EFFECT OF DIETARY PENICILLIN ON SOME B VITAMIN REQUIREMENTS IN THE CHICK

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EFFECT OF DIETARY PENICILLIN ON SOME B VITAMIN REQUIREMENTS IN THE CHICK

by

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INTRODUCTION

The science of nutrition is a relatively new one when compared with the evolution of some of the other sciences such as mathematics, astronomy, physics and chemistry. In its earliest phase, it was generally believed that the nutritional requirements of animals were satisfied by three classes of compounds, namely, carbohydrates, fats and proteins, together with water and necessary minerals. Eijkman (1897) however, was one of the earlier investigators to report the existence of an additional dietary principle. His work showed that rice polishings contained a nutrient which prevented a peculiar kind of paralysis in chickens which was caused by feeding a diet of polished rice.

Hopkins (1906) extended this concept when he stated, "No animal can live on a mixture of pure protein, fat and carbohydrate, and even when the necessary inorganic minerals are carefully supplied, the animal cannot flourish. The animal body is adjusted to live either on plant tissues or other animals, and these contain countless substances other than protein, carbohydrate and fat".

It remained for Funk in 1911 to clarify further these substances as essential factors in the diet of the animal. Funk chemically analyzed the principle reported by Eijkman and located an "amine" group, and chose the name "vital-amine", later shortened to "vitamine" and now referred to as "vitamin".

Later workers discovered essential substances incorporated in the lipid portions of plant and animal tissues indicating that two classes of vitaminic factors were involved; one a fat-soluble principle, and the other a water-soluble principle.

Within the last two decades, progress was rapid in the isolation and identification of most of the water-soluble vitamins. The latest B vitamin to be elucidated was vitamin B_{12} in 1948. Extensive work with the Animal Protein Factor, a complex of which vitamin B_{12} has been shown to be the most important member, disclosed complications indicating a multiple nature of this factor. In addition to vitamin B_{12}, some, but not all animal protein factor supplements were shown to possess extra growth stimulating principles. Independent work by several investigators however, soon showed that the presence of small amounts of antibiotics which were present in certain animal protein factor supplements were responsible for the hitherto unexplained extra growth.

Antibiotics are products of living organisms which inhibit the growth or destroy other living organisms. Examples are penicillin, streptomycin, aureomycin and terramycin. Antibiotics have been shown to increase the rate of growth of poultry and swine, and to increase the efficiency of feed utilization in these animals. Antibiotics will decrease, but not eliminate the vitamin B_{12} and other water-soluble vitamin requirements, exert a "protein-sparing" effect, and increase livability. The exact mode of action of antibiotics is unknown, but it is generally assumed at the present time that the growth stimulation is an indirect effect, mediated in some manner through changes exerted on the intestinal microflora. This may result in a suppression of "unfavorable" types of bacteria which would compete with the host for ingested nutrients, or may also aid in the establishment of "favorable" types which would synthesize vitamins and other factors required for the well being of the animal.

It has also been speculated that antibiotics might stimulate growth as a result of their cleansing action on the intestinal lumen, thereby creating a greater unobstructed surface area, resulting in more efficient absorption of nutrients by the villi.

Regardless of the fact that the exact growth stimulating mechanism of antibiotics remains unknown, antibiotics are playing an important role in the feeding of livestock. It can safely be stated that practically all manufactured feeds prepared for young growing chickens and turkeys today contain one or more antibiotics.

Inasmuch as the exact mechanism of antibiotic growth stimulation is unknown, it seemed desirable to obtain some information which indirectly, at least might lend support to one theory, namely, that antibiotics stimulate growth as a result of limiting the growth of intestinal organisms which compete with the host for ingested nutrients. In such a situation the host animal should be favored because a greater quantity of nutrients would be available for growth. Furthermore, it would appear that if this concept of host-microorganism competition were true then the feeding of an antibiotic should show a marked sparing action on selected vitamins which may be marginal in the diet.

This study was initiated to determine the effect of penicillin on the growth response of chicks to graded levels of calcium pantothenate, niacin, choline and riboflavin, and to determine the extent to which penicillin might reduce the chicks' requirements for these vitamins.

SELECTED REFERENCES ON RECENT ANTIBIOTIC STUDIES IN NUTRITION

Briggs, Luckey Elvehjem and Hart (1944), in work with chicks on purified diets deficient in certain vitamins contained in liver preparations, showed additional growth stimulation due to the inclusion of sulfasuxadine in the diet. Subsequently Moore and co-workers (1946), showed similar growth stimulation in chicks fed purified diets where sulfasuxadine and in addition, streptomycin were fed.

Stokstad and co-workers (1949), reported the animal protein factor to be multiple in nature, containing an unidentified chick growth factor in addition to vitamin B_{12}. The unidentified chick growth factor was contained in a fraction from Streptomyces aureofaciens cultures. In further work on this unidentified chick growth factor, Stokstad and Jukes (1950), reported it to be aureomycin, which was found to increase the rate of growth of chicks.

Groschke and Evans (1950), in an independent study confirmed the work of Stokstad and Jukes (1950), by showing that the growth effects resulting from feeding an animal protein factor supplement could be duplicated by feeding supplements of aureomycin or streptomycin.

Oleson and co-workers (1950), showed aureomycin and vitamin B_{12} to have a sparing action on each other, while Stokstad and Jukes (1951), showed aureomycin to have a sparing effect on vitamin B_{12} in some instances.

The effects of antibiotics on sparing vitamins was further shown by Linkswiler et al (1951). These workers showed aureomycin had a sparing action on the requirement of the rat for pyridoxine.

Lih and Baumann (1951), using rats, demonstrated that aureomycin, penicillin and streptomycin, but not terramycin or chloromycetin would spare the vitamins thiamin, riboflavin and pantothenic acid. The antibiotics were most effective in the diets that contained only enough vitamin for half maximum growth, and the growth responses due to the antibiotics were approximately equal to those observed when the vitamin content of the diet was considered adequate.

Biely and March (1951), using a practical type diet, indicated that sub-optimal levels of folic acid, riboflavin and nicotinic acid may be adequate for chicks when aureomycin is included in the diet.

Procedure

Four experiments were conducted in this study. Day old, straight-run Rhode Island Red chicks were used in each experiment. The maternal diet consisted of a 20 percent protein, practical-type breeder mash complete in all known essential nutrients. A grain mixture of corn and wheat was fed with the mash in an amount to equal one-half of the total daily feed intake.

Twelve groups, of eight chicks, comprised each experiment. The chicks were segregated at random, wingbanded and placed in electrically heated battery brooders with raised wire floors, and fed the experimental diets for a four week period. Feed and water were kept before the chicks at all times. The basal diet used in these experiments is given in Table 1.

The batteries used in this study were equipped on each side with a bank of 40 watt bulbs, vertically suspended at mid-section. This arrangement allowed equal illumination of each deck level. Continuous lighting was provided throughout each experiment. The room temperature was maintained between 80° and 85° Fahrenheit. All chicks were weighed at weekly intervals, kept under daily observation and examined closely for the classical deficiency symptoms.

At the end of the four week period, all chicks were sacrificed by dislocating the neck. The hearts and livers were removed, the livers weighed and both organs were then frozen on dry ice. The frozen tissues were stored in a deep freeze compartment to be assayed for B vitamins at a later date. These assays are part of another study and are not reported in this dissertation. At the end of each experiment

Table 1

Composition of Basal Diet

	Percent
Cerelose	61.0
Gelatin	10.0
Casein (Vitamin Test)	18.0
*Mineral Mixture	6.0
Soy Bean Oil (Crude Degummed)	3.0
Fish Oil (400D, 2000A)	1.0
**Vitamin Mixture	0.4
Methionine	0.3
Choline Chloride	0.2
Inositol	0.1

* Mineral Mixture: (in grams) $CaCO_3$ 1.2100, Ca_3PO_4 1.7000, K_2HPO_4 1.0000, NaCl .9000, Na_2HPO_4 .6000, $MgSO_4 \cdot 7H_2O$ .5000, $FeSO_4 \cdot 7H_2O$ .0570, $MnSO_4 \cdot H_2O$ .0250, KI .0050, $CuSO_4 \cdot 5H_2O$ .0020, $ZnCl_2$ .0009, $CoSO_4 \cdot 7H_2O$ .0001.

** Vitamin Mixture[1]: (mg./kilo) Riboflavin 8.0, Thiamin HCl 4.0, Calcium Pantothenate 20.0, Pyridoxine HCl 6.0, Niacin 100.0, Biotin .2, Folic Acid 1.0, Menadione 1.0, A-tocopheryl Acetate 5.0, B_{12} (NaCl triturate) 30.0, Para Amino Benzoic Acid 2.0.

1 The above vitamins were mixed thoroughly with enough cerelose so as to make up 0.4 percent of the diet.

feed consumption over the four week period was determined, from which feed efficiency values were calculated.

Experiment 1

THE EFFECT OF DIETARY PENICILLIN ON THE RESPONSE OF CHICKS TO GRADED LEVELS OF CALCIUM PANTOTHENATE

Selected References of Pantothenic Acid

The importance of pantothenic acid in chick nutrition has been well established. Norris and Ringrose (1930), were the first to report on a pellagra-like syndrome in chicks. Ringrose, Norris and Heuser (1931), further described this pellagra-like syndrome. Their work indicated a requirement by the chick for a relatively heat-stable growth promoting factor. Present day knowledge of vitamins however, indicates that this early work was complicated with a multiple vitamin deficiency involving pantothenic acid, biotin and riboflavin.

Kline, Keenan, Elehjem and Hart (1932), produced a very severe type of dermatosis by subjecting a purified diet, similar to that of Ringrose and co-workers (1931), to heat in a dry atmosphere.

Ringrose and Norris (1936), working with a pork liver extract found it to be effective in preventing the pellagra-like syndrome in chicks. After various manipulations of the extract in an attempt to identify the active substance, the activity was found to remain in the filtrate. Hence the active principle was desingated the "filtrate factor".

The "filtrate factor" was identified by Jukes (1939) and Wooley and co-workers (1939), as pantothenic acid, and the synthesis of pantothenic acid was reported by Williams et al. (1940).

The manifestations of a pantothenic acid deficiency as described by many workers are very similar in all cases. Ewing (1951) sums up the pantothenic acid deficiency symptoms as poor growth, loss of vitality, poor feathering, inflamation of the skin, sores and incrustations at the corners of the mouth, on the eyelids and sometimes on the feet, cracks on the feet, and scales on the shanks become thickened. Jukes (1943) observed paralytic symptoms in addition to the above mentioned syndrome.

Bauernfiend, Norris and Heuser (1942), reported that Single Comb White Leghorn chicks require 500-550 micrograms percent of pantothenic acid for the prevention of dermatosis, and 600 micrograms percent for maximum growth. In this work, Rhode Island Red chicks were found to require 75 micrograms percent less than the Leghorn chicks.

Using a purified diet, Jukes, Stokstad and Franklin (1943), reported that the requirement of pantothenic acid for New Hampshire chicks appeared to be in excess of 1.0 milligram percent after a depletion period of six days. These results were confirmed in a later study by Jukes and McEloroy (1949), while Hegsted and Riggs (1949), reported 900 micrograms percent of pantothenic acid for maximum growth.

Results and Discussion

Five levels of calcium pantothenate were fed with and without penicillin. These levels ranged from 0.2 milligrams percent to 1.0 milligram percent. On the basis of values reported in the literature, it was believed that the highest value used, namely 1.0 milligram percent of calcium pantothenate would satisfy the chick's requirement for this vitamin.

The results pertaining to growth are graphically presented in Figure 1. The data presented clearly shows that penicillin exerts a sparing action on calcium pantothenate. This action is especially evident in the groups receiving 0.4 milligrams percent pantothate, the group receiving penicillin in addition having a final average body weight 48 percent greater than the non-penicillin group.

As expected, the growth response of the non-penicillin groups increased as the level of pantothenate was increased, with maximum growth appearing to be at the highest level used. The maximum growth response of the groups receiving penicillin was at the 0.8 milligrams percent level, and additional supplements of pantothenate gave essentially the same growth. The greatest percentage increase in growth from penicillin appeared in the groups receiving sub-marginal levels of pantothenate. This confirms the works of Lih and Baumann (1951), and Beily and March (1951), whose work indicated that antibiotics appeared to lower the requirement of rats and chicks for certain vitamins.

Figure 2 compares a normal chick with two chicks from group 9 which exhibit poor growth and symptoms of dermatosis. A close-up of the same two deficient chicks may be seen in Figure 3 which clearly shows the encrustations at the corners of the mouth and the granular condition of the eyelids.

The data on feed utilization, mortality and occurrence of deficiency symptoms together with average body weights are summarized in Table 2.

It will be noted that feed efficiency values were improved by penicillin in those groups receiving the lower levels of pantothenate (compare groups 1, 2, 3, and 4 with groups 7, 8, 9, and 10). Feed efficiency values were not materially affected by penicillin in those groups receiving the higher levels of pantothenate (compare groups 5, and 6 with groups 11, and 12).

In the absence of supplemental pantothenate, penicillin reduced mortality slightly. However, in connection with the occurrence of deficiency symptoms, penicillin tended to accentuate the expression of typical pathological lesions about the beak, eyes, hocks, and feet, especially in those groups fed sub-marginal levels of pantothenate. It is suggested that this accentuation of the deficiency syndrome is probably due to the stress produced by the increased growth stimulating properties of penicillin. This increase is produced on a limited supply of pantothenate, and therefore, the pantothenic acid deficiency symptoms were enhanced.

It should be stated here that the observation of dermatosis in the hock region is a new finding and has not been reported previously in the literature in descriptions of pantothenic acid deficiency in the chick. Moreover, the production of perosis is a hitherto unreported observation which this study has associated with pantothenic acid deficiency. The above mentioned, newly observed syndromes are shown in Figures 4 and 5.

This study confirms the earlier work of Jukes (1943), that some chicks exhibit paralytic symptoms as a result of pantothenic acid deficiency. A greater number of chicks with paralysis were observed, however, in the groups receiving sub-marginal levels of pantothenate and penicillin than in the comparable groups not receiving penicillin (see groups 2 and 8, 9 and 10). Again this shows the tendency of penicillin to enhance the expression of deficiency when a sub-marginal level of the vitamin is fed.

The first pantothenic acid deficiency symptom to appear was diarrhea on the 6th day in groups 1, 2, and 7. On the 10th day, perosis appeared, and by the 13th day the corners of the mouths were inflamed, and on the 14th day, the typical dermatosis appeared. The scales and skin of the shanks and feet became dry and thickened and by the 16th day fissures and cracks appeared on the feet. The eyelids became granular and tended to stick together, and on several occasions it became necessary to open the eyes so that the chicks could see to eat and drink. By the 24th day nervous symptoms were evident, expressed by tremors and ataxia.

The foregoing results tend to support one theory of the growth stimulating mechanism of antibiotics, specifically the host-microorganism competition concept. The sparing action of penicillin might be explained by the limited growth of those intestinal organisms which require pantothenic acid and other vitamins for growth, thereby reducing competition for the vitamin in favor of the host animal. Moreover, the possible establishment of a type of intestinal microorganism which would synthesize vitamins or essential factors can not be overlooked.

The complete absence of the dermatosis syndrome in the group receiving 0.6 milligram percent pantothenate and penicillin, and the occurrence of dermatosis in its domparable non-penicillin group (see groups 4 and 10), further demonstrates the pantothenate sparing action of penicillin.

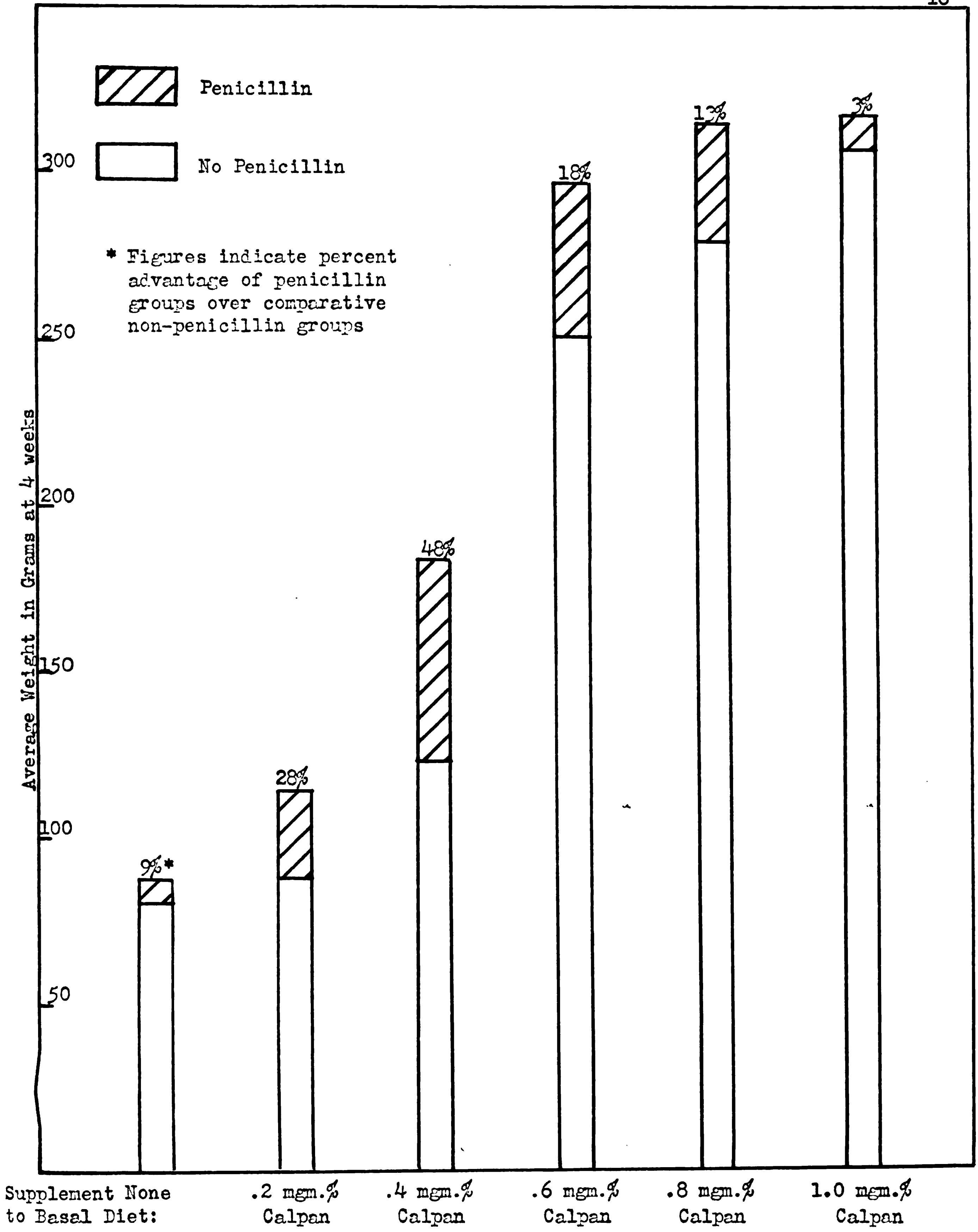

Fig. 1. Response of Chicks To Graded Levels of Calcium Pantothate With and Without Penicillin

Table 2. Effect of graded levels of pantothenic acid with and without penicillin on body weight, feed efficiency, and the incidence of deficiency symptoms. (dermatosis, perosis and paralysis)

Group	Supplement to Basal Diet	Surv-ivors	Average 4 week Body Weight (grams)	Feed / Gain	Dermatosis				Perosis	Paralysis
					Beak	Eyes	Hocks	Feet		
			No Penicillin Added (Groups 1-6)							
1	None	4	80	4.19	7	2	0	0	0	0
2	0.2 mgm.% Calpan*	8	88	3.17	8	4	2	0	0	0
3	0.4 mgm.% Calpan	8	123	2.66	6	3	6	1	0	2
4	0.6 mgm.% Calpan	8	250	1.92	2	0	3	0	2	0
5	0.8 mgm.% Calpan	8	278	1.68	0	0	0	0	0	0
6	1.0 mgm.% Calpan	8	305	1.66	0	0	0	0	1	0
			0.005% Penicillin** Added (Groups 7-12)							
7	None	5	88	3.42	8	3	0	0	1	0
8	0.2 mgm.% Calpan	8	113	2.77	8	7	3	4	3	3
9	0.4 mgm.% Calpan	8	183	2.47	8	4	7	5	3	1
10	0.6 mgm.% Calpan	8	296	1.74	0	0	0	0	3	1
11	0.8 mgm.% Calpan	8	313	1.76	0	0	0	0	1	0
12	1.0 mgm.% Calpan	8	315	1.73	0	0	0	0	1	0

* Calcium Pantothenate

** Procaine Penicillin "G"

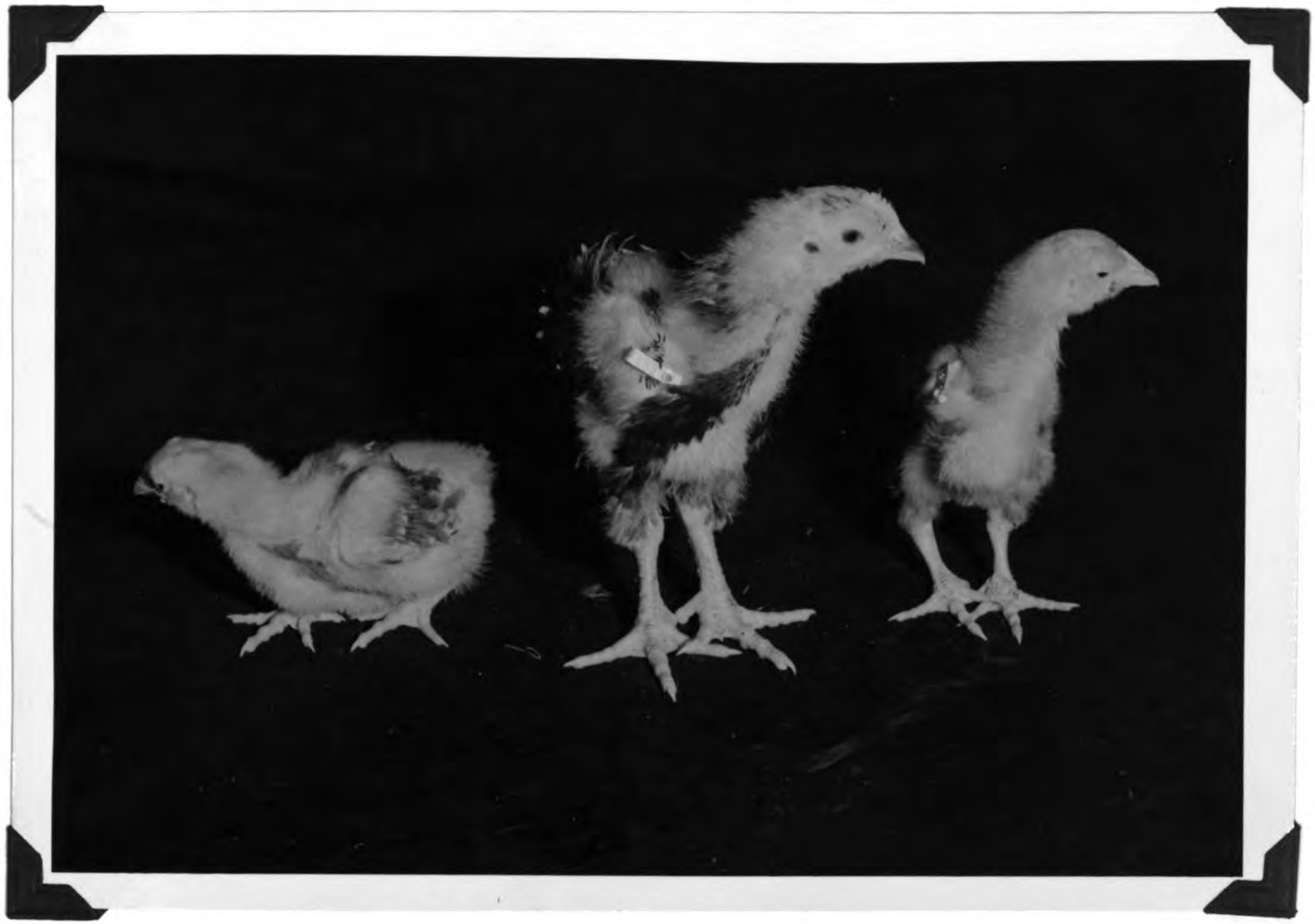

Fig. 2 Control chick (middle) flanked by chicks with panto. acid deficiency. Note Perosis in chick at right, together with poor growth and feathering. All chicks 4 weeks of age.

Fig. 3 Chicks showing pantothenic acid deficiency. Note encrustations around eyes and mouth.

Fig. 4 Dermatosis of the feet and thickening of scales. Note swelling of feather follicles and scaling of skin in region of hocks.

Fig. 5 A typical case of perosis produced by pantothenic acid deficiency.

Experiment 2

THE EFFECT OF DIETARY PENICILLIN ON THE RESPONSE OF CHICKS TO GRADED LEVELS OF NIACIN

Selected References on Niacin

The vitamin, now referred to as nicotinic acid or niacin was first shown to have nutritional significance by Elvehjem (1937). This worker reported the prevention or cure of canine "black-tongue" through the administration of niacin. Bethke (1942), reported that poultry either do not require niacin or that its needs are so low as to be of no significant importance. Jukes and Almquist (1942) were unable to produce a dietary deficiency in poultry. The failure of these early workers to produce a niacin deficiency in poultry is now clear and can be explained by the fact that the diets employed were of a good amino acid pattern high in tryptophane.

Briggs, Mills, Elvehjem and Hart (1942), reported that chicks require a minimum level of 1.8 milligrams percent for maximum growth, but 0.5 milligrams percent was sufficient to prevent chick "black-tongue". Occasional perosis was noted. Further work by Briggs (1946), indicated that 3 to 5 milligrams percent niacin would prevent perosis in turkey poults.

Krehl and co-workers (1946) reported that tryptophane or niacin counteracts a growth retardation in rats caused by the addition of corn grits to a low protein diet.

Briggs (1945), produced typical niacin deficiency symptoms by feeding 10 percent of gelatin as a source of arginine and glycine, and the addition of 5.0 milligrams percent niacin or 200 milligrams percent dl-tryptophane counteracted the symptoms and growth depression. This work indicated tryptophane to be a precursor of niacin.

Scott, Singsen and Matterson (1946) reported that niacin is required to prevent perosis in chicks on a diet high in corn and containing 5.0 percent gelatin. Sarma and Elvehjem (1946) confirmed this report by adding 40 percent corn grits to a purified ration which produced a growth depression which niacin counteracted.

Briggs and co-workers (1943) reported their observations on a niacin deficiency to include chick "black-tongue", decreased feed consumption, poor feather development, occasional perosis and a lowering of the niacin and coenzyme 1 content of the breast muscle.

RESULTS AND DISCUSSION

Five levels of niacin were fed with and without penicillin. The levels ranged from 1.25 milligrams percent to 10.00 milligrams percent based on the values reported in the literature. The highest value used (10.00 milligrams percent) was believed to be more than sufficient to satisfy the chicks' requirements for niacin.

The results pertaining to growth are presented graphically in Figure 6. It may be seen that penicillin produced additional growth at all levels of niacin supplementation. When growth advantages are considered in terms of percent increase, penicillin afforded the greatest increases in growth in the groups receiving 1.25 milligrams percent and 10.00 milligrams percent of niacin. It is doubtful whether the growth advantages shown in groups 11 and 12 represent real differences which can be attributed to penicillin. Rather, it is believed that these differences are fortuitous, and represent the high degree of variability which is known to exist in the strain of Rhode Island Reds used in these studies.

The results indicate that the growth response of the non-penicillin groups reaches its maximum at the 3.75 milligrams percent level of niacin, with additional supplements of the vitamin showing no material advantage.

The data on feed utilization, mortality and the occurrence of deficiency symptoms together with average body weights are summarized in Table 3.

The feed efficiency value for group 1 was not calculated due to the severe mortality (75 percent). Except in groups 3 and 9, penicillin improved the feed efficiency values.

Penicillin exerted a protective action in the absence of supplemental niacin, reducing the mortality significantly (compare group 1 - 75 percent mortality, with group 7 - 12.5 percent mortality).

The niacin deficiency symptoms appeared very early; the chick "black-tongue", and cankers or lingual ulcers being noticed on the fifth day. Chicks of group 1 which died at about 2 weeks were extremely dehydrated and the oral cavities were severely inflamed. By the 18th day, the chicks of groups 1 and 7 (no supplemental niacin) were raggy and wet in appearance caused by excessive salivation. Unlike the effects on pantothenate deficiency symptoms, penicillin had no material effects on the incidence or severity of the chick "black-tongue" or cankers. After the third week, the deficiency syndrome tended to lessen in severity, the chick "black-tonue" and cankers completely disappearing in many cases, and the chicks appeared to make a noticable recovery.

The perosis resulting from this diet was not severe and only ten mild cases in all were noted. This confirms the observations of Briggs and co-workers (1943) who reported "occasional perosis" in their description of the niacin deficiency syndrome. Penicillin exerted no apparent effect on the perosis encountered in this experiment. Two and one-half milligrams percent niacin seemed marginal for the complete prevention of the deficiency syndrome with and without penicillin, while the 3.75 milligrams percent niacin supplement completely prevented the appearance of any symptoms.

In this study, penicillin exhibited its beneficial effects on young growing chicks by increasing the growth rate and by reducing mortality. These effects were especially noted in chicks fed diets totally deficient or sub-marginal in their niacin content. These results definitely show a sparing action of penicillin on niacin in the chick.

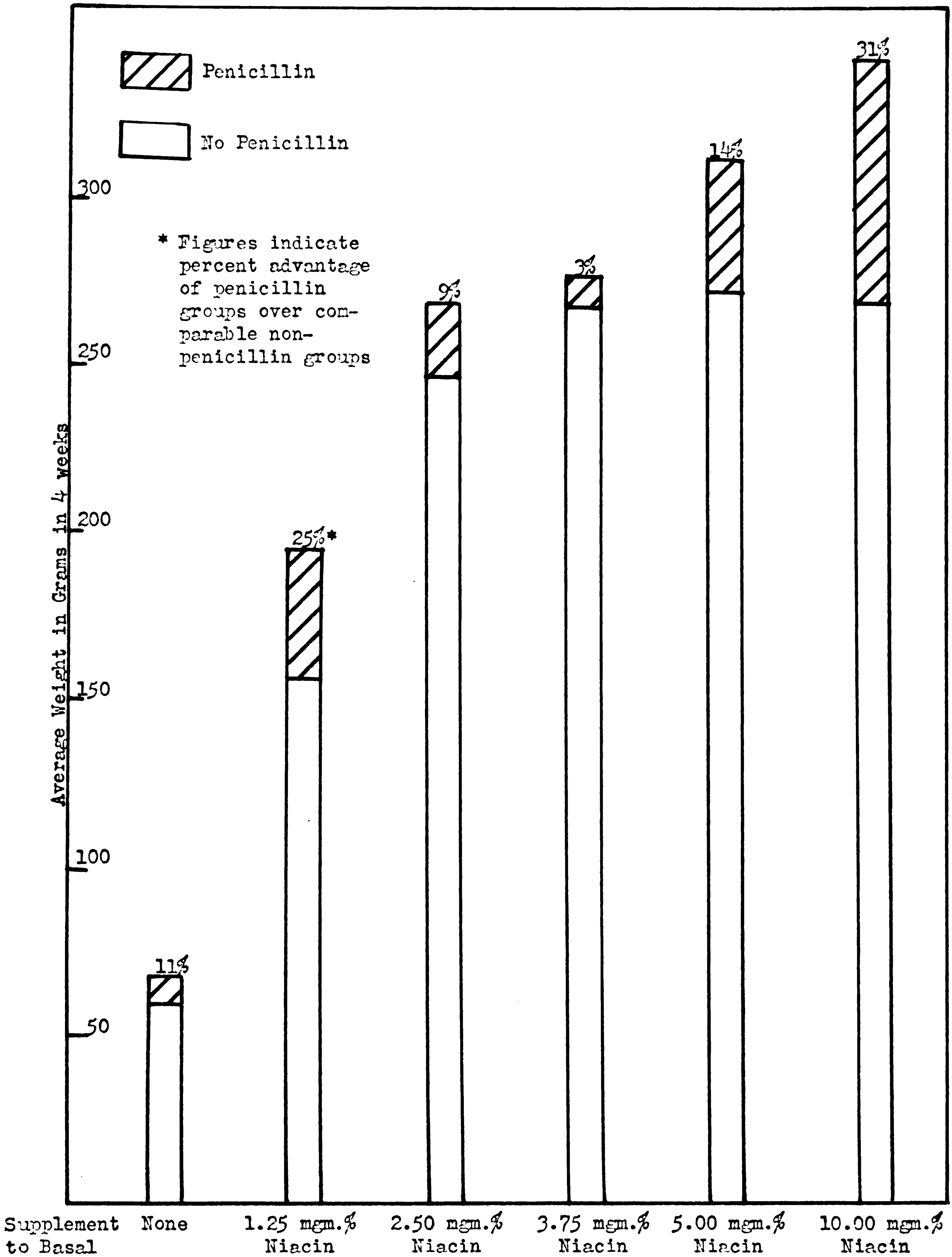

Fig. 6 Response of Chicks to Graded Levels of Nicotinic Acid With and Without Penicillin

Table 3. Effect of graded levels of niacin with and without penicillin on body weight, feed efficiency and the incidence of deficiency symptoms ("chick-black-tongue", mouth canker, and perosis)

Group	Supplement to Basal	Survivors	Average Body Weight 4 weeks (grams)	Feed/Gain	Black-Tongue	Mouth Canker	Perosis
			No Penicillin Added (Groups 1-6)				
1	None	2	59		6	4	0
2	1.25 mgm.% Niacin	8	156	2.07	7	8	4
3	2.50 mgm.% Niacin	8	246	1.58	0	1	1
4	3.75 mgm.% Niacin	8	268	1.68	0	0	0
5	5.00 mgm.% Niacin	8	272	1.75	0	0	0
6	10.00 mgm.% Niacin	8	268	1.70	0	0	0
			0.005% Penicillin Added (Groups 7-12)				
7	None	7	66	4.72	8	7	0
8	1.25 mgm.% Niacin	8	196	1.84	3	6	1
9	2.50 mgm.% Niacin	8	268	1.73	0	1	3
10	3.75 mgm.% Niacin	8	276	1.57	0	0	0
11	5.00 mgm.% Niacin	8	311	1.56	0	0	0
12	10.00 mgm.% Niacin	8	350	1.30	0	0	1

Experiment 3

THE EFFECTS OF DIETARY PENICILLIN ON THE RESPONSE
OF CHICKS TO GRADED LEVELS OF CHOLINE

Selected References on Choline

The elucidation of choline was unique when compared to the discovery and isolation of other vitamins. The biochemical importance of choline was known for a number of years before its nutritional significance was disclosed.

Best and Huntsman (1932) reported the occurrence of fatty livers in rats fed a high fat low choline ration, and the prevention of the deposition of excess liver lipid by dietary supplements of choline.

Elvehjem (1937) suggested that choline be considered a member of the vitamin B complex since it definitely had been shown to play a significant role in the nutrition of several animals.

Jukes (1939), reported a high incidence of perosis on a simplified ration for turkey poults in spite of the addition of as high as 0.4 percent of manganese sulphate. Continuing the work on perosis, Jukes (1940) presented evidence showing choline to prevent a type of perosis often encountered in poults. He also observed choline to be growth-promoting in this work. In further work on perosis Jukes (1940a) found that choline prevented perosis and promoted growth in chicks fed a purified ration. Choline at a level of 0.1 percent was found to be effective for promoting growth and the prevention of perosis in chicks.

Hegsted and co-workers (1941) also reported 0.1 percent choline to be effective in the prevention of perosis and for growth promotion.

Berry and co-workers (1943) reported that the choline requirement for the prevention of perosis is less than the requirement for growth in chickens. One hundred and fifty milligrams percent was sufficient for maximum growth in chicks, while considerably less prevented the occurrence of perosis.

A great deal of work has been carried on to determine the interrelationship of choline, methionine, betaine and vitamin B_{12}, but the work is so voluminous, and since this relationship was not to be studied in this experiment, it was decided not to report on this otherwise interesting phenomen of choline.

Results and Discussion

Five levels of choline were used with and without penicillin. These levels ranged from 40 milligrams percent to 200 milligrams percent. On the basis of values reported in the literature, it was believed that the highest level used in this experiment, namely 200 milligrams percent, supplied enough choline to satisfy the requirements of the chick for this vitamin.

The results representing the growth responses are presented graphically in Figure 7. It will be noted that penicillin exerted a growth advantage in each of the six groups where it was fed. The sparing action of penicillin on choline is most evident in those groups receiving 80 and 120 milligrams percent choline.

Maximum growth among the non-penicillin groups was reached at the 160 milligrams percent level of choline. Maximum growth among the penicillin supplemented groups was attained at 80 milligrams percent choline and the additional supplements of choline did not materially increase growth in the presence of penicillin.

Figure 8 compares two normal chicks with two chicks from group 1 which exhibit the poor growth and feathering resulting from the choline deficiency. The ungainly position of the two deficient chicks is the result of the perosis brought about by the choline deficiency. (See close-up, Figure 9).

The data on feed utilization, mortality and occurrence of deficiency symptoms together with average body weights are summarized in Table 4.

The efficiency of feed utilization values were improved as the supplementation of choline was increased, and the addition of penicillin further improved these values at all supplemented levels of choline. This improvement was not noticed in the groups not receiving a supplement of choline (compare group 1 with group 7).

The mortality which occurred in this experiment was slight and scattered. Although the basal diet employed is deficient in choline, it would appear that sufficient amounts of precursors are available to allow the chick to synthesize enough of this vitamin compatable with maintenance needs.

The incidence of perosis in this trial was one hundred percent in the deficient and sub-marginal groups (1, 2 and 7 and 8). Penicillin did not appear to have any effect on the incidence or severity of perosis while on the other hand, it did show a sparing action on the requirement of choline for growth.

Inasmuch as it has been established in the literature that the requirement of choline for the prevention of perosis is much less than the requirement for growth, the failure of penicillin to sharply affect the incidence of perosis in this study might be explained by reasoning that the levels of choline employed were too wide to allow the sparing action of penicillin to be observed in regard to the perosis pattern.

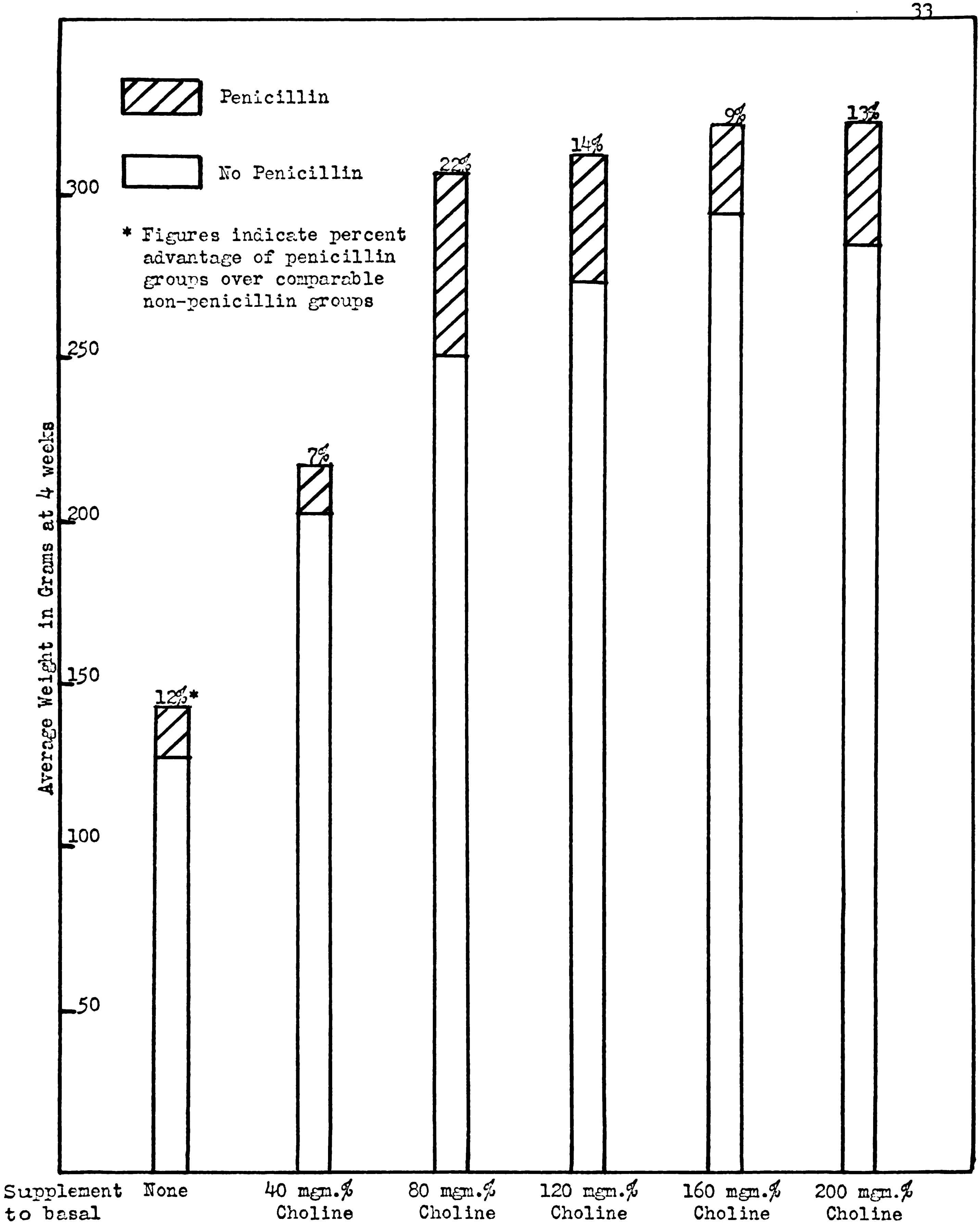

Fig. 7 Response of Chicks to Graded Levels of Choline Chloride With and Without Penicillin

Table 4. Effect of graded levels of choline with and without penicillin on body weight, feed efficiency, and the incidence of deficiency symptoms (perosis)

Group	Supplement to Basal	Survivors	Average Body Weight 4 weeks (grams)	Feed/Gain	Perosis
		No Penicillin Added (Groups 1-6)			
1	None	7	129	2.02	7
2	40 mgm.% Choline	8	202	1.82	8
3	80 mgm.% Choline	7	251	1.81	3
4	120 mgm.% Choline	8	274	1.74	2
5	160 mgm.% Choline	8	294	1.68	0
6	200 mgm.% Choline	8	284	1.67	0
		0.005% Penicillin Added (Groups 7-12)			
7	None	8	144	2.31	8
8	40 mgm.% Choline	8	218	1.78	8
9	80 mgm.% Choline	8	306	1.64	2
10	120 mgm.% Choline	8	312	1.61	0
11	160 mgm.% Choline	7	321	1.62	1
12	200 mgm.% Choline	8	322	1.65	0

Fig. 8. Control Chicks and Choline Deficient Chicks. Note, at 4 weeks of age ungainly position of deficient chicks.

Fig. 9. Typical Perosis Induced by Choline Deficiency.

Experiment 4

THE EFFECTS OF DIETARY PENICILLIN ON THE RESPONSE OF CHICKS TO GRADED LEVELS OF RIBOFLAVIN

Selected References on Riboflavin

Hauge and Carrick (1926) reported results which indicated that the chick required a growth-promoting factor for the maintenance of life and growth. This factor, although unnamed at the time, was probably riboflavin.

These results were confirmed by a number of workers in the following years, and Norris and co-workers (1936) concluded that this growth-promoting factor was flavin or vitamin G.

Heuser and co-workers (1938) further pointed out that the chick's requirement for riboflavin is not constant, but varies for different ages. Evidence was also presented to show that the amount of riboflavin required by the chick is correlated with the rate of growth.

Norris and co-workers (1936) reported that chicks need 290 micrograms percent of riboflavin to attain normal weight at eight weeks of age; 300 micrograms percent at six weeks and 325 micrograms percent at four weeks.

Stokstad and Manning (1938) prevented curled-toe paralysis (a specific riboflavin deficiency syndrome) at six weeks of age in chicks that had been depleted for two weeks by additions of about 275-300 micrograms percent riboflavin to their diet.

Culton and Bird (1940) reported that 300 micrograms percent of crystalline riboflavin added to a ration containing approximately 175 micrograms percent riboflavin was not sufficient to prevent curled-toe paralysis. Likewise, approximately 415 micrograms percent riboflavin in dried skimmilk or dried whey did not fully prevent curled-toe paralysis.

Bethke and Record (1942) found that synthetic and naturally occurring riboflavin were equally effective in preventing curled-toe paralysis and in promoting growth in chicks. They further stated that chicks require greater amounts of riboflavin per unit of feed for the prevention of curled-toe paralysis than for promoting maximum growth, while Bird and associates (1946) stated that less riboflavin was required for the prevention of curled-toe paralysis than was required for optimum growth. They reported that between 275-325 micrograms percent riboflavin was required for optimum growth to four weeks.

Results and Discussion

Five levels of riboflavin were fed with and without penicillin. These levels ranged from 100 micrograms percent to 800 micrograms percent. Based on the values reported in the literature the highest levels used in this experiment, namely 800 micrograms percent riboflavin, was believed to be more than sufficient to satisfy the chick's requirement for growth and the prevention of curled-toe paralysis.

The results pertaining to growth are graphically presented in Figure 10. These results definitely show that penicillin exerts a sparing action on riboflavin. This action is most evident in the lowest riboflavin supplemented groups, (compare groups 1, 2, 3 and 4 with groups 7, 8, 9 and 10) with penicillin showing percent advantages between 29 and 39 percent.

As expected the growth responses of the non-penicillin groups were correlated with the increased supplementation of riboflavin the data indicates however, that the strain of Rhode Island Reds used has a high requirement for riboflavin, needing more than 400 micrograms percent for maximum growth.

With the penicillin supplement, maximum growth was achieved with 300 micrograms percent of riboflavin. In terms of percent advantage, penicillin showed its greatest growth stimulating effect when fed to chicks receiving 100 micrograms percent of riboflavin. The maximum growth response due to the penicillin supplement occurred at the 300 micrograms percent level of riboflavin, and considering that additional supplements of riboflavin resulted in lesser growth responses, this growth advantage is believed to be due to the previously mentioned variability in this strain of Rhode Island Reds rather than attributed to the growth stimulation produced by penicillin.

The data on feed utilization, mortality, and the occurrence of deficiency symptoms, together with the average body weights are summarized in Table 5. It will be noted that penicillin improved the feed efficiency values over all non-penicillin groups. The improved values were very marked at the 100 micrograms percent level of riboflavin, in which case penicillin reduced by almost one-third the amount of feed required per unit-gain.

Severe mortality was experienced among the chicks of group 1 receiving no supplemental riboflavin, 75 percent of the chicks having died before the 4 week period was completed. The protective action of penicillin, noted previously (see experiment 2) increased the livability of the chicks on the riboflavin deficient diet and allowed 100 percent survival.

The incidence and severity of curled-toe paralysis was slight, making its appearance about the end of the fourth week. The curled-toe paralysis did not appear in the deficient or sub-marginal levels of riboflavin. This confirms the work of Stokstad and Manning (1938) that in general, if no riboflavin was added to the basal diet, curled-toe paralysis did not appear, but small supplements of the vitamin increased the incidence and large amounts of riboflavin completely prevented it.

Curled-toe paralysis appeared at the 200 micrograms percent level of riboflavin both with and without penicillin and these results agree with the observations of Bird and associates (1946) that less riboflavin is required for the prevention of curled-toe paralysis than is required for the optimum growth.

Penicillin had but slight effect on the incidence (compare groups 3 and 9) and no effect on the severity of curled-toe paralysis in this experiment.

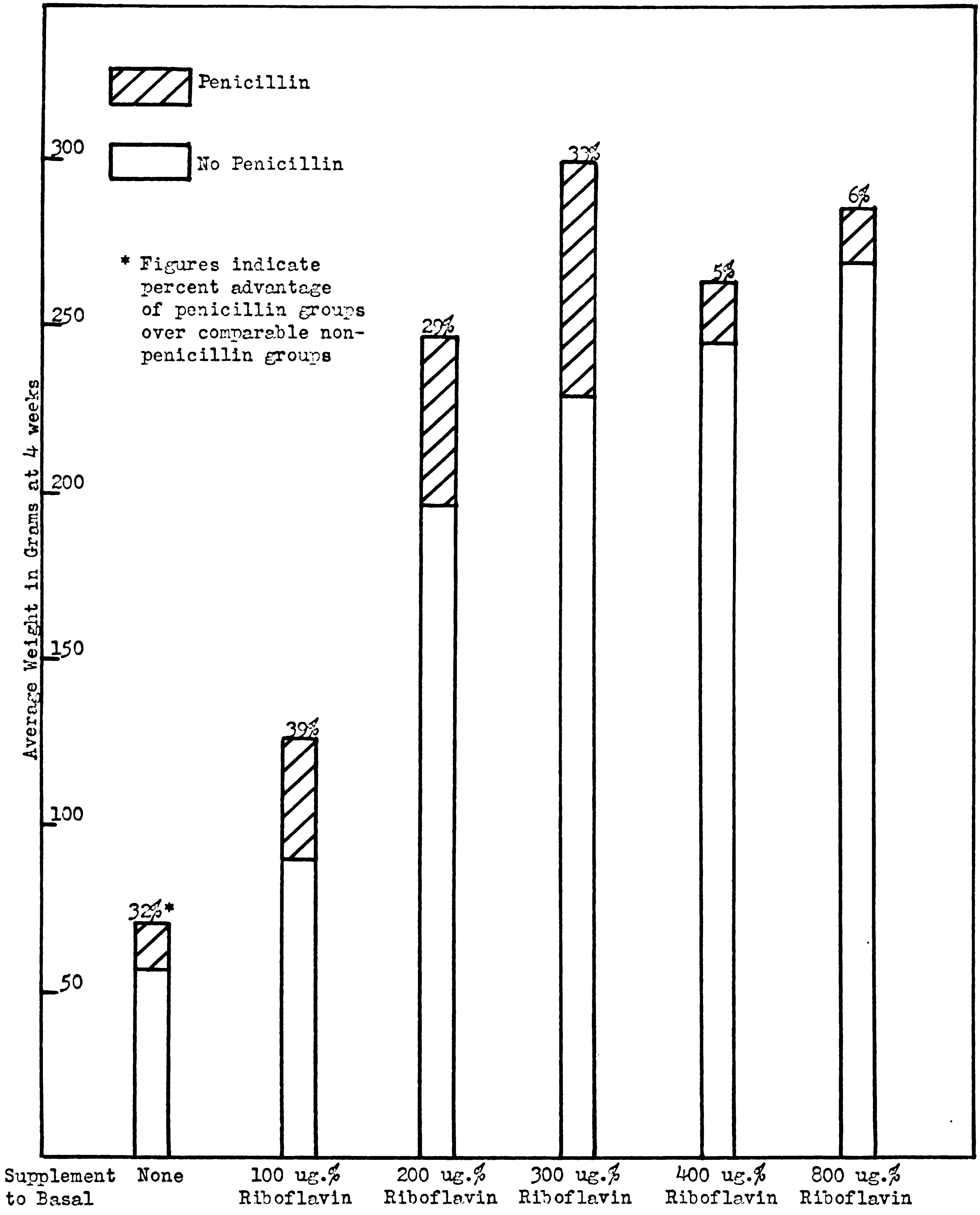

Fig. 10. Response of Chicks to Graded Levels of Riboflavin With and Without Penicillin

Table 5. Effect of graded levels of riboflavin with and without penicillin on body weight, feed efficiency and the incidence of deficiency symptoms (curled-toe paralysis, mouth canker and paralysis)

Group	Supplement to Basal	Survivors	Average Body Weight 4 week (grams)	Feed / Gain	Curled Toes	Mouth Canker	Paralysis
			No Penicillin Added (Groups 1-6)				
1	None	2	53	9.29	0	0	0
2	100 ug.% Riboflavin	8	90	3.33	0	1	0
3	200 ug.% Riboflavin	8	197	1.86	4	0	0
4	300 ug.% Riboflavin	7	229	1.93	0	0	1
5	400 ug.% Riboflavin	6	240	1.83	0	0	0
6	800 ug.% Riboflavin	8	269	1.74	0	0	0
			0.005% Penicillin** Added (Groups 7-12)				
7	None	8	70	2.21	0	1	0
8	100 ug.% Riboflavin	8	125	2.35	0	0	1
9	200 ug.% Riboflavin	8	247	1.71	3	0	0
10	300 ug.% Riboflavin	8	306	1.70	0	0	0
11	400 ug.% Riboflavin	8	253	1.83	0	0	0
12	800 ug.% Riboflavin	8	285	1.67	0	0	0

** Procaine Penicillin G.

SUMMARY

A series of four experiments were carried out to determine the effects of dietary penicillin on the biological response of chicks fed graded levels of calcium pantothenate, niacin, choline, and riboflavin. These experiments were undertaken to obtain information of a positive nature which indirectly might lend support to the theory that antibiotics stimulate growth, in part at least, by altering the intestinal microflora in a manner that would be advantageous to the host.

This speculative approach embraces the concept that the normal animal harbors a countless number of intestinal microorganisms, of various types and species, which compete with the host for ingested nutrients. Should this be true, then it is conceivable that antibiotics might stimulate growth indirectly by limiting the growth of these bacteria with the consequence that competition for food, from the animal's point of view, is reduced and a greater quantity of nutrients is made available to the host. Moreover, it would seem that the amount of any given nutrient that might be spared by an antibiotic would represent a more critical part of the total quantity of that nutrient in diets that were sub-marginal for it as compared with diets that contained an optimum level. Therefore, the feeding of an antibiotic should lead to greater percentage growth increases in chicks fed sub-marginal diets as compared with an adequate diet.

The procedure of feeding graded levels of micro-nutrients (calcium pantothenate, niacin, choline, and riboflavin) with and without penicillin yielded data which may be interpreted as supporting the host-bacteria

competition theory. These data, in general, showed that penicillin exerted a greater growth stimulating effect (percent increase in growth) on chicks receiving very low or sub-marginal levels of vitamins than it did on chicks fed marginal or optimum levels of vitamins. It was also observed that feed efficiency values were improved to a greater extent by penicillin in the groups of chicks fed sub-marginal levels of vitamins as compared with those fed optimum levels. Under conditions of extreme vitamin deficiency penicillin improved the livability of chicks to four weeks of age.

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