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การประเมินระดับไขมันในเลือดหนูทดลองที่เลี้ยงด้วยอาหาร
ที่มีใยอาหารสูงจากธัญพืชและวุ้นน้ำมะพร้าว

(SERUM LIPID LEVEL EVALUATION IN RATS FED HIGH
DIETARY FIBER FROM CEAEAL AND NATA DE COCO)

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สถาบันค้นคว้าและพัฒนาผลิตภัณฑ์อาหาร

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INTRODUCTION

An early definition of dietary fiber described it as the remnants of plant cells remaining after hydrolysis by the enzymes of the mammalian digestive system (1). This "physiological" definition attempts to characterize fiber relative to the process of digestion within the gastrointestinal tract. This definition was understood to include both plant cell wall material, such as cellulose, hemicelluloses, pectin, and lignin, as well as intracellular polysaccharides such as gums and mucilages. A "chemical" definition has been suggested describing fiber as plant nonstarch polysaccharides plus lignin (2). In practice, both definitions encompass essentially the same heterogeneous mixture of plant components.

The major components of dietary fiber are the nonstarch polysaccharides, which include cellulose, mixed – linkage β - glucans, hemicelluloses, pectins and gums. Each of these fractions is characterized by its sugar residues and the linkages among them. Sources of dietary fiber have been classified as providing water soluble and insoluble fiber.

Water soluble fiber

1. **Pectin** The major backbone sugar for pectins is galacturonic acid, and side chains typically include galactose and arabinose. The degree of methoxylation on the uronic acid residues varies. Pectin is highly water-soluble and is almost completely metabolized by colonic bacteria (3).

2. **Gums** The structural features of gums vary according to source. Typically, these are a minor polysaccharide constituent in most foods; however, certain gums are used frequently in research studies (e.g., guar gum and locust bean gum, which are classified as galactomannans). Gums were secreted at site of plant injury by specialized secretory cells.

3. **Mucilages** Synthesized by plant secretory cells, prevent desiccation of seed endosperm (4)

Water Insoluble fiber

1. **Cellulose** Cellulose is found in all plant cell walls, and oats and barley are particularly rich sources of mixed-linkage β - glucans. Cellulose and mixed - linkage β - glucans are glucose polymers with β 1 $\rightarrow$ 4 linkages; in the mixed-linkage β - glucans these linkages are interspersed with β 1 $\rightarrow$ 3 bonds.

2. Hemicellulose The hemicelluloses are a diverse group of polysaccharides with varying degrees of branching. These can be classified according to the monosaccharide in the backbone (e.g., xylans, galactans, and mannans) and in the side chains (e.g., arabinose, galactose)

3. Lignin The noncarbohydrate constituent that is included in most definitions of fiber is lignin, which has a highly complex three – dimensional structure and contains phenylpropane units. Lignin is usually not an important component of human foods because it is generally associated with tough or woody tissue (5).

Physiological Response to Sources of Dietary Fiber

Several physiological responses, such as lowering of plasma cholesterol levels, modification of the glycemic response, improving large bowel function and lowering nutrient availability, have been associated with isolated fiber fractions or diets rich in fiber-containing foods. In mediating these responses it is clear that the physical properties of dietary fibers affect the functioning of the gastrointestinal tract and influence the rate and site of nutrient absorption. Hence, the discussion of the current understanding of these physiological responses will be done in the context of the physical properties of dietary fiber and the effects on gastrointestinal function.

Plasma cholesterol lowering

A tremendous number of studies in both humans and experimental animals have been conducted examining the ability of different types of dietary fiber to lower plasma cholesterol concentrations. From these studies certain generalities can be deduced. Most isolated fibers that are water soluble will lower plasma cholesterol in humans and plasma and liver cholesterol in animals. These include pectins, psyllium, and various gums such as guar gum, locust bean gum, and modified celluloses such as carboxymethylcellulose. Consumption of fiber-rich sources containing water soluble fibers, such as oat bran and barley (sources of mixed-linkage β -glucans), legumes, and vegetables, usually results in a lowering of plasma cholesterol. Reductions in total plasma cholesterol up to 2.5 % have been reported, but most studies find reductions in the range of 5-10 %. Almost invariably the reductions occur in the low-density lipoprotein fraction, with little or no change in high-density lipoprotein cholesterol. In contrast, isolated fibers of fiber sources that are not water

soluble have rarely been found to alter plasma cholesterol. These fibers include cellulose, lignin, corn bran, and wheat bran.

How cholesterol-lowering dietary fibers mediate their action remains a subject of controversy. One hypothesis is that a fiber-induced increase in bile acid excretion leads to an increased demand for bile acid synthesis, resulting in an increased rate of conversion of cholesterol to bile acids (6). If cholesterol synthesis rates do not increase sufficiently to compensate for the loss of cholesterol to bile acids, then cholesterol concentrations will decrease. However, not all fibers that lower cholesterol increase bile acid excretion (7). A corollary to this hypothesis is that cholesterol-lowering fibers alter the bile acid profile by differential binding to bile acids, which could lead to decreases in absorption or synthesis of cholesterol (8). Changes in the profile of the bile acid pool with feeding of cholesterol-lowering fibers have been noted in several studies (9, 10). Future studies with additional fiber types will be necessary to establish the importance of this correlation. Many water-soluble fibers form a viscous matrix within the small intestine (11), which could interfere with cholesterol or bile acid absorption in the small intestine. Guar gum has been found to delay cholesterol disappearance from the small intestine in one study (12), but to have no effect on absorption in another (13). Likewise, pectin has been found to reduce cholesterol absorption in one study (14) but not in another (15). Using a highly viscous but nonfermentable modified cellulose, hydroxypropyl methylcellulose, it has been shown that cholesterol absorption decreases linearly with the logarithm of intestinal contents viscosity (16). Another hypothesis is that sources of fiber will modify cholesterol synthesis. Cholesterol synthesis as measured by ^{14}C -acetate incorporation into cholesterol (17) or hepatic 3-OH-3-methyl glutaryl coenzyme A activity (18) is elevated in rats fed pectin, a hypocholesterolemic fiber source (19). This elevation is undoubtedly due to reduced cholesterol absorption or enhanced bile acid excretion in pectin-fed rats. Studies with isolated hepatocytes have demonstrated that propionate, which can be produced by fermentation of soluble fiber, inhibits fatty acid synthesis and ^{14}C -acetate incorporation into cholesterol but does not inhibit total cholesterol synthesis (20). A preliminary report indicates that in humans in vivo lipogenesis may be suppressed in subjects fed a high-carbohydrate diet that is rich in complex carbohydrates, including starch and dietary fiber (21). These results suggest that the effect of fermentable fibers on hepatic fatty acid synthesis and secretion should be investigated further, especially since hepatic - derived

triglyceride - rich lipoproteins are the precursors of the low-density lipoprotein fraction. Overall, the evidence suggests that more than one mechanism contributes to the cholesterol-lowering effect of dietary fiber. The physical properties of fiber that seem most likely to be responsible are bile acid binding (or entrapment) and viscosity.

Modification of the glycemic response. Numerous studies have demonstrated that consumption of certain water-soluble fibers will reduce the postprandial glycemic and insulinemic responses (22). This effect occurs when the fiber is coadministered with a glucose load or as part of a meal, in both normal and diabetic individuals. The effect of fiber on the rate of gastric emptying has been associated with its ability to blunt the glycemic response to a glucose load and to slow nutrient absorption. Long-term blood glucose control, as measured by glycated hemoglobin, is also improved with guar feeding in both humans (23) and animals (24). Further, guar gum has been shown to reduce the renal enlargement associated with the onset of diabetes in animals (24). The renal enlargement of diabetes is highly correlated with blood glucose concentrations (25).

The postprandial glucose curve flattening ability of various fiber supplements is highly correlated with their viscosity (26, 27). Possible explanations for this effect include a delayed rate of stomach emptying and delayed starch digestion within or a slowing of glucose absorption from the small intestine. These mechanisms are obviously not mutually exclusive. Viscous polysaccharides but not insoluble fiber sources such as cellulose have been reported to delay gastric emptying (28, 29). Comparing oral administration of a guar-containing meal to one that was administered directly into the intestine, Leclerc et al. (30) concluded that a slowing of gastric emptying was the main factor in flattening the postprandial glucose curve. Their study also suggested that guar gum slowed the rate of starch digestion, but had no effect on glucose diffusion. With a steady-state intestinal perfusion technique, guar gum has been shown to slow the rate of glucose uptake in humans (31). Thus, evidence exists in favor of all the mechanisms described, and the predominant action will depend on numerous factors, such as the type and source of fiber used, its rate of hydration, and its ultimate viscosity.

Improving large bowel function. The presence of fiber in the diet can influence large bowel function by reducing transit time, increasing stool weight and frequency, diluting large intestinal contents, and providing fermentable substrate for microflora normally present

in the large intestine. All of these factors are influenced by the source of fiber in the diet as well as other dietary and nondietary factors. Transit time has been shown to decrease because of wheat bran supplementation in 14 studies and decrease because of the addition of fruits and vegetables to the diet. In two studies, cellulose has been reported to decrease transit time and in two other studies to have no effect. Pectin, based on three studies, does not affect transit time (32). Transit time is related to stool weight, but not in a simple linear manner. By examining population data from healthy subjects, Spiller (33) reported that a low stool weight is associated with delayed transit time; as stool weight increases transit time tends to decrease. However, once a transit time of 20-30 hours is achieved, further increases in stool weight do not shorten transit time significantly.

Stool weight can be increased by sources of fiber in a dose-related manner (34). The nonstarch polysaccharides and resistant starch are the primary dietary components that increase fecal bulk. Cummings (35) summarized a number of studies by estimating the increase in fecal weight relative to the weight of fiber fed. Fiber sources that contain insoluble fiber components, such as wheat bran, tend to produce the greatest increase in stool weight. Fruits and vegetables and gums and mucilages also produce a moderate increase in fecal output, whereas legumes and pectin increase stool weight only slightly. An increase in stool weight is typically associated with an increase in the microbial cell mass, in the undigested fecal residue, or the noncellular matrix in the feces. Hence, the fecal bulking ability of a fiber source is related to a change in one or all of these phases. For example, wheat bran is more effective in increasing the amount of undigested residue, whereas the fiber in fruits and vegetables and the soluble polysaccharides can be fermented extensively and are more likely to increase the microbial cell mass of the feces. Differences in particle size of the fiber source have been studied for wheat bran; reducing the particle size reduces fecal weight (36, 37).

The effects of fiber on stool weight and transit time, although inherently variable, are physiological responses important for maintaining large bowel function. A consensus of opinion exists that dietary fiber has an important role in large bowel function (38). Other metabolic consequences of fiber in the large intestine are more poorly understood and, thus, are more difficult to define in terms of their physiological significance. For example, during the fermentation of the polysaccharides associated with dietary fiber, the microflora produce short-chain fatty acids, primarily acetate, propionate, and butyrate. Butyrate can be used by large intestinal cells as an energy source. In vitro, butyrate causes transformed

cells to undergo differentiation (39). Yet, in vivo, all short - chain fatty acids cause large intestinal hypertrophy (40). Feeding of purified, highly fermentable fibers has been found to stimulate large intestinal cell growth (41) and to promote tumor formation in carcinogen-treated animals in some studies, but not in others. In contrast, other investigators have speculated that production of certain short-chain fatty acids may protect against colon cancer (42). The production of short-chain fatty acids in the large intestine is obviously an important consequence of consuming fiber sources that are fermentable; however, the metabolic consequences of short-chain fatty acid production remain poorly understood.

Lowering nutrient availability. Within the small intestine the digestible components of the diet are broken down by hydrolysis and nutrients are absorbed through the mucosal cells. In vitro data indicate that various fiber sources can inhibit the activity of pancreatic enzymes that digest carbohydrates, lipids, and proteins (43). The mechanisms for inhibiting digestive enzyme activity are not clearly established, but in some nonpurified fiber sources, specific enzyme inhibitors exist (44). It is difficult to assess the physiological importance of this inhibition because an excess of digestive enzyme activity is secreted in response to a meal. However, several lines of evidence indicate that specific fibers may reduce the availability of the enzyme for hydrolyzing triglycerides, starch, and proteins within the intestinal contents. Gallaher and Schneeman (45) reported that a diet high in cellulose (20 % by weight) delayed the disappearance of labeled triolein but not labeled cholesterol from the small intestine. The results indicated that the high cellulose content of the diet interfered with triolein breakdown but not with overall lipid absorption. Lairon et al (46, 47) reported that an inhibitor of pancreatic lipase is present in wheat bran and wheat germ. A blunting of the blood plasma increase in triglycerides during the alimentary period has been associated with fiber sources that contain lipase inhibitor (48). The characteristics of this inhibitor suggest that it may be active in the small intestine and capable of slowing the digestion of triglycerides. Legumes have been reported to contain amylase inhibitors that could slow the hydrolysis of starch in the small intestine (44). Inhibition of amylase in human pancreatic or duodenal fluid by wheat bran, xylan, cellulose, guar gum, and psyllium has been reported (43). Many cereals and legumes contain pancreatic protease inhibitors that can decrease protein digestibility. These inhibitors are often inactivated by heat treatment; however, some inhibitor activity can potentially survive normal processing

conditions and remain active in the gut. In patients with pancreatic insufficiency the amylase, trypsin, chymotrypsin, and lipase activity available from pancreatic replacement treatment was significantly reduced when the patients were given a meal containing pectin or wheat bran, which suggests that it is possible for these fiber sources to significantly reduce digestive enzyme capacity in the small intestine (49). In addition to direct inhibition of digestive enzyme activity, the presence of plant cell wall matrix in a food provides a physical barrier to digestion (50-52). An intact cell wall will slow the penetration of digestive enzymes into plant foods. Consequently, grinding of the fiber source to a very fine particle size may disrupt the cell wall structure sufficiently to make digestible nutrients more available for hydrolysis.

Studies on the effect of dietary fiber on vitamin absorption have been conducted for most vitamins. Although differences in the type and amount of fibers fed and the methods for determining uptake make comparisons across studies difficult, it appears that generally fiber has little if any effect on vitamin absorption (53). The effect of fiber on mineral absorption is somewhat less clear. Natural sources of fiber, such as cereals and fruits, generally have a depressing effect on absorption of minerals such as calcium, iron, zinc, and copper (54). However, at least part of this effect is likely to be due to the presence of phytic acid in these foods, which is known to interfere with mineral absorption (55). When isolated fiber sources are examined, such as cellulose, pectin, and gums, the large majority of studies find no detrimental effect on mineral balance or absorption (54). The physical characteristics of the intestinal contents will be changed by the physical properties of the fiber sources in the diet. The bulk or amount of material in the small intestine will increase because the fiber is not digestible and hence remains during the transit of digesta through the small intestine (56). The volume of the intestinal contents can increase because of the water-holding capacity of the fiber source. Sandberg et al. (57, 58) reported that addition of wheat bran or pectin to a low-fiber meal increased the volume of ileostomy fluid by about 20-30 %. In addition, animal data indicate that a greater dry and wet weight of intestinal contents is associated with the addition of a fiber supplement to experimental diets (56). The presence of certain viscous polysaccharides in the fiber source will increase the viscosity of the contents and in particular of the aqueous phase of the intestinal contents from which nutrients are absorbed (59-61). Greater viscosity of the aqueous phase of the intestinal contents will cause an apparent thickening of the unstirred layer at the epithelial surface, which is the theoretical barrier to lipid absorption in the

intestine (62). An increase in the bulk, volume, or viscosity of the intestinal contents is likely to slow diffusion of enzymes, substrates, and nutrients to the absorptive surface, all of which can lead to a slower appearance of nutrients in the plasma following a meal.

The bile acid and phospholipid binding capacities of various fibers are likely to affect micelle formation in the small intestine and consequently the rate and site of lipid absorption. Vahouny and coworkers (63, 64) have demonstrated that addition of fiber supplements to rat diets can slow the appearance of fatty acids and cholesterol in the lymph. The ability of the soluble forms of fiber to slow fatty acid absorption and to interfere with cholesterol absorption undoubtedly contributes to the effect of these fiber sources on plasma lipid levels. Studies in humans have indicated that diets supplemented with oat bran, pectin, or guar gum, but not wheat bran or cellulose, lower plasma cholesterol levels by 5-18 % (32).

The experimental evidence suggests that, through a variety of mechanisms, certain fiber sources, especially those containing viscous polysaccharides, can slow the process of digestion and absorption, although total nutrient absorption is not necessarily reduced. Because of its effect on the rate of absorption, a greater proportion of nutrients from a diet high in fiber will undoubtedly be absorbed from the lower half of the small intestine. This pattern of nutrient absorption is likely to contribute to the physiological responses to various fiber sources. For example, the rate of nutrient absorption will affect the pattern of hormone release in response to diet (65) and the rate of nutrient delivery to the tissues. Evidence also exists that the presence of nutrients in the ileum can influence satiety and food intake, gastric emptying, and the composition and size of chylomicrons (66, 67). The presence of fiber in the gut has an important function in maintaining the gastrointestinal system by regulating the rate and site of nutrient absorption. Sigleo et al. (68) have demonstrated that chronic feeding of fiber supplements will alter the morphology of the small intestine. Both the distribution of nutrient absorption in the small intestine and the influence of bulk in the small intestine on intestinal cell renewal are likely to contribute to this response (69).

The purpose of this study is to recruit both of plant raw material agricultural products and after processing which is source of dietary fiber and plenty in Thailand such as cereal and legume; sweet corn, unpolished rice, mung bean and nata de coco for

producing in form of health food then evaluates the serum lipid lowering effect compare with health food from Bangkok market in the experimental rats.

MATERIALS AND METHODS

Animals

Three-week-old male Sprague-Dawley rats, with a mean initial weight of 50 – 60 g mean body weight within group not different than 10 g and between group not more than 5 gram, were housed individually in metabolic cages and maintained in a controlled environment at 20 – 22°C, 60 % relative humidity and with a 12-h light – dark cycle. Animals were assigned by selection randomization to five groups, ten animals per group with dietary fiber test diets and water freely provided for the 28-d feeding period. Food consumption was measured daily, animals and feces were weighed weekly.

Diets : Preparation of dietary fiber health food

To recruit agricultural plant product of Thailand which was the source of dietary fiber such as unpolished rice, mung bean, sweet corn and nata de coco. Those raw materials were weighed, boiled and mixed together. Water was added into the mixture, blended with electric mixer until homogenous. Food was dried by Drum-dryer at temperature 135°C of roller with speed 50 sec/round, clearance 0.15 mm. Make to powder by Pin Mill.

Apple pectin were bought from supermarket, cellulose and casein were bought from company. The composition of five raw material health foods were shown in table 1.

Table 1 Composition of raw materials health foods (sample) before preparing experimental diet

Formula (Sample)	Unpolished rice (%)	Mung bean (%)	Sweet corn (%)	Nata de coco (%)	Powder milk (%)	Sucrose (%)
1	3.3	9.9	19.8	22	25	20
2	6	18	36	40	-	-
3	Apple pectin					
4	Cellulose					
5	Casein					