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Non-Effect of Aureomycin on Eight Constituents of the Blood Stream of Hens Fed High and Low All-Vegetable-Protein Diets

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IN GUATEMALA the poultry industry is dependent on all-vegetable-protein rations. Recent discoveries of the value of APF (Animal Protein Factor) supplements are important both to Guatemala and to many other areas of the American tropics. Work with baby chicks at this station (the Instituto Agropecuario Nacional of Guatemala) indicates that the efficiency of rations compounded of local indigenous feedstuffs is improved when supplemented with APF concentrates.

No attempt will be made here to review the numerous reports of the use of antibiotics in poultry feeds. Whitehill *et al.* (1950) and Groschke and Evans (1950) have demonstrated the effect of antibi-

otics on baby chicks—on their growth, on the efficiency of their feed utilization, and on their intestinal flora. Halick and Couch (1951) and Couch *et al.* (1951) have shown the effect of antibiotics on production and hatchability of eggs. In the studies presented here, crystalline aureomycin was added to rations for hens to determine its effect on eight constituents of their blood stream.

EXPERIMENTAL

In this experiment 40 New Hampshire hens, 6 months old, were allotted by weight among 4 experimental groups. Before the start of the experiment, these hens had had access to pasture of green kikuyu grass (*Pennisetum clandestinum*). During the 6 weeks of the experiment, they were housed outdoors in individual cages with wire bottoms. Feed and water were provided *ad libitum*. Four rations were fed: 2 were high in protein, one containing aureomycin and the other not; 2 were low in protein, one containing aureomycin and the other not. One of these rations was randomly assigned to each group of 10 hens. Zein was added to the high-protein rations, which contained less corn, in order to equalize the quantity of this protein source between the high- and low-protein rations. The experimen-

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tal rations, weight changes of the hens, and average total feed consumed per hen during the experiment are presented in Table 1.

Approximately 4 ml. of blood was collected from a wing vein of each hen at the start, middle, and end of the experiment. The blood serum of these samples was analyzed for total proteins (Lowry and Hunter, 1945), riboflavin (Burch *et al.*, 1948), vitamin A and carotenoids (Bessey *et al.*, 1946) total tocopherols (Quaife *et al.*, 1949), alkaline phosphatase (Bessey *et al.*, 1946) and ascorbic acid (Lowry *et al.*, 1945; Goodland *et al.*, 1949). The methods for ascorbic acid were modified by using a solution of copper sulfate and thiourea instead of norite as a reducing agent. At each bleeding period an additional 2 ml. of blood was taken from 5 hens of each group, the same hens each time. These samples were collected

in tubes containing an anticoagulant (a mixture of Na and K oxalates and citrates). Hemoglobin content, red-cell count, and the percent of hematocrit were determined on each of these samples by standard methods (Wintrobe, 1946).

RESULTS

As may be observed in Table 1, the hens fed the low-protein rations consumed slightly more feed than the groups fed the high-protein rations. No differences were observed in changes of body weight between the groups fed the different rations.

The analyses of the blood of the hens, which are presented in Table 2, may be summarized as follows:

Serum proteins.—Aureomycin had no apparent effect on the serum proteins. A slight increase of serum protein occurred in all groups except the hens fed the low-protein ration containing aureomycin, which showed no increase.

Riboflavin and ascorbic acid.—The significant reduction of riboflavin and ascorbic acid serum levels, which occurred in all groups, was nonsignificant between groups.

Carotenoids.—The carotenoids increased significantly in all groups; however, the observed increases were greater in the two groups fed aureomycin.

Vitamin A.—The serum vitamin A levels of all groups increased significantly. The greatest increases were observed in the two groups of hens fed the low-protein rations. The effect of aureomycin on vitamin A was nonsignificant.

Hemoglobin.—The grams percent of hemoglobin remained fairly constant for all hens except the groups fed the low-protein ration. In this group there was an increase of hemoglobin. A comparison of the check groups with those fed aureomy-

TABLE 1.—*Experimental rations, total feed consumed, and weight changes of hens fed high and low all-vegetable-protein rations containing aureomycin*

Item	High-protein ration		Low-protein ration	
	Group 1	Group 2	Group 3	Group 4
Ingredients:				
Ground yellow corn	54.2	54.2	81.7	81.7
Zein ¹	2.5	2.5	—	—
Sesame oil meal	40.0	40.0	15.0	15.0
Salt mixture ²	3.0	3.0	3.0	3.0
Cod liver oil ³	.3	.3	.3	.3
Vitamin B complex	4	4	4	4
Aureomycin	5	—	5	—
Vitamin B ₁₂	6	6	6	6
Crude protein (percent)	21.6	21.6	12.0	12.0
Average total feed consumed per hen (pounds)	9.1	9.4	9.9	9.7
Average weight per hen (pounds):				
At start of experiment	5.2	5.2	5.2	5.2
At end of experiment	5.3	5.3	5.3	5.3

¹ Courtesy of the Corn Products Refining Co., N. Y.

² Contains calcium carbonate, 1%; bonemeal, 1%; salt, 1%; and trace minerals.

³ 400 I.U. vitamin D and 2,000 I.U. vitamin A per gram.

⁴ Contains, per 100 gm. of feed: 0.20 mg. thiamine; 0.35 mg. riboflavin; 1.20 mg. calcium pantothenate; 1.50 mg. nicotinic acid; 0.35 mg. pyridoxine; and 125.00 mg. choline.

⁵ Crystalline aureomycin hydrochloride, courtesy of Dr. T. H. Jukes and the Lederle Laboratories. Fed at the rate of 25 mg. per kilogram of diet.

⁶ Crystalline vitamin B₁₂, courtesy of Merck and Co., fed at the rate of 25 micrograms per kilogram of diet.

TABLE 2.—Effect of aureomycin in high and low all-vegetable-protein diets on several constituents of blood stream of hens

Ration	Serum proteins			Riboflavin			Ascorbic acid			Carotenoids			Vitamin A			Red-cell count			Hemoglobin			Hematocrit		
	Start	Middle	End	Start	Middle	End	Start	Middle	End	Start	Middle	End	Start	Middle	End	Start	Middle	End	Start	Middle	End	Start	Middle	End
	gm. %			γ %			mgs. %			γ %			γ %			thousands/cm. ³			mgs. %			%		
High protein ration:																								
Without aureomycin	4.67	4.91	5.21	59.5	32.6	38.1	2.71	2.05	1.60	130	247		36.0	42.2		2,418	2,704		6.7	6.6		30.6	32.0	
With aureomycin	4.71	5.21	4.90	77.6	29.5	28.7	2.45	1.87	1.50	176	336		33.6	47.7		2,544	2,912		7.0	7.6		30.8	36.6	
Low protein ration:																								
Without aureomycin	4.60	4.96	4.82	59.1	29.2	31.5	2.29	1.65	1.46	135	260		27.7	49.9		2,268	2,822		6.3	7.9		31.0	33.8	
With aureomycin	4.72	4.91	4.72	81.7	21.5	22.0	2.36	1.74	1.41	128	426		30.0	51.2		2,548	2,688		6.6	6.6		32.2	31.8	
Pooled standard error of the mean	±0.15	±0.51	±0.16	±11.4	±6.5	±7.4	±0.10	±0.10	±0.10	±32	±71		±6.9	±4.4		±149	±137		±0.4	±0.4		±1.0	±1.7	
Difference due to protein levels	.03	.13	.29	-1.9	5.7	6.7	.26	.27	.12	22	-52		6.0	-5.6		73	53		.4	-2		-9	1.5	
Difference due to aureomycin	.08	.13	-.21	20.4	-5.4	-9.5	-.10	-.05	-.08	20	128		-.1	3.4		203	37		.3	-2		.7	1.3	
Difference due to the interaction protein x aureomycin	.04	.18	-.11	-2.3	2.3	.05	-.17	-.14	-.03	27	-39		-2.4	2.1		-77	171		.0	1.2		-.5	3.3	
* Difference required for significance at the 5-percent level (t=2.03; 36 degrees of freedom)	.43	1.46	.46	32.6	18.6	21.2	.29	.29	.29	92	203		19.7	12.6		426	392		1.1	1.1		2.9	4.9	

*SE_d = $\sqrt{2} + t + SE_n$.

cin showed no apparent effect of the antibiotic on hemoglobin.

Hematocrit.—Some increase of the hematocrit was observed in all groups except the group fed the low-protein ration containing aureomycin. In this group the hematocrit value was slightly reduced. No significant effect of aureomycin was observed among the experimental groups.

Total tocopherols.—The serum tocopherol levels of 18 of the 40 hens sampled at the beginning of the experiment were not determinable by the chemical method used in these studies. The remaining 22 hens averaged 0.10 ± 0.02 milligrams percent total serum tocopherols. At the end of the experiment, again serum tocopherol levels of 18 of the hens were not determinable; the serum tocopherol levels of the remaining 22 hens averaged 0.09 ± 0.02 milligrams percent. Hens that had low or zero levels at the start of the experiment tended to retain these low values. Because of the irregularity of these data, no conclusions may be drawn as to the effect of the different treatments.

Alkaline phosphatase.—The alkaline phosphatase values in all hens were 17.4 ± 0.92 millimoles per liter per hour at the start of the experiment. In all groups these values increased beyond 21.0 millimoles per liter per hour. Simple dilution of the samples used to obtain the initial values did not suffice and further substrate dilution was not made.

Red-cell count.—The red-cell count increased in all groups with no significant differences between groups.

DISCUSSION

The average feed intake per bird was similar among the groups (Table 1). The hens fed the low-protein diets consumed slightly more feed. Because the average feed intake was similar, any significant dif-

ferences of blood levels of essential nutrients between the experimental groups may be attributed to the aureomycin.

It is evident from the data (Table 2) that aureomycin *per se* had no apparent effect on serum proteins, riboflavin, ascorbic acid, vitamin A, red-cell count, hemoglobin, and hematocrit of hens fed all-vegetable-protein rations. Although there was an increase in total serum carotenoids in the hens fed aureomycin, the significance of this phenomenon in terms of vitamin A activity is unknown. The blood data do not explain the growth-promoting and feed-saving mechanisms of aureomycin. The data do not preclude the possibility that certain other essential nutrients in the blood of hens had been influenced by the antibiotic. The observations made on the effect of aureomycin on constituents of the blood stream of these hens were similar to those made in growing and fattening pigs (Squibb *et al.*, 1951) and human beings (Scrimshaw, 1951).

Certain changes and variation in the levels of the blood constituents of the hens (Table 2) are of interest. The data obtained at the start, middle, and end of the experiment indicate that the total proteins remained fairly constant throughout the experiment while the serum ascorbic acid levels became uniformly reduced. The riboflavin values were considerably reduced at the end of 3 weeks and remained so until the end of the experiment. This reduction of riboflavin may be attributed to a lower intake of this vitamin. Unpublished data of the Instituto Agropecuario Nacional indicate that chickens on kikuyu grass pasture maintain high riboflavin blood levels.

Considerable variation was evident among the initial values for several of the blood constituents. Since the hens were randomly distributed it would be logical

to expect these values to be more nearly the same among the experimental groups at the start of the experiment. The standard errors of the means indicate that there were extreme variations of several of the blood constituents between individual hens; therefore, to have had more uniformity between the groups, larger numbers of hens per group would be needed. The variations between hens may have been due to differences among birds in the consumption of the kikuyu grass pasture. Scrimshaw *et al.* (1949) have reported similar variations for serum ascorbic acid for hens in New York. These same authors (unpublished data, 1951) have also observed large variations among individual hens for serum carotenoids, vitamin A, and riboflavin. It is evident that such variabilities will need to be considered when critical studies are made with hens raised under conditions similar to those reported herein.

SUMMARY

The effect of aureomycin on eight constituents of the blood stream of 40 New Hampshire hens fed high and low all-vegetable-protein diets was determined. Under the conditions of these studies, aureomycin had no apparent effect on red-cell count, hematocrit, hemoglobin, total serum proteins, riboflavin, ascorbic acid, and vitamin A. An increase in serum carotenoid levels of hens fed aureomycin was observed, but no interpretation of these data can be made at this time.

The blood data presented indicated considerable variability among individual hens for ascorbic acid, riboflavin, carotenoids, vitamin A, and red-cell count.

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