Effect of Diets High in Fat or Protein on Vitamin B_{12} Deficiency in Non-depleted Chick. (20914)

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Investigations on vit. B_{12} in experimental animals have been hindered by the difficulty experienced in producing a clear-cut deficiency of the vitamin. In studies with chicks, most workers have achieved this end through use of second generation depleted birds or use of thyroid-active substances(1-3). Attempts to enhance the severity of the vit. B_{12} deficiency by dietary means have been successful to varying degrees. Schaefer, Salmon, and Strength(4) obtained marked growth stimulation of non-depleted chicks when vit. B_{12} was added to a diet low in methionine and choline, which contained 19% lard. The effect of varying the level of lard upon the vit. B_{12} deficiency was not studied. Hill and Branion(5) obtained a greater growth response with vit. B_{12} when 5% corn oil and 10% sodium proteinate were both added to an all-vegetable diet than when either was added alone or when both were omitted from the basal diet. Depression of growth in the absence of vit. B_{12} with 1 to 4% glycine, 4% leucine, or 6 to 8% zein has been reported (6,7). This effect was overcome with vit. B_{12}, thus increasing the measurable response attributable to the vitamin.

The present study was concerned with the development of diets for production of a vit. B_{12} deficiency in non-depleted chicks.

Procedure. Female New Hampshire chicks, the progeny of hens fed a standard breeder diet, were obtained from commercial hatcheries. The day-old chicks, which weighed 35 to 40 g, were distributed equally by weight into groups of 6 chicks each. They were kept in electrically heated batteries with screen wire floors. Feed and water were supplied *ad libitum*. The chicks were maintained on the experimental diet for a 4-week period. The principal diet used, diet C8, had the following composition per kg: soybean oil meal 350 g, ground yellow corn 575 g, glucose 10 g (containing riboflavin 8 mg, and vit. B_{12} 0.10 mg), corn oil 5 g (containing vit. D_3 0.02 mg), and chick salts A(8) 60 g. This diet contained approximately 20% protein and 2.5 to 4% fat, by calculation. Both solvent and expeller types of soybean oil meal were used with comparable results. Other vitamins known to be required by the chick were supplied in at least minimal quantities by the corn or soybean oil meal, since growth on the complete basal diet was similar to that obtained with a stock diet. Additions to the diet were made at the expense of corn. Diet C8A, which contained only 0.4% fat, was used in one experiment. The corn was extracted continuously for 8 hours with petroleum ether and solvent extracted soybean oil meal was used. The 0.5% corn oil was omitted and the fat-soluble vitamins were added in alcoholic solutions in the following quantities per kg of diet: vit. A acetate 3 mg, vit. D_3 0.02 mg,

TABLE I. Effect of Imbalanced Diets upon Production of Vit. B_{12} Deficiency in Non-Depleted Chicks (6 Chicks Started per Experimental Group).

Modifications of Diet C8	No. of expts.	4 wk wt (g), S.E., and mortality 0 mg B_{12}	.1 mg B_{12}	Wt difference (g)
None	7	285 ± 7 (4)	343 ± 7 (1)	58*
20% hydrogenated vegetable oil	4	184 ± 14 (0)	301 ± 12 (0)	117*
" lard	4	152 ± 10 (4)	289 ± 12 (1)	137*
" sodium proteinate	2	191 ± 11 (4)	287 ± 9 (0)	96*
" zein	1	156 ± 8 (3)	222 ± 15 (0)	66*
" Cerelose	1	284 ± 22 (1)	301 ± 14 (0)	17
Minus 20% corn	2	316 ± 13 (1)	294 ± 10 (0)	-22

* Significant at 1% level.

α-tocopherol acetate 10 mg, 2-methyl-1,4-naphthoquinone 1 mg.

Results. The effect of the all-vegetable diet and various modifications upon chick growth in the presence and absence of vit. B_{12} can be seen from the data presented in Table I. When all 7 experiments on basal diet C8 were considered together, the omission of vit. B_{12} from the corn-soybean oil meal diet resulted in a highly significant depression of growth as indicated by the "t" test of Fisher(9). In only 4 of the 7 experiments, however, was the difference within each individual experiment significant.

When either hydrogenated vegetable oil* or lard was added to the corn-soybean oil meal diet, a significant depression of growth in the absence of vit. B_{12} was obtained in every experiment. The 2 fats appeared to be similar in this regard. The difference in growth between chicks supplemented with vit. B_{12} and unsupplemented birds was considerably greater in the presence of 20% fat than in chicks fed diet C8.

The addition of 20% protein to the basal diet had a less consistent effect with regard to vit. B_{12} than was observed with the high fat diets. The effectiveness of high vegetable protein in increasing the severity of the vit. B_{12} deficiency is also evident from the high mortality with sodium proteinate and zein in the absence of vit. B_{12} as contrasted with the good survival in the control groups. These data support the observations of others that diets high in protein appear to increase the need for vit. B_{12} by rats(10), mice(11), and chicks(12,5,7).

In the presence of vit. B_{12}, addition of either 20% fat or 20% protein resulted in poorer growth than that obtained with basal diet C8. These imbalanced diets may have increased the chick's requirement for some nutrients above the levels which possibly were already marginal in some instances in these diets. It was desirable to keep at minimal levels those nutrients, such as methionine and choline, which are known to spare vit. B_{12} (as reviewed by Briggs(13)), so that the greatest possible growth difference in the

* Crisco.

TABLE II. Effect of Fat Level of Diet with and without Vit. B_{12} upon 4 Wk Wt of Chicks (6 Chicks Started per Experimental Group).

Diet and additions	Exp. 1			Exp. 2			Exp. 3		
	0 mg B_{12}	.1 mg B_{12}	Diff.	0 mg B_{12}	.1 mg B_{12}	Diff.	0 mg B_{12}	.1 mg B_{12}	Diff.
	g	g	g	g	g	g	g	g	g
C8 (basal)	279	329	50	269	317	48*			
C8A (fat-low)	295	336	41						
C8A + 4% corn oil	259	330	71						
C8 + 10% hydrogenated vegetable oil				246	324	78*			
" 20% *idem*				151	262	111†			
" + 20% lard	155	237	82*	149	301	152†	178	261	83*
" ++ 30% "							122	193	71*
" ++ 40% "							77	111	34†

* Significant at 5% level as determined by "t" test of Fisher.
† Significant at 1% level.

presence and absence of vit. B_{12} would be obtained. These aspects of the problem are being investigated further.

When 20% Cerelose (crude glucose) was substituted for the corn or when 20% corn was omitted from the diet and not replaced with another substance, good growth resulted which was similar in the presence and absence of vit. B_{12}. Therefore, the effect of substituting either protein or fat for 20% corn in depressing growth in the absence of vit. B_{12} is believed to be due to the added constituent and not to the lowered amount of corn or to the altered proportion of soybean oil meal to corn.

The effect of various fat levels up to 44% upon growth of vit. B_{12}-deficient chicks is shown by data presented in Table II. In Exp. 1 growth of chicks fed the fat-low diet C8A and C8A plus 4% corn oil was comparable to that of control groups receiving C8. With none of these diets was the difference in growth attributable to vit. B_{12} statistically significant. In Exp. 2 the 20% level of hydrogenated vegetable oil was more effective than the 10% level in production of a vit. B_{12} deficiency. Also, in this experiment the effect of the lard was somewhat greater than that of the hydrogenated vegetable oil. The addition of either 30 or 40% lard to the all-vegetable diet, which already contained 4% fat, resulted in poor growth (Exp. 3). Improved growth was still obtained with vit. B_{12}, but this effect was less than that obtained with the 20% level of lard.

The relationship of fat to vit. B_{12} observed in this study is quite different from that reported for other species. Bosshardt, Paul, and Barnes(11) found that lowering dietary fat to 0.5% increased the difference in growth between groups of mice with and without vit. B_{12}. Growth in the absence of vit. B_{12} improved with increasing level of fat until the 2 groups grew the same with diets containing 40% fat. McCollum and Chow(14) similarly were able to produce a better vit. B_{12} deficiency in rats fed diets low in fat.

The effect of the imbalanced diets used in this study to depress growth of chicks deprived of vit. B_{12} is important in that a deficient animal may be easily produced for study of the vitamin. Also, this effect of high dietary fat suggests that vit. B_{12} may be involved in some manner in the metabolism of this nutrient.

Summary. A severe vit. B_{12} deficiency, as indicated by poor growth, was produced by dietary means in non-depleted chicks. Replacing 20% of the corn in an all-vegetable corn-soybean oil meal diet with 20% fat increased the weight difference between chicks receiving vit. B_{12} and the unsupplemented birds. A less striking effect was obtained with 20% vegetable protein.

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