碩士學位論文

알팔파, 김 또는 솔잎 粉末을 含有한 飼料의 給與가 쥐의 性別 血中 Cholesterol 含量 및 腸內 Urease Activity에 미치는 影響

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Effects of Sex and Feeding Diets Containing Alfalfa, Laver or Pine-needle meal on the Plasma Cholesterol Level and Cecal Urease Activity in Rats

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摘 要

이 硏究는 알팔파, 김 또는 솔잎 粉末을 含有한 飼料의 給與가 쥐의 性別 Plasma Cholesterol 含量 및 Cecal Urease Activity 에 미치는 影響을 究明하기 위하여 實施되었다. 24마리의 암쥐와 24마리의 숫쥐(平均體重 92 g)를 2×4 要因實驗으로 配置하고 對照區 또는 實驗飼料를 4주동안 給與하였고 모든 實驗飼料에는 0.5% Cholesterol 를 添加하였으며 알팔파, 김 또는 솔잎분말을 Corn Starch에 代置하여 각각 10% 씩 添加하였다. 4주의 飼養實驗이 끝난후 쥐들을 屠殺하여 血液 과 腸 內容物을 採取하여 分析하였다.

ADFI 와 ADG 는 김을 급여한 쥐가 알팔파나 솔잎을 급여한 쥐에서보다 높았으며(P<.05) 또한 숫쥐가 암쥐에 비해 현저히 增加하였고(P<.001) F/G는 낮았다(P<.01). 總 盲腸의 무게 및 盲腸 內容物의 무게는 김이나 솔잎을 給與한 쥐가 對照區나 알팔파를 給與한 쥐보다 增加하였다(P<.001). 김을 給與한 쥐가 對照區에 비해 血中 總 Cholesterol 含量이 떨어졌으며(P<.01) 솔잎을 給與한 쥐는 다른처리구에 비해 血中 總 Cholesterol 含量을 2배 이상 增加시켰다(P<.001). 또한 암쥐가 숫쥐에 비해 2 ~ 3배 정도 總 Cholesterol 含量을 높였다(P<.001). 알팔파를 給與한 쥐가 김이나 솔잎을 給與한 쥐보다 血中 HDL Cholesterol 含量을 增加시켰으며(P<.05) 숫쥐가 암쥐보다 HDL Cholesterol 含量이 높았다(P<.001). 血中 Triacylglycerol 含量은 處理나 性間에 큰 차이가 없었으나 솔잎을 給與한 암쥐에서 현저히 增加하였다(P<.001). 盲腸 內容物中의 그램 당 Urease Activity 는 김이나 솔잎을 給與한 쥐에서 현저

히 떨어졌으나(P<.001) 總 盲腸內容物 中의 Urease Ativity 는 處理間에 有意的인 차이를 보이지 않았다. 그러나 숫쥐가 암쥐에 비해 總 盲腸內容物 중의 Urease Activity 가 높았다(P<.01). 血中 Urea 濃度는 암쥐가 숫쥐에 비해 현저히 增加하였다(P<.001).

本 實驗의 結果를 綜合해보면 김이나 솔잎은 가용성 섬유소가 많이 含有되어 발효과정에서 盲腸의 크기를 增加시킴을 알 수 있고 김은 혈중 Cholesterol 함량을 떨어뜨리나 솔잎은 장내에서 Cholesterol 吸收를 촉진시키거나 體內 Cholesterol 代謝에 큰 影響을 미친다는 것을 알수있다. 암쥐가 숫쥐보다 血中 Cholesterol 含量이 높은 것은 암쥐가 숫쥐보다 體重 增体當 Cholesterol를 많이 攝取했고 또한 飼料中 다량의 Cholesterol 을 添加할때 암쥐가 민감하게 反應함을 보여준다. 따라서 飼料中 特性이 다른 纖維素들이 Cholesterol 이나 Urease Activity에 미치는 影響은 복잡하며 보다 精密하고 다양한 硏究가 必要하다고 思料된다.

I. Introduction

A variety of health-promoting food sources have been used in Korea as well as other countries without knowing well defined effects. These sources include alfalfa, laver and pine-needle, which are used in a form of food additives, pills or drinks, and contain significant amounts of different types of fibers. Dietary fibers, especially soluble fibers are fermented, producing lactic acid and volatile fatty acids (VFA) in the large intestine of nonruminant animals including humans. Dietary cellulose increases bulk and the amount of water in intestinal contents, decreasing transit time and thus the concentration of toxic substances in contact with the intestinal mucosa (Kelsay, 1978). Most of the soluble fibers containing viscous materials, such as guar gum, pectin and psyllium, are known to effectively reduce serum cholesterol levels (Gallaher *et al.*, 1993; Lund *et al.*, 1993; De Deckere *et al.*, 1993). Different types of soluble fibers elicit different hypocholesterolemic responses when fed with diet containing cholesterol, possibly because of the specific action of each fiber type in the intestine (Fernandez *et al.*, 1995).

In addition to an action as a fiber source, pine-needle is known to inhibit estradiol-17β receptor binding (Wagener and

Jackson, 1983). Estrogen concentrations in systemic blood increased progressively after the initiation of pine-needle feeding in cows and the normal rise in estrogen associated with parturition was initiated early by feeding pine-needle (Short *et al.*, 1989).

Sex and sex hormones appear to be major determinants of cardiovascular disease. Women have less cardiovascular disease than men, especially in the premenopausal period. In general population men have higher triglyceride and VLDL and lower HDL concentrations than women (Heiss *et al.*, 1980). Estrogen administration has been reported to decrease triglyceride and low-density lipoprotein cholesterol(LDL) and increase high-density lipoprotein cholesterol (HDL) levels (Matthews *et al.*, 1989). Fernandez *et al.* (1995) showed that female guinea pigs were more susceptible to a dietary cholesterol than males and, while dietary fiber lowered plasma and hepatic cholesterol concentrations, the responses in females were moderate and more scattered than in males. Similarly, a more significant effect of dietary fiber in lowering plasma total and LDL cholesterol levels in men compared to women (Jenkins *et al.*, 1993). These sex effects on cholesterol metabolism has never been reported in rats.

One of the benefits of increasing soluble fibers in diet is to reduce urease activity in the intestine (Wrong *et al.*, 1981). The enzyme urease is produced by certain intestinal bacteria and

serves an essential functions in the process of urea recycling by catalyzing the conversion of urea to ammonia. Ammonia produced by microbial urease may be used for microbial protein synthesis or be absorbed into the blood stream, but is known to be toxic to animals (Visek, 1978). Pigs fed a high fiber diet have lower concentrations of ammonia-N in fecal, cecal and colonic samples (Varel, 1983), and rats fed a high cellulose diet had a lower urease activity (Lee, 1992), compared to the control. Our study was conducted to determine the effect of sex and feeding diets containing alfalfa, laver or pine-needle meal on plasma cholesterol level and cecal urease activity in male and female rats.

II. Literature Review

1. Effects of dietary fibers on animal's digestive function

Numerous studies have shown that nonruminants can digest fibers. Cellulose, one of the major components of crude fiber, is an important dietary factor, although it cannot be termed an essential nutrient. Fiber alters the digestion, absorption or subsequent metabolism of various nutrients (Cummings, 1978; Kelsay, 1978). The amount of cellulose in diet affects not only its digestibility, but also that of other dietary constituents. Gargallo and Zimmerman (1980) reported that the digestibility of dry matter and cellulose varied inversely with the level of fiber in a diet. Pollmann *et al.* (1979) found that the digestibility of energy and nitrogen decreased as the fiber content increased. This reduction may be explained in part by the rate of passage of material through the digestive tract. Fioramonti and Bueno (1980) observed that the rate of passage increased with the fiber content of the diet, possibly the result of increased gut

motility, in particular that of the large intestine. The shorter retention time will reduce the time of contents exposed to digestive enzymes.

The source and level of fiber must also be considered when the effects of fiber on rate of passage are being evaluated (Stanogias and Pearce, 1985). Soybean hulls and wheat bran did not affect the rate of passage when fed at levels from 7% to 15%, but feed transit time increased when fed these fiber sources at levels between 22% and 30%. With less degradable fibrous materials such as ground corn cobs and alfalfa stems, digesta passage increased as a level of increased. Coarse fiber particles may be retained in the cecum longer than more finely ground particles of the same fiber source (Ehle *et al.*, 1982).

In general, hypocholesterolemic properties are ascribed to water-soluble fibers, but there is no consensus on how soluble fibers affect sterol metabolism. It has been proposed that the presence of soluble fibers in the gastrointestinal tract increases viscosity and interferes with micelle formation and lipid absorption (Gallaher *et al.*, 1993). Short-chain fatty acids (acetate, propionate and butyrate) are produced in large quantities from the fermentation of dietary fibers in the colon. Propionate has been shown to inhibit endogenous cholesterol

synthesis (Wright *et al.*, 1990). In contrast, Stark and Madar (1993) found that short-chain fatty acids are not the cholesterol-lowering factor of highly fermentable fiber sources.

Despite the widespread use of dietary fibers, unanswered questions concerning their fate and mechanisms of action in the body still exist. This is in part due to the large number of organic substances included in the definition of dietary fiber and the wide range of physical and chemical properties these materials possess.

2. Effects of fiber on cholesterol metabolism

Cholesterol, a precursor of steroid hormones and bile acids, is synthesized from acetyl Co-A in mitochondria of cells. The amount of its synthesis is reduced when dietary cholesterol intake is increased (Grundy *et al.*, 1969). This mechanism allows body to maintain a relatively constant cholesterol level. A feedback system exists between synthesis and absorption of cholesterol in vivo. However, when absorbed cholesterol exceeds body's need, there is its accumulation

which may be a causing factor of cardiovascular disease including atherosclerosis.

Lipoprotein is classified into chylomicron, very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). The first three (chylomicron, VLDL, LDL) are generally called β-lipoprotein or simply low density lipoprotein (LDL) in total, while high density lipoprotein (HDL) is called α-lipoprotein. The LDL fraction appears to be positively related to coronary heart disease, while the HDL fraction appears to protect against it. Epidemiological, clinical and experimental studies have shown that the HDL cholesterol level is more closely related to lower incidence of atherosclerosis than is total cholesterol, high levels of which increase the incidence of atherosclerosis.

Dietary cholesterol is absorbed through portal vein, and absorbed cholesterol is transported into peripheral tissues (Vahouny *et al.*, 1988). Liver cells synthesize cholesterol from acetyl-CoA and the synthesized cholesterol associated with low density lipoprotein (LDL) is transported to whole body tissue. LDL receptors located in the surface of cells receive LDL, which is degraded into individual components, cholesterol, triacylglycerol, phospholipid and protein inside cells (Goldstein and Broem, 1977). High density lipoprotein (HDL) transports

cholesterol from peripheral tissues to the liver. In the liver, cholesterol transforms into bile salts, which is excreted into the intestine. Part of excreted bile salts is reabsorbed into the liver, and recycled (McCarthy, 1993).

Certain dietary water-soluble fibers such as guar gum, pectin and oat bran have lipid-lowering effects in experimental animals (Chen *et al.*, 1984). Such fibers are viscous and fermentable in the large intestine. Both increased viscosity within the small intestine and increased large bowel fermentation have been implicated in the cholesterol-lowering effect of dietary fiber (Gallaher *et al.*, 1993). Increased fermentation has been hypothesized to lower plasma cholesterol via an inhibition of hepatic cholesterol synthesis, mediated by increased production and absorption of the short chain fatty acid propionate, a fermentation product (Chen *et al.*, 1984). But Stark and Madar (1993) reported that despite significantly higher rates of short chain fatty acid (SCFA) production in pectin-fed rats, cholesterol synthesis was not inhibited, suggesting that SCFA are not the cholesterol-lowering factor of highly fermentable fiber sources.

Vigne *et al.* (1987) reported that low-methoxyl pectin and wheat bran both beneficially modified the serum triglyceride and cholesterol levels. However, the magnitude of the effect of each individual type of fiber was dependent on the fat and cholesterol content of the diet, suggesting the existence of

different mechanisms of action. An increase in intestinal contents viscosity could lower plasma cholesterol concentrations by reducing diffusion of micelles within the intestinal lumen, thereby interfering with absorption of cholesterol or bile acids or both (Judd and Truswell, 1985). Alternatively, the reduced pH in the large intestine that results from increased fermentation may reduce the solubility of bile acids, leading to an increase in their excretion. Plasma cholesterol may subsequently be reduced in order to maintain the bile acid pool.

3. Effects of sex hormones on cholesterol and lipid metabolism.

Sex hormones appear to be major determinants of cardiovascular disease. Women have less cardiovascular disease than men, especially in the premenopausal period. In general population men have higher triglyceride and VLDL and lower HDL concentrations than women (Heiss *et al.*, 1980)

Estrogen administration has been reported to decrease triglyceride and low-density lipoprotein cholesterol (LDL) and increase high-density lipoprotein cholesterol (HDL) levels (Matthews *et al.*, 1989). Estrogen use increases the secretion of

large VLDL particles and also stimulates the take-up of VLDL remnants by the liver (Walsh *et al.*, 1991). In contrast to women, increased androgen concentrations in men do not seem to be associated with increased cardiovascular risk factors, although testosterone concentrations are associated with increased HDL and decreased insulin concentrations (Steven *et al.*, 1995) In animals, the effects of testosterone on insulin sensitivity differ between males and females. Female rats treated with testosterone showed decreased insulin sensitivity and a decreased proportion of red muscle fibers (Holmang *et al*, 1990).

Sex hormones may play a role in LDL composition. Since VLDL is the precursor of LDL, it is possible that differing concentrations or distributions of VLDL may influence the resulting LDL particle size. Sex hormones might influence this conversion of VLDL to LDL, perhaps by affecting hepatic lipase (Kinnunen *et al.*, 1983).

Several enzymes involved in HDL and triglyceride metabolism may be affected by sex hormones. Lipoprotein lipase is higher in women than in men, while hepatic triglyceride lipase is higher in men than in women (Kruis *et al.*, 1974). Tikkanen *et al.* (1982) suggested that the influence of sex steroids on HDL levels is mainly mediated through changes in hepatic triglyceride lipase. In contrast, administration of intramuscular testosterone does not alter the

concentration of hepatic triglyceride lipase (Thompson *et al.*, 1989). Administration of testosterone leads to an increase in hepatic triglyceride lipase and decreases in HDL (Friedl *et al.*, 1990). Sex hormones may also have effects on lipolysis. With age, testosterone levels decline in men and visceral fat increases (Seidell *et al.*, 1990). Older males have increased lipolytic activity in subcutaneous abdominal fat, which is metabolically similar to intraabdominal fat tissue (Rebuffe-Scrive *et al.*, 1987).

4. Effects of fiber on urea and ammonia metabolism in the GI tract

Urea is formed in the liver from dietary and endogenous amino acids, and disposed of either by excretion into the urine or by hydrolysis in the GI tract. Ammonia produced by nitrogen metabolism is normally converted to urea in the mammalian liver, 20 ~ 25% of which is excreted into the GI tract and hydrolyzed to ammonia by microbial urease (Wrong, 1981). High concentrations of ammonia in the intestinal tract can be toxic and increase turnover of intestinal epithelial cells. With the increased turnover, men and animals

are forced to expend greater energy for maintenance of the intestinal tract and thus less energy is available for growth (Visek, 1978; Lin and Visek, 1991). Nitrogen derived from urea hydrolysis in the bowel may either be used for microbial protein synthesis or re-enter the blood stream (Jackson *et al.*, 1990) The large intestine is generally regarded as the main site of urea degradation in nonruminants. The low concentrations of urea in the large bowel are usually attributed to the presence of bacterial urease, which are produced by many microbial species, both aerobes and anaerobes, particularly nonsporing anaerobes, *Peptostreptococcus* and *Proteus* species (Donaldson, 1964; Wozny *et al.*, 1977; Suzuki *et al.*, 1979). Varel *et al.* (1984) reported that concentrations of ammonia from fecal, cecal and colonic samples were all significantly lower in pigs fed the high fiber diet. This, along with the higher acetate to propionate ratio, indicates that high fiber diets modify bacterial metabolism in the large intestine. The lower ammonia concentration suggests that less protein catabolism is taking place or more of the ammonia is used by the intestinal bacteria. It is well established that indigestible polysaccharides cause a bacterial proliferation in the large intestine that is linked with an enlargement of the cecum (Levrat *et al.*, 1991). The increase in cecal weight is fueled by short chain fatty acids (SCFA), especially butyrate, which are the end products of fermentation of

polysaccharides by the flora. Bacterial growth requires a source of nitrogen. This is provided to some extent by dietary protein that escapes digestion and by endogenous proteins such as pancreatic and intestinal secretions and sloughed epithelial cells (MacFarlane and Cummings., 1991). Also, a key supply of nitrogen for bacterial proliferation comes from blood urea.

When the supply of polysaccharides reaching the cecum is substantial, the hypertrophy of the cecum results in a much larger blood flow to the cecum. This allows for the diffusion of urea from the blood to the cecal lumen. (Demigne and Remesy., 1985). Due to the presence of highly ureolytic bacteria in the cecum, the urea concentration gradient favors a net transfer of urea into the cecal lumen. The ammonia generated by bacterial urease is used for bacterial protein synthesis, thus trapping nitrogen for elimination into the feces.

Both dietary fibers and oligosaccharides effectively enhance fecal nitrogen excretion and depress renal nitrogen excretion (Younes *et al.*, 1995). Small amounts of amino acids such as glutamine, glutamate, glycine and taurine are transferred from blood to lumen (Levrat *et al.*, 1993). However, it is blood urea that constitutes the largest and the most readily available sources of nitrogen for bacterial protein synthesis in the cecum (Levrat *et al.*, 1993). When urea is transferred to the cecal lumen, it is broken down to ammonia by bacterial urease and

then used for microbial protein synthesis. These microbial proteins are finally excreted in the feces, where they represent the major part of nitrogen (Meinl and Kreienbring 1985, Mortensen 1992, Viallard 1984).

III. Materials and Methods

1. Animals and diets

A study was conducted to determine the effect of sex and four different diets (control, 10% alfalfa, 10% laver and 10% pine-needle meal diets) in a 2 x 4 factorial design. Twenty-four male and twenty-four female Sprague Dawley rats (mean initial weight, 92 g) were housed individually in suspended wire cages in a room maintained at 20-25℃ with a 12-h light (07:00 to 19:00) and 12-h dark (19:00 to 07:00) cycle. Rats were divided into eight groups (2 x 4), each consisting of six male or female rats. The composition of the experimental diets is shown in Table 1. Diets and water were provided for ad libitum consumption.

After a 5-day adjustment period, rats were fed experimental diets for four weeks. Feed consumption and body weight were recorded every 2 d during a 28-d feeding period.

2. Sample collection and incubation of cecal contents

At the end of the feeding experiment, rats were fasted for 12 hours and eight rats from different treatments were killed daily between day 10:00 and 12:00 until all of the experimental rats were used. Blood samples were centrifuged at 3,000 × g for 15 min and the serum stored at -20℃ for later analysis. Cecal contents were collected in 50-ml centrifuge tubes, weighed and diluted 1:4 (w/v) with 0.2 M phosphate buffer (pH 6.8). The cecal tissue was flushed clean with tap water, blotted on paper towel and weighed (cecal wall weight). Duplicate 1-ml samples of diluted contents were transferred into 15-ml centrifuge tubes and 0.34 ml of 0.44 M urea solution was added to one sample, and 0.34 ml distilled water was added to the other sample and the mixture was inactivated with 0.134 ml of 6 N H_2SO_4 before incubation and served as blank. The former was incubated at 37℃ in a shaking water bath while being flushed with N_2 for the first 1 min and then each unit was clamped sealed. The incubation was initiated within about 10 min after rats were killed.

At the end of the 30-min incubation, an air stream was pulled through the reaction chamber, and 0.134 ml of 6 N H_2SO_4 was added to the inlet tube of the reaction chamber to stop the reaction. The inactivated samples were centrifuged

at 3000 × g for 15 min and supernatants were collected into plastic vials and immediately stored at -20℃ for later analysis.

3. Determination of ammonia production and urease activity

Ammonia concentrations in supernatants stored at -20℃ were determined by using a colorimetric method (Chaney and Marbach, 1962). Net ammonia production during the 30-min incubation was calculated by the difference in ammonia concentration between the blank and incubated sample. Urease activity(μmol of urea hydrolyzed/30 min per g) was calculated by dividing net ammonia production by two.

The urea concentration of plasma was determined by using a colorimetric method (Chaney and Marbach, 1962). Two-hundred μl of urease solution (urease 0.09 g and EDTA 0.04 g/L) and 20 μl of plasma sample were mixed and incubated at 37℃ for 15 min. Ammonia concentration (μmol) determined using the same method used for cecal ammonia concentration and urea concentration (μmol) was calculated by dividing ammonia concentration by two.

4. Analysis of cholesterol and triglycerol in the plasma

Total cholesterol and triglycerol were determined by using respective commercial assay kit (International Reagent Corp., Tokyo, Japan). Ten and 20 μl of plasma were used for determining total cholesterol and triglycerol, respectively. High-density lipoprotein (HDL) cholesterol (in 50 μl of plasma) was also determined by using commercial assay kits (WAKO Pure Chemical Ind., Osaka, Japan). Low-density lipoprotein (LDL) + very low-density lipoprotein (VLDL) cholesterol was calculated by subtracting HDL cholesterol from total cholesterol.

5. Statistical analysis of experimental data

Statistical analysis were carried out by using SAS package (SAS, 1988). Analyses of variance (ANOVA) were calculated in a completely randomized block design (Table 2). Duncan's multiple range test was applied to compare individual means when F-value was significant ($P < 0.05$).

IV. Results and Discussion

1. Effects of diets and sex on weight gain, feed intake and cecal size

Final weights were heavier (P<.001) in male than in female rats, although their weights at beginning were similar. Rats fed the control or diets containing laver meal had higher (P<.01) average daily feed intake and average daily gain, and lower F/G (feed/gain ratio) than rats fed diets containing alfalfa and pine-needle meal (Table 3).

It is not surprising that alfalfa and pine-needle meals in the diet depressed growth because they contained 29.4% and 43.5% ADF as shown in Table 1, respectively. Reduced feed intake in rats fed diets supplemented with alfalfa and pine-needle meals indicates that these components reduced palatability of diet. Over the feeding period, male rats showed higher (P<.01) average daily gain and average feed intake, and lower (P<.01) F/G than females, because the mature size of females is smaller than males, and the feeding experiment was extended over the maturity.

Table 4 shows the effects of diets and sex on total cecal weight, cecal tissue weight and weight of cecal contents.

Significant ($P<0.01$) differences were noted in total cecal weight and cecal contents between sexes also between diets showing laver and pine-needle meal increased the weight of cecum and its contents compared to the control or alfalfa meal diet. Heavier body weight of male rats was considered to be a major factor for the larger cecum size.

Younes *et al.* (1995) reported that when fibers and oligosaccharides were added to semipurified diets at 7.5 g/100 g in place of wheat starch, oat fiber did not cause an enlargement of the cecum. In contrast, gum arabic and oligosaccharides elicited a 35 - 60% enlargement of the cecum. These results indicate that fermentable fibers enlarge the cecum because fermentation products, volatile fatty acids, (especially butyrate) stimulate cell proliferation.

In the present study, rats fed diets containing laver or pine-needle had enlarged cecum and more contents, indicating that fibers in these are more fermentable than that in alfalfa meal.

2. Effects of diets and sex on plasma cholesterol and triacylglycerol of rats.

Plasma total cholesterol, HDL cholesterol, LDL+VLDL cholesterol and triacylglycerol levels are shown in Table 5. Plasma total cholesterol level in rats fed diet containing pine-needle meal was twice ($P<.01$) that in rats fed the other diets. Diet containing laver meal decreased ($P<.01$) the plasma cholesterol level, as compared to the control. Plasma cholesterol level in female rats was two to three folds higher ($P<.001$) than that found in males. Feeding a diet containing alfalfa meal increased ($P<.01$) serum HDL cholesterol compared to that obtained with diets containing laver or pine-needle meal. Interestingly, HDL cholesterol in male rats was significantly ($P<.01$) higher (2.5 to 3.5-folds) than that in female rats. Female rats fed a diet containing pine-needle meal had much higher ($P<.01$) serum triacylglycerol, as compared to male rats fed the same diet or female rats fed the other diets. Male rats fed diets containing alfalfa meal, laver meal or control tended to decrease the serum triacylglycerol, although the difference was not significant.

Certain dietary fibers have been reported to lower plasma cholesterol by binding bile acids and reducing their recycling through the enterohepatic circulation. In addition, some kinds of

fibers may delay the digestion and absorption of fat. Numerous in vitro studies have demonstrated that some, but not all, sources of dietary fiber bind bile acids, leading to the hypothesis that bile acid binding is a mechanism whereby certain dietary fibers lower plasma cholesterol (Story *et al.*, 1976;Vahouny *et al.*, 1981). Story (1986) reported that the response is not consistent, nor is it likely to be sufficient to completely explain the hypocholesterolemic response of dietary fiber. For example, cellulose does not bind bile acids in vitro and in vivo, nor does it lower plasma cholesterol, yet it has been reported to increase fecal bile acid excretion. Nishina *et al.* (1990) reported that cellulose, an insoluble fiber, appeared to have little or no effect on lipid metabolism in rats and fibers, often considered to be physically similar, do not yield the same physiological effects or the same metabolic alterations. Reddy *et al.* (1979) reported that alfalfa did not reduce plasma cholesterol level and decreased the concentration of fecal neutral sterols, but not the bile acids. However, because of a two-fold increase in fecal bulk in rats fed the alfalfa diet, there was an increase in the daily output of total as well as some of the individual bile acids, particularly cholic acid, deoxycholic acid, lithocholic acid and 12-ketolithocholic acid. Cuddeford *et al.* (1992) reported that alfalfa saponins had no consistently significant effects on plasma cholesterol and triglyceride values but might contribute to the negative digestibility of alfalfa ether extract.

Pectin has a significant plasma cholesterol-lowering effect with a high cholesterol diet (Fernandez *et al.*, 1994), while guar gum intake lowers plasma cholesterol more effectively in combination with a low cholesterol rather than with a high cholesterol diet (Fernandez *et al.*, 1995). Such different responses indicate that the primary mechanisms responsible for decreasing plasma cholesterol concentrations are specific for each type of dietary fiber.

Wagner and Jackson (1983) reported that a component of pine-needles could effectively compete with estradiol-17β in a uterine cytosolic binding assay. Estrogen concentrations in systemic blood increased progressively after the initiation of pine-needle feeding (Short *et al.*, 1989). Christenson *et al.* (1992) demonstrated that cows fed pine-needles progressively reduced uterine blood flow to the gravid horn and that this reduction induced the onset of a premature parturition as evidenced by normal prepartum changes in steroid secretion. They suggested that pine-needles may contain a compound similar in structure to an estrogen-catechol estrogen but with antiestrogenic activity.

Unexpectedly, rats fed pine-needle meal had about twofold higher plasma cholesterol level than rats fed the other diets in the present study. We hypothesize that feeding pine-needle meal changes the metabolism of steroid hormones that increase cholesterol synthesis or decrease its degradation.

Sex hormones has been known to be major determinants of the cardiovascular disease. Women have less cardiovascular disease than men, especially in the premenopausal period. In general population men have higher triglyceride and very-low-density lipoprotein cholesterol(VLDL) and lower high-density lipoprotein cholesterol(HDL) concentrations than women (Heiss *et al*, 1980). Most cross-sectional studies have reported that sex hormone-binding globulin (SHBG) and testosterone levels correlate positively with HDL cholesterol levels. Thus SHBG by modulating the balance in the biodisposal of testosterone and estradiol, might have a profound effect on the risk of cardiovascular disease (Pugeat *et al*, 1995). Estrogen use increases the secretion of large VLDL and also stimulates the uptake of VLDL by the liver and increases the catabolism of LDL in the liver. Sex hormones may affect several enzymes involved in the metabolism of HDL and triglyceride and may also affect lipolysis (Steven *et al*., 1995).

Godin *et al*. (1995) reported that when control or cholesterol-supplemented diet (1% by weight) to male and female birds, plasma cholesterol levels in controls were comparable in both sexes although females showed higher triglyceride levels. Cholesterol supplementation increased plasma cholesterol in both males (8.1-fold) and females (2.5-fold) but triglycerides were significantly elevated (3.4-fold) by cholesterol feeding only in males. In contrast, Fernandez *et al*., (1995)

showed that female guinea pigs had a higher plasma cholesterol level than males when fed a high cholesterol diet (0.25% of diet), but not when fed a low cholesterol diet (0.04% of diet). They also found that dietary soluble fibers lowered plasma and hepatic cholesterol concentrations, the response being moderate and more scattered in females than in males.

The present study demonstrated that female rats had twice as much plasma cholesterol as males when they were fed a high cholesterol (0.5%) diet. Some of this difference may be accounted for by more feed intake (or cholesterol intake) per gram body weight by female than male rats, showing higher feed/gain ration. But other factors are evident in increasing plasma cholesterol level in female compared to male rats because the cholesterol intake per unit body weight by female rats was no more than 150% of that by males, while the plasma cholesterol level in females was more than 200% of that of males.

Certain dietary fibers and their components could also affect the enterohepatic circulation of bile salts and cholesterol. In addition, different kinds of fibers can also alter the activity of gut microflora, which influence the metabolism of bile acids and cholesterol. Thus, the effect of dietary fiber and on cholesterol and lipid metabolism is complex and needs to be further studied.

3. Effects of diets and sex on urease activity and ammonia production in the cecal contents of rats

Net ammonia production (or urease activity), serum urea and fecal nitrogen concentration are presented in Table 6. Ammonia concentration(μmol of ammonia per g contents) in the contents before incubation was higher ($P<.05$) in the control than in the other groups, but there were no significant differences between sexes. Urease activity(μmol of urea hydrolyzed/30 min per g content) in rats fed diets containing laver and pine-needle meal was much lower($P<.01$) than that in rats fed the control or alfalfa meal diet, but was not different between sexes. However, total urease activity in the contents was not significantly different between the dietary treatments or sexes. But urease activity (per total contents) in rats fed laver and pine-needle meal tended to be lower than in the control or rats fed alfalfa meal.

Interestingly, male rats showed higher ($P<.01$) urease activity (per total contents) compared to female rats, but male rats had much lower ($P<.01$) blood urea concentration than male rats. No significant differences in urea concentration were found between dietary treatments.

Feeding a diet containing fiber in rats (Lupton and Marchant, 1989) or a diet containing lactulose in humans (Weber

and Veach, 1979) decreased urease activity or ammonia production in the GI tract. Bacterial urease activity could be directly inhibited by ammonia concentration as shown with ureolytic ruminal bacteria (Cheng and Wallace, 1979). If oligosaccharides were present in the diet at a relatively low level together with other soluble and insoluble fibers, they could be useful in stimulating urea diffusion and ureolysis in the large intestine without inducing excessive nitrogen recycling (Younes *.et al*, 1995).

In the present study, the consumption of diets containing laver and pine-needle meal decreased urease activity (per g collected contents). A significant difference in blood urea concentration was found between male and female rats. Tsumura *et al.* (1995) reported that blood urea nitrogen in female rats were higher than in male rats. In the present study, F/G of male rats was much lower ($P<.01$) than that of female rats and thus female rats consumed more protein per unit body weight than did male rats.

In conclusion, results of the present study indicates that dietary fermentable fibers such as that in laver decrease plasma cholesterol and cecal urease urease activity. The cholesterol-increasing effect of pine-needle meal and much higher response in plasma cholesterol in female than in male rats to the dietary cholesterol is interesting, but how these happens is yet to be studied.

V. Summary

This study was conducted to investigate the effect of sex and feeding diet containing alfalfa, laver or pine-needle meal on the plasma cholesterol level and cecal urease activity in rats. Four groups of Sprague Dawley rats (initial mean weight, 92 g), each group consisting of six male and 6 females rats, were fed a control or experimental diets, respectively. All the diets contained 0.5% added cholesterol, 5% corn oil and 5% lard. After a 4-week feeding period, rats were killed and cecal contents and plasma samples were collected. Rats fed the control or diets containing laver meal had higher ($P<.01$) average daily feed intake (ADFI) and average daily gain (ADG) and lower feed/gain ratio (F/G) than rats fed diets containing alfalfa and pine-needle meal. Plasma total cholesterol level in rats fed diet containing pine-needle meal was twice ($P<.01$) that in rats fed the other diets. Diet containing laver meal decreased ($P<.01$) the plasma cholesterol level, as compared to the control. A significant difference ($P<.001$) in plasma cholesterol was found between male and female rats. Female rats showed two to three-fold higher plasma cholesterol levels than male rats, regardless of diet. Diet containing alfalfa meal increased ($P<.01$) the serum HDL cholesterol compared to diets containing

laver and pine-needle meal. Interestingly, HDL cholesterol was significantly ($P<.01$) higher (2.5 to 3.5-fold) in male than in female rats. Female rats fed diet containing pine-needle meal had higher ($P<.01$) serum triacylglycerol than their male counter parts and rats (male+female) fed the other diets. Ammonia concentration (μmol ammonia per g collected contents) in the contents (blank) was higher ($P<.05$) in the control than in the other groups. Urease activity (μmol ammonia produced from urea/30 min per g contents) in rats fed diets containing laver and pine-needle meal was much lower ($P<.01$) than that in rats fed the control or alfalfa meal diet, but was not different between sexes. However, total urease activity in the contents was not significantly different between the dietary treatments or sexes. The present studies suggest that dietary soluble fibers such as that in laver decreases plasma cholesterol and cecal urease activity. Feeding a diet containing pine-needle meal increases plasma cholesterol level for unknown reasons. Female rats respond to dietary cholesterol much greatly, compared to male rats.

Table 1. Composition of experimental diets (% on an air-dry basis)

Ingredient	Control	Alfalfa	Laver	Pine-needle
Casein[a]	20.0	18.0	18.0	20.0
L-methionine[a]	.3	.3	.3	.3
Corn oil[b]	5.0	5.0	5.0	5.0
Lard[c]	5.0	5.0	5.0	5.0
Choline chloride[a]	.2	.2	.2	.2
Vitamin mix[d]	1.0	1.0	1.0	1.0
Salt mix[d]	3.5	3.5	3.5	3.5
Cholic acid[a]	.2	.2	.2	.2
Sucrose[b]	20.0	20.0	20.0	20.0
Cholesterol[e]	.5	.5	.5	.5
Corn starch[f]	44.3	36.3	36.3	34.3
Alfalfa meal[g]	-	10.0	-	-
Laver meal[h]	-	-	10.0	-
Pine-needle meal[i]	-	-	-	10.0
Total	100.0	100.0	100.0	100.0

[a] United States Biochemical Co., Cleveland, Ohio.

[b] Jeil Jedang Co., Seoul, Korea.

[c] Samlip Yugi Co., Seoul, Korea.

[d] AIN-76A, Harlan, Madison, WI.

[e] Fluka Chemie, Switzerland.

[f] Sunil Pododang Co., Seoul, Korea.

[g] Alfalfa(*Medicago sativa* L.) containing 22.5% CP, 42.3% NDF and 29.4% ADF dried and ground.

[h] Laver(*Porphyra spp.*) containing 22.1% CP, 4.6% ADF, 45% NDF dried and ground.

[i] Pine-needle (*Pinus densiflora* S.) containing 7.12% CP, 60.4% NDF and 43.5% ADF dried and ground.

Table 2. Analysis of variance(ANOVA) of the data.

Item	MS		
	Treat	Sex	Treat × Sex
Final Weight, g	1370.93**	122937.76**	570.02
ADFI. g	6.05*	264.09***	.64
ADG, g	1.66**	145.60***	.60
F/G	.34**	14.14**	.36**
Total Cecal Weight, g	3.47***	3.57**	.37
Cecal Wall Weight, g	.07**	.09*	.02
Cecal Contents, g	2.10***	3.46**	.47
Total Cholesterol, mg/ dl	579687.36***	2575067.77***	89973.77**
HDL Cholesterol, mg/dl	82.66*	2260.91***	37.21
LDL + VLDL, mg/dl	584480.77***	2729761.32***	89578.39**
Triglycerol, mg/dl	16524.53***	17973.82***	15315.65***
Ammonia Production, μmol			
Before incubation	.05**	.0001	.0005
After incubation	8.36***	.002	.215
Net production	7.66***	.002	.218
Urease Activity, μmol			
per g contents	.174***	.0001	.004
per total contents	.037	.186**	.011
Plasma urea, mg/dl	.205*	2.90***	.042

* P<.05

** P<.01

*** P<.001

Table 3. Effects of feeding diets containing alfalfa, laver or pine-needle meal on body weight gain and feed efficiency.

Item	Control			Alfalfa			Laver			Pine-needle			Sex class		SE
	male	female	mean	male	female	mean	male	female	mean	male	female	mean	male	female	
Initial weight, g	93.9	91.6	92.7	94.9	89.7	92.2	94.9	91.2	93.1	95.3	90.3	92.7	94.7	90.6	4.29
Final weight, g	307.1	213.8	260.4^{ab}	305.2	194.3	249.7^{b}	311.3	225.2	268.2^{a}	301.8	187.1	244.4^{b}	306.3^{A}	205.1^{B}	7.52
ADFI, g	18.9	14.9	16.9^{ab}	19.3	14.3	16.7^{b}	20.2	15.7	17.9^{a}	18.9	13.8	16.3^{b}	19.3^{A}	14.6^{B}	.53
ADG, g	7.6	4.4	6.0^{ab}	7.5	3.7	5.6^{bc}	7.7	4.8	6.2^{a}	7.4	3.5	5.4^{c}	7.5^{A}	4.0^{B}	.21
F/G[1]	2.5	3.4	2.9^{b}	2.6	3.8	3.2^{a}	2.6	3.3	3.0^{b}	2.6	4.0	3.3^{a}	2.5^{A}	3.6^{B}	.08

Values are means of 6 rats.

Values in the same row with different superscripts differ significantly($P<.05$).

^{A,B} Significantly different($P<.05$) between sexes.

[1]Significant($P<.01$) interaction between treatment and sex.

Table 4. Effects of feeding diets containing alfalfa, laver or pine-needle meal on cecal size and cecal contents.

Item	Control			Alfalfa			Laver			Pine-needle			Sex class		SE
	male	female	mean	male	female	mean	male	female	mean	male	female	mean	male	female	
Total cecal weight, g	1.82	1.63	1.72b^{c}	1.47	1.10	1.28^{c}	2.50	1.87	2.18^{ab}	3.01	2.01	2.57^{a}	2.20^{A}	1.65^{B}	.20
Cecal wall weight, g	.57	.55	.56b^{c}	.60	.46	.53^{c}	.65	.65	.65^{ab}	.80	.60	.70^{a}	.65^{A}	.56^{B}	.04
Cecal contents, g	1.25	1.08	1.17b^{c}	.86	.63	.75^{c}	1.83	1.05	1.44^{ab}	2.21	1.25	1.73^{a}	1.54^{A}	1.09^{B}	.18

Values are means of 6 rats.
Values in the same row with different superscripts differ significantly($P<.05$).
^{A,B} Significantly different($P<.05$) between sexes.

Table 5. Effects of feeding diets containing alfalfa, laver or pine-needle meal on the plasma cholesterol or triacylglycerol content in rats.

Item	Control			Alfalfa			Laver			Pine-needle			Sex class		SE
	male	female	mean	male	female	mean	male	female	mean	male	female	mean	male	female	
							mg/dl								
Total cholesterol[1]	239.8	669.8	454.5^{b}	201.0	620.8	410.9^{bc}	177.7	473.9	325.7^{c}	470.3	1176.6	823.4^{a}	272.0^{A}	735.3^{B}	45.7
HDL cholesterol	22.0	6.1	14.0^{ab}	25.5	7.9	16.7^{a}	16.2	6.0	11.1^{b}	17.0	5.7	11.3^{b}	20.2^{A}	6.4^{B}	1.5
LDL+VLDL[2]	217.3	663.8	440.5^{b}	175.5	612.9	394.2^{bc}	161.5	467.8	314.7^{c}	453.3	1170.9	812.1^{a}	251.9^{A}	728.8^{B}	46.2
Triacylglycerol[3]	21.9	26.6	24.2^{b}	21.4	31.8	26.6^{b}	16.6	20.1	18.3^{b}	24.2	169.7	96.5^{a}	22.1^{A}	60.8^{B}	5.2

Values are means of 6 rats.

Values in the same row with different superscripts differ significantly($P<.05$).

^{A,B} Significantly different($P<.05$) between sexes.

^{1,2,3}Significant($P<.01$) interaction between treatment and sex.

HDL=high density lipoprotein.

LDL+VLDL=low density lipoprotein + very low density lipoprotein.

Table 6. Effects of feeding diets containing alfalfa, laver or pine-needle meal on ammonia producton and urease activity in the cecal

Item	Control			Alfalfa			Laver			Pine-needle			Sex class		SE
	male	female	mean	male	female	mean	male	female	mean	male	female	mean	male	female	
Ammonia production[1]															
Before incubation	.44	.46	.45^{a}	.35	.35	.35^{b}	.29	.29	.29^{b}	.34	.33	.33^{b}	.35	.35	.03
After incubation	2.54	2.58	2.56^{b}	3.34	2.94	3.13^{a}	1.41	1.51	1.47^{c}	1.35	1.56	1.46^{c}	2.16	2.14	.25
Net production	2.10	2.12	2.11^{b}	2.98	2.58	2.78^{a}	1.12	1.21	1.17^{c}	1.00	1.24	1.12^{c}	1.80	1.79	.24
Urease activity[2]															
per g contents	.32	.32	.32^{b}	.44	.38	.41^{a}	.16	.17	.17^{c}	.15	.18	.16^{c}	.27	.26	.03
per total contents	.41	.34	.37^{a}	.38	.23	.30^{a}	.34	.14	.24^{a}	.31	.23	.27^{a}	.36^{A}	.23^{B}	.05
Plasma urea, mg/dl	.88	1.35	1.11^{b}	1.11	1.53	1.32^{ab}	.85	1.51	1.17^{ab}	1.19	1.61	1.40^{a}	1.00^{A}	1.50^{B}	.12

Values are means of 6 rats.

Values in the same row with different superscripts differ significantly($P<.01$).

^{A,B} Significantly different($P<.05$) between sexes.

[1] μmol of ammonia per g contents.

[2] μmol of urea hydrolyzed / 30 min at 37℃.

References

Abraham, Z.D. and T. Mehta. 1988. Three-week psyllium-husk supplementation: Effect on plasma cholesterol concentrations, fecal steroid excretion, and carbohydrate absorption in men. Am. J. Clin. Nutr. 47:67-74.

Arjmandi, B.H., J. Craig, S. Nathani and R.D. Reeves. 1992. Dietary soluble fiber and cholesterol affect serum cholesterol concentration, and hepatic portal venous short-chain fatty acids concentrations and fecal sterol excretion in rats. J. Nute. 122:246-253.

Beaulieu, K.E. and M.I. McBurney 1992. Changes in pig serum lipids, nutrient digestibility and sterol excretion during cecal infusion of propionate. J. Nutr. 122:241-245.

Bedbury. H.P. and G.E Duke. 1983. Cecal microflora of turkeys fed low or high fiber diets: Enumeration, identification, and determination of cellulotic activity. Poult. Sci. 62:675-683.

Boila, R.J., M.O. Salomons, L.P. Milligan and F. Aherne. 1981. The effect of dietary propionic acid on cholesterol synthesis in swine. Nutr. Rep. Int. 23:1113-1121.

Chaney, A.L. and E.P. Marbach, 1962. Modified reagents for determination of urea and ammonia. Clinical Chemistry 8:130-132.

Cheng, K.-J. and R.J Wallace. 1979. The mechanism of passage of endogenous urea through the rumen wall and the role of ureolytic epithelial bacteria in the urea flux. Br. J. Nutr. 42:553-557.

Chen W-J.L., F.W. Hays and J.R. Overfield. 1970. Effects of dietary ascorbic acid on performance and plasma cholesterol levels of growing swine. J. Anim. Sci. 31:63-66.

Christenson, L.K., R.E. Short and S.P. Ford. 1992. Effects of ingestion of Ponderosa pine needles by late-pregnant cows on uterine blood flow and steroid secretion. J. Anim. Sci. 70:531-537.

Cromwell, G.L., V.W. Hays and J.R. Overfield. 1970. Effect of dietary ascorbic acid on performance and plasma cholesterol levels of growing swine. J. Anim. Sci. 31:63-66.

Cuddeford D., A. Woodhead, R. Muirhead. 1992. A comparison between the nutritive value of short-cutting cycle, high temperature-dried alfalfa and timothy hay for horses. Equine Veterinary Journal. 24(2): 84-9.

Cummings, J.H. 1978. Nutritional implication of dietary fiber. Am. J. Clin. Nutr. 31:S21-S29.

De Deckere, E. A. M., W.J. Kloots, and J.M.M. Van Amelsvoort. 1993. Resistant starch decreases serum total cholesterol and triacylglycerol concentrations in rats. J. Nutr. 123:2142-2151.

Demigne, C. and C. Remesy. 1985. Stimulation of absorption of volatile fatty acids and minerals in the cecum of rats adapted to a very high fiber diet. J. Nutr. 115:53-60.

De Schrijver, R., D. Fremaut and A. Verheyen. 1992. Cholesterol-lowering effects and utilization of protein, lipid, fiber and energy in rats fed unprocessed and baked oat bran. J. Nutr. 122:1318-1324.

Donaldson, R.M. 1964. Normal bacterial populations of the intestine and relation to intestinal function. N. Engl. J. Med. 270:938-1056.

Eastwood, M.A. and E.R. Morris. 1992. Physical properties of dietary fiber that influence physiological function: a model for polymers along the gastrointestinal tract. Am. J. Clin. Nutr. 55:436-442.

Eaton, K.A., C.L. Brooks, D.R. Morgan, and S. Krakowka. 1991. Essential role of urease in pathogenesis of gastritis induced *Helicobacter pylori* in gnotobiotic piglets. Infec. Immun. 59:2470-2475.

Ebihara, K. and B.O. Schneeman. 1989. Interaction of bile acids, phospholipids, cholesterol and triglyceride with dietary fibers in the smalll intestine of rats. J. Nutr. 119:1100-1106.

Ebihara, K., T. Miyada and A. Nakajima. 1993. Hypocholesterolemic effect of cecally infused propionic acid in rats fed a cholesterol-free casein diet. Nutr. Res. 13:209-217.

Fernandez, M.L., D-M. Sun, M.A. Tosca, and D.J. Mcnamara. 1994. Citrus pectin and cholesterol interact to regulate hepatic cholesterol homeostasis and lipoprotein metabolism: a dose response study in guinea pigs. Am. J. Clin. Nutr. 59: 869-878.

Fernandez, M.L., D-M. Sun, M.A. Tosca, and D.J. Mcnamara. 1995. Guar gum effects on plasma low-density lipoprotein and hepatic cholesterol metabolism in guinea pigs fed low- and high-cholesterol diets; a dose response study. Am. J. Clin. Nutr. 61:127-134.

Fernandez, M.L., N.R. Ruiz, K. Conde, D-M. Sun, S.K. Erickson, and D.J. Mcnamara. 1994. Psyllium reduces plasma LDL in guenia pigs by altering hepatic cholesterol homeostasis. J. Lipid Res. 36:1128-1138.

Fioramonti, J. and L. Bueno. 1908. Motor activity in the large intestine of the pig related to dietary fiber and retention time. Br. J. Nutr., 43:155-162.

Friedl K.E., R.E. Jones, C.J. Jr. Hannan, S.R. Plymate. 1989. The administration of pharmacological doses of testosterone or 19-nortestosterone to normal men is not associated with increased insulin secretion or impaired glucose tolerance. J. Clin. Endocrinol Metab. 68: 971-5.

Friedl K.E., C.J. Jr. Hannan, R.E. Jones, S.R. Plymate. 1990. High density lipoprottein cholesterol is not decreased if an aromatizable androgen is administered. Metabolism 39: 69-74.

Gallaher, D.D., C.A. Hassel, K-J. Lee and C.M. Gallaher. 1993. Viscosity and fermentability as attributes of dietary fiber responsible for the hypocholesterolmic effect in hamsters. J. Nutr. 123:244-252.

Gallaher, D.D., C.A. Hassel and K-J. Lee. 1993. Relationships between viscosity of hydroxypropyl methylcellulose and plasma cholesterol in hamsters. J. Nutr. 123:1732-1738.

Gargallo, J. and D.R. Zimmerman. 1980. Effects of dietary cellulose and neomycin on function of the caecum of pigs. J. Anim.Sci. 51:121-126.

Gibson, J.A., N.J. Park, G.E. Sladen, and A.M. Dawson. 1976. The role of the colon in urea metabolism in man. Clin. Sci. Mol. Med. 50:51-59.

Godin D.V., M.E. Garnett, K.M. Cheng and C.R. Nichols. 1995. Sex-related alterations in antioxidant status and susceptibility to atherosclerosis in Japanese quail. Canadian Journal of Cardiology. 11(10) : 945-51.

Goldstein, J.L. and M.S. Brown. 1977. The low-density lipoprotein pathway and its relation to atherosclerosis. Ann. Rev. Biochem. 46:897-930.

Grundy, S.M., E.H. Ahrens and J. Davignon. 1969. The interaction of cholesterol absorption and cholesterol synthesis in man. J. of Lipid REs. 10:304-315.

Haffner S.M., R.S. Kushawaha, D. Applebaum-Bowden, W.R. Hazzard. 1983. Studies on the metabolic mechanism of reduced hig density lipoproteins during anabolic steroid therapy. Metabolism. 32:413-20.

Heilbrum, L.K., A. Nomura, H.H. Hankin, and G.N. Stemmermann. 1989. Diet and colorectal cancer with special reference to fiber intake. Int. J. Cancer. 44:1-6.

Heiss G., I. Tamir, Ce. Davis *et al.* 1980. Lipoprotein-cholesterol distributions in selected North American populations: the Lipid Research Clinics Program Prevalence Study. Circulation 61:302-15.

Holmang A., J. Svedberg, E. Jennische, and P. Bjornto. 1990. Effects of testosteron on muscle insulin sensitivity and morphology in female rats. Am. J. Physiol. 259: E555-60.

Hutagalung, R.I., G.L. Crowell, V.W. Hays and C.H. Chaney. 1969. Effect of dietary fat, protein, cholesterol and ascorbic acid on performance, serum and tissue cholesterol levels and serum lipid levels of swine. J. Anim. Sci. 29:700-705.

Illman, R.J. and D.L. Topping. 1995. Effect of dietary oat bran on fecal steroid excretion, plasma volatile fatty acids and lipid synthesis in rats. Nutr. Res. 5:839-846.

Jackson, A.A., J. Doherty, M.H. De Benoist, J. Hibbert, and C. Persaund. 1990. The effect of the level of dietary protein, carbohydrate and fat on urea kinetics in young children during rapid catch-up weight gain. Br. J. Nutr. 64:371-385.

Jenkins, D.A., T.M.S. Wolever, A.V. Rao *et al.* 1993. Effect on blood lipids of very high intakes of fiber in diets low in saturated fat and cholesterol. N. Engl. J. Med. 329:21-26.14.

Jonnalagadda, S.S., F.W. Thye and J.L. Robertson. 1993. Plasma total and lipoprotein cholesterol, liver cholesterol and fecal cholesterol excretion in hamsters fed fiber diets. J. Nutr. 123:1377-1382.

Judd, P.A. and A.S. Truswell. 1985. The hypocholesterolmic effects of pectins in rats. Br. J. Nutr. 53:409-425.

Judd, P.A. and A.S. Truswell. 1982. Comparison of the effects of high- and low- methyl pectin on blood and fecal lipids in man. Br. J. Nutr. 48:451-458.

Kass, M.L., P.J. Van soest, W.G. Pond, B. Lewis and R.E. McDowell. 1980. Utilization of dietary fiber from alfalfa by growing swine. 1. Apparent digestibility of diet components in specific segments of the gastrointestinal tract. J. Anim. Sci. 50:175-191.

Kelley, J.J. and A.C. Tsai. 1978. Effeect of pectin, gum arabic and agar on cholesterol absorption, synthesis and turnover in rats. J. Nutr. 108:630-639.

Kelsay, J.K. 1978. A review of research on effects of fiber intake on man. Am. J. Cln. Nutr. 31:142-159.

Kim, K.L., N.J. Benevenga and R.H. Grummer. 1978a. Estimation of the fraction of lactose in a high lactose diet available for fermentation in the cecum and colon of the rat. J. Nutr. 108:79-89.

Kim, K.L., N.J. Benevenga and R.H. Grummer. 1978b. The fraction of dietary lactose available for fermentation in the cecum and colon of pigs. J. Anim. Sci. 46:1658-1665.

Kim, K.I., N.J. Benevenga and R.H. Grummer. 1978c. Lactase activity and VFA production in the cecum and colon of pigs fed a corn-soy or 40% whey diet. J. Anim. Sci. 46:1648-1657.

Kim, K.I., N.J. Benevenga and R.H. Grummer. 1979. In vitro measurement of the lactase activity an fermentation products of lactose in the cecal and colonic contents of rats fed a control or 30% lactose diet. J. Nutr. 109:856-863.

Kim, K.I. and J.M. Yeo. 1995. Effects of dietary antimicrobial agents, probiotics or yucca extract on urease activity and ammonia production in the chicken intestine. K. J. Poult. Sci. 22(2):105-115.

Kim, T.W. and K.I. Kim. 1992. Effects of feeding diets containing probiotics or antimicrobial agent on urease activity and ammonia production in the intestinal contents of rats. Korean J. Anim. Sci. 34:167-173.

Kinnunen P.K.H., J.A. Vitanen, P. Vianio. 1983. Lipoprotein lipase and hepatic endothelial lipase: their roles in plasma lipoprotein metabolism. Atherosclerotic Rev. 11: 65-105.

Krauss R.M., R.I. Levy, D.S. Fredrickson. 1974. Selective measurement of two lipase activities in postheparin plasma from normal subjects and patients with hyperlipoproteinemia. J. Clin. Invest. 54: 1107-24.

Levrat, M.A., C. Remesy, and C. Demigne. 1991. Very acidic fermentation in the rat cecum during adaptation to a diet rich in amylase-resistant starch (crude potato starch). J. Nutr. 119:235-241.

Lee, J.J. and I.K. Han. 1985. Effects of dietary pectin levels and kinds of lipid on serum and tissues cholesterol levels in rats. Korean J. Anim. Sci. 27:724-727.

Lee, W.S. 1992. Effects of feeding diets containing probiotics, cellulose or lactose on urease activity and ammonia production in the intestine or rats. Dissetation for the Degree of Master of Science. Cheju National University, Republic of Korea.

Lehninger, A.L. 1982. The biosynthesis of lipids. In: Principles of Biochemistry. pp. 583-611. Worth Publishers, Inc., New York, N.Y.

Lin, H.C. and E.J. Visek. 1981. Colon mucosal cell damage by ammonia in rats. J. Nutr. 121:887-893.

Lund, E.K., K.L. Salf and I.T. Johnson. 1993. Baked rye products modify cholesterol metabolism and crypt cell proliferation rates in rats. J. Nutr. 123:1834-1843.

Lupton, J.R. and L.J. Marchant. 1989. Independent effects of fiber and protein on colonic luminal ammonia concentraion. J. Nutr. 119:235-241.

MacFarlane, G.T. and J.H. Cummings. 1991. The colonic flora, fermentation and large bowel digestive function. In: The Large Intestine: Physiology, Pathophysiology and Diease (Phillips, S.F., Pemberton, J.H. and Shoter, R.G., eds.), pp. 51-92. Raven Press, New York, NY.

Matthews K.A., E. Meilahn, L.H. Kuller, S.F. Kelsey, A.W. Caggiula, R.R. Wing. 1989. Menopause and risk factors for coronary heart disease. N. Engl. J. Med. 321:641-6.

McCarthy, P.A. 1993. New approaches to atherosclerosis: an overview. Med. Res. Rev. 13:139-159.

Meinl, M. and K. Kreienbring. 1985. Untersuchungen zum Bacterialanteil im Schweinkot. Arch. Trerernahr. 35:33-44.

Mortensen, P.B. 1992. Effects of oral-administered lactulose on colonic nitrogen metabolism and excretion. Hepatology 16:1350-1356.

Nelson, R. L., J.C. Tanure, G. Andrianopoulos, G. Souza, and W.E.M. Lands. 1988. A comparison of dietary fish oil and corn oil in experimental colorectal carcinogenesis. Nutr. Cancer 11:215-220.

Nishina, P.M. and R.A. Freedland. 1990. The effects of dietary fiber feeding on cholesterol metabolism in rats. J. Nutr. 120:800-805.

Nishina, P.M. and R.A. Freedland. 1990. The effects of dietary fiber feeding on cholesterol metabolism in rats. J. Nutr. 120:800-805.

Nishina, P.M., B.D. Schneeman and R.A. Freedland. 1991. Effects of dietary fibers on nonfasting plasma lipoprotein and apoprotein levels in rats. J. Nutr. 121:431-437.

Pollman, D.S., D.M Danielson, and E.R. Poe. 1979. Value of high fibre diets for gravid swine. J. Anim. Sci. 48:1385-1393.

Pugeat M., P. Moulin, P. Cousin *et al.* 1995. Interrelations between sex hormone-binding globulin(SHBG), plasma lipoproteins and cardiovascular risk. Journal of Steroid Biochemistry and Molecular Biology. 53(1-6) : 567-72

Rebuffe-Scrive M., P. Lonnroth, C. Esslau, P. Bjorntorp, U. Smith. 1987. Regional adipose tissue metabolism in men and postmenopausal women. Int. J. Obesity. 11: 347-55.

Reddy, B.S., K. Watanabe, and A. Sheinfil. 1980. Effect of dietary wheat bran, alfalfa, pectin and carrageenan on plasma cholesterol and fecal bile acid and neutral sterol excretion in rats. J. Nutr. 110:1247-1254.

SAS. 1988. SAS User'guide: statistics(version 6.03). SAS Inst. Inc., Cary, NC.

Seidell J., P. Kvist H. Bjorntorp, R. Sannerstedt. 1990. Viceral fat accumulation in men is positively associated with insulin, glucose, and C-peptide levels, but negatively with testosterone levels. Metabolism. 39: 897-901.

Short, R.E., L.F. James, R. B. Staigmiller, and K.E. Panter. 1989. Pine-needle abortion in cattle: associated changes in serum cortisol, estradiol and progesterone. Cornell Vet. 67:294.

Shoupe D. and R.A. Lobo. 1984. The influence of androgens on insulin resistence. Fertil Steril. 41: 385-8

Smoot, D.T., H.L.T. Mobley, G.R. Chillendale, J.F. Lewison, and J.H. Resau. 1990. *Hellicobacter pylori* urease activity is toxic to human gastric epithelial cells. Infec. Immun. 58:1992.

Stark, A.H. and Z. Mardar. 1993. In vitro production of short-chain fatty acids by bacterial fermentation of dietary fiber compared with effects of those fibers on hepatic sterol synthesis in rats. J. Nutr. 123:2166-2173.

Steven M., M.D. Haffner, A. Rodolfo, ph.D. Valdez. 1995. Endogenous sex hormones: Impact on lipids, lipoproteins, and insulin. The American Journal of Medicine. Volume 98 (suppl 1A)

Story, J.A. 1986. Modification of steroid excretion in response to dietary fiber. In Dietary Fiber, Basic and Clinical Aspects (Vahouny, G.V. and Kritchevsky, D., eds), pp. 153-264, Plenum Press, New York.

Story, J.A. and D. Kritchevsky. 1976a. Comparison of the binding of various bile acids and bile salts in vitro by several types of fiber. J. Nutr. 106:1292-1294.

Story, J.A. and D. Kritchevsky. 1976b. Dietary fiber and lipid metabolism. In: Fiber in Human Nutrition (Spiller, G.A. and Amen, R.J., eds), pp. 171-184, Plenum Press, New York.

Suzuki, K., Y. Benno, T. Mitsucka, S. Takebe, K. Kobashi, and J. Hase. 1979. Urease-producing species of intestinal anaerobes and their activities. Appl. Environ. Microbiol. 37:379-382.

Taggert H., D. Applebaum-Bowden, S.M. Haffner et al. 1984. Reduction in high density lipoproteins by anabolic steroids(stanozolol) therapy for postmenopausal osteoporosis. Metabolism. 31: 1147-52.

Thacker, P.A., M.O. Salomons, F.S. Aherne, S.P. Milligan ann J.P. Bowland 1981. Influence of propionic acid on the cholesterol metabolism of pigs fed hypercholesterolemic diets. Can. J. Anim. Sci. 61:969-975.

Thompson P.D., E.M. Cuillinane, S.P. Sady *et al.* 1989. Contrasting effects of testosterone and stanozolol on serum lipoproteins levels. JAMA. 261: 1165-8.

Tikkanen M.J., E.A. Nikkila, T. Kuusi, S.U. Sipinen. 1983. High density lipoprotein-2 and hepatic lipase: reciprocal changes produced by estrogen and norgesrol. J Clin Endocrinol Metab. 54: 1113-7.

Topping, D.L., R.J. Illman, J.M. Clarke, R.P. Trimble, K.A. Jackson and Y. Marsono 1993. Dietary fat and fiber alter large bowel and portal venous volatile fatty acids and plasma cholesterol but not biliary steroids in pigs. J. Nutr. 123:133-143.

Tsuchiya N, Y. Harada, M. Taki et al. 1995. Age-related changes and sex differences on the serum chemistry values in Sprague-Dawley rats- I . 6-30 weeks of age. Jikken Dobutsu. Experimental Animals. 43(5) : 671-8.

Turley, S.D., B.P. Daggy and J.M. Dietschy. 1991. Cholesterol lowering action of psyllium mucilloid in the hamster: sites and possible mechanisms of action. Metabolism. 40:1063-1073.

Vahouny, G.V., R. Tomas, M.M. Cassidy, D. Kritchevsky and L.L. Gallo 1981. Dietary fibers. VI. Binding of fatty acids and monolein from mixed micelles containing bile salts and lecithin. Proc. Soc. Exp. Biol. Med. 166:12-16.

Vahonuny, G.V., S. Satchithanandam, I. Chen, S.A. Tepper, D. Kritchevsky, F.G. Lightfoot and M.M. Cassidy. 1988. Dietary fiber and intestinal adaptation: effects on lipid absorption and lymphatic transport in the rat. Am. J. Clin. Nutr. 47:201-206.

Varel, V.H., W.G. Pond and J.T. Yen. 1984. Influence of dietary fiber on the performance and cellulose activity of growing-finishing swine. J. Anim. Sci. 59:388.

Viallard, V. 1984. Endogenous urea as a nitrogen source for microorganisms of the rabbit digestive tract. Ann. Nutr. and Metab. 28:151-155.

Vigne, J.L., D. Lairon, P. Borel, H. Portugal, A. Pauli, J.C. Hanton and H. yLafont. 1987. Effect of pectin, wheat bran and cellulose on serum lipids and lipoprotein in rats fed on a low- or high-fat diet. Br. J. Nutr. 58:405-413.

Vince, A., A.M. Dawson, N. Park, and F.W. O'Gray. 1973. Ammonia production by intestinal bacteria. Gut. 12:171-177.

Vince, A., P.J Down, J. Murison, F.J. Twigg and O.M. Wrong. 1976. Generation of ammonia from non-urea sources in a fecal incubation system. Clin. Sci. Mol. Med., 51:313-322.

Vince, A. and S.M. Burridge. 1980. Ammonia production by intestinal bacteria: The effects of lactose, lactulose and glucose. J. Med. Microbiol. 13:177-191.

Visek, W.J., 1978. The mode of growth promotion by antibiotics. J. Anim. Sci. 46:1447-1469.

Wagner, W.D., and L.L. Jackson. 1983. Phytoestrogen from pinus ponderosa assayed by competitive binding with 17β-estradiol to mouse uterine cytosol. Theriogenology. 19:507.

Walsh, B.W., I. Schiff, B. Rosner, L. Greenberg, V. Ravnikar, F.M. Sacks. 1991. Effects of postmenopausal estrogen replacement on the concentration and metabolism of plasma lipoproteins. N. Engl. J. Med. 325:1196-204.

Willet, W. 1989. The search for the causes of breast and colon cancer. Nature (Lond.) 338:389-394.

Willet, W.C. 1989. The search for the causes of breast and colon cancer. Nature(Lond.). 338:389-394.

Willet, W.C., M.J. Stamfer, G.A. Colditz, B.A. Rosner and F.E. Speizer. 1980. Relation of meat, fat, and fiber intake to the risk of colon cancer in a prospective study among women. N. Engl. J. Med. 323:1664-1672

Wozny, M.A., M.P. Bryant, L.V. Holdman, and W.E.C. Moore. 1977. Urease assay and urease-producing species of anaerobes in the bovine rumen and human feces. Appl. Environ. Microbiol. 33:1097-1104.

Wright , R.S., J.W. Anderson and S.R. Bridges. 1990. Propionate inhibits hepatocyte lipid synthesis. Pro. Soc. Exp. Biol. Med. 195: 26-29.

Wrong, O.M., A. Metcalfe-Gibson, R.B.I. Morrison, S.T. Ng, and A.V. Howard. 1965. In vivo dialysis of feces as a method of stool analysis. I. Technique and results in normal subjects. Clin. Sci. 28:357-375.

Wrong, O.M. 1981. Nitrogen compounds. In: The large intestine: Its role in mammalian nutrition and homeostasis (O.M. Wrong, C.J. Edmonds and V.S. Chadwick, ed), pp. 133-211. John wiley and Sons, New York, NY.

Yang, Y.H. 1994. Effects of feeding diets containing tangerine pulp meal or pectin on the cholesterol contents or cholesterol absorption in the serum, Liver and feces of rats. Dissertation for the Degree of Master of Science. Cheju National University, Republic of Korea.

Younes, H., K. Garleb, S. Behr, C. Remesy and C. Demigne. 1995. Fermentable fibers or oligosaccharides reduce urinary nitrogen excretion by increasing urea disposal in the rat cecum. J. Nutr. 125:1010-1016.

Xu X., G. De Pergola and P. Bjorntorp. 1990. The effects of androgens on the regulation of lipolysis in adipose precusor cells. Endocrinology. 126: 1229-34.

감사의 글

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이 논문이 만들어지기까지 저의 부족함을 채워주시고 항상 바른 길을 걷게하여 주신 지도교수 김규일 박사님께 깊은 감사를 드립니다. 보잘것없는 제 논문을 정성껏 다듬어주시고 충고를 아끼지 않으신 양영훈 교수님 과 강정숙 교수님께 감사드립니다. 그리고 저에게 많은 용기를 주신 강태숙 학과장님을 비롯한 축산학과 모든 교수님들께도 진심으로 고마움을 표합니다.

아울러 저를 배움의 길에 매진할 수 있게 하여주신 제주농업시험장 정선부 장장님과 고서봉 과장님을 비롯한 모든 직원 여러분께 감사드리며 특히 힘들때마다 많은 조언과 충고를 아끼지 않으신 이왕식 연구사님께 특별히 감사드립니다. 그리고 제 실험을 성심껏 도와준 이성연, 최승준, 김경호 후배와 영양학 실험실 모든 후배님들게도 감사합니다.

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