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Effect of Certain Adsorbents and Mineral Mixtures on the Availability of Riboflavin and other B-vitamins in Rations

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The Effect of Certain Adsorbents and Mineral Mixtures on the Availability of Riboflavin and Other B-Vitamins in (Synthetic and Natural) Rations

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INTRODUCTION

The question is often raised as to what effect mineral mixtures or fillers in mineral mixtures have on the overall utilization of livestock feeds. True, mineral supplementation is necessary but this is only one factor in good nutrition. In recent years a host of mineral supplements, even trace mineral mixtures, have appeared on the market to meet these requirements and to insure an adequate intake for economical production.

However, Rubin, *et al.* (1948) have shown that corn grit premixes containing "ferrum reductum" when incorporated into foods caused a loss of thiamine and riboflavin, as determined by the microbiological method. Kandutsch and Bauman (1953) found that the stability of vitamin B_1 in a laboratory diet depended upon the fat, salts, and the temperature at which it was stored.

The studies reported herein are concerned with the availability of riboflavin, niacin, and pantothenic acid when fed to laboratory animals in the presence of vitamin carriers, minerals, and trace mineral mixtures and from these results a plan may be found for recommendations to all who are in touch with livestock feeders. The microbiological and rat growth methods were used to determine vitamin availability. The results should be interpreted only from the data as they are presented in this type of study.

EXPERIMENTAL PROCEDURE

Preparation of Materials. Riboflavin. The riboflavin was dissolved in water (acidified) and then mixed and evaporated to dryness on weighed amounts of adsorbents, such as Norite A, fuller's earth, bone black, bone meal, etc. This procedure was carried out in subdued light at room temperature. After drying the materials were finely ground in an agate mortar and then mixed in the otherwise complete synthetic ration. Alfalfa meal and dried skimmilk were used as natural sources of riboflavin. These products were mixed in synthetic rations with different adsorbents as the only source of riboflavin.

Niacin and Pantothenic Acid. Niacin and pantothenic acid as calcium pantothenate were mixed separately with weighed amounts of two brands of commercial trace mineral mixtures of approximately the same composition (from label) and then incorporated into adequate synthetic rations free from the vitamin under study.

Microbiological Method. Riboflavin. Suitable samples were prepared by adding 50 ml. 0.1 N HCl to weighed amounts of the material to be assayed and then autoclaved for 15 minutes at 15 pounds pressure. To the cooled sample was added 3.3 ml. 2.5 molar sodium acetate and made to volume. The samples were then filtered and the filtrate adjusted to pH 6.6–6.8. Suitable aliquots were then taken for assay, using a modified microbiological method of Snell and Strong (1939) using *Lactobacillus caseii.* The modification consisted of adding 100 γ calcium pantothenic per liter to the basal medium.

Pantothenic Acid. The samples (known weight) were prepared by adding 75 ml. distilled water and autoclaving for 30 minutes at 15 pounds pressure, then cooled and made to volume, filtered and suitable aliquots were assayed according to the method previously used in this laboratory (Hunt *et al.*, 1947), using *Lactobacillus arabinosus* 17-5 as the assay organism.

Niacin. Known weight of the samples were placed in 250 ml. Erlenmeyer flasks, to which were added 25 ml. 1 N NaOH and autoclaved 30 minutes at 15 pounds pressure. The solutions were cooled, neutralized to pH 6.6–6.8, made to volume, and then allowed to stand one hour or longer to allow precipitate to settle. Suitable aliquots were taken and assayed by the method used in this laboratory (Hunt *et al.* 1947).

Rat Growth Method. Rats (40-50 gm. weight) from the Station colony were placed on certain vitamin deficient rations (depending upon the vitamin under study) for two weeks. The rations had the following composition: sucrose, 74.5; casein (vitamin free), 18.0; salt mixture, 4.0; corn oil, 3.25; A and D oil, 0.25. The vitamin B-complex requirements, except the vitamin under study, were incorporated into the diet. At the end of the depletion period all the lots of experimental animals continued to receive the vitamins as added to the ration and in addition the vitamins under study in various amounts and from different sources as the nature of the study indicated. The amount of feed in which the vitamins were added was based upon the experiences that the rat will consume about 10 gms. per day when given this type of diet. However, the amount of feed consumed will depend upon how near their vitamin requirements are being met. In the later experiments the vitamin B-complex requirements were given to the animal daily and in a separate dish in order to make sure that the only limiting factor was the availability of the vitamin under study.

RESULTS AND DISCUSSION

The results of the microbiological assay of the riboflavin containing adsorbent are given in table 1. Riboflavin adsorbed on Norite A (decolorizing carbon), spent bone black, and two brands of fuller's earth was very poorly eluted by the method used and hence it was not available for the use of the test organism. On the other hand, steamed bone meal, limestone (finely ground), dicalcium phosphate, and one brand of fuller's earth were very satisfactory carriers in that the riboflavin eluted by the same method was available to the test microorganisms. The results also show that in the assays where bone meal, limestone, dicalcium phosphate were the carriers more riboflavin was

TABLE 1.—The Effect of Adsorbents on the Availability of Riboflavin (Microbiological Method)

	Riboflavin available, γ/gm.	Riboflavin available, %	Riboflavin γ/gm in feed		Riboflavin available, %
			Total	Available	Total
Norite A	8 (1)	8	6.6 (4)	6.3	95
Norite A	13 (2)	3	12.9 (5)	7.6	59
Bone black	16 (1)	16	6.6 (4)	6.3	95
Bone black	84 (2)	21	12.9 (5)	9.1	71
Fuller's earth—C-80	8 (3)	16	6.2 (6)	3.4	55
Fuller's earth—P-59	6 (3)	12	6.2 (6)	3.2	52
Fuller's earth—W-a	58 (3)	116	6.2 (6)	6.8	110
Bone meal	400 (2)	100	12.9 (5)	12.7	98
Limestone	116 (1)	116	6.6 (4)	7.6	115
$Ca\ H\ PO_4 . 2H_2O$	108 (1)	108	6.6 (4)	8.0	121

(1) 100 γ riboflavin per gm. adsorbent.
(2) 400 γ riboflavin per gm. adsorbent.
(3) 50 γ riboflavin per gm. adsorbent.
(4) 2.5 γ riboflavin on adsorbent mixed with feed (Feed contains 4.1 γ/gm)
(5) 10.0 γ riboflavin on adsorbent mixed with feed (Feed contains 2.9 γ/gm)
(6) 2.5 γ riboflavin on adsorbent mixed with feed (Feed contains 3.7 γ/gm)

recovered than was added. Errors due to method of assay may partially supply the answer but on further investigation it was found that the addition of a calcium compound was also responsible for additional growth of the microorganisms. Since then dicalcium phosphate and/or calcium pantothenate has been added to the basal medium to supply this need.

It appears from the results (table 1) that mixing the riboflavin-carrying substance—Norite A, bone black, and fuller's earth—with a stock rat ration, improved the recovery of the riboflavin as determined microbiologically. There is experimental evidence that fatty acids stimulate microbial growth but these factors appear to be ruled out here for it has been shown that these acids can be removed by the addition of 2.5 M. sodium acetate (pH 4.5) and filtering through filter paper. The stock ration contained no added fat, and $CaCO_3$ was part of the minerals added.

TABLE 2.—The Growth Rate of Rats on Various Levels of Riboflavin and the Effect of Adsorbents With and Without Riboflavin on Its Availability

Supplements to basal ration, including adsorbents (carriers)*	Average gain/rat/wk. gms.	Feed consumed /gram gain	Riboflavin available, %
1. No riboflavin	3.0	13.3	
2. 5 γ riboflavin in 10 gm. feed	9.7	4.9	
3. 10 γ riboflavin in 10 gm. feed	15.5	3.9	
4. 20 γ riboflavin in 10 gm. feed	26.0	3.3	
5. 40 γ riboflavin in 10 gm. feed	29.0	3.3	
6. 20 γ riboflavin on Norite A in 10 gm. feed	4.0	9.5	15.4
7. 20 γ riboflavin on Fuller's earth in 10 gm. feed	24.5	3.1	94.2
8. 20 γ riboflavin on bone black in 10 gm. feed	21.5	3.7	82.7
9. 20 γ riboflavin on bone meal in 10 gm. feed	29.0	3.3	111.5
10. 20 γ riboflavin/10 gm feed + Norite A	22.2	3.4	85.4
11. 20 γ riboflavin/10 gm. feed + Fuller's earth	22.5	3.3	86.5
12. 20 γ riboflavin/10 gm. feed + bone black	26.0	3.1	100.0
12. 20 γ riboflavin/10 gm feed + bone meal	25.0	3.1	96.1

*All carriers—5.0 gm./kilogm. of feed.

The results of the rat assay (table 2) show that 15.4 percent of the riboflavin adsorbed on Norite A was available, while only 3-8 percent was available microbiologically. Bone black, fuller's earth and steamed bone meal were satisfactory carriers in supplying riboflavin for rat growth. The recovery of 82.7 percent from bone black would place it in a questionable position as a suitable carrier. Since the accuracy of rat assay is no better than ± 10 percent, recoveries of 90 percent or better were considered within the normal range.

The effect of the available riboflavin adsorbed on Norite A and noted as growth, compared to the growth of the controls (Lot 4) was very significant. The addition of 5.0 gm/kilo of Norite A or fuller's earth to a ration adequate in riboflavin (Lots 10 and 11) reduced the rat growth somewhat.

Alfalfa meal and dried skimmilk are considered to be good sources of riboflavin in practical rations. The effect of additions such as bentonite, soft clay rock phosphate, and spent bone black on the availability of riboflavin, the sole source of which was alfalfa meal and dried skimmilk, is shown in table 3. The alfalfa meal and dried skimmilk were each fed at the 5 percent level or 0.5 gm. for each 10 gm. of basal ration.

The results show that the biological method of assay for riboflavin in alfalfa was 14.3 γ/gm compared to 14.0 γ/gm by the microbiological method, while the assay of dried skimmilk was 21.9 γ/gm compared to 17.0γ/gm by the microbiological method. Therefore, any great difference in the biological value due to the additive must be due to the non-available riboflavin.

The results show (table 3) that bentonite and bone black not only affected the availability of the riboflavin of these two ingredients but also increased the feed consumption per gram of gain.

It is not known how much bentonite or spent bone black is now used as a carrier of vitamins or as a filler in mineral mixtures, yet they are obtainable. It is also known that bentonite is used as a binder in pelleting feeds. The results show that there was a loss of 25-30 percent and 18-36 percent of riboflavin from alfalfa meal and dried skimmilk, respectively, compared to the control when bentonite and bone black were added (1 percent) to these rations. That is an appreciable loss.

Soft clay rock phosphate is at the present time a common source of calcium and phosphorus in feeds. The results show that when it is added to this type of ration where dried skimmilk was the source of riboflavin, there was a loss of about 23 percent of the riboflavin. However, the feed consumption per gram of gain was very little different from that of the control. When the diet contained 10 percent of alfalfa meal or dried skimmilk, as the source of riboflavin, the effect of bone

TABLE 3.—The Effect of Additives on the Availability of Riboflavin in Alfalfa Meal and Dried Skimmilk

	Gain/rat per week gms.	Grams feed consumed/ rat/day	Alfalfa meal consumed/ rat/day gms.	Ribo-flavin assay γ/gm.	Ribo-flavin avail-able, %	Feed consumed per gram gain gms.
1. Alfalfa meal—5% . . .	12.5	7.98	0.3990	14.3		4.46
2. Alfalfa meal + 1% bentonite	9.7	7.17	0.3585	10.6	74.1	5.17
3. Alfalfa meal + 1% bone black	10.5	8.69	0.4345	9.9	69.2	5.79
			Dried skimmilk consumed/ rat/day gms.			
4. Dried skimmilk—5% . .	19.0	11.34	0.5670	21.9		4.18
5. Dried skimmilk + 1% bentonite	12.5	8.01	0.4005	14.2	64.4	4.48
6. Dried skimmilk + 1% bone black	16.2	10.03	0.5015	18.1	82.6	4.33
7. Dried skimmilk + 1% soft rock phosphate . .	14.5	8.69	0.4345	17.0	77.1	4.20

black and soft clay rock phosphate were nil (Data not shown). Bentonite, on the other hand, caused a loss of 30 percent and 20 percent of riboflavin in alfalfa meal and dried skimmilk, respectively. Bentonite appears to effect the availability of the riboflavin in these two products, while bone black and soft clay rock phosphate have less effect in that by feeding larger amounts of the riboflavin-carrying substances the resulting tieup can be overcome.

An indication that charcoal may interfere with the availability of vitamins to chicks has been reported by Almquist and Zander (1940), and Grummer, *et al.* (1951) reported that feeding coal to pigs decreased the feeding efficiency, although there was no change in the growth performance. These published data and the results presented herein would appear to confirm these reports.

Trace Minerals. The question is frequently raised as to the efficiency in livestock feeding of one commercial trace mineral mixture compared to that of another. Two such mixtures were selected, called No. 1 and No. 2. According to the label, these two mixtures were quite similar as to their elemental composition. This part of the study was to note the effect of these mineral mixtures on the availability of niacin and pantothenic acid as they effect growth when mixed with the same in an otherwise adequate synthetic ration.

In the first experiment, the B-complex vitamins, except the one under study, were mixed in the synthetic rations (described earlier). The composition of the B-complex vitamins and the amount added are shown in table 4. In addition, 10 mgs. pantothenic acid and 50 mgs. niacin each were mixed (wet) with 5.0 gm. of the respective trace mineral mixtures and then added to one kg. of the basal ration as the sole source of that particular vitamin.

The results of the study (table 4) show that when the pantothenic acid was mixed (wet) with mineral mixture No. 1 the gain per week, the feed consumed per gram gain are all very similar (Lot 4) to the control (Lot 1).

When the same amount was mixed with mixture No. 2 the results are quite different, showing that something has happened to the pantothenic acid when fed in conjunction with this mixture. It is true that mineral mixture No. 2 produced a highly rancid feed of low palatability, as indicated by the low feed consumption (table 4, Lot 5).

A similar effect was noted when niacin was mixed with trace mineral mixture Nos. 1 and 2 but with less extreme effect. In addition, it is shown that rancidity was not the sole cause of low feed consumption and poor growth. If rancidity was the determining factor, then rancidity should cause a low feed consumption when niacin is adsorbed on mixture No. 2.

TABLE 4.—Effect of Mineral Mixtures on the Availability of Pantothenic Acid and Niacin in Synthetic Rations

Modification of basal ration	Feed consumed rat/day	Gain/week /rat	Feed consumed /gram gain
	Gms.	Gms.	Gms.
1. Basal complete ration	11.6	23.5	3.5
2. Basal complete—only 5 mgs. pantothenic acid/kilo	9.6	18.9	3.6
3. Basal—pantothenic acid free	6.8	3.9	12.2
4. Basal—pantothenic acid free + (a)	10.6	21.3	3.5
5. Basal—pantothenic acid free + (b)	1.7	6.6	1.8
6. Basal—niacin free	9.3	18.4	3.5
7. Basal—niacin free + (c)	9.1	19.7	3.2
8. Basal—niacin free + (d)	8.5	12.9	4.6

(a) 10 mgs. pantothenic acid absorbed on 5 gm. mineral mixture No. 1/kilo feed.
(b) 10 mgs. pantothenic acid absorbed on 5 gm. mineral mixture No. 2/kilo feed.
(c) 50 mgs. niacin absorbed on 5 gm. mineral mixture No. 1/kilo feed.
(d) 50 mgs. niacin absorbed on 5 gm. mineral mixture No. 2/kilo feed.

Vitamin mixture added to all rations. Thiamine, 1.25 mgs.; riboflavin, 2.5 mgs.; pyridoxine, 1.0 mgs.; choline chloride, 10 gms./kilo. In addition, 10 mgs. pantothenic acid and 50 mgs. niacin/kilo to basal complete ration

The data do not support this (table 4, Lot 8). On the other hand, it could be said that the difference in the effect on growth (table 4, Lots 5 and 8) was due not to palatability alone but to the fact that pantothenic acid is more a limiting factor for growth than is niacin. Therefore mixture No. 2 limited the availability of pantothenic acid more so than niacin and this in turn limited the feed intake and the resulting growth.

In order to overcome the question of rancidity due to the oxidation of the fat and a probable loss of vitamin A, the next experiment was devised so that a few drops of corn oil and vitamins A and D oil was fed daily in addition to the amount already in the feed. The niacin and pantothenic acid were fed separately as indicated in table 5.

A comparison of the growth response of rats in Lots 1, 6, 7, and 8 indicates that niacin is essential for the growing rat and even the 24 percent casein diet did not supply sufficient precursoral substance for niacin to equal the control Lot 1.

The effect of mineral mixture No. 2 on the 18 percent casein diet (Lot 9) is very evident from the growth response. The destructive effect or unavailability due to adsorption or oxidation of some factor by mixture No. 2, even in a complete ration where all of the vitamins were fed separately, as noted in Lot 2, is noticeable. When pantothenic acid and niacin were mixed with mixture No. 2 and then incorporated in the

TABLE 5.—Effect of Different Levels of a Mineral Mixture on the Availability of Pantothenic Acid and Niacin in a Synthetic Diet

Modification of basal ration	Feed consumed rat/day Gms.	Gain/week /rat Gms.	Feed consumed /gram gain Gms.
1. Basal complete ration—18% casein	12.2	23.5	3.6
2. Basal complete ration + 5 gms. mineral mixture No. 2/kilo	11.0	16.3	4.7
3. Basal—pantothenic acid free + (a)	7.6	11.4	4.7
4. Basal—pantothenic acid free + (b)	9.1	15.0	4.3
5. Basal—pantothenic acid free + (c)	10.4	16.7	4.4
6. Basal—niacin free—18% casein	10.8	20.7	3.7
7. Basal—niacin free—18% casein + 5 gm. mineral mixture No. 2/kilo	9.5	15.1	4.4
8. Basal—niacin free—24% casein	10.3	21.3	3.4
9. Basal—niacin free—18% casein + (d)	8.7	14.7	4.1

(a) 10 mgs. pantothenic acid absorbed on 5 gm. mineral mixture No. 2/kilo feed.
(b) 10 mgs. pantothenic acid absorbed on 2.5 gm. mineral mixture No. 2/kilo feed.
(c) 10 mgs. pantothenic acid absorbed on 1.25 gm. mineral mixture No. 2/kilo feed.
(d) 50 mgs. niacin absorbed on 5.0 gm. mineral mixture No. 2/kilo feed.

Basal complete ration same as in Table 4. Additional corn oil and A and D oil fed daily and separately, as well as the B-complex vitamins.

diet, still further reduction in rate of growth is noted. This seems to indicate that a chemical (catalytic) or physical reaction has taken place making these vitamins not fully available for the use of the animal.

Mineral mixture No. 2 appears to have less effect on the availability of niacin and pantothenic acid when the reaction takes place in the intestinal tract, than in the laboratory. One observes (Lots 3, 4, and 5) that the rate of gain, due to a constant amount of pantothenic acid, increases as the amount of the mineral mixture is decreased. This indicates a stoichiometric relationship between pantothenic acid and the mineral element (s) of the mixture or other oxidative substances. This offers a possible explanation for the results obtained in the previous experiments.

In the third experiment, a fat-free ration was used and the amounts of the vitamin B-complex, including pantothenic acid, were considerably increased in order to overcome the question of inadequate intake due to the chemical or physical reaction that appears to be taking place. The amounts fed are shown in table 6.

TABLE 6.—Effect of Different Amounts of a Mineral Mixture on the Availability of High Levels of Pantothenic Acid and Niacin in a Synthetic Diet

Modification of basal ration	Feed consumed rat/day Gms.	Gain/week rat Gms.	Feed consumed gram gain Gms.
1. Basal complete ration—corn and A and D oil in ration	12.8	28.7	3.1
2. Basal complete ration—corn and A and D oil fed daily	11.9	27.4	3.0
3. Basal complete ration—corn and A and D oil fed daily + (*)	12.1	26.1	3.3
4. Basal—pantothenic acid free + (a)	12.5	26.6	3.3
5. Basal—pantothenic acid free + (b)	13.8	29.8	3.3
6. Basal—pantothenic acid free + (c)	11.3	28.4	2.8
7. Basal—niacin free + (d)	10.8	28.2	2.7
8. Basal—niacin free + (e)	11.4	28.1	2.8
9. Regular stock ration	14.5	26.0	3.9
10. Regular stock ration + 5 gm mineral mixture No. 2/kilo	16.2	27.1	4.2

*5 gms. mineral mixture No. 2/kilo.

(a) 15 mgs. pantothenic acid absorbed (wet) on 5 gm. mineral mixture No. 2/kilo.
(b) 15 mgs. pantothenic acid absorbed (dry) on 5 gm. mineral mixture No. 2/kilo.
(c) 15 mgs. pantothenic acid absorbed (wet) on 1.25 gm. mineral mixture No. 2/kilo.
(d) 50 mgs. niacin absorbed (wet) on 5.0 gm. mineral mixture No. 2/kilo.
(e) 50 mgs. niacin absorbed (wet) on 2.5 gm. mineral mixture No. 2/kilo.

Each rat received daily 30 γ thiamine, 40 γ riboflavin, 30 γ pyridoxine, 150 γ pantothenic acid, and 150 γ niacin except where indicated. No vitamins were added to Lots 9 and 10.

When the consumption of these vitamins, including pantothenic acid was increased there was an increase in feed consumption, a gain in weight and feed consumed per gram gain in all lots compared to the results shown in table 5. This type of experiment completely eliminated the effect of mineral mixture No. 2 on the growth of rats. The fact that no fat was in the ration which could be oxidized and thus effect the availability of these vitamins, may be part of the answer.

It is observed that there are some slight differences in the feed efficiency, especially the lots receiving our regular stock ration. The mineral mixture No. 2 in this case lowered the efficiency somewhat. There is also a slight difference between the gains per week in the lots in which the pantothenic acid was mixed, wet or dry, with the mineral mixture.

The microbiological assay for pantothenic acid was made on the rations from Lots 4 (wet mix), 5 (dry mix), and 6 (1.25 gm. No. 2) with the recovery results of 69.3, 90.6, and 77.3 percent, respectively. The assay of the feces (moisture free) gave a pantothenic acid content for Lot 4 of 50.8 γ/gm; Lot 5, 40.5 γ/gm; Lot 6, 52.4 γ/gm. The niacin content of the feces from the same lots were 76.6 γ/gm; 66.6 γ/gm, and 78.3 γ/gm, respectively. This may not be a true picture of the vitamin content of the feces, because no measure of the microbiological synthesis in the intestinal tract is known.

Results show that when the pantothenic acid was mixed dry with the mineral mixture a larger percentage of the pantothenic acid was found in the dry mixed feed than where it was mixed wet, indicating a physical or chemical change has taken place and more so when water was a factor in this change. Since there was no fat or vitamin A and D oil in the ration the results indicate that the cause of rancidity was indirectly a factor in the results obtained.

What was in the mineral mixture No. 2 that caused the feed to become rancid and reduced the rate of gain? The fourth experiment was designed to determine whether or not one or more of the minerals listed on the tag as contained in the feed was responsible for the development of the rancid synthetic feed. The amount of pantothenic acid adsorbed on the various mineral salts was equivalent to that in the first two experiments which was incorporated into the feed at the concentration of 10 mgs. per kilo. The A and D oil was added to the synthetic ration, but the other essential vitamins were fed separately daily. The results substantiate the previous experiments which indicated that mineral mixture No. 2 lowered the rate of gain and increased feed consumption per gram gain.

TABLE 7.—Effect of Minerals and Storage on the Availability of Pantothenic Acid and on the Rancidity of the Ration

Modification of basal ration	Gain /rat /week Gms.	Feed consumed gram grain Gms.	Peroxide No. of oil in feed at end of 4-week period	Total pantothenic acid in feces two weeks γ
Basal—corn and A and D oil in ration + (a)	22.2	3.6	128	919
Basal + (b) stored at room temperature. .	14.9	4.9	4286	1062
Basal + (b) stored in refrigerator.	22.1	3.5	636	730
Basal + (c) stored at room temperature .	20.1	3.7	500	1616
Basal + (d) stored at room temperature. .	19.9	4.1	150	1467
Basal + (e) stored at room temperature	21.5	3.6	300	2032
Basal + (f) stored at room temperature.	20.4	4.0	171	1121

(a) 100 γ pantothenic acid per rat per day added separately.
(b) 10 mgs. pantothenic acid absorbed on 5 gms. mineral mixture No. 2 per kilo feed.
(c) 10 mgs. pantothenic acid absorbed on 921 mgs. $FeSO_4 . 7H_2O$ per kilo feed.
(d) 10 mgs. pantothenic acid absorbed on 886 mgs. $MnSO_4 . H_2O$ per kilo feed.
(e) 10 mgs. pantothenic acid absorbed on 37.3 mgs. $CuSO_4 . 5H_2O$ + 11.9 mgs. $CoSO_4 . H_2O$ per kilo feed.
(f) 10 mgs. pantothenic acid absorbed on 921 mgs. $FeSO_4 . 7H_2O$ + 886 mgs. $MnSO_4 . H_2O$ + 37.3 mgs. $CuSO_4 . 5H_2O$ + 11.9 mgs. $CoSO_4 . H_2O$ per kilo feed.

The basal ration contained 745 gm. sucrose, 180 gm. casein, 40 gm. salts IV mixture, 32.5 gms. corn oil and 2.5 g. cod liver oil/kilo.

In addition, each rat received 12.5 γ thiamine, 25 γ riboflavin, 10 γ pyridoxine, 500 γ niacin, and 10 mgs. choline chloride daily and separately.

When pantothenic acid was adsorbed on ferrous sulfate, manganese sulfate, or copper and cobalt sulfate, or a mixture of the four salts, no significant decrease in the rate of gain occurred and the rations did not become appreciably rancid, as determined by observation and peroxide number. However, the ration, Lot 2, containing the mineral mixture No. 2 became quite rancid as evidenced by observation and peroxide number determination. It was observed that when the ration, Lot 3, containing the mineral mixture No. 2 was refrigerated, no rancidity developed.

In addition, there was no decrease in the rate of gain or increase in feed consumed per gram of gain. The feces were collected for a period of two weeks and analyzed for pantothenic acid. The mineral mixture had no effect on the amount of pantothenic acid excreted. However, when iron, manganese, copper or cobalt were added singly to the ration there was an increase in the excretion of pantothenic acid, but not when they were added as a mixture.

These results indicate that the added trace mineral mixture No. 2 caused rancidity in a synthetic ration when stored at room temperature —approximately 27° C, but not when stored in the refrigerator. When the trace minerals listed on the tag were added singly or as a mixture to the ration, no rancidity or growth depression occurred. Therefore some other substance or substances incorporated into mineral mixture No. 2 was responsible for the development of rancidity and the depression of growth.

SUMMARY AND CONCLUSIONS

Solutions of riboflavin when mixed with bone meal, limestone, dicalcium phosphate and one brand of fuller's earth was readily available for the growth of microorganisms and laboratory animals. Norite A, bone black and two brands of fuller's earth were unsatisfactory in that the riboflavin was not available to an appreciable extent. When these same unsatisfactory carriers were mixed with natural feeds, the availability of the riboflavin was improved but still was not satisfactory.

Bentonite, bone black and soft clay rock phosphate interfered with the availability of riboflavin as found in alfalfa meal and dried skimmilk when these constituted 5 percent of the rat ration. When these natural vitamin sources constituted 10 percent of the ration only bentonite interfered with the availability of riboflavin; 69 and 82 percent, respectively, was available for the growth of the animals. This would suggest that the deleterious effect of certain adsorbents may be overcome by supplying higher levels of vitamins in the feed.

Two trace mineral mixtures produced different effects on the stability of pantothenic acid and niacin when these were incorporated with the vitamins in a synthetic rat ration. These vitamins were stable as measured by growth response in rats when mixture No. 1 was used. Mixture No. 2 caused a decreased availability of pantothenic acid and niacin. The effect of the latter (No. 2) was eliminated when the amounts of the vitamin-B complex were increased and the fat was removed from the ration.

Refrigeration prevented the early development of rancidity in feeds containing mineral mixture No. 2 and the peroxide number of the fat in this feed remained low. Storage at room temperature increased the peroxide number and the loss of pantothenic acid in feeds containing mineral mixture No. 2.

Salts of the trace minerals, iron, manganese, copper and cobalt individually or as a group in a simulated trace mineral mixture did not effect the availability of pantothenic acid.

These results suggest the importance of carefully testing mineral feeds and adsorbents (carriers) before they are offered or recommended as feed ingredients.

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