Proceedings of the Iowa Academy of Science

Volume 57 | Annual Issue | Article 17

1950

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Effect on Growth and Efficiency of Food Utilization of Quantity of Protein and Vitamin B_{12} Supplementation *

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INTRODUCTION

Certain nutritional and therapeutic values of liver extracts had been demonstrated experimentally again and again prior to 1948. Most of the early studies were related mainly to hematopoiesis (Subbarow et al., 1945), yet others were concerned with their capacity to augment the growth promoting properties of rations for chicks (Nichol et al., 1948), mice (Bosshardt et al., 1946) and rats (Jaffe, 1946; Sporn et al., 1947; Ershoff, 1947; Zucker, 1948). More recently the beneficial effects of liver extracts for hogs and poultry have been attributed to an unknown factor(s) tentatively identified as the "animal protein factor, APF." The identity of the APF is unknown. However, one of the markedly effective constituents of APF concentrates is vitamin B_{12}.

In 1948 Rickes *et al.* (1948) isolated from liver a crystalline product highly specific for the treatment of pernicious anemia and named it vitamin B_{12}. At the same time Shorb (1948) assayed this vitamin for Lactobacillus lactis Dorner activity and found it to be either wholly or partially responsible for the LLD growth obtained from liver. Shortly thereafter Ott et al. (1948) demonstrated that when vitamin B_{12} was added to purified basal diets containing 40-70 percent soybean meal as the only source of protein, it exerted "animal protein factor" activity in chicks which had been hatched from eggs laid by hens restricted to rations composed largely of ingredients of plant origin. The growth promoting activity of vitamin B_{12} likewise was demonstrated in rats by Emerson 1949; Register et al., 1949; Hartman et al., 1949; and Frost et al., 1949; and in the hog by Neumann et al., 1950.

Insofar as can be ascertained no one has determined whether, other things being equal, efficiency of food utilization is dependent upon a specific relationship between level of protein in the ration and amount of vitamin B_{12} supplementation. A recent report by Cunha et al., (1950) gives some indication of such a relationship. They showed that efficient utilization of protein by pigs was obtained only when the ration contained APF supplement, and that

* Journal Paper No. J-1807 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1131.

pigs fed rations containing 15.9 and 17.9 percent protein supplemented with APF gained weight at a faster rate than similar pigs fed a ration containing 19.6 percent protein without APF supplementation. The data collected in the present investigation focus additional light on this relationship. They point to the desirability of maintaining a specific relationship between quantity of protein and amount of vitamin B_{12} supplement in the ration.

Experimental Procedure

The animals used in this investigation were albino rats obtained from Sprague-Dawley, Inc. Forty-eight females and 48 males of the same age, recently weaned, were conditioned to ration and surroundings during a two-week pre-experimental period. During this time all rats were fed the same basal ration. Each rat had access at all times to ample ration and distilled water. During the two-week experimental period a record was kept of all food eaten and wasted by each rat. All rats were weighed several times during the experiment and at the beginning and end of it.

During the two-week experimental period 16 groups of six rats each received 16 different dietary treatments. Four groups of 24 rats each received the pre-experimental basal ration modified to meet the requirements of the test by adjusting the percentages of dextrin and casein so that the rations contained 8, 12, 18 and 36 percent casein. The 24 rats which received each level of casein were divided into four smaller groups of 6 rats each. The rats in these groups were given different amounts of crystalline vitamin B_{12} in aqueous solution injected intraperitoneally.

The composition of each ration fed is given in Table 1.

The experiment was set up so the data could be readily analyzed statistically by currently acceptable procedures (Snedecor, 1946). Accordingly, the experiment was carried out in an air-conditioned room maintained at a temperature of approximately 78°F. The 96 rats were caged individually in metal cages with wire bottoms suspended well above the droppings-pan. The cages were located in rows at five levels on the four sides of two movable metal racks. The rat which was to occupy each cage during the experiment was determined randomly. Similarly, the specific experimental treatment which each rat was to receive was determined randomly. All rats were weighed at the same time each day several times during the experiment and at the beginning and end of it. All weights made of the rats and of ration fed to each were expedited to avoid adding variability unnecessarily. The time and method of giving the intra-peritoneal injections were standardized.

Table 1

Composition of Rations Fed to Control Rats and Those Injected with Crystalline Vitamin B_{12}

Ingredient	Pre-experimental ration	Rations fed during experimental period			
		1* a, b, c, d	2* a, b, c, d	3* a, b, c, d	4* a, b, c, d
	%	%	%	%	%
Vitamin test casein	18	8	12	18	36
Dextrin	69	79	75	69	51
Cystine	0.2	0.2	0.2	0.2	0.2
Salt mixture (U.S.P. No. 2)	4	4	4	4	4
Ruffex	2	2	2	2	2
Agar	1.5	1.5	1.5	1.5	1.5
Crisco	5	5	5	5	5
Cod liver oil	1	1	1	1	1
Choline	0.1	0.1	0.1	0.1	0.1
Sulfaguanidine	0.5	0.5	0.5	0.5	0.5
Protamone	0.05	0.05	0.05	0.05	0.05
Vitamin supplement †					

* Four groups, namely, a, b, c, and d of six rats each received none, 0.025 γ, 0.050 γ and 0.100 γ respectively of crystalline B_{12} daily per rat via intra-peritoneal injections.

† The vitamin supplemental mixture was included in the ration as follows: riboflavin 3, thiamine 3, inositol 20, niacin 3, pyridoxine 5, biotin 0.01, folic acid 0.01, calcium pantothenate 5, para-aminobenzoic acid 5, and menadione 0.5 mg. per 100 g. of ration.

Results and Discussion

The environmental conditions which prevailed during the experiment and the behavior of the rats during this time were especially favorable for collecting these data. All rats adjusted readily to their rations from the start and, with very few exceptions, continued to eat well and maintained a thrifty condition. Only one rat died. Death occurred at the start of the test from causes unrelated to the experiment.

The rats wasted variable amounts of their ration, but these were relatively small in all instances. However, all feed wasted by each rat essentially was recovered completely. The amount of feed wasted in each instance was not correlated with any particular experimental treatment.

A study of the data in table 2 reveals readily several important similarities and contrasts in results. The average initial weights of all lots of rats were essentially the same. Intra-peritoneal injections of crystalline vitamin B_{12} at all levels used increased the average gain in live weight at all feeding levels of casein; even at the very lowest level of 8 percent. Though the total intake of essential

Table 2

Effect of Vitamin B_{12} on Growth and Food Consumption of Rats Fed Different Levels of Casein

Lot No.	Treatment: Casein	Treatment: B_{12}	Av. live weight and gain: Initial	Final	Total gain	Av. total feed eaten	Av. amount ration required per 100 gm. gain in wt.
	%	γ/day	gm	gm	gm	gm	gm
1 a	8	none	64.7	81.8	17.1	142.1	831
1 b	8	0.025	67.8	90.5	22.7	164.0	722
1 c	8	0.05	68.2	95.7	27.5	168.9	614
1 d	8	0.1	66.6	89.8	23.2	162.6	701
2 a	12	none	65.0	86.3	21.3	140.8	661
2 b	12	0.025	64.5	93.8	29.3	162.0	553
2 c	12	0.05	65.2	97.0	31.8	167.8	528
2 d	12	0.1	66.7	99.2	32.5	173.2	533
3 a	18	none	66.2	93.7	27.5	158.5	576
3 b	18	0.025	65.7	103.2	37.5	174.3	465
3 c	18	0.05	66.8	103.5	36.7	180.3	491
3 d	18	0.1	65.0	108.0	43.0	186.3	433
4 a	36	none	64.7	86.9	22.2	145.3	655
4 b	36	0.025	65.7	94.0	28.3	149.0	527
4 c	36	0.05	65.7	97.5	31.8	154.0	484
4 d	36	0.1	68.3	108.8	40.5	172.5	426

amino acids may have been insufficient for normal growth at this low level of protein, daily injections of B_{12} as small as 0.025 micrograms and larger, increased consumption of ration, rate of gain in live weight, and efficiency of ration. At all levels of casein vitamin B_{12} injections increased the average total ration eaten. The rations consumed by all the B_{12} treated lots b, c and d, regardless of their content of casein, were metabolized significantly more efficiently than the rations eaten by all lots which did not receive vitamin B_{12}. However, the differences between the utilization of ration by the lots which received B_{12} injections (lots 1b, c, and d; 2b, c, and d; 3b, c, and d; and 4b, c, and d), regardless of level of protein were not significant since the magnitude of the variations within and between lots was so large.

Other less obvious comparisons which may be noted from the data in table 2 seem to warrant the following comments. At the 8 percent level of casein, the rats which received 0.025 and 0.05 γ of B_{12} (lots 1 b and 1 c) produced the same total live weight gain as those which received the 12 and 36 percentages of casein without B_{12}

injections (lots 2 a and 4 a). Similarly, the 12 per cent casein, B_{12} supplemented lots 2 b, c, and d produced as much or more total gain than the rats in Lot 3 a which received 18 per cent casein without B_{12}. Additional comparisons of a similar nature are evident from the data in table 2; viz, lots 1 c and 3 a, and lots 3 a, 4 b and 4 c.

When the data of averages involving amount of ration consumed per 100 grams of gain in live weight are plotted on three dimensional graph paper (not shown here) against levels of casein in the ration, and amount of vitamin B_{12} injected intra-peritoneally, a striking similarity in trends in apparent. Thus, the slopes of the curves of ration efficiency are remarkably similar for those rations containing like percentages of casein supplemented with increasing amounts of vitamin B_{12}, regardless of the percentage of casein in the ration. Judging from a composite analysis of the data in table 2 there appears to be an optimal effect between level of casein in the ration, amount of ration eaten, and amount of vitamin B_{12} furnished by intra-peritoneal injections. However, this optimum is not specifically indicated by these data. For the type of ration used the optimum combination would appear to be one containing about 18 percent casein and about 0.5 micrograms of vitamin B_{12} daily. Additional data are needed to determine more accurately the optimum relationship.

SUMMARY

Ninety-six weanling albino rats were randomized into 16 lots of 6 rats each. The rats were caged individually in an air-conditioned room and received semi-synthetic rations containing either 8, 12, 18 or 36 percent "vitamin-test" casein, and intra-peritoneal injections of either none, 0.025, 0.050, or 0.100 micrograms daily of crystalline vitamin B_{12}. Precise records of gain in live weight and ration consumed were kept for each rat.

The average growth response of those lots of rats which received intra-peritoneal injections of vitamin B_{12} was significantly and markedly greater than those lots which did not receive B_{12}.

Those lots of rats which did not receive injections of vitamin B_{12} required significantly more ration to produce 100 grams gain in live weight.

Those lots of rats fed rations containing casein supplemented with injections of vitamin B_{12} grew as well and utilized their rations as well or better than rats fed similar rations containing much larger percentages of casein unsupplemented with B_{12}.

Composite analyses of the data indicate, up to a certain point, that the growth promoting efficiency of these rations is correlated with level of protein, casein in the case of this experiment, and quantity of injected supplemental B_{12}. Whereas, the optimum relationship for producing maximum efficiency was not determined

specifically, the data indicate this to be a combination of about 18 percent casein and 0.05 micrograms of vitamin B_{12}.

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