CHOLINE IN CHICK RATIONS

Thesis for the Degree of M. S.
MICHIGAN STATE COLLEGE

Albert Wade Brant

1942

CHOLINE IN CHICK RATIONS

by

Albert Wade Brant

A THESIS

Submitted to the Graduate School of Michigan State College of Agriculture and Applied Science in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

Department of Poultry Husbandry

1942

ACKNOWLEDGEMENT

The author is indebted to Dr. P. J. Schaible, Research Associate in Chemistry; J. A. Davidson, Associate Professor of Poultry Husbandry; and C. G. Card, Professor of Poultry Husbandry, Michigan State College. Their helpful guidance and cooperative efforts are greatly appreciated.

TABLE OF CONTENTS

INTRODUCTION

The existence of choline in the living organism has been recognized for almost a century. Its importance as a dietary essential, however, was demonstrated only within the last ten years. At the present time there is evidence that choline functions in at least three ways; to stimulate the formation of phospholipids, to make possible the production of acetylcholine, or to supply labile methyl groups.

The experimental work reported herein has been along two distinct lines: (1) an attempt to produce choline deficiency symptoms in the chick, (2) the adaption of a chemical procedure to the analysis of choline in poultry feedstuffs.

LITERATURE REVIEW*

It had been known for some years that depancreatized dogs developed fatty livers. However, it was not possible to study this phenomonen properly until after the discovery of insulin. Allen and co-workers (1924) and Fisher (1924) reported that depancreatized dogs, even though maintained with insulin, developed fatty livers after some months. A nephrosis was also produced, secondary to the liver damage. The addition of fresh beef pancreas or crude egg-yolk "lecithin" (Hershey, 1930; and Hershey and Soskin, 1931) to the diet prevented the development of these pathological conditions.

Best et al (1932) produced dietary fatty livers in normal rats and found lecithin to be lipotropically active. Further investigation by Best, Hershey, and Huntsman (1932) and by Best and Hershey (1932) revealed that the component of the lecithin molecule responsible was the nitrogenous base choline. Later Best, Ferguson and Hershey (1933) found that choline would also prevent fatty livers in depancreatized dogs. Fatty livers, preventable by choline, have also been developed in mice (Best et al, 1932) and chickens (Abbott and DeMasters, 1940).

The condition referred to as "fatty" liver is characterized by an accumulation of large amounts of neutral fat in the liver. As a result the organ becomes light in color,

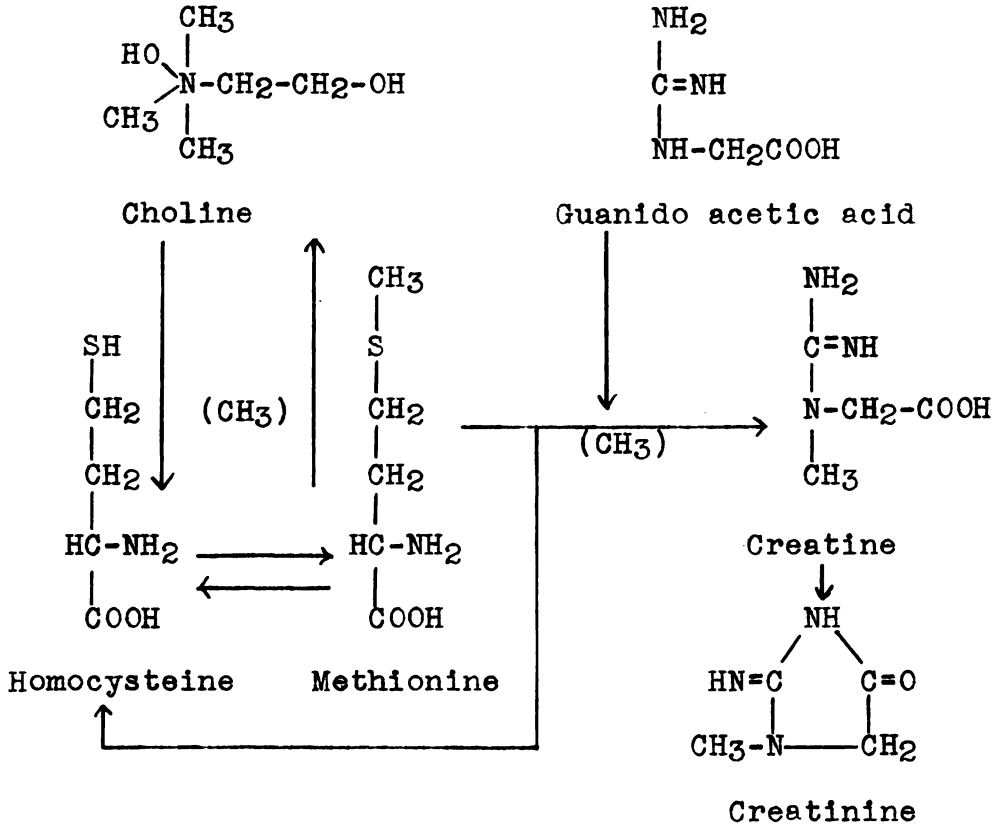
*The literature on this subject has also been reviewed by Best and Ridout (1939), Griffith (1941), McHenry (1941), Best (1941).

soft, and much enlarged. Diets high in fat or sucrose, but containing no added choline, result in "fat" type fatty liver; those containing considerable cholesterol produce the "cholesterol" type (Best et al, 1934; Channon and Wilkinson, 1934). A large quantity of choline is necessary to prevent or cure the "cholesterol" fatty liver. The effect of choline is on the glyceride fraction of the fat in the liver (Best Ridout, 1939). Choline is considered lipotropic because it promotes the formation of phospholipids (McHenry, 1941; Welch, 1936; Perlman and Chaikoff, 1939).

Choline, however, is only one of many lipotropic compounds (Moyer and du Vigneaud, 1942). It is necessary that cystine (Beeston and Channon, 1936) and thiamin (McHenry, 1937; Best and Ridout, 1938) be present in a low-choline diet to produce the greatest concentration of fat in the liver.

Another important biological function of choline is its ability to afford a labile methyl supply to the organism (du-Vigneaud, 1941). Homocystine will support growth of rats fed a methionine-free diet only in the presence of choline (du - Vigneaud et al, 1939, 1939a; Welch, 1941; Klose and Almquist, 1941). The transfer of methyl groups from choline to homocystine was theorized since triethylcholine does not support growth in the presence of homocystine (du Vigneaud et al, 1939, 1939a). Labelling the methyl groups of choline and methionine with deuterium, these methyl groups were traced throughout the body of the rat.

The results of these methylation studies may be represented schematically as follows (du Vigneaud, 1941).



By using N_{15} in aminoethanol it has been shown that this compound may be a precursor of choline *in vivo* if labile methyl groups are available (Stetten, 1941, 1941a).

Rats, 21 to 26 days of age, maintained on a diet adequate in all respects except choline developed severe hemorrhagic renal degeneration within 10 days (Griffith and Wade, 1939, 1940; Griffith, 1940). The renal hemorrhage disappeared later but renal pathology persisted. Hemorrhagic degeneration was prevented by choline, methionine and betaine (Griffith and Mulford, 1941). By restricting food intake Griffith (1941) prevented signs of choline deficiency on a diet that produced severe deficiency symptoms when fed *ad libitum*.

Low-choline diets also produced deficient vagus function in rats and a deficient formation of acetylcholine at the nerve endings (Solandt and Best, 1939). The administration of choline depresses hematopoiesis induced in dogs by cobalt (Davis, 1939). It prevents papillomatous lesions in the fore-stomach of rats on a diet containing 89% white flour (Sharpless, 1940). It is indispensable for lactation in adult rats and for growth and prevention of paralysis in suckling rats (Sure, 1940). Small amounts of lecithin prevents the occurrence of experimental arteriosclerosis caused by high-cholesterol feeding of rabbits (Downs, 1935).

Choline and lecithin are concerned in maintaining acid-base equilibria and excretion of phosphate by the kidney (Bloor, 1940). Sharpe (1923) suggested that choline is used in the elaboration of guanidine in the hens' egg during incubation since choline decreased proportionately as guanidine increased. Choline chloride, injected subcutaneously in rabbits and dogs, caused hyperglycemia but no glycosuria in rabbits with no effect on dogs (Underhill and Petrelli, 1929).

The earliest work indicating that choline was an accessory food factor in the diet of the domestic fowl was presented by Abbott and DeMasters (1940) who employed a basal diet consisting of skimmilk and alcohol-extracted rice plus vitamin supplements. Rhode Island Red pullets, when placed on this basal diet at 5 to 6 months of age, laid an average number of 16 eggs; mortality was high. When birds were given one gram of lecithin per day in addition, egg production was improved and mortality decreased. Chicks reared from 2 weeks

of age on the basal diet matured very slowly and many did not come into production. Autopsy showed they had large fatty livers and many aborted yolks. Additions of 25 mg., 50 mg., and 75 mg. of choline were given daily to three other groups of birds on the basal diet. The last group produced the most eggs, aborted the fewest yolks, and showed apparantly normal livers. The 25 mg. and 50 mg. improved the basal diet, dut not to the same extent. Males reared on the lowest amount of choline showed normal testes and produced live and numerous sperm as contrasted to the underdeveloped testes and fewer sperm produced in cockerels on the basal diet alone.

Additions of choline caused a marked reduction in the fatty acid content of the livers. The smallest quantity of fatty acids was found in the livers of birds fed the 75 mg. of choline per day. It was also noted that the concentration of fatty acids in the liver of males was lower than that in females, on the same diet, with or without choline.

Jukes (1940) noted that choline stimulated the growth of chicks on a simplified diet. In earlier experiments, he was unable to prevent perosis in poults receiving as high as 1440 parts per million of manganese(Jukes, 1939). The diet was composed of yellow corn meal, dried skimmilk, washed casein, and alfalfa meal, plus vitamin and mineral supplements. Since Hogan et al (1940) had reported that liver contained an alcohol-soluble organic factor which prevented a perosis, an examination of the known B-vitamins was prompted. When a mixture of vitamins including thiamin, riboflavin, nicotinic

acid, pyridoxine, and pantothenic acid plus choline chloride was added to the basal diet (Jukes 1939, 1940a, 1940b), no perosis occurred. Testing the various ingredients separately he found that choline was the active agent. Using a more simplified diet consisting of glucose ("cerelose"), washed casein, dried yeast, and soy bean oil plus vitamin and mineral supplements, Jukes (1940a, 1940b) confirmed his earlier findings. Betaine accelerated the occurrence of perosis and arsenocholine exhibited limited anti-perotic activity; neither biotin nor inositol gave any preventive action. Table I summarizes the compounds known at present to have antiperotic and growth-promoting activity in poultry.

Table I

Compounds similar to choline which have been experimentally tried in the diet of the fowl. Taken from a compilation by Moyer and du Vigneaud (1942).

Compound	Prevention of Perosis	Fowl Growth
Aminoethanol	-	-
Arsenocholine	+	+
Betaine	-	-
Betaine aldehyde	-	±
Choline	+	+
Creatine	-	±
Diethylmethylhydroxyethylammonium chloride	+	-
a,a-dimethylcholine	-	-
Dimethylethylhydroxyethylammonium chloride	+	+
Lecithin	+	+

Table I (Cont.)

Compound	Prevention of Perosis	Fowl Growth
Methionine	-	-
B-methylcholine	±	-
Triethylcholine	-	-

Jukes (1940a, 1940b) reported that choline stimulated growth in poults and that .1% of choline added to the basal ration was sufficient to promote growth but .3% was required to prevent perosis. Jukes (1941) tested various feedstuffs to determine their anti-perotic activity, concerned with choline content, with the results as shown in Table II.

Table II
Anti-perotic Activity (Choline Content) of Various Feedstuffs Tested by Jukes

Material	Level Fed %		Anti-perotic activity of level fed	Comparative rating
Yellow corn	65	a*	None	Very poor
	50	b**	None	
Wheat	65	a	Very slight	Poor
	50	b	Slight	
Barley	65	a	Fair	Fair
	40	b	Fair	
Oats	50	b	None	Very poor
Rice bran	25	a	Fair	Poor
	20	b	None	
Wheat bran	25	a	Very slight	Poor
Soybean meal	25	a	Good	Good
	40	b	Very good	
Sardine meal	17	a	Fair	Good
	40	b	Good	
Alfalfa meal	15	a	Very slight	Poor
Cottonseed meal	40	b	Fair	Fair
Dried pork liver	5	a	Fair	Very good
Dried skimmilk	15	a	None	Very poor
Fish oil blend	2	a	None	Very poor
Cane molasses	10	a	Slight	Poor

*a. Tested (Jukes, 1940b) with a basal diet consisting principally of yellow corn, skimmilk, and casein and containing 0.1 percent of added $MnSO_4$.
**b. Tested, (Jukes, 1941) on a basal diet consisting principally of glucose, casein, and yeast, and containing 0.05 percent of added $MnSO_4$.

It was observed (Jukes, 1941) that wheat and especially corn increased the severity of perosis. Lecithin prepared from egg yolk had an effect similar to choline, but methionine, inositol, and creatine were ineffective. The anti-perotic activity was removed from soy bean meal and meat scrap by extraction with boiling alcohol (Jukes, 1941b).

Using a diet similar to diet b, Table II, Jukes (1940b) produced perosis also in White Leghorn chicks. Choline prevented perosis and stimulated growth on this ration. The "rice factor"(Almquist et al, 1940) was supplied by adding gelatin and sodium alginate to the basal diet since if it were not included, no perosis resulted. Jukes (1941a) used gum arabic to supply the carbohydrate component of the "rice factor" and found creatine just as effective as gelatin. Since Almquist and Mecchi (1940) had shown that a dietary deficiency of creatine or its precursors resulted in muscular dystrophy, it was speculated that in the absence of creatine, muscular tension was so reduced that the hock joint remained intact.

McElroy and Jukes (1940) have observed perosis accompanying the egg-white syndrome in chicks. Choline was ineffective in preventing this condition but biotin, when injected, completely protected chicks (Jukes and Bird, 1942).

Hogan et al (1940) reported a perosis occurring on a simplified diet in the presence of an adequate supply of manganese. The factor, provisionally termed B_p, was present in the hot alcohol extract of dried liver but not in the residue nor the hot water extract. Choline prevented the perotic conditions developed (Hogan et al, 1941). The ration employed consisted

of casein, corn starch, cane molasses, lard and cellulose plus vitamin and mineral supplements. Hogan et al (1941) describe the following conditions as indicative of this type of perosis: (1) the tendon of Achilles is out of position, and the tarsometatarsal bone is rotated in such a way as to point the toes to one side; (2) the tibial-tarsometatarsal joint is greatly enlarged; (3) the tarsometatarsal bone is markedly short and thick; (4) because of curvature of the tarsometatarsal bone the chicks are bow legged. Curvature of the tibia is less pronounced; (5) the long bones are all shorter and thicker than is normal, but the deformity would pass unnoticed on casual examination. The fifth set of symptoms was found to be the most common. In addition, choline deficiency causes a "frayed" condition of the feathers (Warren, 1938; Jukes, 1941b).

In studying growth factors, Hegsted et al (1941) encountered a perosis in chicks fed a ration of dextrin, casein, soy bean oil, cartilage, and yeast plus vitamin and mineral supplements. The addition of .05% choline to the ration was sufficient to prevent perosis but .2% choline gave the maximum growth. These chicks did not show fatty livers nor have a low bone phosphatase (as reported for manganese-deficient chicks by Wiese et al, 1939).

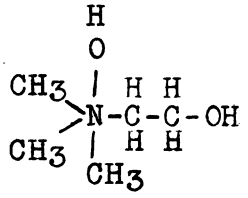
Norris (1941) reported that chicks fed a simplified diet deficient in choline would develop perosis. However, the work at Cornell indicates that there is another organic antiperotic factor found in yeast. This conclusion was based on

the finding that all the anti-perotic activity of brewers' yeast could not be due to its very low choline content alone.

Record and Bethke (1942) also reported that choline prevents perosis in chicks fed a simplified diet of corn meal, peanut meal, casein, cane molasses, and soy bean oil plus vitamin and mineral supplements. Growth was stimulated by choline; soybean lecithin was found effective to the extent of its choline content. The feathering condition of the chicks was improved by added choline. It was found that .15% choline in the ration gave maximum effects.

CHEMICAL DETERMINATION OF CHOLINE

Choline is an organic homologue of ammonium hydroxide having the following structural formula:



Choline has been determined by its oxidation to trimethylamine, the formation of an insoluble complex with iodine, the isolation of the chloroplatinate or chloroaurate, and its precipitation as the reineckate. In the latter method choline is separated from the other reineckates by virtue of its insolubility in water and ethyl alcohol. When dissolved in acetone it forms a red solution which can be measured colorimetrically. The procedure finally adopted was that reported by Jacobi et al (1941), modified as follows:

About 2 grams of finely ground sample is weighed and transferred to a dried Gooch crucible with an asbestos mat. The crucible with the sample is placed in a Bailey-Walker extraction flask containing 30 ml. of 99% methyl alcohol;

a condensor is placed in the neck of the flask and the material is extracted overnight on an electric hot plate. The alcohol containing the extract is transferred to a 100 ml. evaporating dish, covered with a watch glass, and the extract evaporated to near dryness. Care must be exercised to prevent the material from becoming completely dry. Fifteen ml. of saturated $Ba(OH)_2$ are added to the evaporating dish, the watch glass replaced and the mixture hydrolyzed for 2 hours at 80° C. on a steam bath. The excess $Ba(OH)_2$ is then neutralized with glacial acetic acid using phenolphthalein as indicator. The residue is filtered by suction on hardened filter paper in a small Büchner funnel and washed with a few ml. of distilled HOH. The filtrate is directed into a 50 ml. centrifuge tube and 5 ml. of a freshly prepared 2% solution of ammonium reineckate in methanol is added.

The centrifuge tubes are covered with small watch glasses and placed overnight in the refrigerator to insure complete precipitation. The precipitate is filtered on to hardened filter paper in a small Büchner funnel and washed with 15 ml. of 95% ethyl alcohol at 10° C. or less. A 25 ml. volumetric flask is then placed in the suction flask to receive the colored solution. A few ml. of acetone is then poured into the funnel. After most of the choline reineckate is in solution, suction is applied and the filter paper washed free of the red precipitate by directing a stream of acetone from a wash bottle upon it. The volume of the acetone solution in the volumetric flask is brought to exactly 25 ml. and the color intensity measured in a photelometer (Sheard-Sanford

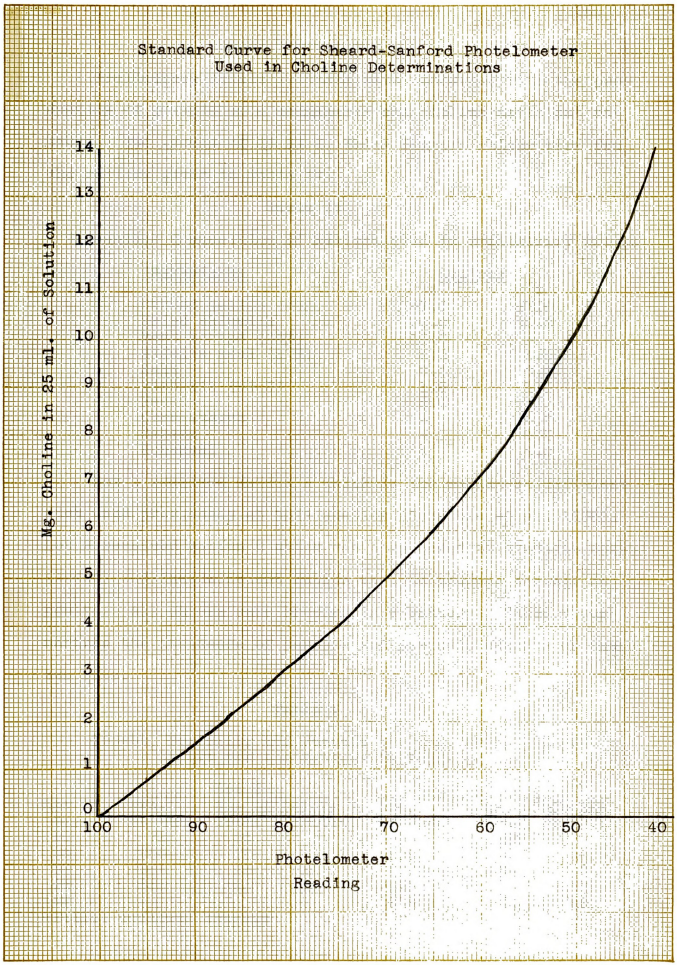
photelometer, filters #352 and #401). In a similar manner the reference curve on the next page was prepared by using known quantities of choline. Table III lists a number of poultry feedstuffs analyzed for choline.

Table III

The Choline Content of Certain Poultry Feedstuffs

Material Analyzed	Mg. Choline per Gm.
Ground yellow corn	0.40
Ground whole oats	0.45
Ground wheat	0.70
Wheat bran	0.85
Flour middlings	0.95
Dehydrated alfalfa meal	1.00
Dehydrated leafy alfalfa meal	1.10
Soy bean oil meal (Expeller)	2.10
Dried skimmilk	0.85
Dried buttermilk	0.75
Sardine meal	2.00
White fish meal	2.55
Meat Scraps	1.50
Yeast*	2.00
Blood meal	1.35
Tankage	2.00
Corn gluten meal	0.00

*Fleischmann's, type 2019


Standard Curve for Sheard-Sanford Photelometer
Used in Choline Determinations
Mg. Choline in 25 ml. of Solution
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
100
90
80
70
60
50
40
Photelometer
Reading

CHICK EXPERIMENTS

Preliminary trials -- These were concerned with the production of perosis upon manganese- and choline-deficient rations. Two groups of 25 S. C. White Leghorn chicks were fed the rations given in Table IV.

Table IV

Percentage Composition of Rations Used in Preliminary Trials

Ingredients	Rations	
	A	B
Ground yellow corn	67.0	67.0
Dried skimmilk	10.0	10.0
Meat scraps (55%)	10.0	-
Alfalfa meal (17%)	6.5	1.0
Salt	0.5	0.5
Fish oil (85 D)	1.0	1.0
Yeast	5.0	-
Corn starch	-	5.5
Casein	-	9.0
Bonemeal	-	6.0

Lot 1 received Ration A which is the manganese-deficient diet used previously at this station (Schaible et al, 1938). Lot 2 received Ration B which was compounded to simulate Jukes' (1940a) choline-deficient ration used to produce perosis in poults. All chicks were brooded in a battery. Table V summarizes the incidence of perosis and periodic weights of these two lots.

Table V

Growth, Mortality and Perosis in S. C. White Leghorn Chicks Fed the Rations in Table IV

Age in days	Lot 1			Lot 2		
	Ave. Wt.-Gms*	No. of Chicks		Ave. Wt.-Gms*	No. of Chicks	
		Total	Perotic		Total	Perotic
10	71	25	0	59	25	0
24	156	23	0	108	23	0
38	264	23	12	177	21	9
45	330	23	13	190	21	7
52				238	20	16

*Pullet cockerel average

Ration A produced a considerably faster growth rate than did Ration B. The incidence of perosis suddenly increased as the chicks reached an average weight of 200 grams. This indicates that the weight supported by the "hock" joint has a direct bearing on its ability to remain in a normal state.

Three lots of 18 Barred Plymouth Rock chicks were treated in a similar manner. Lot 3 received Ration A, lot 4 received Ration B, and lot 5 received Ration B plus 50 p.p.m. of manganese. Table VI gives the periodic weights of these birds and records the incidence of perosis.

Table VI

Growth, Mortality, and Perosis in Barred Plymouth Rocks Fed the Rations in Table IV

	Lot 1			Lot 2			Lot 3		
Age in Days	Ave. Wt. Gms.	No. chicks Total	No. chicks Perotic	Ave. Wt. Gms.	No. chicks Total	No. chicks Perotic	Ave. Wt. Gms.	No. chicks Total	No. chicks Perotic
1	38	18	0	38	18	0	39	18	0
8	63	18	0	59	16	0	63	18	0
15	94	18	0	79	16	0	96	18	0
22	143	18	8	103	15	0	143	18	1*
29	203	18	14	143	14	5	209	18	1*
36	270	17	15	187	13	7	292	17	1*
43	342	17	16	230	12	10	340	16	0

*Very mild cases

As in the trial with Leghorns, Ration B did not produce nearly as much growth as Ration A. However, the addition of 50 p.p.m. of manganese to Ration B resulted in growth as good as that produced by Ration A. Ration B, therefore, was lacking in some unknown growth requirement since Ration A was known to be deficient in manganese. Ration B did not produce perosis from choline deficiency since the added manganese gave practically complete protection. Inasmuch as perosis was not obtained by choline deficiency, it was decided to follow exactly Jukes' rations and technic.

Experiment I

Six lots of day-old Barred Plymouth Rock chicks were placed in battery brooders. Fifteen chicks were included in each lot. The composition of the basal diet and of the salt mixture are given in Table VII.

Table VII

The Basal Diet and Salt Mixture of Jukes Used in Experiment I

Basal Diet		Salt Mixture	
Ingredient	Parts	Ingredient	Parts
Cerelose	53.0	Bone ash	38.0
Vit.-free casein	18.0	$CaCO_3$	20.0
Yeast*	6.0	Iodized NaCl	22.0
Gum arabic	5.0	$MgSO_4$	3.9
Soy bean oil	5.0	KH_2PO_4	7.5
Salt mixture	5.0	Ferric citrate	3.5
Fish oil (400 D)	0.3	$MnSO_4$	2.0
		$CuCO_3$	0.1
*Anheuser-Busch, Strain G		ZnO	0.1

Lots 1 and 2 received no gelatin but lot 2 received added choline chloride. The remaining four lots received gelatin plus different amounts of added choline chloride. Table VIII records the kinds and amounts of supplements added to the basal ration for each lot as well as the weights at weekly intervals. No perosis occurred.

Table VIII

Growth of Barred Plymouth Rock Chicks Fed the Basal Ration in Table VII Plus Certain Supplements

Age in days	Average Weight in Grams					
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
1	33	32	33	33	33	33
8	44	44	39	50	47	48
15	53	54	55	71	51	51
22	66	70	68	95	89	87
29	88	82	90	123	111	102
36	107	122	106	151	126	116
43	131*	150*	132*	182*	162*	169*

Lot 1 - Basal Ration
" 2 - Basal Ration + 0.1% Choline Chloride
" 3 - Basal Ration + 8% Gelatin
" 4 - Basal Ration + 8% Gelatin + 0.1% Choline Chloride
" 5 - Basal Ration + 8% Gelatin + 0.2% Choline Chloride
" 6 - Basal Ration + 8% Gelatin + 0.3% Choline Chloride
*Pullet cockerel average

Barred Plymouth Rock chicks did not develop perosis upon any of these Rations. The chicks, however, did not reach an average weight of 200 grams in any case. It was noted in the preliminary trials that, upon reaching this weight, the incidence of perosis was markedly increased. Added choline, with or without gelatin, produced some increase in weight. There was some improvement in feathering and feather quality resulting from added choline.

Experiments II and III

In Experiment II, lots of 15 S. C. White Leghorn chicks were fed the same diets fed lots 3 and 4 of Experiment I. Mortality was so high no quantitative data are given. No explanation for this mortality was apparent. No perosis was observed.

Upon the recommendation of Jukes (1942a) 30 parts of corn meal were substituted for an equivalent amount of cerelose in the basal ration given in Table VII. This modified basal ration was fed to two lots of 15 S. C. White Leghorn chicks. One lot received the basal ration and the other 0.1% of added choline chloride. Mortality of unknown origin was again so high that any interpretation of the results would be questionable and therefore data is not given. However, the addition of corn meal tended to increase the growth rate slightly. A few cases of perosis were noted in the group receiving no choline.

Experiment IV

Since mortality terminated Experiments II and III before the chicks were old enough to show a definite reaction to the

ration, it was decided to repeat the Experiment again. Two lots of 20 S. C. White Leghorn chicks were placed in batteries and fed the modified basal ration of Jukes'. However, neither lot received any added choline. Lot 1 received the basal ration and lot 2 received the basal ration plus certain synthetic vitamins. The added vitamins in mg./kg. were thiamin, 2; riboflavin, 2; pyridoxine, 2; alpha tocopherol, 80; 2 methyl-1, 4-napthoquinone, 10. Table IX records the perosis and weekly weights of the birds.

Table IX

Growth, Mortality, and Perosis in S. C.
White Leghorn Chicks Fed Jukes' Modified Ration

	Lot 1			Lot 2		
Age in days	Average Wt.in gm.	Total No.	Number Perotic	Average Wt.in gm.	Total No.	Number Perotic
1	35	20	0	36	20	0
9	57	19	0	60	19	0
16	73	15	1	78	14	2
23	110	14	4	113	14	4
30	153*	14	6	159*	14	11

*Pullet cockerel average

The added vitamins did not produce any increase in growth; there was, however, an increase in the incidence of perosis. The incidence of perosis and the growth rate were much lower than that reported by Jukes using the basal ration containing no corn meal.

Experiment V

The slow chick growth and infrequent occurrence of perosis on the rations employed by Jukes prompted Experiment V. The ration used was similar to that reported by Hegsted et al (1941). The composition of this ration and of the salt mixture are given in Table X.

The cartilage was prepared by removing the cartilagenous tips of the scapulae from beef cattle and grinding them as fine as possible in an ordinary meat grinder. This material was dried overnight at about 45° C. over steam pipes. The dried material was then ground and incorporated in the ration.

Table X

Hegsted's Basal Ration and Salt Mixture

Basal Ration		Salt Mixture	
Ingredient	Parts	Ingredient	Parts
Cerelose	46.7	$CaCO_3$	30.0
Vit.-free casein	18.0	KH_2PO_4	32.0
Salt mixture	5.0	$CaHPO_4 \cdot 2H_2O$	7.6
Soy bean oil	5.0	$MgSO_4 \cdot 7H_2O$	10.0
Cartilage	15.0	NaCl	17.0
Yeast*	10.0	$Fe(C_6H_5O_7) \cdot 6H_2O$	2.7
Fish oil (400 D)	0.3	KI	0.1
Thiamine mg/kg	2.0	$MnSO_4 \cdot 4H_2O$	0.5
Riboflavin mg/kg	2.0	$ZnCl_2$	0.04
Pyridoxine mg/kg	2.0	$CuSO_4 \cdot 5H_2O$	0.06

*Anheuser-Busch, Strain G

Two lots of 20 S. C. White Leghorn chicks were placed in a battery and fed Hegsted's ration for 30 days. Lot 1 received the basal ration unsupplemented; lot 2 received the basal ration plus 0.1% of added choline chloride. The weekly weights and incidence of perosis in these two lots are recorded in Table XI.

Table XI

Growth, Mortality, and Perosis in S. C. White Leghorn Chicks Fed Hegsted's Ration

	Lot 1			Lot 2		
Age in days	Average Wt.in gm.	Total No.	Number Perotic	Average Wt.in gm.	Total No.	Number Perotic
1	36	20	0	36	20	0
9	61	18	0	61	20	0
16	91	14	6	121	13	0
23	143	14	6	185	13	0
30	191*	14	11	261*	13	1**

*Pullet cockerel average

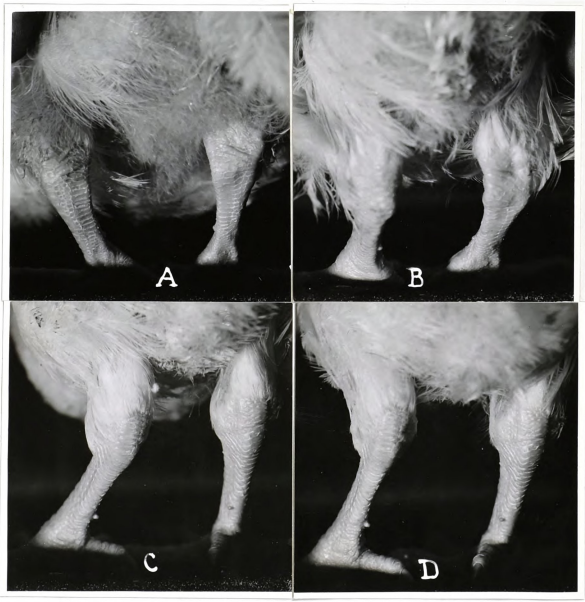
**Very mild case

This ration produced a very marked perosis preventable by choline. The percentage of perotic birds was greater and the growth rate of the chicks fed Hegsted's ration far exceeded that of any chicks on Jukes' rations. In addition to completely preventing perosis, 0.1% of added choline gave a very marked increase in growth.

In Figure I are the photographs of two typically perotic chicks and two normal ones. The perotic chicks received Hegsted's basal ration, whereas the normal chicks received 0.1% of choline in addition.

Figure I

Perotic Chicks Developed on Hegsted's Choline-deficient Ration and Normal Chicks Produced When Choline Was Added



A & B are typical cases of severe perosis.
C & D are normal chicks with sturdy legs.

DISCUSSION

The preliminary Trials confirmed Jukes' findings that chicks would not develop perosis when fed the choline-deficient ration which Jukes found produced perosis in poults. The growth of Barred Plymouth Rock chicks in Experiment I was much lower than that reported by Jukes (1940b) for S. C. White Leghorn chicks fed the same rations. On normal rations, Barred Plymouth Rock chicks weigh more than S. C. White Leghorn chicks of the same age. No reason for the difference noted was apparent. However, the slow growth of the chicks in Experiment I may account for the lack of perosis, since perosis does not seem to occur as frequently in underdeveloped chicks.

The high mortality in Experiments II and III did not appear to result from the rations fed, since in subsequent identical trials livability was very good. Although conclusive results were not obtained, the substitution of corn meal for part of the cerelose stimulated growth and increased the incidence of perosis.

The addition of the synthetic vitamins to the modified diet of Jukes did not materially accelerate growth, but the incidence of perosis was increased. Two possible explanations may apply here. One or more of the added vitamins may aggravate choline deficiency or the added vitamins might result in a slight increase in metabolic rate resulting in more acute choline-deficiency symptoms. The growth of the S. C. White Leghorns in Experiment IV compares very closely with that obtained by Jukes (1940b, 1941a, 1941b) upon his

ration containing no corn meal or added vitamins. The data does not explain why the chicks in these Trials grew at a slower rate than that reported by Jukes for chicks fed the same or even more simplified rations.

It was difficult to record accurately the conditions of feathers and feathering encountered during these Trials. However, an improvement in feather quality and condition of feathering appeared to result from choline added to the deficient ration of Jukes'. The feather quality and feathering condition of choline-supplemented birds was still much below that of birds on ordinary rations. However, no difference was noted between the two groups in Experiment V. Cartilage may also contain a factor involved in feathering of chicks. Such a conclusion is purely speculative, however, since Record and Bethke (1942) reported an improvement in feathering obtained by adding choline to a diet much different from any used in these Trials.

The growth of chicks in Experiment V slightly exceeded that reported by Hegsted et al (1941) for S. C. White Leghorns and was far greater than any reported by Jukes for a like period of time. The very marked increase in growth of chicks fed Hegsted's ration indicates very strongly that cartilage contains a growth factor or factors other than choline not present in Jukes' ration. Hegsted's ration was further improved by added choline and perosis was completely prevented by this addition. Cartilage must contain the factor or factors because Hegsted's ration differed but little from Jukes' ration in all other respects.

On the basis of the choline content of the various poultry feedstuffs analyzed, it appears that poultry rations composed of naturally occurring materials will contain a large quantity of this factor. The chick starting mash developed at the Michigan Agricultural Experiment Station (Spartan Chick Starter) contains approximately 0.1% of choline. This quantity was more than sufficient in all of the experimental rations. The most potent sources of choline in poultry rations are soy bean oil meal, fish meals, and meat scraps.

SUMMARY

The chemical method for determining choline reported by Jacobi et al (1941) has been modified in order to determine choline in poultry feedstuffs. The quantity of choline in certain poultry feedstuffs is reported. On the basis of the analyses presented, the Spartan Chick Starter was found to contain an ample supply of naturally occurring choline.

A choline-deficient ration similar to that used by Jukes to produce perosis in poults was found to be deficient in undetermined growth requirements of chicks. Cartilage was found to improve this ration, possibly because it contains growth factors not present in gelatin or gum arabic.

Choline improved the growth of chicks fed the simplified rations reported by Jukes and by Hegsted. Neither Barred Plymouth Rock nor S. C. White Leghorn chicks developed perosis on the diets reported by Jukes. S. C. White Leghorn chicks did, however, develop perosis when this diet was modified to contain 30 parts of corn meal. Added choline improved the feathering condition of chicks fed Jukes' rations, but no improvement was noted in the chicks fed Hegsted's ration.

BIBLIOGRAPHY

Abbott, C. U. and O. D. DeMasters, 1940. Choline in the diet of chickens. J. Nutrition 19:47.

Allan, F. N., D. J. Bowie, J. J. R. Macleod, and W. L. Robinson, 1924. Behaviour of depancreatized dogs kept alive with insulin. Brit. J. Exp. Path. 5:75.

Almquist, H. J. and E. Mecchi, 1940. Identification of the rice factor. The essential nature of the glycine component. J. Biol. Chem. 135:355.

Almquist, H. J., E. Mecchi, E. L. R. Stokstad, and P. D. V. Manning, 1940. Identification of the rice factor. The carbohydrate component. J. Biol. Chem. 134:465.

Beeston, A. W. and H. J. Channon, 1936. Cystine and the dietary production of fatty livers. Biochem. J. 30:280.

Best, C. H., 1941. The significance of choline as a dietary factor. Science 94:523.

Best, C. H., H. J. Channon, and J. H. Ridout, 1934. Choline and the dietary production of fatty livers. J. Physiol. 81:409.

Best, C. H., G. C. Ferguson, and J. M. Hershey, 1933. Choline and liver fat in diabetic dogs. J. Physiol. 79:94.

Best, C. H. and J. M. Hershey, 1932. Further observations on the effects of some component of crude lecithin on depancreatized animals. J. Physiol. 75:49.

Best, C. H., J. M. Hershey, and M. E. Huntsman, 1932. The effect of lecithine on fat deposition in the liver of the normal rat. J. Physiol. 75:56.

Best, C. H. and J. H. Ridout, 1938. Undernutrition and liver fat. J. Physiol. 94:47.

Best, C. H. and J. H. Ridout, 1939. Choline as a dietary factor. Ann. Rev. Biochem. VIII:349.

Bloor, W. R., 1940. Fat transport in the animal body. Physiol. Rev. 19:557.

Channon, H. J. and H. Wilkinson, 1934. Cholesterol feeding in the production of dietary fatty livers. Biochem. J. 28:206.

Davis, J. E., 1939. Depression of experimental polycythemias by choline hydrochloride or liver administration. Am. J. Physiol. 127:322.

Downs, W. G. Jr., 1935. Lecithin and the cure of arteriosclerosis in rabbits. Amer. Med. 41:460.

Fisher, N. F., 1924. Attempts to maintain the life of totally pancreatectomized dogs indefinitely by insulin. Am. J. Physiol. 67:634.

Griffith, W. H., 1940. Choline metabolism III. The effect of cystine, fat, and cholesterol on hemorrhagic degeneration in young rats. J. Biol. Chem. 132:639.

Griffith, W. H., 1941. The nutritional importance of choline. J. Nutrition 22:239.

Griffith, W. H. and D. J. Mulford, 1941. Choline metabolism VI. Hemorrhagic degeneration and the labile methyl supply. J. Amer. Chem. Soc. 63:929.

Hegsted, D. M., R. C. Miller, C. A. Elvehjem, and E. B. Hart, 1941. Choline in the nutrition of chicks. J. Biol. Chem. 138:459.

Hershey, J. M., 1930. Substitution of lecithin for raw pancreas in the diet of the depancreatized dog. Am. J. Physiol. 93:657.

Hershey, J. M. and S. Soskin, 1931. Substitution of "lecithin" for raw pancreas in the diet of the depancreatized dog. Am. J. Physiol. 98:74.

Hogan, A. G., L. R. Richardson, and H. Patrick, 1940. Relation of perosis to unrecognized vitamins. J. Nutrition, Proc. 19:14.

Hogan, A. G., L. R. Richardson, H. Patrick, and H. L. Kempster, 1941. Perosis due to a vitamin deficiency. J. Nutrition 21:327.

Jacobi, H. P., C. A. Baumann, and W. J. Meek, 1941. The choline content of rats on various choline free diets. J. Biol. Chem. 138:571.

Jukes, T. H., 1939. Ineffectiveness of manganese in preventing slipped tendon in turkey poults. Poultry Sci. 18:405.

Jukes, T. H., 1940. Effect of yeast extract and other supplements on the growth of chicks fed simplified diets. J. Biol. Chem. 133:631.

Jukes, T. H., 1940a. Prevention of perosis by choline. J. Biol. Chem. 134:789.

Jukes, T. H., 1940b. Effect of choline and other supplements on perosis. J. Nutrition 20:445.

Jukes, T. H., 1941. Studies of perosis in turkeys. I. Experiments related to choline. Poultry Sci. 20:251.

Jukes, T. H., 1941a. Effects of choline, gelatin, and creatine on perosis in chicks. Proc. Soc. Exp. Biol. and Med. 46:155.

Jukes T. H., 1941b. The effect of certain organic compounds and other dietary supplements on perosis. J. Nutrition 22:315.

Jukes, T. H. and F. H. Bird, 1942. Prevention of perosis by biotin. Proc. Soc. Exp. Biol. and Med. 49:231.

Jukes, T. H., 1942a. Personal communication.

Klose, A. A. and H. J. Almquist, 1941. Methionine in the diet of the chick. J. Biol. Chem. 138:467.

McElroy, L. W. and T. H. Jukes, 1940. Formation of the anti-egg-white-injury factor (biotin) in the rumen of the cow. Proc. Soc. Exp. Biol. and Med. 45:296.

McHenry, E. W., 1937. Vitamin B_1 and fatty livers. J. Physiol. 89:287.

McHenry, E. W., 1941. Choline, the B vitamins and fat metabolism. Biological Symposia V:177.

Moyer, A. W. and V. du Vigneaud, 1942. The structural specificity of choline and betaine in transmethylation. J. Biol. Chem. 143:373.

Norris, L. C., 1941. Choline in poultry nutrition. A paper presented at the Cornell nutrition conference. Oct., 1941.

Perlman, I. and I. L. Chaikoff, 1939. Radioactive phosphorus as an indicator of phospholipid metabolism V. On the mechanism of the action of choline upon the liver of the fat fed rat. J. Biol. Chem. 127:211.

Record, P. R. and R. M. Bethke, 1942. Further observations on choline and yeast in chick nutrition. Poultry Sci. 21:271.

Schaible, P. J., S. L. Bandemer, and J. A. Davidson, 1938. The manganese content of feedstuffs and its relation to poultry nutrition. Mich. Agr. Exp. Sta. Tech. Bul. 159:5.

Sharpe, J. S., 1923. Choline as a precursor of guanidine. The decrease of choline in the egg during incubation. Biochem. J. 18:151.

Sharpeless, G. R., 1940. Choline and epithelial hyperplasia in the forestomach of rats. Proc. Soc. Exp. Biol. and Med. 45:487.

Stetten, D., Jr., 1941. Biological relationships of choline, ethanolamine, and related compounds. J. Biol. Chem. 138:437.

Stetten, D., Jr., 1941a. Biological relationships of choline, ethanolamine and related compounds. J. Biol. Chem. 140:143.

Sure, B., 1940. The essential nature of choline for lactation and growth of the albino rat. J. Nutrition 19:71.

Underhill, F. P. and J. Petrelli, 1929. Effects of choline on blood sugar content. J. Biol. Chem. 81:159.

du Vigneaud, V., 1941. Interrelationships between choline and other methylated compounds. Biological Symposia V:234.

du Vigneaud, V., J. P. Chandler, A. W. Moyer, and D. M. Keppel, 1939. The ability of homocystine plus choline to support growth of the white rat on a methionine free diet. J. Biol. Chem. 128:CVIII.

du Vigneaud, V., J. P. Chandler, A. W. Moyer, and D. M. Keppel, 1939a. The effect of choline on the ability of homocystine to replace methionine in the diet. J. Biol. Chem. 131:157.

Warren, D. C., 1938. A heritable variation of feather structure in the fowl. J. Heredity 29:91.

Welch, A. D., 1936. Utilization of the arsenic analogue of choline chloride in the bio-synthesis of phospholipids. Proc. Soc. Exp. Biol. and Med. 35:107.

Welch, A. D., 1941. The preparation of a casein hydrolysate for the study of the relationship between choline and homocystine. J. Biol. Chem. 137:173.

Wiese, A. C., B. C. Johnson, C. A. Elvehjem, E. B. Hart, and J. G. Halpin, 1939. A study of blood and bone phosphatase in chick perosis. J. Biol. Chem. 127:411.