INTRODUCTION

In recent years, dietary fiber (DF) has received considerable attention with regard to its influence on nutrient digestion and utilization. DF has been defined as plant materials that are resistant to mammalian digestive enzymes. With the exception of lignin, all of the materials called DF are carbohydrates in nature (Kritchevsky, 1988). It is well documented that dietary fiber is thought to mediate protective effects on the colonic epithelium through their fermentation products and fecal bulking capacity (Bach Knudsen and Hansen, 1991; Wang et al., 2002; Wang et al., 2003). Some metabolic characteristics of colonic epithelial cells are closely related to the digestive events in nutrition of the large intestine. Resistant starch, non-starch polysaccharides and residual protein are broken down by colonic microflora to produce short-chain fatty acids. The major products are acetate, propionate and butyrate, which are rapidly absorbed through the colonic mucosa (Macfarlane and Cummings, 1991). Acetate serves primarily as a fuel for muscle, and propionate has been implicated in the control of both cholesterol and carbohydrate metabolism in the liver. In general, butyric acid, which has important effects on epithelial cell growth and differentiation and the concentrations of long-chain fatty acids, may control cell turnover in the gut (Lupton, 1995).

Gaiva et al. (2001) found that ATP citrate lyase and malic enzyme activities are contributed to alterations of in vivo lipogenesis rate. In the study of lipogenesis in adipose tissue in pigs, malic enzyme was recognized to play a key role in the lipogenic process and the activity of malic enzyme is thought to be a good indicator of lipogenesis (Lee and Kauffman, 1974; Leveille, 1970). In addition, fatty acids are degraded by oxidation at the β-carbon in mitochondria. Ketogenesis occurs mainly in the hepatocytes, but also in the intestines of suckling mammals (Thumelin et al., 1993). In pigs, ketone-body metabolism is much lower than in other mammals. One earlier study shows that the limitation of ketogenesis in newborn-pig liver is due to a very low amount and activity of mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (3-HMG-CoA synthase) (Duee et al., 1994). However, there is little information on investigating the enzyme activities involved in fatty acid metabolism in the colon mucosa of pigs fed on fiber diets.

The aim of the present work was to study the activities of enzymes involved in fatty acid metabolism in the colon mucosa of piglets fed diets with various fiber levels. It was of interest to investigate whether the activity of enzymes involved in fatty acid metabolism in the colon mucosa of pigs was influenced by either the different fiber diets supplied or different ages between animals.
DIETARY FIBER AND ACTIVITIES OF ENZYMES FOR PIGLETS 1525

MATERIALS AND METHODS

Animals

Thirty-six crossbred (Yorkshire × Swedish Landrace) piglets originating from twelve litters were randomly allotted by litter, weight and gender to three groups with four individuals per group. Each group was fed on one of the three experimental diets. One piglet from each litter was selected in one of the different age groups: 5-week (suckling), 6-week (1st week post-weaning) and 7-week (2 weeks post-weaning) old, respectively. The mean body weight (BW) at birth (n=36) was 1.6 ± 0.2 kg, and the mean BW of piglets (n=12) in each age group, i.e. 5, 6, and 7 weeks old, was 9.9 ± 0.2, 10.9 ± 2.2 and 12.2 ± 2.5 kg, respectively.

Piglets were given the three different experimental diets ad libitum from 3 weeks old until the study ended. The piglets used for sample collection were weighed and killed by an injection of 30-40 ml of sodium pentobarbitone (100 mg/ml, i.p.) per pig at 5, 6 and 7 weeks, respectively. The gastrointestinal tract was exposed by a midline laparotomy and removed immediately.

Preparation of intestinal tissues samples

About 30 cm large intestine samples between the apex of the colon spiral and the rectum were rapidly excised, opened longitudinally and rinsed twice with ice-cold 0.9% sodium chloride solution. Immediately, the segments were divided finely into about 10 cm specimen and snap-frozen on dry ice. All samples were stored at -80°C for further analysis of enzyme activities.

Preparation of homogenates

After thawing, a 1/3 (w/v) mucosal homogenate was made by gently scraping the luminal surface of the colon tissue with a small spatula, transferring a small quantity of mucosa to ice-cold homogenizing buffer (1 g mucosa/3 ml) and homogenizing in a Potter-Elvehjem all-glass homogenizer. The homogenate was sonicated for 60 seconds using an ultrasonic bath (Bandelin Sonorex RK100H, Germany) and then centrifuged at 15,800 g for 30 min. All operations were conducted at 4°C. Homogenizing buffer contained 50 mM triethanolamine/HCl, 2 mM magnesium chloride and 1 mM EDTA, pH 7.5 (Crabtree et al., 1979). The supernatant fluid was considered to contain the total soluble protein of the cell (cytoplasm and mitochondria matrix), and was assayed without further

Chemicals

Micro BCA™ Protein Assay Reagent Kit was purchased from Pierce (Rockford, IL, USA). EDTA and Triton X-100 were from Merck (Darmstadt, Germany) and the other chemicals were from Sigma Chemical (St. Louis, MO, USA).

Table 1. Composition of the experimental diets (g/kg DM as fed) fed to examine the activities of enzymes involved in fatty acid metabolism in the colon epithelium of piglets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Low fiber</th>
<th>Control</th>
<th>High fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolizable energy (MJ/kg)</td>
<td>15.59</td>
<td>14.43</td>
<td>14.31</td>
</tr>
<tr>
<td>DM (%)</td>
<td>92.0</td>
<td>86.0</td>
<td>87.3</td>
</tr>
<tr>
<td>Crude fat</td>
<td>41.1</td>
<td>27.0</td>
<td>63.6</td>
</tr>
<tr>
<td>Crude protein</td>
<td>179.6</td>
<td>171.3</td>
<td>171.8</td>
</tr>
<tr>
<td>Lysine</td>
<td>10.3</td>
<td>8.1</td>
<td>7.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>3.8</td>
<td>3.2</td>
<td>2.8</td>
</tr>
<tr>
<td>Threonine</td>
<td>5.8</td>
<td>5.6</td>
<td>4.7</td>
</tr>
<tr>
<td>Ash</td>
<td>38.6</td>
<td>34.5</td>
<td>44.1</td>
</tr>
<tr>
<td>Calcium</td>
<td>9.1</td>
<td>8.5</td>
<td>9.0</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>7.9</td>
<td>7.0</td>
<td>8.2</td>
</tr>
<tr>
<td>Starch</td>
<td>538.2</td>
<td>549.4</td>
<td>276.2</td>
</tr>
<tr>
<td>Total NSPa</td>
<td>99.8</td>
<td>147.0</td>
<td>199.6</td>
</tr>
<tr>
<td>I-NSPb</td>
<td>75.7</td>
<td>107.4</td>
<td>149.0</td>
</tr>
<tr>
<td>S-NSPc</td>
<td>24.1</td>
<td>40.2</td>
<td>50.6</td>
</tr>
</tbody>
</table>

a NSP, non-starch polysaccharides; b I-NSP, insoluble non-starch polysaccharides; c S-NSP, soluble non-starch polysaccharides.
preparation. The resulting supernatant is referred to as “homogenates”.

**Assay of enzyme activities**

Malic enzyme activity was assayed as described by Wise and Ball (1964) by measuring the rate of NADPH formation by monitoring at 340 nm and 37°C. One unit of enzyme activity causes 1 µmol NADPH to be formed.

Citrate lyase activity was assayed as described by Bergmeyer (1974). Citrate lyase activity was determined by the malate dehydrogenase-catalysed reduction of oxaloacetate. One unit of enzyme activity is defined as the consumption of 1 µmol NADH.

The activities of these enzymes were measured by using a spectrophotometer (UV-2101PC UV-VIS Scanning Spectrophotometer, SHIMADZU, Japan). All measurements were performed at 37°C and the unit of the activity of enzyme is nkat per g tissue. The relationship between nkat and unit is 1 unit =16.67 nkat.

**Protein determination**

The protein assay was based on the spectrophotometer on a microwell plate reader (Labsystems Multiskan RC) method by using Micro BCA™ Protein Assay Reagent Kit (U.S. Patent No. 4,839,295). Protein concentration in the homogenate was adjusted to 1-20 µg/ml before measurement.

**Statistical analysis**

The results were presented as the least squares means value±standard error of the mean (SEM) and were tested statistically in an analysis of variance test using a General Linear Model (GLM) procedure. The model used was:

\[ Y = \mu + T_i + A_j + T A_{ij} + e_{ijk} \]

Where \( \mu \) is the mean value for all treatments, \( T_i \) (a=3) is the effect of diet, \( A_j \) (b=3) is the effect of age, TA\(_{ij}\) is the effect of interaction between diet and age and e\(_{ijk}\) is the residual variance. If the interaction was not significant, pairwise comparisons were then used to compare treatment groups and age groups, respectively.

**RESULTS**

**Activity of enzymes and protein content in the epithelium of the large intestine**

Pigs fed on the control diet showed a significantly higher (p<0.05) activity (nkat/g tissue) of malic enzyme in week 6 (38.25±3.57) and week 7 (34.36±3.57) than in week 5 (20.7±3.57) (Figure 1). Also with the high and low fiber diets, the activity tended (p<0.10) to increase in pigs aged 6 weeks and 7 weeks as compared with that in pigs aged 5 weeks. At week 6, the activity of malic enzyme in pigs fed on the low fiber diet (23.9 nkat/g tissue) was significantly (p<0.01) lower than in pigs fed on the high fiber (31.7 nkat/g tissue) and the control diets (38.3 nkat/g tissue), respectively. Also, the activity of malic enzyme in pigs fed on the high fiber diet was lower than in pigs fed on the control diet at week 6 and week 7, respectively, although there were no significant differences in the activity of malic enzyme observed.

After weaning the activity of citrate lyase tended to decrease at week 6 whereupon it gradually increased at week 7 (Figure 2). However, there were no significant differences between either pigs of different ages or pigs fed on the different fiber diets.

There were no differences (p>0.05) in the protein concentration (mg/g wet tissue) at week 5 (43.5, 51.5 versus 52.3), week 6 (47.3, 56.5 versus 57.2) and week 7 (50.0, 57.5 versus 56.6) between diets based on low fiber, control
and high fiber. The protein content in the colonic epithelium gradually increased with age for all experimental diets.

**DISCUSSION**

Malic enzyme is now recognized to be one of the important enzymes involved in supplying NADPH for the reductive biosynthesis of fatty acids (Mourot et al., 1995). Oxaloacetate is reduced by cytosolic malate dehydrogenase and malate is formed. Malate is oxidatively decarboxylated by malic enzyme with the formation of NADPH (Wise and Ball, 1964; Bergmeyer, 1974). Thumelin et al. (1993) reported that small intestine mucosa of suckling rats had the capacity for ketone-body synthesis. Malic enzyme was recognized to play a key role in the lipogenic process and the activity of malic enzyme is also a good indicator of lipogenesis (Lee and Kauffman, 1974; Leveille, 1970). Malic enzyme activity remained high in areas where lipogenesis was high, and it decreased in adipose tissues where lipogenesis was low. The present data show that malic enzyme activities in pigs fed on the high fiber diet had a tendency to decrease as compared with that in pigs fed on the control diet. These results indicate that in the colonic epithelium of pigs given the high fiber diet a diminished rate of lipogenesis and concomitant decrease in the activity of malic enzyme took place. Similarly, earlier reports on liver metabolism by Arbeeny et al. (1992) and Gibbons (1990) indicated that dietary fiber could decrease lipogenic flux in the liver.

In contrast, Raju et al. (2001) reported that the activities of the lipogenic enzymes, especially the activity of malic enzyme, were increased in the liver and kidney of diabetic rats treated with Trigonella seeds powder which is rich in fibers.

The present study demonstrates that there were significant differences in the activity of malic enzyme between different weeks, and that the activity of malic enzyme was increased with age, irrespective of type of diet. Earlier findings reviewed by Leskanich and Noble (1999) indicated that the capacity for in vivo lipogenesis at birth was low.

The present study shows that the activity of citrate lyase was very low, indicating that there was a limited capacity to synthesize fatty acids from carbohydrate derivatives in colonocytes of piglets during the suckling-weaning transition. This situation is similar to that described by Le Dividich et al. (1994) who found that suckling newborn piglets were unable to synthesize fatty acids from carbohydrate in the adipose tissue. The general apprehension is that fatty acid synthesis occurs in the cytosol. However, acetyl-CoA is produced by pyruvate dehydrogenase in the mitochondrion. Fatty acid synthesis consists of a set of reactions, and the general principle is consuming NADPH in biosynthetic reactions (Stryer, 1995). In the high energy state the mitochondrial has large quantities of ATP and NADH. Consequently, citrate is formed in the mitochondrial matrix by the condensation of acetyl-CoA with oxaloacetate. When citrate formed in the mitochondrial matrix arrives at high level, it is transported to the cytosol, and meanwhile is cleaved by ATP-citrate lyase in the cytosol to form oxaloacetate and acetyl-CoA. The latter is the precursor for fatty acid synthesis (Salway, 1994). Several studies have shown that glucose-6-phosphate dehydrogenase and malic enzyme were the main enzymes involved in supplying NADPH for biosynthesis of fatty acids (Mourot et al., 1995; Bee, 2000). In the current study, we could not find significant differences in the activity of citrate lyase in the colon epithelium of piglets between either different ages or different diets.

Other studies (Cecilia et al., unpublished data) have shown that the activity of citrate synthase in pigs fed on the high fiber diet tended to increase as compared with that in pigs fed on the low fiber and control diets. This indicated that feeding high fiber caused a tendency of an increased capacity to utilize acetyl-CoA in pigs. A high fiber content in the diet resulted in an increased amount of short-chain fatty acids absorbed by the colonocytes. Acetate, which is the predominant short-chain fatty acids produced, can be rapidly absorbed by the colonocytes. In order to get energy, citrate synthase regulates the acetyl-CoA entry into the TCA-cycle by catalysing the condensation with oxaloacetate to citrate (Stryer, 1995).

Fatty acids are degraded by oxidation at the β-carbon in mitochondria. The hydration of enoyl CoA is the prelude to the second oxidation reaction of fatty acids, which converts the hydroxyl group at C-3 into keto groups and generates NADH. This oxidation is catalyzed by 3-hydroxyacyl CoA dehydrogenase. Villaverde et al. (unpublished data) clearly showed that the activity of 3-hydroxyacyl CoA dehydrogenase in the colon of pigs was very low. Their data suggested that long chain fatty acids were not the major substrates for the energy metabolism in the colonocytes of pigs.

In conclusion, piglets during the suckling-weaning transition had a limited capacity to synthesize fatty acids from carbohydrate in colonocytes. In addition, lipogenesis in colonocytes was enhanced with age during the suckling-
weaning transition. Moreover, the present work indicates that dietary fiber resulted in a lowered rate of lipogenesis and a reduced activity of malic enzyme. However we cannot completely explain how fiber constituents affect the enzyme activity in the colon mucosa. Further studies are required to investigate the mechanisms by which dietary fiber influences the activities of enzymes involved in fatty acid metabolism in the colon mucosa.

REFERENCES


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