the present study for the same or closely related strains.

Alderks	%	Present Study	%
A3-176 (Adams)[a]	1.43	Adams	1.45
A4-107-12 (Hawkeye)[b]	1.34	Hawkeye	1.36
Chief	1.48	Chief	1.44
Earlyana	1.37	Earlyana	1.31
Lincoln	1.40	Lincoln	1.34
Richland	1.37	Richland	1.37

[a] Parent strain from which Adams was selected.
[b] One of component strains of Hawkeye.

The values reported by Alderks were determined by microbiological methods by Kuiken and Lyman (*7*), while the present authors' values were obtained by chemical methods. It is evident that the two methods give comparable results and that there are some consistent varietal differences. Most of the varieties which the authors found to be superior in methionine content in the present study had not yet been developed at the time of the Alderks report.

Literature Cited

(1) Alderks, O. H., *J. Am. Oil Chemists' Soc.* **24,** 126–32 (1949).
(2) Almquist, H. J., Merritt, J. B., *Arch. Biochem. and Biophys.* **35,** 352–5 (1952).
(3) Block, R. J., Bolling, D., "Amino Acid Composition of Proteins," Chas. C Thomas, Springfield, Ill., 1951.
(4) Flodin, N. W., J. AGR. FOOD CHEM. **1,** 222–35 (1953).
(5) Horn, M. J., Jones, D. B., Blum, A. E., *J. Biol. Chem.* **166,** 313–320 (1946).
(6) Huge, W. E., *Soybean Digest* **14** (11), 36–8 (1954).
(7) Kuiken, K. A., Lyman, C. M., *J. Biol. Chem.* **177,** 29–36 (1949).
(8) Liener, I. E., Pallansch, M. J., *Ibid.*, **197,** 29–36 (1952).
(9) Lipke, H. F., Liener, I. E., J. AGR. FOOD CHEM. **2,** 410–414 (1954).
(10) Morse, W. J., Cartter, J. L., Williams, L. F., "Soybeans: Culture and Varieties," U. S. Dept. Agr. Farmers' Bull. **1520,** 6, 7 (rev. August 1949).
(11) Prescott, J. M., J. AGR. FOOD CHEM. **1,** 894–6 (1953).
(12) Rose, W. C., *Science* **86,** 298 (1937).
(13) Sheldon, V. L., Blue, W. G., Albrecht, W. A., *Plant and Soil* **3,** 361–5 (1950).
(14) Shelton, D. C., Beeson, W. M., Mertz, E. T., *J. Animal Sci.* **10,** 57–64 (1951).

Received for review July 11, 1955. Accepted January 21, 1956. Publication 264, U. S. Regional Soybean Laboratory.

MINOR ELEMENTS IN NUTRITION

Effect of Dietary Cobalt on Growing Chicks and Rats

M. J. BURNS and W. D. SALMON
Department of Animal Husbandry and Nutrition, Alabama Polytechnic Institute, Auburn, Ala.

Experiments were conducted to determine the effect of dietary cobalt on the growth of chicks and weanling rats fed diets with and without adequate choline and vitamin B_{12}. When cobalt was added at the rate of 12 mg. per kg. to a diet deficient in vitamin B_{12} and low in choline, growth of chicks increased significantly when the diet contained only 5% fat; when the diet contained 20% fat, a significant depression in growth resulted. When folacin was omitted from the diet for rats, cobalt addition failed to produce a growth response; when folacin was added, an average of 18% increase in gain and an increase in the vitamin B_{12} content of the liver resulted from addition of 12 mg. of cobalt per kg. of vitamin B_{12}–deficient diet containing 0.1% choline chloride. Cobalt did not improve growth when added to diets that contained adequate amounts of choline, methionine, or vitamin B_{12}. The results indicate that dietary cobalt is nutritionally important for nonruminants fed diets inadequate in choline and vitamin B_{12}.

VITAMIN B_{12} contains cobalt (*7*, *9*) and rumen bacteria use cobalt in the synthesis of this vitamin (*1*). These facts elucidate the role of cobalt in ruminant nutrition, which was known to be important long before the discovery of vitamin B_{12}.

Many attempts to show a role for dietary cobalt in nonruminants have been unsuccessful. However, Klosterman and others (*5*) demonstrated a 13 to 18% increase in daily gains of swine on addition of approximately 2 mg. of cobalt per kg. of ration. Jaffe (*4*) observed a beneficial effect of cobalt when added to an all-vegetable ration for breeding rats at the rate of 9 mg. per kg. This ration was as good as a ration containing vitamin B_{12} for breeding and lactation, but was inferior to a ration containing vitamin B_{12} for young, growing rats. Davis and his associates (*3*) demonstrated an increase of 8 to 21% in the growth of female chicks when 2 to 10 mg. of cobalt was added per kg. of vitamin B_{12}–deficient ration containing penicillin.

The object of this work was to determine the effect of dietary cobalt on the growth of chicks and weanling rats fed diets with and without adequate amounts of choline and vitamin B_{12} and to show a relationship of folacin to the utilization of cobalt. A preliminary report has been presented (*2*).

Experimental

Method for Chicks. One-day-old New Hampshire chicks of mixed sex were distributed among various modifications of the basal diet according to weight. From 10 to 14 chicks were used per treatment and they were kept on experiment for 4 weeks. They were

Table I. Effect of Dietary Cobalt on Chick Growth and Perosis

Additions/Kg. Basal Diet			Experiment No.[a]					
			1		2		3	
Co, Mg.	B_{12}, γ	Choline, grams	Gain, grams[b]	Perosis No.[c]	Gain, grams[b]	Perosis No.[c]	Gain, grams[b]	Perosis No.[c]
0	0	0	241 ± 8	2	163 ± 11	3	164 ± 29	0
6	0	0	...	...	176 ± 9	5	268 ± 25	0
12	0	0	220 ± 17	7	131 ± 6	4	258 ± 22	0
0	30	0	291 ± 18	2	...	...	...	...
12	30	0	232 ± 23	7	...	...	...	...
0	0	2	...	...	252 ± 12	3	...	...
12	0	2	...	...	206 ± 13	3	...	...
Least significant difference (P = 0.05)			50		30		74	

[a] 1. 20% fat in basal diet, 14 chicks/treatment. 2. 20% fat in basal diet, 13 chicks/treatment. 3. 5% fat in basal diet, 10 chicks/treatment.
[b] Average gains after 4 weeks on experiment ± standard error of mean.
[c] An animal was considered to have perosis when it was unable to stand with its joints in a normal position.

kept in heated batteries, fed fresh food and water *ad libitum*, and weighed weekly. The basal diet consisted of the following ingredients: methanol-extracted soybean meal (50% protein) 40%, lard 19, cod liver oil 1, choline chloride 0.1, cystine 0.1, calcium phosphate 2.5, calcium hydrogen phosphate monohydrate 1, magnesium sulphate heptahydrate 0.6, potassium chloride 0.62, sodium bicarbonate 0.71, manganese sulfate monohydrate 0.031, zinc carbonate 0.005, potassium iodide 0.003, ferric citrate 0.130, cupric carbonate 0.001, and sucrose to 100. The following vitamins were added: riboflavin 6 mg. per kg. of diet, thiamine 3, pyridoxine 4, calcium pantothenate 20, niacin 50, inositol 1000, α-tocopherol 50, α-tocopherol acetate 50, menadione 5, folacin 2, and biotin 0.25.

The fat content was reduced from 20% to 5% in the third experiment. When this change was made, 2.25 grams of sucrose was used to replace each gram of lard to make the two diets isocaloric. Other modifications of the basal diet are shown in Table I.

Method for Rats. Weanling rats of either the Alabama Experiment Station strain (AES) or Sprague Dawley strain (SD), 45 to 60 grams in weight, were placed in individual cages and grouped uniformly with respect to weight, litter, and sex. Four or more rats were used per treatment, and they were kept on experiment for 4 weeks or longer. Fresh food and water were supplied *ad libitum* and the animals were weighed weekly.

The basal diet used for rats was similar to the one used for chicks. It contained adequate amounts of all known vitamins except choline, vitamin B_{12}, and folacin. The basal diet was modified as shown in Tables II and III.

Determinations. A microbiological assay for vitamin B_{12} (*8*) was conducted on the livers from four rats that received the basal diet, four that received the basal diet plus cobalt, and four that received the basal diet plus vitamin B_{12} for 30 weeks. (The choline was reduced to 0.05% during the second 4 weeks of the experiment and no choline was added during the last 22 weeks.)

Hemoglobin and red cell counts were determined on about four animals from each dietary treatment.

Results

First Experiment with Chicks. The results of this experiment are shown in Table I. Diets containing 20% fat were compared with and without cobalt and vitamin B_{12}. The addition of 12 mg. per kg. of cobalt to the soybean meal diets depressed growth and increased the incidence of perosis, whether or not vitamin B_{12} was added to the diet. The presence of vitamin B_{12} accentuated the depressing effect of the cobalt, so that a significant difference resulted.

Second Experiment with Chicks. This experiment was designed to test a lower level of cobalt and to determine the effect of cobalt when added to a diet containing additional choline. When 6 mg. per kg. of cobalt was added, a slight increase in growth was obtained; 12 mg. per kg. depressed growth whether

Table II. Effect of Folacin, Cobalt, and Vitamin B_{12} on Growth of Weanling Rats

Additions/Kg. Basal Diet			Av. Gain/4 Weeks[a], Grams
Co, mg.	Folacin, mg.	B_{12}, γ	
0	0	0	87.7
0	2	0	84.8
12	0	0	82.0
12	2	0	101.5
0	2	30	131.5
12	2	30	140.3

[a] Four rats per treatment.

Table III. Effect of Vitamin B_{12} and Level of Cobalt on Growth of Weanling Rats

Group No.	Additions/Kg. Basal Diet		No. Rats/Treatment	Av. Gain/4 Weeks, Grams
	Co, mg.	B_{12}, γ		
1	0	0	24	76.0 ± 5.2
2	1	0	4	75.5
3	6	0	4	91.2
4	12	0	20	90.0 ± 4.5
5	48	0	4	88.3
6	0	30	24	120.0 ± 3.2

Least significant difference (P = 0.05) = 12.5 grams.

or not the diet contained additional choline, as was observed in the preceding experiment with vitamin B_{12}.

Third Experiment with Chicks. This experiment was designed to determine the effect of a diet that contained only 5% fat on the utilization of cobalt. The addition of cobalt (6 or 12 mg. per kg.) caused a significant increase in growth.

Experiment with Rats. These experiments were conducted to determine the effect of cobalt, folacin, and vitamin B_{12} on the growth of weanling rats when fed diets with and without adequate amounts of choline and vitamin B_{12}.

In the first experiment (Table II), neither folacin nor cobalt alone caused an increase in growth; but when both were added, the increase in growth was about half of that obtained with vitamin B_{12}.

When different levels of cobalt were tested in the presence of adequate folacin (Table III), 1 mg. per kg. was found to be without effect and there was little difference between 6, 12, and 48 mg. per kg., each giving a barely significant effect. The results of six different trials in which cobalt was added to vitamin B_{12}-deficient diets containing 0.1% choline and folacin are shown in Table III. An average growth increase of about 18% was obtained from the addition of cobalt in this series of experiments. These data were analyzed statistically and found to be significant.

Experiments were conducted to determine the effect of cobalt on growth when adequate amounts of choline or methionine were present; the results were negative. A diet containing 5% fat was also tested with rats and was found not to improve the utilization of cobalt.

The results of the vitamin B_{12} assay on the livers from rats that received diets with and without cobalt and vitamin B_{12} for 30 weeks are given in Table IV. The vitamin B_{12} content of the livers from rats that received cobalt was intermediate between those not receiving cobalt and those receiving vitamin B_{12}. The degree of cirrhosis was also significantly decreased by dietary cobalt and no cirrhosis occurred in rats that received vitamin B_{12}. Further studies are being conducted to determine the effect of dietary cobalt on chronic choline-deficient rats and chickens.

Table IV. Effect of Cobalt and Vitamin B_{12} on Cirrhosis and Vitamin B_{12} Content of Livers from Chronic Choline-Deficient rats[a]

Additions/Kg. Basal Diet		Liver B_{12}, γ/Gram	Incidence of Cirrhosis
Co, mg.	B_{12}, γ		
0	0	0.005 ± 0.0030	All severe
12	0	0.011 ± 0.0029	3 mild, 1 severe
0	30	0.020 ± 0.0040	None

[a] Four rats per treatment.

Discussion

The results indicate that the function of cobalt in the rat and chick is similar to that in ruminants, but that the chick and rat are much less efficient in the utilization of cobalt and the synthesis of vitamin B_{12}. Cattle and sheep require less than 0.1 mg. of cobalt per kg. of dry ration for the synthesis of ample vitamin B_{12}, while nonruminants require more than 10 times this amount to produce a fraction of the vitamin B_{12} required.

The effect of dietary fat on the utilization of cobalt in the chick is probably related to action of the microflora of the intestinal tract. A low-fat diet was used in the previous experiments with cobalt and a stimulation in growth usually was produced. No depression in growth has been reported unless toxic levels were fed.

The variations in the amount of growth obtained in the different chick experiments was large; this may have been the result of difference in vitamin B_{12} stores in the chicks (*6*).

When folacin was omitted from the diet for rats, cobalt addition failed to produce a growth response; however, folacin alone was without effect on growth. Folacin and cobalt probably acted together as a stimulant for the bacterial synthesis of vitamin B_{12}.

It is apparent that under certain conditions cobalt may improve the nutrition of nonruminants fed diets deficient in vitamin B_{12}; however, the growth obtained with cobalt was definitely inferior to that obtained with vitamin B_{12}.

Summary

Under certain conditions, the growth of chicks and weanling rats was moderately increased by dietary cobalt, but not so much as with vitamin B_{12}.

The fat content of the diet definitely affected the utilization of cobalt in the chick but not in the rat. In the experiment with chicks in which cobalt was added to a diet that contained 5% fat, there was a significant increase in growth; in trials with a 20% fat diet, a significant depression in growth resulted. This effect of the fat content of the diet is presumably due to its effect on the microbial synthesis of vitamin B_{12}.

When folacin was omitted from the diet for rats, cobalt addition failed to produce a growth response; however, when folacin was added, an average of about 18% increase in gain and an increase in the vitamin B_{12} content of the liver resulted when 12 mg. per kg. of cobalt was added to a vitamin B_{12}-deficient ration that contained 0.1% choline chloride.

Cobalt did not improve growth when added to diets that contained adequate amounts of choline, methionine, or vitamin B_{12}.

Acknowledgment

The folacin used in these studies was kindly furnished by Lederle Laboratories, the methionine by the Dow Chemical Co., the inositol by the A. E. Staley Manufacturing Co., and the other vitamins by Merck & Co., Inc.

Literature Cited

(1) Abelson, P. H., Darby, H. H., *Science* **110,** 566 (1948).
(2) Burns, M. J., Salmon, W. D., *Federation Proc.* **13,** 452 (1954).
(3) Davis, R. L., Briggs, G. M., Sloan, H. J., *Proc. Soc. Exptl. Biol. Med.* **82,** 175–7 (1953).
(4) Jaffe, W. G., *Arch. venezolanos nutric.* **2,** 19–25 (1951); *Nutrition Rev.* **10,** 238–9 (1952).
(5) Klosterman, E. W., Dinusson, W. E., Lasley, E. L., Buchanon, M. L., *Science* **112,** 168–9 (1950).
(6) O'Neil, J. B., *Poultry Sci.* **31,** 764–8 (1952).
(7) Rickes, E. K., Brink, N. G., Koniuszy, F. R., Wood, T. R., Folkers, K., *Science* **108,** 134 (1948).
(8) Scheid, H. E., Andrews, M. M., Schweigert, B. S., *Proc. Soc. Exptl. Biol. Med.* **78,** 558–60 (1951).
(9) Smith, E. L., *Nature* **162,** 144 (1948).

Received for review October 21, 1955. Accepted January 8, 1956. Published with approval of the Director, Alabama Agricultural Experiment Station. Investigation supported in part by research grants from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council, and from the National Cancer Institute, of the National Institutes of Health Service.