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THE EFFECT OF ALKALI-DECOMPOSED AUREOMYCIN ON THE GROWTH OF CHICKS[1]

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ABSTRACT

A series of 4 experiments, using 3-day-old white Leghorn cockerels as the material, was conducted to test for the growth-promoting effect of aureomycin. An increase of body weight gain in chicks supplemented with alkali-decomposed aureomycin was demonstrated. The result was comparable to the growth effect due to untreated aureomycin. Since stability of aureomycin is strictly a function of pH and temperature, it is likely that when aureomycin in minute quantity is administered to chicks, the antibiotic would be degraded *in vivo*. The decomposed aureomycin prepared *in vitro* was found to possess no antibacterial potency. Therefore the results of the present experiments lend weight to the suggestion that the current theory of antibacterial action of the drug on intestinal microflora is dubious. The increased thyroid index obtained in the present study on chicks supplemented with untreated or decomposed aureomycin indicated that hypothyroidism might possibly have played a role in the weight gain effect.

INTRODUCTION

In animal husbandry, it is a usual practice to supply minute amounts of aureomycin in the feed to promote growth. The current theory suggests that the antibiotic growth-promoting effect is due to the control of intestinal microflora so that pathogenic bacteria are suppressed and vitamin-B producing microbes enhanced, resulting in better nutrition (1-6). However, the mechanism by which such a benefit is produced has not yet been convincingly demonstrated, since the bacteriological findings were contradictory and perplexing in some cases (7, 8). Further more, evidence at variance with the antibacterial concept of promotion of growth was found both in this laboratory (9-12) and by other workers (13-15).

Studies in this laboratory showed that alkali-decomposed aureomycin promoted tadpole growth while intact aureomycin retarded growth (10); that the growth-promoting effect was found in the precipitate portion of the

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decomposed aureomycin (11); and that the precipitate possessed no antibiotic potency as shown by bacteriological assays (12). It was then suggested that the growth-promoting effect of aureomycin fed to birds and mammals might not result from the antibacterial activity of the antibiotic *per se* but from its degraded product(s) after passing through the alkaline medium of the small intestine at body temperature. Therefore, it is imperative to verify our hypothesis on the growth of warm-blooded species, the chicks.

MATERIALS AND METHODS

I. *Preparation of the feed*:

1. Basal ration—There were 2 formulae for the composition of the basal ration, varying in Ca content, as follows:

TABLE I.
Composition of No. 1 basal ration, high Ca diet

Ingredients	%
Ground yellow corn	42.00
Soybean oil meal	28.00
Rice bran	9.00
Wheat bran	7.00
Dehydrated green feed	5.00
Skim milk powder	3.60
Yeast powder	1.00
Bone meal	2.40
Oyster shell powder	1.20
NaCl	0.68
+Rovimix	0.12
Total	100.00

2. Untreated aureomycin feed—Crystalline chlortetracyline HC1* in the proportion of 25 and 50 parts per million parts of the basal ration was used in the present experiments as the untreated aureomycin.

3. Alkali-decomposed aureomycin feed—30 gm of crystalline chlortetracycline HC1 were dissolved in 4477.5 ml of distilled water. To this aureomycin solution 22.5 ml of 6N NaOH were added to make up the final solution to pH 8.35. The alkalinized mixture was incubated at 37 C for a week and then allowed to stand in the dark at 18 C for another 10 days. During this period grayish-yellow colored crystals appeared on the wall and at the bottom of the glass container. The precipitate was then separated from the mother liquid, washed 3 times with distilled water and dried in an incubator at 25 C for 2 days. The yield was 3120 mg of decomposed substance. The result was consistantly reproducible. The decomposed product was mixed with the basal ration in the proportion of 1, 2, 8, 10 and 50 ppm respectively.

TABLE II.
Composition of No. 2 basal ration, low Ca diet

Ingredients	%
Ground yellow corn	42.00
Soybean oil meal	28.00
Rice bran	9.00
Wheat bran	7.00
Dehydrated green feed	5.00
Skim milk powder	3.40
Yeast powder	3.00
Bone meal	1.00
NaCl	1.00
$MnSO_4$	0.05
KH_2PO_4	0.50
+Rovimix	0.05
Total	100.00

+ Manufactured by F. Hoffmann-La Roche & Co., Ltd., Basle, Switzerland. Each gram contains 40,000 IU vitamin A, 40 mg vitamin B_{12} and 10,000 IU vitamin D_3.

The ingredients of each of the 3 kinds of rations were mixed respectively in an electric mixer for 30 minutes in order to ensure an even distribution of the ingredients in the ration.

* Kindly supplied by the Cyanamid Taiwan Corporation, Taipei, Taiwan.

II. *The animals*:

3-day-old, single-combed, white Leghorn cockerels of the Hyline strain were used. 30 baby chicks were selected around the mean body weight of each 100 chicks. 160 such selected birds were used for the 4 experiments run from December, 1965 to July, 1966. Each group of 10 chicks was reared in an electrically heated brooder with raised screen floor of 74 × 60 cm. All chicks in one experiment were kept under identical environmental conditions except the feed which consisted of 8 different kinds of rations. Feed and water were supplied *ad libitum*.

Body weight increment of each chick and food consumption for each group of chicks were recorded weekly. Experiment No. 3 was run for 6 weeks and the other 3 experiments for 4 weeks. At the end of experimentation all birds in Experiment Nos. 4, 5 and 6 were sacrificed by bleeding with a cut around the throat. Their thyroids after dissecting free from connective tissues and fat were weighed to the nearest 0.05 mg with a microtorsion balance.

RESULTS

I. *Growth response*

1. Experiment using high Ca ration—Basal ration No. 1 which contained not less than 3.9% of Ca was used. The result of body weight gain of the cockerels is tabulated as follows:

TABLE III.

Body weight gained by the 6 groups of cockerels at the age of 6 weeks (Experiment No. 3)

Ration	No. of cockerels	Initial wt mean±SE, gm	Final wt mean±SE, gm	Wt gained mean±SE, gm	Difference of wt gained between chicks on basal & other rations mean±SE, gm
BR* No. 1	10	31.1±0.07	485.9±11.87	454.8±11.80	—
BR+25 ppm UA*	10	31.1±0.07	494.3±15.53	463.2±15.54	8.4±19.51
BR+50 ppm DA*	10	31.2±0.08	461.3±11.88	430.1±12.52	—24.7±17.20
BR+10 ppm DA	10	31.2±0.08	509.2± 7.49	478.0± 7.43	23.2±13.94
BR+ 2 ppm DA	10	31.2±0.08	497.9±12.01	466.7±11.96	11.9±16.80
BR+ 1 ppm DA	10	31.2±0.08	540.9± 4.54	509.7± 4.54	54.9±12.64 ($p<0.001$)

* BR=Basal ration, UA=Untreated aureomycin, DA=Decomposed aureomycin.

The result in TABLE III indicated that the cockerels fed with either untreated aureomycin or decomposed aureomycin of the lower dosages gained more weight than the controls on basal ration. Statistical analysis with t-test showed that cockerels had a highly significant increase of weight gain after supplementation of 1 ppm of decomposed aureomycin, thus indicating a positive growth-promoting effect of the degraded antibiotic.

TABLE III also shows the trend that the growth-promoting effect of decomposed aureomycin may lie in the range of lower doses between 1 and 10 ppm which served as a reference for the next experiments.

2. Experiments using low Ca ration—Basal ration No. 2 containing 1.3% of Ca in the form of bone meal and skim milk powder and 0.5% of P as inorganic phosphate was used in the following 3 experiments, 2 of them were duplicates. The results are presented in TABLES IV to VI.

TABLE IV.

Body weight gained by the 3 groups of cockerels at the age of 4 weeks (Experiment No. 4)

Ration	No. of cockerels	Initial wt mean±SE, gm	Final wt mean±SE, gm	Wt gained mean±SE, gm	Difference of wt gained between chicks on basal & other rations mean±SE, gm
BR No. 2	10	37.2±0.08	314.2±5.54	277.0±5.49	—
BR+25 ppm UA	10	37.2±0.07	330.0±6.68	292.8±5.75	15.8±7.95 P=0.06
BR+ 2 ppm DA	10	37.1±0.07	318.1±7.28	281.0±7.23	4.0±9.07

Abbreviations as for Table III.

TABLE IV shows that low Ca ration rendered the growth-promoting effect of untreated aureomycin evident with p=0.06 but that of decomposed aureomycin did not show up. However, the effect was obvious in Experiment No. 5 (TABLE V) with p=0.05. Therefore the 2 duplicate experiments complemented each other in showing the growth-promoting effect.

TABLE V.

Body weight gained by the 3 groups of cockerels at the age of 4 weeks (Experiment No. 5)

Ration	No. of cockerels	Initial wt mean±SE, gm	Final wt mean±SE, gm	Wt gained mean±SE, gm	Difference of wt gained between chicks on basal & other rations mean±SE, gm
BR No. 2	10	37.8±1.00	294.1±6.10	256.3±6.03	—
BR+25 ppm UA	10	37.6±0.90	297.1±6.67	259.5±6.61	3.2±9.80
BR+ 2 ppm DA	10	37.8±0.97	308.9±3.33	271.1±3.17	14.8±7.46 P=0.05

Abbreviations as for Table III.

TABLE VI.

Body weight gained by the 4 groups of cockerels at the age of 4 weeks (Experiment No. 6)

Ration	No. of cockerels	Initial wt mean±SE, gm	Final wt mean±SE, gm	Wt gained mean±SE, gm	Difference of wt gained between chciks on basal & other rations mean±SE, gm
BR No. 2	10	41.0±0.26	305.0±5.85	264.0±5.86	—
BR+50 ppm UA	10	41.0±0.26	318.1±6.34	277.1±6.28	13.1± 8.59
BR+25 ppm UA	10	41.0±0.26	318.9±8.46	277.9±8.74	13.9±10.52
BR+ 8 ppm DA	10	41.0±0.26	316.5±4.74	275.5±4.76	11.5± 7.55

Abbreviations as for Table III.

TABLE VI presents data of another experiment in which the chicks, fed with either untreated aureomycin in the doses of 25 and 50 ppm or decomposed aureomycin of 8 ppm, showed some appreciably greater weight gain than the controls, but the difference was not

statistically significant. This might be due to the increase of environmental temperature. However, the result did show a similar and comparable effect of untreated and decomposed aureomycin on growth.

TABLE VII.

Thyroid indices of different groups of chicks at the age of 4 weeks

Experiment No. Environmental temperature, mean±SE, C	Ration	Thyroid index, mean±SE, mg/100 gm BW	Gp difference, mean±SE, mg/100 gm BW	P
No. 4	BR No. 2	6.97±0.27	—	
22.8±0.70	BR+25 ppm UA	8.05±0.30	1.08±0.49	P=0.05
	BR+ 2 ppm DA	9.80±0.86	2.83±1.33	0.05>P>0.025
No. 5	BR No. 2	5.43±0.31	—	
23.1±0.66	BR+25 ppm UA	5.70±0.33	0.27±0.46	
	BR+ 2 ppm DA	6.08±0.33	0.65±0.44	0.20>P>0.10
No. 6	BR No. 2	7.80±0.36	—	
29.2±0.17	BR+50 ppm UA	7.66±0.37	—0.14±0.52	
	BR+25 ppm UA	7.70±0.46	—0.10±0.59	
	BR+ 8 ppm DA	8.26±0.38	0.46±0.52	

Abbreviations as for Table III.

II. *The thyroid index*

TABLE VII indicates that the chicks in Experiment No. 4 showed a significantly higher thyroid index after supplement of untreated or decomposed aureomycin; whereas the increase was not so significant in Experiment No. 5 and not at all in Experiment No. 6. This decrease was probably attributable to temperature factor. The environmental temperature rose from 22.8 C to 29.2 C when Experiments No. 4, No. 5 and No. 6 were conducted consecutively in that order from winter to summer.

DISCUSSION

The present experiments demonstrated that decomposed aureomycin stimulated body weight gain in chicks which was comparable to the growth-promoting effect due to untreated aureomycin. However, the weight gain effect could be influenced by the composition of the basal ration and the environmental temperature.

It is clear from the result in TABLE III that high Ca diet (basal ration No. 1 containing not less than 3.9% of Ca in the form of bone meal, oyster shell power and skim milk powder) inhibited the expression of growth response to untreated aureomycin while that of decomposed aureomycin was not affected. The reason is that high Ca content in tetracycline-supplemented diet would prevent the antibiotic from being utilized due to the formation of a complex between Ca and aureomycin (16-18). On the other hand, decomposed aureomycin was already degraded *in vitro* and thus there was no occasion for Ca to bind aureomycin.

The presence of casein in the form of skim milk powder is another diet factor to affect weight gain. According to Hill *et al.*, growth response to aureomycin was decreased or even eliminated when casein was added to the ration (19). Both basal ration Nos. 1 and 2 in this study contained skim milk powder. Body weight gain due to aureomycin

feeding could have been more evident if skim milk powder were omitted from the present diet.

Hoffmann and Shaffner demonstrated the effect of environmental temperature on chick thyroids as that cold made the gland more active than warm environment (20). An active winter gland would be more susceptible to drug treatment than a reluctant one. The result in TABLE VII might illustrate such an example. *Pari passu* with the decrease of thyroid response to aureomycin when temperature rose, there might be a weakening or even an abolition of the growth-promoting effect.

Since aureomycin is the most unstable compound among the 4 members of the tetracycline family and its stability is strictly a function of pH and temperature (21 24), it is most likely that when aureomycin is administered to chicks in minute amount together with the basal ration the antibiotic will be degraded by the alkaline medium of the small intestine and high body temperature at 39 C.

The *in vitro* preparation to decompose aureomycin was intended to simulate the condition of the small intestine. Preliminary chemical study of the alkali-decomposed aureomycin indicated that the substance was different from chlortetracycline base by the analysis of infra red electrophotometry. Bacteriological analysis in our laboratory showed a total loss of antibacterial potency after alkaline degradation (12). The same result was also reported by other workers (21, 25).

In view of the present evidence that the stimulating effect of decomposed aureomycin on weight gain in chicks was similar to that of untreated aureomycin, it is justifiable to presume that weight gain effect of aureomycin may not be due to its antibiotic property.

The augmented thyroid index of the chicks after aureomycin feeding in the present experiments was in accord to the findings of Mullen and Waller (26) and Wu and Ma (27). These results suggest a possibility that hypothyroidism in a mild degree plays a role in the antibiotic weight gain effect. However, histological study and iodine uptake of the thyroid should be undertaken.

Therefore, the results of the present study does not lend support to the current theory that antibacterial action of aureomycin on intestinal microflora is responsible for weight gain.

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