BREED DIFFERENCES IN RESISTANCE TO A DEFICIENCY OF VITAMIN B_1 IN THE FOWL[1]

By W. F. LAMOREUX, *assistant poultry husbandman,* and F. B. HUTT, *poultry husbandman and animal geneticist, New York (Cornell) Agricultural Experiment Station*

INTRODUCTION

The breeds and varieties of domestic fowls differ in size and shape of body, in structure and color of plumage and skin, in variation of the skeleton, and in the modification of structures, such as the comb and spurs, arising from the skin. Apart from colors, the differentiation of the breeds at the time of their establishment was apparently based upon morphological characters. A few differences between breeds in physiological characters, such as broodiness and color of egg, are now known, but it is not impossible that the present comparative freedom of Leghorns from broodiness and the ability of the same breed to lay white-shelled eggs may have resulted largely from artificial selection for those physiological characters as for other distinguishing breed characteristics. This paper reports an association between breed characteristics and an interesting physiological character—resistance to a deficiency of vitamin B_1.

The first indication that White Leghorns require comparatively little vitamin B_1, or through differential storage of it, or by other means, are markedly resistant to polyneuritis, was put forward by Nichita and Iftimesco (*9*).[2] On a diet deficient in vitamin B_1, or lacking it entirely, one of three White Leghorns, 10 months old at the start of the experiment, showed symptoms of polyneuritis after 41 days and died in 49 days. Another was entirely unaffected and was removed from the experiment at 78 days. A third showed no symptoms till the one hundred and seventh day and for 3 weeks thereafter maintained a subacute chronic state of polyneuritis, but quickly recovered when placed on a normal diet. On the other hand, Nichita, Tuschak, and Calcef (*10*) found that on diets deficient in vitamin B_1 six Rhode Island Reds about 10 months old showed symptoms of polyneuritis at from 7 to 19 days. Three of them were given yeast and quickly recovered, but the other three died of acute polyneuritis in 9, 18, and 21 days. In both experiments, controls on the experimental diet plus yeast were entirely normal.

The experiments of Nichita and his coworkers were not set up primarily to test the two breeds as to their requirements of vitamin B_1, and unfortunately the diets used differed considerably. The diet of the Rhode Island Reds included fresh beef, glucose, and starch, whereas that the White Leghorns did not. The latter received their carbohydrates from decorticated rice. Hence, while the experiments within each breed were well controlled, the fact that the diets were not identical makes any comparison of the two breeds with respect to their

[1] Received for publication May 2, 1938. This is No. 8 in the series by the junior author entitled "Genetics of the Fowl."

[2] Italic numbers in parentheses refer to Literature Cited, p. 316.

requirement of vitamin B_1 less accurate than might be desired. Accordingly, experiments were planned by the present writers to determine in chicks of these two breeds their resistance to a deficiency of vitamin B_1. The results are reported in this paper.

MATERIALS AND METHODS

In four separate trials Single Comb White Leghorn chicks were compared with Rhode Island Red chicks of the same age upon a diet deficient in vitamin B_1. Barred Plymouth Rocks were also included in the fourth trial. Environmental conditions were made identical for all chicks in any one experiment by brooding both lots together in the same brooder. To obtain samples of each breed affected as little as possible by differences between individuals and strains, the chicks used in each test were picked at random. Their dams were selected entirely at random. The population from which these chicks came included three strains of Rhode Island Reds and three of White Leghorns. The fowls used to produce chicks for any one test were not the same as those used for the other experiments. Since all the adult breeding stock received the same diet, which was made as complete as possible according to present knowledge of requirements for reproduction in the fowl, any possibility of differential viability in the chicks resulting from differences in the "carry-over" of essential nutrients in the egg was reduced to a minimum.

A diet lacking vitamin B_1 was obtained by the method of Keenan, Kline, Elvehjem, and Hart (*6*) who showed that by autoclaving a mixture of ground grains the vitamin B_1 could be completely destroyed without much loss of vitamin B_2 (riboflavin), or of B_4. The diet used was as follows:

	Pounds per 100
Ground yellow corn	40
Standard wheat middlings	20
Ground buckwheat	15
Wheat-germ meal	2
Soybean oil meal	5
Fish meal	7
Meat scrap	2
Liver meal	3
Alfalfa-leaf meal	3
Ground limestone	2
Salt (NaCl)	.5
High potency cod-liver oil	.5

This diet was used with uniformly good results for all chicks in the regular hatches of the department in 1936. It was autoclaved at 120° C. and 15 pounds pressure for 6 hours. An additional 0.25 percent of high potency cod-liver oil was added after autoclaving. This oil contained 250 units of vitamin D and 3,000 units of vitamin A per gram. The records for the controls proved that the diet not thus treated was entirely satisfactory.

That the data reported are an accurate measure of the requirements for vitamin B_1 and are not complicated by error due to the destruction of other nutrients was shown in a separate experiment with Rhode Island Red chicks. Twenty-five chicks receiving the autoclaved ration died at the mean age of 11 days, and all were dead 14 days after hatching. Of 23 chicks receiving the same ration plus 120 micrograms

of synthetic vitamin B_1 (Betabion, Merck) per 100 g of feed, none had died. Of 25 chicks receiving 240 micrograms of synthetic vitamin B_1 per 100 g of feed 2 died at 4 and 5 days of age. The remainder of the chicks appeared quite healthy at the end of the experiment.

The four experiments were so planned that in each one the dietary deficiency was initiated at a different age and (except in experiment 1) in chicks which, before introduction of the deficient diet or afterwards (experiment 2), had been for different periods upon the normal (unautoclaved) diet, with consequent different storages of vitamin B_1 as temporary defence against the deficiency. Only strong, vigorous chicks were used at the start of each trial. The four tests of the two breeds were as follows:

Experiment 1, initiated February 8, 1936. Deficient diet from hatching.
Experiment 2, initiated January 25, 1936. Deficient diet first 2 days; normal feed on the third day; deficient diet thereafter.
Experiment 3, initiated February 1, 1936. Normal feed to 14 days of age; deficient diet thereafter.
Experiment 4, initiated June 10, 1936. Normal feed to 21 days of age; deficient diet thereafter.

In experiments 3 and 4 all the chicks that died and those alive at the end of experiments were sexed. Since no evidence was found that either sex is more susceptible than the other, data for the two sexes were combined for all analyses.

RESULTS

The findings in the first three experiments (table 1) show conclusively that the White Leghorns are more resistant to a deficiency of vitamin B_1 than are the Rhode Island Reds.

TABLE 1.—*Comparative survival of Single Comb White Leghorn and Rhode Island Red chicks on diets lacking vitamin B_1*

Diet and breed	Chicks	Average body weight at—		Age at death	
		1 day	1 week	Range	Mean
	Number	*Grams*	*Grams*	*Days*	*Days*
Experiment 1. B_1-free diet from hatching:					
White Leghorn	21	35.0	41.1	5–26	12.86
Rhode Island Red	20	36.1	39.0	9–16	10.85
Experiment 2. B_1-free diet from hatching, normal diet on third day only:					
White Leghorn	15	32.0	46.5	15–62	22.13
Rhode Island Red	15	32.3	40.3	13–21	16.93
Experiment 3. Normal diet to 2 weeks, B_1-free diet thereafter:					
White Leghorn	12	36.1	44.1	29–49	34.17
Rhode Island Red	13	35.1	40.8	26–34	29.0

As was to be expected, the mean age at death is lowest for the chicks which never received any normal feed, highest for those on normal feed to 2 weeks of age, and intermediate for those which got normal feed on 1 day only. In each experiment, however, the mean age at death was higher for the White Leghorns than for the Rhode Island Reds. It is noteworthy also that the variation in susceptibility among individuals, as measured by the range in age at death, was much greater in the former than in the latter. In experiments 1, 2, and 3, chicks of the latter breed all died within 8, 9, and 9 days, respectively, whereas

deaths in the White Leghorns in the same trials were spread over 22, 48, and 21 days. The most resistant White Leghorn outlived the last surviving Rhode Island Red by 10, 41, and 15 days in experiments 1, 2, and 3, respectively. An exceptional White Leghorn chick in experiment 2 survived to 62 days of age.

It is commonly believed that animals growing most rapidly are more susceptible to nutritional deficiencies than are those in which growth proceeds more slowly. Weights of these chicks at 1 day (table 1) show the samples of the two breeds to be almost identical in size at the

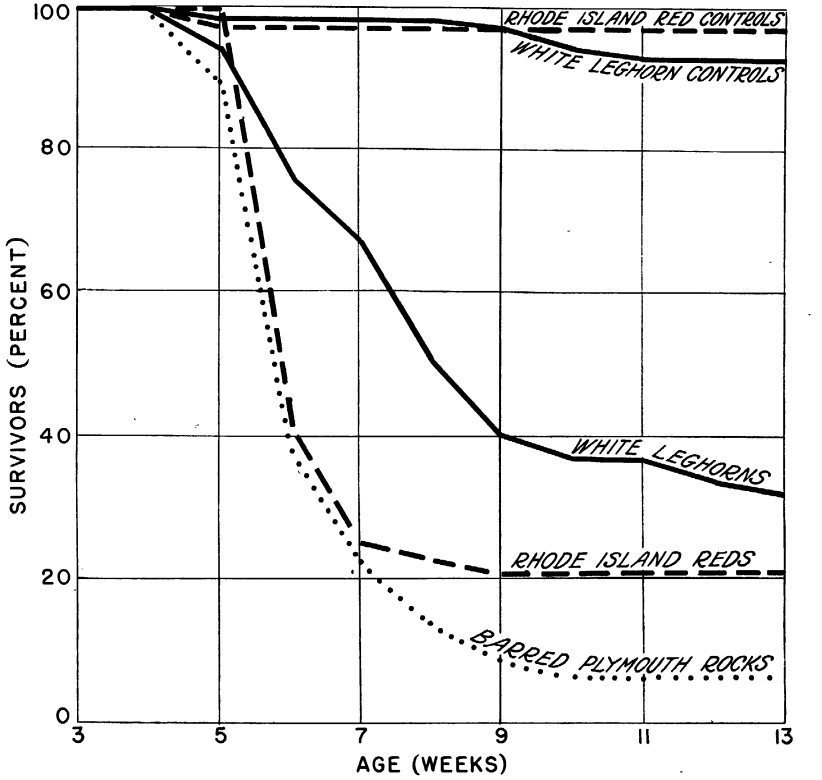

FIGURE 1.—Survival data for 59 White Leghorns, 39 Rhode Island Reds, and 38 Barred Plymouth Rocks placed on vitamin B_1-deficient feed at 3 weeks of age. The controls include 128 White Leghorns and 67 Rhode Island Reds.

beginning of the three tests. The increases in weights of these chicks from hatching to 1 week of age indicate that in all three experiments the White Leghorns grew somewhat more rapidly than the Rhode Island Reds. Ordinarily White Leghorns do not grow more rapidly than Rhode Island Reds in the first week. The differences observed may have resulted from chance or from adverse effects of the vitamin deficiency on the Rhode Island Reds. In any case, the greater resistance of the White Leghorns to a deficiency of vitamin B_1 cannot be ascribed to relatively slow growth in that breed.

Similar results were obtained in experiment 4. Since all the chicks did not die, the results in this test are not strictly comparable with

those in the first three and are therefore presented separately (figs. 1 and 2). Birds in this experiment were kept in battery brooders till 6 weeks old and confined thereafter in a 10-by-12-foot colony house with access to a wire-floored sun porch. Several cases of cannibalism occurred at from 8 to 12 weeks of age, and it is probable that some of the birds still alive after 10 weeks on the vitamin B_1-free diet (at 13 weeks of age, when the experiment was discontinued) were able to survive because of having received an extra supply of the vitamin from the birds eaten. Since these birds went out of the battery brooders and into the colony house at 6 weeks of age, it is possible that after that time some of the birds which survived the longest acquired a supply of the vitamin by coprophagy. It has not yet been proven,

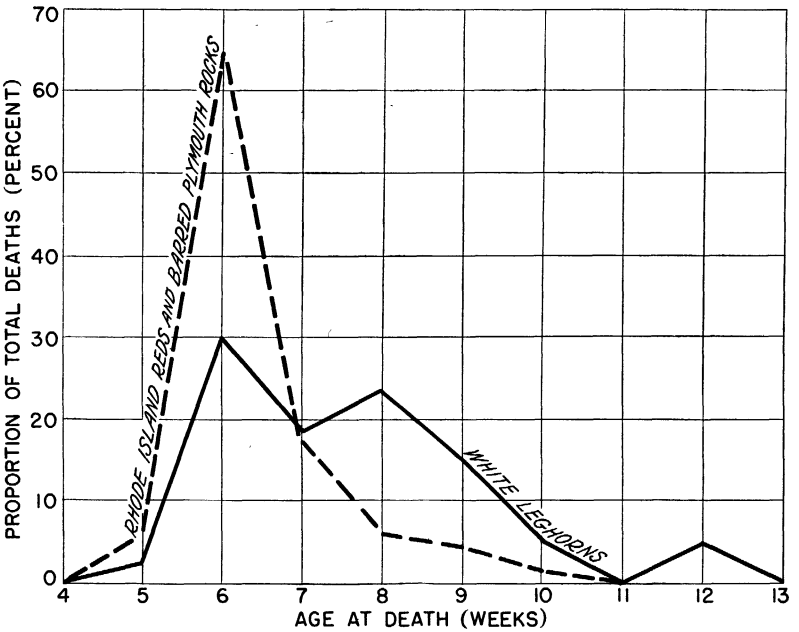

FIGURE 2.—Distribution of ages at death in 39 White Leghorns and 65 Rhode Island Reds and Barred Plymouth Rocks that died from deficiency of vitamin B_1.

however, that in the fowl, as in the rat (*3*) vitamin B^1 is synthesized in the digestive tract and voided with the feces. However, the critical period in this experiment was when the birds were from 5 to 7 weeks of age, at which time the greatest mortality was experienced by all three breeds. Since cannibalism was not serious till the ninth week, it is improbable that error from this source could have affected in any way the breed difference so conclusively demonstrated at from 5 to 8 weeks (fig. 1).

It is quite evident that whatever storage of vitamin B_1 had been accumulated by these chicks during 3 weeks on normal feed it was quickly exhausted (fig. 1). Some mortality occurred in the fourth week, but in the sixth week (third week on the deficient diet) mortality was extremely high in both the Barred Plymouth Rocks and the Rhode Island Reds. Only 39.5 and 41 percent, respectively, of these sur-

vived at the end of the sixth week of the 37 Rhode Island Reds and 34 Barred Plymouth Rocks placed on the deficient diet at 3 weeks of age. In contrast to these, 76.3 percent of the 59 White Leghorns put on the B_1-free diet at 3 weeks were still alive at the end of the sixth week. The percentages of survivors in the Rhode Island Reds, Barred Plymouth Rocks, and White Leghorns at 8 weeks of age were 23.1, 15.8, and 50.8, and at 13 weeks 20.5, 7.9, and 32.2, respectively, of the original numbers in each breed.

The greater resistance of the White Leghorns to a deficiency of this vitamin is demonstrated not only by the lower mortality in that breed but also by the fact that even those that did succumb were able to survive for a longer period than the Rhode Island Reds which died. Analyses of the ages at death up to 13 weeks show that, whereas 64.6 percent of the Plymouth Rocks and Rhode Island Reds that died did so during the sixth week, only 30.8 percent of the White Leghorns that succumbed did so during the sixth week and the rest lived for periods varying up to 7 weeks after the first peak of mortality. As a result the distributions of the ages at death in the two breeds differ markedly (fig. 2). Both curves are skewed; but the curve for the Plymouth Rocks and Rhode Island Reds is leptokurtic, while that for the White Leghorns is platykurtic.

Most of the chicks that died exhibited symptons of vitamin B_1 deficiency including unsteady gait, other signs of neuromuscular incoordination, and, in extreme cases, retraction of the head in characteristic opisthotonos. The birds appeared not to relish the autoclaved feed. This may have resulted from the fact that autoclaving made the mash dark in appearance and (presumably) less palatable, particularly to those chicks which had received normal feed before going on the autoclaved diet. On the other hand, the condition of anorexia may have resulted, in part or entirely, directly from lack of vitamin B_1 as Cowgill (*2*) has shown may happen in other species.

Other evidence of a deficiency of vitamin B_1 was provided by the retardation in growth of the experimental chicks. At 5 weeks of age, just prior to the onset of heavy mortality, the mean weights of the White Leghorns and Rhode Island Reds were respectively 87 and 97 g less than the normal weights for these breeds at 5 weeks, as determined by Card and Kirkpatrick (*1*). Since at 3 weeks of age, when these chicks were placed on the deficient diet, they had exceeded Card and Kirkpatrick's normal weights by 16.5 g in the White Leghorns and 13.7 g in the Rhode Island Reds, the experimental chicks obviously showed at 5 weeks a marked retardation of growth such as is commonly associated with a deficiency of vitamin B_1.

It seems reasonable to conclude, therefore, that the deaths in these chicks resulted primarily from polyneuritis attributable to the deficiency of vitamin B_1.

BREED DIFFERENCES ON A NORMAL DIET

It seemed possible that the differences between breeds with respect to mortality rate might be present even in birds raised under optimum conditions. If this were so, the differences found in experiments 1 to 4 might reflect a general debility of the Rhode Island Reds in comparison with White Leghorns, rather than a special susceptibility to a deficiency of vitamin B_1. To answer this question a comparison was made

of control populations of each breed drawn at random from the several thousand chicks hatched for other purposes from the same strains as those providing the chicks for these experiments.

The mortality curves for these populations show that the strains of White Leghorns and Rhode Island Reds used did not differ in this respect when on normal feed (fig. 1). Up to 3 weeks of age none of either breed died, and at 13 weeks the deaths were 1.5 percent in the Rhode Island Reds and 4.8 percent in the White Leghorns. The diet used for these controls was the same as that of the experimental birds except that it was not autoclaved. It was obviously satisfactory.

It seems safe to conclude that chicks of the two breeds used did not differ in mortality from other causes and that the differences observed in them on the experimental diets can be attributed solely to a difference between the breeds in susceptibility to a deficiency of vitamin B_1.

INDEPENDENCE OF SUSCEPTIBILITY AND BODY SIZE

The demonstration by Nichita et al. (*9, 10*) that adult Rhode Island Reds are more susceptible than adult White Leghorns to a lack of vitamin B_1 indicates a difference between breeds but raises the question whether or not that difference depends upon anything other than the difference in size of those two breeds. Cowgill (*2*) has presented some data indicating that in the larger species the amount of vitamin B_1 required per animal per day is somewhat less in proportion to body weight than in small ones. If this same principle were applicable to intraspecific races differing markedly in size, one would expect a somewhat lower requirement per unit of weight for Rhode Island Reds than for White Leghorns since the former breed exceeds the latter in weight at maturity by an average amount (in females) of around 900 g. This would be the reverse of what has actually been found.

However, the validity of Cowgill's formulas, which are based upon rather hypothetical "maximum normal weights" of the species considered, is open to question. Direct proof that the lower requirement of vitamin B_1 by White Leghorns is independent of their smaller body size at maturity is provided when the experimental animals are of the same size in the breeds compared. It was with this fact in mind that the experiments reported in this paper were carried out with young chicks. Comparisons of growth in White Leghorns and Rhode Island Reds made by Card and Kirkpatrick (*1*) and by Kempster and Parker (*7*) have shown that chicks of the two breeds do not differ in size till after 4 weeks. The present writers' data agree with their findings.

It follows, therefore, that in experiments 1 and 2, where all chicks but one died earlier than 4 weeks of age, and in experiment 3, where the mean age at death in the resistant white Leghorns was not quite 5 weeks, the markedly superior ability of the White Leghorns to withstand a deficiency of vitamin B_1 is a distinct racial characteristic entirely independent of body size. As it happened, the White Leghorns were slightly heavier than the Rhode Island Reds at 1 week of age in these three tests (table 1). In experiment 4, where the deficient diet was not introduced till the chicks were 3 weeks of age, the chicks of the two breeds on hand at 5 weeks of age weighed practically the same, 142.9 g for the Leghorns and 136.4 g for the Rhode Island

Reds. It was in the succeeding 2 weeks that both breeds experienced their heaviest losses (figs. 1 and 2). Obviously the superiority of the White Leghorns in this test was in no way related to body size.

CROSS OF RESISTANT × SUSCEPTIBLE BREEDS

While the primary object of this investigation was to determine whether or not there is a difference between White Leghorns and Rhode Island Reds with respect to their requirement of vitamin B_1, a test was also made to get some idea of the genetic basis for the lower requirement of the White Leghorns. The chicks used were from a cross of Rhode Island Red ♀♀ × White Leghorn ♂. These were tested concurrently with the purebreds in experiment 4 and in exactly the same manner. Mortality in the 53 cross-bred chicks was 73 percent up to 13 weeks of age, when the test was discontinued. The mortality in the same period was 68 percent for purebred White Leghorns and 79.5 percent for purebred Rhode Island Reds. It would appear, therefore, that the crossbred chicks were intermediate between the parent breeds in requirement of vitamin B_1. While further data are necessary to prove the point, this is some indication that the comparatively low requirement of this vitamin by the White Leghorns depend upon multiple factors.

DISCUSSION

These experiments with chicks yielded the same results with respect to breed differences in requirement of vitamin B_1 as did those of Nichita and Iftimesco (*9*) and Nichita et al (*10*) with adult birds. Since the strains of White Leghorns and of Rhode Island Reds used by the Rumanian workers were quite different from those used by the present authors, and since the birds of each breed used by the present writers came from more than one strain, there can be no doubt that the difference observed is really one between breeds and not merely between strains. The low requirement of vitamin B_1 is as truly a genetic breed character as are the dominant white of White Leghorns and the restricted black of the Rhode Island Reds. Whether it is peculiar to White Leghorns or occurs in all Leghorn varieties regardless of color remains to be determined.

The physiological basis for this special attribute of the White Leghorns is as yet quite unknown, and little or no explanation of its persistence as a breed characteristic can be offered. It is obviously different from practically all other distinguishing breed characteristics in that the latter are maintained by artificial selection, whereas it is impossible to see how the poultrymen, either modern or primitive, who helped to establish the White Leghorn breed, could have consciously selected for a lower requirement of vitamin B_1 as they did for morphological characteristics.

The character under consideration is not the only physiological one in which White Leghorns and Rhode Island Reds differ. Hutt (*4*) has shown that these two breeds differ in the rates at which the body temperature rises during the first 9 days after hatching. He (*5*) has also shown that adult White Leghorns are much more resistant to extreme heat than are Rhode Island Reds and Barred Plymouth Rocks. In a discussion of these and other physiological differences

between breeds, the junior author (*5*) has suggested ways in which such differences might have become established, but none of these can yet be considered as anything more than conjecture. The differences are probably between all Leghorns as one class and the so-called heavy breeds as another, rather than merely between the White Leghorns and the Rhode Island Reds.

So far as the writers can ascertain, apart from the work of Nichita and his coworkers, confirmed in this paper, it has not previously been demonstrated that breeds of the domestic fowl vary in their requirement of certain vitamins. Since most recent investigations of the nutritional requirements of fowls are based upon experiments with White Leghorns, there is much doubt about the extent to which findings reported for that breed are applicable to Rhode Island Reds and to other heavy breeds. There has been a tendency in recent years to establish standard requirements of various vitamins for poultry, i. e., so many units of vitamin A, of vitamin D, or of riboflavin per gram of feed. It is obvious that, with respect to vitamin B_1, any such requirement determined from experiments with White Leghorns only would not be accurate for Rhode Island Reds and vice versa.

Assay of vitamin B_1 with pigeons, according to the method of Kinnersley, Peters, and Reader (*8*) is now a standard procedure. These investigators tested pigeons of several different colors, but, so far as the present writers are aware, no experiments have yet been made to determine whether all of the many different breeds of pigeons have the same requirement of this vitamin, or whether some would yield entirely different results in such an assay. It is not impossible that some of the differences in such assays reported from different laboratories have arisen because the pigeon is not yet so well standardized as the commonly used rats of the Wistar Institute strain.

SUMMARY

In each of four experiments the ability of White Leghorn chicks to survive on a diet deficient, or lacking, in vitamin B_1 was greater than that of Rhode Island Reds. When chicks received the deficient diet from hatching, the mean ages at death were 12.9 days for the White Leghorns and 10.8 days for the Rhode Island Reds. For chicks similarly treated but given nondeficient feed on the third day only, the corresponding mean ages were 22.1 and 16.9 days. When deficient feed was supplied after the chicks had been for 2 weeks on the normal diet, the mean ages at death were 34.1 days in the White Leghorns and 29 days in the Rhode Island Reds. Typical symptoms of polyneuritis were observed.

Among chicks on normal feed to 3 weeks of age and the deficient diet thereafter, the percentages surviving to 13 weeks of age were 32 for the White Leghorns, 20.5 for the Rhode Island Reds, and 8 for Barred Plymouth Rocks.

In the White Leghorns deaths were delayed and spread out over a longer period, while in the heavy breeds they occurred earlier and were clustered somewhat narrowly around the mean age at death.

The superior resistance of the White Leghorns to this dietary deficiency is shown to be independent of body size and is not caused by differential mortality from causes other than polyneuritis.

F_1 progeny from a cross of Rhode Island Red ♀♀ × White Leghorn ♂ were intermediate between the parent breeds with respect to resistance to a deficiency of this vitamin.

These findings, along with those of Nichita and his coworkers in Rumania, prove that White Leghorns differ from Rhode Island Reds and Barred Plymouth Rocks in possessing as a hereditary breed characteristic a marked resistance to a deficiency of vitamin B_1.

The significance of this fact in poultry feeding, in experimental work in nutrition, and in the use of birds for quantitative assays of vitamin B_1 is discussed.

LITERATURE CITED

(1) Card, Leslie E., and Kirkpatrick, Wm. F.
1918. rearing chickens. part i. normal rate of growth in white leghorns and rhode island reds. Conn. (Storrs) Agr. Expt. Sta. Bull. 96: [355]–372, illus.

(2) Cowgill, George R.
1934. the vitamin b requirement of man. 261 pp., illus. New Haven, Conn., and London.

(3) Guerrant, N. B., Dutcher, R. Adams, and Tomey, L. F.
1935. the effect of the type of carbohydrate on the synthesis of the b vitamins in the digestive tract of the rat. Jour. Biol. Chem. 110: 233–243, illus.

(4) Hutt, F. B.
1935. on the physiological basis of genetic resistance to salmonella pullorum in the fowl. Amer. Nat. 69: 66–67.

(5) ———
1938. genetics of the fowl. vii. breed differences in susceptibility to extreme heat. Poultry Sci. 17: 454–462, illus.

(6) Keenan, J. A., Kline, O. L., Elvehjem, C. A., and Hart, E. B.
1935. the stability of vitamins b (B_1), g (B_2) and B_4. Jour. Nutrition 9: 63–74, illus.

(7) Kempster, H. L., and Parker, Jesse E.
1936. the normal growth of chickens under normal conditions. Mo. Agr. Expt. Sta. Research Bull. 247, 47 pp., illus.

(8) Kinnersley, Henry Wulff; Peters, Rudolph Albert; and Reader, Vera.
1928. antineuritic yeast concentrates. iii. the curative pigeon test: a critique. Biochem. Jour. 22: [276]–291.

(9) Nichita, G., and Iftimesco, G.
1934. recherches sur l'avitaminose du complexe b chez les poules leghorn blanches. Ann. Inst. Natl. Zootech. Roumanie 3: 79–109, illus. [Also in Compt. Rend. Soc. Biol. [Paris] 117: 289–292. 1934.]

(10) ——— Tuschak, N., and Calcef, Ch.
1934. recherches sur l'avitaminose du complexe b chez les poules rhode island red. Ann. Inst. Natl. Zootech. Roumanie 3: 35–78, illus. [Also in Compt. Rend. Soc. Biol. [Paris] 117: 283–286. 1934.]

U. S. GOVERNMENT PRINTING OFFICE: 1939