碩士學位論文

生菌劑, 셀룰로스 또는 乳糖을 含有한 飼料의 給與가 쥐의 腸內 尿素分解酵素 活性 및 암모니아 生産에 미치는 影響

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Effects of Feeding Diets Containing Probiotics, Cellulose or Lactose on Urease Activity and Ammonia Production in the Intestine of Rats

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Contents

摘　　要

30마리의 成熟한 스프라그 달리 쥐(平均體重 200g, 處理當 6마리)에게 商業用飼料(對照區)또는 商業用飼料에 0.1% 生菌劑(試驗 1), 試驗 2에서는 對照飼料, 10% 셀룰로스나 20% 乳糖을 含有한 飼料를 최소 14日 간 給與하였다.

飼養期間中 總增體量, 日當增體量, 飼料攝取量 및 飼料效率을 測定하였고, 飼養試驗 後 屠殺하여 小腸(점막층 包含) 및 大腸 內容物을 採取하여 尿素分解酵素의 活性과 암모니아 生産率을 測定하였다. 採取된 腸 內容物은 pH 6.5인 燐酸緩衝液으로 稀釋한 後 ^{14}C尿素를 含有한 尿素溶液을 添加하여 37℃에서 30분간 培養, 尿素分解酵素의 活性 (內容物 g당 또는 全體 內容物當 分解된 尿素 μmol/30분)과 암모니아 生産率(內容物 g당 μmol/30분)의 測定에 利用하였다.

【試驗 1】에서 商業用飼料와 商業用飼料에 生菌劑를 添加하여 增體率과 飼料效率을 測定하였는데 生菌劑 添加에 의한 增體率과 飼料效率 改善效果는 없었다. 그리고 尿素分解 酵素의 活性과 암모니아 生産率 모두 處理間에 有意差가 없었으나 生菌劑 添加區에서 減少하는 傾向을 보였다.

【試驗 2】에서 純粹飼料(對照區), 셀룰로스나 乳糖을 含有한 飼料를 給與한 境遇에 對照區와 他 處理間에 總增體量, 日當增體量 및 飼料效率에서는 有意差를 볼 수 없었으나, 飼料 攝取量은 셀룰로스 添加區에서 현저히 많았다(P<0.05). 小腸 및 大腸 內容物의 尿素分解酵素 活性과 암모니아 生産率은 小腸에서는 飼料에 따른 有意差가 없었으나 大腸에서는 셀룰로스 添加區가 他 處理區에 비해 현저히(P<0.05) 낮았다. 本試驗結果는 셀룰로스 添加가 大腸 內容物 중의 尿素分解酵素의 活性과 암모니아 生産을 抑制한다는 것을 提示해 준다.

I. Introduction

Ammonia, one of the principal products of nitrogen metabolism, is normally converted to urea in the mammalian liver, 20 - 25% of which is excreted into the gastrointestinal(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). Therefore, many urease-producing bacteria are considered to be harmful to men and animals.

Microflora growing in the GI tract are important factors influencing animal health in both positive and negative ways. Changes in the intestinal microflora of men and animals are known to take place following dietary change or weaning (Kenworth and Crabb, 1963). The addition of certain probiotics, such as Lactobacillus acidophilus, to diets for young pigs decreased the number of pathogenic E. coli in the digestive tract (Smith, 1971; Muralidhara et al., 1977) and improved weight gain and feed efficiency (Pollmann et al., 1980).

Dietary cellulose has long been known to increase bulk and water of intestinal contents, shorten transit time, and to decrease the concentration of toxic substances in contact with the intestinal mucosa (Kelsay, 1978). Feeding diets containing lactose has been known to result in depressed weight gain and enlarged caecum, and changes in intestinal microflora and proportions of acetate, propionate, butyrate and lactate in the large intestine (Kim et al., 1979). However, no substantial amounts of work on the effect of the dietary components, such as cellulose and lactose, on urease activity or ammonia production in the large intestine have been published to date.

The objective of this study was to determine the effect of feeding diets containing probiotics(Biocerin), cellulose or lactose on urea degradation and ammonia production in the intestinal contents of growing adult rats.

II. Literature Review

1. Urea and ammonia metabolism in the gastrointestinal tract

Although urea is generally regarded as an end product, significant urea destruction has been found in all mammals (Wrong, 1981). There is strong evidence that urea destruction is caused by the activity of urease-producing bacteria in the GI tract.

The large intestine is generally regarded as the main site of urea destruction in nonruminants. In man, urea concentrations in ileostomy fluid are close to plasma levels (Cummings et al., 1976; Gibson et al., 1976), but in feces the concentrations are very low, of the order of 0.1mmol(Wrong et al., 1965; Owens and Padovan, 1976) except during oral administration of broad spectrum unabsorbed antibiotics. Urea concentrations are about 0.8mmol in the cecum of rabbits (Knutson et al., 1977) and could not be demonstrated at all in cecal contents of normal rats (Combe et al., 1965).

The low concentrations of urea in the large intestine are usually attributed to the presence of bacterial urease, which are produced by many colonic organisms, both aerobes and anaerobes, particularly nonsporing anaerobes, Peptostreptococcus productus and Proteus species (Donaldson, 1964; Wozny et al., 1977; Suzuki et al., 1979).

Demigne and Remesy (1979) have compared the contributions of the ileum and cecum to urea destruction in the rat, by measuring ammonia concentrations in the lumen and the rate of disappearance of urea from the blood supplying the organ. More urea was taken up by the cecum than by the ileum, and ammonia concentrations in the cecum were double those in the ileum (mean 4.8mmol). This work suggests that the small intestine contributes significantly to urea destruction, although on a smaller scale than the large intestine.

Large intestinal bacteria are also known to produce ammonia from non-urea sources, particularly from the amine and amide nitrogen of proteins, peptides and amino acids. In 1915, Gamble showed that feces outside the body generate large amounts of ammonia, and because more recent studies have demonstrated that normal feces contain virtually no urea (Wrong et al., 1965; Owens and Padovan, 1976), it is clear that this ammonia must be derived from some other sources. Vince et al. (1976) have carried these studies further and have shown that feces incubated anaerobically with good survival of their bacterial flora convert up to 25% of their total nitrogen to ammonia (an amount much greater than the normal fecal ammonia) and that this process is not prevented by sterilization with gamma-irradiation, so that it must be dependent on the activity of bacterial enzymes rather than living bacteria. Comparison of

different intestinal bacteria shows that Gram-negative anaerobes and clostridia are the most active ammonia producers (Vince and Burridge, 1980).

Weber and Veach(1979) measured blood ammonia coming from various parts of the dog gut and simultaneously determined tissue uptake from the blood of both urea and glutamine. They found that half the ammonia released into the portal blood stream was derived from the small intestine (cleansed before study) and could be accounted for by the disappearance of glutamine from the perfusing blood. The remaining ammonia came from the uncleansed large intestine: of this 42% could be accounted for by urea and 9% by glutamine taken up from the blood stream, leaving 49% derived from other sources-including mucosal ammonia derived from metabolites other than glutamine, and bacterial sources other than urea. This study has elegantly elucidated the various origins of portal venous ammonia.

The available evidence therefore indicates that the ammonia in the large intestine is mainly of bacterial origin, partly derived from urea but probably mainly from other nitrogenous substrates, with a minor contribution from non-bacterial sources. This ammonia in the GI tract may be used for microbial protein synthesis or may reenter the blood stream.

2. Effects of dietary probiotics on animal growth

The term probiotics originated from two Greek words meaning "for life" and contrasted with the term antibiotics which means "against life", and was first used by Parker (1974) to describe "Organisms and substances which contribute to intestinal microbial balance".

Over the last several years, considerable attention has been given to the use of probiotics in animal feeds (Fuller, 1989). Much of this interest has been due to the increased public awareness of, and objection to, the use of antibiotics as a growth promotant. Certain probiotics, such as lactic acid-producing bacteria, can help maintain a healthy microbial balance in the GI tract by "competitive exclusion" against pathogenic organisms (Nurmi and Rantala, 1973).

The beneficial effect of probiotics comes from Metchnikoff's (1910) original contention that the longevity of Bulgarian peasants was related to their consumption of large amounts of milk fermented with organisms such as Lactobacillus acidophilus. Nurmi and Rantala (1973) demonstrated the protective effect of some gut flora in young chickens. By dosing newly hatched chicks with feces from adult chickens, colonization of the cecum by Salmonella infantis was restricted. The responsible microorganism was anaerobic but was not specifically identified out of the 48 strains containing predominantly lactobacilli and streptococci (Rantala, 1974).

The inclusion of Latobacillus acidophilus in the feed or drinking water causes changes in the intestinal microflora to occur (Sandine et al., 1972; Speck, 1976). Gilliland (1979) showed that feeding humans unfermented milk containing cells of Lactobacillus acidophilus caused significant increase in the number of lactobacilli in their feces.

The beneficial effect of probiotics may be mediated by a direct antagonistic effect against specific groups of organisms, resulting in a decrease in numbers or by an effect on their metabolism or by stimulation of immunity.

Possible modes of action of probiotics (Fuller, 1989) are:

1) Suppression of viable count by ;
   (a) production of antibacterial compounds
   (b) competition for nutrients
   (c) competition for adhesion sites
2) Alteration of microbial metabolism
   (a) increased enzyme activity
   (b) decreased enzyme activity
3) Stimulation of immunity
   (a) increased antibody levels
   (b) increased macrophage activity

Features of a good probiotics (Fuller, 1989) should be:

1) A strain which is capable of exerting a beneficial effect on the host animal, e.g., increased growth or resistance to disease.
2) Non-pathogenic and non-toxic
3) Present as viable cells, preferably in large numbers, although we do not know the minimum effect dose.
4. Capable of surviving and metabolizing in the gut environment, e.g., resistant to low pH and organic acids
5. Stable and capable of remaining viable for long periods under storage and field conditions.

Improvement in the body weight gain and feed efficiency was found when turkey poults were fed a lactobacillus culture, and coliform and total aerobe counts in the intestine were reduced by feeding lactobacillus (Francis et al. 1978).

Chapman (1988) reported that probiotics, such as Lactobacillus acidophilus and Streptococcus faecium, can exert beneficial effects through "competitive exclusion". This means that probiotics actually compete for receptor sites or space along the intestinal wall with certain types of harmful bacteria. This "competitive exclusion" theory has recently been elaborated in a symposium on "Colonization Control of Human Bacterial Enteropathogens in Poultry" (see Mulder and Bolder, 1991).

3. Effects of dietary cellulose on animal growth and health

Cellulose is generally a major component of crude fiber. During the last two decades, there has been a revival of interest in the fiber content of foods and the effects of its intake on humans and animals. In addition to the increasingly well-documented effects fiber has on colonic function, it is clear that fiber is a nutrient of importance in its own, although it cannot be termed an essential nutrient. Fiber alters the digestion, absorption or subsequent metabolism of various nutrients (Cummings, 1978; Kelsay, 1978).

Several reports indicate that the microbial enzyme systems are inducible and can vary in different human populations (Goldin and Gorbach, 1976; Mastromarino et al., 1976; MacDonald et al., 1978). Intestinal ammonia concentration is known to be dependent on both protein level and fiber type. Feeding a 24%-casein diet containing 8% cellulose significantly decreased ammonia concentration in the proximal colon of rats over feeding a control or a diet containing 8% pectin (Lupton and Marchant, 1989).

Fiber is being used with increasing frequency to regulate gastrointestinal function in variety of disorders. This include an increase in stool output, dilution of colonic contents, a faster rate of passage through the gut and changes in the colonic metabolism of minerals and nitrogen (Kelsay, 1978).

It is thought that nonruminant animals derive some food energy from poorly digested polysaccharides by fermentation to VFA (Volatile Fatty Acids) in the large intestine. Microbial degradation of fiber is of particular interest. A mixed culture from turkeys (Bedbury and Duke, 1983) or pigs (Varel and Pond, 1985; Varel et al., 1987) fed a high fiber diet showed a significantly greater cellulolysis than that obtained from animals fed a low fiber diet.

4. Effects of dietary lactose on animal growth and intestinal fermentation

Lactose, a disaccharide which occurs only in mammalian milk, is hydrolyzed into its monosaccharides galactose and glucose by lactase secreted by the brush border of small intestinal mucosa. After hydrolysis galactose and glucose are actively absorbed. People belonging to northern European ethnic groups, including their descendants overseas (Caucasians) and to some isolated African and Indian tribes, maintain high intestinal lactase activity throughout life and these people can drink milk at all ages without any gastrointestinal problems (Scrimshaw and Murray, 1989). On the other hand, in the majority of the world's population, lactase activity falls to low levels at some time between early childhood and adolescence (Saavedra and Perman, 1989).

People with lactose intolerance generally consume less dairy products than those with no intolerance (Goodenough and Kleyn, 1976; Scrimshaw and Murray, 1989). Kim et al. (1978a, b) observed that more than 30% of dietary lactose was available for fermentation in the large intestine of rats and pigs, when they were fed a diet containing 30% lactose. The capacity of the large intestine to ferment lactose was tripled by feeding rats a diet containing 30% lactose (Kim et al., 1979) or almost doubled by feeding pigs a diet containing 40% dried whey (Kim et al., 1978c), as compared to that found in rats and pigs fed diets containing no lactose, respectively. Lactose is fermented into lactic acid in the large intestinal tract, in turn, lowering pH of the contents in pigs. The lowered pH reduced ammonia production by intestinal bacteria (Vince et al., 1973).

III. Materials and Methods

1. Animals and diets

Thirty male Sprague Dawley rats(mean initial weight, about 200g) were housed individually in suspended cages in a room maintained at 20 ~ 25℃ with 12-hr light (07:00 to 19:00) and 12-hr dark (19:00 to 07:00) cycle. Rats were fed a commercial diet supplemented with or without 0.1% probiotics(Biocerin; Bayer Vetchem (Korea) Ltd., Seoul, Korea) in Experiment 1, or were fed control (purified) or diets containing 10% cellulose or 20% lactose in Experiment 2. The composition of the experimental diet used for Experiment 2 is shown in Table 1. Six rats were assigned to each diet and were given ad libitum access to diets in feed cups for at least 14 days prior to being killed. They also had free access to water.

2. Incubation of intestinal contents

At the end of feeding period, five rats from different treatments were killed daily between day 15:00 and 19:00 until all of the experimental rats were used. The contents of small intestine (including mucosa) and cecum+colon were collected in 50-ml centrifuge tubes, weighed and diluted 1:2 (w/v) with 0.2M phosphate buffer (pH 6.5). (The small intestine was slit and homogenized with a Potter-Elvehjem homogenizer in ice and the homogenate, excluding the tough serosal tissue, was used for incubation).

Two 3-ml samples of diluted contents were transferred into 15-ml centrifuge tubes and 1ml of 0.4M urea solution containing 0.1μci of ^{14}C urea (Amersham International PK., Amersham, UK) was added to one sample, and 1ml of 0.4M urea solution was added to another sample and the mixture was inactivated with 0.4ml of 6N 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 2 min and then each unit was clamped sealed. The incubation was initiated within about 10 and 15 min after rats were killed for the contents of cecum + colon and small intestine, respectively.

At the end of the 30-min incubation, an air stream was pulled through the reaction chamber and CO_2 trap (5ml of 1:2, by volume, mixture of ethanolamine and ethylene glycol monomethylether), and 0.4ml of 6N H_2SO_4 was added to the inlet tube of the reaction chamber to stop the reaction and release CO_2. Over a 20-min period, CO_2 released was trapped by use of a gas dispersion tube. This technique allowed more than 98% recovery of the $^{14}CO_2$ generated from $Na_2{}^{14}CO_3$ instilled in the reaction chamber.

The $^{14}CO_2$ release from ^{14}C[urea] in the incubation was linear over the 30-min incubation when the $^{14}CO_2$ release was determined at 15min and 30 min. The inactivated samples were centrifuged at 600 x g for 1hr and the supernatants were collected into plastic vials and stored at 4℃ for ammonia analyses.

3. Determination of urease activity

The radioactivity in the CO_2 trap (1ml) was determined in 15ml of Aquasol (Du Pont, Boston, MA) using a Liquid Scintillation Counter (Wallac Dy, Turku, Finland). Total radioactivity in each sample was calculated. Urease activity (μmol of urea hydrolyzed/ 30 min per g or per total contents) was calculated by dividing radioactivity (dpm) recovered in CO_2 during the 30-min incubation by specific radioactivity (dpm/ mol) of urea added to samples, assuming that no significant amounts of urea from the contents contributed to the incubation. This value was divided by 0.9 to correct for unrecoverable CO_2 during a 30-min incubation and the corrected value was used as urease activity. The unrecoverable CO_2(10%) was observed in a preliminary experiment in which a known amount(radioactivity) of $Na_2{}^{14}CO_3$ was added to the large intestinal content sample and incubated for 30 min. This unrecoverable CO_2 may have been incorporated into the bacterial cells.

4. Determination of ammonia production

After the samples and blanks were centrifuged, supernatants of both blanks and incubated samples were collected. Ammonia concentrations in supernatants were determined by using a

colorimetric method (Weatherburn, 1967) with a miner modifications. Net ammonia production during the 30-min incubation was calculated by the difference in ammonia concentration between the blank and the incubated sample.

5. Statistical analysis

Data were subjected to analysis of variance (AGRISP, 1986). Significant differences were determined according to LSD (Steel and Torrie, 1960).

IV. Results and Discussion

Exp. 1. Effects of feeding a commercial diet with or without supplementary probiotics on urease activity and ammonia production in the intestinal contents of rats

No significant difference in weight gain or feed efficiency was found among the dietary treatments during a 2-wk feeding period (Table 2). By contrast, probiotics supplementation in chicken diets have been reported to have significant effects on growth (Dilworth and Day, 1978) and egg production (Miles et al., 1981). Baird (1977) obtained an increase in daily weight gain and an improvement in feed conversion in separate experiments with feeder pigs and growing-finishing pigs using a lactobacillus supplement. With the same probiotics, Pollmann et al. (1980) obtained a positive result with starter pigs but not with growing -finishing pigs.

Urease activity and ammonia production in the intestinal contents were not significantly ($P>0.05$) different between rats fed a commercial diet supplemented with or without 0.1% probiotics,

although urease activity tended to be lower in the large intestinal contents of rats fed diet containing probiotics (Tables 3). In contrast, Kim and Kim (1992) found that urease activity in the large intestinal contents of rats fed a purified diet containing probiotics was significantly lower than that found without supplementary probiotcs. The difference between results of the two studies may have been due to the difference in experimental animals, seasons or types of diets used.

Urease activity (μmol of urea hydrolyzed/30 min per total contents) in the large intestinal contents was much higher than in the small intestinal contents (Table 3). Similar results have been reported by Stutz and Metrokotsas (1972), who showed that urease activity in the cecal contents accounted for 99% of the total activity in the GI contents of the chicken. Demigne and Remesy (1979) compared the contributions of the ileum to urea destruction in the lumen and the rates of disappearance of urea from the blood circulating the organ. A greater amount of urea was taken up by the cecum than by the ileum, and ammonia concentration in the cecum was twice that in the ileum.

Exp 2. Effects of dietary cellulose or lactose on urease activity and ammonia production in the intestinal contents of rats

Average daily gain was not significantly different among rats fed the control or diets containing 10% cellulose and 20% lactose during a 2-week feeding period, but feed consumption was significantly ($P<0.05$) increased by feeding diet containing 10% cellulose (Table 5). A similar result has been shown when rats were fed a control or a diet containing 30% lactose (Kim et al., 1978a).

Urease activity and ammonia production in the large intestinal contents were significantly ($P<0.05$) lower in rats fed diet containing 10% cellulose than in rats fed the control diet or diet containing 20% lactose (Tables 6 and 7, respectively). Although feeding diet containing lactose seemed to decrease urease activity and net ammonia production per g of large intestinal contents over the control, only net ammonia production was significantly ($P<0.05$) decreased.

Attempts have been made to reduce urease activity or ammonia production in the GI tract. Feeding a diet containing fiber to rats (Lupton and Marchant, 1989) or a diet containing lactulose to humans (Weber and Veach, 1979) has been known to decrease urease activity or ammonia production (or increase ammonia utilization by bacteria) in the GI tract.

As shown in Figure 1, net ammonia production (y), expressed as μmol/30 min per g large intestinal content was highly correlated with 2 x urease activity (x): $y = 1.78x + 90.3$ ($r = 0.78$); $y = 1.23x + 73.7$ ($r = 0.60$); and $y = 1.26x + 79.7$ ($r = 0.83$) for rats fed the control diet and diet containing cellulose or lactose, respectively.

Urease is produced by intestinal microflora; e.g., bacteroides, bifidobacteria, clostridia, proteus spp, and klebsilla spp. The growth-promoting effects of subtherapeutic levels of antibiotics used for animal feeds have been attributed to inhibited urea hydrolysis and subsequently reduced ammonia production in the GI tract of animals (Visek, 1978).

Interestingly, urease has been known to play an essential role in pathogenesis of gastritis induced by Helicobacter pylori and a urease-negative strain did not cause gastritis in gnotobiotic piglets (Smoot et al., 1990; Eaton et al., 1991). Similarly, the generation of ammonia in the rat stomach following instillation of urea in the presence of urease resulted in deleterious influence on the rat gastric mucosa, including stasis of microcirculation, disruption of the surface epithelial cells and necrosis of the mucosa (Murakami et al., 1990). These reports suggest that ammonia locally produced by ureolysis in the intestinal mucosa can exert a significant damage to the surface cells, disturbing nutrient absorption and metabolism.

Ammonia production in the GI tract results from hydrolysis of urea, and deamination of proteins (amino acids) and other nitrogenous compounds. Normal feces contain no urea (Wrong et al., 1965; Owens and Padovan, 1976), but they produce large amounts of ammonia (Gamble, 1915), indicating that ammonia is derived from some sources other than urea. Peptides and amino acids are known to be deaminated by intestinal bacteria (Phear and Ruebner, 1956; Francois and Michel, 1964; O'Grady, 1966; Vince et al., 1973; Vince and Burridge, 1980).

Although intestinal bacteria produce ammonia from many nitrogenous sources, they are also capable of utilizing ammonia as a nitrogen source for their own amino acid and protein needs. All bacteria have this potentiality, the primary reaction being one of reductive fixation of ammonia under the action of glutamate dehydrogenase (Dawes and Large, 1973). In most circumstances the utilization of ammonia by bacteria in the large intestine proceeds more slowly than the bacterial generation of ammonia.

Results of the present studies together with other studies do suggest that dietary cellulose help maintain healthy intestinal environment, enhancing the growth of cellulolytic bacteria and maybe inhibiting the urease-producing bacteria and ammonia production.

V. Summary

Experiments were done to determine the effect of feeding a diet supplemented with or without 0.1% probiotics (Experiment 1), or a control diet or diets containing 10% cellulose or 20% lactose (Experiment 2) on weight gain and feed efficiency, and urease activity and ammonia production in the intestine of rats.

Average daily gain of rats fed a commercial diet supplemented with or without probiotics, or a purified diet or diets containing cellulose or lactose, over a two-week period were not different. No differences were found in urease activity and net ammonia production between the intestinal contents of rats fed a commercial diet supplemented with or without probiotics, although urease activity and net ammonia production in the large intestinal contents tended to be lower in rats fed a diet supplemented with probiotics.

Feeding a diet containing cellulose significantly ($P<0.05$) depressed urease activity (13.9 vs 48.4 μmol of urea hydrolyzed/30 min per g content of the control) in the large intestinal contents over the control. Regression analysis showed that net ammonia production was highly correlated with urease activity in the large intestinal contents.

The results of the present experiments indicated that feeding diets containing cellulose reduces urease activity and ammonia production in the gastrointestinal tract, and thus can be beneficial for animals to maintain healthy microbial balance in their gastrointestinal tract.

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Table 1. Composition of experimental diets(% on an air-dry basis) - Exp. 2.

Ingredient	Control	Cellulose	Lactose
Casein(1)	20.0	20.0	20.0
L-Methionine(1)	0.3	0.3	0.3
Corn oil(2)	5.0	5.0	5.0
Choline chloride(3)	0.2	0.2	0.2
Vitamin mix(4)	0.5	0.5	0.5
Salt mix(4)	5.0	5.0	5.0
α-Cellulose(5)	-	10.0	-
Lactose(1)	-	-	20.0
Corn starch(6)	69.0	59.0	49.0

(1) United States Biochemical Corp., Cleveland, OH.
(2) Jeil Jedang Co., Seoul, Korea
(3) Fisher Scientific Co., Fair Lawn, NJ.
(4) Rodgers, Q.R. & A.E. Harper. 1965. J. Nutr. 87:267
(5) Sigma Chemical Co., St Louis, MO.
(6) Sunil Pododang Co., Seoul, Korea.

Table 2. Effects of feeding diets supplemented with or without probiotics on body weight gain and feed efficiency '- Exp. 1

Item	Control	Probiotics
Initial weight, g	199.5 ± 18.8	201.7 ± 13.3
Final weight, g	277.1 ± 16.6	272.0 ± 15.4
Avg daily gain, g	5.5 ± 0.6	5.0 ± 0.7
Feed consumption, g	300.1 ± 13.9	290.3 ± 12.9
Feed efficiency	0.26 ± 0.02	0.24 ± 0.03

'Values are means ± SEM of 6 rats.

Table 3. Effects of feeding diet supplemented with or without probiotics on urease activity in the intestinal contents of rats ' - Exp. 1

Diet	Small intestine		Large intestine	
	per g	per total	per g	per total
	μmol of urea hydrolyzed / 30min at 37℃			
Control	2.23±0.65	7.04±2.59	68.5±18.7	269±86
Probiotics	3.47±0.99	11.43±3.60	48.1± 7.5	164±10

'Values are means ± SEM of 6 rats.

Table 4. Effects of feeding diet supplemented with or without probiotics on ammonia production in the intestinal contents of rats '— Exp. 1

Diet	Small intestine			Large intestine		
	Before incubation	After incubation	Net production	Before incubation	After incubation	Net production
	μmol of urea hydrolyzed / 30 min at 37℃					
Control	12.0 ± 1.84	24.6 ± 3.59	12.6 ± 2.46	32.1 ± 5.58	234.4 ± 40.3	202.0 ± 39.2
probiotics	12.2 ± 2.29	30.4 ± 5.04	18.7 ± 2.94	27.8 ± 4.62	202.0 ± 26.9	174.5 ± 27.4

'Values are means ± SEM of 6 rats.

Table 5. Effects of feeding diet containing cellulose and lactose on body weight gain and feed efficiency '— Exp. 2

Item	Control	Cellulose	Lactose
Initial weight, g	198 ± 10	201 ±14	202 ± 8
Final weight, g	253 ± 14	261 ± 9	238 ±14
Avg daily gain, g	3.8 ± 0.5	4.2 ± 0.5	2.5 ± 0.5
Feed consumption, g	218 ± 12[b, 2]	269 ± 6[a]	197 ± 9[b]
Feed efficiency	0.25± 0.02	0.22± 0.03	0.18± 0.04

' Values are means ± SEM of 6 rats.

' Values in the same raw with different superscript letters differ significantly ($P<0.05$).

Table 6. Effects of feeding diets containing cellulose and lactose on urease activity in the intestinal contents of rats '- Exp. 2

Diet	Small intestine		Large intestine	
	per g	per total	per g	per total
	μmol of urea hydrolyzed/30min at 37℃			
Control	1.25±0.65	2.46±1.31	48.4± 8.1[a,2]	106.8±15.8
Cellulose	1.27±0.27	2.87±0.60	13.9± 1.9[b]	37.0± 7.9
Lactose	1.08±0.25	2.21±0.45	32.6±11.3[ab]	132.8±65.0

' Values are means ± SEM of 6 rats.

' Values in the same column with different superscript letters differ significantly ($P<0.05$).

Table 7. Effects of feeding diets containing cellulose and lactose on ammonia production in the intestinal contents of rats '— Exp. 2

Diet	Small intestine			Large intestine		
	Before incubation	After incubation	Net production	Before incubation	After incubation	Net production
	μmol of urea hydrolyzed / 30 min at 37℃					
Control	10.5 ±0.46	16.1 ±1.82	5.52 ±0.53	58.6 ±18.25	269[a, 2] ±21.40	210[a, 2] ±30.5
Cellulose	10.1 ±1.06	18.3 ±2.93	8.24 ±2.00	42.8 ± 7.42	129[b] ± 6.93	86.4[b] ± 5.30
Lactose	11.04 ±1.23	17.4 ±1.52	6.32 ±1.73	45.2 ± 2.90	175.2[b] ±34.7	130[ab] ±32.1

'Values are means ± SEM of 6rats.

'Values in the same column with different superscript letters differ significantly ($P < 0.05$).

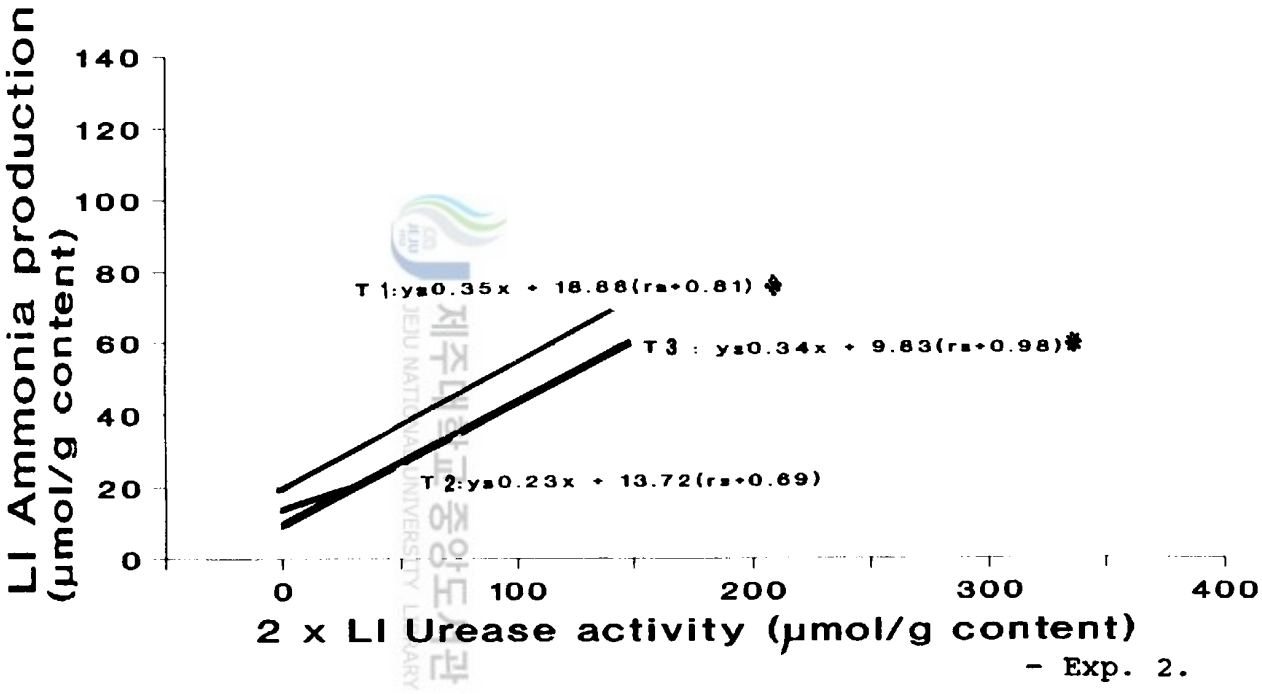

Figure 1. Relationship between net ammonia production and 2 x urease activity per g of the large intestinal contents - Exp. 2.
*: P<0.05

T1:control diet T2:10% cellulose diet T3:20% lactose diet

謝　　辭

大學生活을 통하여 얻었던 조그마한 知識과 結實을 엮어서 한편의 論文을 완성함으로서 더 깊은 學問의 세계로 나아가기위한 발판으로 삼으려고 합니다.

이 論文은 부모님의 각별하신 관심과, 단점투성이인 저에게 정성어린 가르침을 주셨던 指導教授 김규일 박사님의 도움의 結果라고 생각합니다. 그리고 이論文이 만들어지기 까지 많은 激勵와 忠告를 주셨던 정창조 교수님과 강정숙 교수님께 감사드리며, 實驗室에서 同苦同樂하며 實驗을 도와주었던 營養學 實驗室의 김태욱씨, 박학문씨, 양용호씨 에게도 辭意를 표합니다.

아울러 濟州試驗場 姜泰洪 場長님 이하 모든 職員에게도 감사드리며, 특히 원고정리에 힘써준 양 진씨, 김영주씨, 김양희씨에게도 고마움을 전합니다.

끝으로 이 論文이 어려운 여건중에도 온갖 精誠을 쏟아준 아내 김향선, 사랑스런 아들 승조에게 조그만 선물이 되었으면 합니다.

1992년 2 월