CERTAIN NUTRIENT RELATIONSHIPS INVOLVED IN THE SUPPLEMENTATION OF DIETS FOR SWINE AND RATS

By

Ronald Edward Bloss

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies
of Michigan State University of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Husbandry

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Approved J. A. Hoefer

ABSTRACT

PART I. SUPPLEMENTATION OF A CORN-MEAT AND BONE SCRAP RATION FOR WEANLING PIGS

In recent years considerable evidence has accumulated to indicate that meat and bone scraps are an unsatisfactory sole source of supplemental protein in corn based diets for growing swine. The chemical analysis of various feed ingredients in swine rations revealed the fact that meat and bone scraps are quite variable in their content of the amino acid tryptophan, suggesting that this amino acid might be responsible for the poor results often noted.

A corn-meat and bone scrap diet supplemented with minerals and vitamins was found to be inadequate for the growth of weanling pigs in drylot in two separate trials. The addition of 0.06% of DL-tryptophan to the above diet allowed growth to proceed at the same rate as that observed with a corn-soybean oil meal diet - the positive control. On the basis of this study it appears that for growing pigs 30 to 70 pounds in weight, the requirement for tryptophan lies close to 0.13% with a diet containing 18% protein and 75% total digestible nutrients. The antibiotic Aureomycin when included in a tryptophan deficient diet had no significant effect on rate of growth.

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ABSTRACT Continued

PART II. SUPPLEMENTATION OF A BABY PIG DIET WITH A VITAMIN B_{13} CONCENTRATE AND ISONICOTINIC ACID HYDRAZIDE

The anti-tubercular compound, isonicotinic acid hydrazide, had been shown to stimulate both appetite and weight gains in tubercular patients. If this effect held true for pigs it would have considerable significance in the production of pork. Since its discovery in 1948, vitamin B_{13} had been shown to stimulate the growth of rats, chicks and hogs. It remained to be determined if the baby pig would show a similar response.

A "synthetic milk" composed of "vitamin-free" casein, Cerelose, lard, minerals and vitamins supported excellent growth and feed efficiency in baby pigs during a 26 day trial. The inclusion of a "vitamin B_{13} concentrate" in this milk at a level of 0.05 ml. per 100 gm. of dry matter had no effect on the pigs. Isonicotinic acid hydrazide or Aureomycin hydrochloride included in the "synthetic milk" at 5 mg. per 100 gm. of dry matter had no effect on the performance of the pigs. No toxic side effects from the isonicotinic acid hydrazide were noted.

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ABSTRACT Continued

PART III. SOME INTERRELATIONSHIPS OF PANTOTHENIC ACID, METHIONINE AND PROTEIN IN THE RAT

Research work with growing rats had suggested that protein or methionine has a sparing effect on the need for pantothenic acid. Since there is good evidence to indicate that vitamin B_{12} is interrelated in the metabolism of both pantothenic acid and methionine it appeared that this point should be checked using diets containing a known and adequate amount of vitamin B_{12}.

Weanling rats were fed a pantothenic acid deficient purified diet composed of a soybean protein preparation, DL-methionine, Cerelose, lard, vitamins and minerals and containing 18% of protein. Animals receiving this diet exhibited relatively poor growth with no mortality during a 35 day trial. Increasing the level of protein in the diet to 28% appeared to depress growth. The addition of supplementary amounts of DL-methionine to either of the basal diets also reduced growth. Supplementation of the low and high protein basal diets with a suboptimal level of pantothenic acid resulted in a significant improvement in weight gains and a definite improvement in feed utilization. Adding both pantothenic acid and DL-methionine to the basal diets showed no further improvement in growth over that achieved with pantothenic acid alone.

Under the conditions of this trial neither protein nor methionine in excess of the rat's requirements spared the need for pantothenic acid.

Ronald E. Bloss

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PART I. SUPPLEMENTATION OF A CORN-MEAT AND BONE SCRAP RATION FOR WEANLING PIGS

INTRODUCTION

For many years protein by-products of the animal packing plant industry have been an important part of most swine rations. Early studies demonstrated that tankage or meat scrap aided in balancing the nutritional deficiencies of corn for growing swine. These products provided, in addition to essential amino acids, significant quantities of essential minerals and vitamins so that early studies comparing various protein supplements were complicated with factors other than protein quality or amino acid balance.

Since the early 1940's there has developed a belief among most animal and poultry nutritionists that meat scraps and tankages have not produced the uniformly good results previously obtained. Robison (1943) reported that in nine experiments conducted with swine up to 1920, pigs fed corn and tankage in dry-lot made an average daily gain of 1.30 pounds and required 389 pounds of feed per 100 pounds gain. In 16 similar experiments conducted later, the average daily gain was only 1.02 pounds and 410 pounds of feed were consumed per 100 pounds gain. It is a generally accepted view that the reason for this reduction in performance through the years is due to the fact that the packing plant by-products now contain lesser amounts of hearts, livers, kidneys, beef trimmings, and glandular materials, all of which supply good quality protein.

The analysis of various feed ingredients in swine rations indicated that meat and bone scrap was variable in its content of the amino acid tryptophan. Very often it was found that samples of meat and bone scrap contained insufficient tryptophan to meet the needs of the growing pig if used as the only protein supplement in a corn based ration. It may be that the poor results sometimes obtained with a corn-meat scrap ration could be explained on the basis of a tryptophan deficiency.

REVIEW OF LITERATURE

As early as 1935 Almquist *et al.* (1935) found that meat scrap, when used as a protein supplement in chick rations, gave unsatisfactory results. These workers stated that the cystine and tryptophan content of this animal protein concentrate could not be used to predict its nutritive value. Kraybill and Wilder (1947) carried out investigations with rats which indicated that some samples of meat scrap may be deficient in methionine and tryptophan. Using swine-type rations Kraybill (1947) fed different samples of meat scrap to rats and concluded that the growth response was related to the tryptophan content of the meat scrap, however, this was not found to be true with chicks. March *et al.* (1949) investigated four market samples of meat meal on the basis of a protein quality index, amino acid assays, and chick biological tests. According to the results of all three methods there was considerable variation in the nutritive value of the meat meals. The relatively low nutritive value of the meat meal protein, when compared with fish meal, was considered to be partly the result of deficiencies of tryptophan or methionine or a combination of these amino acids. Later March *et al.* (1950) fed meat scrap as the only source of animal protein to chicks and observed no growth response when the diets were supplemented with either methionine or tryptophan. The addition of lysine did result in improved growth, and adding methionine in conjunction with the lysine resulted in an additive response.

Beeson et al. (1949) using a tryptophan deficient purified ration showed that tryptophan was an essential amino acid for the weanling pig. The lack of tryptophan in the diet of the pig resulted in a decrease in feed efficiency and feed consumption and also caused weight loss. Subsequently Shelton et al. (1951) observed that a level of 0.2% DL-tryptophan, when added to a tryptophan deficient purified diet containing 24.5% protein and adequate niacin, supported maximum growth under the conditions of the experiment. A level of .1% DL-tryptophan added to the basal diet proved to be inadequate.

In studies with chickens Wilkening and Schweigert (1947) concluded that D-tryptophan is utilized to an extent of from 17% to 40%. The percentage of utilization appeared to be slightly higher at lower levels of the test compound. Kratzer et al. (1951) found that D-tryptophan was approximately 30% as active as L-tryptophan in promoting growth of young turkey poults. Utilizing baby pigs and employing a synthetic milk deficient in tryptophan Reber et al. (1951) studied the utilization of D-tryptophan by a nitrogen balance technique. On the basis of their findings it was concluded that little if any use was made of the D-isomer of tryptophan in promoting N retention on a diet deficient in this amino acid. Thompson et al. (1952) conducted similar studies and conversely concluded that partial utilization of D-tryptophan was effected but they were unable to assign a value to the degree of utilization.

Luecke et al. (1947) and Powick et al. (1948) demonstrated the importance of the niacin-tryptophan relationship in swine receiving diets high in corn. The latter authors concluded that nicotinic acid did not appear to be completely effective as a substitute for tryptophan, but tryptophan appeared to be, in large measure at least, a satisfactory substitute for nicotinic acid.

Cunha and coworkers (1949) reported a sparing action upon the methionine needs of the pig when a crude APF supplement (containing an antibiotic) was added to a corn-peanut meal basal ration. Using battery reared chicks fed practical type rations suboptimal in lysine and tryptophan Jones and Combs (1951) observed that Aureomycin supplementation appeared to spare the dietary requirement for tryptophan but not for lysine. Another study suggesting a sparing effect of an antibiotic on an amino acid was reported by Wilder et al. (1951). The addition of either 0.2% DL-tryptophan or 0.25% of a B_{12}-antibiotic supplement (Lederle's) to a corn-meat scrap ration stimulated the growth of rats to about the same extent but a combination of these compounds did not produce an additive effect.

It has been shown quite clearly by Grau (1948) and Grau and Kamei (1950) that the lysine requirement of the chick varies more or less directly with the protein level of the diet. Kratzer et al. (1949) and Bird (1950) showed that this relationship held true for turkeys. Brinegar et al. (1950), working with weanling pigs showed that the lysine requirement was 0.5% and 1.2% of the ration when the ration contained 10.6% and 22% of protein respectively. The same type of relationship has been shown to exist with chicks for methionine (Grau and Kamei, 1950; Almquist, 1949) and for arginine (Almquist et al., 1950). According to Almquist (1952) it seems almost certain that these findings will apply to all indispensible amino acids. In view of these results, therefore, it would seem logical to assume that amino acid allowances should be stated in reference to a certain protein level.

EXPERIMENTAL PROCEDURE

This investigation consisted of two experiments. In the first trial thirty-six weanling Duroc pigs about eight weeks of age and averaging approximately thirty-three pounds in weight were randomly allotted into six drylot pens of six pigs each. Allotment was based on initial weight and sex, and the test period was of six weeks duration. The animals were kept in adjoining concrete-floored pens bedded with wood shavings and were self-fed a mixed ground feed. All pigs were weighed individually at the start of the test and thereafter at two week intervals. Feed placed in the self-feeders was weighed and recorded and feed weigh backs followed the weighing of the pigs. Water was available to the pigs at all times from automatic waterers. The pens were cleaned daily.

In the second trial thirty-two weanling Duroc and Chester White pigs, approximately six weeks of age, were randomly allotted into four drylot pens of eight pigs each. Allotment was based on initial weight and sex, and the test period was of eight weeks duration. These pigs were housed and handled in a manner similar to the pigs in the first trial.

Diets A, B and C, as shown in table 1, used in the first trial, were formulated to contain, in so far as possible, similar quantities of energy, total crude protein and

TABLE 1

COMPOSITION OF BASAL DIETS

Ingredient	Diets			
	A	B	C	D
	%	%	%	%
Ground corn	78.5	75.8	73.0	79.7
Meat & bone scrap (50%)[1]	20.0	11.0	-	20.0
Soybean oil meal (44%)	-	11.0	24.0	-
Limestone	0.4	0.4	0.5	-
Dicalcium phosphate	0.5	1.0	1.5	-
Iodized salt	0.5	0.7	0.9	0.2
Trace mineral	0.1	0.1	0.1	0.1
Tryptophan[2]	0.09	0.13	0.16	0.08

All diets were supplemented with calcium, pantothenate, niacin, riboflavin and vitamin B_{12} (Merck's APF Supplement #3) in amounts of 10 mg., 15 mg., 2 mg. and 6.25 mcg. respectively per pound of diet. Vitamins A and D were added at levels to provide 2000 I.U. and 200 USP units respectively per pound of feed.

[1]The meat and bone scrap used in diet D was a composite of four different brands in equal amounts.

[2]As determined microbiologically. Kuiken *et al.* (1947).

vitamins. The levels of crude protein, minerals and vitamins were designed to meet or exceed the amounts recommended by Hughes et al. (1950) for the size of pigs used.

Diet A, which served as the negative control diet fed group 1, contained a regular commercial source of meat and bone scrap as the only form of supplemental protein. Three different levels of DL-tryptophan, namely 0.02%, 0.06% and 0.12%, were added to diet A to form the experimental diets for groups 2, 3 and 4 respectively and this is presented in table 2. The tryptophan, as well as supplemental vitamins and antibiotic, were added in a premix form at the time of mixing the diets. In diet B, fed group 5, the supplemental protein was provided by equal parts of meat and bone scrap and soybean oil meal to determine if this amount of soybean oil meal would improve the amino acid balance of the meat and bone scrap, since soybean oil meal is considered to be a good source of tryptophan. All of the supplemental protein in diet C was provided by soybean oil meal and group 6 fed this diet served as the positive control.

In the second trial diet D, as shown in table 1, served as the basal diet. On the basis of chemical determinations made of diet A in the first trial, it was decided that supplemental additions of calcium and phosphorus were unnecessary in this type of diet and consequently were not included. The meat and bone scrap used in diet D was a composite of

TABLE 2

TRIAL 1 - SUMMARY OF RESULTS
OF TRYPTOPHAN SUPPLEMENTATION
OF MEAT AND BONE SCRAP

(6 pigs in each lot on trials lasting 6 weeks)

Group	Diet and supplement	Average initial weight	Average daily gain	Average daily feed	Feed per lb. gain
		lbs.	lbs.	lbs.	lbs.
1	A	32	0.39	1.7	4.31
2	A + 0.02% DL-tryptophan	32	0.46	1.8	3.91
3	A + 0.06% DL-tryptophan	33	1.03*	3.6	3.49
4	A + 0.12% DL-tryptophan	34	1.09*	3.7	3.37
5	B	33	0.96*	3.1	3.24
6	C	33	0.96*	2.9	3.03

* Difference in daily gain highly significant ($P < 0.01$) over group 1.

four different brands purchased on the open market, namely Swift, Hygrade, Wilson and Armour. As shown in table 3, group 7 served as the negative control and received diet D. Two levels of DL-tryptophan, 0.04% and 0.06%, were added to diet D to form the experimental diets for groups 8 and 9 respectively. Group 10 was fed diet D supplemented with 10 mg. of crystalline Aureomycin HCl per pound of feed.

The gains were tested for significance by the method of Snedecor (1946).

All diets used in Trials 1 and 2 were analyzed for various nutrients as shown in tables 4 and 5. The crude protein, calcium and phosphorus determinations were carried out by the A.O.A.C. methods (1950). Methods employed for the determination of vitamins in the feeds were: riboflavin, Snell and Strong (1939); niacin, Krehl et al. (1943); and pantothenic acid, Skeggs and Wright (1944) and Buskirk et al. (1948). Tryptophan in the meat and bone scraps and the mixed feeds was determined by microbiological assay after alkaline hydrolysis using the method of Kuiken et al. (1947). The nine other amino acids required by the growing pig were determined by microbiological assay after acid hydrolysis according to the methods suggested by Henderson and Snell (1948).

TABLE 3

TRIAL 2 - SUMMARY OF RESULTS
OF TRYPTOPHAN AND AUREOMYCIN SUPPLEMENTATION
OF MEAT AND BONE SCRAP

(6 pigs in each lot on trials lasting 8 weeks)

Group	Diet and supplement	Average initial weight	Average daily gain	Average daily feed	Feed per lb. gain
		lbs.	lbs.	lbs.	lbs.
7	D	27	0.29	1.8	6.16
8	D + 0.04% DL-tryptophan	26	0.64*	2.6	4.02
9	D + 0.06% DL-tryptophan	27	0.93**	3.3	3.55
10	D + Aureomycin[1]	26	0.34	1.6	4.68

[1] Crystalline Aureomycin HCl added at rate of 10 mg. per pound of feed.

* Difference in daily gain significant ($P < 0.05$) over group 1.

** Difference in daily gain highly significant ($P < 0.01$) over group 1.

TABLE 4

TRIAL 1 - RESULTS OF FEED ANALYSIS

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
	%	%	%	%	%	%
Crude protein	17.00	17.94	17.19	17.13	18.06	17.56
Calcium	1.60	1.64	2.01	1.93	1.77	0.71
Phosphorus	1.08	1.11	1.29	1.26	1.18	0.71
Amino Acids						
Tryptophan	.09	.12	.15	.20	.13	.16
Methionine	.26	.27	.27	.27	.25	.25
Lysine	.91	.98	.96	.99	1.08	1.02
Arginine	1.08	1.22	1.12	1.10	1.26	1.26
Histidine	.42	.43	.38	.41	.49	.52
Threonine	.62	.62	.61	.61	.70	.74
Leucine	1.31	1.34	1.29	1.30	1.36	1.42
Phenylalanine	.65	.68	.66	.65	.75	.82
Valine	.97	1.01	.98	.98	1.06	1.09
Isoleucine	.69	.67	.66	.66	.81	.93
Vitamins	mg/lb.	mg/lb.	mg/lb.	mg/lb.	mg/lb.	mg/lb.
Riboflavin	2.58	3.05	2.91	2.86	2.70	2.83
Niacin	30.28	30.29	29.86	31.10	27.51	26.35
Pant. Acid	13.92	14.77	14.95	13.40	14.09	15.16

TABLE 5

TRIAL 2 - RESULTS OF FEED ANALYSIS

	Group			
	7	8	9	10
	%	%	%	%
Crude protein	17.88	17.63	17.69	17.50
Calcium	1.95	1.84	1.84	1.91
Phosphorus	1.26	1.20	1.19	1.23
Amino Acids				
Tryptophan	.08	.11	.12	.08
Methionine	.26	.26	.26	.26
Lysine	.74	.73	.72	.75
Arginine	1.25	1.20	1.20	1.21
Histidine	.36	.52	.46	.49
Threonine	.70	.69	.65	.67
Leucine	1.34	1.34	1.35	1.31
Phenylalanine	.67	.67	.67	.66
Valine	.96	.95	.84	.83
Isoleucine	.76	.76	.72	.72
Vitamins	mg/lb.	mg/lb.	mg/lb.	mg/lb.
Riboflavin	3.06	3.07	2.79	2.99
Niacin	28.30	27.10	24.78	26.85
Pant. Acid	13.77	13.73	11.84	14.38

RESULTS

TRIAL 1

Growth

The average daily gains of the pigs in the various groups of this test are shown in summary form in table 2. Individual weight of the pigs during the course of the test are presented in table 6. The addition of DL-tryptophan at three different levels to basal diet A resulted in improved growth. At the 0.02% added level of DL-tryptophan growth rate was improved about 18% over the control group. Increasing the level of added DL-tryptophan to 0.06% resulted in further improvement in rate of growth amounting to approximately 164% over the control group. When the level of DL-tryptophan was further increased to 0.12% no further increase in growth over the 0.06% level was observed.

Group 5, receiving diet B, showed essentially the same rate of growth as that obtained in groups 3 and 4. Likewise, the pigs in group 6 receiving diet C grew at about the same rate as did those in groups 3, 4 and 5.

When pig weight gains for each group were subjected to an analysis of variance it was found that the difference in gains made by groups 3, 4, 5 and 6 over group 1 were highly significant ($P \leq 0.01$).

The divergence in growth among groups was evident at

TABLE 6

TRIAL 1 - INDIVIDUAL WEIGHTS OF PIGS
IN POUNDS BY PERIODS

Pig No.	Sex[1]	Days on Test 0	14	28	42	Total gain per pig
GROUP 1						
46	F	32	38	43	48	16
17-5	F	34	40	42	45	11
12-5	F	36	39	42	48	12
15-6	F	29	34	41	45	16
00	B	32	36	44	57	25
44	B	29	32	39	48	19
Average		32.0	36.5	41.8	48.5	16.5

GROUP 2						
18	F	33	38	41	53	20
49	F	30	35	39	49	19
15-7	F	26	29	33	39	13
45	B	34	37	42	44	10
12-2	B	38	48	58	72	34
41	B	36	41	47	56	20
Average		32.5	38.0	43.3	52.2	19.3

TABLE 6 - Continued

GROUP 3						
		Days on Test				Total gain
Pig No.	Sex[1]	0	14	28	42	per pig
16	F	33	43	59	79	46
24	F	35	48	66	89	54
87	F	36	45	63	84	48
15-1	B	26	33	45	64	38
16-1	B	31	37	45	62	31
18-2	B	37	48	56	79	42
Average		33.0	42.3	55.7	76.2	43.2

GROUP 4						
2-10	F	32	41	60	79	47
2-11	F	32	40	55	77	45
19	F	37	51	68	84	47
3-11	F	36	51	77	99	63
32	B	37	48	61	64	27
57	B	30	39	54	76	46
Average		34.0	45.0	62.5	79.8	45.8

TABLE 6 - Continued

GROUP 5 Pig No.	Sex[1]	Days on Test 0	14	28	42	Total gain per pig
15	F	30	40	49	68	38
26	F	36	49	67	74	38
88	F	32	46	66	88	56
13	B	28	36	44	46	18
12-4	B	36	46	61	78	42
12	B	34	47	63	85	51
Average		32.7	44.0	58.3	73.2	40.5

GROUP 6						
12-9	F	36	48	66	85	49
16-4	F	28	34	46	63	35
17	F	37	54	80	104	67
55	F	30	44	54	61	31
17-7	F	32	44	47	50	18
17-3	B	33	46	60	76	43
Average		32.7	45.0	58.8	73.2	40.5

[1] F = female, B = barrow

the time of the first 14 day weigh period and continued for the remainder of the trial. Groups 1 and 2 showed poor growth and at the end of the experiment were uniformly small, but exhibited no other outward signs of a tryptophan deficiency.

Feed Consumption

The average daily feed consumption per pig for each group is shown in table 2. Total feed consumption per group by periods can be found in table 7. It is evident from table 2 that feed consumption for groups 1 and 2 was significantly less than for any of the other groups and in this respect shows some correlation with growth rates. In examining table 7 it is of interest to note that groups 1 and 2 actually consumed less feed during the second 14 day period, despite a greater body weight, than they did during the first 14 day period. It is quite possible that this reduction in feed intake reflects an adjustment in body metabolism to compensate for an inadequate supply of tryptophan in the diet, and this is born out to some extent by the fact that both of these groups showed better feed utilization during the second period than they did during the first period.

Feed wastage was minimal in all groups and could not be considered as an interfering factor in the interpretation of the feed figures.

TABLE 7

TRIAL 1 - FEED CONSUMPTION

Group	Days on Test			Total
	14	28	42	
	lb.	lb.	lb.	lb.
1	142	122	163	427
2	144	128	182	454
3	215	282	406	903
4	216	312	400	928
5	189	258	341	788
6	194	235	307	736

Feed Efficiency

Feed consumed per pound of gain figures for each group are presented in table 2. For the first four groups receiving diet A, it is evident that as the level of tryptophan in the diet was increased feed utilization was improved. While these differences appear fairly large they would no doubt have been much greater had the pigs in groups 1 and 2 been fed out to the same final weight as those in groups 3 and 4. A comparison of feed efficiences among pigs or groups of pigs of differing weights is not a valid one.

In comparing feed efficiencies among groups 4, 5 and 6 it will be noted that each succeeding group showed a somewhat better figure than the preceding group. It does not appear that these differences can be readily explained on the basis of amino acid balance or energy contents of the diets. This will be discussed in more detail in another section of the thesis.

Feed Analysis

The results of chemical analysis and microbiological assay for various nutrients as contained in these diets are shown in table 4. Crude protein values are fairly close to the calculated levels and fall within the normal expectancy range. The values reported for calcium and phosphorus for diets A and B (groups 1 - 5) are well in excess of the pig's requirements for these minerals and may explain in part the somewhat poorer feed efficiency obtained in those groups receiving diets A or B as compared with group 6 receiving diet C which contained calcium and phosphorus at levels close to the pig's requirement. Reported values for riboflavin, niacin and pantothenic acid for all diets are well in excess of the pig's requirements and consequently were not limiting factors. Values for the ten essential amino acids required by the pig are shown for each of the diets employed. It should be pointed out that in the procedure for determining tryptophan racemization of this amino acid occurred as a result of alkaline hydrolysis of the feed. Thus the assayed value was only one-half of the total tryptophan since the test organism could utilize only the L-form. This assayed value was then doubled in order to obtain the total tryptophan content of the diet which is the reported figure. The tryptophan values reported for groups 2, 3 and 4 include all of the added DL-tryptophan and show good agreement with calculated values based on the assayed value of 0.09% tryptophan in diet A plus the added amounts. The meat and bone scrap used in this trial was found to contain 0.26% tryptophan.

TRIAL 2

Growth

Average daily gains for the pigs in each group are shown in summary form in table 3. Individual weights of the pigs during the course of the test are presented in table 8. Again in this trial as in the first trial the addition of DL-tryptophan to the basal diet resulted in an improvement in rate of growth. The pigs in group 8 grew about twice as fast as those in group 7 as a result of the addition of 0.04% DL-tryptophan to diet D. When the level of DL-tryptophan added to diet D was increased to 0.06% the growth of the pigs was further improved with the result that they exhibited a growth rate about three times as fast as the control group.

The addition of Aureomycin HCl to diet D at a level of 10 mg. per pound of diet resulted in a very slight improvement in growth which was not statistically significant.

An analysis of variance conducted on the weight gains showed that the difference in gain between groups 7 and 8 was significant ($P \leq 0.05$) and between groups 7 and 9 was highly significant ($P \leq 0.01$).

In general the growth rates of the individual pigs within the groups were not as uniform as those in the first trial. Part of this may have been due to an enteritis which was in evidence in all groups to some degree.

TABLE 8

TRIAL 2 - INDIVIDUAL WEIGHTS OF PIGS IN POUNDS BY PERIODS

Pig No.	Sex	Days on Test 0	14	28	42	56	Total gain per pig
GROUP 7							
21-7	F	23	17	15[1]			
18-9	F	24	25	25	26	29	5
16-9	F	27	30	33	38	45	18
16-3	B	25	28	30	34	36	11
16-2	B	29	34	40	43	48	19
15-8	F	26	27	29	32	34	8
13-8	F	33	39	51	64	77	44
10-2	B	24	27	27	30	33	9
Average		26.9	30.0	33.6	38.1	43.1	16.3
GROUP 8							
17-6	F	22	23	25[1]			
19-10	F	24	33	42	58	75	51
18-6	F	25	30	35	47	55	30
18-3	B	26	28	31	36	42	16
14-2	B	27	35	44	59	78	51
10-3	F	28	31	36	44	53	25
15-6	F	25	29	41	55	66	41
14-1	B	33	44	58[2]			
Average		25.8	31.0	38.2	49.8	61.5	35.7

TABLE 8 - Continued

Pig No.	Sex	Days on Test 0	14	28	42	56	Total gain per pig
GROUP 9							
21-8	F	23	22	23[1]			
19-6	F	25	37	45	59	66	41
16-6	F	31	43	55	71	78	47
21-2	B	24[3]					
18-4	B	26	33	35	50	63	37
17-2	B	28	42	58	81	94	66
15-4	F	23	28	38	57	75	52
14-2	B	31	47	61	79	101	70
Average		27.3	38.3	48.7	66.2	79.5	52.2
GROUP 10							
18-5	F	25	26	26	26	31	6
19-8	F	25	31	35	42	47	22
19-3	B	23	26	27	29	30	7
21-1	B	25	22	22[1]			
18-1	B	26	33	36	41	46	20
15-5	F	24	28	30	32	37	13
15-7	F	30[4]					
14-3	B	33	39	49	63	79	46
Average		26.0	30.5	33.8	38.8	45.0	19.0

1 Pigs removed on 29th day of test for autopsy. All data excluded from averages.
2 Pig died 41st day of test. All data adjusted for removal.
3 Pig died 10th day of test. All data adjusted for removal.
4 Pig died 8th day of test. All data adjusted for removal.

Feed Consumption

The average daily feed consumption per pig for each group is shown in table 3. Total feed consumption per group by periods can be found in table 9. Both levels of added tryptophan improved feed consumption over the control group, with the 0.06% level of DL-tryptophan showing the higher feed consumption figure. It is interesting to note that group 10 receiving Aureomycin in their diet consumed less feed than did group 7, the control group.

Feed wastage was minimal in all groups with the result that feed consumption values are fairly accurate. All feed data were adjusted for pigs that died or were removed from test. The growth rate of the individual pig involved was used as the criteria to estimate how much feed should be charged to that pig during the time on experiment and this amount was then subtracted from the total feed consumption for that group.

Feed Efficiency

The figures pertaining to feed consumed per pound of gain for each group are shown in summary form in table 3. The addition of 0.04% and 0.06% DL-tryptophan to the basal diet resulted in a considerable improvement in feed efficiency with the higher level giving the greatest improvement. Adding Aureomycin to the basal diet also resulted in a substantial improvement in feed utilization.

TABLE 9

TRIAL 2 - FEED CONSUMPTION

Group	Days on Test				Total
	14	28	42	56	
	lb.	lb.	lb.	lb.	lb.
7	118	138	154	181	591
8	142	182	219	318	861
9	187	249	317	357	1110
10	115	110	139	170	534

Feed Analysis

Feed analysis data are shown in table 5. The reported values for crude protein for all diets agree rather closely with the levels calculated on the basis of formulation. Calcium and phosphorus levels are approximately twice the pig's requirement for these elements and it is now well known that these excessive levels can reduce both the rate of growth and feed efficiency of growing pigs. The values reported for riboflavin, niacin and pantothenic acid for all diets are well in excess of the pig's requirements and consequently were not limiting factors. Values for the ten essential amino acids required by the pig are shown for each of the diets fed the four groups. Information regarding tryptophan values is the same as previously mentioned under the Feed Analysis Results for Trial 1.

The name of the brand of meat and bone scrap with the amount of tryptophan found is as follows: Armour, 0.24%, Hy-Grade, 0.24%, Swift, 0.18% and Wilson 0.25%. An equal mixture composite of these four sources of meat scrap was calculated to contain 0.23% tryptophan.

Disease and Mortality

After two weeks on experiment it became apparent that all groups had at least one or two pigs which were not growing normally. In an attempt to determine the origin of the trouble one poor-doing pig from each group was sacrificed and autopsied by the Animal Pathology Department. The diagnosis in each case was enteritis, however, the cause was undetermined. Three of the four pigs were found to be littermates from a nine pig litter that was exceptionally large and healthy at birth.

One case of intussusception of the colon with resulting peritonitis and death occurred in each of groups 8, 9 and 10. Post mortem examination failed to reveal the cause for this condition.

After about five weeks on experiment two of the pigs in group 9 showed evidences of a mild dermatitic condition and by the eighth week four of the six pigs remaining in this group were so affected. At no time during the course of the experiment did any of the other three groups show evidence of this disease. It is almost certain that this disease was the same as has since been described by Kernkamp and Ferrin (1953) and who termed the syndrome parakeratosis.

DISCUSSION OF RESULTS

At the present stage of our knowledge of swine nutrition it appears that no class of nutrients is more important than proteins or more specifically their components, the amino acids. Until such time as the amino acid requirements of swine and the amino acid composition of feed ingredients used to supply these needs are thoroughly known and understood the formulation of satisfactory swine diets necessarily involves considerable guess work.

The tryptophan values for the five different samples of meat and bone scrap used in this test are of interest not only because of their variation but also because they are lower than the values presented in most amino acid tables from various sources. Amino acid tables prepared by Almquist (1954), Block and Weiss (1956), Sievert and Fairbanks (1958) and Bird et al. (1954) all assign 50% meat and bone scrap a value of 0.35% tryptophan. On the other hand, Morrison (1957) in his tables on the amino acid content of feed ingredients gives meat and bone scrap a value of 0.20% tryptophan, which is actually in closer agreement with the results reported herein.

Perhaps one of the greatest limitations to the use of meat scraps as the only source of supplemental protein in a corn based ration for swine is its variable tryptophan content. Following is a list of tryptophan values reported for samples of meat meals, meat scraps or meat and bone scraps:

Tryptophan in crude protein %	Crude Protein %	Source
1.12	56.8	Almquist *et al.* (1935)
0.62	56.2	"
1.30	58.2	"
0.70	-	Block and Bowling (1943)
0.70	-	Kratzer (1944)
0.84	-	Wilder *et al.* (1948)
0.62	50.8	March *et al.* (1949)
0.44	49.7	"
0.56	57.1	"
0.54	49.7	"
0.41	51.7	March *et al.* (1950)
0.60[1]	-	Henson *et al.* (1954)

[1]Based on reported value of 0.30% tryptophan and assumed crude protein content of 50% in sample.

Assuming a crude protein content of 50% in the samples of meat and bone scrap used in this trial their tryptophan content based on the crude protein would be: Trial 1 - 0.52%; Trial 2 - Armour, 0.48%; Hygrade, 0.48%; Swift, 0.36%;

Wilson, 0.50%, and the composite of the four brands used, 0.46%. Obviously, some of the variations among the reported values may be due to differences in the assay procedures used, however, it should be noted that considerable variation occurred within any one series of assays performed in a particular laboratory. Of considerable interest and perhaps significance is the fact that the tryptophan values reported in the above list show a general downward trend from the first reference in 1935 to those reported for the 1950's. It seems quite likely that this trend parallels the general reduction in quality of meat and bone scrap that has occurred as the result of including lesser amounts of hearts, livers, kidneys, beef trimmings and glandular materials.

In view of this trend toward a lower tryptophan content of the meat meals it would appear that the average value of 0.35% tryptophan for meat and bone scrap as shown in most amino acid tables is too high and should be revised downward on the basis of more recent information.

The results obtained in these trials indicate that the corn-meat and bone scrap diets employed were deficient in tryptophan for growing pigs. In a similar type study Terrill, et al. (1954) using a composite blend of four samples of meat and bone scrap as the supplemental protein in a corn based diet, improved the growth of pigs significantly by the addition of 0.1% DL-tryptophan. It is unfortunate that these authors did not present a tryptophan assay value for the basal diet since the reported growth and feed efficiency of the basal fed group were clearly superior to those reported herein, suggesting the possibility of a higher tryptophan content. In this Illinois work the inclusion of an antibiotic in the diet may have been responsible, at least in part, for the superior performance. On the basis of studies involving the amino acid supplementation of a corn-meat scrap diet for growing pigs Henson et al. (1954) concluded that tryptophan is probably the most limiting amino acid in this type ration.

Since the degree of utilization of D-tryptophan by the pig is unknown at this time and since only a limited number of tryptophan levels were tested it is impossible to state an exact tryptophan requirement for this animal on the basis of the information presented. In spite of these limitations, it would appear that these data warrant an approximation of the growing pig's requirement for this amino acid. In Trial 1, group 5 receiving an 18.1% protein diet containing 0.13% of naturally occurring tryptophan, exhibited a growth rate equal to that shown for group 6 where the level of naturally occurring tryptophan in a diet containing 17.6% protein was 0.16%. If the assumption is made that no appreciable amount of the D- form of tryptophan is utilized then a level of 0.12% L-tryptophan in the 17.2% protein diet fed group 3 was sufficient to allow for normal growth. A level of 0.10% L-tryptophan in diets containing 17.9% and 17.6% protein (groups 2 and 8 respectively) appeared to be inadequate from the standpoint of growth. Thus on the basis of this work it appears that for growing pigs 30 to 70 pounds in weight the requirement for tryptophan lies close to 0.13% with a diet containing 18% protein and 75% total digestible nutrients.

In early studies with weanling pigs Shelton, et al. (1951) observed that 0.2% of DL-tryptophan permitted more rapid growth than 0.1% level when added to a deficient diet. Becker et al. (1954b) estimated the tryptophan requirement of the 20 pound pig at 0.15% in a diet containing 12% of protein. Utilizing growing pigs from 40 to 100 pounds Becker et al. (1954a) obtained satisfactory performance with diets containing 0.13% tryptophan and 14% of protein. On the other hand, in studies concerned with the nutritive deficiency of a corn-fish meal diet for the weanling pig Becker et al. (1955d) showed that 0.10% tryptophan in a diet containing 17.5% protein was inadequate for normal performance. The addition of 0.05% DL-tryptophan increased growth rate to that obtained on a corn-soybean oil meal diet which contained 0.17% tryptophan and 18% of protein.

In an attempt to determine more accurately the quantitative tryptophan requirement of the weanling pig Becker et al. (1955c) supplemented diets containing protein levels of 15.4% and 19.6% with graded levels of L-tryptophan. According to the authors, the diets contained by assay

0.075% of tryptophan, although no mention is made of the type of assay used. The growth results indicated that the pig required no more than 0.115% of L-tryptophan in a diet containing 15.3% protein. This tryptophan level constituted 0.75% of the dietary protein as compared with a value of 0.72% of the dietary protein for the estimated requirement suggested on the basis of the trial reported herein. Thus it would appear that the tryptophan requirement of the growing pig, as reported by the Illinois workers, agrees rather closely with our own, recognizing the possible limitation to expressing and comparing an amino acid requirement as percent of dietary protein.

Grau and Kamei (1950) reported that as the protein level of the chick's diet was increased, the lysine and methionine plus cystine requirements also increased but at a slower rate. In 1954 Salmon was able to show that the tryptophan requirement of the rat was not a constant value, but increased as the level of dietary nitrogen increased, although the relationship did not appear to be a linear one. On the basis of studies designed to determine the isoleucine requirement of the weanling pig at two different levels of protein Becker et al. (1957) concluded that as the level of protein in the diet was increased the isoleucine requirement was also increased but at a slower rate. At a protein level of 13.35% the isoleucine requirement was 3.4% of the dietary protein while at a protein level of 26.70% the isoleucine requirement was only 2.4% of the dietary protein. In a similar type experiment McWard *et al.* (1959) found that the lysine requirement of weanling pigs varied with the level of protein fed. At 12.8% protein the pig required 0.71% lysine or 5.55% of dietary protein; and at 21.7% protein the lysine need was 0.95% of the diet or 4.38% of the dietary protein. While it appears likely that level of protein affects the tryptophan requirement of the pig this point has not been satisfactorily answered to date. In a study reported by Becker *et al.* (1955c) in which two different protein levels were supplemented with graded levels of L-tryptophan the results did not seem to conform with the idea that the tryptophan requirement of the pig is higher with a higher protein diet. The authors point out, however, that "the level of available protein varied less than anticipated" since the commercial zein used to increase the protein level was only 44.8% digestible. Thus, in effect, the range of available protein was so small as to seriously limit the results. It would appear that a fairly wide range in protein levels would have to be employed to adequately determine whether level of protein influences the tryptophan requirement.

Whether the pig is capable of utilizing the D-isomer of tryptophan, and if so, to what degree is still open to question, based on conflicting reports from several laboratories coupled with the lack of sufficiently critical studies. As already pointed out Reber *et al.* (1951) concluded that the pig made little or no use of D-tryptophan while Thompson *et al.* (1952) conversely concluded that partial utilization of the D-isomer was effected. Quite different results were reported by Becker *et al.* (1955b) in which the presence of the D-isomer of tryptophan in a racemic mixture appeared to impair the utilization of L-tryptophan. In contrast with these results Becker *et al.* (1955c) found no tendency for DL-tryptophan to depress growth. On the basis of this study these workers concluded that the pig can utilize a considerable share of the D-isomer of tryptophan when it is fed in a racemic mixture. The results reported herein are such as to preclude the possibility of stating how much if any of the D-isomer of tryptophan was utilized by the pig.

At this point it may be well to mention that the use of crystalline amino acids in determining the requirement of an animal may be subject to serious limitations. Amino acids in crystalline form may be more digestible than the naturally occurring amino acids in feedstuffs. Thus, the use of a crystalline amino acid might result in under-estimation of the requirement when natural feeds are used. For example, Brinegar *et al.* (1950) reported that lysine from certain natural sources is only 88 to 90% digestible. On the other hand, crystalline amino acids may be more rapidly absorbed, and hence undergo catabolism before the total ingested amino acids are available for synthesis into tissue protein. This might result in an over-estimation of the minimum requirement.

Theoretically the caloric density of the diet should influence the amino acid requirement. As the caloric density decreases, the diet is consumed in increasing quantities, so that the amino acids required in the diet would also decrease. All other factors being equal the amino acid needs should be a constant percent of the available energy. Mitchell and Hamilton (1935) demonstrated with rats that an upward adjustment of the protein level, to maintain the proper relation to total energy, was necessary to realize the full benefit of increased energy in the diet. Donaldson et al. (1955) and Sunde (1956), among others, have also demonstrated this with chicks. The relationship between energy and protein must necessarily apply to a similar type relationship between energy and amino acids. Rosenberg and Culik (1955) have been able to show that the lysine requirement of the growing rat is a function of the productive energy of the diet. A similar relationship in growing pigs is suggested by the work of Abernathy et al. (1958). In this study a highly significant depression in rate of gain resulted when L-lysine was added at a level of 0.1% of the ration. As the caloric density of the ration was increased this inhibitory effect appeared to be reduced. Using values as set forth by Beeson et al. (1953) the TDN content of the diet employed by Becker et al. (1955c) in suggesting the tryptophan requirement of the pig at 0.115% (protein 15.3%) calculates to be very close to 75%. Using these same values the diets used in the study reported herein also contained 75% TDN. The similarity of the diets in this regard may be further reason for the similarity of results achieved at these stations and lends further support to the proposed tryptophan requirement of the pig as mentioned above.

The inclusion of the antibiotic Aureomycin HCl at a level of 10 mg. per pound of feed in a diet shown to be deficient in tryptophan resulted in only a slight improvement in growth but a rather significant improvement in feed utilization (group 10, trial 2). If one defines a sparing effect as the ability of an antibiotic to improve growth on a tryptophan deficient diet, then it seems questionable that the results presented here demonstrate this. However, since a sparing effect might also be evidenced by improved utilization of feed then these data might suggest a sparing effect of an antibiotic on the tryptophan requirement of the pig. Braude _et al._ (1953), in a review of the literature concerning the use of antibiotics in swine nutrition, concluded that pigs fed a ration with better over-all nutrient balance will respond less to antibiotic supplementation than with a poorer ration. In 1951 Jones and Combs reported that antibiotics exert a tryptophan sparing action in the chick. Likewise, Sauberlich (1954) has suggested the occurrence of a tryptophan-sparing action by antibiotics in the rat. In a study designed to compare two different sources of supplemental protein upon the response of pigs to antibiotics, (Becker *et al.* 1955d) the results suggest that chlortetracycline has a sparing effect on the tryptophan requirement of the pig. In addition, a mixture of antibiotics (chlortetracycline, penicillin and streptomycin) appeared to exert an even greater sparing action than the single antibiotic. In a subsequent experiment of more critical design Becker *et al.* (1955c) reported that there was no significant effect of antibiotics on the tryptophan requirement of the pig, although the antibiotics did increase body weight gains and feed efficiency when added to rations either low or high in tryptophan. A point of interest in this experiment is that on the various levels of supplementary tryptophan the improvement in feed efficiency produced by antibiotics decreased as the level of tryptophan increased. A similar lack of interaction between dietary antibiotics and an amino acid requirement of the pig has been reported by Becker *et al.* (1955a). In this study when a mixture of three antibiotics was added to a diet, growth rate was considerably improved in both the basal and methionine supplemented groups. No significant interaction of antibiotics with methionine requirement was noted although the absolute increase in daily weight gains tended to decrease with increasing levels of methionine. In the presence of the antibiotic mixture best growth (1.21 lbs. gain per day) was obtained with a total of 0.42% sulfur amino acids in the ration, the same value as was obtained in the absence of antibiotics.

Several factors that may influence the tryptophan requirement of the pig, when expressed as a percentage of the diet, have already been discussed. Perhaps other factors as yet untested may have considerable influence on the pig's requirement for amino acids. For example, is the pig's requirement for an amino acid a function of body size or age or a combination of both? Do different breeds or strains of breeds vary in their requirement for amino acids? Does the inherent growth potential of a pig affect its amino acid requirement? Does environmental temperature indirectly influence the amino acid needs of the pig by affecting caloric intake? Certainly the establishment of the pig's requirement for any particular amino acid is not a simple and uncomplicated problem.

Recently Becker (1959) has summarized in tabular form the amino acid requirement of the weanling pig based on the best information available. For comparative purposes part of this table along with the amino acid assay results obtained in the trial reported herein is shown in table 10. An examination of this table would indicate that with only a few exceptions the diets fed were adequate in all amino acids except tryptophan. The values shown for methionine appear to be suboptimal or borderline even if 40% of the methionine requirement was supplied by cystine. The values reported for methionine agree quite closely with the calculated

TABLE 10

A COMPARISON OF AMINO ACID REQUIREMENTS OF SWINE WITH THE AMINO ACID CONTENT OF EXPERIMENTAL DIETS EXPRESSED AS PERCENT OF DIET

Amino Acid	Requirements[1]	Diet			
		A[3]	B	C	D[4]
Tryptophan	.13	.09	.13	.16	.08
Methionine	.54[2]	.27	.25	.25	.26
Lysine	.88	.96	1.08	1.02	.74
Arginine	.28	1.13	1.26	1.26	1.21
Histidine	.24	.42	.49	.52	.46
Threonine	.49	.62	.70	.74	.68
Leucine	.74	1.31	1.36	1.42	1.33
Phenylalanine	.59	.66	.75	.82	.67
Valine	.50	.99	1.06	1.09	.89
Isoleucine	.57	.67	.81	.93	.74
Protein content of diet, %	18.0	17.3	18.1	17.6	17.7

[1] Becker (1959)

[2] Cystine can satisfy 40% of the total need for methionine.

[3] With exception of tryptophan, values presented represent average of diets fed groups 1, 2, 3 and 4.

[4] With exception of tryptophan, values presented represent average of diets fed groups 7, 8, 9 and 10.

values for these diets based on methionine values given for the ingredients (Bird et al., 1954). Cystine content was calculated at 0.24%, 0.25%, 0.27% and 0.25% for diets A, B, C and D respectively. Even assuming full utilization of the cystine to meet the requirements for methionine the total amount of sulfur bearing amino acids does not quite meet the requirement of 0.54%. There is, however, considerable evidence to suggest that the methionine requirement of the pig was met by the values given above. Catron et al. (1953) found that corn-soybean oil meal diets formulated to yield either 12 or 14% protein were not improved by supplementary methionine. In agreement with these findings Becker et al. 1954a) found that a corn-soybean oil meal ration containing 14% of protein and 0.23% of methionine supported satisfactory growth of weanling pigs. It seems logical to assume that if sufficient methionine and cystine are supplied in a 14% protein ration composed of corn and soybean oil meal then proportionately larger amounts of these amino acids should also be supplied in a similarly composed ration containing 18% protein, since soybean oil meal contains over four times as much methionine and cystine as does corn. Becker et al. (1955a) state that "From the practical viewpoint, the studies presented here tend to emphasize the lack of need for supplementary methionine in practical rations" for swine. Likewise Sewell and Keen (1958) concluded that failure to obtain a response in pigs to methionine supplementation on rations which contained high protein oil meals, generally recognized to be limiting in methionine content, indicates that there is little need for methionine supplementation of practical swine rations.

In table 10 the values shown for lysine appear to be adequate in all diets except D. While it is quite probable that the lysine content of this diet was suboptimal the growth results suggest that tryptophan was the primary limiting amino acid.

It will be noted that groups 1 and 2 in Trial 1 (table 2) and groups 7 and 10 in Trial 2 (table 3) showed relatively poor feed consumption. While these feed consumption figures correlate with the poor rates of gain a possible mechanism for at least part of this effect has been suggested by Almquist _et al._ (1947). He points out that when an amino acid is not supplied in sufficient amounts, tissue protein synthesis slows down with a resultant accumulation of the other free amino acids in the circulation to be removed by catabolism or excretion. Such an accumulation may result in depressed appetite which served to further curtail protein digestion.

As previously mentioned under the Results for Trial 1 the differences in feed efficiency shown for groups 4, 5 and 6 cannot readily be explained on the basis of amino acid balance or energy contents of the diets. The calcium and possibly phosphorus contents of these diets as shown in table 4 may be of significance in this regard. Groups 4, 5 and 6 received diets containing 3.19, 2.95 and 1.42% of combined calcium and phosphorus respectively. While the relationship is not entirely clear because of the similarity of growth in these groups the most plausible explanation is that as the level of minerals was increased in the diet a mineral imbalance of some type, possibly a calcium-zinc relationship, had an adverse effect on feed utilization. Because of the high levels of calcium contained in the diets fed groups 4 and 5 it is perhaps surprising that none of the pigs showed evidences of a skin disease known as parakeratosis which has been shown by a number of workers to be caused, at least in part, by excessive dietary calcium and insufficient zinc (Tucker and Salmon, 1955; Luecke et al., 1956; Stevenson and Earle, 1956; and Lewis et al., 1956). The Michigan workers (Luecke et al., 1956) reported that meat and bone scrap contain considerably more zinc than a number of other feedstuffs which were analyzed. This may in part explain why the pigs in groups 4 and 5 failed to develop parakeratosis. However, four out of six pigs in group 9, Trial 2 showed evidences of this disease. The diet fed this latter group contained essentially the same level of calcium as the diets fed groups 4 and 5 and the same amount of meat and bone scrap as contained in the diet fed group 4. At this time the reason for the difference in the incidence of parakeratosis between these groups showing essentially the same rates of gain and consuming similar diets is not apparent.

SUMMARY

1. A corn-meat and bone scrap diet supplemented with minerals and vitamins was found to be inadequate for the growth of weanling pigs fed in drylot.

2. The addition of 0.06 percent of DL-tryptophan to the above ration allowed growth to proceed at the same rate as that observed on a corn-soybean oil meal diet.

3. When half of the meat and bone scrap protein was replaced with soybean oil meal protein growth equaled that obtained in the corn-soybean oil meal group.

4. On the basis of this study it appears that for growing pigs 30 to 70 pounds in weight, the requirement for tryptophan lies close to 0.13 percent with a diet containing 18% protein and 75% total digestible nutrients.

5. The inclusion of the antibiotic Aureomycin hydrochloride at a level of 10 mg. per pound of feed in a diet shown to be deficient in tryptophan had no significant effect on rate of growth of pigs but did improve feed efficiency.

PART II. SUPPLEMENTATION OF A BABY PIG DIET WITH A VITAMIN B_{12} CONCENTRATE AND ISONICOTINIC ACID HYDRAZIDE

INTRODUCTION

One of the significant factors limiting swine production is the high rate of pig mortality that occurs during the suckling period. It has long been generally believed that approximately one third of the pigs farrowed in the United States die before they are weaned. A part of these losses can undoubtedly be attributed to infectious diseases or parsitism aggravated in all probability by faulty nutrition. It follows that knowledge in the area of baby pig nutrition and management is fundamental to the reduction of these early losses.

With the discovery that certain antibiotics were capable of increasing the growth rate of animals an entirely new vista in experimental nutrition was opened. As a result many investigators initiated studies to determine if other antibacterial agents possessed growth stimulating properties. In general the response of growing swine to the antibiotics has been somewhat greater than that observed with chickens or other farm livestock and generally the younger the pig the greater the response. For these reasons the young pig appears to be an excellent subject for the evaluation of promising growth promoting antibacterial agents.

Early in 1952 reports appeared in the literature describing a new anti-tubercular drug for man called isonicotinic acid hydrazide. The fact that many tubercular patients treated with this compound showed remarkable weight gains, prompted the idea that this compound might likewise exert a beneficial effect on the growth of pigs similar to that obtained with antibiotics.

Since the discovery of vitamin B_{12} considerable attention has been given to the search for still unidentified animal growth factors. The presence of an unidentified growth factor in distillers' dried solubles has been indicated by numerous studies with both chickens and rats since this product was introduced as a feed ingredient in 1939. Attempts to identify and purify this active component resulted in 1948 in the isolation of a highly purified fraction tentatively named vitamin B_{13}. While this "vitamin B_{13} concentrate" had been shown to possess activity for the growing rat, chick and weanling pig it remained to be determined if the baby pig would show a similar response.

REVIEW OF LITERATURE

In 1948 Novak and Hauge reported a growth factor for rats in distillers' dried solubles. This factor which was obtained in a highly purified state was reported to be distinct from all other known factors and was designated vitamin B_{13}.

Synold *et al.* (1943) were the first to report that dried distillers' solubles contain an unidentified growth factor for chickens. The results indicated that the unidentified growth factor in distillers' dried solubles was the same as that contained in commercial casein as well as other growth factors found in dried skim milk, however, these solubles did not appear to contain all the necessary growth factors found in meat and bone scraps. Similarly, reports published by Hill *et al.* (1944) and Nelson *et al.* (1944) provided additional evidence that dried distillers' solubles promote growth beyond that expected from their known nutrient content. In somewhat more refined studies with chickens, designed to further evaluate and compare the growth factor in distillers' dried solubles, Novak and coworkers (1947) presented evidence that the factor or factors in solubles is distinct from vitamin A, vitamin D, thiamin, riboflavin, pyridoxine, pantothenic acid, niacin, choline, biotin, folic acid, para amino benzoic acid, 2-methyl-naphthoquinone and inositol. Their results indicated that both distillers' dried solubles and condensed fish solubles contain an unidentified growth factor or factors which is necessary for chicks. Further studies directed toward purifying and identifying this factor were reported by Novak and Hauge in the following year (1948b). They reported that the factor was stable to heat, acid and alkali and soluble in ether, ethanol and water at widely different pH values. Using the rat as the test animal they presented evidence which indicated that the factor was distinct from all known vitamins as well as a number of other postulated growth factors. Additional work reported by these same investigators (Novak and Hauge 1948a) indicated that they had been successful in preparing a highly purified but non-crystalline extract from distillers' dried solubles. After concentrating the factor they determined solubility, adsorption on various adsorbents, and the potency or assay value with rats. The yellow amorphous compound which was obtained promoted rat growth at a level as low as 2 mcg. per rat per day indicating its vitamin-like nature. The factor was tentatively called vitamin B_{13}. In the following year Austin and Boruff (1949) demonstrated that vitamin B_{13} could be extracted from distillers' dried solubles with methanol, and that an active fraction could be extracted from the methanol extract with chloroform. The concentrate obtained by this simplified purification procedure was reported to give a growth response in chicks.

Using pigs as the test animals Cunha *et al.* (1950) reported that a vitamin B_{13} concentrate prepared in the laboratories of Hiram Walker and Sons was effective in promoting growth. The basal diet employed was composed essentially of corn, peanut meal, minerals and vitamins A, D plus seven B-vitamins. In the first experiment with weanling pigs no response from the B_{13} concentrate was observed during the first 16 days of the trial but during the following 46 days the B_{13} supplemented pigs gained 25% faster than the controls. In a second experiment similar results were obtained with the exception that no response was observed from the B_{13} for a period of 45 days.

Soon after its isolation in 1948 vitamin B_{12} was shown to account for much of the activity of the animal protein factor, and the addition of the vitamin to diets from only vegetable sources enhanced their value for pigs and poultry. The value of vitamin B_{12} in all vegetable diets led to a search for suitable sources of the vitamin, and they were found in the residues from the industrial preparation of antibiotics, especially aureomycin and streptomycin. The crude residues from *Streptomyces aureofaciens* proved better for poultry than an equivalent quantity of pure vitamin B_{12} as reported by Stokstad *et al.* in 1949. Subsequently Stokstad and Jukes (1950) showed that the superiority was due to the presence in the residues of traces of aureomycin and that aureomycin itself promoted the growth of chicks. Jukes et al. (1950a) showed that additions of crystalline aureomycin to a vitamin B_{12}-containing ration produced the same type of extra weight gains in pigs that had been observed in chicks. In a similar type experiment Luecke et al. (1950), using crystalline streptomycin, demonstrated that this growth stimulating effect in swine was not specific for aureomycin.

In 1950 Nesheim and Johnson reported that streptomycin promoted the growth of young pigs receiving a semi-synthetic milk containing alpha-protein derived from soybean oil meal. Subsequent work using alpha-protein based "synthetic milks" for baby pigs has confirmed that antibiotics improve the performance of pigs fed this diet, and under most conditions aureomycin and terramycin proved superior to other antibiotics which were tested (Wahlstrom et al., 1950; Noland et al., 1951; Wahlstrom et al., 1952).

Using a casein "synthetic milk" diet, Nesheim et al. (1950) reported no statistically significant response from the addition of aureomycin, streptomycin or penicillin when the baby pigs were pair fed. In another test, however, Sheffy et al. (1952) using vitamin-free casein as the source of protein in a "semi-synthetic milk" fed ad libitum, demonstrated a growth response from both aureomycin and streptomycin.

This property of growth stimulation resulting from the addition of small quantities of a non-nutrient compound to animal feeds is not confined to the antibiotics or anti-bacterial substances of biological origin. As early as 1946 Moore et al. reported that sulfasuxidine stimulated the growth of chicks on purified diets. Results obtained by Grumbles et al. (1948) suggested that sulfaquinoxaline improved the growth of chicks. Several reports covering chickens (Bird et al., 1948), pigs (Carpenter, 1951; Becker et al., 1952) and calves (Graf and Holdaway, 1952) have shown that certain arsonic acids are capable of stimulating the growth of animals. Chemically, these various compounds have little in common and because of this pronounced lack of chemical similarity their effect on growth can scarcely be ascribed to their direct utilization in the growth processes. It seems more likely that their similar effect on growth is somehow mediated through their common antibacterial effect on the intestinal flora. Thus it would seem plausible to assume that other compounds with anti-bacterial properties might possess growth stimulating properties.

Early in 1952 there appeared in the medical literature several reports (Bosworth et al., 1952; Selikoff and Robitzek, 1952; Robitzek and Selikoff, 1952; Elmendorf et al., 1952) regarding clinical trials with a new and promising oral anti-tubercular drug-isonicotinic acid hydrazide. In each report it was noted among the findings that shortly after the administration of the drug to patients with advanced cases of tuberculosis there was an improvement in appetite followed by a remarkable gain in weight. Selikoff and Robitzek (1952) gave considerable emphasis to this point in their report covering the treatment of 92 patients. In this regard they summarized their results by stating that: "Appetite increase and restored sense of well-being were matched and overshadowed by weight gains which varied up to 64 pounds and averaged 18 pounds at an average of nine weeks of treatment."

EXPERIMENTAL PROCEDURE

Twelve four- and five-day old Duroc baby pigs which had received colostrum from their dams, were used in this experiment. These pigs were allotted as equally as possible among four groups on the basis of initial weight, litter and sex. Each group of three pigs was housed in a wire bottomed cage which was placed in a heated and well ventilated room. A heat lamp was provided for each cage for the first four days of the experiment. The cages were scrubbed and flushed daily to maintain sanitation. All groups were fed a synthetic milk four times daily (8 A.M., 1 P.M., 5 P.M. and 9 P.M.) in amounts sufficient to provide an *ad libitum* feeding program. For the first six days of the experiment the refrigerated milk was warmed to room temperature prior to feeding but thereafter, and for the remainder of the experiment, the milk was fed at the refrigerated temperature. The milk was offered in a shallow metal trough from which the pigs learned to drink within the first day of the experiment.

The composition of the synthetic milk employed as the basal diet is shown in tables 1, 2 and 3. This milk was prepared fresh each week in the following manner: A measured amount of tap water was placed in a 30 liter milk can and brought to a temperature of 80°C. Sodium bicarbonate

TABLE 1. COMPOSITION OF BASAL DIET[1]

Ingredient	%
Casein, Labco Vitamin-Free	30
Cerelose	54
Lard	10
Mineral Mix[2]	6
Vitamins[3]	+

[1] An emulsion containing 17% solids was prepared and then homogenized.

[2] See table 3.

[3] See table 4.

TABLE 2. COMPOSITION OF MINERAL MIXTURE[1]

Ingredient	Grams
NaCL	594.
K_2HPO_4	1288.
$CaHPO_4$	1690.
Ca lactate	1144.
$MgSO_4 \cdot 7H_2O$	177.
$FeSO_4 \cdot 7H_2O$	96.50
KI	3.00
$MnSO_4 \cdot H_2O$	5.00
$Zn\ Cl_2$	1.00
$CuSO_4 \cdot 5H_2O$	1.00
$CoCl_2 \cdot 6H_2O$	0.50
	5000.00

[1] Similar to mixture described by Johnson *et al.* (1948).

TABLE 3. VITAMINS ADDED PER LITER OF MILK

Thiamin hydrochloride	1.0	mg.
Riboflavin	2.0	mg.
Niacin	4.0	mg.
Calcium pantothenate	12.0	mg.
Pyridoxine hydrochloride	2.0	mg.
Inositol	40.0	mg.
Choline	400.0	mg.
Biotin	16.0	mcg.
Ascorbic acid	16.0	mcg.
Folic acid	8.0	mcg.
Vitamin B_{12}	4.36	mcg.
Vitamin A	2333.	I.U.
Vitamin D	233.	I.U.
Alpha-Tocopherol	1.17	mg.
2-Methyl 1-4 naphthoquinone	0.33	mg.

equal to 4.75% of the water weight was then added. While this solution was agitated by an automatic stirrer the casein was slowly added, followed by the cerelose. The fat soluble vitamins were added to the melted lard and then these components were incorporated in the mixture. Hot water was added to the mineral mixture and this was then stirred in a Waring Blender prior to adding to the can. The remaining vitamins were then mixed in and the entire emulsion was then homogenized. To this milk the various experimental components as shown in table 4, were added and mixed and the resulting milks were then placed in large glass bottles and refrigerated.

The pigs were weighed individually at the start of the test and thereafter at intervals of three or four days as shown in table 6. A record of milk consumption for each group was maintained and the pigs were closely observed for any abnormal conditions during the 26 day experimental period.

The pig weight gains were tested for significance by the method of Snedecor (1946).

TABLE 4. DESIGN OF EXPERIMENT

Group	Dietary Treatment
1	Basal
2	Basal + 5 mg. Aureomycin hydrochloride per 100 gm. of dry matter
3	Basal + 0.05 ml. vitamin B_{13} concentrate[1] per 100 gm. of dry matter
4	Basal + 5 mg. isonicotinic acid hydrazide per 100 gm. dry matter

[1] Supplied by R. A. Rasmussen, Hiram Walker and Sons, Inc.

RESULTS

The overall results of this experiment are shown in summary form in table 5. An examination of this table indicates that perhaps the most outstanding aspect is the uniformity of performance among the groups. The similarity of gains, food consumption and food utilization indicates that there were no differences resulting from the various experimental treatments.

An analysis of variance conducted with the weight gains of the pigs indicated the variation within the groups far exceeded the variation between groups as evidenced by an F value of 0.012 (table 6). The average daily gains of the pigs can be considered very good and especially so in view of the necessary adjustment to a new diet, a new method of food intake and a totally different environment, after weaning. It would appear that these gains are evidence of the nutritional adequacy of the synthetic milk employed. The average daily gains of the pigs in each group during the course of the experiment are shown in table 7. It is of interest to note that for three of the four groups weight gains made during the first three days of the experiment exceeded the gains made during the following three days. A probable explanation for these results is that food fill rather than

TABLE 5. SUMMARY OF RESULTS

(3 pigs per group on test 26 days)

Group	1	2	3	4
Treatment	None	Aureo-mycin	Vit. B_{13}	INH[2]
Ave. initial age/pig, days	4.3	4.7	4.3	4.7
Ave. initial wt./pig, lbs.	4.6	4.3	4.2	4.4
Ave. final wt./pig, lbs.	23.2	22.9	22.2	22.6
Ave. daily gain/pig, lbs.	0.72	0.72	0.69	0.70
Ave. daily milk cons./pig, liters	2.0	2.0	2.0	1.9
Ave. daily DM[1] cons./pig, lb.	.75	.75	.73	.73
DM[1] cons./lb. gain	1.04	1.06	1.05	1.03

[1] Dry Matter

[2] Isonicotinic acid hydrazide

TABLE 6. ANALYSIS OF VARIANCE OF WEIGHT GAINS

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	11	191.63	
Between means	3	.86	.29
Within error	8	190.77	23.85

TABLE 7. AVERAGE DAILY GAIN IN POUNDS PER PIG DURING COURSE OF EXPERIMENT

Interval Days	Group			
	1	2	3	4
3	0.71	0.52	0.71	0.62
3	0.35	0.67	0.47	0.43
4	0.51	0.54	0.32	0.39
4	0.55	0.48	0.57	0.53
4	0.82	0.86	0.79	0.81
4	0.81	0.74	0.63	0.75
4	1.16	1.14	1.30	1.28

increase in tissue mass accounted for a part of these gains. In general all groups showed a similar steady increase in daily gains as the test progressed.

As indicated above both food consumption and food utilization were remarkably similar among the groups. The food efficiency values are excellent giving evidence of the high degree of digestibility and nutritional balance of the diet.

The pigs in all groups appeared quite normal during the course of the test. A mild and intermittent type of diarrhea was in evidence during the first few days of the test but this condition then subsided and the droppings assumed a firm consistency for the remainder of the experimental period.

DISCUSSION OF RESULTS

The lack of response to Aureomycin in this trial is perhaps not too surprising when one considers both the conditions under which this test was conducted along with data available regarding the mode of action of antibiotics.

It is first of all necessary to establish several facts regarding the area in which the pig cages were housed as well as the cages themselves. At the time this test was initiated the room in which the trial was conducted was newly completed and had not housed pigs prior to this time. Likewise the cages were newly constructed and were provided with wire screened floors which tended to remain relatively clean of both urine and feces. As already pointed out the floors of these cages were scrubbed and flushed daily. The metal feeding troughs were also cleaned daily. Supplies of milk were refrigerated between feedings in order to keep any bacterial growth at a minimum.

Coates _et al._ (1951 and 1952) in well controlled experiments observed that chicks reared in an old environment showed a marked growth response to antibiotics while such an effect was not seen when they were reared in new quarters or in a clean environment. Moreover, in the clean environment, the growth of the chicks on the control or unsupplemented diet was as good, or better than, that of the birds in the old environment receiving the antibiotic supplemented feed. These observations were confirmed in other studies conducted by Bird *et al.* (1952) and Hill *et al.* (1952). The results of these studies suggested that the growth promoting action of antibiotics appears to be associated with the control of deleterious microorganisms in the animal's environment. Further evidence in support of this theory has been shown by antibiotic studies with animals raised under germ-free conditions (Luckey *et al.*, 1956 and Forbes *et al.*, 1958) and a comparison of germ-free chicks with those experimentally infected (Forbes and Park, 1959). In another study Anderson *et al.* (1956) were able to demonstrate that feeding a high level of a mixed enterococci culture retarded the growth of chicks and that this growth retardation could be prevented by including Aureomycin in the diet. In view of the above data it is not surprising that no response to Aureomycin was noted in the trial reported herein.

It is quite clear that the pigs in this trial showed no response to the vitamin B_{13} concentrate used. One possible explanation for this lack of response is based on the fact that a "vitamin-free" casein was used as the source of protein in the milk. In their vitamin B_{13} studies with rats Novak and Hauge (1948b) found that neither casein extracted with ethanol nor commercial "vitamin-free" casein was satisfactory as a source of protein because of the presence of this factor. In order to obtain a response from their vitamin B_{13} concentrate it was necessary to employ in the diet a casein which had been rigorously treated by a series of extraction procedures.

It is also entirely possible that the pig's storage of this factor accounted for the lack of response to the B_{13} concentrate. In two separate trials with weanling pigs Cunha _et al._ (1950) observed no growth response from a vitamin B_{13} concentrate during the first 16 and 45 days of these tests. These results would suggest that the pig is capable of storing this factor. Since the trial reported herein ran for a period of 26 days it is conceivable that the pig's storage of vitamin B_{13} was sufficient to support normal growth. Evidence that this factor can be stored by rats has been reported by Manna and Hauge (1953).

The question might logically be raised as to whether the amount of vitamin B_{13} concentrate used was sufficient to ellicit a response. Cunha and coworkers (1950) using a similarly prepared concentrate from the same laboratory (Hiram Walker & Sons) incorporated 3 ml. of this concentrate per 100 pounds of feed. Calculated on a dry matter basis the amount of vitamin B_{13} concentrate used in the test reported herein was almost seven times greater than the amount used by the above mentioned investigators. From this limited information it would appear that the amount of active material used was not a limiting factor.

Although several years have elapsed since the reported discovery of vitamin B_{13} and despite a considerable amount of investigation with regard to this factor there still remains considerable confusion as to its significance in animal nutrition as well as its relationship to orotic acid. There have been data to suggest that vitamin B_{13} and orotic acid are related. Manna and Hauge (1953) advanced the idea that orotic acid may be a decomposition product of vitamin B_{13}. On the other hand Rasmussen et al. (1954) were unable to find a direct relationship between orotic acid and vitamin B_{13} and concluded that the two are different nutritional entities. More recently Ott et al. (1958) were unable to observe a significant growth promoting effect in chicks receiving "vitamin B_{13}" concentrates prepared from distillers' solubles by the method of Novak and Hauge (1948a) or a "vitamin B_{13}" concentrate prepared in the Hiram Walker and Sons' laboratory.

The results, or rather lack of results, obtained in this test with isonicotinic acid hydrazide (INH) agrees with now published information regarding the ineffectiveness of this compound with regard to improving appetite and weight gain in non-tubercular humans. Mudie et al. (1954) found that normal healthy individuals receiving INH for an eight week period showed weight gains similar to a non-treated group. A somewhat different approach to this same problem was made by Hollander (1955). This worker used patients who were not suffering from tuberculosis and whose weight had been constant for a period of weeks, but whose appetites were poor or fair. He observed no appetite stimulating properties from the administration of 300 mg. of isonicotinic acid hydrazide daily. It is therefore probable that the effect on appetite and weight gain in humans as reported by Robitzek and Selikoff (1952) and others was secondary to the suppression of the effects of tuberculosis and not an independent effect of the isonicotinic acid hydrazide on the appetite.

Based on the average weights of the pigs and their average daily consumption of dry matter the average daily intake of isonicotinic acid hydrazide calculated to be 3 mg. per kg. of body weight. In studies with infected mice the minimum effective daily dose was found to be 2 mg. of INH per kg. of body weight (Berstein _et al._, 1952). In INH studies with tubercular humans daily oral dosage levels of 4 mg. per kg. of body weight (Selikoff _et al._, 1952b) and 3 to 6 mg. per kg. of body weight (Pitts _et al._, 1953) were found to be effective. On the basis of this information the amount of isonicotinic acid hydrazide used in this trial should have been adequate.

Selikoff _et al._ (1952a) noted certain toxic effects in patients receiving INH including constipation and involuntary twitching. Neither of these symptoms nor other visible untoward effects were observed in any of the pigs receiving INH during the course of the experiment.

SUMMARY

1. A "synthetic milk" composed of "vitamin-free" casein, cerelose, lard, minerals and vitamins supported excellent growth and feed efficiency in baby pigs.

2. The addition of Aureomycin hydrochloride to the above milk at a level of 5 mg. per 100 gm. of dry matter had no effect on rate of growth, consumption of milk or feed utilization.

3. The inclusion of a vitamin B_{12} concentrate in this milk at a level of 0.05 ml. per 100 gm. of dry matter likewise had no effect on the pigs.

4. Isonicotinic acid hydrazide included in the "synthetic milk" at 5 mg. per 100 gm. of dry matter had no effect on the performance of the pigs and no toxic side effects were noted.

PART III. SOME INTERRELATIONSHIPS OF PANTOTHENIC ACID, METHIONINE AND PROTEIN IN THE RAT

INTRODUCTION

That the dietary requirement for a single nutrient can be rather variable and dependent upon the character and quality of the accompanying nutrients is becoming increasingly evident. Such interplay of nutrients is classically exemplified by the sparing action of fat on thiamin and the increased dietary riboflavin requirement for the rat on high fat rations.

It is well known that pantothenic acid is a dietary essential for the rat. As a result of numerous investigations during the early 1940's, it became generally accepted that the requirement of the growing rat for this vitamin was at or close to 100 mcg. per day. Subsequently it became evident, however, that the protein level of the diet could have a profound influence on the rat's need for pantothenic acid. Further study suggested that it was the amino acid methionine which was responsible for this sparing effect on the need for pantothenic acid. In the studies concerned with these interrelationships casein or blood fibrin was used as the protein source and likely contributed to a variable and perhaps inadequate source of vitamin B_{12} in the diets.

There is good evidence to suggest that vitamin B_{12} is interrelated in the metabolism of both pantothenic acid and methionine - a point not mentioned in previous investigations concerned with the relationship of pantothenic acid and protein or methionine. In light of this information it appeared desirable to re-examine this latter relationship using soybean protein based diets containing a known and adequate amount of vitamin B_{12}.

REVIEW OF LITERATURE

In 1940 Unna reported the pantothenic acid requirement of the growing rat to be 80 mcg. per day. This same investigator in conjunction with coworkers (Unna and Sampson, 1940; Unna and Richards, 1942) conducted additional studies along this line and found the daily requirement of the growing rat for pantothenic acid to be between 80 and 100 mcg. Numerous other investigators including Emerson and Evans (1941), Elvehjem *et al.* (1941), Henderson *et al.* (1942), Bacon and Jenkins (1943) and Slanetz (1943) reported confirmation of the findings of Unna and coworkers with the result that the value of 100 mcg. per day or 1 mg. per 100 gm. of ration became generally accepted as the amount needed by the young growing rat (Brown and Sturtevant, 1949). It is perhaps of considerable significance that in each of the above cited reports casein was used as the sole source of protein in the diet, and furthermore, in seven of the eight reports the level of casein in the diet was either 18 or 20%. The similarity of the diets in these respects may well account for the similarity of results.

The above mentioned studies involving the pantothenic acid requirement of the rat were conducted utilizing diets containing a high proportion of carbohydrate with minimal levels of protein and fat. On these high carbohydrate diets it has been shown that pantothenic acid deficient rats grow poorly and usually survive only one to three months (Mills et al., 1940; Salmon and Engel, 1940; Unna, 1940). Wright, Skeggs and Sprague (1945) found that rats receiving liberal protein diets were less susceptible to the deficiency syndrome induced by feeding succinylsulfathiazole in highly purified diets. Such animals had higher hepatic stores of pantothenic acid than control rats receiving the usual amount of protein in the diet. As a result of increasing the casein level in a pantothenic acid deficient diet from 24 to 64% Nelson and Evans (1945) were able to demonstrate improved growth and livability in young rats. In the following year Wright and Skeggs (1946) reported that in rats fed ad libitum the fecal elimination of the B-vitamins studied closely paralleled the level of dietary protein. In equalized feeding the fecal elimination of pantothenic acid was directly proportional to the protein (casein) content of the diet. These results prompted several hypotheses regarding the actual mechanism(s) involved. The superiority of the high protein diet in these studies could have been due to the decreased level of dietary carbohydrate since there is considerable evidence that pantothenic acid is involved in carbohydrate metabolism (Williams, 1943). Traces of pantothenic acid or other factors in the casein might have accounted for the improved results or the amino acid content of the casein in some way spared the need for this vitamin. In later studies Nelson *et al.* (1947) confirmed that growth and survival of rats fed pantothenic acid deficient diets were improved when the casein content was increased from 24 to 64%. That more vitamin was actually available to the rat on the higher protein diets was indicated by a higher urinary excretion of pantothenic acid on diets containing 64% casein. The results were interpreted as indicating a sparing action of high protein diets on the pantothenic acid requirement, either by decreasing the need for the vitamin or by producing a limited biosynthesis from one or more of the amino acids. An analysis of the purified casein used in the diets indicated that its pantothenic acid content was not sufficient to fully account for the protective action of the high protein diets. As further proof that the pantothenic acid residues in the washed casein were not entirely responsible for these results the same authors (Nelson and Evans, 1947) used washed beef blood fibrin, which was even lower in its pantothenic acid content than casein, as the protein component of the diet. Despite an accentuation of a pantothenic acid deficiency produced by substituting this fibrin for casein at the 25% level, doubling the fibrin level resulted in significantly better growth and survival. The pantothenic acid requirement of the pig has also been shown by Luecke and coworkers (1952) to be similarly dependent on the level of protein in the diet.

Further studies by Nelson and Evans (1949) were directed toward determining if amino acids were responsible for the pantothenic acid sparing action of high protein diets. Levels of the single amino acids - DL-methionine, L-cystine, L-tryptophan, L-histidine, L-glutamic acid and glycine - equivalent to 10% to 30% casein were added to a pantothenic acid deficient diet containing 24% casein. The data showed that methionine had a marked sparing action, improving both growth and survival of the deficient rats. Ludovici _et al._ (1951) in a similar type experiment were unable to confirm these findings of Nelson and Evans. Utilizing levels of 2.7% and 1.4% of DL-methionine in a basal diet containing 25% vitamin-free casein there appeared to be no effect from methionine on the growth of either pantothenic acid deficient rats or normal controls. However, these workers were able to demonstrate that the methionine exerted a significant sparing action upon the pantothenic acid requirement for antibody production. In their work neither Nelson and Evans, (1949) nor Ludovici _et al._ (1951) mentions the possible influence of vitamin B_{12} on their results. None of the diets used contained added vitamin B_{12} per se but since casein was used as the protein source it is likely that some B_{12} was provided.

Yacowitz _et al._ (1951) in studies with chicks found that vitamin B_{12} spared the pantothenic requirement for growth, survival and the prevention of dermatosis. Further indication that vitamin B_{12} and pantothenic acid are related in the chick was reported by Evans _et al._ (1951). These authors observed that in the absence of sufficient vitamin B_{12}, pantothenic acid accumulated in the liver indicating that the vitamin B_{12} aids in the transfer of pantothenic acid from the liver for use elsewhere in the body.

There is ample evidence to suggest that methionine and vitamin B_{12} are interrelated in metabolism. Patton _et al._ (1946) noted that supplementation of a corn-soybean diet with methionine produced an extra growth response in chicks. When sardine meal was added to the ration at a level of 2%, no additional growth response to methionine was obtained even though the sardine meal did not supply sufficient methionine to account for the effect in terms of this supplement. In studies with rats György and Rose (1950) found that vitamin B_{12} exerted a lipotropic effect in animals receiving a low protein - low fat ration limited in its choline-methionine content. A partial clarification of this interrelationship was reported by Jukes _et al._ (1950). These authors concluded that their results gave clear indication that vitamin B_{12}-deficient chicks, in contrast to normal chicks, are unable to transform homocystine into methionine, and consequently, that vitamin B_{12} is somehow involved in this transformation. A similar conclusion was reached by Oginsky (1950).

EXPERIMENTAL PROCEDURE

Fifty-six weanling male rats of Sprague-Dawley strain averaging approximately 40 gm. in weight were placed on a pantothenic acid depletion diet for a period of ten days prior to the start of the test. This procedure was designed to serve a two-fold purpose, including the depletion of body stores of pantothenic acid as well as a means of allotting the animals at the start of the test on the basis of both original weight and percentage gain for the period rather than the single criteria of original weight.

The rats were individually caged and at the start of the test, in order to avoid or equalize a possible position effect, the animals within a group were assigned to various levels in the cage rack so that all groups were essentially equal in this respect. Feed and water were provided ad libitum and a record of feed consumption was maintained. Weights of the individual rats were taken at weekly intervals at which times feed weighbacks were made.

The ingredients and percentage composition of the basal diets used in this study are shown in table 1. Since one of the objectives of this experiment was to determine if protein had a sparing effect on the pantothenic acid requirement of the rat it seemed advisable to equalize the pantothenic

TABLE 1. COMPOSITION OF BASAL DIETS

Ingredient	Diet	
	A %	B %
Sodium Proteinate[1]	21.0	32.6
Cerelose	69.74	58.0
Lard[2]	4.0	4.0
Mineral Mix[3]	5.0	5.0
Vitamins[4,5]	+	+
DL-Methionine	0.26	0.40

[1] Obtained from The Archer-Daniels-Midland Co., Minneapolis

[2] Swift'ning

[3] See table 2.

[4] Calcium pantothenate was added to diet A in the amount of 0.76 mg. per 1000 grams of mixed diet in order to equalize the pantothenic acid content of both diets.

[5] See table 3.

TABLE 2. COMPOSITION OF MINERAL MIXTURE[1]

Ingredient	Grams
NaCl	594.0
K_2HPO_4	1288.0
$CaHPO_4$	1690.0
Ca lactate	1144.0
$Mg\ SO_4 \cdot 7H_2O$	177.0
$FeSO_4 \cdot 7H_2O$	96.5
KI	3.0
$MnSO_4 \cdot H_2O$	5.0
$Zn\ Cl_2$	1.0
$Cu\ SO_4 \cdot SH_2O$	1.0
$Co\ Cl_2 \cdot 6H_2O$	0.5
	5000.0

[1] Similar to mixture described by Johnson et al. (1948).

TABLE 3. VITAMIN MIXTURE

Vitamin	Amount/1000 gm. of diet
Thiamin hydrochloride	5.0 mg.
Riboflavin	10.0 mg.
Niacin	20.0 mg.
Pyridoxine hydrochloride	5.0 mg.
Inositol	400.0 mg.
Choline chloride	1000.0 mg.
Folic acid	0.2 mg.
Para-amino benzoic acid	10.0 mg.
Biotin	0.2 mg.
Vitamin B_{12}	20.0 mcg.
Vitamin A	10,010. I.U.
Vitamin D	1,500. I.U.
Alpha-tocopherol	0.1 gm.
2-Methyl 1-4 naphthoquinone	2.5 mg.

acid content of both basal diets. It will be noted in table 4 that these diets were calculated to provide identical quantities of pantothenic acid. The level of 0.19 mg. of pantothenic acid per 100 grams of diet should have been well below the rat's requirement for this vitamin as already cited in the literature review.

Table 4 shows that the sodium proteinate provided 0.21% and 0.33% of methionine in diets A and B respectively. A level of 0.47% total methionine was contained in diet A through the addition of synthetic DL-methionine. This level of methionine was designed to just meet the growing rat's requirement based on the work of Womack and Rose (1941). Since studies with chickens had shown that their methionine requirement was approximately a constant percentage of the protein level (Almquist 1949, Grau and Kamei 1950) this concept was applied in arriving at the rat's requirement for methionine in diet B. It can be noted that both diets A and B contained the same amount of methionine when expressed as percent of protein.

The design of the experiment is shown in table 5. Additions of DL-methionine were again made on the same basis as indicated above. All diets containing the added methionine were calculated to provide 3.32% of methionine expressed as percent of protein. The amount of pantothenic acid added together with the amount contained in the basal diets was

TABLE 4. CALCULATED NUTRIENT LEVELS

	Diet	
	A	B
Protein, %[1]	18.	28.
Methionine, %[2]	.21	.33
DL-methionine added, %	.26	.40
Total methionine, %	.47	.73
Methionine as % of protein, %	2.60	2.60
Pantothenic acid, mg/100 gm.[3]	.12	.19
Pantothenic acid added, mg/100 gm.	.07	-
Total pantothenic acid, mg/100 gm.	.19	.19

[1] Does not include nitrogen from added methionine.

[2] Based on methionine content of 1.02% in sodium proteinate (Schultze 1950).

[3] Based on 5.72 mg. pantothenic acid per kilogram of sodium proteinate.

TABLE 5. DESIGN OF EXPERIMENT

Group	Basal Diet	Supplement
1	A, low protein	none
2	A, " "	0.129% DL-methionine
3	A, " "	0.21 mg. of pantothenic acid/100 gm. diet
4	A, " "	combined group 2 + group 3 treatments
5	B, high protein	none
6	B, " "	0.2% DL-methionine
7	B, " "	0.21 mg. of pantothenic acid/100 gm. diet
8	B, " "	combined group 6 + group 7 treatments

calculated to provide 0.4 mg. of pantothenic acid per 100 grams of diet - a level considered to be well below the growing rat's requirement.

All diets were prepared just prior to the start of the experiment, stored in covered glass jars and refrigerated until used.

The rat weight gains were tested for significance by the method of Snedecor (1946).

RESULTS

The results of this study are shown in summary form in tables 6 and 8. It is evident that the additions of DL-methionine to either of the basal diets did not improve growth. If anything there is some suggestion of impaired growth since groups 2, 4 and 6 (but not group 8) showed slightly poorer growth than their respective reference groups 1, 3 and 5. In each case where pantothenic acid was added to the diet (groups 3, 4, 7 and 9) there was a marked and highly significant improvement in weight gains over the controls. Of particular interest is the fact that groups 5, 6, 7 and 8 receiving the higher protein level in their diets showed poorer weight gains than their respective counterpart groups 1, 2, 3 and 4 receiving the lower protein level. The latter groups outgained the former by an average of 20%.

An examination of table 7 clearly indicates that those groups receiving supplemental additions of pantothenic acid to their diets showed a growth response the first week of the test and consistently outgained the unsupplemented groups throughout the five week experimental period. During the fifth and final period all groups continued to show weight gains although groups 5 and 6 exhibited small gains which might suggest that body stores of pantothenic acid were almost depleted.

TABLE 6. SUMMARY OF RESULTS

(7 rats per group on trials lasting 5 weeks)

Group	Initial[1] wt.	Final[1] wt.	Wt.[1] gain	Feed[1] Consumption	Gm. feed per[1] gm. gain
	gm.	gm.	gm.	gm.	
1	72.9	130.0	57.1	305	5.34
2	74.1	129.7	55.6	289	5.20
3	72.9	192.4	119.5*	453	3.79
4	74.6	178.3	103.7*	383	3.69
5	74.3	125.3	51.0	276	5.41
6	73.1	118.6	45.5	313	6.88
7	74.7	165.7	91.0*	376	4.13
8	73.3	165.7	92.4*	328	3.55

[1] Average figures per rat.

* Difference in gain highly significant ($P < 0.01$) over groups 1, 2, 5 and 6.

TABLE 7. AVERAGE GAINS PER RAT BY PERIOD

GROUP	PERIOD[1]				
	1	2	3	4	5
	gm.	gm.	gm.	gm.	gm.
1	19.4	8.1	12.2	5.8	11.6
2	17.6	7.3	12.3	8.8	9.6
3	21.5	22.5	23.8	24.6	27.1
4	19.3	16.2	23.3	22.7	22.2
5	15.7	11.3	8.6	9.7	5.7
6	17.5	6.8	10.2	8.3	2.7
7	21.6	14.6	16.4	21.3	17.1
8	23.1	18.6	20.1	16.9	13.7

[1] Each period of 7 days duration.

TABLE 8. A COMPARISON OF THE EFFECTS OF DIETARY TREATMENTS ON WEIGHT GAINS[1] OF RATS

Supplement to basal diets	Low Protein		High Protein		Average	
	no methionine	added methionine	no methionine	added methionine		
	gm.	gm.	gm.	gm.	gm.	gm.
No pant. acid	57.1		51.0		54.1	52.3
No pant. acid		55.6		45.5	50.5	
Added pant. acid	119.5		91.0		105.3	101.7
Added pant. acid		103.7		92.4	98.1	
Average	88.3	79.7	71.0	69.0		
Average	84.0		70.0			

1 Average gain per rat.

In each case where pantothenic acid was added to the basal diets there was an improvement in both feed consumption and feed utilization. In group 2 the addition of methionine appeared to depress feed consumption but improved the utilization of feed, when compared with the control group. A comparison of groups 5 and 6 shows that the latter, receiving supplemental methionine, showed improved feed consumption but a poorer feed efficiency value. In comparing groups 4 and 8 with groups 3 and 7 it is apparent that the addition of methionine resulted in a reduction in feed consumption and an improvement in feed efficiency. These results tend to confirm the same effect shown by group 2 and raise a question as to the validity of feed results shown for group 6.

None of the rats in any of the groups showed evidence of spectacle alopecia, scaling of the skin or dermatitis (Follis, 1948) which might be indicative of a pantothenic acid deficiency. There was no mortality during the 35 day experimental period.

DISCUSSION OF RESULTS

The results obtained in this study do not indicate a sparing action of either protein or methionine on the pantothenic acid requirement of the growing rat. These findings are in contrast to those of Nelson _et al._ (1947), Nelson and Evans (1947), Nelson and Evans (1949), Dinning _et al._ (1954) and Kaunitz _et al._ (1955), but are in accord with the report of Ludovici _et al._ (1951).

On the basis of the data obtained it is evident that both basal diets, containing 18% and 28% protein, were deficient in pantothenic acid. That these diets were not severely limiting in pantothenic acid is evidenced by the fact that the rats continued to show some growth and no mortality occurred. The basal diets were calculated to contain 0.19 mg. of pantothenic acid per 100 grams of diet, a value which while well below the reported requirement of the growing rat is well above the amount contained in diets used by the above mentioned investigators. Nelson _et al._ (1947) reported that the alcohol extracted caseins as used in their experimental diets were found to contain by analysis 1.5, 1.8 and 2.8 mcg. of pantothenic acid per gram. Thus in diets containing 24% casein the pantothenic acid level could not have exceeded 0.07 mg. per 100 grams of diet and in some instances may have been only one-half this value. It is conceivable that in order to demonstrate the sparing effect of protein or methionine on the pantothenic acid requirement of the rat it is necessary to employ diets more severely limiting in this vitamin than those used in this study. The report by Nelson and Evans (1947) partially supports this contention. By utilizing water washed beef blood fibrin in place of casein these workers were able to devise a diet considerably more deficient in its pantothenic acid content. In a comparison of diets containing 24% and 48% of casein or fibrin they observed that the differences in growth and survival were more marked between fibrin levels than they were between casein levels - suggesting that the greater the deficiency of pantothenic acid the more dramatic the response to a higher protein level.

Failure to observe a growth response from the methionine or higher protein level may also be partially attributable to the short duration of this experiment. Nelson and Evans (1945) and Nelson and coworkers (1947) reported that the pantothenic acid sparing effect from a high casein diet became more pronounced as the experimental period was extended beyond 60 days. In another study Nelson and Evans (1947) were able to show that the greater the deficiency of pantothenic acid in the diet the shorter the period of time required to demonstrate a sparing effect from a high protein level.

Similar data on the time required for the sparing action of methionine to become apparent were not presented by Nelson and Evans (1949) or Dinning et al. (1954). Ludovici et al. (1951) reported a lack of growth response to methionine in a pantothenic acid deficient diet in trials conducted for a period of seven weeks - a length of time similar to the 45 days used in the trial reported herein.

The question might logically be raised as to whether the methionine content of the basal rations was in excess of the rat's requirement. If this were the case then it is conceivable that the excess methionine exerted a pantothenic acid sparing effect to an extent that it prevented the development of a deficiency in the basal fed groups. This might also account for the lack of response to supplemental methionine.

As already cited, the work of Womack and Rose (1941) indicated the methionine requirement of the growing rat to be approximately 0.50% in the presence of adequate cystine and in a diet containing 18% protein. Basal diet A was calculated to contain 0.47% methionine and it was assumed that the cystine content was adequate. The data of Wretling and Rose (1950) indicate that the young rat requires 2.0 grams of methionine per 16 grams of nitrogen in a diet containing adequate cystine and presumably adequate vitamin B_{12} supplied in the form of a liver extract. Thus in diets containing 18% and 28% protein, as used in this study, the methionine needs would be met by 0.36% and 0.56% respectively. If the assumption is made that the above data on methionine requirements are correct then diets A and B would have supplied methionine in excess of the rat's requirement of 0.12% and 0.17% respectively.

In a study conducted by Dinning _et al._ (1954) a basal diet reportedly devoid of pantothenic acid was supplemented with pantothenic acid, graded levels of DL-methionine and combinations of these two. The 16.2% protein basal diet was reported to contain 0.27% methionine which would be somewhat less than the rat's requirement of 0.33%, based on 2.0 grams of methionine per 16 grams of nitrogen. When 0.1% of DL-methionine was added to the diet, for a total of 0.37% methionine, growth and survival of the rats was improved to a degree comparable with that achieved when the rat's requirement for pantothenic acid was met. The addition of 0.43% DL-methionine further improved growth and prevented mortality. From the above data it seems likely that the methionine content of basal diets A and B was in excess of the rat's requirement and to this extent exerted some pantothenic acid sparing effect and was at least partially responsible for the lack of response to supplements of this amino acid.

As already cited, there is good evidence to suggest that vitamin B_{12} has a sparing effect on both the pantothenic acid (Yacowitz _et al._, 1951; Evans _et al._, 1951) and methionine (Patton et al., 1946; György and Rose, 1950; Jukes et al., 1950) requirements of several species. At this writing it appears possible that the vitamin B_{12} added to the basal diets may have contributed to some sparing action on either or both the pantothenic acid and methionine and thus influenced the reported relationship between these latter two nutrients. Unfortunately the design of this test was such as to preclude the possibility of assessing the effect of vitamin B_{12} on the results. The work of Dinning et al. (1954) indicates that in diets devoid of vitamin B_{12} the sparing effect of methionine on the pantothenic acid requirement of the growing rat can be shown.

Of particular interest is the fact that there was an apparent slight growth depression resulting from the additions of DL-methionine. This can probably best be explained on the basis that the addition of methionine to a diet already adequate in this amino acid has a tendency to depress growth in the rat (Kade and Sheperd, 1948; Brown and Allison, 1948; Russell et al., 1949; and Wretlind and Rose, 1950). While the above investigators utilized methionine levels in excess of those used in the experiment reported herein the degree of growth retardation which they observed was of a much greater magnitude.

Those groups receiving the higher protein level (diet B) exhibited poorer growth than the correspondingly treated groups receiving the lower protein level (diet A). The reason or reasons for this rather surprising effect are not known. It is conceivable that this result is related to the above discussion on the inhibitory effects of excess methionine. According to the calculations for methionine requirements and contents of the basal diets as set forth above, diet B provided 0.17% methionine in excess of the rat's requirement while diet A provided an excess of 0.11% methionine. Thus it can be seen that the higher protein diet B contained a greater excess of methionine than did diet A. The experimental diets fed groups 6 and 8 contained a total of 0.93% methionine or 0.37% methionine in excess of requirements while groups 2 and 4 received diets containing 0.60% methionine or an excess of 0.24%. This small but perhaps important excess of methionine in the diet may have created an amino acid imbalance capable of depressing growth to the degree observed.

SUMMARY

1. Weanling rats fed a pantothenic acid deficient purified diet composed of a soybean protein preparation, DL-methionine, Cerelose, lard, vitamins and minerals and containing 18% of protein exhibited relatively poor growth with no mortality during a 35 day trial.

2. Increasing the level of protein in the diet to 28% appeared to depress growth and feed utilization thus giving no evidence that the extra protein spared the growing rat's requirement for pantothenic acid.

3. The supplementation of both the high and low protein containing diets with a suboptimal level of pantothenic acid resulted in a significant improvement in weight gains and a definite improvement in feed utilization.

4. The addition of DL-methionine to both the 18% and 28% protein diets appeared to depress rate of growth and gave no evidence that this amino acid spared the need for pantothenic acid.

5. The inclusion of both pantothenic acid and DL-methionine in the 18% and 28% protein diets did not improve growth over that achieved with pantothenic acid alone, however, there was some improvement in the utilization of feed apparently attributable to the added methionine.

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