抗生物質のブロイラー腸内菌叢に及ぼす影響

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Effects of Dietary Antibiotics on Intestinal Microflora in Broiler Chickens

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Changes were examined in the intestinal microflora in broiler chickens fed a diet containing antibiotics to obtain fundamental information on the mechanisms of beneficial effect of the antibiotics upon livestock production. Three antibiotics (colistin, bacitracin, and enramycin) were employed as feed additives. Experiments were conducted with broiler chickens in two ways. In one way dietary antibiotics were fed continually at levels approved for use as feed additives for a long term. In the other they were fed the same antibiotics for a short term. Significant changes in microflora were observed mainly in such bacterial groups as aerobic bacteria and *Lactobacillus*. In the long term administration, three possible modes of variance in the bacterial flora were postulated: (1) Changes directly related to the antibacterial spectrum of antibiotics. (2) Antagonistic changes related to an ecological balance in the bacterial flora. (3) Changes in quantitative balance of bacteria constituting each bacterial group. The change in the intestinal microflora during administration of the antibiotic diet was expressed as a complex form of these transition modes. In the short term administration, it was demonstrated that the effect of the antibiotic diet lingered even 7 days after administration. This suggests that antibiotics used as feed additives may possibly affect the stability of the intestinal microflora.

Antibiotics are now widely used as feed additives to promote growth or to increase feed efficiency besides prophylatic and therapeutic purpose in livestock production. Many hypotheses have been proposed on the mechanism of growth promotion by using antibiotics as feed additives. It is evident that much interplay exists among the models of action.

Hays[7] summarized them as follows. In his review: (1) A metabolic effect. (2) A nutrient-sparing effect. (3) A disease-control effect. He suggested the comparative importance of the disease-control effect of antibiotics; that is, the major effects of antibiotics used as routine feed additives might be the suppression or control of subclinical or nonspecific diseases. On the basis of these hypotheses, the significance of the relationship between the host and its intestinal microflora was emphasized.

In his review, Schaedler[20] summarized

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the presumptive role of the microflora in the growth and development of the animal. With this kind of view, it is necessary to clarify the significance of intestinal microorganisms when the growth-promoting effect of antibiotics was displayed.

The hypotheses proposed on the role of feed additive antibiotics by Visek[22] in 1978 are as follows:(1) Microorganisms responsible for infections too mild to be recognized may be suppressed. (2) The microbial production of growth-depressing toxins may be reduced. (3) Antimicrobial agents may reduce the microbial destruction of essential nutrients in the gastrointestinal tract, or there may be an increase in synthesis of vitamins or other growth factors. (4) The efficiency of absorption of nutrients may be enhanced, because the intestinal wall is rather thin. Many workers[1,4,9,11,18,21] evaluated the significance of the microflora in poultry during the administration of dietary antibiotics. Most of them, however, committed two common defects. One defect is that the methods of culture employed for anaerobic microorganisms which are predominant in the intestinal microflora, were inadequate. The other is that the experimental period used was comparatively short.

In the present experiment, an attempt was made to reveal changes in the intestinal microflora in broiler chickens fed a diet containing antibiotics for 6 weeks. At the same time, observation was made on the effects of these antibiotics administered for a short time.

Three antibiotics, colistin, bacitracin and enramycin, were used. They are not absorbed from the intestinal wall, being now widely used as feed additives in Japan.

MATERIALS AND METHODS

Experimental design: Four experiments were conducted. In experiments I to III antibiotics were fed for a long time to investigate changes in the intestinal microflora over a period from 24 hours after hatching to 6 weeks of age. In experiment IV antibiotics were administered over a period from 10 to 12 days of age to observe their effects in short-term administration. The former experiments were carried out with the lowest and highest dosage levels of each antibiotic that had been approved for practical use as a feed additive in poultry raising. The latter experiment was performed with a dosage level of each antibiotic which was twice as high as the highest dosage level used in the former experiments. The experimental design used is shown in Table 1.

Table 1. *Experimental design*

1. *Long term administration*

		Dosage (mg/kg)		
Experiment I	CL	0,	5,	50
Experiment II	BC	0,	4.8,	48
Experiment III	ER	0,	2,	20

Weeks after hatching	0	1	2	3	4	6
Administration of antibiotics	←	·····	·····	·····	·····	····→
Examination of microflora	*	*	*	*	*	*

2. *Short term administration*

		Dosage (mg/kg)
Experiment IV	None	0
	CL	100
	BC	96
	ER	40

Days after hatching	0	10	12	13	19
Administration of antibiotics		←····→			
Examination of microflora		*		*	*

CL: colistin sulfate
BC: zinc bacitracin
ER: enramycin

Birds: Female broiler chicks (Meat nick) were purchased from a local hatchery. In experiments I to III, 120 chicks were assigned for each antibiotic. They were divided randomly into 3 groups (control, low dose and high dose) for experimental feeding of 6 weeks, as shown in Table 1. In experiment IV, 4 groups of 20 chicks each were used. Three of them were fed a diet containing each antibiotic over a period from 10 to 12 days of age. Before and after

this period they were given a basal diet which contained no antibiotics. The other group was fed the basal diet to serve as a control. All the birds were raised in electrically heated battery brooders till 21 days of age, when they were transferred to wire-floored cages in an air-conditioned room. In experiments I to III, the birds were allowed to have a free access to the medicated diet and water throughout the experimental period beginning with 24 hours after hatching. Body weight and feed consumption were recorded every week.

Antibiotics: The antibiotics administered were colistin sulfate (CL) (Meiji Seika Co., Ltd.), zinc bacitracin (BC) (Asahi Chemical Industry Co., Ltd.) and enramycin (ER) (Takeda Chemical Industries, Ltd.). They were mixed with basal feed distributed for broiler chickens as experimental birds by the Nippon Formula Feed Manufacturing Co., Ltd. The levels of these antibiotics in feed (mg/kg) were 5 and 50 and for CL, 4.8 and 48 for BC, and 2 and 20 for ER in experiments I to III. In experiment IV, they were 100 for CL, 96 for BC, and 40 for ER. The mixed feed was stored in a refrigerator at 4°C until use.

Media employed and cultivation of intestinal microflora: Bacteriological examination was performed by essentially the same method as that of Mitsuoka et al.[12,14,15] The media employed and culture conditions are given in Table 2. The plate-in-bottle method devised by Mitsuoka et al.[13] was adopted for the cultivation of fastidious anaerobes.

Five chickens of each group were sacrificed by inhalation of chloroform 1, 2, 3, 4 and 6 weeks after hatching and subjected to the examination of intestinal microflora in experiments I to III. In experiment IV, the chickens were examined at 10 days of age just before administration with the antibiotic diet, and at 13 and 19 days of age (one and, 7 days respectively, after administration).

About 1 g of intestinal contents was collected from jejunum (near Meckel's diverticulum) and cecum. The sample was weighed and homogenized with 9 ml of sterile anaerobic diluent. Appropriate

Table 2. *Media and culture methods employed*

Medium	Organisms cultured mainly	Incubation method	Incubation time (days)
Non-selective media			
Medium 10[5]	Fastidious anaerobes	Plate-in-bottle method[13]	4
BL agar[12]	Anaerobes	Steel wool method with air replaced by CO_2[17]	3
EG agar[12]	Anaerobes		
Trypticase soy blood agar[14]	Aerobes	Air	1
Selective media			
BS agar[15]	Bifidobacterium	Steel wool method with air replaced by CO_2	3
ES agar[15]	Eubacterium		
NBGT agar[12]	Bacteroidaceae		
NN agar[12]	Lecithinase-positive Clostridium		
PNC agar[15]	Clostridium		
Modified LBS agar[12]	Lactobacillus		
DHL agar[12]	Enterobacteriaceae	Air	2
TATAC agar[12]	Streptococcus		
PEES agar[12]	Micrococcaceae		
Potato dextrose agar[12]	Yeast and molds	Air	3

dilutions were prepared from it and 0.05 ml of sample was spread on each agar medium. After incubation, colonies were differentiated macroscopically from one another and colonies of each type picked up and identified on all the plates. These organisms were subjected to Gram's staining and classified into families or genera on the basis of morphological and biochemical characteristics. *Lactobacillus* was included into the anaerobic bacterial group.

The number of organisms of the same bacterial group was compared with the number of colonies grown on selective medium and that on non-selective medium. The number on non-selective medium was considered as the accurate count of the corresponding bacterial group when it was larger than that on selective medium. Data on the number of each bacterial group recovered were analyzed statistically by Student's *t* test. As for the occurrence of recovery, they were analyzed by the chi-square test.

Groups fed a diet containing 5 and 50 mg/kg of colistin were expressed with the abbreviations, CL-5 and CL-50, respectively. In the same manner as this, BC-4.8 and BC-48 were used for bacitracin, and ER-2 and ER-20 for enramycin.

RESULTS

1. *Changes in intestinal microflora in long-term administration (experiments I to III)*: The changes in microflora in experiments I to III are shown in Tables 3, 4 and 5, respectively, by using the logarithmic number of bacteria corresponding to each bacterial group. Statistically significant changes are shown in Table 6, where arrows indicate increase and decrease confirmed by Student's *t* test ($p<0.05$). The significant occurrence of recovery confirmed by the chi-square test ($P<0.05$) is also shown by arrows in the same way.

Experiment I: In the CL-5 and CL-50 groups, significant changes in the microflora were seen in bacterial groups, including *Enterobacteriaceae, Streptococcus,* and *Lactobacillus,* total aerobes, total anaerobes, and total bacteria.

In the small intestine *Enterobacteriaceae* increased in CL-5, at 4 weeks of age, but decreased in CL-50 at 6 weeks of age. *Lactobacillus* increased in CL-5 at 1 week of age, but decreased in CL-5 at 3 weeks of age. *Streptococcus* decreased in CL-5 at 6 weeks of age. Total aerobes increased in CL-5 at 2 weeks of age. On the other hand, total anaerobes increased in CL-5 at 1 week of age and decreased at 3 weeks of age.

In the cecum *Enterobacteriaceae* increased in CL-5 at 2 weeks of age. An increase in *Streptococcus* was obvious in CL-5 and CL-50 at 1 week of age. *Lactobacillus* increased in CL-5 and CL-50 at 1 week of age, but decreased in CL-5 at 3 weeks of age. No changes were observed in the number of total aerobes, total anaerobes, or total bacteria.

Experiment II: Changes in the microflora of the BC-4.8 and BC-48 were observed mainly in *Enterobacteriaceae, Streptococcus, Lactobacillus,* total aerobes, total anaerobes and total bacteria.

In the small intestine *Streptococcus* and *Lactobacillus* decreased in BC-48 at 1 week of age. As the reflection of these changes, total aerobes, total anaerobes and total bacteria decreased in this group. A similar variation was observed at 3 weeks of age. *Enterobacteriaceae* increased in BC-48 at 2 weeks of age. *Lactobacillus* increased in BC-4.8 at 2 weeks of age. A decrease in *Streptococcus* was obvious in BC-48 at 4 weeks of age.

In the cecum *Enterobacteriaceae* increased in BC-48 throughout the experimental period. *Lactobacillus* increased in BC-4.8 and BC-48 at 2 weeks of age. An increase in total aerobes was observed in BC-48 at 2 and 3 weeks of age.

Experiment III: Essentially variation in bacterial groups was seen in ER-2 and ER-20 during the experimental period as in the groups CL and BC.

In the small intestine *Streptococcus,* total aerobes and total bacteria increased in

ER-20 at 1 week of age. *Lactobacillus*, total anaerobes and total bacteria decreased in ER-20 at 3 weeks of age. An increase was observed in total anaerobes and total bacteria in ER-2 at 4 weeks of age. *Lactobacillus* decreased in ER-20 at 3 weeks of age. The occurrence of recovery of *Micrococcaceae* decreased in ER-2 and ER-20 at 3 weeks of age.

In the cecum *Enterobacteriaceae* increased

Table 3. *Changes in microflora during administration of colistin diet (Experiment I)*

Weeks after hatching		1			2			3			4			6		
Dosage of antibiotic (mg/kg)		0	5	50	0	5	50	0	5	50	0	5	50	0	5	50
Enterobacteriaceae	J*1	5.2*2 (5)*3	4.9 (4)	4.6 (4)	4.8 (5)	5.6 (5)	3.7 (4)	4.1 (5)	4.4 (5)	3.6 (4)	3.5 (5)	5.1 (5)	3.5 (5)	4.3 (5)	4.3 (3)	2.5 (5)
	C	9.4 (5)	9.1 (5)	7.5 (5)	8.0 (5)	9.0 (5)	8.4 (5)	8.7 (5)	8.5 (5)	8.5 (5)	8.0 (5)	7.8 (5)	7.6 (5)	8.4 (5)	7.9 (5)	6.1 (5)
Streptococcus	J	7.7 (5)	8.0 (5)	7.9 (5)	5.1 (4)	5.5 (5)	5.2 (5)	5.0 (5)	4.9 (5)	5.9 (5)	6.6 (5)	6.0 (5)	6.7 (5)	6.6 (5)	5.2 (5)	6.6 (5)
	C	7.0 (5)	8.7 (5)	8.5 (5)	8.3 (5)	8.3 (5)	8.7 (5)	7.7 (5)	7.5 (5)	7.8 (5)	7.8 (5)	7.9 (5)	8.0 (5)	8.0 (5)	7.7 (5)	8.4 (5)
Micrococcaceae	J	ND*4	ND	ND	4.1 (5)	4.3 (5)	3.8 (5)	4.5 (5)	4.0 (4)	3.7 (5)	3.5 (5)	3.7 (5)	3.8 (4)	3.2 (2)	ND	ND
	C	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.7 (3)	2.8 (2)	2.6 (2)
Bacillus	J	3.3 (5)	3.0 (5)	3.2 (4)	2.9 (5)	3.5 (5)	3.1 (4)	3.1 (4)	3.3 (3)	3.0 (3)	3.3 (5)	3.2 (5)	3.2 (4)	4.2 (1)	2.7 (1)	ND
	C	2.5 (1)	ND	ND	2.3 (1)	ND	2.3 (1)	2.7 (3)	2.9 (2)	2.3 (1)	2.3 (4)	2.6 (4)	2.7 (2)	3.5 (5)	3.7 (5)	3.3 (4)
Molds	J	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	2.3 (2)	ND	ND	ND	ND	2.6 (1)	2.1 (1)	2.3 (2)	ND	ND	ND	2.1 (2)	ND	2.2 (3)	2.1 (1)
Total aerobes	J	7.7	8.0	7.9	5.3	6.1	5.2	5.1	5.1	5.9	6.6	6.0	6.7	6.6	5.3	6.6
	C	9.4	9.2	8.8	9.1	9.1	8.9	8.8	8.6	8.6	8.2	8.2	8.2	8.7	8.2	8.2
Lactobacillus	J	7.3 (5)	7.8 (5)	7.9 (5)	7.6 (5)	7.5 (5)	7.5 (5)	7.6 (5)	5.3 (5)	6.4 (5)	5.9 (5)	6.3 (5)	6.7 (5)	6.2 (5)	5.9 (5)	6.9 (5)
	C	7.1 (5)	8.9 (5)	8.7 (5)	9.0 (5)	8.6 (5)	8.8 (5)	9.0 (5)	8.3 (5)	8.7 (5)	8.2 (5)	8.2 (5)	8.6 (5)	8.9 (5)	8.6 (5)	8.9 (5)
Bifidobacterium	J	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	ND	ND	9.7 (1)	ND	ND	ND	ND	ND	ND	8.4 (2)	8.6 (2)	9.2 (5)	9.3 (1)	8.1 (1)	ND
Eubacterium	J	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	9.4 (2)	9.5 (1)	9.0 (2)	9.7 (2)	9.5 (5)	9.6 (4)	9.2 (5)	9.6 (5)	9.4 (5)	9.3 (5)	9.2 (4)	9.5 (4)	9.2 (5)	9.5 (5)	9.7 (5)
Bacteroidaceae	J	4.1 (4)	4.7 (5)	4.2 (5)	4.4 (5)	5.2 (5)	4.3 (5)	3.8 (2)	4.0 (2)	4.5 (2)	3.9 (2)	4.0 (3)	3.5 (3)	4.2 (3)	3.9 (3)	2.3 (1)
	C	9.8 (5)	9.9 (3)	8.8 (3)	10.0 (5)	10.1 (5)	10.0 (5)	9.6 (5)	9.7 (5)	9.9 (5)	9.7 (5)	9.4 (5)	9.8 (5)	9.5 (5)	9.7 (5)	9.4 (5)
Peptococcaceae	J	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	10.1 (4)	9.6 (4)	9.9 (4)	9.8 (5)	9.8 (3)	9.7 (5)	9.9 (5)	10.0 (5)	10.0 (5)	9.3 (5)	9.7 (5)	9.7 (5)	9.1 (5)	9.2 (5)	9.1 (5)
Clostridium	J	ND	ND	4.5 (2)	ND	3.0 (1)	3.0 (1)	ND	ND	4.4 (2)	ND	ND	ND	ND	ND	ND
	C	9.9 (5)	8.7 (5)	9.6 (5)	9.8 (5)	9.8 (5)	9.8 (5)	9.3 (5)	9.2 (5)	9.9 (5)	9.1 (5)	9.2 (5)	9.6 (5)	9.0 (5)	8.3 (5)	8.7 (5)
Total anaerobes	J	7.3	7.8	7.9	7.6	7.5	7.5	7.6	5.3	6.4	5.9	6.3	6.7	6.2	5.9	6.9
	C	10.5	10.0	10.1	10.4	10.5	10.5	10.3	10.4	10.5	10.1	10.2	10.4	10.0	10.1	10.1
Total bacteria	J	7.8	8.2	8.2	7.6	7.5	7.5	7.6	5.7	6.5	6.7	6.6	7.1	6.7	6.0	7.1
	C	10.5	10.0	10.1	10.4	10.5	10.5	10.3	10.4	10.5	10.1	10.2	10.4	10.0	10.1	10.1

*1 J: Jejunum, C: cecum.

*2 Mean $\log_{10}$ No. of organisms/g of content.

*3 In parentheses is shown the number of birds harboring corresponding bacteria out of five chickens examined.

*4 Not detected (showing a count below about 10^2 organisms/g of content)

in ER-2 and ER-20 at 1 week of age, and in ER-20 at 2 weeks of age. An increase in *Streptococcus* was observed in ER-20 at 1 week of age and in ER-2 at 3 weeks of age. *Micrococcaceae* decreased in ER-2 at 4 weeks of age. Changes in microflora induced by the antibiotics were focussed on such bacterial groups as *Enterobacteriaceae*, *Streptococcus*, *Lactobacillus*, total aerobes, total anaerobes and total bacteria. They were comparatively evident over a period from 1 to 3 weeks of age in this experiment.

Body weight and feed conversion ratio: Transition of body weight and feed conversion ratio are shown in Fig. 1 and Table 9, respectively. Body weight showed

Table 4. *Changes in microflora during administration of bacitracin diet (Experiment II)*

Weeks after hatching		1			2			3			4			6		
Dosage of antibiotics (mg/kg)		0	4.8	48	0	4.8	48	0	4.8	48	0	4.8	48	0	4.8	48
Enterobacteriaceae	J*1	5.1*2 (5)*3	4.0 (5)	5.6 (5)	4.7 (5)	4.8 (5)	6.5 (5)	4.0 (4)	4.5 (4)	4.5 (5)	3.7 (5)	3.0 (5)	3.7 (5)	4.3 (4)	4.2 (5)	3.8 (3)
	C	8.7 (5)	8.9 (5)	9.6 (5)	8.7 (5)	9.0 (5)	9.4 (5)	7.9 (5)	8.3 (5)	9.1 (5)	6.7 (5)	7.5 (5)	9.0 (5)	7.7 (5)	7.4 (5)	9.1 (5)
Streptococcus	J	7.7 (5)	6.0 (5)	4.4 (5)	4.8 (4)	5.5 (5)	5.2 (5)	4.9 (5)	5.0 (5)	5.6 (5)	6.3 (5)	5.6 (5)	5.2 (5)	6.2 (5)	6.9 (5)	6.3 (5)
	C	7.8 (5)	6.8 (5)	8.8 (5)	8.2 (5)	7.9 (5)	8.6 (5)	7.7 (5)	8.3 (5)	8.2 (5)	8.6 (5)	8.4 (5)	8.6 (5)	8.2 (5)	7.9 (5)	8.7 (5)
Micrococcaceae	J	ND*4	ND	ND	3.6 (4)	3.3 (3)	3.3 (5)	3.4 (5)	3.0 (5)	3.1 (3)	3.9 (1)	3.2 (3)	3.8 (3)	2.8 (2)	3.5 (4)	3.2 (3)
	C	ND	ND	ND	2.1 (2)	3.0 (2)	3.2 (4)	ND	2.9 (1)	2.6 (3)	2.0 (2)	2.7 (1)	ND	3.0 (4)	3.0 (2)	2.3 (2)
Bacillus	J	3.2 (5)	3.6 (5)	3.8 (5)	3.6 (5)	3.4 (5)	3.7 (5)	3.6 (5)	2.9 (3)	3.0 (4)	ND	3.1 (5)	3.3 (5)	3.1 (5)	3.1 (5)	3.2 (5)
	C	ND	ND	ND	2.7 (3)	3.1 (4)	2.4 (3)	2.6 (1)	2.6 (3)	2.8 (2)	3.1 (5)	2.8 (5)	2.3 (3)	2.8 (5)	3.0 (4)	2.9 (3)
Molds	J	ND	ND	ND	ND	ND	ND	2.7 (1)	ND	ND	2.4 (1)	ND	ND	2.2 (1)	ND	ND
	C	2.3 (1)	ND	ND	2.0 (1)	2.5 (2)	2.6 (2)	2.3 (1)	ND	ND	2.3 (1)	ND	ND	2.2 (2)	2.3 (5)	2.2 (1)
Total aerobes	J	7.7	6.1	5.8	5.1	5.8	6.7	5.0	5.1	5.7	6.3	5.7	5.3	6.2	6.9	6.3
	C	8.8	8.9	9.7	8.9	9.1	9.5	8.5	8.8	9.2	8.8	8.6	9.2	8.3	8.1	9.2
Lactobacillus	J	8.1 (5)	6.3 (5)	4.3 (5)	7.5 (5)	8.3 (5)	8.3 (5)	7.8 (5)	5.7 (5)	6.5 (5)	6.6 (6)	5.9 (5)	5.4 (5)	6.7 (5)	7.4 (5)	6.7 (5)
	C	8.7 (5)	8.8 (5)	8.9 (5)	8.3 (5)	9.3 (5)	9.4 (5)	8.8 (5)	8.6 (5)	8.4 (5)	9.3 (5)	9.0 (5)	8.9 (5)	9.2 (5)	8.2 (5)	8.3 (5)
Bifidobacterium	J	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	ND	ND	9.6 (1)	ND	ND	ND	8.5 (3)	8.6 (1)	8.2 (1)	ND	ND	ND	ND	ND	ND
Eubacterium	J	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	9.6 (3)	9.7 (3)	9.9 (4)	9.3 (2)	9.3 (3)	9.3 (4)	9.5 (5)	9.3 (5)	9.2 (5)	9.1 (5)	9.9 (4)	9.4 (3)	9.8 (4)	9.6 (5)	9.4 (5)
Bacteroidaceae	J	4.4 (4)	3.9 (4)	4.9 (5)	3.7 (5)	4.1 (5)	5.9 (5)	4.4 (4)	3.2 (5)	3.9 (3)	3.2 (2)	2.4 (1)	3.5 (3)	4.7 (2)	4.0 (4)	2.3 (1)
	C	10.1 (5)	10.0 (4)	10.0 (4)	10.0 (5)	10.1 (5)	9.2 (5)	9.7 (5)	9.9 (5)	9.6 (5)	9.3 (5)	10.2 (5)	9.9 (5)	9.9 (5)	9.9 (5)	9.9 (5)
Peptococcaceae	J	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	10.2 (4)	10.0 (4)	9.9 (5)	9.5 (4)	9.9 (4)	9.8 (5)	9.5 (5)	9.9 (5)	10.1 (5)	9.5 (5)	9.9 (3)	9.8 (5)	9.7 (4)	9.8 (3)	9.9 (5)
Clostridium	J	4.6 (2)	ND	3.3 (1)	ND	3.6 (3)	ND	5.0 (2)	ND	ND	2.8 (3)	ND	ND	3.6 (2)	ND	ND
	C	9.5 (5)	9.8 (5)	8.4 (4)	9.5 (5)	9.7 (5)	9.8 (5)	9.5 (5)	9.7 (5)	9.6 (5)	9.0 (5)	9.0 (5)	9.7 (5)	9.5 (5)	9.6 (5)	9.3 (5)
Total anaerobes	J	8.1	6.3	5.1	7.5	8.3	8.3	7.8	5.7	6.5	6.6	5.9	5.4	6.7	7.4	6.7
	C	10.6	10.5	10.5	10.4	10.5	10.4	10.4	10.4	10.5	10.3	10.5	10.5	10.4	10.4	10.5
Total bacteria	J	8.3	6.6	5.9	7.5	8.3	8.3	7.9	5.9	6.6	6.8	6.2	5.7	6.9	7.5	6.9
	C	10.6	10.5	10.6	10.4	10.5	10.4	10.4	10.4	10.5	10.3	10.5	10.5	10.4	10.4	10.5

For symbols *1~*4 see the footnote of Table 3.

a significant decrease in CL-5 at 6 weeks of age and a significant increase in BC-48 at 6 weeks of age. The feed conversion ratio was imporved in BC-48 and ER-2 as a whole. It showed a tendency to rise in the early stage of administration.

2. *Changes in intestinal microflora in short-term administration (experiment IV).*

One day after administration (13 days of age): In the small intestine *Enterobacteriaceae, Streptococcus,* and *Entrobacteriaceae* and *Lactobacillus* decreased in the CL, BC, and ER groups respectively.

In the cecum *Streptococcus* increased in the CL groups, and *Enterobacteriaceae* in the BC groups. A decrease in *Lactobacillus* was noticed in the ER diet.

Seven days after administration (19 days of

Table 5. *Changes in microflora during administration of enramycin diet (Experiment III)*

Weeks after hatching		1			2			3			4			6		
Dosage of antibiotics (mg/kg)		0	2	20	0	2	20	0	2	20	0	2	20	0	2	20
Enterobacteriaceae	J*4	5.5*2 (5) *3	5.3 (5)	6.3 (5)	4.4 (5)	4.1 (5)	5.3 (4)	4.0 (5)	4.1 (5)	4.8 (5)	4.5 (5)	4.1 (5)	4.4 (5)	4.4 (5)	3.4 (5)	5.7 (4)
	C	8.8 (5)	9.8 (5)	10.0 (5)	8.0 (5)	8.5 (5)	8.8 (5)	7.2 (5)	7.9 (5)	7.6 (5)	8.7 (5)	7.3 (5)	8.4 (5)	7.5 (5)	7.7 (5)	7.6 (5)
Streptococcus	J	7.7 (5)	8.5 (5)	9.0 (5)	4.4 (5)	4.7 (5)	3.7 (5)	4.3 (5)	4.1 (5)	3.8 (5)	6.2 (5)	6.0 (5)	5.3 (5)	6.2 (5)	7.0 (5)	6.3 (5)
	C	9.5 (5)	9.0 (5)	10.3 (5)	6.6 (5)	7.2 (5)	7.5 (5)	7.2 (5)	8.2 (5)	7.9 (5)	8.5 (5)	8.6 (5)	8.0 (5)	9.1 (5)	8.8 (5)	9.6 (5)
Micrococcaceae	J	ND*4	ND	ND	3.9 (5)	4.4 (5)	4.0 (4)	3.3 (5)	2.7 (1)	2.7 (1)	4.2 (2)	ND	3.5 (2)	2.9 (3)	ND	3.1 (4)
	C	ND	ND	ND	ND	ND	ND	3.8 (5)	3.6 (4)	2.8 (2)	4.6 (5)	3.0 (2)	4.9 (5)	2.9 (4)	3.0 (4)	3.0 (4)
Bacillus	J	3.1 (4)	3.6 (5)	3.2 (4)	3.0 (5)	3.6 (5)	3.4 (5)	3.9 (4)	3.8 (5)	ND	3.2 (5)	ND	ND	4.1 (2)	ND	2.8 (3)
	C	4.9 (5)	4.3 (5)	5.1 (5)	4.2 (5)	4.2 (5)	4.1 (5)	3.9 (5)	4.3 (5)	4.3 (5)	3.8 (5)	2.3 (1)	3.8 (5)	3.7 (5)	3.9 (5)	3.7 (4)
Molds	J	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	ND	ND	ND	2.9 (1)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total aerobes	J	7.7	8.5	9.0	4.8	5.0	5.3	4.6	4.5	4.8	6.2	6.0	5.4	6.2	7.0	6.4
	C	10.0	10.4	10.5	8.7	8.8	8.0	8.4	8.2	7.8	8.8	8.5	9.5	9.1	9.6	9.2
Lactobacillus	J	7.9 (5)	8.6 (5)	8.9 (5)	7.2 (5)	7.4 (4)	6.0 (5)	7.9 (5)	6.7 (4)	5.0 (4)	6.5 (5)	7.3 (5)	5.6 (5)	6.2 (5)	7.0 (5)	6.4 (5)
	C	3.9 (4)	6.2 (5)	5.6 (5)	4.0 (2)	7.1 (3)	6.6 (3)	6.6 (5)	6.1 (5)	4.9 (5)	8.3 (5)	9.2 (5)	8.0 (5)	9.4 (5)	9.5 (5)	9.7 (5)
Bifidobacterium	J	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	ND	ND	ND	ND	7.5 (2)	9.1 (1)	ND	9.0 (1)	ND	9.9 (1)	9.8 (1)	10.9 (1)	9.7 (4)	9.3 (2)	9.7 (3)
Eubacterium	J	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	9.7 (5)	10.0 (5)	9.5 (4)	10.1 (5)	9.1 (5)	9.8 (2)	9.3 (5)	9.7 (5)	10.5 (5)	9.6 (5)	10.1 (5)	10.2 (5)	10.1 (5)	10.3 (5)	9.9 (4)
Bacteroidaceae	J	5.1 (4)	5.0 (5)	4.9 (5)	3.3 (2)	3.4 (4)	3.9 (3)	3.8 (3)	3.9 (3)	4.3 (4)	3.3 (4)	3.3 (3)	3.0 (5)	3.3 (4)	2.7 (3)	2.7 (1)
	C	10.0 (5)	9.6 (5)	10.2 (5)	10.4 (5)	10.1 (5)	9.7 (5)	10.3 (5)	9.9 (5)	10.2 (5)	10.2 (5)	9.8 (5)	9.8 (5)	6.1 (5)	5.3 (5)	4.8 (5)
Peptococcaceae	J	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	10.6 (5)	10.3 (5)	10.8 (5)	10.3 (5)	10.1 (5)	10.3 (5)	10.5 (5)	10.4 (5)	10.6 (5)	10.5 (5)	9.7 (5)	10.4 (5)	10.2 (5)	10.2 (5)	10.1 (5)
Clostridium	J	4.6 (1)	3.2 (2)	6.0 (1)	ND	ND	ND	ND	3.7 (1)	ND	ND	ND	ND	ND	ND	ND
	C	9.6 (4)	10.0 (5)	10.1 (5)	9.2 (5)	9.9 (5)	9.7 (4)	9.2 (5)	9.5 (5)	9.9 (5)	8.8 (3)	9.4 (3)	8.0 (2)	9.7 (5)	9.6 (4)	8.5 (5)
Total anaerobes	J	7.9	8.6	8.9	7.2	7.4	6.0	7.9	6.7	5.0	6.5	7.3	5.6	6.9	7.4	7.7
	C	10.6	11.0	10.8	10.8	10.7	10.8	10.8	11.1	10.9	10.6	10.8	10.7	10.6	10.5	10.6
Total bacteria	J	8.1	8.9	9.3	7.2	7.4	6.1	7.9	6.7	5.2	6.5	7.3	5.9	7.0	7.5	7.9
	C	10.7	11.2	10.8	10.8	10.7	10.8	10.8	11.1	10.9	10.6	10.8	10.7	10.6	10.5	10.6

For symbols *1~*4 see the footnote of Table 3.

age): In the small intestine *Streptococcus, Streptococcus* and *Lactobacillus,* and *Enterobacteriaceae* and *Streptococcus* decreased in the CL, BC, and ER groups, respectively.

In the cecum Streptococcus decreased and *Clostridum* increased in the CL groups. A decrease in *Bacteroidaceae* and an increase in *Clostridium* were obvious in the ER group. The effect of the antibiotic on the intestinal microflora was seen even 7 days after administration.

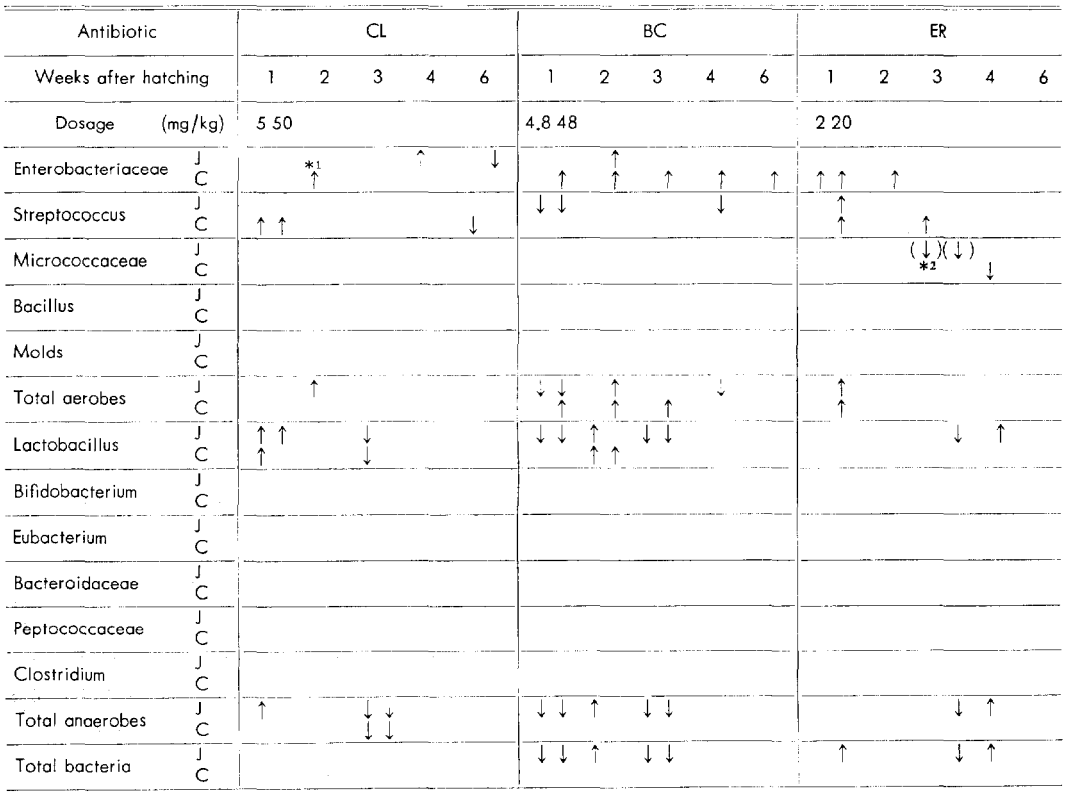
DISCUSSION

In the long-term administration with the antibiotics, the intestinal microflora showed significant changes mainly over a period from 1 to 3 weeks of age. It is well-known that a microflora is established 2 or 3 weeks after hatching.[2,9,16] A significant variance showed a tendency to diminish at 4 and 6 weeks of age. Antibiotics contained in feed may affect the establishment of intestinal microflora in broiler chickens.

The antibacterial spectra of the antibiotics administered were as follows.[23] CL: gram negative bacteria, BC: gram-positive bacteria, gram-negative cocci, leptospires and actinomycetes, and ER: gram-positive bacteria.

As shown in Table 6, the changes in microflora were not consistent with the antibacterial spectrum of the antibiotic concerned. In the CL groups, an increase in *Enterobacteriaceae* was observed at 2 and 4 weeks of age. Kikuchi et al.[10] obtained similar results from their experimental system constituted by gnotobiotic mice and *Escherichia coli*. They also demonstrated

Table 6. *Significant changes in microflora during the administration of antibiotics (Experiments I to III)*



*1 Arrow indicates a significant change confirmed by Student's t test ($P<0.05$).

*2 Arrow in parentheses indicates a significant occurence of recovery confirmed by chi-square test ($P<0.05$).

that some strains of *E. coli* were not decreased in number by administration of colistin, but were still sensitive to the antibiotic *in vitro*. An antagonistic variance of bacteria was presumed to occur during administration of dietary antibiotics.

Saloma et al.[19] proposed one hypothesis that an antagonistic change might be induced by microorganisms resistant to the drug in question. This change may be a reasonable factor. The results obtained from the present experiments suggest the significance of floral variation related to the ecological balance in the intestine. Incomprehensible changes, such as an increase in *Streptococcus* in the ER groups at 1 week of age and an alternative change in *Lactobacillus* in the BC groups over a period from 1 to 3 weeks of age, were observed. They may be due to the difference in antibiotic susceptibility between species or strains in the corresponding bacterial group. Changes in *Streptococcus* at a species level were described by Barnes et al.[3] in animals fed a diet containing bacitracin.

The variance of intestinal microflora induced by the dietary antibiotic is assumed to be composed of the following three phenomena.

1. Changes directly related to the antibacterial spectrum of the antibiotic concerned. 2. Antagonistic changes related to an ecological balance in the bacterial flora. 3. Changes in the quantiatative balance of bacteria which constitute each bacterial group.

By these regards it will be concluded that the changes in intestinal microflora during the administration of an antibiotic diet will be expressed as a complicated form of the three possible modes of transition of microflora described above.

Few fastidious anaerobes varied apparently in the present experiments. Detailed studies at a species level are necessary to evaluate the significance of them.

In experiment IV, the changes in microflora

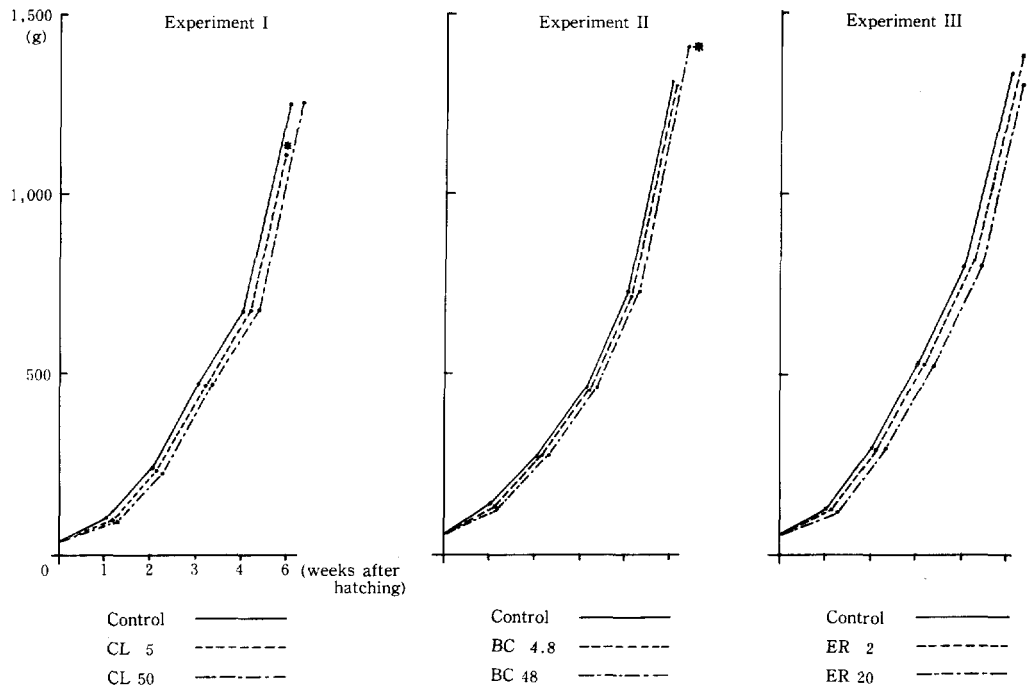


Fig. 1. Changes in body weight in experiments I to III

were partly similar to those in experiments I to III. It seemed that they might be more consistent with the antibacterial spectrum of the antibiotic administered. It should be noted that the effect of the antibiotic on the intestinal microflora still lingered 7 days after administration. Since it is a common characteristic of the antibiotics employed that these substances are not absorbed from the intestial wall, this result is very significant. Once the intestinal microflora is modified by an antibiotic or some other drug, it will take a rather long time for it to be re-formed. In this case, there may occur the multiplication of pathogenic bacteria elicited by the modification of the intestinal flora. Attention should be paied to the administration of an

Table. 7 *Changes in microflora in short-term administration of dietary antibiotics (Experiment IV)*

Days after hatching		10	13				19			
Dosage of antibiotic	(mg/kg)	0	0	CL 100	BC 96	ER 40	0	CL 100	BC 96	ER 40
Enterobacteriaceae	J*1	2.8*2 (5)*3	5.7 (5)	3.3 (2)	4.2 (4)	4.4 (5)	5.4 (5)	3.9 (3)	4.3 (5)	3.6 (3)
	C	8.2 (5)	8.4 (5)	7.8 (5)	9.6 (5)	9.3 (5)	8.4 (5)	7.9 (5)	9.0 (5)	8.6 (5)
Streptococcus	J	7.2 (5)	7.1 (5)	6.4 (5)	5.0 (5)	4.4 (5)	8.1 (5)	5.7 (5)	5.1 (5)	5.3 (5)
	C	9.0 (5)	8.1 (5)	8.8 (5)	8.2 (5)	8.5 (5)	9.3 (5)	7.8 (5)	8.0 (5)	8.4 (5)
Micrococcaceae	J	2.4 (1)	3.3 (4)	ND	2.7 (1)	3.2 (2)	3.0 (5)	3.4 (2)	3.3 (3)	3.3 (3)
	C	3.5 (4)	3.2 (4)	3.2 (2)	2.6 (2)	3.0 (1)	3.4 (5)	2.8 (1)	3.3 (5)	2.9 (4)
Bacillus	J	2.7 (5)	2.8 (2)	2.8 (1)	2.6 (1)	ND	2.9 (4)	3.5 (2)	2.8 (2)	2.9 (2)
	C	2.8 (2)	3.0 (4)	2.8 (4)	3.0 (2)	3.0 (2)	3.4 (5)	2.7 (4)	3.4 (3)	3.5 (4)
Molds	J	ND*4	ND	ND	ND	ND	ND	ND	ND	ND
	C	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total aerobes	J	7.2	7.1	6.4	5.3	5.5	8.1	5.8	5.4	5.3
	C	9.2	8.9	9.0	9.7	9.4	9.4	6.7	9.1	9.1
Lactobacillus	J	8.4 (5)	7.2 (5)	6.6 (5)	6.1 (5)	5.7 (4)	8.2 (5)	7.4 (5)	6.2 (5)	7.4 (5)
	C	9.5 (5)	9.5 (5)	8.9 (5)	6.3 (3)	6.9 (5)	9.5 (5)	9.0 (5)	9.3 (5)	8.9 (5)
Bifidobacterium	J	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	ND	9.1 (1)	9.1 (2)	9.0 (1)	ND	8.8 (2)	9.6 (2)	9.7 (1)	ND
Eubacterium	J	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	10.6 (1)	10.2 (5)	9.8 (4)	9.8 (3)	10.0 (5)	9.9 (4)	9.9 (5)	10.4 (5)	10.2 (5)
Bacteroidaceae	J	ND	4.4 (2)	3.3 (1)	ND	3.5 (1)	2.7 (2)	ND	ND	ND
	C	10.0 (5)	8.8 (5)	10.1 (5)	9.9 (5)	9.9 (5)	10.0 (5)	9.3 (5)	9.9 (5)	9.0 (5)
Peptococcaceae	J	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C	10.2 (5)	10.5 (4)	9.9 (5)	9.9 (5)	10.5 (5)	10.0 (5)	10.2 (5)	10.5 (5)	10.3 (5)
Clostridium	J	3.5 (2)	ND	ND	ND	ND	ND	ND	ND	ND
	C	9.0 (3)	9.6 (5)	9.5 (5)	9.7 (4)	9.8 (4)	9.5 (5)	10.2 (5)	9.8 (4)	10.4 (5)
Total anaerobes	J	8.4	7.2	6.6	6.1	5.7	8.2	7.4	6.2	7.4
	C	10.7	10.8	10.5	10.5	10.8	10.6	10.7	10.9	10.9
Total bacteria	J	8.4	7.4	6.8	6.3	5.8	8.5	7.4	6.4	7.4
	C	10.7	10.8	10.5	10.6	10.8	10.7	10.7	10.9	10.9

For symbols *1~*4 see the footnote of Table 3.

antibiotic, especially over a period of 2 to 3 weeks after hatching, which is a critical stage for the establishment of an intestinal microflora in the chicken.

Body weight showed a significant increase in BC-48 and a decrease in CL-5 at 6 weeks of age. The feed conversion ratio was improved in BC-48 and ER-2. Coates et al.[6] and Hill et al.[8] demonstrated that well-nourished health chicks responded to no antibiotic supplements when they were housed in a carefully cleaned and disinfected place. In the present experiments, chicks were reared in a disinfected air-conditioned room and fed formulated feed prepared for experimental use and consisting of selected clean ingredients. At the same time, judgeing from the number of birds examined, the body weight and feed conversion ratio obtained may be insufficient to estimate the efficiency of the antibiotics employed, which may be explained to some extent from the results of the present experiments.

Table 8. *Singificant changes in microflora in short-term administration of dietary antibiotics (Experiment IV)*

Antibiotic		CL		BC		ER	
Days after hatching		13	19	13	19	13	19
Dosage (mg/kg)		100		96		40	
Enterobacteriaceae	J*	↓*1				↓	↓
	C			↑			
Streptococcus	J		↓	↓	↓		↓
	C	↑	↓				
Micrococcaceae	J						
	C						
Bacillus	J						
	C						
Molds	J						
	C						
Total aerobes	J				↓	↓	↓
	C						
Lactobacillus	J				↓	↓	
	C					↓	
Bifidobacterium	J						
	C						
Eubacterium	J						
	C						
Bacteroidaceae	J						
	C						↓
Peptococcaceae	J						
	C						
Clostridium	J						
	C		↑				↑
Total anaerobes	J				↓		
	C						
Total bacteria	J				↓	↓	
	C						

For symbol *1 see the footnote of Table 6.

* Abbreviation: J, jejunum; C, cecum

Table 9. *Cumulative feed conversion ratio in experiments I to III*

Antibiotics (mg/kg)	CL			BC			ER		
	0	5	50	0	4.8	48	0	2	20
0–1week	1.24	1.20	1.21	1.20	1.27	1.32	1.18	1.18	1.16
–2week	1.48	1.36	1.50	1.35	1.29	1.40	1.30	1.33	1.24
–3week	1.63	1.61	1.70	1.61	1.47	1.49	1.46	1.43	1.49
–4week	1.73	1.71	1.80	1.80	1.70	1.70	1.58	1.50	1.58
–6week	1.99	2.00	2.06	1.95	2.06	1.85	1.96	1.90	2.02

From the present experiment, some possible modes of changes were postulated on the transition of intestinal microflora during the administration of dietary antibiotics. It is apparent that the beneficial effects of feed additive antibiotics on livestock production are deeply connected with the metabolism of the host, circumstantial condition and intestinal microflora. Further experiments are needed with these aspects involved.

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