Effect of Terramycin and Vitamin B_{12} on Hatchability.* (19338)

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(Introduced by E. R. Norris.)

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Terramycin has been shown to support growth of swine and poultry when added to a complete diet including vit. B_{12}(1,2). When fed to lactating females, it also increased the weaning weights of rats as shown by the work of Stern and McGinnis(3). Elam *et al.*(4) using cross-bred chickens, concluded that penicillin improved egg production. Their results showed that the injection of 1 μg vitamin B_{12} per bird per week gave 38% egg production as compared with 50% for the combination of vit. B_{12} and penicillin (33 mg/kg). These rates of production are not very outstanding. Hatchability of fertile eggs for the 2 treatments was 72 and 80%, respectively. The vit. B_{12} content of the liver, kidney, and egg yolk for the 2 treatments in their study was not appreciably different, but hatchability and, to some extent, egg production were improved by the penicillin supplement.

The purpose of the present study was to determine the effect of terramycin on egg production and hatchability when fed alone and in combination with vit. B_{12}.

Procedure. White Leghorn pullets, 8 weeks of age, were divided into 4 groups of approximately 90 birds each. The pullets were kept on range and fed mash and whole wheat free-choice during the pre-laying period to 22 weeks of age. The pre-laying basal diet is shown in Table I. During this pre-laying period, all groups received vit. B_{12}‡ at a level of 12.5 μg per pound of feed. Groups 2 and 4 received terramycin§ at a level of 15 ppm.

At 22 weeks of age when the pullets showed signs of approaching sexual maturity, they were distributed into lots of 16 at random and housed in individual cages with wire floors. Only one group of 16 hens from each of the pre-laying treatments was used in this particular study. The basal diet fed after this time is also shown in Table I. Vit. B_{12} supplementation was withdrawn from Groups 1 and 2, but the terramycin supplementation for Group 2 was continued as before. Records of the age when the first egg was laid. egg production, hatchability, mortality and body weight were kept. The experiment was continued for approximately 10 months. During the latter part of the experiment, eggs were saved from Groups 1, 2, and 4 and were assayed for vit. B_{12} content by a modification of the method of Skeggs *et al.*(5) using *Lactobacillus leichmanii* (ATCC 4797).

Eggs were saved for hatching and incubated at weekly intervals. The total number of fertile eggs incubated from each group is shown in Table II. Eggs were saved from Groups 1 and 2 for a 15-week period beginning April 24, 1951; eggs from the other 2 groups were saved only during a 4-week period beginning July 13, 1951, since we were primarily interested in determining whether a terramycin supplement would increase the severity of a vit. B_{12} deficiency as suggested by preliminary work with swine.

TABLE I. Composition of Basal Diets.

Ingredients	Pre-laying, %	Laying, %
Ground yellow corn	24.7	16.6
" barley	15	2.1
" oats	10	2.1
Millrun	10	13.4
Soybean oil meal (solvent)	30	15.9
Dehydrated alfalfa	5	3
Whole wheat	—	40
Ground limestone	2	2.6
Dicalcium phosphate	2	2.9
Salt	1	.6
Riboflavin supplement (500 μg/g)	.3	.6
A and D supplement (4000 I. U. vit. A, 1000 A.O.A.C. units of vit. D/g)	—	.2
$MnSO_4$	8 oz/ton	8 oz/ton

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‡ Merck vit. B_{12} supplement containing 12.5 mg/lb.

§ Terramycin hydrochloride.

TABLE II. Effect of Terramycin and Vitamin B_{12} on Mortality, Body Weight Gain, Egg Production and Hatchability.

Group No.	Dietary supplements — Pre-laying period: Vit. B_{12}, μg per lb	Dietary supplements — Pre-laying period: Terramycin, ppm	Dietary supplements — Laying period: Vit. B_{12}, μg per lb	Dietary supplements — Laying period: Terramycin, ppm	Avg body wt* — Pre-laying period: Initial wt (8 wk), g	Avg body wt* — Pre-laying period: Gain in 14 wk, g	Avg body wt* — Laying period: Initial wt (22 wk), g	Avg body wt* — Laying period: Avg gain, g	Mortality during laying period, %	Avg age at first eggs, days	Avg egg production, %	Hatchability: No. fertile eggs incubated	Hatchability: Chicks hatched, %
1	12.5	—	—	—	725	883	1608	490	18.8	177.8	62.5	346	43.1 (20.7– 68.9)†
2	12.5	15	—	15	697	881	1578	604	12.5	184.5	60.1	361	78.1 (63.6–100)
3	12.5	—	12.5	—	725	883	1841	446	12.5	179.2	61.6	85	84.7 (78.6– 90.9)
4	12.5	15	12.5	15	697	881	1633	527	6.3	183.5	63.3	104	95.2 (94.3–100)

* 90 pullets/treatment during pre-laying period; 16 pullets/treatment during laying period.
† Range of individual hatches.
Difference in hatchability between Groups 3 and 4 was significant at the 5% level.

Results. The results of the experiment are summarized in Table II. Body weight data showed no differences in gain during the developing season. When the initial weights are taken into consideration, it is apparent that the vit. B_{12} and terramycin supplements had no effect on gain during the laying period, either. All 4 groups reached sexual maturity at about the same age. While the data on egg production indicate little or no effect of the terramycin and B_{12} supplements, there appeared to be a greater drop in egg production toward the end of the experiment in the groups not receiving vit. B_{12}. Terramycin markedly increased hatchability, even in the absence of a vit. B_{12} supplement. The vit. B_{12} content of the egg yolk from Groups 1, 2, and 4 was 9.3, 11.83, and 12.2 mμg per gram, respectively. Hatchability of fertile eggs for these groups was 43, 84.7 and 95.2%, respectively.

Discussion. It is difficult to explain the increased hatchability given by the terramycin supplement in Group 2, since no great increase in vit. B_{12} content of the egg was observed. Furthermore, the amount of B_{12} in eggs from the basal treatment should have been sufficient for hatchability, based on the findings of Olcese *et al.*(6) who showed that eggs containing as little as 5 mμg B_{12}/g egg yolk gave satisfactory hatchability. Elam *et al.*(4) using crossbred chicks, showed an effect of penicillin in the reproduction of the fowl in addition to that of vitamin B_{12}. Although the small number of eggs involved does not permit definite conclusions, there was a suggestion in these experiments that the addition of terramycin to a diet already containing vitamin B_{12} further improved hatchability.

Elam *et al.*(4) reported that optimal hatchability occurred when the vit. B_{12} content of the yolk was 3.7 mμg per gram. Olcese *et al.* (6) found that, with a purified diet containing no added vitamin B_{12}, hatchability dropped to 0 after 6 weeks, although the vit. B_{12} of the eggs from these hens contained between 5 and 10 mμg per gram of yolk. The eggs from hens fed a practical diet which supported optimal hatchability also contained between 5 and 10 mμg of vit. B_{12} per gram of yolk. In the present experiment, it was observed that eggs from group 1 hatched poorly

even though the amount of vit. B_{12} in the yolk was greater than that found by Elam *et al.*(4) in eggs that hatched satisfactorily. There is, thus, a contradiction between the vit. B_{12} content of the egg yolk by microbiological analysis and the amount required for hatchability.

Summary. An experiment was conducted with White Leghorn pullets to determine the effect of feeding terramycin alone and in combination with vit. B_{12} on egg production and hatchability. The results obtained indicate that terramycin had little or no effect on egg production and gain in weight. In the absence of vit. B_{12}, hatchability of fertile eggs was definitely improved by feeding terramycin. There was an indication that terramycin fed in combination with vit. B_{12} further improved hatchability.

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Proteinuria in Experimental Renal Hypertension.* (19339)

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Recently the rate of development of proteinuria in three-quarters nephrectomized rats was reported(1). Removal of both adrenals after the proteinuria was well-developed did not significantly alter the rate of protein excretion although Addis and his coworkers have shown that adrenalectomy abolishes the proteinuric action of renin and decreases the rate and amount of excretion of intraperitoneally injected protein. Administration of cortical hormones restores the proteinuric effect of renin and increases the rate and amount of excretion of intraperitoneally injected protein(2-4). The purpose of the present paper is to report on the rate of development of proteinuria in rats made hypertensive by the removal of one kidney and constriction of the other, and its relationship to growth of the remaining renal tissue, development of hypertension and development of renal lesions. Since the renin-angiotonin pressor system is probably involved in the pathogenesis of constrictive renal hypertension and since not only renin but angiotonin as well(2) will induce proteinuria in the rat the possibility exists that the proteinuria of the hypertensive rat is to be explained on this basis. On the other hand the proteinuria might be a consequence of a renal lesion. Study of the time relationships should throw some light on the pathogenesis of the proteinuria.

Methods. 24 male albino rats of the Stanford colony, weighing close to 150 g each at the start of the experiments were divided into 2 equal groups. A base line of protein excretion was established by 4, and a base line of blood pressure by 2, observations. Following one-stage operative procedures on the kidneys these measurements were repeated bi-weekly. In the first group of rats one kidney was removed and the other exposed, handled and replaced. In the second group one kidney was removed and the other constricted by a figure-of-eight silk ligature, according to the technic of Grollman(5). Measurements of protein excretion were carried out by placing the rats in metabolism cages at 4 p.m., col-

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