purpose of study 1 was to measure the quantitative absorption to the portal vein of ENL. A total of four male castrated pigs (Danish Institute of Agricultural Sciences Swine herd, Foulum, Denmark) with an initial body weight of 44.6 ± 2.4 kg were used in the study. Each pig was fitted under anesthesia with two catheters, one in the portal vein (size: I.D. 0.050; O.D. 0.090; wall 0.02; Buch & Holm, Denmark) and the second in the mesenteric artery (size: I.D. 0.040; O.D. 0.070; wall 0.13; Buch & Holm), and with an ultrasonic blood-flow probe (14 mm; Transonic System, Ithaca, NY) around the portal vein. A flowmeter (T201D flowmeter with P-option; Transonic System) was used for measuring the flow rate. The pigs were given penicillin after surgery for 4 d. Before the surgery, the pigs were fed a habitual diet (barley, wheat and soybean meal), which is naturally rich in plant lignans. During the 10-d recovery, the pigs were again on the same diet rich in lignans and then, from d 7 to d 10, gradually introduced to the test diet, which was then fed as the sole diet for 1 wk in a repeated 2 × 2 Latin square design without any washout periods between the dietary interventions. The bread was cut into pieces, mixed 1:2.5 (w/w) with water and fed in equal amounts three times daily, at 0700, 1500 and 2200 h. Portal and arterial blood samples were collected twice weekly. The pigs were given penicillin after surgery for 4 d.

TABLE 1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>WD</th>
<th>RD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat crisp bread, g</td>
<td>602</td>
<td>2465</td>
</tr>
<tr>
<td>Wheat soft bread, g</td>
<td>370</td>
<td>30480</td>
</tr>
<tr>
<td>Rye crisp bread, g</td>
<td>593</td>
<td>666</td>
</tr>
<tr>
<td>Rye soft bread, g</td>
<td>379</td>
<td>12</td>
</tr>
<tr>
<td>Vitamins/minerals, g</td>
<td>28</td>
<td>53</td>
</tr>
<tr>
<td>Chemical composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ash, g</td>
<td>33</td>
<td>56</td>
</tr>
<tr>
<td>Protein (N×6.25), g</td>
<td>119</td>
<td>127</td>
</tr>
<tr>
<td>Fat, g</td>
<td>68</td>
<td>73</td>
</tr>
<tr>
<td>Total carbohydrates, g</td>
<td>773</td>
<td>699</td>
</tr>
<tr>
<td>Sugars, g</td>
<td>24</td>
<td>40</td>
</tr>
<tr>
<td>Fructans, g</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>Starch, g</td>
<td>529</td>
<td>455</td>
</tr>
<tr>
<td>Total NSP, g</td>
<td>220 (23)</td>
<td>203 (55)</td>
</tr>
<tr>
<td>β-glucan, g</td>
<td>131</td>
<td>20</td>
</tr>
<tr>
<td>AX, g</td>
<td>12 (1)</td>
<td>19 (7)</td>
</tr>
<tr>
<td>Mammalian lignans precursors, μg</td>
<td>53 (13)</td>
<td>127 (36)</td>
</tr>
<tr>
<td>SECO, μg</td>
<td>161</td>
<td>754</td>
</tr>
<tr>
<td>MAT, μg</td>
<td>33</td>
<td>813</td>
</tr>
<tr>
<td>LAR, μg</td>
<td>52</td>
<td>1689</td>
</tr>
<tr>
<td>PINO, μg</td>
<td>230</td>
<td>235</td>
</tr>
<tr>
<td>SYR, μg</td>
<td>3131</td>
<td>35177</td>
</tr>
</tbody>
</table>

1 The mineral and vitamin mixture provided the following (mg/kg diet): CaC2PO4, 14,000; NaCl, 4000; CaCO3, 8000; FeSO4·5H2O, 50; ZnO, 80; MnO, 27; CuSO4·5H2O, 20; KI, 0.2; Na2SeO3, 0.3; retinyl acetate, 1.1; cholecalciferol, 0.01; dl-α-tocopherol, 60; menadione, 2; riboflavin, 4; pantothenic acid, 11; cobalamin, 0.02; niacin, 22; biotin, 0.06.

2 Abbreviations used: WD, wheat diet; RD, rye diet; NSP, nonstarch polysaccharides; AX, arabininoxylans; SECO, secoisolariciresinol; MAT, matairesinol; I-LAR, isolariciresinol; LAR, lariciresinol; PINO, pinosylvin; SYR, syringaresinol.

3 Values in parentheses are soluble-NSP, soluble β-glucan and soluble AX.
then deprived of food for 24 h and then, on d 6, fed one dose of diet RD, after which blood samples were taken at −30, 0, 30 and 60 min, then at 60-min intervals to 960 min after the morning feeding. The purpose of study 3 was to quantify the urinary excretion of ENL, the fecal excretions of SECO, MAT, ENL and END, and to follow the changes in ENL in the jugular vein after introduction to diets containing plant lignans. A total of six male castrated pigs (Danish Institute of Agricultural Sciences Swine herd, Foulum, Denmark) with an initial body weight of 38.7 ± 2.7 kg were used for the study. The study was designed as a crossover experiment with washout periods before, between and after the dietary interventions. In periods 1, 3 and 5, the pigs were fed for 1 wk with the purified diet (diet SSD), whereas diets WD and RD were fed for 2 wk in periods 2 and 4. Chromic oxide (2 g/kg dry matter) was incorporated into the diets (Table 1) and used as an indigestible marker. The pigs were surgically fitted with a catheter (size: I.D. 0.050; O.D. 0.090; wall 0.015; Bach & Holm) for blood sampling in the jugular vein. Blood samples were taken in the morning and in the evening the first 3 d in the dietary intervention period, thereafter once daily in connection with the morning feeding. Unfortunately, however, the catheter worked properly in only three of the pigs that were fed diet RD, and in one of the pigs fed diet WD only during the first 2 wk of the experiment, and it was therefore decided to take blood samples by venipuncture 2 d before the pigs switched diet. The pigs were fed twice daily (at 0930 and 1930) throughout the whole experiment. In the diet intervention periods (periods 2 and 4), the pigs were equipped with a urinary bladder catheter for urine collection (size: I.D. 0.200; O.D. 0.500; wall 0.015; Ricch AG, Germany). Urine was collected in benzoic acid and the stool samples in plastic bags the last 3 d of diet intervention. Furthermore, stool samples where taken (from rectum) in periods 1, 3 and 5. Stool samples were analyzed for plant and mammalian lignans and the plasma for ENL.

**Analytical methods.** The diets were analyzed in duplicate for DM, ash, protein (Kjeldahl method), fat (hydrochloric acid-fat), sugars (glucose, fructose and sucrose), fructans, starch, total non-starch polysaccharides, cellulose, soluble and insoluble mixed linked β-(1→4)-d-glucan (β-glucan), soluble and insoluble arabinoxylans (AX) and Klasson lignin. These methods are all described in detail by Bach Knudsen (24). Chromic oxide in diet and feces was analyzed by colorimetry (25). The 24-h net absorption was calculated as three times the measured 8-h net absorption.

The gas chromatography–mass spectrometry (GC-MS) method, used for analysis of MAT and SECO, and the newly found food plant lignans I-LAR, LAR, PINO and SYR in the pig diets, was optimized from the previously published method for analysis of isoflavonoids and lignans in food matrices (26). The optimization and full validation of the method will be presented elsewhere. The method is briefly as follows. Duplicate analyses were carried out for each sample. To 50 μg of dry sample, deuterated internal standards (3H2-matairesinol, 3H2-secoisolariciresinol and 3H2-anhydrosecoisolariciresinol) and 500 μL of distilled water were added. Enzymatic hydrolysis was carried out with H. pomatia in 0.15 mol/L acetate buffer, pH 5. The sample was incubated first overnight at 37°C and then 1 h at 60°C. A hydrolyzed sample was extracted twice with 6 mL of diethyl ether. The combined organic phases were evaporated completely, dissolved in 0.5 mL of methanol and stored in a refrigerator. The water phase was subjected to acidic hydrolysis by adding 200 μL of 0.6 mol/L HCl followed by incubation of the sample 2 h at 70°C. The sample was extracted twice with diethyl ether:ethyl acetate, 1:1 (v:v). The organic phases were combined with the organic phases obtained after enzymatic hydrolysis. The sample was evaporated completely, dissolved in 0.5 mL of methanol and stored in a refrigerator. The water phase was further hydrolyzed with 1.5 mol/L HCl 2 h at 100°C, followed by extraction with diethyl ether:ethyl acetate, 1:3 (v:v). The water phase was discarded. The organic phases were combined with the stored organic phases, obtained after enzymatic and mild acid hydrolyses. The sample was evaporated completely under nitrogen, and dissolved in 200 μL of methanol.

The sample was applied in 2 μL of methanol on a Lipidex 5000 column (0.3 μm, 2 cm, phase: MeOH:CHCl3:H2O, 4:1:1). Lignans were eluted with 4 mL of MeOH:CHCl3:H2O, 4:1:1 (v:v:v). The fraction was evaporated completely under nitrogen, and dissolved in 0.5 mL of MeOH. Further purification of the sample was carried out with chromatographies on DEAE-OH and QAE-Ac, as described by Mazur et al. (26). The sample was derivatized with 100 μL of silanization reagent (pyridine:HMD/S:TMCS, 9:3:1) by incubating 30 min at room temperature, transferted to a microvial and analyzed by GC-MS. Deuterated internal standards were not available for new plant lignans, so quantification was done using deuterated MAT for analysis of LAR, PINO and SYR, and deuterated SECO and anhydrosecoisolariciresinol for analyses of isolariciresinol and anhydroisolariciresinol, respectively.

The analytical procedure for time-resolved fluorimunonassay of ENL in plasma (27,28) and in urine (29) was as described previously. All intra- and interassay CV were <10% in the present experiment. Because the immunoassay used for the urine ENL gives 30% higher values than the GC-MS method used in earlier studies (29), the values for urine ENL excretion and concentration were corrected to correspond with the values for the GC-MS method using the formula: Y(GC-MS) = 0.821X − 0.148.

**Calculations and statistical analysis.** The quantitative absorption of ENL in study 1 was calculated from the porto-arterial differences and the portal flow measurement as described by RéÔrat et al. (30):

\[
q = (C_t - C_p) F (dt)
\]

\[
Q = \sum q
\]

where \( q \) is the amount of ENL absorbed within the time period \( dt \), \( C_t \) is the concentration of ENL in the portal vein, \( C_p \) is the concentration of ENL in the mesenteric artery, \( F \) is the blood flow in the portal vein and \( Q \) is the amount of ENL absorbed from \( t_t \) to \( t_f \). The 24-h net absorption was calculated as three times the measured 8-h net absorption.

The 24-h net absorption of ENL in study 2 pigs after a pulse dose of diet RD was calculated as

\[
Q = \beta 1440
\]

where \( \beta \) is the slope (nmol/min) of the cumulated ENL absorption in individual pigs over 360–660 min after feeding.

The urinary excretion of ENL in pigs in study 3 was calculated as the collected daily amount of urine multiplied by the concentration of ENL in the urine, expressed in μmol/d. Fecal excretion of plant and mammalian lignans in study 3 pigs was calculated as follows:

\[
\text{Fecal excretion} = \frac{\text{Lignan}_{\text{feces}} \times \text{Cr}_{2} \text{O}_{3} \text{diet} \times \text{Food}}{\text{Cr}_{2} \text{O}_{3} \text{feces}}
\]

where \( \text{Lignan}_{\text{feces}} \) is the concentration of plant and mammalian lignans in feces, \( \text{Cr}_{2} \text{O}_{3} \text{diet} \) and \( \text{Cr}_{2} \text{O}_{3} \text{feces} \) are the concentrations of chromic oxide in the diet and feces, respectively, and Food is the amount of food consumed.

The bioavailability of mammalian lignan precursors (lariciresinol, matairesinol, pinoresinol, secoisolariciresinol and syringaresinol) was calculated as

Bioavailability of mammalian lignan precursors

\[
\text{Bioavailability} = \frac{\text{Urine}_{\text{ENL}}}{\text{Intake}_{\text{SECO} + \text{MAT}} \times \text{LAR} \times \text{PINO} \times \text{SYR}}
\]

where \( \text{Urine}_{\text{ENL}} \) and \( \text{Intake}_{\text{SECO} + \text{MAT}} \) are the quantitative excretion in urine of ENL and quantitative intake of mammalian lignan precursors, respectively. The bioavailability of plant lignans SECO and MAT was calculated as

Bioavailability of SECO + MAT

\[
\text{Bioavailability} = \frac{\text{Intake}_{\text{SECO} + \text{MAT}} - \text{Feces}_{\text{SECO} + \text{MAT}}}{\text{Intake}_{\text{SECO} + \text{MAT}}} \times 100
\]

where \( \text{Intake}_{\text{SECO} + \text{MAT}} \) and \( \text{Feces}_{\text{SECO} + \text{MAT}} \) are the quantitative intake and fecal excretions of plant lignans SECO and MAT, respectively. The mean bioavailability of plant lignans LAR, PINO and SYR was calculated as
Table 2 shows the composition of the diets. The breads were formulated to be almost similar with regard to macronutrients but to vary in the content of plant lignans. Diet RD contained 35,177 μg/kg DM of total lignans and 30,480 μg/kg DM of mammalian lignan precursors, whereas the concentration in diet WD was only 31,31 μg/kg DM of total lignan and 2,465 μg/kg DM of mammalian lignan precursors and with a much higher proportion of SECO than of MAT. The dietary fiber level was 230–235 g/kg DM in the two diets but the proportion of different dietary fiber components varied; diet WD contained more cellulose than diet RD, whereas β-glucan, AX and Klason lignin were higher in diet RD than in diet WD. Diet RD also had a higher level of soluble components, predominantly as soluble AX and β-glucan.

Study 1. The blood-flow rate in the portal vein was not influenced by the dietary treatment but varied with time after feeding. The flow rate was about 1.4 L/min before feeding, increasing to ~1.7 L/min 60 min after feeding, after which it decreased to 1.4 L/min before the next feeding (data not shown). The mean blood flow was 30.3 mL kg⁻¹ min⁻¹.

The intake of food was 1250 g DM/d for both diets (Table 2). The intake of dietary fiber was ~290 g/d, whereas there was a significant difference in the intake of the two plant lignans (10.0 μmol/d for diet WD and 10.87 μmol/d for diet RD). The mean concentration of ENL in the portal vein of pigs fed diet WD was 74 nmol/L and in the mesenteric artery, 56 nmol/L, whereas it was significantly higher in pigs fed diet RD: 451 nmol/L in the portal vein and 343 nmol/L in the mesenteric artery.

The concentrations of mammalian lignans ENL and END in feces from the pigs in studies 1 and 3 were analyzed by a two-way ANOVA model (31):

\[ X_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + e_{ijk} \]

where \( e_{ijk} \) (0, σ²), \( \alpha_i \) is the diet (\( i = 1, 2 \)), \( \beta_j \) is the week (\( j = 1, 2 \)), and \( \gamma_k \) represents the experimental pigs (\( k = 1, \ldots, 4 \)).

The portal and arterial ENL concentrations and the cumulated net absorption were analyzed by a second-degree polynomial (31):

\[ X_i = \mu + \beta_1 X + \beta_2 X^2 + e_{i} \]

where \( e_i \) (0, σ²), \( \mu \) is the intercept and \( \beta_1 \) and \( \beta_2 \) are the slopes for time \( X \), after pigs were fed the pulse dose of diet RD.

The concentrations of mammalian lignans ENL and END in feces from the pigs in studies 1 and 3 were analyzed by a two-way ANOVA model (31):

\[ X_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + e_{ijk} \]

where \( e_{ijk} \) (0, σ²), \( \alpha_i \) is the diet (\( i = 1, 2 \)) and \( \beta_j \) is the duration of intervention (\( j = 1, 2 \)). All statistical calculations were done using SuperAnova or Statview packages (Abacus Concepts, Berkeley, CA). Differences with \( P < 0.05 \) were considered significant.
Study 2. The blood-flow rate in the portal vein on the day when the pigs were fed one dose of diet RD was 32.7 mL kg\(^{-1}\) min\(^{-1}\), with a variation from 1.4 L/min at the time of feeding to 1.8 L/min 120 min after feeding, and with a steady decline to 1.5 L/min 600 min after feeding, after which it was constant. Portal and arterial levels of ENL in pigs fed the diet SSD for 5 d were 4.3 and 3.5 nmol/L in the portal vein and the mesenteric artery, respectively, around the time pigs were fed diet RD on d 6. This level was kept almost constant up to 300 min postfeeding, after which portal and arterial concentrations of ENL increased progressively to 28 nmol/L in the portal vein and 20 nmol/L in the mesenteric artery 960 min after feeding (Fig. 2). The total net absorption of ENL in pigs fed a pulse dose of 50.2 \(\mu\)mol of plant lignans was estimated to be 5.3 \(\mu\)mol in the period 0–960 min. In the period 360–660 min, when the contribution from the enterohepatic circulation is negligible, the mean rate of absorption was 0.27 nmol/h, corresponding to 6.5 \(\mu\)mol/d.

Study 3. The concentration of ENL in the jugular vein of pigs before the dietary intervention was 1.1 nmol/L. For the three pigs that started on diet RD, there was a gradual increase in the plasma level of ENL, which reached the plateau level of 350 nmol/L on d 4 (Fig. 3). The mean plasma level of ENL of all pigs on diet RD was 310 nmol/L, which was significantly higher than the mean plasma level of pigs on diet WD, 56 nmol/L (Fig. 4). In the washout periods, the concentration was 3.5 nmol/L, with no difference in pigs that previously had been fed different diets. The urinary concentration of ENL was 5–6 times higher than the plasma levels in pigs fed both diets. The correlation between the concentration of plasma and urine was \(\tau = 0.69\) (\(P < 0.01\)).

The mean intake of the two plant lignans in pigs in study 3 was 12.5 and 137.5 \(\mu\)mol/d for diet WD and RD, respectively. At this level of intake, 2.3 \(\mu\)mol/d of mammalian lignans was excreted in the urine and 2.3 \(\mu\)mol/d excreted in feces of diet WD–fed pigs and 16.6 \(\mu\)mol/d was excreted in the urine and 53.9 \(\mu\)mol/d was excreted in feces of diet RD–fed pigs (Table 3). The majority of mammalian lignans in the feces was in the form of ENL. At present there is no method available to quantify the new plant lignans in fecal material, although the excretion of plant lignans MAT and SECO was 0.2 \(\mu\)mol/d for diet WD–fed pigs and 1.6 \(\mu\)mol/d for diet RD–fed pigs, corresponding to a bioavailability of the two lignans of 75–77%. Assuming that SECO, MAT, PINO, LAR and SYR represent all plant lignans that can be converted to mammalian lignans, the mean conversion of LAR, PINO and SYR can be estimated to be 19% for the lignans in diet WD–fed pigs and 10% for the lignans in diet RD–fed pigs.

The profile of mammalian lignans in fecal material was different in catheterized pigs (study 1) where the dietary intervention lasted 1 wk and in the pigs in study 3 where the dietary intervention lasted 2 wk (Table 4). The concentration of mammalian lignans, in particular ENL, was much higher in feces of diet RD–fed pigs in study 3 than in feces of pigs in study 1. This was, however, not the case for diet WD–fed pigs.

**DISCUSSION**

In the present study we found a strong association between the dietary intake level of plant lignans and the formation, absorption, excretion and circulating levels of ENL. Thus, the

**FIGURE 2** Portal and arterial blood concentrations of enterolactone (A) and cumulated uptake of enterolactone (B) in pigs after the intake of one dose of the rye-based diet. Values are means ± SEM, \(n = 4\).

**FIGURE 3** Jugular vein plasma concentrations of enterolactone in pigs after introduction of the rye-based diet. Values are means ± SEM, \(n = 3\).
\(~12\) times higher intake of mammalian lignan precursors in diet RD-fed pigs than in diet WD-fed pigs resulted in a substantially higher gut formation, fecal and urinary excretion of mammalian liginans, higher absorption and higher circulating levels of ENL. It is also clear that the identification of the new precursors that potentially may be converted to mammalian lignans explains the discrepancy between plant lignan input and mammalian lignan output in human rye-consumption experiments (15,32), in the pig study of Glitsø et al. (7) and in this study. Thus, the amounts of LAR, PINO and SYR are 12 (diet WD) and 18 (diet RD) times higher than the sum of MAT and SECO. In vitro studies revealed a substantial variation in the conversion of plant to mammalian lignans, from 4% for SYR to 101% for LAR. For MAT and SECO, the conversions were 62 and 72% (9), respectively, which is slightly lower than our in vivo data of 75–77%. The bioavailability of SECO and MAT is thus much higher than the mean bioavailability of LAR, PINO and SYR, which was 19% in diet WD-fed pigs and 10% in diet RD-fed pigs. Of the mammalian lignan precursors, we found that 48% were converted into mammalian liginans in diet WD-fed pigs and 60% in diet RD-fed pigs; however, the excretory route differs. When pigs were fed diet WD, the excretion was equally distributed between the urine and the feces, whereas the excretion in the urine represented only 14% of the intake, and excretion in feces, 46% of the intake in pigs fed diet RD. The reason for this divergence between the two experimental diets is unknown, although it may be that when the body pool is saturated, as may be the case when pigs are fed diet RD, the excretory route changes from urine to feces, suggested by the much higher levels of ENL in the feces in study 3 than in study 1. Excretion of ENL through feces was somewhat higher than that found in humans, where it was found that 34–35% (mean) of the mammalian liginans were excreted through the fecal route (13,14). This discrepancy between humans and pigs is most likely a consequence of the higher dietary fiber intake in pigs than in humans, given that the excretion in this study and the study of Glitsø et al. (7) showed that the fecal excretion of mammalian liginans per g dietary fiber was comparable in both species.

The pigs in study 1 were treated with antibiotics for 3 d because of the surgery, and it can thus be questioned whether the period of adaptation was sufficient for the microflora to fully retain the capacity for the conversion of plant to mammalian liginans. This concern is accentuated by a recent study of Kilkkinen et al. (33), who found that because of the pronounced impact of the antimicrobials on the intestinal microflora, oral intake of antimicrobials had a significantly negative influence on plasma ENL concentration. In contrast, Horn-Roos et al. (34) did not find any difference in the ENL level of antibiotic users and nonusers. Our results are in concert with the results of the second study, given that we found a high degree of similarity in plasma levels of ENL in pigs fed the wheat and rye-based diets, respectively.

<table>
<thead>
<tr>
<th>Diet</th>
<th>WD</th>
<th>RD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake of food, g DM</td>
<td>1538 ± 24.0</td>
<td>1534 ± 24.0</td>
</tr>
<tr>
<td>Intake of dietary fiber, g</td>
<td>360 ± 5.6</td>
<td>353 ± 5.4</td>
</tr>
<tr>
<td>Total, (\mu)mol</td>
<td>12.5 ± 0.19*</td>
<td>137.5 ± 2.14</td>
</tr>
<tr>
<td>I-LAR, (\mu)mol</td>
<td>2.9 ± 0.04*</td>
<td>20.3 ± 0.31</td>
</tr>
<tr>
<td>Mammalian lignan precursors, (\mu)mol</td>
<td>9.6 ± 0.15*</td>
<td>117.2 ± 1.83</td>
</tr>
<tr>
<td>SECO, (\mu)mol</td>
<td>0.7 ± 0.01*</td>
<td>3.4 ± 0.05</td>
</tr>
<tr>
<td>MAT, (\mu)mol</td>
<td>0.1 ± 0.01*</td>
<td>3.5 ± 0.05</td>
</tr>
<tr>
<td>LAR, (\mu)mol</td>
<td>0.2 ± 0.01*</td>
<td>7.3 ± 0.11</td>
</tr>
<tr>
<td>PINO, (\mu)mol</td>
<td>1.7 ± 0.03*</td>
<td>12.3 ± 0.19</td>
</tr>
<tr>
<td>SYR, (\mu)mol</td>
<td>6.8 ± 0.11*</td>
<td>90.7 ± 1.41</td>
</tr>
<tr>
<td>Wet stool weight, g</td>
<td>859 ± 33*</td>
<td>1047.0 ± 19</td>
</tr>
<tr>
<td>Dry stool weight, g</td>
<td>297 ± 13</td>
<td>286 ± 6</td>
</tr>
<tr>
<td>Excretion of lignans</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SECO + MAT, (\mu)mol</td>
<td>0.2 ± 0.01*</td>
<td>1.6 ± 0.06</td>
</tr>
<tr>
<td>ENL + END, (\mu)mol</td>
<td>2.3 ± 0.8*</td>
<td>53.9 ± 6.2</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, \(n = 6\). * Different from control, \(P < 0.05\).
The extent to which the bacterial microflora present in the pig stomach and small intestine can deconjugate the plant lignans was previously discussed (7). As was concluded in the previous study with pigs and supported by results from the urinary excretion of mammalian lignans in ileostomy patients (8), plant lignans probably cannot be biologically available in the small intestine. This conclusion is further substantiated by the results from cathereterized pigs given a pulse dose of diet RD. In pigs, the mouth-to-cecum transit time is 240 min, as judged from the increase in butyrate concentration in the portal vein (37), and it is not until 360 min postfeeding that we start to see an increase in the portal and, consequently, arterial levels of ENL.

A common feature in studies on lignans is the substantial variation of plasma levels of ENL found in controlled interventions studies (15) and descriptive studies (33). It is also striking that intake of antibiotics has a detrimental influence on the urinary and plasma levels of ENL (23,33). In the present study, we also saw a much higher between-individual variation in plasma level of ENL than was seen for the metabolites deriving from carbohydrate fermentation (SCFA; A. Serena, Danish Institute of Agricultural Sciences, personal communication) or reported in earlier studies (35). The reason for that is unknown, but one possibility would be that the microbial deconjugation of recycled mammalian lignans and the demethylation and dehydroxylation of plant lignans are carried out by minor groups of microorganisms, sensitive to external factors; that is, antibiotic treatment and/or the growth rate of these microbes cannot keep up with the major groups of microorganisms that perform the conversion of carbohydrates to SCFA in the large intestine.

There is no doubt that the most important factor for determining the concentration of mammalian lignans in the gut lumen, the circulation and the urine in pigs is the dietary concentration of plant lignan precursors. The present study shows that it takes time to fill up the body pool. Given the condition of a constant intake of plant lignans, however, the plasma concentration of ENL will be little influenced, whether blood samples are taken in the fed or nonfed stage.

In conclusion, the results of the present study showed that the rye-based diet that contained ~11 more plant lignans compared with the wheat-based diet enhanced the formation,
circulating levels and fecal and urinary concentrations and excretions of mammalian lignans. Of the total plant lignans, 37% of the lignans in diet WD-fed pigs and 51% of the lignans in diet RD-fed pigs were converted into mammalian lignans, with some differences in the excretory routes of mammalian lignans in the two diets. Furthermore, the enterohepatic circulation of lignans is substantial and presumably responsible for the low diurnal variation in plasma levels of ENL.

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LITERATURE CITED