STUDIES ON CELLULOSE IN PURIFIED DIETS FOR CHICKS

By

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INTRODUCTION

From time immemorial man has not only fed fiber to himself but also to his domesticated livestock. During times of stress and famine, his interest in such feeding was intensified. The end of World War I ushered in many patents on inexpensive fiber mixes not only for animals but also for dietary disease conditions of man.

Nutritional findings leading to the development of the so called "synthetic" animal diet excluded any fiber, with a resulting decrease in scientific interest in its utilization as a nutrient. World War II and the tremendous increased demand for feedstuffs has again directed attention to the possibility of including greater percentages of fiber in animal diets. The tools for a better understanding of its role in the organism were now available. Fiber, purified from many plant sources, could readily be obtained. Cellulose which makes up the prime part of crude fiber had been diligently and intensely studied chemically and physically and could easily be included in a purified diet. The recent studies on the elucidation of the interrelationships of the nutrients with the resulting greater clarification of their metabolic roles have been additional aids in the experimental approach to this study. Finally the rapid advances in the field of bacteriology contributed valuable information as to the part played by intestinal microorganisms in the overall nutrition of the animal. With these methods of experimental attack at hand, studies have been made on the need for fiber by chickens with the use of "synthetic" diets supplemented with purified cellulose and degradation products of and substances associated with or structurally similar to cellulose.

LITERATURE REVIEW

There has been for some time much controversy as to the need of fiber by poultry. The results and conclusions of previous workers, in all cases using practical-type rations, varied with the type of diet and source of fiber. Natural sources of feedstuffs all contain some woody material which is supposedly non-utilizable by some species. Yet the composition of the purified diet, used in laboratory studies for the chick, completely omits plant fiber without any deleterious effects on growth and feathering. Some laboratories (Bird, 1945 and Campbell, Brown, Bird and Emmett, 1946) have included 3 to 5 percent of cellulose in purified diets for chicks without any published reasons. To date, there have been no studies on the effect of chemically purified cellulose in "synthetic" rations on the nutrition of the chick.

Radeff (1928) reported that chickens can use 7 percent of oat fiber and 17 percent of corn fiber. He believed that actual digestion of the fiber took place in the ceca because there was only a slight decrease in the crude fiber of the hen droppings after cecectomy when compared with the fiber content of the feed.

In the same year, Fraps (1928) found that the percentage of digestibility of crude fiber of certain grains to be as high as 65 percent. He concluded though that, "poultry have little ability to digest crude fiber, and that the digestibility of feeds containing much fiber was low." Further work by Fraps (1931) showed that the sugars and starches were highly digested by the chicken, whereas the pentosans had low digestibilities especially when fed in roughage materials.

Henning (1929) observed that 5 to 21 percent of the fiber of the various chicken feeds was digested, but filter paper was not attacked at all. He believed that this was due to the enzymatic action of the grain itself, as fiber of cooked grain was not as well absorbed.

Continuing the study of fiber digestion, Halnan (1926,30) reported the coefficients of digestibility of the crude fiber for corn, Sussex ground oats, and wheat to be 8, 7.6 and 11 percent, respectively. He further stated that crude fiber depressed the digestion of organic matter, and that its principle function was to regulate the amount of feed consumed. He also expressed the opinion that feed passed through the digestive system so rapidly that there was little time for active enzymation except in the crop and the ceca. He felt that the acid condition of the crop would in general prevent bacterial action there. In addition, he was unable to find a cellulose-digesting enzyme in the alimentary tract of the fowl.

A group of investigators at Oklahoma Agricultural and Mechanical College, Stillwater, Oklahoma made an extensive study of fiber in poultry rations over a period of 10 years. White Leghorn and Rhode Island Red chickens were used in their experiments which are reviewed in the following paragraphs.

A. Morris, Thompson and Heller (1932) found that fiber can be increased as much as 8 to 9 percent without harmful effects on chick growth, mortality, feed consumption and egg production. It was found that chicks up to 21 weeks of age consumed the same average amount of feed whether on a high fiber ration or not. The experimental period was for two years. During the first year, the source of fiber was oat hulls and during the second, alfalfa stems. The rations contained from 3 to 13 percent of crude fiber.

B. Using 4, 7, 10 and 12 percent of alfalfa stems and cottonseed hulls as sources of crude fiber in chick rations, Penquite and Thompson (1932) found that mortality increased and rate of growth decreased as the percentage of fiber in the diet increased. Chick mortality was higher on the cottonseed hull rations. The harmful effects were probably due to the presence of the toxic substance, gossypol. Autopsies after 5 weeks showed no significant effects on the intestines, gizzards or proventriculi.

C. The fiber content of a basal all-mash ration was then increased by adding peanut hulls, alfalfa stems, cotton burrs or dried wood pulp (Loblolly pine). The percentage of crude fiber ranged from 3.65 to 18 percent. Employing such diets, Penquite (1934) found that the viability and growth decreased with increased amounts over 10 percent of all the types of fibrous substances fed except alfalfa stems. With the alfalfa stems, the mortality was irregular and the viability did not decrease markedly. It was found that 3.51, 4.30 and 6.45 pounds of a low, medium and high fiber wood pulp diet, respectively, were required per pound of gain in 12 weeks, whereas peanut hull supplemented rations required 3.90, 4.10 and 4.94 pounds. These experiments showed that the source of fiber was a factor to be considered.

D. Penquite (1936) later reported that in addition to decreased growth and higher mortality, there was a retardation in feather development when the fiber content of chick rations exceeded 10 percent by chemical analysis. Rhode Island Red chicks were used in the 12 week experiments. He felt that the increased fiber acted as a dilution factor.

E. At about the same time, Heller and Penquite (1936) determined the pH of the chicken crop, proventriculus, gizzard, duodenum, upper ileum, lower ileum, caecum, colon and bile when fed fiber from old alfalfa stems, cottonseed burrs, and peanut hulls at levels from 3.65 to 14.92 percent. No differences in pH were found at any level of fiber.

F. Milby (1940) reported that rations containing large amounts of crude fiber from peanut hulls gave good growth in turkeys but only at the expense of heavy feed consumption per pound of gain. The turkeys fed oat hulls and peanut hulls grew faster and were heavier at market age and had the lowest mortality when compared with those fed oat straw, alfalfa stem meal and low fiber (control) rations. Percentages of fiber and feed consumption were not given.

A report by Sheehy (1939) stated that introduction of extra fiber in a concentrated diet does not reduce and probably enhances its nutritive value. He found that 7 percent was excessive and that it was a factor in preventing feather picking and cannibalism. The source of fiber was wheat bran and beet pulp. He also pointed out that the source of fiber determined the effect.

The utilization of sawdust by chickens was investigated by Guisti (1939), who reported that the coefficient of digestibility of the cellulose constituent averaged 2.33 percent, and that of concentrates such as corn meal, barley and wheat bran was 42 percent.

Miller and Bearse (1937) reported that Single Comb White Leghorn chicks and pullets fed 80.8 percent of oats as the only cereal consistently and significantly reduced cannibalism when compared with that observed on rations containing solely corn. The order of growth value of the grain at 8 weeks was first oats, followed by corn, wheat and barley, respectively.

Further experimentation by Miller and Bearse (1938) showed that when a ration was supplemented with 30 percent of oat hulls, 32 percent of oat mill feed, or replaced with an oat basal ration, practically no cannibalism appeared. Oat hull ash, oat ash, or manganese sulfate were not effective in preventing picking. Two lots of 65 birds each, fed supplements of 15 percent of spruce sawdust, developed feather picking at 14 weeks of age, but the condition disappeared at 32 weeks of age. The sawdust used was not in a fine state and tended to make the ration bulky and unpalatable, with a resulting slightly lower average weight when compared with the other lots.

In the following year, Bearse, McClary and Miller (1939) found that if the spruce wood was fed in flour form in place of corn at a level of 20.2 percent in a practical-type diet, slight increases of growth were obtained at 28 weeks of age when compared with the control birds fed a ration containing 80.9 percent of ground yellow corn. No detrimental effects were observed. Cannibalism mortality was high in both groups.

Bearse, Miller and McClary (1940), continuing their studies of oat hull fiber, observed that the fiber obtained by dilute acid digestion, when fed at a level sufficient to produce a diet of 11 percent of crude fiber, controlled cannibalism and improved the quality of the feathers. The oat hull was effective also, but neither the ash of a dilute acid extract nor the ash of a water extract were of any value in these respects. The feeding trials involved duplicate lots of 64 White Leghorn pullets, each from 2 to 40 weeks old.

That much better results could be obtained with 6 to 7 percent of fiber than 2 to 3 percent was observed by Wilcke and Hammond (1940). Feeding of oat hulls seemed to improve growth and feathering in both rate and quality. Perosis was also reduced.

Writing for a volume on poultry diseases, Wilcke (1945) states that with finely ground feeds, the addition of bulky materials such as sawdust, paper pulp or regenerated cellulose will increase the efficiency of feed utilization.

Harding (1942) reported that cellulose from rice hulls added in amounts of 5 percent to the diet of laboratory animals is effective in the prevention of constipation.

Oat hulls, at 20 percent of a ration composed of oat groats, minerals, cod-liver oil and dried skim milk, was found by Record (1943) to increase Barred Rock and New Hampshire chick growth and also improve feather quality. One of 2 lots fed 6.6 percent of spruce pulp and another group fed 6 percent of regenerated cellulose grew faster than the lots fed the basal ration.

Heuser, Norris, Peeler and Scott (1945) found that feeds of little fibrous nature were more efficient for growth, egg production, and maintenance of body weight than rations containing oats and wheat by-products. The calculated fiber contents of the rations were 5.35 percent and 2.65 percent. They believed that the reason they obtained poorer results with wheat by-products and pulverized oats than reported by previous workers was that the Cornell ration used in their experiments contained less fat.

In determining the effect of diet on efficiency of egg production, Bird and Whitson (1946) concluded that the efficiency with which a laying hen (yearling Rhode Island Red) utilized a diet containing 2.56 percent of fiber was greater than one containing 5.89 percent. The added fiber was obtained from oats, wheat bran, wheat middlings, and alfalfa meal.

Carrick and Roberts (1947) reported that 10 percent of wheat bran and 10 percent of wheat middlings used in place of corn decreased the relative efficiency of chick rations. When wheat products were included in the diet more feed was required per unit of gain.

Studying the effect of ration on cannibalism, Schaibel, Davidson and Bandemer (1947) found that the replacement of corn in a diet containing 2.56 percent of fiber, 13.5 percent of protein and 0.534 percent of phosphorus with 4 percent of alfalfa leaf meal, 1 percent of wheat bran, 6 percent of paper pulp or 0.7 percent of ground wheat straw was beneficial to feathering. Rice hulls at 4 and 8 percent supplemental levels gave a small reduction in cannibalism. Casein and certain other protein supplements were most effective in controlling cannibalism and improving feather quality.

Nestler, Coburn and Titus (1945) observed that picking occurred among pen-reared Bobwhite quail on all levels of oat hull fiber from 1 through 11 percent.

From the foregoing literature review survey, dealing entirely with the avian specie, it can be concluded that low levels of fiber can safely be included in practical-type poultry rations without any harmful effects. Actually, depending on its source, fiber may even be beneficial as to growth, mortality and cannibalism. It should be noted though that in much of the older work, the results were complicated by the fact that the basal rations were low in essential factors such as vitamins which may have been supplied by the crude fiber source. There have also been many interesting studies on cellulose in the diets of other species.

Growth responses were obtained when 8 percent of cellulose replaced starch in diets for mice (McCay, Ku, Woodward and Sehgal, 1934), and when 20 percent of cellulose of Cellu-flour or powdered cellophane was supplied in purified rations for guinea pigs (Woolley and Sprince, 1945). The utilization of wood cellulose by horses had been reported by Hvidsten (1945) and digestion of straw by ruminants (McAnally, 1942). Extensive rumen digestive studies in the bovine were reported by Hale, Duncan and Huffman (1947,47a).

Correlation coefficients of -0.978, -0.808 and -0.944 connecting digestibilities and percentage content of lignin, cellulose and crude fiber, respectively, to organic matter in feeds for sheep were calculated by Lancaster (1943). He observed that these exerted a depressing effect upon the digestibility of organic matter and individual cell wall constituents. McMeekan (1943) verified Lancaster's calculations.

Hume and Smith (1926) found that irradiated sawdust exerted a beneficial action on growth and calcification when consumed by rats. Ershoff and McWilliams (1946) did not obtain any growth effects but did get slightly better feed efficiency values when 10 percent of Cellu-flour was included in diets for rats. Finally, attention is directed to an excellent review by Hoelzel (1947) on the effect of cellulosic bulk-formers on rat longevity and human hunger.

PROCEDURE

Day-old New Hampshire chicks of mixed sexes were used in all but one of these experiments. They were progeny of the University of Maryland flock and their dams had received a good practical breeder ration. All chicks were reared in electrically heated batteries with raised screen floors. Feed and water were supplied ad libitum throughout the 4-week experimental periods. In the turkey experiment, crosses of a Broad-Breasted Bronze male and Beltsville Small-type White females were used. All chicks and poults were individually weighed and uniformly divided before being put on experiment. A set procedure was strictly adhered to in order to keep the sampling error at a minimum. If one of the chicks showed even the slightest sign of illness, the entire group was discarded. Occasionally runts would be encountered, and these would be culled as early as possible. Six to 10 chicks were put into each group and the results were recorded as summations of a series of experimental runs.

The basal rations 113, 113a, 114 and 24, used in the experiments, are given in Tables I and II. They contained all the nutrients known to be required by the chick. Rations 113 and 114 were purified diets and differed only in that the latter contained vitamins A and D_3 in a stearate carrier whereas the use of 113 required these vitamins to be fed weekly by dropper. Diet 113a contained L-cystine in place of DL-methionine. Ration 24 was a typical all-purpose practical mash which contained 21.0 percent of protein and 5.9 percent of crude fiber.

TABLE I

PURIFIED BASAL DIETS USED IN EXPERIMENTS

Dietary ingredient	Basal diets		
	113a	113	114
	%	%	%
Glucose ("Cerelose")	61.4	61.4	59.1
Casein (crude)	18.0	18.0	18.0
Gelatin	10.0	10.0	10.0
Salts 1 M*	6.0	6.0	6.0
Soybean oil	4.0	4.0	4.0
DL-Methionine	—	0.3	0.3
L-Cystine	0.3	—	—
(Vitamins-MG./100 gms. diet)			
Thiamine HCl	0.4	0.4	0.4
Riboflavin	0.8	0.8	0.8
Pyridoxine HCl	0.6	0.6	0.6
Biotin	0.02	0.02	0.02
Ca pantothenate	2.0	2.0	2.0
Niacin	5.0	5.0	5.0
p-Aminobenzoic acid	0.2	0.2	0.2
Folic acid	0.2	0.2	0.2
Choline chloride	200.0	200.0	200.0
i-Inositol	100.0	100.0	100.0
2-Methyl-1,4 naphthoquinone	0.1	0.1	0.1
Alpha-tocopherol	0.5	0.5	0.5
Vitamins A and D_3	1200 I.U. and 170 A.O.A.C. units, respectively by dropper weekly		—
Vitamins A (500 units per gram)**	—	—	2.0
D_3 (500 units per gram)	—	—	0.3

*Salts 1 M are composed of the following ingredients by weight:

$CaCO_3$	150.0	$MgSO_4 \cdot 7H_2O$	50.0	$ZnCl_2$	0.2
K_2HPO_4	90.0	$Fe(C_6H_5O_7)_2 \cdot 6H_2O$	14.0	$CuSO_4 \cdot 5H_2O$	0.2
Na_2HPO_4	73.0	$MnSO_4 \cdot 4H_2O$	4.1	H_3BO_3	0.09
$Ca_3(PO_4)_2$	130.0	KI	0.4	$CoSO_4 \cdot 7H_2O$	0.01
NaCl	88.0				600.0

When fed at a level of 6 percent, the mixture supplies 1.11 gms. of calcium, 0.58 gm. of phosphorus, and 0.01 gm. of manganese per 100 gms. of diet.

**The vitamins were added in SPAN-65, a stearate carrier.

TABLE II

COMPOSITION OF RATION 24, AN ALL-PURPOSE POULTRY MASH

Dietary ingredient	Percent
Ground yellow corn	25.25
Ground heavy oats	12.50
Wheat middlings	10.00
Wheat bran	12.00
Soybean oil meal	10.00
Corn gluten meal	5.00
Alfalfa leaf meal	5.00
Fish meal	5.00
Meat scrap	5.00
Dried whey or other milk products	5.00
Riboflavin concentrate (80 gamma per gram)	1.25
Oyster shell	2.00
Bone meal	0.75
Salt, iodized	0.75
Vitamin A and D feeding oil (2000 units A per gram (400 units D per gram	0.50
Manganese Sulphate	0.025

In preparing the purified basal rations, thiamine HCl, riboflavin, pyridoxine HCl, biotin, calcium pantothenate, p-aminobenzoic acid, and choline chloride were added in aqueous solutions. Vitamin K additions were made from a 95 percent alcoholic solution, and niacin, i-inositol and DL-methionine or L-cystine were mixed directly with the other dry ingredients of the diets. The soybean oil, fortified with alpha-tocopherol, was mixed directly with the casein. Because of its tendency to gum up, gelatin was added last. In addition, for ration 114, the stearated vitamins A and D_3 were added to the dry components of the diets. Finally, all the dietary ingredients, excluding the glucose ("Cerelose") were thoroughly mixed by hand and passed through a 14 mesh screen. The glucose ("Cerelose") was included when the various supplements were added to make up aliquot quantities of the rations for each experimental group of chicks.

The batteries employed were all metal 5-tier type. False bottoms and feed catch-troughs were always used to minimize feed wastage. More than one control group was usually set up in each experimental series, and these were distributed so that their positions in the battery would not be a possible added source of error. The water trough was checked frequently to insure an adequate, clean, fresh supply of water at all times.

All chicks were weighed individually at weekly intervals, and feed consumption data were collected at the end of each experimental run in order to calculate feed efficiency values (Total weight gained/Total feed consumed). At weighing times, each chick was examined for any external abnormalities. From time to time, at the end of experimental trials, chicks were killed and autopsies performed. Occasionally blood samples were taken, and hemoglobin determinations made by use of the Evelyn photoelectric method.

All additions of cellulose ("Ruffex", Table III) to the basal rations were made at the expense of glucose ("Cerelose") unless otherwise stated. Supplemental substances were first pre-mixed, and then also added to the basal rations at the expense of the utilizable carbohydrate. Mixed sawdust, composed of white and yellow pine, was passed through a 10 mesh screen before incorporating into the diets.

Treated cellulose, used in the cellulose-niacin studies, was a product obtained after chemical treatment. It was digested for one half hour with 1.25 percent of sulfuric acid and then washed with boiling water until the effluent was neutral to litmus paper. Another half hour of digestion was carried out with 1.25 percent of sodium hydroxide which was followed by re-washing with hot water. The product was then dried in an oven at 90° C. and passed through a 14 mesh screen. Analyses of both treated and untreated cellulose gave a value of 75.02 and 75.38 percent of crude fiber, respectively.

Apparent crude fiber digestibility was determined by difference. Crude fiber analysis of the "Ruffex" was made, and the amount of crude fiber in the ration fed was calculated. A crude fiber value was also obtained from representative samples of chick droppings for a 1-week experimental period with 6 chicks per group. Crude fiber in the feed minus the crude fiber in the droppings have an approximation of the crude fiber digestibility.

Bacteriological studies of the cecal and intestinal contents of a chick fed a purified ration supplemented with 61.4 percent of cellulose were made employing a medium reported successful in isolating

TABLE III

DATA ON "RUFFEX"[1]

Source		Processed rice hulls
Size		Through 40 mesh
Proteins		None
Fats		None
Vitamins		Destroyed or removed
		(percent)
Alpha cellulose		70
Simple and hydrocelluloses		30
Total Ash	less than	1.00
Nitrogen	less than	0.05
Calcium	less than	0.03
Aluminum	less than	0.02
Silica	less than	0.50
Iron	approx.	0.001
Phosphorus		nil

[1]A roughage product distributed by Fisher Scientific Co., Pittsburgh, Pa.

cellulose-decomposing microorganisms from the intestinal tract of wild gallinaceous birds by Soumalainen and Arhimo (1945). The composition of this medium is given in Table IV. Shake and stab cellulose-agar tubes were prepared by adding 4 grams of cellulose to 110 cubic centimeters of a 1 percent nutrient agar solution. All media were sterilized at 120 pounds of pressure for 15 minutes. Innocula were obtained as aseptically as possible from the chick ceca and intestinal tracts. Samples were taken immediately after a chick was killed.

For the cellulose enzyme investigation, a crude extract was prepared by grinding up the ceca and their contents with washed sand. The chick used was fed ration 113 supplemented with 61.4 percent of cellulose. The macerated product was then filtered and the resulting neutral enzyme-filtrate poured into a test tube containing loosely packed cellulose. A drop of phenol or toluol was added, and the mixture incubated at 37° C. At the end of 24 hours, the demarcation of the enzyme extract and cellulose was noted, and the incubation continued for a week. Fehling's reducing test was applied to the enzyme-filtrate before and after incubation with the cellulose. Fehling's solution is a mixture of copper sulfate solution and alkaline tartrate solution.

To study the effects of a mixture of cellulases and hemicellulases in the purified ration 114 containing cellulose, the newly hatched chicks were fed ration 24 for 2 weeks. At the end of this preliminary feeding period, the chicks were individually weighed and uniformly divided into experimental groups. The choice of experimental chicks was determined by the closeness of their rates of growth. Those with the highest and lowest weights were discarded. The purified

TABLE IV

COMPOSITION OF BACTERIOLOGICAL MEDIUM USED FOR ISOLATION OF CELLULOSE-DECOMPOSING MICROORGANISMS

Ingredient	Gram
Peptone	3.0
$NH_4H_2PO_4$	2.0
KH_2PO_4	1.0
$MgSO_4 \cdot 7H_2O$	0.2
NaCl	0.1
$FeSO_4$	traces
Yeast extract (200 gm./800 cc. H_2O)	20 cc.
Ascorbic acid (1 percent solution)	1.0 cc.
H_2O	to 1000 cc.
$CaCO_3$ buffered	

experimental diets were then fed the selected chicks until 4-weeks of age. Weighings were made the third and fourth weeks and feed consumption data were collected in order to calculate feed efficiency values.

Specific gravity of the feed in the nutrient dilution experiments was obtained by weighing the contents of a 2 inch cubic cardboard box filled with the various cellulose and non-supplemented diets. The cube was filled in the same way each time and the same procedure was repeated on two successive days. The average of the two determinations was recorded as the weight per unit volume.

The sawdust acid extract was prepared by digesting the screened wood with dilute hydrochloric acid for one half hour. The digest was then cooled, filtered and brought to a pH of 2.

The furfural (C_4H_3OCHO) and levulinic acid ($CH_3COCH_2CH_2COOH$) used in the experiments were both purchased from the research laboratory of the Eastman Kodak Co., Rochester, New York.

RESULTS, PART I

A. Cellulose Experiments with Chicks and Poults

The results of feeding purified diets supplemented with various levels of cellulose to chicks are summarized in Table V. The greatest response in growth and calculated feed efficiency value was at the 5 percent level of cellulose supplementation. Analysis of variance, given in Table VI, showed that chick growth at the 5 through 15 percent supplemental levels of cellulose was statistically significant to the 0.05 level when compared with the control groups. Individual t tests of significance for the treatments showed that the 5 percent level was highly significant and the 10 and 15 percent levels were non-significant. With increasing additions of cellulose over the 15 percent level to basal ration 113a, growth and feed efficiency decreased. Although growth was poor, mortality was negligible at the 50 percent of cellulose supplementation. However, there was 100 percent mortality when all the glucose was replaced by cellulose.

No difference in feathering and no feather picking or cannibalism were observed in any of the experimental groups. As much as 50 percent of cellulose in the ration was tolerated without ill effects. The chicks fed rations supplemented with 20 percent or more of cellulose seemed to be continually eating in what seemed to be a vain attempt at increasing their caloric intake.

The droppings of the chicks on all levels of cellulose supplements were of a drier, firmer consistency than those of the controls. Feed consumption values and chemical analyses of the droppings for a 1-week

TABLE V

SUMMARY OF THE EFFECT OF CELLULOSE SUPPLEMENTS IN PURIFIED RATIONS

Group No.	Level of cellulose added to basal diet 113a	No. chicks started	No. dead at 4 wks.	Av. wt. at 4 wks.	Feed efficiency
	CHICKS			gm.	
1	None	133	8	312	0.550
2	2.5 %	10	0	351	0.532
3	5 %	94	2	349	0.565
4	10 %	22	1	344	0.550
5	15 %	22	1	327	0.526
6	20 %	16	0	288	0.434
7	30 %	12	0	268	0.311
8	40 %	12	0	200	0.312
9	50 %	12	1	140	0.215
10	61.4%	20	20	—	—
	TURKEYS				
11	None	6	0	268	0.464
12	5%	6	2	322	0.506

TABLE VI

ANALYSIS OF VARIANCE FOR CHICK GROWTH OBTAINED BY CELLULOSE ADDITIONS TO RATION 113

Source of variation	D. f.	Variance
Treatment	3	25,422.00[1]
Error	255	7,401.60

[1]Significant to 0.05 level.

t TEST OF SIGNIFICANCE FOR TREATMENTS

Supplement to R-113a	t	P
5% Cellulose	2.93	greater than 0.01
10% Cellulose	1.33	less than 0.1*
15% Cellulose	0.62	less than 0.5

*P value for d. f. of 20.

experimental period gave apparent digestibilities of 53.9, 27.9 and 34.3 percent of the crude fiber fed at the 5, 10 and 15 percent levels of cellulose, respectively.

The results of feeding ration 113a alone and with additions of cellulose at the 5 percent supplemental level to turkey crosses are given in Table V. There was a definite increase in poult growth and feed efficiency on the cellulose supplemented ration when compared with the control group. The poults fed purified ration 113a developed sticky heads and feathers resulting in a bald head-like appearance. The presence of cellulose seemed to somewhat diminish this sticky effect. "Pendulous crops" were not observed in these experiments.

Records of feed consumption for the 4-week experimental period showed that the control poults consumed 464 grams each while those on the cellulose supplement ate 517 grams.

The results of feeding sulfasuxidine, chick droppings, cotton flocks and ground corn in diet 113a are given in Table VII. Crude fiber analyses of droppings of chicks fed 1 percent of sulfasuxidine plus ration 113a at the 5 and 15 percent cellulose supplemental levels were 23.06 and 38.85 percent, respectively. The corresponding values for chicks receiving cellulose supplements without sulfasuxidine were 15.72 and 33.67 percent. The effects of feeding corn starch in place of glucose and cellulose plus ration 24, the practical ration, are summarized in Table VIII. Little or no growth response was obtained with these supplements. Actually, 5 percent of cellulose plus ration 24 produced a slight depression in chick growth.

TABLE VII

EFFECT OF SULFASUXIDINE, COTTON FLOCKS, GROUND YELLOW CORN AND CHICK DROPPINGS SUPPLEMENTS ON CHICK GROWTH AND FEED EFFICIENCY

Group No.	Supplement to R-113a	No. chicks at 4 weeks	Av. wt. at 4 weeks	Feed efficiency
			gm.	
1	None	11	347	0.586
2	5% Cellulose	11	379	0.601
3	10% Cellulose	5	354	0.532
4	15% Cellulose	10	327	0.526
5	50% Cellulose	5	167	0.239
6	As (1) + 1% sulfasuxidine	5	347	0.553
7	As (2) + 1% sulfasuxidine	5	355	0.520
8	As (3) + 1% sulfasuxidine	6	358	0.534
9	As (4) + 1% sulfasuxidine	6	291	0.549
10	As (5) + 1% sulfasuxidine	6	151	0.285
11	As (1) + 5% cellulose droppings	6	344	0.569
12	As (1) + 5% sulfa-cellulose droppings	6	360	0.580
13	0.1% Acid extract of pine sawdust	9	325	0.582
14	None	20	311	0.538
15	5% Cellulose	9	365	0.549
16	2.5% Cotton flocks	9	319	0.504
17	5% Cotton flocks	10	327	0.547
18	As (4) + 25% ground yellow corn	6	338	0.517

TABLE VIII

CHICK GROWTH AS EFFECTED BY CELLULOSE IN A CORN STARCH BASAL AND IN A PRACTICAL-TYPE POULTRY RATION

Group No.	Supplements to	No. chicks at 4 weeks	Av. wt. at 3 weeks	Av. wt. at 4 weeks	Feed efficiency
	RATION 113a		CORN STARCH BASAL gm.	GLUCOSE BASAL gm.	4-WEEK
1	None	6	208	345	0.592
2	5% Cellulose	5	212	375	0.630
3	10% Cellulose	5	208	354	0.532
	RATION 24				
4	None	6	—	287	0.425
5	5% Cellulose	6	—	259	0.380

B. Nutrient Variation Experiments with Chicks

In order to determine the effect of cellulose additions to purified rations for chicks on the concentrations of the nutrients in the diet, experiments diluting and concentrating the nutrients were set up. Tables IX and X illustrate the effects obtained: (1) when cellulose was added to the basal ration 113 at the expense of glucose, (2) when cellulose was added to the complete basal ration and, (3) when diets with graded omissions of glucose were fed. Concentration of the nutrients by graded omissions of glucose did not result in greater chick growth. In fact, 10 percent of glucose omission produced a slight growth depression. Replacement of the same levels of glucose omission by cellulose resulted in an increase in chick growth when compared with the control groups.

Dilution of the effective nutrients with 20 percent of more of cellulose resulted in decreased chick growth and feed efficiency values. Calculation of 4-week feed consumption per chick showed that with increased dilution of the nutrients with 20 percent or more of cellulose, more feed was eaten per unit weight gained per chick than when a concentrated ration was fed. The data showed that for the 4-week experimental period those groups on the diluted diets received less of the nutrients, and thereby gained proportionately less weight. In other words, growth was dependent on the caloric content of the ration. With increased dilution, the specific gravity of the ration obviously decreased.

TABLE IX

SUMMARY OF THE EFFECT OF GRADED GLUCOSE OMISSIONS FROM BASAL RATION 113 AND ITS REPLACEMENT BY CELLULOSE ON CHICK GROWTH AND FEED EFFICIENCY

Group No.	Ration 113		No. chicks at 4 weeks	Av. wt. at 4 weeks	Feed efficiency	Av. 4 week feed consumption per chick
	% GLUCOSE OMITTED	% CELLULOSE		gm.		gm.
1	None	0	15	311	0.553	490
2	2.5	0	8	318	0.562	495
3	5.0	0	14	317	0.552	459
4	7.5	0	4	304	0.564	467
5	10.0	0	6	265	0.559	404
6	2.5	2.5	10	351	0.532	567
7	5.0	5.0	10	350	0.576	538
8	10.0	10.0	16	340	0.560	547

TABLE X

SUMMARY OF THE EFFECT OF CELLULOSE DILUTION ON CHICK GROWTH AND FEED EFFICIENCY

Ration R-113	Ration Cellulose	No. chicks at 4 wks.	Av. wt. at 4 wks.	Feed eff.	4-Wk. feed consumption per chick	Feed consumed per unit wt. gained per chick	Wt. feed per 2" cube
gm.	gm.		gm.		gm.	gm.	gm.
100	None	15	311	0.572	477	1.53	75.5
100	5	8	296	0.542	472	1.59	72.0
95	5	6	296	0.544	471	1.59	66.5
100	10	5	327	0.555	517	1.58	63.5
90	10	4	313	0.513	517	1.65	61.0
80	20	5	253	0.464	490	1.90	52.0
70	30	5	248	0.420	498	2.01	43.5
60	40	6	176	0.337	403	2.29	36.5
50	50	5	116	0.198	383	3.90	31.5

C. Sawdust Experiments with Chicks

As an inexpensive source of cellulose for laboratory diets, mixed pine sawdust was added to ration 113. The summary of results, given by Table XI, showed that the 10, 15 and 20 percent supplemental levels resulted in the greatest growth responses when compared with the control groups. The increased growth was found to be highly significant by analysis of variance and t tests of significance of the treatments. The results are given in Table XII. Further additions of sawdust with correspondingly greater reductions of the available metabolizable simple carbohydrate resulted in poor growth and lowered feed efficiency values. Autopsies revealed partial packing of the gizzards with sawdust particles. The weights of the gizzard contents ranged from 4 to 5 grams each. No wood particles were observed in the ceca but could be readily detected in the droppings, which were of a firm consistency. Crude fiber analysis of the sawdust was 60.62 percent.

At 4 weeks, the feathering of the chicks on the sawdust supplemented rations appeared to be of a better quality than those of the control groups. No satisfactory scoring system could be set up. Feather picking, perosis, and mortality were negligible at the supplemental levels fed in these experiments.

Table VII shows that no comparable increase in chick growth was obtained with 0.1 percent of the sawdust acid extract supplement.

D. Cellulose-Niacin Studies with Chicks

Ration 113a, made up with 5 and 20 mg. of niacin per kilogram of feed, which is less than the dietary requirement, was found to give growth

TABLE XI

SUMMARY OF THE EFFECT OF MIXED PINE SAWDUST SUPPLEMENTS IN PURIFIED RATIONS FOR CHICKS

Group No.	Level of sawdust added to R-113	No. chicks at 4 weeks	Av. wt. at 4 weeks	Feed efficiency
			gm.	
1	None	32	331	0.594
2	5%	9	339	0.496
3	10%	30	373	0.553
5	15%	10	366	0.520
6	20%	9	367	0.491
7	30%	10	345	0.345

TABLE XII

ANALYSIS OF VARIANCE FOR CHICK GROWTH OBTAINED BY PINE SAWDUST ADDITIONS TO RATION 113

Source of variation	D. f.	Variance
Treatment	3	10,143.00[1]
Error	77	866.32

[1]Significant to 0.01 level.

t TEST OF SIGNIFICANCE FOR TREATMENTS

Supplement to R-113	t	P
10% Sawdust	5.71	greater than 0.01
15% Sawdust	2.78	0.03*
20% Sawdust	2.85	0.03**

*P value for d.f. of 9.

**P value for d.f. of 8.

responses proportional to additions of graded amounts of cellulose. The non-supplemented diet produced the typical inflamed tongue and buccal cavity, salivation and diarrheal symptoms. With increased cellulose supplementation of such low niacin diets, the black tongue syndrome was found to be less severe and resulted in lowered mortality. Supplemental levels of cellulose range from 5 through 50 percent. A completely deficient diet in niacin supplemented with 15 percent of cellulose resulted in only 33 percent mortality at 4 weeks. Treated and untreated cellulose gave similar effects in offsetting the syndrome in completely deficient niacin diets. Table XIII gives the results obtained with these diets.

E. Enzymatic and Bacteriological Studies

Fehling's reduction test was negative for the freshly prepared crude enzyme extract and for the enzyme-cellulose mixture before and after 1 week of incubation at 37° C. Neither was there an observable change in the level of demarcation (scratch line on side of test tube) between the cellulose and enzyme extract.

Slides were prepared (Baker, 1942) directly from the ceca and intestinal tracts of cellulose fed chicks. Gram staining showed large gram positive (Lactobacilli) and smaller gram negative rods and cocci (Coliform). Giant gram positive cocci were also observed.

The cellulose-agar stab tubes and plate dilutions showed no conspicuous discolored areas. Incubation, at 37° C. for 1 week, of the cultured broth with filter paper in petri dishes resulted in no breakdown of the cellulose.

TABLE XIII

SUMMARY OF THE EFFECTS OF ADDING CELLULOSE TO LOW- AND DEFICIENT-NIACIN DIETS

Group No.	Supplement to R-113a	No. chicks started	No. dead at 4 wks.	Av. 4 week weight
				gm.
NIACIN - 20 MG. PER KILOGRAM OF RATION				
1	None	8	0	216
2	5% Cellulose	8	1	234
3	10% Cellulose	8	0	258
4	15% Cellulose	8	2	254
5	20% Cellulose	8	1	286
NIACIN - 5.0 MG. PER KILOGRAM OF RATION				
6	None	8	8	—
7	10% Cellulose	8	1	197
8	20% Cellulose	8	1	215
9	50% Cellulose	8	1	112
NO NIACIN				
10	None	6	2	66*
11	0.05% Niacin	6	0	333
12	15% Cellulose	6	2	216
13	0.05% Niacin	6	0	358*
14	5% Treated cellulose	6	2	135*
15	As (14) + as (13)	6	0	380*
16	As (13) + 5% cellulose (untreated)	6	1	400*

*Mean chick weights for 29 days.

The results obtained by the addition of 5 percent of distane, a crude source of diastesic enzyme, to ration 113a, given in Table XIV, showed that at the 50 percent level of cellulose supplementation, there was no chick growth response and 58 percent mortality. The enzyme product was obtained from mold growth on wheat bran.

Additions of a purified mixture of cellulases and hemicellulases to ration 114 at the 2.5, 5 and 10 percent levels resulted in slight growth responses when compared with the non-supplemented group and those fed diets containing the inactive forms of the enzymes.

TABLE XIV

THE EFFECT OF ENZYME SUPPLEMENTS IN PURIFIED RATIONS FOR CHICKS

Group No.	Supplement to	No. chicks at 4 weeks	Av. wt. at 4 weeks	Feed efficiency	Av. feed consumed per chick
			gm.		gm.
	R-113a				
1	None	11	347	0.586	——
2	5% Cellulose	11	379	0.601	——
3	50% Cellulose	5	167	0.239	——
4	As (3) + 5% diatase	5	137	0.202	——
	R-114				
5	None	4	257	0.531	305
6	As (5) + 10% inact. 19AP*	10	262	0.510	334
7	As (6) - 5% glucose	10	261	0.510	325
8	As (7) + 5% cellulose	10	252	0.454	342
9	As (8) but 10% act. 19AP	10	287	0.518	373
10	As (8) but 5% act. 19AP	9	274	0.467	371
11	As (8) but 2.5% act. 19AP	10	278	0.503	368

*19AP is a mixture of purified cellulases and hemicellulases. Its reported protein content is approximately 25 percent.

DISCUSSION

Cellulose added to a purified chick diet free of fiber at the expense of glucose was effective as a growth stimulant at the 5 through 15 percent supplemental levels. The greatest effect was obtained with 5 percent supplementation. If the cellulose additions were made at low levels to the complete basal ration, no such growth responses were obtained, but it should be noted that these nutrient dilution results were obtained with very small groups. Continuing these dilution studies, it was found that when the ration contained 80 percent or less of the nutrients, the intake of the nutrients was clearly inadequate to maintain normal chick growth. The chicks ate more per unit weight gained with increased dilution with cellulose, but their physiological capacity prevented ingestion of sufficient nutrients for growth. Rats, as reported by Adolph (1947), began to lose weight when the nutrients were 33 percent or less. Furthermore, if graded amounts of glucose were omitted from the ration and not replaced with cellulose, no growth activity was obtained. The purified cellulose could be replaced by mixed pine sawdust but not by cotton flocks. The data show that it is not the increase of effective nutrients other than glucose which is responsible for the increased growth, but that it is rather something within the cellulose itself which is released when mixed with the nutrients in the gastro-intestinal tract.

Crude fiber analyses further show that the fiber in its original form is broken down to some degradation product(s). This is shown by a decrease in the crude fiber value from 75.38 percent to 63.88 percent after three hour acid-alkali digestion. The product(s) is soluble in

1.25 percent of both sulfuric acid and sodium hydroxide. The filtrate from such treatment still gave a negative Fehling's test. It can therefore be concluded that there is no, or a negligible, increase in the available utilizable carbohydrate component of the diet, i.e. glucose.

The results of the enzyme and microbiological studies show also that cellulose fed to chicks is neither broken down completely to a reducing substance nor an associated reducing substance which can readily be detected by standard reducing tests. A positive test for levulinic acid, a weak reducing substance, and furfural, a strong reducing substance, discussed in Part II could have easily been masked by the cecal contents.

It is possible that a partial hydrolysis of the alpha cellulose and of the simple and hydro-celluloses in the ceca and/or intestinal tract gives rise to small amounts of a growth stimulatory product(s). Enzymes of microbiological or intestinal origin, or both, may be active in such hydrolyses. These biocatalysts do not necessarily have to carry the hydrolysis to completion. The short period of time that nutrients remain in the chick digestive tract (Hauser, 1945,46) may be a factor in producing incomplete hydrolysis.

It is known that certain microorganisms produce cellulose-splitting enzymes. Baker (1942a) and Hungate (1944,46) have shown that various organisms possess the ability to metabolize cellulose. Elvehjem and Krehl (1947) have pointed out that the type of carbohydrate used in a purified diet will greatly influence intestinal synthesis of growth factors. One or more of the decomposition or liberated products derived from cellulose metabolism or microbial synthesis may be responsible for the growth responses.

From the data obtained, it appears that the presence of the released stimulant(s) can readily be detected by increased chick growth only when cellulose replaces the glucose of the diet at low levels, and there is a correspondingly proportionate increase of the effective nutrients other than glucose.

It is also possible that the mere physical presence of cellulose may be beneficial in some manner. For instance, its presence in restricted amounts could aid in the intestinal absorption. This too may be a factor in the sawdust results. However, the feed efficiency values for the 5, 10 and 15 percent levels of cellulose show that cellulose is probably not functioning simply as a bulk factor. Non-effectiveness of dilution with low levels of cellulose further enhances this view.

The retarded growth and lowered feed efficiency values obtained with the feeding of the 20 through 61.4 percent levels of cellulose were probably caused by a decrease in the availability of metabolizable simple carbohydrate, since the supplements were fed at the expense of glucose.

The source of cellulose may be another factor since no growth response was obtained when cellulose was replaced by cotton flocks. The structure of the fiber from rice hulls and pine sawdust may be of such a nature that growth stimulatory substances are possible. These fibers may possibly be favorable for the growth of a particular type of intestinal flora which may produce effective growth stimulants as by-products of their metabolism.

In order to determine whether microorganisms were instrumental in producing chick growth responses with cellulose supplements, 1 percent of sulfasuxidine was added to the diets. Sulfasuxidine is non-absorbable and is supposed to be effective in reducing the bacterial flora of the intestinal tract. Campbell et al. (1946) did not obtain any chick growth responses with a purified diet containing 0.025 mg. percent of crystalline vitamin B_c supplemented with 0.1 percent of sulfasuxidine. Moore et al. (1947) reported that 1 percent of sulfasuxidine in a purified ration containing 0.5 mg. percent of folic acid, even though not absorbed, did not destroy all the intestinal flora, but by restricting its multiplication, less toxic substances were synthesized which resulted in increased chick growth. In the experiments described here, the drug was added to cellulose supplemented diets in order to determine whether there would be a depression of growth if the organism population of the digestive tract were controlled. Under these experimental conditions, the addition of 1 percent of sulfasuxidine to basal ration 113, which contains 0.2 mg. percent of folic acid did not result in increased chick growth. That intestinal synthesis of folic acid is a factor to be considered was shown by Skeggs and Wright (1946) who found that the inclusion of 2 percent of sulfasuxidine in purified diets, deficient in folic acid and biotin, irrespective of carbohydrate source, caused a combined folic acid and biotin deficiency in rats. The effect of cellulose-supplemented diets plus sulfasuxidine on chick growth shows that the intestinal flora cannot entirely be controlled this way. The slight growth depressions at the 5, 15 and 50 percent cellulose supplemented levels may partially be attributed to a decrease in the microbial population which is possibly synthesizing the growth stimulant(s).

Chick droppings from 5 percent cellulose supplemented rations, and droppings from chicks fed the same ration plus 1 percent of sulfasuxidine did not augment normal growth when fed at 5 percent supplemental levels. The presence of urates may have counteracted any stimulatory effect from the cellulose degradation. Crude fiber values for the droppings of chicks fed the sulfasuxidine supplements indicate that cellulose-decomposing organisms were probably present. Rubin et al., (1946) reported that no growth responses were obtained when 5 percent of manure (urine plus feces) of hens or of growing chickens was fed to day-old chicks on a diet free of animal protein. McGinnis et al., (1947) found that if hen feces (manure) were incubated for 72 hours at 30° C., there was a stimulation of the synthesis of unidentified factor(s) necessary for maximum chich growth. The rations used by these workers were of a practical type containing approximately 5 percent of crude fiber. The findings reported here further substantiate that non-incubated chicken droppings do not increase chick growth. Independently, it was found that chick droppings from 1 percent of sulfasuxidine plus 5 percent cellulose supplemented rations was also non-effective as a growth stimulant.

Elvehjem and Krehl (1947) reviewed the effect of the type of carbohydrate in the diet on microbial synthesis in the gastro-intestinal tract. The results in general showed that the more insoluble the carbohydrate in the diet, the greater was the bacterial activity. This is consistent with the results obtained when a corn starch basal diet was used for 3 weeks, and no growth response resulted with cellulose supplementation. It can be inferred that there was maximum microbial activity of a particular type of flora on the corn starch basal and no additive effect was induced by cellulose.

The addition of 25 percent of ground yellow corn to a 15 percent of cellulose supplemented ration did not give a very large growth response, probably because the overall fiber content of the combined supplements offset the beneficial effects.

Ration 24 contained 5.9 percent of crude fiber and the addition of 5 percent of cellulose diluted the nutrients and increased the fiber content of the ration sufficiently to make its growth promoting activity negligible.

Jukes et al., (1947) reported that corn starch replacement of glucose in a purified turkey ration would prevent the occurrence of stickiness of head and wings which usually resulted with such diets. It was found that on a cellulose supplemented purified chick diet there was an increase in poult growth. There was also somewhat less of the sticky effect on the cellulose supplemented ration. The cellulose probably acted as a drying agent in the feed. In addition, there is the possibility that the cellulose may have contained minute quantities of the unidentified factor referred to by Jukes et al., (1947).

Mixed pine sawdust as a source of cellulose was found to give highly significant increases in chick growth at the 10 through 20 percent supplemental levels. The 10 percent level resulted in the greatest effect. It was felt that possibly a concentrated acid extract of the wood would replace the 10 percent of the sawdust, but no growth response resulted. The weights of the gizzard contents were approximately 4 to 5 grams and thereby could not account for the increased growth. Since pine sawdust, according to Ritter and Fleck (1923), contains about 52 percent of alpha cellulose, a growth response was expected at the 10 percent supplemental level.

The effect of nutrient concentration in purified chick rations was strikingly shown by supplementing low-niacin basal diets with graded amounts of cellulose. As increasing amounts of glucose through 50 percent were replaced by cellulose, the syndrome symptoms appeared less intense and there was a definite decrease in mortality. This was probably the combined result of three effects: (1) replacement of glucose by cellulose in the diets proportionately increased the niacin content; (2) replacement of the glucose by cellulose in the diets raised proportionately the level of casein, and therefore the tryptophane content also; (3) insoluble carbohydrates in the diet activated microbial synthesis in the digestive tract. Possibly some niacin was synthesized in this way. Elvehjem and Krehl (1947) reviewed the niacin-tryptophane relationship and observed that added tryptophane to a diet might spare or decrease the metabolic requirement for the vitamin. Elvehjem (1948) later pointed out that tryptophane would not be converted to niacin until all the requirements of the amino acid itself are first met. He also drew attention to the fact that the type of carbohydrate in the diet would determine the level of intestinal synthesis of the B vitamins. Ration 113a, used in these experiments, is adequate in all the nutrients known to be required by the chick.

Guerrant and Dutcher (1934) found that CellU flour added at 2, 4, 6 and 8 percent levels to a rat diet at the expense of sucrose produced a sparing effect on vitamins B and G. They believed this was due to more favorable conditions for the growth of microorganisms. These findings can now further be explained by a decrease in the utilizable carbohydrate and a proportionate increase of the vitamins in the ration.

In the experiments reported here, both the treated and untreated cellulose were equally effective in offsetting the black tongue-syndrome symptoms. This showed that the originally used cellulose was free of niacin.

Hastings (1946) reported that there was some indication that a diastasic enzyme added to a practical-type poultry ration high in fiber would make the fiber metabolically available and thereby promote growth. Diatase, such an enzyme, possessing about the same potency as the product used in Hastings' work was non-effective as a growth-promoting agent when fed at the 5 percent level of a 50 percent cellulose supplemented diet. It was thought that the enzyme might possess cellulase or/and hemicellulase activity. The high mortality obtained cannot be explained.

The growth responses obtained with ration 114 supplemented with a purified mixture of cellulases and hemicellulases were not appreciable. It is possible that the enzymes were partially destroyed in the gastro-intestinal tract and that additions greater than 10 percent would have been more effective. The mean weights of the control chicks fed equivalent amounts of inactivated enzymes showed that the somewhat greater growth was not caused by the increased level of protein or the slightly altered levels of the nutrients other than glucose. Only further work with these enzymes will determine their effectiveness in making available possible cellulosic growth stimulants for the chick.

RESULTS, PART II

A. Levulinic Acid, Furfural, Furfuramide, Xylans, d-Xylose and d + Cellobiose Experiments with Chicks

When it was found that diets which were considered complete in all the known essential nutrients required by the chick could further be improved by the addition of 5 through 15 percent of cellulose, substances associated with and degradation product(s) of cellulose were investigated.

In reviewing the literature, it was found that studies reported by Stokstad et al., (1941) indicated that additions of 1 and 3 percent of d-xylose to a simplified chick basal diet resulted in growth responses. Later, Hegsted et al., (1941), using a purified diet, found that d-xylose plus arginine stimulated the growth of White Leghorn chicks. Roe et al., (1948) developed a photometric method for the determination of pentoses in animal tissues. Their studies show that d-arabinose is physiologically available to the rabbit. In vitro studies seem to suggest that there is an enzyme system in the liver which brings about a metabolic transformation of d-arabinose. The exact nature of these transformations is unknown.

Using diet 113, it was found that 0.05 through 0.1 percent of xylans and 0.05 through 2 percent supplemental levels of d-xylose gave no chick growth responses. The results are summarized in Table IV.

Levulinic acid was fed to chicks at 0.05 through 2 percent supplemental levels in ration 113. The results, given in Table XVI, show that 0.1 percent of levulinic acid supplementation stimulated chick growth. Comparisons of the 0.1 percent of levulinic acid fed chicks with the

TABLE XV

EFFECT OF XYLANS AND d-XYLOSE ADDITIONS TO A PURIFIED DIET ON CHICK GROWTH AND FEED EFFICIENCY

Group No.	Supplement to R-113	No. chicks at 4 weeks	Av. wt. at 4 weeks	Feed efficiency
			gm.	
1	None	45	312	0.589
d-XYLOSE				
2	0.05%	10	310	0.590
3	0.1%	26	301	0.580
4	0.3%	24	307	0.583
5	0.5%	32	303	0.589
6	0.8%	10	320	0.599
7	1.0%	16	317	0.519
8	2.0%	10	325	0.608
XYLANS				
9	0.05%	12	300	0.572
10	0.1%	6	306	0.549

TABLE XVI

SUMMARY OF THE EFFECT OF LEVULINIC ACID SUPPLEMENTS IN PURIFIED RATIONS FOR CHICKS

Group No.	Level of levulinic acid added to R-113	No. chicks at 4 weeks	Av. wt. at 4 weeks	Feed efficiency	Hemo-globin
			gm.		gm./100 cc.
1	None	60	301	0.568	8.70
2	0.005%	21	301	0.514	
3	0.05%	21	307	0.559	
4	0.1%	47	342	0.548	8.98
5	0.5%	26	263	0.549	
6	0.8%	30	285	0.540	
7	1.0%	13	258	0.542	8.82

controls show statistically that the weight differences are highly significant. The statistical analysis is given in Table XVII. Hemoglobin values at the 0.1 and 1 percent of levulinic acid supplementation were within the normal range. Calculation of the feed consumption values per unit weight per chick resulted in the same magnitude for both the levulinic and control fed chicks.

Furfural was also found to be effective as a growth stimulant at the 0.1 and 0.3 percent supplemental levels. The greatest response was obtained at the 0.1 percent level as shown in Table XVIII. No statistical analysis was attempted. If the furfural supplemented feed remained in the battery room for more than 3 days, it would take on a yellowish color. This appeared to be an oxidative effect.

Little or no growth activity was obtained with d+cellobiose or furfuramide. Summations of the results are given in Table XIX. Autopsies of chicks on all levels of furfuramide showed extensive gizzard erosions.

To determine whether there was augmentation of chick growth, with the chemicals tested, furfuramide, levulinic acid, and furfural were individually added to cellulose supplemented ration 113. The results, given in Table XX, showed that 0.5 percent of furfural was partially effective when added to the diet supplemented with 15 percent of cellulose, and that 0.1 percent of levulinic acid and 0.1 percent of furfuramide when added to the ration supplemented with 5 percent of cellulose were non-effective in growth augmentation.

TABLE XVII

t TEST OF SIGNIFICANCE FOR COMPARISON OF GROWTH OBTAINED BY LEVULINIC ACID SUPPLEMENTATION TO RATION 113 WITH THAT OF CONTROL CHICKS

Supplement to R-113	Number of chicks	D. f.	Mean wt. at 4 weeks	Sum of squares
			gm.	
None	60	59	301	87,024
0.1% Levulinic acid	47	46	342	90,590
TOTAL	107	105	d = 41	177,614

t = 5.12 P = greater than 1% level.

TABLE XVIII

SUMMARY OF THE EFFECT OF FURFURAL SUPPLEMENTS IN PURIFIED DIETS FOR CHICKS

Group No.	Level of furfural added to R-113	No. chicks at 4 weeks	Av. wt. at 4 weeks	Feed efficiency
			gm.	
1	None	41	309	0.588
2	0.05%	10	319	0.578
3	0.1%	18	346	0.566
4	0.3%	14	335	0.577
5	0.5%	22	277	0.517
6	0.8%	10	292	0.555
7	1.0%	9	295	0.507
8	2.0%	10	227	0.524

TABLE XIX

EFFECT OF d+CELLOBIOSE AND FURFURAMIDE SUPPLEMENTATIONS TO PURIFIED RATIONS FOR CHICKS

Group No.	Supplement to R-113	No. chicks at 4 weeks	Av. wt. at 4 weeks	Feed efficiency
			gm.	
d+CELLOBIOSE				
1	None	37	315	0.573
2	0.005%	6	314	0.553
3	0.05%	15	303	0.575
4	0.10%	21	325	0.578
5	0.30%	10	321	0.553
FURFURAMIDE				
6	None	19	317	0.549
7	0.05%	8	314	0.534
8	0.1%	19	318	0.570
9	0.3%	23	306	0.556
10	0.5%	9	305	0.525
11	0.8%	10	227	0.526
12	1.0%	10	210	0.526

TABLE XI

THE EFFECT OF CELLULOSE SUPPLEMENTED WITH FURFURAMIDE, LEVULINIC ACID OR FURFURAL IN PURIFIED RATIONS FOR CHICKS

Group No.	Supplement to B-113	No. chicks at 4 weeks	Av. wt. at 4 weeks	Feed efficiency
			gm.	
1	None	6	311	0.571
2	5% Cellulose	6	346	0.565
3	0.1% Furfuramide	5	285	0.589
4	As (3) + 5% cellulose	6	360	0.598
5	None	9	315	0.538
6	5% Cellulose	9	365	0.549
7	0.1% Levulinic acid	8	336	0.556
8	As (7) + 5% cellulose	10	337	0.559
9	None	10	312	0.577
10	15% Cellulose	10	327	0.526
11	As (10) + 0.5% furfural	5	354	0.538

B. Levulinic Acid in Vitamins A- and B_1- Deficient Rations

One of the first experimental groups of chicks fed a purified ration supplemented with 0.8 percent of levulinic acid exhibited leg weakness and general emaciation. To determine if possibly the acid was involved in vitamins A or B_1 utilization, these experiments were conducted.

The results of feeding 0.8 percent of levulinic acid to chicks deficient in vitamins A and B_1, recorded in Table XXI, showed that the gamma keto acid neither aggravated the deficiency symptoms nor increased the mortality. The data also showed that levulinic acid did not replace the vitamins or alleviate the syndrome symptoms. Ration 114 was used in these experiments.

TABLE XXI

THE EFFECT OF LEVULINIC ACID IN DIETS DEFICIENT IN VITAMINS A AND B_1

Group No.	Supplement to diets def. in vitamins A and D	No. started	No. dead at 4 wks.	Av. wt. at 4 wks.	Feed efficiency
				gm.	
	RATION 113				
1	A (1200 units) and D_3 (170 units) by dropper weekly	20	0	319	0.556
2	0.1% Delsterol	10	7	160	0.482
3	As (2) + 0.8% levulinic acid	10	8	182	0.477
	RATION 114				
4	2% A + 0.3% D_3 in stearate carrier	20	1	302	0.547
5	As (4) minus vitamin B_1	6	6	—	—
6	As (5) + 0.8% levulinic acid	6	6	—	—

DISCUSSION

The exact reason for the growth response with 0.1 percent of levulinic acid supplementation to a purified chick ration which does not contain cellulose is as yet unknown. The fact that the feed consumed per unit weight gained per chick was approximately the same for levulinic acid and control fed chicks indicates that there was an increase in palatability of the levulinic acid supplemented feed with a resulting increase in feed consumption. From the experimental data, it can be concluded that the physiological capacity of the chick gastro-intestinal tract is not completely utilized when fed non-supplemented ration 113. Levulinic acid then may be a factor in increasing the utilization of the capacity of the digestive system.

As chicks individually vary in their wastage of feed, consumption figures may have been affected by a chance factor. Therefore, other possibilities should be considered. Levulinic acid may be synthesized to some heterocyclic metabolite which directly stimulates growth or in some way increases the utilization of the nutrients. Morton (1946) reported that levulinic acid was an excellent starting substance for the synthesis of heterocyclic compounds in vitro. He pointed out that such a product was an analogue of thiamine, but the results reported here showed that there was no observable replacement of the vitamin by levulinic acid.

The furfural growth response could have also been caused by an increase in the palatability of the feed. As with levulinic acid, the experimental feed consumption per unit weight gained per chick was approximately the same as that of the control chicks.

A possibility to be considered in both the levulinic acid and furfural experiments to explain the obtained chick growth responses is that they both, structurally, contain 5 carbon atoms. It is known that certain essential metabolites and cell constituents contain the pentose, ribose. Mitchel (1946) states that 2-desoxyribose can be converted to levulinic acid by mineral acids. Possibly the reverse takes place in the body under enzyme stimulus.

Contrary to earlier work by other workers, d-xylose under these experimental conditions did not stimulate chick growth. In the early work with pentoses, the diets used were not complete in all the chick nutrient requirements. The presence of d-xylose may have stimulated the bacterial synthesis of essential metabolites. The pentoses may be physiologically available, but there is a preference for the absorption of the hexoses (Best and Taylor, 1945). Therefore, in a diet containing more than ample amounts of glucose, it would seem that none of the xylose would be utilized.

Most likely the absence of bacterial and digestive enzymes could explain why the chick is unable to utilize xylans. Obviously, even if such enzymes were present, the non-effectiveness of d-xylose would explain the lack of chick growth response with the pentose polymer.

Furfuramide and d-cellobiose were not active as growth stimulants either. Rice and Bean (1942) showed that amide nitrogen is not readily available to the growing chick. Furthermore, furfuramide appears to be a gizzard irritant. The fact that no reducing substances could be demonstrated in the enzyme and bacteriological studies showed that cellobiose was not the responsible growth agent. The cellobiose experiments were an added check on these studies.

If levulinic acid is the growth stimulant or, possibly, one of the active substances released from the partial degradation of the cellulose, additional supplements of the keto acid would not augment the growth-promoting effect of 5 percent of cellulose. The data obtained by levulinic acid supplementation over 0.1 percent to ration 113 support these conclusions.

Furfuramide was found to possess no growth activity when added to ration 113. Therefore, as was expected, no additive effects were obtained when the compound was incorporated in a cellulose supplemented diet.

Partial growth augmentation of 15 percent cellulose supplemented rations with 0.5 percent furfural is difficult to explain. Using the same reasoning as for the explanation of the levulinic acid effect, the growth response should have been negative. Sampling may have been instrumental in the partial activity obtained. Only experimental repetition will aid in arriving at an answer to this phase of the problem.

Continuation of such studies with chemically pure substances would add much in understanding the role of fiber in poultry nutrition. From a practical point of view, the finding that inexpensive and prevalent substances such as plant fibers can be incorporated in a purified diet at the expense of a higher priced dietary ingredient such as glucose ("Cerelose") with beneficial effects on the growth and general well-being of poultry is of great value. At some future date, the "synthetic diet" for chickens and turkeys may possibly be in wide use and these findings will then be very applicable.

CONCLUSIONS

A study has been made with young chicks on the nutritive value of cellulose. Purified diets composed of all the nutrients known to be required by the chick were used.

1. The 5, 10 and 15 percent cellulose supplemental levels in a purified ration for chicks added at the expense of glucose resulted in statistically significant growth responses. The t test of significance showed that only the 5 percent level was highly significant.

2. Additions of cellulose to the complete diet and graded omissions of glucose did not give any appreciable growth stimulation.

3. Chick growth and feed efficiencies decreased when the nutrient concentration was diluted with 20 percent or more of cellulose. Increased dilution with cellulose resulted in greater feed consumption per unit weight gained per chick at 4 weeks. When the nutrient concentration was 80 percent or less, growth was dependent upon the potential energy of the ration.

4. Additions of mixed pine sawdust at the 10, 15 and 20 percent supplemental levels at the expense of glucose resulted in highly significant increases in chick growth due probably to its cellulose content.

5. Crude fiber analyses of both feed and droppings and the absence of detectable reducing substances indicate incomplete degradation of the cellulose in the chick alimentary tract. The data obtained indicate

that the stimulatory substance(s) is released during this partial breakdown of the cellulose.

6. Cellulose additions to niacin-deficient rations offset the severity of the syndrome symptoms. Mortality decreased and greater growth than the negative controls was obtained.

7. Sulfasuxidine, chick droppings, cotton flocks and ground yellow corn supplements in a purified ration did not produce growth responses. Neither was there any growth stimulation obtained with a corn starch basal nor with a practical-type ration both supplemented with cellulose.

8. Crossbred turkey poults fed a purified diet supplemented with 5 percent of cellulose grew better and showed greater feed utilization than those fed the non-supplemented ration.

9. Levulinic acid at 0.1 percent supplemental level gave highly significant growth response compared with the controls. Furfural at 0.1 and 0.3 percent levels was also effective as a growth stimulant.

10. Furfuramide, d-xylose, xylans and d-cellobiose did not possess any growth activity.

11. Levulinic acid at the 0.8 percent level did not aggravate or counteract the deficiency symptoms of either vitamins A or B_1.

12. Levulinic acid and furfuramide, both at the 0.1 percent level, did not augment the growth effect of a ration supplemented with 5 percent of cellulose. Furfural at the 0.5 percent level gave a partial additive growth effect.

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