碩士學位論文

쥐에서 有機酸, 生菌劑 또는 抗菌劑를 含有한 飼料의 給與가 腸內 尿素分解酵素 活性 및 암모니아 生産에 미치는 影響

濟州大學校 大學院

畜 産 學 科

金　　泰　　旭

1991年　12月

A Thesis

for the Degree of Master of Science

EFFECTS OF FEEDING DIETS CONTAINING ORGANIC ACIDS, A PROBIOTICS, OR AN ANTIMICROBIAL AGENT ON UREASE ACTIVITY AND AMMONIA PRODUCTION IN THE INTESTINAL CONTENTS OF RATS

by

TAEWOOK KIM

DEPARTMENT OF ANIMAL SCIENCE

GRADUATE SCHOOL

CHEJU NATIONAL UNIVERSITY

1991

EFFECTS OF FEEDING DIETS CONTAINING ORGANIC ACIDS, A PROBIOTICS, OR AN ANTIMICROBIAL AGENT ON UREASE ACTIVITY AND AMMONIA PRODUCTION IN THE INTESTINAL CONTENTS OF RATS

Tae-Wook Kim

(Under the Supervision of Professor Kyu-Il Kim)

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

DEPARTMENT OF ANIMAL SCIENCE

GRADUATE SCHOOL

CHEJU NATIONAL UNIVERSITY

1991. 12

쥐에서 有機酸, 生菌劑 또는 抗菌劑를 含有한 飼料의 給與가 腸內
尿素分解酵素 活性 및 암모니아 生産에 미치는 影響

指導敎授 金 圭 鎰

金 泰 旭

이 論文을 農學碩士學位 論文으로 提出함.

1991年 12月 日

金泰旭의 農學碩士學位 論文을 認准함.

審査委員長 鄭 昌 朝
委 員 金 圭 鎰
委 員 梁 榮 勳

濟州大學校 大學院

1991年 12月

CONTENTS

요 약

성숙한 Sprague Dawley 잡종쥐 (평균 체중 212g, 처리당 6마리)에게 대조, 또는 대조사료에 유산, fumaric acid, 생균제 (Biocerin), 항균제 (Mecadox)를 각각 첨가한 사료를 최소 21일간 먹인후 소장 (점막층 포함) 및 대장 내용물을 채취, pH 6.5 완충액으로 희석한 후, C^{14}요소를 함유한 요소용액을 첨가하여 37℃에서 30분간 배양, 요소분해효소의 활성 (요소 μmol/30분 per g 또는 전체 내용물)과 암모니아 생산율 (μmol/30분 per g 내용물)을 측정하였다. 대조구에 비교하여 유기산 첨가구에서는 요소분해효소의 활성과 암모니아 생산율 모두 유의차를 볼 수 없었다. 그러나 생균제 또는 항균제 첨가구에서는 대장내용물에서의 요소분해효소의 활성이 억제되었다 ($P<0.05$). 암모니아 생산율의 경우에는, 항균제 처리구가 약간의 감소효과를 보였으며 ($P<0.07$) 그 외의 처리구는 대조구와 유의차가 없었다. 생산된 암모니아중 요소분해로 인하여 발생한 암모니아가 차지하는 비율은 대조구, 또는 유산, fumaric acid, 생균제, 항균제 첨가구에서 각각 28.3, 34.2, 26.4, 18.3, 24.5% 이였다. 생균제는 대장 내용물중 요소분해효소를 약 41% 억제하였고, 암모니아 생산율을 약 8% 억제하여 요소분해효소를 생산하는 세균을 선택적으로 억제하였음을 말해준다. 쥐에게 일반사료를 급여하여 하루동안의 요소분해효소의 활성을 측정해 본 결과, 22:00에 측정한 대장 내용물중 요소분해효소의 활성이

하루중의 다른 시간에서 측정한 것보다 낮았다.

본 실험 결과는, 쥐의 대장 내용물중 요소분해효소의 활성이 사료 섭취후 경과시간에 따라 변한다는 사실과, 생균제 또는 항균제는 쥐의 대장 내용물중 요소분해와 암모니아 생산을 억제하며 이는 장내 유해균의 번식을 억제한다는 사실을 암시해준다.

Ⅰ. INTRODUCTION

Ammonia produced from the amino acid degradation in the body is 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). This ammonia together with that produced by bacteria acting on nitrogenous substrates in the GI tract may be used for microbial protein synthesis or may re-enter the blood. Ammonia is one of the microbial products that is a recognized toxin in warm-blooded animals (Lin and Visek, 1991).

The enzyme urease involved in the degradation of urea is produced by some species of microbes in the GI tract, which are considered to be harmful to the host. Attempts have been made to reduce urease activity or ammonia production in the GI tract by dietary manipulations. The additidn of organic acids, such as lactic, fumaric, acetic, and citric, has been shown to be of benefit for improving both growth rate and feed efficiency by lowering pH of the GI contents in pigs (Easter, 1988a). Certain probiotics, such as lactic acid producing bacteria, can help maintain a favorable microbial profile in the gut by "competitive exclusion" (as described later on) against

pathogenic organisms (Chapman, 1988). Adding Aureo SP250, one of the antimicrobial agents, reduced the number of ureolytic oganisms in animals fed a basal diet (Varel et al., 1987).

The present studies were conducted to determine the diurnal variations of urease activity (Experiment 1) and to determine the effects of feeding diets containing organic acids (lactic acid, fumaric acid), a probiotics or an antimicrobial agent on urea degradation and ammonia production (Experiment 2) in the intestinal contents of adult rats.

Ⅰ. LITERATURE REVIEW

1. Urea and ammonia metabolism in the GI tract

The normal metabolic pathways for urea are as follows: Urea formation takes place in the liver from dietary and endogenous amino acids. The urea is disposed of either by excretion in the urine or by hydrolysis in the GI tract. Nitrogen derived from urea hydrolysis in the bowel may either be used for microbial protein synthesis or re-enter the blood, then re-formed into urea or used for synthetic activities by being incorporated into amino acids and other compounds (Jackson et al., 1990). Ammonia generated from the hydrolysis of urea causes damages in the GI mucosa (Murakami, 1990). Therefore, in aspects of its toxicity and decreasing energy efficiency, ammonia is a harmful substance to animals and environments. In an attempt to decrease urease activity and ammonia production in the GI contents, many feed additives have been examined (Visek, 1978).

2. Effects of supplementary organic acids on animal growth

The dietary inclusion of organic acid was effective in lowering the pH of the GI contents and improving performance of early-weaned pigs (Burnell et al., 1988). Addition of lactic acid (Easter, 1988b) or fumaric acid (Giesting and Easter, 1991a; Giesting et al., 1991b) to diets improved weight gain and feed efficiency. In general, young pigs can not produce sufficient amount of hydrochloric acid to maintain proper gastric pH. Thus, acidification of diets is known to be beneficial for young pigs.

The relationship between organic acid and urease activity is still not clear. The effects of fermentable carbohydrates not digestible in the small intestine on urease activity or ammonia production have been reported. Vince and Burridge (1980) reported that feeding lactose or lactulose decreased ammonia production and lowered pH of the intestinal contents of humans. Weber (1979) suggested that lactulose might alter bacterial flora in the gut, reducing the number of urea-splitting organisms.

3. Effects of supplementary probiotics on animal growth

Over the last several years, considerable attention has been given to the use of probiotics for animal feeds. Much of this interest has

been due to the increased public awareness of and objection to the use of antibiotics as growth promotants.

Beneficial actions of probiotics have been suggested as follows (Pollman, 1986):

(1) change in enteric flora and reduction of E. coli;

(2) synthesis of lactate with subsequent reduction in intestinal pH;

(3) adhesion or colonization of the microbes in the digestive tract;

(4) production of antibiotic substances; and

(5) reduction of toxic amines and ammonia levels in the GI tract and blood.

Probiotics can be classified into two major types - viable microbial cultures and microbial fermentation products. Most of the research has been conducted with *Lactobacillus* species, *Bacillus subtilis* and *Streptococcus faecium*. Macbeth et al. (1965) reported that supplement of *Lactobacillus acidophilus* lowered fecal urease activity and blood ammonia levels in humans.

4. Effects of supplementary antimicrobial agents on animal growth

Antimicrobial agents have been significant in reducing costs of animal production and have given new insights into the influence of the intestinal flora upon the host. A number of mechanisms that have been proposed to explain the growth stimulatory effects of antimicrobial agents are as follows (Visek, 1978):

(1) microorganisms responsible for mild but unrecognized infections are suppressed;

(2) microbial production of growth depressing toxins is reduced;

(3) antimicrobial agents reduce microbial destruction of essential nutrients in the GI tract or that there is increased synthesis of vitamins or other growth factors; and

(4) there is enhanced efficiency of absorption and utilization of nutrients because the wall of the intestinal tract becomes thinner when fed antibiotics.

When antibiotics (sulfaguanidine, terramycin, and penicillin) were added to diets, urease activity of the GI tract of mice was significantly decresed (Dintzis and Hastings, 1953), and Aureo SP250 (chlorotetracycline, sulfamethazine, and penicilline) markedly reduced the number of *Streptococcus spp.* which made 74% of the ureolytic isolates from the pigs (Varel et al., 1987). Monensin caused a large

decrease in ammonia production in mixed ruminal microorganisms *in vitro* (Chen and Russel, 1991).

Ⅰ. MATERIALS AND METHODS

1. Animals and diets

Thirty male Sprague Dawley × Fisher rats (mean initial weight 212g) were housed individually in suspended wire 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 chow (see footnote1 of Table 2) *ad libitum* in Experiment 1 or were fed a control or diets containing 2.4% lactic acid, 2.8% fumaric acid, 0.1% probiotics (Biocerin; Bayer Vetchem(Korea) Ltd., Seoul, Korea), or 1% antimicrobial agent (Mecadox, Pfizer Agricultural Division, New York, NY) in Experiment 2. The composition of the experimental diets used for Experiment 2 is shown in Table 1. In Experiment 1, diurnal variations of urease activity were determined by killing 5 rats at 04:00, 10:00, 16:00, and 22:00 hours. For Experiment 2 done to determine the effects of dietary organic acids, probiotics, and antimicrobial agents on urease activity and ammonia production in the rat intestinal contents, 6 rats were assigned to each diet and fed in feed cups *ad libitum* for at least 21 days prior to being killed. They

had free access to water.

2. Incubation of the intestinal contents

At the end of feeding period, three rats from different treatments were killed each day until finished. 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 0.2 M phosphate buffer (pH 6.5). Two 3-ml samples of diluted contents were transferred into 15-ml centrifuge tubes and 1 ml of 0.4 M urea solution containing 0.1 μC_i of C^{14}-urea (Amersham International Plc., Amersham, UK) was added to one sample, and 1 ml of water was added to another sample and the mixture was inactivated with 0.4 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 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 (5 ml of 1:2, by volume, mixture of ethanolamine and ethylene glycol monomethylether), and 0.4 ml of 6 N 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 CO_2 generated in the reaction chamber. The CO_2 release was linear over the 30-min incubation . The inactivated samples were centrifuged at 600 × g for 1 hr and the supernatants were collected into plastic vials and stored at 4℃ for later analyses.

3. Determination of urease activity

The radioactivity in the CO_2 trap (1 ml) was determined in 15 ml of Aquasol (Du Pont, Boston, MA) using a Liquid Scitillation Counter (Wallac Oy., 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/μmol) of urea added to samples assuming that no significant amount of urea contributed to the incubation by urea from the contents.

4. Determination of ammonia production

After the samples and blanks were centrifuged, supernatants of both blank and incubated samples were collected only for Experiment 2. Ammonia concentrations in supernatants were determined by using a colorimetric method (Weatherburn, 1967) with a minor modification.

5. Statistical analysis

Data were analyzed by analysis of variance using MINITAB (Minitab Inc., 1987). Significant differences were reported according to Student's t - test ($P<0.05$).

Ⅳ. RESULTS AND DISCUSSION

1. Diurnal variations of urease activity in the intestinal contents of rats - Experiment 1

Urease activity in the rat intestinal contents (per g) is given in Table 2. Urease activity was significantly lower at 22:00 than that measured at the other times of a day. Since 22:00 was 3 hr after the start of the dark period, the GI tract was filled with digesta because rats usually consume most of their feed during the dark. We assume that the lower urease activity was due to the dilution of the bacteria with the digesta and also due to the sufficient supply of nutrients needed by intestinal microflora without hydrolysis of urea.

2. Effects of dietary organic acids, probiotics, and antimicrobial agents on urease activity and ammonia production - Experiment 2

Significant differences in urease activity in the contents of the large intestine were found between the control and rats fed diets

containing Biocerin or Mecadox, but diets containing either lactic acid or fumaric acid had no effect, compared with the control diet (Table 3).

Bacteria are the main source of ammonia in the GI tract (Phillips et al., 1952), and contribute significantly to the portal blood ammonia concentration through deamination of ingested protein and urea hydrolysis illustrated by experiments with antibiotics (Silen et al., 1955). The probiotics (Biocerin) and the antimicrobial agent (Mecadox) appeared effective in depressing urea-degrading bacteria in the intestinal tract.

The result that diets containing either lactic acid or fumaric acid had no effect, might be due in part to an already low urease activity in the control rats (only 3.65% of urea added were hydrolyzed in the contents of large intestine). Karasawa (1989) reported that 43% of urea-nitrogen or 25% of uric acid-nitrogen was converted to ammonia-nitrogen in the cecal contents of cockrel.

Urease activity (μmol of urea hydrolyzed / 30 min per total contents) in the contents of the large intestine was much higher than that of the small intestine (24.38 ± 4.4 vs. 1.63 ± 0.27). Stutz and Metrokotsas (1972) reported that urease activity of cecal contents accounted for 99% of the total urease activity in the contents of the

digestive tract of the chicken. Demigne and Remesy (1979) have compared the contribution of the ileum and cecum to urea destruction in the rat, measuring ammonia concentration 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 concentration in the cecum was twice that in the ileum. In the present studies, ammonia concentration (μmol per g contents) before incubation (blank) was 4.50 ± 0.73 and 28.37 ± 3.65, and net ammonia production during the 30-min incubation was nearly zero and 95.5 ± 15.2 for the contents of small and large intestine, respectively.

As shown in Table 4, net ammonia production was slightly decreased in the contents (per g) of the large intestine by feeding diets containing fumaric acid, Biocerin, or Mecadox as compared to that in the control. Among them, the Mecadox diet showed the greatest effect ($P<0.07$). Wolpert et al. (1970) showed that administration of neomycin halved the ammonia concentration in the colon of patients with cirrhosis. No differences in net ammonia production in contents of small intestine were found between the control and the treatments (data not shown).

The difference between the net ammonia production and two times the urease activity (one mole of urea releases two moles of ammonia

when hydrolyzed) is ammonia from non-urea-N origin. In these studies, ureolysis accounted for 28.3, 34.2, 26.4, 18.3, or 24.5% of net ammonia production in the large intestinal contents of rats fed the control, lactic acid, fumaric acid, Biocerin, or Mecadox diet, respectively. Ammonia production in the intestinal tract results from either hydrolysis of urea or from deamination of proteins (amino acid) and other nitrogenous compounds. Many intestinal microflora, e.g., bacteroids, bifidobacteria, clostridia [some species of this are known to depress growth of ckickens (Lev et al., 1957) and the growth depression is counteracted by addition of 45 mg penicillin per kg of diet (Coates et al., 1963)], *Proteus spp.*, and *Klebsilla spp.* produce urease. Others notably E. coli do not, so that ammonia produced by E. coli will be by deamination rather than ureolysis (Lev and Forbes, 1959). Studies on ammonia production from non-urea sources, e.g., peptides and amino acids, by intestinal bacteria have been reported (Vince et al., 1973; O'Grady, 1966). Because feces outside the body produce large amounts of ammonia (Gamble, 1915) and normal feces contain no urea (Owens and Padovan, 1976), it is obvious that this ammonia in the feces must be derived from some other sources than urea. Intravenous infusions of N^{15}-urea have been used to determine what proportion of intestinal ammonia is derived from urea. From the

results of a single injection experiment, Nolan et al. (1976) calculated that only 30% of the ammonia in the sheep cecum was derived directly from the plasma urea.

Intestinal bacteria produce ammonia from many different nitrogenous sources and they are also capable of utilizing ammonia as a nitrogen source for their own amino acid and protein synthesis. 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 which is occurring simultaneously.

Interestingly, when the Biocerin diet was compared with the control, the percentage of decrease in urease activity (41.0%) was much higher than that in net ammonia production (8.5%), and ureolysis accounted for only 18.3% (vs. 28.3%) of net ammonia production. The result suggested that feeding diets supplemented with Biocerin depressed more selectively urea-degrading bacteria rather than ammonia-producing bacteria. Chapman (1988) reported that certain probiotics, such as *L. acidophillus* and *S. faecium* can exert beneficial effects through "competitive exclusion". This means that the

probiotics actually compete for receptor sites or space along the intestinal wall with certain types of pathogenic organisms. This theory was supported by the result found in the present study that probiotics selectively depressed intestinal bacteria.

The results of the present studies collectively indicate that supplementary probiotics or antimicrobial agent in diets reduces ureolysis and perhaps ammonia production in the intestinal tract especially in the large intestine, but organic acids do not show significant effects. Such effects can vary depending on animals and environments, e.g., how heavily animals are contaminated with bacteria that produce urease, and what levels of dietary protein (Levenson and Tennant, 1963) and what kinds and perhaps levels of additives in diets (Francois and Michel, 1964) were used.

Table 1. Composition of experimental diets (%) - Experiment 2

Ingredient	Diet				
	Control	Lactic acid	Fumaric acid	Biocerin	Mecadox
Casein[a] (vitamin-free)	20	20	20	20	20
L-methionine[a]	0.3	0.3	0.3	0.3	0.3
Corn oil[b]	5	5	5	5	5
Choline chloride[c]	0.2	0.2	0.2	0.2	0.2
Vitamin mix[d]	0.5	0.5	0.5	0.5	0.5
Salt mix[d]	5	5	5	5	5
Lactic acid[e]	--	2.4	--	--	--
Fumaric acid[e]	--	--	2.8	--	--
Biocerin[f]	--	--	--	0.1	--
Mecadox[g]	--	--	--	--	1
Corn starch[h]	69	66.6	66.2	68.9	68

Table 1. Composition of experimental diets (%) - *Continued.*

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

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

[c] Fisher Scientific Co., Fair Lawn, New Jersey.

[d] Rodgers, Q.R. & A.E. Harper. 1965. J. Nutr. 87 : 267.

[e] Sigma Chemical Co., St.Louis, MO.

[f] *Bacillus toyoi* spore, Bayer Vetchem(Korea) Ltd., Seoul, Korea.

[g] 0.02% carbodox, Pfizer Agricultural Division, New York, NY.

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

Table 2. Diurnal variations of urease activity in the intestinal contents of rats - Experiment 1[a]

	Large intestine		Small intestine	
Time	per g	per total	per g	per total
	μmol of urea hydrolyzed / 30 min at 37℃			
04:00	64.22 ± 4.74[b]	225.8 ± 28.0[b]	2.19 ± 1.13	9.16 ± 5.32
10:00	62.58 ± 9.70[b]	165.2 ± 40.6[b]	0.98 ± 0.24	3.06 ± 0.57
16:00	62.43 ± 6.57[b]	158.7 ± 17.6[b]	1.53 ± 0.33	4.24 ± 0.98
22:00	42.99 ± 4.67[c]	104.2 ± 18.6[c]	0.94 ± 0.24	3.87 ± 1.19

[a] Values are means ± SEM of 5 rats fed a commercial chow (crude protein 15%; Daeje Feed Co., Cheju, Korea).

[bc] Values in the same column with different superscripts differ significantly ($P<0.05$).

Table 3. Effects of feeding diets containing organic acids, a probiotics, or an antimicrobial agent on urease activity in the intestinal contents of rats - Experiment 2[a]

	Small intestine		Large intestine	
Diet	per g	per total	per g	per total
	μmol of urea hydrolyzed / 30 min at 37℃			
Control	0.593±0.14	2.13±0.62	14.60±1.6[b]	29.62±2.7[b]
Lactic acid	0.540±0.8	1.65±0.28	20.16±3.1[b]	34.60±6.7[b]
Fumaric acid	0.540±0.09	1.68±0.13	12.61±3.4[b]	25.10±7.1[b]
Biocerin	0.503±0.09	1.60±0.27	8.61±2.0[c]	16.22±3.7[c]
Mecadox	0.382±0.02	1.11±0.07	8.14±0.8[c]	16.34±1.8[c]

[a] Values are means ± SEM of 6 rats.

[bc] Values in the same column with different superscripts differ significantly ($P<0.05$).

Table 4. Effects of feeding diets containing organic acids, a probiotics or an antimicrobial agent on ammonia production per g of the large intestinal contents of rats - Experiment 2[a]

Diet	Before incubation	After incubation	Net production[b]
	μmol of ammonia per g contents		
Control	30.12 ± 4.09	133.2 ± 14.1	103.1 ± 13
Lactic acid	29.22 ± 2.92	147.2 ± 26.4	118.0 ± 24
Fumaric acid	28.78 ± 4.48	124.5 ± 16.3	95.7 ± 15
Biocerin	30.37 ± 4.58	124.7 ± 19.0	94.3 ± 15
Mecadox	23.39 ± 2.21	89.8 ± 9.75	66.4 ± 9

[a] Values are means ± SEM of 6 rats.

[b] After - before incubation (30 min at 37℃).

V. ABSTRACTS

Studies were done to determine diurnal variations of urease activity and to determine effects of feeding diets containing organic acids (lactic acid, fumaric acid), a probiotics (Biocerin), or an antimicrobial agent (Mecadox) on *in vitro* urease activity and ammonia production in the rat intestinal contents. Adult Sprague Dawley × Fisher male rats (mean initial weight, 212g) were fed a commercial diet for determination of the diurnal variations of urease activity, or a contol or diets containing 2.4% lactic acid, 2.8% fumaric acid, 0.1% Biocerin, or 1% Mecadox for determination of the dietary effects on urease activity and ammonia production. Urease activity was significantly lower at 22:00 than that measured at the other times of a day. Feeding the organic acid diets did not show significant effects on urease activity (μmol of urea hydrolyzed / 30 min per g or per total contents) or net ammonia production (μmol / 30 min per g contents). But urease activity per g or per total of large intestinal contents was significantly ($p<0.05$) decreased by feeding diets supplemented with Biocerin or Mecadox. No significant effect of the supplementary Biocerin or Mecadox on net ammonia production was found.

Ureolysis accounted for 28.3, 34.2, 26.4, 18.3, or 24.5% of net ammonia production in the large intestinal contents of rats fed the control, lactic acid, fumaric acid, Biocerin, or Mecadox diet, respectively. Feeding diet supplemented with Biocerin reduced urease activity more selectively than ammonia production. The results showed that urease activity in the rat intestinal contents varies during a day possibly with time after feeding, and that dietary probiotics and antimicrobial agent reduce urease activity and perhaps ammonia production in the rat intestinal contents.

REFERENCES

Burnell, T.W., G.L. Cromwell, and T.S. Stahly. 1988. Effects of dried whey and copper sulfate on the growth response to organic acid in diets for weanling pigs. J. Anim. Sci. 66 : 1100 - 1108.

Chapman, J.D.. 1988. Probiotics, acidifiers and yeast culture: A place for natural additives in pig and poultry production. In: Biotechnology in the feed industry (Lyons, T.P., ed.), pp. 219 - 223, Alltech Technical Publications, Kentucky.

Chen, G., and J.B. Russell. 1991. Effect of monensin and a protonophore on protein degradation, peptide accumulation, and deamination by mixed ruminal microorganisms *in vitro*. J. Anim. Sci. 69 : 2196 - 2203.

Coates, M.E., R. Fuller, G.F. Harrison, M. Lev, and S.F. Suffolk. 1963. A comparison of the growth of chicks in the Gustafsson germ-free apparatus in a conventional environment, with and without dietary supplements of penicillin. Br. J. Nutr. 17 : 147 - 153.

Dawes, E.A. and P.J. Large. 1973. Class II reactions: Synthesis of small molecules. In: Biochemistry of Bacterial Growth

(Mandelstam, J. and K. Mcquillen, ed.), pp. 202 - 256, Blackwell Scientific Publications, Oxford.

Demigne, C and C. Remesy. 1979. Urea cycling and ammonia absorption *in vivo* in the digestive tract of the rat. Ann. Biol. Anim. Biochim. Biophys. 19 : 929 - 935.

Dintzis, R.Z., and A.B. Hastings. 1953. The effect of antibiotics on urea breakdown in mice. Proc. N.A.S. 39 : 571 - 578.

Easter, R.A.. 1988a. Acidification of diets for pigs. In: Recent Adv. Anim. Nutr. (Haresign, W., ed.), PP. 61 - 72, Butterworth, London.

Easter, R.A.. 1988b. Biochemical aids in gastrointestinal development and function. In: Biotechnology in the feed industry (Lyons, T.P., ed.), PP. 205 - 216, Alltech Technical Publications, Kentucky.

Francois, A.C., and M. Michel. 1964. Flora of the digestive tract and nutrition of the host: The role of antibiotics. In: The role of the gastrointestinal tract in protein metabolism (Munro, H.M., ed.), PP. 239 - 266, Blackwell Sci. Publ., Oxford.

Gamble, J.L.. 1915. The ammonia and urea content of infants' stool. Am. J. Dis. Child 9 : 519 - 532.

Giesting, D.W., and R.A. Easter. 1991a. Effect of protein source and

fumaric acid supplementation on apparent ileal digestibility of nutrients by young pigs. J. Anim. Sci. 69 : 2497 - 2503.

Giesting, D.W., M.A. Roos, and R.A. Easter. 1991b. Evaluation of the effect of fumaric acid and sodium bicarbonate addition on performance of starter pigs fed diets of different types. J. Anim. Sci. 69 : 2489 - 2496.

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.

Karasawa, Y.. 1989. Ammonia production from uric acid, urea, and amino acids and its absorbtion from the ceca of the cockerel. J. Exp. Zool. Supplement 3 : 75 - 80.

Levenson, S. M. and B. Tennant. 1963. Some metabolic and nutritional studies with germ-free animals. Fed. Proc. 22 : 109 - 119.

Lev, M., C.A.E. Briggs, and M.E. Coates. 1957. The gut flora of the chick. 3. Differences in cecal flora between 'infected', uninfected and penicillin-fed chicks. J. Anim. Sci. 16 : 364 - 372.

Lev, M. and M. Forbes. 1959. Growth response to dietary penicillin of germ-free chicks and of chicks with defined intestinal flora.

Br. J. Nutr. 13 : 78 - 84.

Lin, H.C. and W.J. Visek. 1991. Colon mucosal cell damage by ammonia in rats. J. Nutr. 121 : 887 - 893.

Macbeth, W.A.A.G., E.H. Kass, and W.V. McDermott, Jr.. 1965. Treatment of hepatic encephalopathy by alteration of intestinal flora with *Lactobacillus acidophilus*. Lancet 1 : 399 - 403.

Murakami, M., J.K. Yoo, S. Teramura, K. Yamamoto, H. Saita, K. Matuo, T. Asada, and T. Kita. 1990. Generation of ammonia and mucosal lesion formation following hydrolysis of urea by urease in the rat stomach. J. Clin. Gastroenterol. 12 : 104 - 109.

Nolan, J.V., B.W. Norton, and R.A. Leng. 1976. Further studies of the dynamics of nitrogen metabolism in sheep. Br. J. Nutr. 35 : 127 - 147.

O'Grady, F.W.. 1966. Differences in ammonia production by fecal bacteria of patients with hepatic encephalopathy. Proc. Royal Soc. Med. 59 : 1246 (abst.).

Owens, C.W.I. and W. Padovan. 1976. Limitations of ultracentrifugation and *in vivo* dialysis as methods of stool analysis. Gut 17 : 68 - 74.

Phillips, G.B., R. Schwart, G.J. Gabuzda, and C.S. Davidson. 1952.

The syndrome of impending hepatic coma in patients with cirrhosis of the liver given certain nitrogenous substances. New Engl. J. Med. 247 : 239 - 246.

Pollman, D.S.. 1986. Probiotics in pig diets. In: Recent Adv. Anim. Nutr. (Haresign, W., and D.J.A. Cole, ed.), PP. 193 - 205, Butterworth, London.

Silen, W., H.A. Harper, D.L. Mawdsley, and W.L. Weirich. 1955. Effect of antibacterial agents on ammonia production within the intestine. Proc. Soc. Exp. Biol. Med. 88 : 138 - 140.

Stutz, M.W., and M.J. Metrokotsas. 1972. Urease activity in the digestive tract of the chick and metabolism of urea. Poult. Sci. 51 : 1876 (abst.).

Varel, V.H., I.M. Robinson, and W.G. Pond. 1987. Effect of dietary copper sulfate, Aureo SP250, or clinoptilolite on ureolytic bacteria found in the pig large intestine. Appl. Environ. Microbiol. 53 : 2009 - 2012.

Vince, A., A.M. Dawson, N. Park, and F. O'Grady. 1973. Ammonia production by intestinal bacteria. Gut 14 : 171 - 177.

Vince, A.J., 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. : 1447 - 1469.

Weatherburn, M.W.. 1967. Phenol-hypochlorite reaction for determination of ammonia. Analyt. Chem. 39 : 971 - 974.

Weber, F.L., Jr.. 1979. The effect of lactulose on urea metabolism and nitrogen excretion in cirrhotic patients. Gastroenterol. 77 : 518 - 523.

Wolpert, E., S.F. Phillips, and W.H. Summerskill. 1970. Ammonia production in the human colon: Effects of cleansing, neomycin and acetohydroxamic acid. New Engl. J. Med. 283 : 159 - 164.

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

ACKNOWLEDGEMENT

The author wishes to express his most sincere appreciation to Dr. Kyu-Il Kim, his major professor, for his unceasing guidance and profound advice throughout the course of his graduate studies. The author also wishes to express thanks to Dr. Chang-Jo Cheong and Dr. Young-Hoon Yang for their tedious work for the judgement on this thesis. He wishes to return his thanks to Hak-Moon Park, Dong-Jin Kang, Bong-Joo Kang, Kyeong-Lyong Kim, and Young-Nam Ko of Laboratory of Animal Nutrition and Feeding, Cheju National University. The deepest gratitute must be offer to his parents and wife for their unceasing affection and devoted helps.