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NUTRITIVE STUDIES ON DISTILLERS' BY-PRODUCTS

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NUTRITIVE STUDIES ON DISTILLERS' BY-PRODUCTS

Augustino D. D'Ercole

Thesis submitted for
the Degree of
Master of Science

MASSACHUSETTS STATE COLLEGE
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INTRODUCTION

The utilization of by-products from various food and beverage industries has progressed tremendously in the past ten years. The nutritive values of many of these foods have been carefully studied. While distillers' by-products have long been used as animal feeds, very little has been published on their vitamin content or on the quality of their protein.

The grain used in alcoholic fermentation is sprouted and dried. This malting process transforms starch to sugar by means of the liberated diastase. The sugar is then fermented by yeasts and is converted into ethyl alcohol. In the process, some of the salts and protein are used by the yeasts themselves. After the fermentation of the mash has been completed, the spent grains are pressed out. The product is then dried and constitutes the distillers' grains of commerce. This grain is a dark brown fibrous material having a bitter taste and a pH of approximately 4.5. The extract, or wort, is then distilled, the alcohol recovered, and the residue, which is an amber colored liquid containing yeast cells and the soluble solids not utilized by the fermentation, is concentrated and constitutes the distillers' slop under investigation. It is of a lighter brown color than the grain. The slop is concentrated to a heavy consistency under vacuum and has a pH of approximately 4.5. Normally,

in the past, the slop has been wasted. Its feed value, after vacuum concentration was one of the objects of this investigation. The sample of distillers' grains contained 31 percent protein and the sample of concentrated slop 15 percent.

PURPOSE

The main objective of the study was to determine the relative feed value of the by-products. Vitamins A, B, C, D, and G, as well as protein quality tests were made. The work was done with special reference to utilization of the by-products in poultry rations. Both rat and chick methods were used for the vitamin G determination. Since poultry requirements for vitamin G are relatively high, a good source of this factor must be used in their ration. At the present time milk by-products such as powdered skim-milk and buttermilk are often used as sources of vitamin G. A comparison of the rat and chick as laboratory animals for vitamin G, and protein quality determinations was also afforded.

REVIEW OF LITERATURE

Broughton, Frey, and Carmichael (1933) investigated an industrial alcoholic by-product resulting from cane molasses fermentation. With rats the results were negative when the

by-product was fed as the only source of protein. That is, the protein was incomplete and did not support growth. It was believed, however, that palatability of the by-product played an important part, since the rats did not seem to relish the food, although no toxic effects were noted even at the highest levels fed. However, their results show that the by-products may with certain precautions be used as a supplement for stock grains in feeding. They state that the amount of vitamin B_1 present was high, although no quantitative results were reported. Their findings suggested the use of the by-product as a supplement to corn in the feeding of hogs. It had a definitely laxative effect when fed to hogs with corn and minerals. In their first experiment with hogs, the by-product seemed to be highly palatable to the animals as compared with shelled corn. When self-fed separately, or in a mixture with fishmeal, the by-product used in the second experiment was less palatable than was fish meal. In the third experiment mixtures of ground corn and by-product 8:1, and of ground corn, by-product, and fishmeal 24:1:1, were readily eaten. The difference in palatability of the various lots suggested to the investigators that this factor might be controlled in the process of manufacturing. In the second experiment the addition of the by-product did not result in as great gains in weight as were secured from fishmeal, corn, and mineral. Substitution of the by-product for fishmeal resulted in lowered efficiency of the ration. In the third

experiment the addition of the by-product was associated with increased efficiency. Although the addition of the by-product to a ration of ground corn, fishmeal, and mineral, improved the rations, the by-product is less valuable than fishmeal as a single protein supplement for corn. They concluded that if the by-product was to be used to supplement corn in hog feeding, the corn should be ground and mixed with the by-product.

Investigations carried out by Tomhave (1936) also showed the limitations of distillers' dried grain as a supplement for hog feeding to be due to palatability. His experiments were carried out on fattening pigs. Shelled yellow corn was fed as the basal feed and was supplemented with other feeds to give a ration with a protein content of 14.5 to 14.7 percent. Comparing tankage and brewers' dried grains when alfalfa meal and linseed oil meal were used with each supplement, the pigs on the tankage ration made an average daily gain of 1.1 pounds as compared with 0.9 pounds for the pigs fed brewers' dried grains. The tankage fed pigs required 114 pounds less feed to produce 100 pounds of gain in weight. Supplementing brewers' dried grains with linseed oil meal and alfalfa meal increased daily gains 0.2 of a pound, and decreased the feed requirement to produce 100 pounds of pork 19.4 pounds. Tankage was decidedly superior to dried distillers' grains, producing 0.3 pounds greater daily gains, and requiring 117 pounds less total feeds to produce 100 pounds of gain. When the ration contained 37 percent of brewers' dried grains on 42 percent of dried distillers'

grains, approximately 45 percent of the feeds were refused. It would appear from this experiment that when dried distillers' or brewers' dried grains were used in greater amounts than 22 percent of the daily rations, the palatability of the ration was decreased.

Morrison, "in the 20th Edition (1936) of his well known book "Feeds and Feeding" states that while distillery slop is sometimes fed to fattening cattle, it is too watery to promote satisfactory gains in weight and should be fed with hay or other dry roughage in addition to at least one-third of their usual allowance of grain. Distillers' dried grains are not usually fed to swine, since they are much better suited for cattle feeding especially, dairy cows.

The literature on the nutritional value of distillers' by-products is thus very scanty. However, the investigations quoted above point to a rather poor quality protein which, when fed without supplements, did not support growth in rats and swine. The only reference to vitamin content was that of Broughton, Frey, and Carmichael (1933) who found a high (unstated) value of the vitamin B complex.

VITAMIN A

Vitamin A assays were made by the Sherman and Munsell method (1935), as described by the U. S. Pharmacopeia X (1934). Fourteen white rats not exceeding twenty -eight days of age

and weighing not less than 39 grams, nor more than 50 grams, were placed in individual metabolism cages and fed ad libitum the vitamin A-free test diet and distilled water.

VITAMIN A FREE DIET

Starch	65 percent
Vitamin free casein	18 percent
Irradiated yeast	8 percent
Crisco	5 percent
Salt mixture	4 percent

Osborne and Mendels' salt mixture was used. This mixture contains all the known mineral salts essential to the rat. At the end of a 35 day period the rats showed evidence of vitamin A deficiency characterized by weight losses, sniffles and xerophthalmia. At that time they were fed a supplement of one gram of distillers' grain per rat per day. The assay period was continued for three weeks, this period was sufficient since the animals showed continuous loss in weight. Some of the rats developed severe xerophthalmia and were killed. The total loss of the fourteen animals over the three weeks period was 71 grams, or an average loss of 5 grams per rat over a three weeks period. In order to substantiate the results of the first finding a second assay

was attempted. This time twenty rats were used; the procedure followed was the same as the above. Some difficulty was encountered in depleting the rats of their vitamin A, probably due to an overabundance of the vitamin stored in the body, Sherman and Smith (1931). However, four of the 20 rats were depleted satisfactorily of their reserve of vitamin A within the 45 days set for depleting vitamin A rats by the U. S. Pharmacopoeia, Tenth Revision Method of assay for vitamin A (1934). Of the four, two died from xerophthalmia. The observation from this experiment was that distillers' grain contained less than one Sherman unit or less than 1.4 international units of vitamin A, since only one gram levels were used. The international unit of vitamin A is 0.6 microgram of beta-carotene, whereas the Sherman unit is defined as that amount of vitamin A which when fed daily to suitable depleted rats just suffices to support a rate of weight gain of three grams per week in a standard test animal during an experimental feeding period of from four to eight weeks. Since no measurable amount of vitamin A was found to be present in one gram of the distillers' by-product, it cannot be considered even a fair source of this vitamin. Some of the sources of this vitamin A are given in Table I.

TABLE I. VITAMIN A CONTENT OF SOME FOODS

Product	Investigator	Vitamin A international units per gram
Asparagus	Fellers and Isham	1.7
Banana	Eddy	2.8
Butter	Sherman and Smith	70
Carrot	" " "	46.2
Cod liver oil	Harrison	1400
Halibut liver oil	Harrison	87200
Liver	Sherman and Smith	137
Milk	" " "	3.2
Dried peppers	Fellers and Jancik	56
Canned spinach	DeFelice	550
Chinook salmon	Harrison	8
Yellow corn	Fraps and Treichler	3.5 - 11.2
White corn	" " "	0
Dried whole milk	" " "	14
Alfalfa leaf meal	" " "	14
Yellow hominy feed	" " "	2.1
White " "	" " "	0
Lard	" " "	0
Lemons	Mellanby	0
Cottonseed oil	"	0
Kidney	"	11.2
Mushrooms		0
Sorghum silage	Fraps and Treichler	14
Wheat bran	" " "	1.4
Distillers' grains	D'Ercole	less than 1.4

VITAMIN B_1

The method used for vitamin B_1 assay was that of Chase and Sherman (1936). This method depends upon the growth of young white rats. Rats about five weeks old and weighing approximately 50 grams are used.

BASAL DIET

Autoclaved brewers' yeast	15 percent
Casein, free from vitamin-B complex	18 percent
Salt mixture (Osborne and Mendel)	4 percent
Cod liver oil	2 percent
Butter fat, washed	8 percent
Corn starch	53 percent

Twenty young white rats were placed in individual metabolism cages and fed the basal ration until they lost weight for several days. Then seven were fed 0.5 gram of spent grain and seven others fed 0.5 gram of concentrated slop for a period of five weeks. Six rats were kept as controls, all of which developed polyneuritis convulsions and died within two weeks. It required 20 days to deplete the rats. Those fed 0.5 gram of the spent grain made an average gain of 30 grams over the five week assay period. While those fed 0.5 gram of the slop made an average gain of 16 grams for the first

two weeks of the assay period. It was then thought best to decrease the level to 0.25 gram; and after two weeks on this level the average gain was only 9 grams. The animals were placed back on the 0.5 gram level for the balance of the assay period. It may be concluded that distillers' spent grains contain 4 Sherman units per gram, while distillers' concentrated slop contains 6.7 Sherman units per gram. The Sherman unit is the weight of a daily dose of vitamin B_1 preparation necessary to maintain such B_1-deficient animals and to induce in them a gain in weight of three grams per week over a period of five weeks (see Harris 1936). The relatively high amount of vitamin B_1 present in the concentrated slop is most likely due to the yeast cells present. A microscopic examination of the slop showed the presence of a great many yeast cells in various stages of disintegration.

For purpose of comparison Table II is given showing vitamin B_1 content of some foods.

VITAMIN C

The vitamin C content of the by-product was determined by the Tillmans, Hirsch and Hirsch titration method (1932) as modified by Bessey and King (1933). There was no significant amount of vitamin C present in the grain when 5 gram and 10 gram samples were used. After a series of tests the slop

TABLE II. VITAMIN B_1 CONTENT OF FOODS

Source	Investigator	Sherman-Bourquin units per gram
Dried distillers' grains	D'Ercole	4.0
Dried distillers' slop	D'Ercole	6.7
Milk	Roehm	0.2 - .5
Pasteurized milk	Todhunter	0.74
Evaporated milk	"	1.32
Whole eggs	"	1.30
Dried prunes	Poe	1.2
Canned tomato juice	Coe and Gawbill also Hanning	0.21 - .25
Beets	"	0.12
String beans	"	0.30
Spinach	"	0.40
Peas	"	0.40
Pineapple juice	"	0.10
Lettuce (dry)	Munsell and Kennedy	0.24 - 1.18
Cranberries	Fellers and Isham	trace
Grapefruit	Roehm	0.4
Broccoli	Munsell and Kifer	3 - 4
Rabbit, lean pork, chicken	Sebrell, Wheeler and Hunt	good
Cottonseed meal	"	poor
Haddock	Wheeler	poor
Apples	Day and Darby	poor
Pears	" " "	fair
Dried Brewer's yeast	Quinn, Whalen and Hartley	10 - 15
" " "	Aykroyd and Roscoe	15 - 25
Fish, beef steak	Stiebeling also Aykroyd and Roscoe	0.5 - 1.0
Beef liver	Day	3.5 - 8
Whole wheat	Bourquin	1.5
Corn	Goldberger, Wheeler, Lillie and Rogers	poor
Skim milk powder	Aykroyd and Roscoe	3 - 5

was found to contain between 0.035 and 0.06 milligrams of ascorbic acid per gram. The international unit of vitamin C is 0.05 milligrams of ascorbic acid, therefore the concentrated distillers' slop contained between 0.7 and 1.2 international units of this vitamin. Vitamin C is of little significance in the nutrition of poultry, cattle, hogs, and dogs. Guinea pigs and humans require it in relatively large quantities.

Inasmuch as no published reports on the vitamin C content of distillers' by-products were found, it was deemed of scientific interest to evaluate these products. For comparative purposes the vitamin C (ascorbic acid) content of some foods and feeds is given in Table III.

VITAMIN D

The U. S. P. method of assay was employed for vitamin D assay (1934). Thirty-two young white rats weaned at from 21 to 23 days and weighing approximately 40 grams were placed on the Steenbock rickets-producing rations No. 2 consisting of:

Whole yellow maize, ground	76 percent
Ground gluten	20 percent
Calcium carbonate	3 percent
Sodium chloride	1 percent

Rats were kept on this diet for 20 days at which time they manifested evidence of rickets as shown by enlarged joints and wabbly gait. At this time the rats were divided

Table III. VITAMIN C CONTENT OF SOME FOODS AND FEEDS

Product	Vitamin C content international units
	per gram
Distillers' grains	none
Distillers' concentrated slop	0.7-1.2
Dry beans	none
Fresh spinach	10-13
Green corn fodder	2-5
Dried corn fodder	trace
Fresh milk	.2
Powdered skim milk	trace
Fresh apples (25 varieties)	0.4-3
Wheat, barley, oats, soybeans	none
Malted barley	.5-2
Raw cabbage	12
Orange juice	6-8
Meat scrap and fish meal	none
Fresh liver	5
Soybeans, sprouted	3
Green alfalfa	5-10
Dried fruits	trace

Note: Data obtained from the following sources:
Vitamins, A Survey of Present Knowledge. Med. Research Council Gt. Britain 1932, 332 p. and
Sherman, H. C. and Smith, S. L. The Vitamins. Chem. Catalog Co., New York. 575 p.

into four groups of eight each and individually housed. One group was then fed the same basal ration supplemented by 0.25 gram of the distillers' slop for eight days, two groups of rats received 0.5 and 1.0 gram, respectively, of distillers' grains, while the fourth group served as a positive control and received daily 28 milligrams of U. S. P. Reference oil. This amount of Reference oil contains 2.7 international units of vitamin D. On the eleventh day of the assay proper, the rats were killed and the tibiae were removed and placed in 95 percent alcohol overnight. They were then treated by washing in distilled water, and the bones were split longitudinally on the proximal end so that a plane surface was exposed through the junction of the epiphysis and diaphysis. One section of the bone was again rinsed in distilled water and allowed to stand in a solution of 2 percent silver nitrate for three minutes. They were then exposed to a strong light until the calcified areas turned dark. Records were then made immediately as to the extent and degree of calcification of the rachitic bones. The rats were weighed at the beginning and end of the 10-day assay period. All the rats gained weight. The results are given in Table IV.

Table IV is self-explanatory. There seems to be a definite calcifying action resulting from the feeding of both the distillers' grains and concentrated slop. Whether this action is due to vitamin D alone cannot be definitely stated. It is true that some factor present acted like vitamin D.

Table IV. VITAMIN D CONTENT OF DISTILLERS' GRAIN AND CONCENTRATED SLOP

Group and level	No. of rats	Av. wt. gain	Av. degree of calcification	Steenbock units per g.	Intern.* units per g.
0.25 gram distillers' concentrated slop	8	10	1.9	0.6	6.4
0.5 gram distillers' grains	8	12	2.4	0.8	4.2
1.0 gram distillers' grains	8	11	2.3	0.8	2.1
28 mgs. U. S. P. Reference oil	8	9	3.0	1.0	95.0

* 1 Steenbock unit is equivalent to 27 international units of vitamin D.

The amount present is small and corresponds to that found in average milk. Vitamin D is not widely distributed in nature, particularly in the plant world. Grasses, grains, and legumes have been reported to contain only very small amounts. The best sources are fish liver oils, egg yolk, irradiated yeast, butter and ultraviolet light.

In conclusion, it is possible to state that there is sufficient vitamin D in the distillers' by-products to be of some slight feeding value to poultry and animals.

VITAMIN B_2 (G)

The method for vitamin G assay used was that of Sherman and Bourquin (1931). It is essentially a method for the assay of the growth-promoting factor of the vitamin B_2 or G. White rats weighing 40 to 50 grams, and about 28 days old, were placed in individual cages and fed the following vitamin B-complex free diet:

Casein (free from vitamin B complex)	18 percent
Salt mixture (Osborne and Mendel)	4 percent
Cod liver oil	2 percent
Butter fat (washed)	8 percent
Corn starch	68 percent

Each rat was fed one drop of tiki-tiki daily as a source of vitamin B_1. The rats were kept on this diet until they ceased to gain weight, usually from four to five weeks. Then,

the curative period of the assay was started, by feeding them a definite amount of the product under study. This was continued for a period of five weeks.

The Sherman-Bourquin unit for vitamin G is the daily dose of a material containing this dietary supplement which will induce a gain of three grams per week for a period of five weeks. There is no international unit of vitamin G. In testing the by-products this method was followed. It took approximately four weeks to deplete 20 white rats of their vitamin G reserve. Then, eight were fed 0.5 grams of distillers' grain daily, eight more were fed 0.5 grams of concentrated slop daily, and the remaining four were used as control rats. Those fed 0.5 grams of concentrated slop made an average gain of 4.5 grams per rat per week, while those on the grain made an average gain of 3.6 grams per rat per week. In Sherman-Bourquin units the distillers' grains contain 2.4 units, whereas the concentrated slop contains 3 Sherman units.

These results are summarized in Table V. Both these by-products are good sources of vitamin G and should be valuable additions to poultry and animal diets in supplying vitamin G. A comparison with other foods and feeds is given in Table VI.

Table VI. VITAMIN G CONTENT OF FOODS

Source	Investigator	Sherman-Bourquin units per gram
Milk	Roehm	0.2-0.5
Pasteurized milk	Todhunter	0.74
Evaporated milk	"	1.32
Whole eggs	"	1.30
Dried prunes	Poe	1.20
Canned tomato juice	Coe and Gawbill	0.21-0.25
Beets	Hanning	0.12
String beans	"	0.30
Spinach	"	0.40
Peas	"	0.40
Pineapple juice	"	0.10
Lettuce, dry	Munsell and Kennedy	0.24-1.18
Cranberries	Fellers and Isham	trace
Asparagus	Fellers	0.62
Grapefruit pulp	Roehm	0.40
Broccoli	Munsell and Kifer	3-4
Lean pork and rabbit	Sebrell and Wheeler	good
Cottonseed meal	" " "	poor
Haddock flesh	Wheeler	poor
Apples	Day and Darby	poor
Pears	" " "	fair
Dried brewers' yeast	Quinn, Whalen and Hartley	10-15
Dried brewers' yeast	Aykroyd and Roscoe	15-25
Fish and beefsteak	Stiebeling, also Aykroyd	0.5-1.0
Beef liver	Day	3.5-8.0
Whole wheat	Bourquin	1.5
Corn	Goldberger, Wheeler and Lillie	poor
Skim milk powder	Aykroyd	3-5
Distillers' feed	D'Ercole	2.4
Distillers' concentrated slop	D'Ercole	3.0

Table V. VITAMIN G CONTENT OF DISTILLERS' BY-PRODUCTS

Product	No. of rats	Amt. supplement fed daily	Av. wt. change per week	Sherman-Bourquin units per gram
Distillers' grains	8	0.5	3.6	2.4
Distillers' concentrated slop	8	0.5	4.5	3.0
Negative control	4	0	*	---

* 3 rats died within 10 days and 1 rat showed severe pellagra symptons.

VITAMIN B_2 (G) NORRIS-WILGUS CHICK METHOD

The method for vitamin G chick assay used was that developed by Norris and co-workers (1936) which determines the relative growth promoting vitamin G content of feed stuffs based upon the gain produced over the negative control diet by adding 5 to 15 percent of the supplement under study. Sixty Hubbard Strain New Hampshire Red Chicks were grown to two weeks of age on a ration deficient in vitamin G. Preliminary work by Norris and co-workers (1936) showed that this period was adequate to deplete chicks of their natural reserve of this factor. The chicks were then distributed into five groups of 11 to 15 chicks, all weighing from 88 to 95 grams each. After the distribution, the chicks were individually banded, reweighed, and then fed the basal ration, supplemented by the materials under study. One group was continued on the basal ration to serve as the negative control group. The basal ration was nutritionally complete except for the vitamin G complex. It was markedly deficient in the anti-pellagric one. Nutritional paralysis occurred to a moderate extent but was not severe enough to affect growth results. Norris and co-workers showed that an assay period of six weeks was too long since growth rates slowed up toward the end of that time and the need for vitamin G was reduced. However, they found four weeks to be suitable as the slowing up effect was not evident at the end of that period.

The basal diet had a protein content of 25 percent.

NEGATIVE CONTROL BASAL DIET

	Percent	Percent
Ground yellow corn	60	6
Wheat flour middlings	20	2.6
Purified casein (vitamin G-free)	15.5	14.2
Steamed bone meal	2	slight amt.
Pulverized limestone	1	0
Salt, iodized	0.5	0
Cod liver oil	1	0

This diet contains approximately 33 percent of the vitamin G requirement of chicks. The supplement supplies the balance of the vitamin G. The supplements were incorporated in the other diets in such a way as to keep the protein content approximately 25 percent. This was accomplished by making adjustments in the corn and casein which would compensate for the protein content of the supplements under study.

The vitamin G-free casein was prepared in the following manner:

Six kilograms of technical casein were placed in a wash boiler which was filled with tap water. To this was added 100 grams of NaOH and mixed with the casein. The boiler was heated in a water bath at 124°F. for 24 hours

with frequent stirring. When 226 cc. of HCl were added to neutralize the NaOH present. The casein was recovered by filtration through cheese cloth, washed four times with large volumes of cold water, and again filtered through cheese cloth. The casein was then placed in shallow pans and dried in a tunnel dryer at 140°F. When dry it was ground to a fine powder. Norris (1936), found that dried pork liver contained approximately 100 micrograms of flavin per gram. It was taken as a standard and given the value of 100 units of vitamin G per gram. The vitamin G content of the distillers' by-products was determined by comparing their potency with that of the reference dried pork liver as recommended by Norris (1936). The composition of the diets of the several groups of chicks are given in Tables VII, VIII, IX, and X. The protein content of the several ingredients was obtained from the Feed Control Laboratory.

After the two weeks depletion period, the chicks were grouped into five lots, with 12 chicks to the lot. They were fed their respective rations as given above, and weighed each week for the four week assay period. The results are given in Table XI, and Figure 1.

A correlation between Sherman-Bourquin vitamin G rat units and Norris-Wilgus chick units for vitamin G has not been established. However, from this limited comparison it would seem that five Norris-Wilgus chick units of vitamin G are approximately equal to one Sherman-Bourquin rat unit.

Table VII.

Positive Control Diet Containing Dried Pork Liver

	Per-cent	g. per 100 g. of diet	Per-cent protein	g. protein per 100 g. diet	Morris-Wilgus chick units vit. G per 100 g. diet
Ground yellow corn	57.0	57.0	10.0	5.7	57
Wheat flour middlings	20.0	20.0	13.0	2.6	40
Purified casein (vitamin G-free)	16.2	16.2	97.0	15.6	0
Steamed bone meal	2.0	2.0	slight amount	-	0
Calcium carbonate	1.0	1.0	0	0	0
Salt (iodized)	0.5	0.5	0	0	0
Cod liver oil	1.0	1.0	0	0	0
Dried pork liver	2.3	2.3	76.0	1.7	230
Total	100.0	100.0	-	25.6	327

Table VIII.

Diet Containing Distillers' Grains as a Source of Vitamin G

	Per-cent	g. per 100 g. of diet	Per-cent protein	g. pro-tein per 100 g. diet	Norris-Wilgus chick units vit. G per 100 g. diet
Ground yellow corn	51.5	51.5	10.0	5.4	54.5
Wheat flour middlings	20.0	20.0	13.0	2.6	40.0
Purified casein, (vitamin C-free)	14.0	14.0	97.0	13.5	
Steamed bone meal	2.0	2.0	slight amount	-	
Calcium carbonate	1.0	1.0	0	0	
Iodized salt	0.5	0.5	0	0	
Cod liver oil	1.0	1.0	0	0	
Distillers' grain	10.0	10.0	31.0	3.1	to be determined
Total	100.0	100.0	-	24.6	-

Table IX.

Diet Containing Concentrated Distillers' Slop as a Source of Vitamin G

	Per-cent	g. per 100 g. of diet	Per-cent protein	g. pro-tein per 100 g. diet	Norris-Wilgus chick units vit. G per 100 g. diet
Ground yellow corn	44.5	44.5	10.0	4.4	44.5
Wheat flour middlings	20.0	20.0	13.0	2.6	40.0
Purified casein, (vitamin G-free)	16.0	16.0	97.0	15.5	
Steamed bone meal	2.0	2.0	slight amount	-	
Calcium carbonate	1.0	1.0	0	0	
Iodized salt	0.5	0.5	0	0	
Cod liver oil	1.0	1.0	0	0	
Concentrated distillers' slop	15.0	15.0	15.0	2.4	to be determined
Total	100.0	100.0	-	24.9	-

Table X.

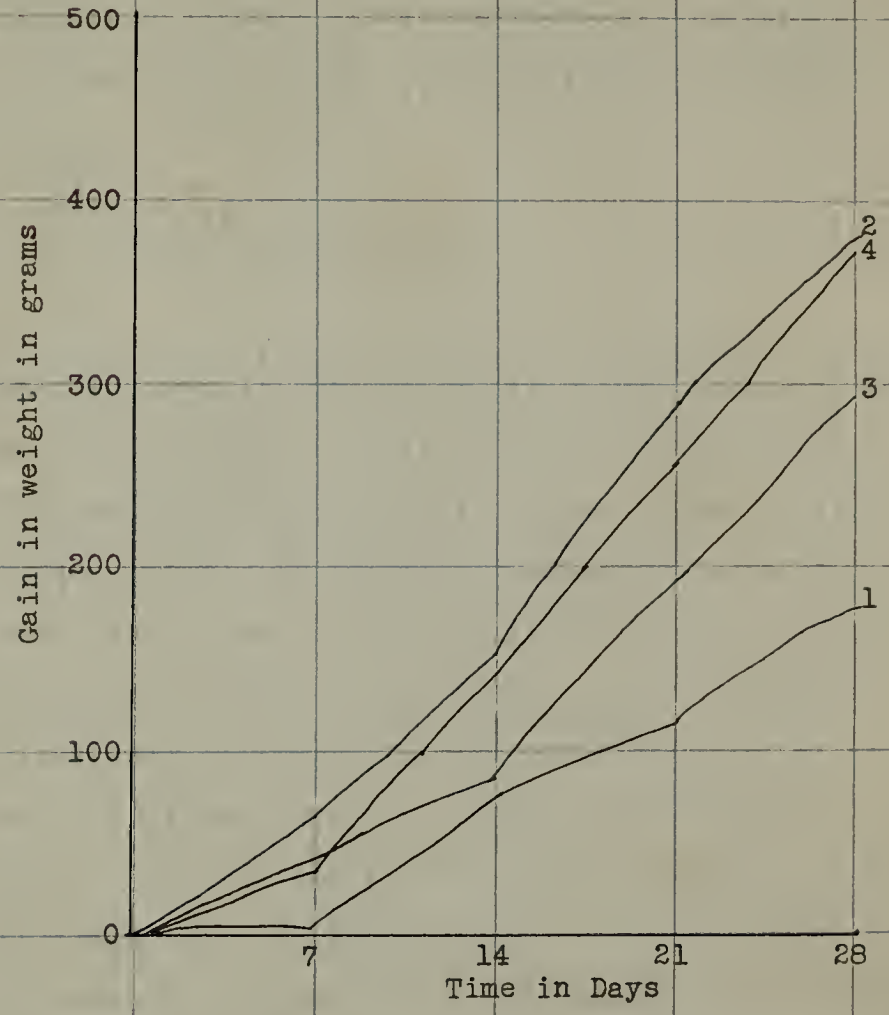
Diet Containing Dried Buttermilk as a Source of Vitamin G

	Per-cent	g.per 100 g. of diet	Per-cent protein	g. protein per 100 g. diet	Norris-Wilgus chick units vit. G. per 100 g. diet
Ground yellow corn	51.1	51.1	10.0	5.1	51
Wheat flour middlings	20.0	20.0	13.0	2.6	40
Purified casein, (vitamin G-free)	13.4	13.4	97.0	13.0	
Steamed bone meal	2.0	2.0	slight amount	-	
Calcium carbonate	1.0	1.0	0		
Iodized salt	0.5	0.5	0		
Cod liver oil	1.0	1.0	0		
Dried buttermilk	11.0	11.0	33.8	3.4	to be determined
Total	100.0	100.0	-	24.1	-

Table XI. Results of Vitamin G Chick Assays.

Product	Feeding level of ration	Av. total Wt. at 6 weeks	Av. gain wt. over negative control.	Norris-Wilgus chick units per gram
	percent	grams	grams	units
Negative control (basal diet only).	-	269	-	0
Positive control (dried pork liver).	2.3	468	199	100
Distillers' grains.	10	391	122	14
Distillers' slop.	15	471	202	15.6
Dried buttermilk.	11	419	241	15.3

Figure 1. Influence of Amount of Source of Vitamin G on Chick Growth



1. Negative control - 100 units vitamin G per 100 grams.
2. Positive control - 325 units vitamin G per 100 grams
3. Distillers' grain (spent) fed 10% of the diet.
4. Distillers' slop - fed 15% of the diet.

Product	Norris-Wilgus Vitamin G Chick Units per Gram	Sherman-Bourquin Vit. G Rat Units per Gram
Distillers' grains	14	2.4
Concentrated slop	15.6	3

More work must be done before any definite statement can be made concerning a factor for converting rat units to chick units.

OTHER OBSERVATIONS

As stated by Norris (1936), paralysis of the legs does occur in chicks on vitamin G assay to a moderate extent, but it is not severe enough to affect normal growth in young chicks a few weeks old. No slipped tendons were noted in the positive control group, the distillers' grains, nor in the concentrated slop groups. Three chicks on the negative control and one chick on the dried buttermilk diet did show evidence of slipped tendons. Severe leg paralysis was noted in the negative group and slight paralysis in the dried buttermilk group. Gizzard erosion occurred in many cases, this, however, was not due to a vitamin G deficiency. Bird (1936) and others concluded that the anti-gizzard erosion factor occurs abundantly in pork lung, liver and kidney, and moderately in oats, wheat and corn. It is insoluble in ether and in ethyl alcohol, but follows the alkali-soluble precipated proteins in the fractionation of lung tissue.

RAT PROTEIN QUALITY TESTS

The method for protein assay used was that taken from Daniel and McCallum (1931), and Cleveland (1934). The method of preparing the rations was taken from the work done by Cleveland (1934). The ration common to all the diets including the control diets:

Salt mixture	4 percent
Cod liver oil	2 percent
Agar agar	2 percent
Treated starch	12 percent
Butter fat (washed)	3 percent

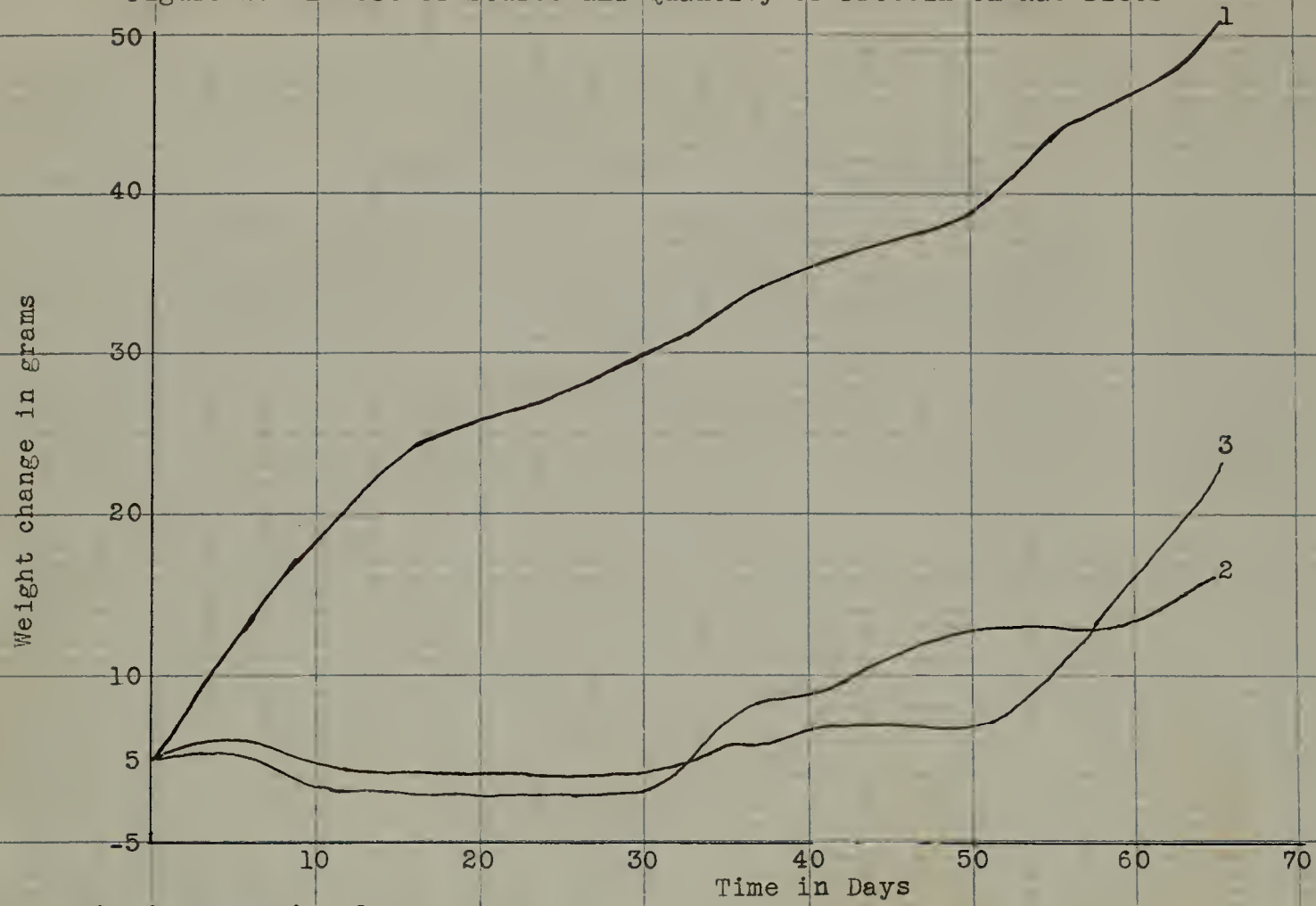
The Osborne and Mendel salt mixture was used as given in the U. S. Pharmacopeia X (1934). Alcoholic wheat germ extract was dried on cornstarch as described by Daniel and McCallum (1931). The control rations contained casein as the sole source of protein. The control diet supplement contained 10 percent casein and 67 percent corn starch.

The distillers' grains diet was fed at two levels, as the sole source of protein. The distillers' grains diet at the 15 percent protein level contained 53.2 percent distillers' grains and 23.8 percent corn starch. The distillers' grain diet at 10 percent protein level contained 35.5 percent distillers' grains and 41.5 percent corn starch.

Three groups of rats, six rats to a group, were used in this experiment. They were kept in individual cages and

fed their respective rations and distilled water for a period of two months. They were weighed every five days and records of their food intake were taken. The rats fed on the 10 percent distillers' grain diet gradually lost weight, so, after the thirtieth day, the ration was supplemented with 5 percent protein from skim milk powder. They were left on this diet for 10 days. At the end of 10 days the ration was changed to 5 percent level from the distillers' grains together with 5 percent skim milk powder (not skim milk protein). Those rats fed the distillers' grains at a 15 percent protein level also lost weight, and after the fortieth day their ration was supplemented with one percent skim milk powder. They failed to make normal growth gains. After the fiftieth day, the rations were supplemented with 8 percent skim milk powder. The rats promptly gained weight. Figure 2 shows the growth rates more clearly. The data show that the protein from distillers' grains was incomplete, that is, it lacked some of the essential amino acids. As indicated in Figure 2, growth was merely maintained, but weight gains were not made unless the distillers' grains were supplemented with complete proteins such as those present in skim milk powder. In conclusion, from this experiment it appears that it is probably unsafe to feed distillers' grains to animals as a sole source of protein, or even as the principal source. Fed in amounts of up to 33 percent of the total protein, the distillers' grains may be used as a satisfactory protein supplement.

Figure 2. Effect of Source and Quantity of Protein on Rat Diets



1. 10% protein from casein alone.
2. 10% protein from distillers' grains alone for 30 days; 30-40 days, ration supplemented with 5% skim milk powder; 40-65 days ration changed to 5% protein from distillers' grains + 5% skim milk powder.
3. 15% protein from distillers' grains alone for 40 days; 40-50 days, ration was supplemented with 10% skim milk powder; 50-65 days' ration was supplemented with 8% skim milk powder.

CHICK PROTEIN QUALITY TESTS

Distillers' by-products were not used in this chick experiment as a sole source of protein, as was done in the rat experiment, but was planned to test the value of the by-products as a supplementary source of protein for growing chicks. This method was used because chick rations are normally varied in character and consist of several different sources of protein. The method used was patterned after the work of Cleveland (1934). The New England College Conference Chick Mash was used as a control, and the distillers' by-products were incorporated into a modification of this mash, leaving out the meat scrap of the experimental diets completely, and making such changes in the fish meal and dried skim milk content as would allow a total protein content of 18 percent, with the distillers' by-products contributing about one-third of the total protein. Both the distillers' grain and the concentrated slop were assayed in this experiment. Since both the slop and grains were found to be good sources of vitamin G, information regarding the protein value was necessary to ascertain whether the by-products could furnish satisfactory quality protein as well as serving as a good source of vitamin G.

Thirty of the M. S. C. strain Rhode Island Red chicks, two to three days old and weighing between forty and fifty grams, were distributed into three groups, ten to a group.

They were individually weighed, banded, placed in heated battery brooders, and fed their respective rations. The diets are shown in Table XII. The chicks were weighed weekly. The results were clear cut, and after four weeks the feeding was discontinued. Sufficient data were at hand to show rather conclusively that distillers' by-products cannot be used as a principal source of protein in chick feeds, at least at so high a level as one third of their total protein requirements. It was evident even at the end of the second week that their growth rates slowed up to a considerable degree as compared with those fed on the New England College Conference Chick Mash. As a result of this chick growth experiment, it is evident that the protein of distillers' by-products is not a complete protein, and that it does not promote growth in young chicks when fed at a level of one third of their total protein requirements. In this regard, the chick data are in close agreement with the results just reported on rats. The results are given in Table XIII and Figure 3.

Table XII. Composition of Chick Rations Used in Protein Quality Experiment

Ingredient	Control ration N.E. college conference chick mash	Distillers' concd. slop constituting 7% of the total protein	Distillers' grains constituting 7% of total protein
	percent	percent	percent
Corn meal	29.54	1.4	23.20
Oat groats	14.77	14.77	14.77
Wheat middlings	14.77	14.77	14.77
Meat scrap	7.39	-	-
Fish meal	3.69	4.00	-
Dried skim milk	7.39	7.31	5.0
Alfalfa meal	3.70	3.7	3.7
Limestone	2.21	2.21	2.21
Salt	.74	.74	.74
Cod liver oil	1.03	1.03	1.03
Distillers' concd. slop	-	40.00	-
Distillers' grains	-	-	20.00

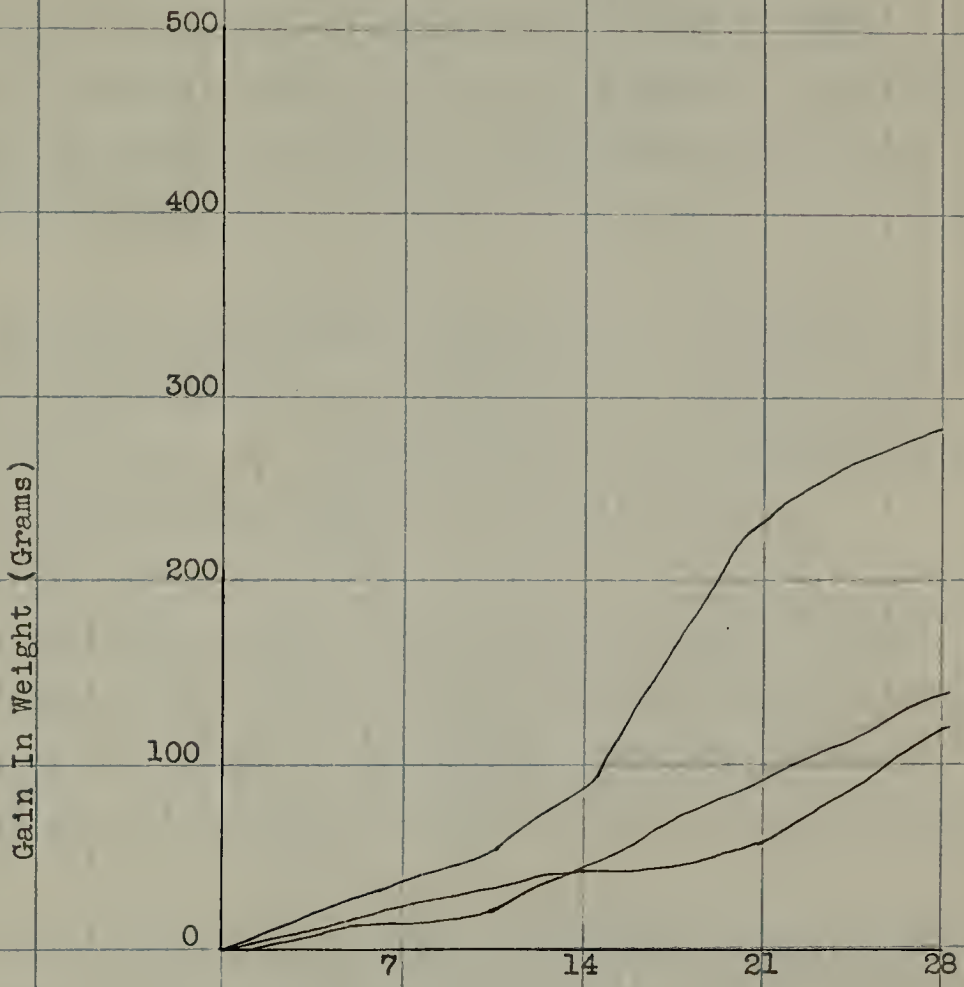
Table XIII. Weight Gains in Chicks Fed Distillers' By-products so as to Furnish 33 percent of the Total Protein (18 Percent).

Ration	7 days	14 days	21 days	28 days
	wt. grams	wt. grams	wt. grams	wt. grams
N. E. College Conference Ration	82	135	280	325
Distillers' grains to furnish 1/3 of total protein	60	89	139	185
Distillers' concd. slop to furnish 1/3 of total protein	68	89	102	173

Figure 3--Effect of Source of Protein on Chick Growth

Distillers' By-Products Constitute 33

Percent of the Total Protein



1. Positive control-Fed-N.E. College Conference Chick Mash.
2. Fed distillers' grains at one third of total proteins.
3. Fed distillers' slop at one third of total protein.

Total protein content of diets was 18 percent.

GENERAL SUMMARY

1. A summary of the vitamin results are given in Table XIV. Distillers' grains are very good sources of vitamin B and G, and poor sources of vitamin A. Both products contain small quantities of vitamin D. The grains contain no vitamin C, though the concentrated slop had a moderate amount.

2. There is a relationship between the Sherman-Bourquin rat unit and the Norris-Wilgus chick unit of vitamin G. This work showed that one Sherman-Bourquin unit is approximately equivalent to 5 to 6 Norris-Wilgus chick units.

3. Distillers' grains used as the sole source of protein for rats did not promote growth, but merely maintained the original body weight of the animal. In order to promote growth in rats supplements of other proteins were necessary.

4. Distillers' grain and concentrated slop cannot be used as a supplementary source of protein for growing chicks, when fed at a level of one third of their total protein requirements. If the percentage of distillers' by-products in a chick is small, it is probable that normal growth will occur.

5. The concentrated slop when mixed in rations definitely reduced the palatability of the ration.

Table XIV. Summary of Vitamin Results

Determination	Distillers' Grain	Distillers' Slop
	international units per gram	international units per gram
Vitamin A	less than 1.4	less than 1.4
Vitamin D	approximately 4	approximately 4
Vitamin C	none found	0.7 - 1.2
Vitamin B	4 Sherman units	6.7 Sherman units
Vitamin G (rat)	2.4 Sherman units	3.0 Sherman units
Vitamin G (chick)	14.0 chick units	15.6 chick units

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