Effects of Feeding Diets Containing Probiotics or Antimicrobial Agent on Urease Activity and Ammonia Production in the Intestinal Contents of Rats

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生菌劑, 또는 抗菌劑를 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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摘 要

쥐에서, 1일중 장내 요소분해효소의 활성 변화와 생균제 또는 항균제를 함유한 사료의 급여가 장내 요소분해효소의 활성과 암모니아 생산에 미치는 영향을 측정하였다. 22:00시에 측정한 대장내용물중 요소분해효소의 활성이 하루중의 다른 시간에서 측정한 것보다 낮았다. 대조구에 비교하여 생균제 또는 항균제 첨가구에서는 대장내용물에서의 요소분해효소의 활성이 억제되었다($P<0.05$). 암모니아 생산율의 경우에는 항균제 처리구가 약간의 감소효과를 보였으며($P<0.07$) 생균제 처리구는 대조구와 유의차가 없었다. 생산된 암모니아중 요소분해로 인하여 발생한 암모니아가 차지하는 비율은 대조구, 생균제, 항균제 첨가구에서 각각 28.3, 18.3, 24.5% 이었다. 생균제는 대장 내용물중 요소분해효소를 약 41% 억제하였고, 암모니아 생산율을 약 8% 억제하여 요소분해효소를 생산하는 세균을 선택적으로 억제하였음을 말해준다. 이 실험결과는 생균제 또는 항균제를 첨가한 사료의 급여가 장내에서 요소분해효소를 생산하는 유해세균의 번식을 억제하여 동물의 건강증진과 성장촉진의 가능성이 있음을 제시해 준다.

(**Key words** : urease activity, ammonia production, probiotics, antimicrobial agent, intestine, rat)

I. INTRODUCTION

Ammonia produced from animo acid degradation in the body is converted to urea in the mammalian liver, 20 to 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 reenter the blood stream. Ammonia has been recognized as a toxin in animals (Lin and Visek, 1991).

Urease is produced by intestinal microbes; some species of the urease-producing bacteria were known to decrease the growth of chickens (Lev *et al.*, 1957) and the lack of growth was counteracted by addition of 45 mg of penicillin per kg of diet (Coates *et al.*, 1963). Attempts have been made to reduce urease activity or ammonia production in the GI tract by feeding fermentable carbohydrates, such as lactulose (Bircher *et al.*, 1966; Vince *et al.*, 1978; Weber, 1979), or by supplementing diets with antibiotics (Francois and Michel, 1964).

Urease immunization (Visek, 1978) or dietary urease inhibitors (Whitelaw *et al.*, 1991) have been implicated as methods to improve growth of farm

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animals.

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 growth-promotants. 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). 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) for one of the papers presented).

Antimicrobial agents have been significant in reducing costs of animal production and have given new insights into the influence of intestinal flora on the host (Gedek, 1984). Feeding a diet supplemented with the antimicrobial agent Aureo SP250 reduced the number of ureolytic organisms in animals (Varel *et al.*, 1987). However, no such effects were examined with dietary probiotics.

Our studies were conducted to determine diurnal variations of urease activity in the intestinal contents (Exp. 1) and to determine effects of feeding diets containing probiotics or an antimicrobial agent on urease activity and net ammonia production in the intestinal contents (Exp. 2) of adult rats.

Ⅱ. MATERIALS AND METHODS

Animals and diets.

Twenty (Exp. 1) or eighteen (Exp. 2) male Sprague Dawley × Fisher rats (mean initial weight, 212 g) were housed individually in suspended wire cages in a room at 20 to 25℃ with a 12 h light (07:00 to 19:00) and 12 h dark (19:00 to 07:00) cycle. Rats were allowed to consume a commercial chow diet (see footnote a to Table 2) on an *ad libitum* basis in Exp. 1 or were given *ad libitum* access to a control diet or to diets containing the probiotics *Bacillus toyoi* (Biocerin™; Bayer Vetchem (Korea) Ltd., Seoul, Korea) or the antimicrobial agent carbodox (Mecadox™; Pfizer Agricultural Division, New York, NY) in Exp. 2. The composition of the experimental diets used for Exp. 2 is shown in Table 1.

In Exp. 1, diurnal variations of large intestinal urease activity were determined by killing 5 rats at 04:00, 10:00, 16:00, and 22:00. For Exp. 2, conducted to determine effects of the dietary

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

Ingredients	Diets		
	Control	Probiotics	Antimicrobial agent
Casein[a] (vitamin-free)	20	20	20
L-methionine[a]	0.3	0.3	0.3
Corn oil[b]	5	5	5
Choline chloride[c]	0.2	0.2	0.2
Vitamin mix[d]	0.5	0.5	0.5
Salt mix[d]	5	5	5
Probiotics[e]	–	0.1	–
Antimicrobial agent[f]	–	–	1
Corn starch[g]	69	68.9	68

[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] *Bacillus toyoi* spore, Bayer Vetchem (Korea) Ltd., Seoul, Korea.
[f] 0.02% carbodox, Pfizer Agricultural Division, New York, NY.
[g] Sunil Pododang Co., Seoul, Korea.

probiotics and antimicrobial agent on urease activity and net ammonia production in the intestinal contents, 6 rats were assigned to each diet and fed the experimental diets in feed cups for at least 21 d before they were killed. All rats had free access to water.

Incubation of the intestinal contents

Beginning 21 d after receiving diet, a rat from each treatment (total 3 rats) was killed daily between 15:00 and 16:00 until all the experimental animals were used. The contents of cecum + colon and small intestine (including mucosa) were collected in 50-ml centrifuge tubes, weighed, and diluted 1:2 (w/v) 0.2 M sodium 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, where enough samples were available, were transferred into 15-ml centrifuge tubes and 1 ml of 0.4 M urea solution containing 0.1 μCi of ^{14}C [urea] (Amersham International Plc, Amersham, UK) was added to one sample; 1 ml of water was added to the other sample, which served as a blank, and the mixture was inactivated with 0.4 ml of 6 N H_2SO_4 before incubation. When the samples were not enough, only 1.5 ml of diluted samples were used as blank after 0.5 ml of water and 0.2 ml of 6 N H_2SO_4 were added.

The samples were incubated in a shaking water bath at 37℃ while being flushed with N_2 for the first 2 min. Then each unit was clamped sealed. Incubation of the contents of the cecum + colon and the samll intestine was initiated within approximately 10 and 20 min, respectively, after the rats were killed.

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 monomethy), 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 to 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 $^{14}CO_2$ 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 15 min and 30 min. The samples were contrifuged at 600 × g for 1 h and the supernatants were collected into plastic vials and stored at 4℃ for ammonia analyses.

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 Scintillation Counter (Model 1220 Quantulus; 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) recoved in CO_2 during the 30-min incubation by specific radioactivity (dpm/μmol) 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.

Determination of ammonia production

In Exp. 2, after the incubated samples and blanks were centrifuged, the supernatants were collected. Ammonia concentrations in the supernatants were determined by the colorimetric method (Weatherburn, **1967**) with minor modifications. Net ammonia production during the 30-min incubation was calculated by the difference in ammonia concentration between the blank and the incubated sample.

Statistical analysis

Data were subjected to analysis of variance. When differences were significant at less than 5 %, the Newaman-Keuls test (Snedecor and Cochran, 1980) was used to compare mean values of individual treatments.

Ⅲ. RESULTS AND DISCUSSION

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

Urease activity in the large intestinal contents was lower ($P<.05$) at 22:00 than at other times

(Table 2). In rats killed at 22:00, the 3 h after the start of the dark period, the GI tract, especially the stomach, was filled with digesta. Rats usually consume most of their daily feed during the early period of a dark cycle. We assumed that the lower urease activity was due to the dilution of bacteria with the digesta and also due to the sufficient supply of nitrogen sources needed by intestinal microflora without hydrolyzing urea. It has been known that many urease-producing bacteria do not produce urease when a growth medium contains sufficient nitrogen sources, such as yeast extract and trypticase (Wozny *et al.*, 1977). It is also known that little urease is produced by a rumen population not exposed to urea (Czerkawski and Breckenridge, 1982).

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

Time	Large intestine		Small intestine	
	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). [b,c] Values in the same column with different superscripts differ significantly ($P<0.05$).

Effects of dietary probiotics and an antimicrobial agent on urease activity and net ammonia production - Experiment 2.

Urease activity in the large intestinal contents was different ($P<.05$) between the control and rats fed diets containing probiotics or an antimicrobial agent (Table 3). The probiotics (*Bacillus toyoi*) and the antimicrobial agent (carbodox) tested in our study seemed to be effective in decreasing urea degradation in the intestinal tract.

Urease activity (μmol of urea hydrolyzed/30 min per total contents) in the large intestinal contents was much higher than that in the small intestinal contents (e.g., 32.9 vs 2.4). Stutz and Metrokotsas (1972) reported that urease activity in cecal contents accounted for 99% of the total urease activity in the GI contents of the chicken. Demigne and Remesy (1979) have also 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 circulation in 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.

Net ammonia production in the large intestinal contents (per g) was slightly ($P=0.07$) influenced by the dietary treatments (Table 4). The antimicrobial agent seemed to reduce net ammonia production more than the probiotics did. Wolpert *et al.* (1970) reported that administration of

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

Diet	Small intestine		Large intestine	
	per g	per total	per g	per total
		μmol of urea hydrolyzed/30 min at 37℃		
Control	0.59±0.14	2.13±0.62	14.6±1.6[b]	29.6±2.7[b]
Probiotics	0.50±0.09	1.60±0.27	8.6±2.0[c]	16.2±3.7[c]
Antimicrobial agent	0.38±0.02	1.11±0.07	8.1±0.8[c]	16.3±1.8[c]

[a] Values are means±SEM of 5 rats.
[b,c] Values in the same column with different superscripts differ significantly ($P<0.05$).

Table 4. Effects of feeding diets containing 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±13
Probiotics	30.37±4.58	124.7±19.0	94±15
Antimicrobial agent	23.39±2.21	89.8± 9.7	66± 9

[a] Values are means±SEM of 6 rats.
[b] After - before incubation (30 min at 37℃)

neomycin halved the ammonia concentration in the colon of patients with cirrhosis. 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, as illustrated in experiments with antibiotics (Silen *et al.*, 1955).

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 the amount of ammonia produced from non-urea nitrogen sources. Ureolysis accounted for 31.4, 20.3, or 27.2% of net ammonia production in the large intestinal contents of the control rats or rats fed diets containing probiotics or the antimicrobial agent, respectively (calculated from the data shown in Tables 3 and 4).

Studies on ammonia production from non-urea nitrogen sources, e.g., peptides and amino acids, by intestinal bacteria have been reported (Vince *et al.*, 1976). 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 production in the feces must be derived from some sources other than urea. Intravenous infusions of ^{15}N[urea] have been used to determine the proportion of intestinal ammonia that 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 potential, the primary reaction being 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, as shown in Table 4.

Interestingly, feeding a diet containing probiotics decreased urease activity by 41.0% but reduced net ammonia production by only 8.5%, compared with the control. The result suggests that feeding diets supplemented with probiotics decreases urea-degrading bacteria more than it decreases bacteria that produce ammonia from non-urea nitrogen sources. This suggestion is supported by the aforementioned "competitive exclusion" theory. It also has been demonstrated that probiotics can influence microbial metabolism in the GI tract. When human subjects were fed *L. acidophilus*, the activities of β-glucuronidase, nitroreductase, and azoreductase in the intestinal contents were suppressed (Goldin and Gorbach, 1984).

The results of our studies indicate that supplementing diets with probiotics or antimicrobial agents reduces ureolysis and perhaps net ammonia production in the intestinal tract, possibly by modifying microflora. Such effects can vary, depending on animals and environments (e.g., how heavily animals are stressed, or contaminated with bacteria that produce urease), and on kinds and levels of dietary protein, probiotics or antimicrobial agents included in diets. These variations can lead to variable effects of supplementary probiotics or antimicrobial agents on growth and feed efficiency in feeding trials. Therefore, we propose that urease activity in the GI tract be used as a measure of growth-promoting potential (which is not always reflected in

growth and feed efficiency) of feed additives that act on animals through the modification of intestinal microflora.

Ⅳ. SUMMARY

Adult Sprague Dawley × Fisher male rats (mean initial weight, 212 g) were *ad libitum* fed a commercial chow diet to determine the diurnal variations of urease activity in the large intestinal contents, or fed a control or diets containing probiotics (*Bacillus toyoi*) or an antimicrobial agent (carbodox) to determine dietary effects on urease activity and ammonia production in the small and large intestines. Urease activity measured at 22:00 was lower ($P<.05$) than that measured at 04:00, 10:00, or 16:00. Urease activity (μmol of urea hydrolyzed/30 min) per g or per total of large intestinal contents was decreased ($P<.05$) by feeding diets supplemented with probiotics or antimicrobial agent. A slight decrease ($P=.07$) in net ammonia production (μmol/30 min per g contents) was observed in rats fed diets supplemented with probiotics or antimicrobial agent, compared with the control. Ureolysis accounted for 31.4, 20.3 or 27.2% of net ammonia production in the large intestinal contents of rats fed the control or diets containing probiotics or antimicrobial agent, respectively. Feeding a diet supplemented with probiotics reduced urease activity more significantly than net ammonia production. The results of our studies indicate that urease activity in the intestinal contents of rats varies during the day, possibly with time after feeding, and that dietary probiotics and antimicrobial agent reduce urease activity and perhaps ammonia production in the rat intestine.

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Ⅴ. REFERENCES

1. Bircher, J., Muller, J., Guggenheim, P. and Haemmerli, U. P. 1966. Treatment of chronic portal systemic encephalopathy with lactulose. Lancet 1966(1):890.
2. Chapman, J. D. 1988. Probiotics, acidifiers and yeast culture: A place for natural additives in pig and poultry production. In: T. P. Lyons (Ed.) Biotechnology in the Feed Industry. pp. 219-223. Alltech Technical Publications, Nicholasville, KY.
3. Coates, M. E., Fuller, R., Harrison, G. F., Lev, M. and Suffolk, S. F. 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.
4. Czerkawski, J. W. and Breckenridge, G. 1982. Distribution and changes in urease (EC 3.5.1.5) activity in rumen simulation technique (Rusitec). Br. J. Nutr. 47:331.
5. Dawes, E. A. and Large, P. J. 1973. Class II reactions: Synthesis of small molecules. In: Mandelstam, J. and McQuillen, K. (Ed.) Biochemistry of Bacterial Growth (2nd Ed.). pp. 202. Blackwell Scientific Publications, Oxford.
6. Demigne, C., and Remesy, C. 1979. Urea cycling and ammonia absorption in vivo in the digestive tract of the rat. Ann. Biol. Anim. Biochim. Biophys. 19:929.
7. Francois, A. C. and Michel, M. 1964. Flora of the digestive tract and nutrition of the host: The role of antibiotics. In: H. M. Munro (Ed.) The Role of the Gastrointestinal Tract in Protein Metabolism. pp. 239. Blackwell Scientific Publications, Oxford.
8. Fuller, R. 1989. A review: Probiotics in man and animals. J. Appl. Bacteriol. 66:365.
9. Gamble, J. L. 1915. The ammonia and urea conent of infants' stool. Am. J. Dis. Child 9:519.
10. Gedek, B. 1984. Contemporary concepts of growth promoter use. In: M. Woodbine (Ed.) Proc. 4th International Symp. Antibiotics in Agriculture: Benefits and Malefits. pp. 315. Butterworths, London.
11. Goldin, B. R. and Gorbach, S. L. 1984. The effect of milk and lactobacillus feeding on human intestinal bacterial enzyme activity. Am. J. Clin. Nutr. 39:756.
12. Lev, M., Briggs, C. A. E. and Coates, M. E. 1957. The gut flora of the chick. 3. Differences in cecal flora between 'infected', uninfected and penicillin-fed chicks. J. Anim. Sci. 16:364.
13. Lin, H. C., and Visek, W. J. 1991. Colon mucosal cell damage by ammonia in rats. J. Nutr. 121: 887.
14. Mulder, R.W.A.W., and Bolder, N. M. 1991.

Experience with competitive exclusion in the Netherlands. In: Blankenship L. C. (Ed.) Colonization Control of Human Bacterial Enteropathogens in Poultry, pp. 77. Academic Press, Inc., New York.

15. Nolan, J. V., Norton, B. W. and Leng, R. A. 1976. Further studies of the dynamics of nitrogen metabolism in sheep. Br. J. Nutr. 35:127.
16. Nurmi, E., and M. Rantala. 1973. New aspects of Salmonella infection in broiler production. Nature 241:210.
17. Owens, C. W. I., and Padovan, W. 1976. Limitations of ultracentrifugation and in vivo dialysis as methods of stool analysis. Gut 17:68.
18. Phillips, G. B., Schwart, R., Gabuzda, G. J. and Davidson, C. S. 1952. The syndrome of impending hepatic coma in patients with cirrhosis of the liver given certain nitrogenous substances. New Engl. J. Med. 247:239.
19. 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. 88:1384.
20. Snedecor, G. W., Cochran, W. G. 1980. Statistical Methods (8th Ed.). The Iowa State University Press, Ames.
21. Stutz, M. W., and Metrokotsas, M. J. 1972. Urease activity in the digestive tract of the chick and metabolism of urea. Poult. Sci. 51:1876 (Abst.).
22. Varel, V. H., Robinson, I. M. and Pond, W. G. 1987. Effect of dietary copper sulfate, Aureo SP 250, or clinoptilolite on ureolytic bacteria found in the pig large intestine. Appl. Environ. Microbiol. 53:2009.
23. Vince, A., Down, P. F., Murison, J. Twigg, F. J. and Wrong, O. M. 1976. Generation of ammonia from non-urea sources in a fecal fecal incubation system. Clin. Sci. Molec. Med. 51:313.
24. Vince, A., Killingley, M. and Wrong, O. M. 1978. Effect of lactulose on ammonia production in the fecal incubation system. Gastroenterol. 74:533.
25. Visek, W. J. 1978. The mode of growth promotion by antibiotics. J. Anim. Sci. 46:1447.
26. Weatherburn, M. W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Anal. Chem. 39:971.
27. Weber, F. L., Jr. 1979. The effect of lactulose on urea metabolism and nitrogen excretion in cirrhotic patients. Gastroenterol. 77:518.
28. Whitelaw, F. G., Milne, J. S. and Wright, S. A. 1991. Urease (EC 3.5.1.5) inhibition in the sheep rumen and its effect on urea and nitrogen metabolism. Br. J. Nutr. 66:209.
29. Wolpert, E., Phillips, S. F. and Summerskill, W. H. 1970. Ammonia production in the human colon: Effects of cleansing, neomycin and acetohydroxamic acid. New Engl. J. Med. 283:159.
30. Wozny, M. A., Bryant, M. P., Holdeman, L. V. and Moore, W. E. C. 1977. Urease assay and urease-producing species of anaerobes in the bovine rumen and human feces. Appl. Environ. Micribiol. 33:1097.
31. Wrong, O. M. 1981. Nitrogen compounds. In: Wrong, O. M., Edmonds, C. J. and Chadwick V. S. (Ed.). The Large Intestine: Its Role in Mammalian Nutrition and Homeostasis. pp. 133. John Wiley and Sons, New York.

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