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Effects of Dietary Antimicrobial Agents, Probiotics or Yucca Extract on Urease Activity and Ammonia Production in the Chicken Intestine

K.I. Kim and J.M. Yeo[1]

Department of Animal Science, Cheju National University, Cheju, Korea 690-756

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김 규 일 · 여 진 모[1]

제주대학교 축산학과

ABSTRACT

The balance of microbial populations in the gastrointestinal (GI) tract of all warm-blooded animals is critical to the maintenance of health and resistance to disease. The composition of the populations can be altered by diet and environment, making the host animal susceptible to disease, and reducing growth rate and feed efficiency. Some feed additives including antimicrobial agents, probiotics or yucca extract have been used to promote growth and feed utilization. There is evidence that part of growth-promoting effect of those feed additives results from the suppression of microbial urease activity or ammonia production in the GI contents of animals. Over 200 microbial species have been known to produce urease and the product of urea hydrolysis, ammonia, is toxic to animals. Carefully tested probiotics or other urease-suppressing agents can be a possible alternative to antimicrobial agents including antibiotics as growth promotants used for animals feeds.

(Key words: chickens, urease activity, ammonia production, antimicrobial agents, probiotics, yucca extract)

INTRODUCTION

Since Dubos et al. (1965) attempted to explain intestinal microflora in adversarial terms, the microbiology of the GI tract has gained much attention. Microflora inhabiting the GI tract of animals interacts with host animals and their populations vary with animal species, sites along the tract, age, diet and environment. Healthy animals within species generally maintain a balanced microbial population which plays an important role in the growth and health of animals, inhibiting the growth of bacteria harmful to the host. For example, intestinal bacteria metabolize nutrients in the gut contents, produce short-chain fatty acids and lactic acid, and synthesize some vitamins as well as toxic subst-

[1] 삼양사료(Samyang Feed Co.)

ances. Some of these activities can be beneficial to the host animals.

Ammonia (including ammonium ion) produced from the amino acid degradation in the body is converted to urea in the mammalian liver, or to uric acid in the chicken liver. A significant amount of the compounds(e.g., 20~25% of urea) is excreted into the gastrointestinal (GI) tract, and hydrolyzed into ammonia by microbial enzymes (Wrong et al., 1981). This ammonia together with that produced by bacteria acting on other nitrogenous substrates may be used for microbial protein synthesis or may enter the blood stream. Ammonia is one of the microbial products, which is toxic to animals. Many intestinal bacteria (e.g., bacteroides, bifidobacteria, clostridia, *Proteus* spp. and *Klebsiella* spp.) produce urease. *Escherichia coli* produce no urease but other ammonia-generating enzymes. However, most lactobacilli strains have been known to produce little urease or other ammonia-generating enzymes (Phear and Ruebner, 1956; Macbeth et al., 1965; Agostini et al., 1972).

It has been reported that cecal ammonia concentration is relatively high in the domestic fowl (Bell and Bird, 1966), and cecal microbial activity is great (Barnes and Impey, 1974) compared with other parts of the intestine. These facts suggest that the cecum of the chicken is the most active site in the digestive tract for ammonia production by microflora. The urine of chicken contains uric acid as a major end product of nitrogen metabolism and also urea, amino acids and creatine (O'Dell et al., 1960). There are many types of bacteria in the cecum that have the hydrolytic activity of uric acid (Barnes and Impey, 1974) and urea (Stutz and Metrokotsas, 1972), and deaminating activity (Fujita, 1968). Urease-producing bacteria inhabiting the GI tract are involved in the nitrogen recycling and the resulting product ammonia can be harmful to animal health. Karasawa (1989) found that 77% of uric acid injected into a cecal sac disappeared within one hour, with a concomitant increase in ammonia concentration.

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). Feeding diets containing probiotics also reduced urease activity and possible ammonia production in the rat (Kim and Kim, 1992) and in the chicken (Yeo, 1992). The growth-promoting effects of subtherapeutic-level antibiotics (Visek, 1978), or probiotics (Kim and Kim, 1992; Yeo, 1992) used in animal feeds as growth promotants have been ascribed to suppression of urea hydrolysis and subsequently reduced ammonia production in the GI tract. Jackbean urease immunization in chickens (Dang and Visek, 1960) or dietary urease inhibitors in sheep (Whitelaw et al., 1991) have also been suggested as a means of improving growth. Chicks hatched from urease-injected hens grew better than those from non-immunized hens and feeding these chicks a diet supplemented with penicillin (50 ppm) showed additive effects (Pimentel and Cook, 1988).

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" (Nurmi and Rantala, 1973). 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"(Mulder and Bolder, 1991). Yucca extract, major active gredient being sarsaponin, has also been used as an animal feed supplement, especially to reduce ammonia concentration in the barn (Johnston et al., 1981; Preston et al., 1987).

This review will focus on the effect of dietary antimicrobial agents, probiotics or yucca extract on urease activity and ammonia production in the intestinal contents of chickens because the reduced ammonia production in the intestine may well be part of the effect of these compounds on improved growth and feed efficiency.

EFFECTS OF DIETARY ANTIMICROBIAL AGENTS ON UREASE ACTIVITY AND AMMONIA PRODUCTION IN THE GI TRACT

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 (Kim and Kim, 1992).

Bacteria 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; Dintzis and Hastings, 1973). Francois and Michel (1955) were among the first to suggest that reduction of bacterial ammonia production may be related to growth-stimulatory effects of antibiotics. Subsequently, Visek et al. (1959) showed that three antibacterial agents, penicillin, chlortetracycline and arsanilic acid, when fed to rats at 100 mg/kg diet, decreased *in vivo* hydrolysis of ^{14}C-urea. Oral administration of antibiotics and sulfonamide in sufficient dosage would virtually eliminate the gastrointestinal microflora and abolish urea hydrolysis (Kornberg and Davies, 1955; Dintzis and Hasting, 1973). Similarly, Karasawa et al. (1994) showed that 20 mg penicillin/kg diet decreased urease activity in cecal contents to 17% of that found in the control group fed no penicillin. These chickens were provided with 10 mL urea solution (60 g/L) per day along with the experimental diets.

Whether decreased urea hydrolysis and, in turn, decreased ammonia production, had any causal relationship to growth stimulation was tested in immunity experiments with crystalline jackbean urease (Dang and Visek, 1960). The initial growth experiments employing urease immunity were in rats fed diets marginal in protein or vitamin A, but free of urease activity (Dang and Visek, 1960; Harbers et al., 1963). Following immunization, sera, feces and urine of the immunized animals contained substances which cross-reacted with jackbean urease, but non-immunized rats showed no cross-reactivity which was assumed to be due to jackbean urease antibodies. The immunized rats expired less $^{14}CO_2$ after the injection of ^{14}C-urea, and their gastrointestinal contents had less *in vitro* urease

activity and chicks grew faster than the non-immunized controls. Thus the observed changes in urea hydrolysis were qualitatively similar to those obtained with the feeding of antibacterial substances, and there was a concomitant increase in feed efficiency and growth rate.

Portal blood ammonia concentrations of germ-free guinea pigs were about 25% of those in conventional animals (Warren and Newton, 1959) and were reduced in conventional animals by oral intake of antibiotics (Warren and Newton, 1959; Stahl, 1963). The studies by Levenson and Tennant (1963) and Evrard et al.(1964) showed a higher excretion of nitrogen in the feces of germ-free rats. Feeding urea to chicks causes alterations in histology in localized areas of gastrointestinal tissues, and similar but more uniform changes were found with the feeding of deconjugated bile acids. Some of the changes were reversed with 100 ppm of chlortetracycline in the diet (Visek, 1969).

The question of whether antibiotics spare proteins as measured by increased nitrogen retention has been debated. The nitrogen content of the experimental diet is certainly one factor which adds to the confusion, the other possible factor in the variable findings being too short a balance period used by some of the investigators (Francois and Michel, 1964). This protein-sparing effect of antibiotics may result from the inhibition of decarboxylation (Larson and Hill, 1960) and deamination (Michel, 1958) of amino acids in the intestine by microflora.

Microflora inhabiting the GI tract has a metabolic activity which can cause degradation of a large number of substances, especially sugars, bile acids, amino acids and urea. Antibiotics, by inhibiting this metabolic activity, can reduce their breakdown. Consequently, there is a sparing of nutrients and less microbial metabolites such as ammonia, amines, phenols and other microbial toxins are expected to be produced. In regard to toxins, Lev and Forbes (1955) have shown that *Clostridium perfringens* is an organism capable of depressing the growth of the chicken. Penicillin, which counteracts this depressing action, has the property of eliminating this organism from the intestinal flora or at least of suppressing one of its essential biochemical properties, namely, production of toxin, lecithinase.

The overall picture provided by the experimental findings shows that the intestinal flora undoubtedly plays a significant nutritional role. It is certain that antibiotics which are capable of stimulating growth also affect the microflora of the digestive tract. Part of this effect is considered to be exerted through the inhibition of urease-producing bacteria. Generally, more significant effects have been reported in younger than older animals and in on-farm than university trials (Zimmerman, 1986).

EFFECT OF DIETARY PROBIOTICS ON UREASE ACTIVITY AND AMMONIA PRODUCTION IN THE GI TRACT

The term probiotics meaning "for life" was first used by Richard Parker, a microbiology professor at the Oregon Medical School (Wren, 1987). Probiotics are products containing viable, lactic acid-producing bacteria (primarily lactobacilli and streptococci) that are administered orally, either alone or in feed, with the intent of establishing a favorable intestinal microflora (Fox, 1988; Fuller, 1991).

The probiotics that are currently available contain strains of lactic acid-producing organisms. The most common strains are *Lactobacillus*

acidophilus, L. bulgaricus, L. plantarum, L. casei, Streptococcus faecium, S. lactis, S. thermophilus, and *S. diacetilactus* (Montes et al., 1993). These bacteria are used either alone or in combination, and are available in a variety of forms, including powders, pastes, boluses, capsules, and drenches. Probiotics are not yet classified as drugs, so there are no regulations regarding drug residues and withdrawal periods.

Some authors, preferring the more generic term of "direct-fed microbial products," also include in the concept of probiotic components such as *Bacillus* spp. (not a normal GI tract inhabitant), yeasts and yeast cultures, enzymes, by-products of industrial fermentation, chemicals for diet acidification, and biomass (i.e., addition of nonviable bacterial cells to the gut). Although *Bacillus* spp. do not adhere to the intestinal microvilli, these bacteria do grow in the mucous biofilm over the intestinal villi and render the mucus more suitable as food for other probiotic bacteria.

Probiotics for chickens are designed either to replace beneficial organisms that are not present in the alimentary tract or to provide the chicken with the effects of beneficial bacteria (Barrow, 1992). Probiotics are thought to colonize the crop and small intestine in ways described by Fuller (1978). They are thought to exert antibacterial effect against potential pathogens (Fuller, 1978) and are also considered to increase performance by unknown mechanisms.

Several species of lactobacillus including *L. acidophilus* are normal inhabitants of the GI tract of healthy chickens (Morishita et al., 1971; Barnes et al., 1980; Sarra et al., 1985). Antagonistic factors that may contribute to colonization and continuous presence of lactobacilli in the digestive tract of chickens have been recently reviewed by Juven et al.(1991). These factors include presence of organic acids, hydrogen peroxide, reuterin, bacteriocins and other compounds. Because of the broad spectrum of activity of bacteriocins, Vincent et al. (1959) concluded that *L. acidophilus* might occupy an important position in controlling undesirable microflora in the intestinal tract. It is also known that volatile fatty acids exert their inhibitory activity on *Salmonella* infections in their undissociated state, which is determined by pH (Meynell, 1963; Bohnhoff et al., 1964a,b; Pjescak, 1970; Barnes et al., 1980a,b).

During periods of stress, this balance can be altered, generally resulting in a reduced lactobacilli population in the GI tract, which concomitantly eliminates the restraint on pathogenic microorganisms. The purpose of administering probiotics is to try to re-establish the ideal relationship between beneficial and pathogenic microorganisms that constitute the intestinal flora.

Results of our studies (Kim and Kim, 1992; Yeo, 1992) suggest that dietary probiotics and also antibiotics possibly suppress the growth of bacteria that produce urease. The effect of the probiotic on suppressing urease activity was remarkable in the small intestine, suggesting a benefit of feeding probiotics, such as lactic acid bacteria or products containing such bacteria. This effect may be partly responsible for the increased weight gain during the first three weeks of feeding in chicks fed diet supplemented with a probiotics (Yeo, 1992). A similar effect was found when broiler chicks were fed diet supplemented with *Lactobacillus acidophilus* (Tortuero, 1973; Francis et al., 1978).

In contrast, Watkin and Kratzer(1983) reported that *Lactobacillus* strains fed in drinking water reduced growth rate and did not improve feed efficiency in broiler chicks. Similarly, no

effects were found in large white turkey hens (Damron et al., 1981). Such differences reported among studies may be attributable to differences in bacteria used, species or age of animals, diet fed and environment.

Some bacteria, especially lactic acid bacteria, inhibit others, such as pathogenic bacteria by so-called "competitive exclusion" (Nurmi and Rantala, 1973). Interestingly, lactose or mannose administered in drinking water decreased counts of *Salmonella typhimurium* inoculated (10^8 cells) into broiler chicks at 3 days of age to 53 and 27% of the control, respectively (Oyofo et al., 1989). However, dextrose, sucrose or maltose had no effect. Lactose at this concentration was considered to possibly stimulate lactose-fermenting bacteria (Kim et al., 1979) which is inhibitory for Salmonella. Additional studies (DeLoach et al., 1990) supported the original findings by demonstrating similar inhibitory effects in the ceca with 5% lactose in drinking water or with 5% whey in the feed. Feeding diet containing 20% lactose or 40% skim milk to chickens also reduced the severity of infection with *Eimeria*, an avian protozoan (Beach and Davies, 1925). The antibacterial mechanism of probiotics, although not completely known, may include pH, low redox potential, competition for nutrients and adhesion to receptor sites, and miscellaneous inhibitory substances such as H_2S, bacteriocins, fatty acids and deconjugated bile acids (Barrow, 1992). The growth-promoting effects of subtherapeutic-level antibiotics (Visek, 1978a) or probiotics (Kim and Kim, 1992; Yeo, 1992) used in animal feeds as growth promotants have also been ascribed to suppression of urea hydrolysis and subsequently reduced ammonia production in the GI tract.

EFFECTS OF DIETARY YUCCA EXTRACT ON UREASE ACTIVITY AND AMMONIA PRODUCTION IN THE GI TRACT

Yucca saponin is a natural product prepared by drying and pulverizing the stems of Yucca schidigera. The plant extract contains several steroid saponins, of which sarsaponin is the predominant member (Johnston et al., 1981). The terms yucca extract and the molecular entity sarsaponin are often used interchangeably, which can lead to some confusion. Johnston et al.(1981) reported that dietary yucca saponin (63 ppm extract or 0.9 ppm active steroid) improved growth of broilers over 28 or 51 days after hatching, compared to the control. However, feed efficiency, mortality, shank pigmentation or environmental ammonia levels were not influenced. By contrast, in their later study, yucca saponin did not increase growth at 51 days of age, but improved feed efficiency of broilers when fed with 121 ppm monensin (Johnston et al., 1982). Poults exposed to 200 ppm atmospheric ammonia had reduced feed intake and growth rate during exposure, and subsequently showed reduced egg production and increased mortality (Deaton et al., 1984).

In poultry, high concentrations of ammonia from the litter causes harm to the respiratory tract and increases susceptibility to respiratory tract infections. Turkeys exposed to 10 and 40 ppm ammonia had more virulent inhaled pathogenic *E. coli* in their lungs, air sacs, and livers than those poults not exposed to ammonia (Nagaraja et al., 1984). It was hypothesized that one mechanism of action of yucca saponin in improving performance in poultry might be the reduction of ammonia concentration in the atmos-

phere of the poultry house.

Yucca extract have been used as a component of the feed or added directly to lagoons and tanks or sprayed on litter in the pits. With regard to the control of ammonia levels in the atmosphere the generally held view is that the natural yucca extract exerts its action through inhibition of urease (Ellenberger et al., 1985; Gibson et al., 1985; Preston et al., 1987).

However, Headon and Dawson (1990) reported that dietary yucca extract reduces ammonia in the atmosphere by binding (or absorbing) ammonia molecules. They found that yucca extract added to a ammonium chloride solution reduced the level of ammonia detected due to the binding of ammonia by some components of yucca extract thereby reducing the level of free ammonia or by the conversion of ammonia to some other compound(s). The levels of ammonia detected decreased with increasing concentration of yucca extract. This result was further confirmed by measuring $^{14}CO_2$ production from ^{14}C-urea as a means of determining jackbean urease activity. At higher concentrations of yucca extract (>5 mg/mL), the urease activity was even stimulated. This increased activity was assumed to be associated with the decreased ammonia concentration resulting from absorption by yucca extract, although no known effect of ammonia on purified urease activity has been reported. Our study also showed that urease activity in the cecal contents measured by $^{14}CO_2$ production from ^{14}C-urea was not influenced by dietary yucca extract (Yeo, 1992).

Interestingly, experiments employing ion-exchange resin capable of binding ammonia in the gastrointestinal tract also increased growth. The resin did not alter urea hydrolysis, but, in accord with its known action, undoubtedly bound free ammonia in the gastrointestinal lumen (Holtzman and Visek, 1965).

적 요

모든 항온 동물의 장내 세균총의 균형은 건강 유지와 질병에 대한 저항성에 매우 중요한 역할을 한다. 세균총의 조성은 사료와 환경에 의해서 변화될 수 있고, 이 변화는 숙주동물의 질병에 대한 감수성을 증가시키고 성장율과 사료효율을 저하시킨다. 항균제, 생균제, 및 유카추출물 등을 포함한 일부 사료 첨가제들이 성장을 증가시키고 사료효율을 개선하기 위하여 사용되어 왔다. 그러한 사료첨가제들의 성장촉진 효과는 일부 장내 미생물에 기인하는 요소 분해효소 활성을 감소시키고 암모니아 생성을 억제하는 데서 나타난다는 증거가 있다. 약 200여종의 미생물들이 요소 분해효소를 생산한다고 알려져 있으며, 요소분해 생성물인 암모니아는 동물에게 유독하다. 주의깊게 검정된 생균제나 요소 분해효소 억제물질들은, 동물사료에 쓰이는 성장 촉진제로 항생제를 포함한 항균제의 대체품으로 사용될 가능성이 있다.

(색인: 닭, 요소분해효소, 암모니아 생산, 항균제, 생균제, 유카추출물)

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