Effects of Various Factors on Vitamin B_{12} Content of Cows' Milk

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Vitamin B_{12} was determined in milk samples from herds and from individual animals maintained at three distinct physiographic regions in the state of Georgia. In milk from individual animals, differences in vitamin B_{12} production associated with breed and season at one location and with quality or type of feeding regime at all locations were statistically significant by covariance analyses. The correlations between concentrations of fat and vitamin B_{12} were positive and statistically significant at all locations, and the negative correlation between concentration of the vitamin and quantity of milk produced at one location was significant. Differences in vitamin B_{12} content of herd milk brought about by differences in location were significant. Variations within animals and among animals of each breed at each location contributed greatly to the over-all variation found in vitamin B_{12} content of milk.

Milk is an important source of vitamin B_{12} in human diets in the United States. Reviews of studies conducted in various parts of the world indicate that the concentration of vitamin B_{12} in cows' milk is highly variable (*3, 8, 9, 13*). Almost all studies show great variation in vitamin concentration of milk obtained at various times from individual animals. Generally, no significant differences have been observed between breeds of animals maintained under the same environmental conditions (*9*). Conflicting evidence on effect of season of the year, associated with feeding regimes, has been published. Some workers have reported no effect of season (*6*), while others have reported either increases or decreases of vitamin concentration when animals were transferred from pasture to stall feeding in the autumn (*8*). Treatment of ewes (*5, 15*) or of cows (*16*) subjected to cobalt-deficient feeding regimes with cobalt bullets resulted in increased concentration of vitamin B_{12} in milk produced by these animals, but addition of cobalt to the ration of cows showing no signs of cobalt deficiency on pasture or stall feeding did not increase vitamin B_{12} potency of milk as measured by rate growth assay (*6*).

The upper portion of the digestive tract probably is the site of activity of cobalt, since cobalt given to lambs by mouth is much more effective than that given by injection or introduced directly into the duodenum in relieving symptoms of cobalt deficiency (*1*). Smith and Loosli (*17*) reported that cobalt-deficient lambs respond to vitamin B_{12} therapy, and suggested that cobalt deficiency is essentially a vitamin B_{12} deficiency.

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The studies reported here were undertaken to determine the amount of vitamin B_{12} in milk produced in various locations in Georgia, and to assess the effects of breed, feeding regime, season, length of lactation, quantity of milk produced, and content of some other milk constituents on vitamin content of the milk.

Procedures

Pooled herd milk samples were obtained from herds located at Experiment, Blairsville, and Tifton; and samples from individual animals were obtained from herds at Experiment, Tifton, and Reidsville. These locations represent the three physiographic regions of the state, Blairsville being in the Mountain area, Experiment in the Piedmont section, and Tifton and Reidsville in the Coastal Plain region. The herd at Experiment was composed primarily of Guernseys but contained some Holsteins. The herd at Reidsville contained Holsteins, Jerseys, and Brown Swiss. The animals at Blairsville were Holsteins, and those at Tifton were Jerseys.

Samples of milk from the herd at Experiment were collected first at weekly intervals, then at monthly intervals for two years. Milk from the herds in Blairsville and Tifton was sampled monthly during the last of these years. Samples from individual animals were obtained at monthly intervals for 37 months from animals located at Experiment, 29 months from those at Tifton, and 17 months from those at Reidsville. Samples of milk, but not colostrum, were obtained from the animals during normal lactation periods. The animal feeding program at all locations was in accordance with good dairy practice. Quality of roughages fed at Experiment and Reidsville was rated as poor, fair, good, or very good by the animal husbandmen.

For microbiological analyses of vitamin B_{12} content, samples of milk were obtained from individual animals or from herds immediately after the morning milking and centrifuged. Portions of the nonfat layer were taken for analysis and frozen, if assays could not be performed within a few hours. Preliminary work indicated that most consistent results were obtained when the skim milk was diluted with water and assayed without further treatment; therefore, this method was used. *Lactobacillus leichmannii* A.T.C.C. No. 7830 was the test organism used (*11, 12, 14*), and the nutrient medium was a dehydrated preparation obtained from Difco Laboratories, Detroit, Mich.

Fat content of whole milk from individual animals was determined by the Babcock method (*7*). Nonfat-solids content was calculated from the specific gravity of the milk produced by animals located at Reidsville only. Other data obtained for some animals included in the study were quantity of whole milk and skim milk produced at the morning milking, age of cows, length of lactation, type of feed, and amount of grain consumed.

Samples of roughage and grain fed to the animals at Tifton and Reidsville were analyzed for cobalt content by a modification of the method of Ellis and Thompson (*2*).

Results

Mean values of the herd samples obtained were 2.88, 2.74, and 1.04 μg. of vitamin B_{12} per liter of whole milk from Blairsville, Experiment, and Tifton, respectively. These means are significantly different statistically at the 1%

level, but in view of the confounding differences in breed and environmental factors associated with the herds, the biological significance of variance in the means is difficult to interpret. Generally, the herd samples contained more vitamin B_{12} during the fall and winter than during spring and summer.

The large variation among individual cows in production of vitamin B_{12} is indicated by the frequency distribution (Figure 1) of the mean values obtained for the animals from which at least five samples were obtained. The distribution of the coefficients of variation of the samples taken from each animal (Figure 2) shows the great differences in concentration of vitamin B_{12} in milk produced at different times by one animal.

Data obtained on samples of milk from individual animals located at Experiment, Reidsville, and Tifton were subjected to covariance analyses (Table I). Neither concentration nor total content of vitamin B_{12} in the milk produced by animals at any of the locations was significantly correlated with length of lactation. Samples were not taken during the first few days post partum, when vitamin B_{12} content is usually high (*8*, *9*). Percentage of nonfat milk solids was not significantly correlated with vitamin B_{12} content of milk produced by animals in the Reidsville herd.

In data obtained from individual animals at all locations, the correlations between concentrations of fat and vitamin B_{12} in milk were statistically significant at the 5 or 1% level, though the correlation coefficients were only about 0.2. The total quantity of vitamin produced was not correlated significantly with percentage of fat in the milk. In samples from the animals located at Tifton only, concentration of vitamin B_{12} in the milk was highly significantly and negatively correlated ($r = -0.47$) with quantity of whole milk produced.

Differences among breeds of animals in vitamin B_{12} production were statistically significant at the 1% level in the Reidsville herd but not in the Experiment herd. Seasonal differences in production of the vitamin were statistically significant for the animals located at Reidsville but not for those maintained at Tifton or Experiment.

Differences in quality of nutrition, as assessed subjectively, resulted in differences significant at the 1 and 5% levels in total production of vitamin B_{12} by animals located at Experiment and Reidsville, respectively, and in concentration of the vitamin in milk produced by animals maintained at Reidsville. There appeared to be no consistent trend, however, between quality of nutrition and amount of the vitamin in the milk. Consideration of the various components of the diets of these animals seemed to indicate that inclusion of oat silage in the feeding programs was associated with higher levels of vitamin B_{12} in the milk than was inclusion of corn silage. Therefore, in the statistical analysis of data obtained from animals located at Tifton, the feeding regimes were grouped according to those containing oat silage, those containing corn silage, and those containing neither oat nor corn silage: nutrition levels one, two, and three, respectively. Differences among those feeding regimes at Tifton were significant at the 1% level.

The covariant adjusted means of the three discrete variables are in Table II. In the herd located at Reidsville, both total quantity and concentration of the vitamin were greater in milk produced by animals of the Holstein and Brown Swiss breeds than by those of the Jersey breed. Concentration and total quantity of vitamin B_{12} were greater in milk produced in the fall and least in milk produced in the spring in herds located at Reidsville and at Experiment. (Data from milk produced by the animals in Tifton in the summer were not included in the analysis of variance since the animals were fed oat silage at all sampling

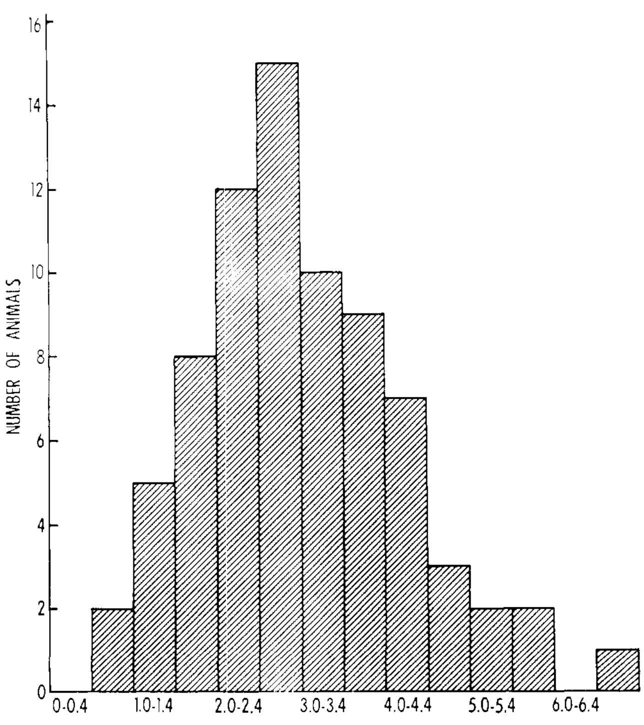

Figure 1. Frequency distribution of mean vitamin B_{12} content of five or more samples of milk obtained from individual cows

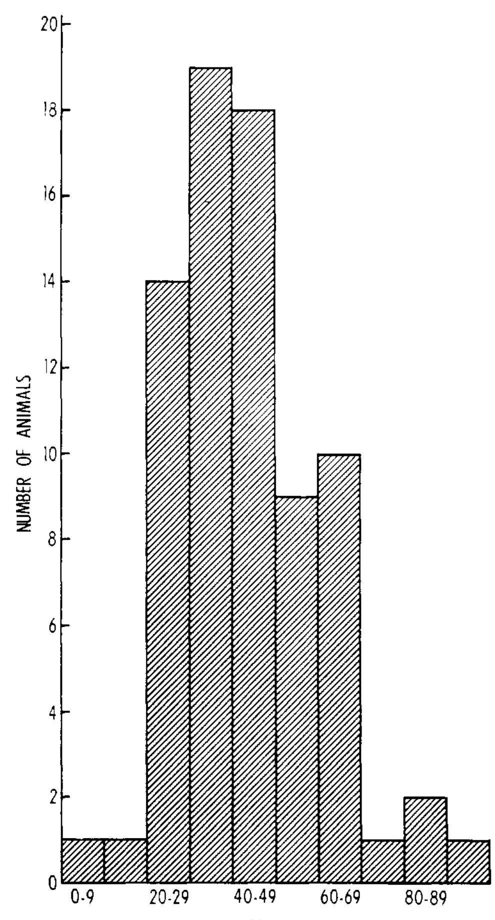

Figure 2. Frequency distribution of coefficient of variation of vitamin B_{12} content of samples of milk obtained from individual cows

Table I. Covariance Analyses of Concentration and Total Content of Vitamin B_{12} in Milk Obtained in the Mornings from Individual Animals at Three Locations

	μg. per Liter						Total Content, μg.					
	Experiment		Reidsville		Tifton		Experiment		Reidsville		Tifton	
Source of Variation	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Length of lactation	1	6.35	1	1.41	1	0.21	1	155.64	1	99.66	1	1.56
% SNF			1	9.77					1	185.10	1	
% Fat	1	31.45[a]	1	12.55[b]	1	5.91[b]	1	72.98	1	286.82	1	39.81
Whole milk produced	1	9.35	1	0.07	1	35.15[a]	1	43.01	1	161.17	1	64.27
Skim milk produced	1	8.86	1	0.12			1	35.14	1	95.08		
Breed	1	6.76	2	27.41[a]			1	279.54	2	1299.12[a]		
Season	3	4.67	3	9.69[b]	2	1.83	3	225.37	3	549.21[a]	2	2.92
Nutrition	2	7.24	3	7.14[b]	2	16.72[a]	2	338.21[a]	3	434.24[b]	2	540.76[a]
Error	350	2.97	322	2.73	178	1.47	350	86.65	322	133.52	178	47.23
R^2[c]		0.116		0.227		0.439		0.188		0.556		0.277

[a] Significant difference at 1% level.
[b] Significant difference at 5% level.
[c] Square of multiple correlation coefficient indicating portion of total variation explained by model.

periods.) In the Tifton herd, production of vitamin B_{12} was greater when oat silage was fed than when it was not fed. The interaction between seasons and types of feed was statistically significant, however, and in spring vitamin B_{12} content of the milk was greater when the animals were fed corn silage than when they were fed oat silage.

In the last phase of this study, cobalt bullets were given to 10 Jersey animals in the two herds at Tifton and Reidsville. Samples from these animals and from 10 other control animals in each herd were collected just before the bullets were given and at intervals of one, three, and seven weeks after they were given. No effect on vitamin B_{12} content of milk that could be attributed to the presence of cobalt bullets in the rumens of the animals was found. Analyses of samples of roughage and grain obtained from the two locations during this phase of the study indicated no difference between locations in concentration of cobalt in the feed used. The air-dried roughage contained less than 0.1 p.p.m. of cobalt, but the grain mixtures were supplemented with minor elements and contained about 1.5 p.p.m. of cobalt on an air-dried basis.

Table II. Covariant Adjusted Means by Breed, Season, and Nutrition Level of Vitamin B_{12} Content of Milk Obtained in the Morning from Individual Animals at Three Locations

	μg. per Liter			Total Content, μg.		
	Experiment	Reidsville	Tifton	Experiment	Reidsville	Tifton
	2.87	3.94	2.09	16.11	27.15	12.04
Breed						
Guernsey	2.60			14.36		
Holstein	3.14	4.61		17.86	31.40	
Brown Swiss		4.44			30.98	
Jersey		2.77	2.09		19.07	12.04
Season						
Spring	2.53	3.34	2.18	13.47	21.86	11.80
Summer	2.78	3.76		15.58	25.57	
Fall	3.35	4.67	1.82	19.29	31.39	12.08
Winter	2.82	3.99	2.28	16.13	29.78	12.24
Nutrition						
1[a]		3.45	2.80		23.20	15.92
2	2.96	4.19	1.97	16.69	29.74	10.85
3	3.20	3.40	1.51	18.03	23.28	9.36
4	2.30	4.70		11.95	32.38	

[a] For Experiment and Reidsville the nutrition ratings of roughages are: 1. very good, 2. good, 3. fair, and 4. poor. For Tifton the roughages were classed as: 1. oat silage, 2. corn silage, and 3. neither oat nor corn silage.

Discussion

The statistically significant differences among breeds in the Reidsville herd in production of vitamin B_{12} has little precedent in the literature. For example, Kon and Henry (*9*) noted that no significant breed difference had been observed in the vitamin B_{12} content of milk, and Hartman, Dryden, and Riedel (*6*) observed no difference in vitamin potency of milk produced by Holstein and Jersey animals as determined by rat growth assay. These latter authors reported an average vitamin B_{12} content of 7.1 μg. per liter of whole milk, which is considerably higher than the mean values obtained in the present study. One possible explanation for the observance of breed differences in this study is that the means tested are covariance adjusted means in which differential responses of the various breeds to effects of season, quality of nutrition, length of lactation, milk production, and fat concentration in the milk have been accounted for statistically.

The increase in vitamin content of milk produced in the fall by animals located at Experiment and Reidsville is in accord with several published reports—for example, Karlin and Portafaix (*8*)—in which it has been suggested that concentration of vitamin B_{12} in milk increases when animals are changed from pasture to stall feeding and decreases when the opposite change in feeding regimen is made. In this study, however, the vitamin content decreased again in winter when animals in Georgia would have had even less access to pasture than in fall. No evidence was found in the literature to substantiate the evidence of these data that inclusion of oat silage in the feed leads to secretion of greater quantities of vitamin B_{12} in milk than does inclusion of corn silage.

Though the correlations between concentrations of fat and vitamin B_{12} in the milk were statistically significant, the coefficients were too low to indicate that a direct association exists between production of fat and vitamin B_{12}. It may be that the quantities of all solid constituents of milk produced are influenced similarly by the general physiological and nutritional status of the animals or that the factors which regulate the relative amount of water secreted with the nonaqueous components are responsible for a general correlation among all solid constituents of milk.

The negative correlations between concentration of vitamin B_{12} and quantity of milk produced by the animals located at Tifton and Experiment indicate that the capacity of these animals to produce the vitamin was relatively more limited than their capacity to produce milk. Since animals located at Reidsville secreted milk of higher vitamin B_{12} concentration than did animals of the same breed located in Tifton or in Experiment and since there was no correlation between concentration of vitamin and quantity of milk produced by the animals at Reidsville, the factors limiting production of the vitamin are probably environmental rather than hereditary. The failure of cobalt bullets in animals located at Tifton and Reidsville to result in increased production of vitamin B_{12} suggests that the supply of cobalt is not the primary factor limiting secretion of the vitamin in milk elaborated by animals situated at these two locations.

In this study and in the majority of reports in the literature of vitamin B_{12} content of cows' milk, major portions of the variation observed are associated with differences in milk produced by one animal at different times and with differences in milk produced by various animals maintained under the same environmental conditions. For example, Gregory, Ford, and Kon (*4*) observed that day-to-day and animal-to-animal variations in concentrations in milk were greater for vitamin B_{12} and biotin than for thiamine, riboflavin, pantothenic acid, or vitamin B_6. This extreme variation indicates that secretion of the vitamin into milk is ultimately influenced to a great extent by conditions within the animal which can change markedly in a short period of time or which would vary among different animals maintained under the same environmental conditions.

Little is known of the actual mechanisms by which vitamin B_{12} is synthesized in the rumen or of the factors which control its secretion into milk. Investigations of types of microorganisms which synthesize vitamin B_{12} and of conditions in the rumen which would support growth of these organisms, of substrates and cofactors required in the biosynthetic pathways, and of factors involved in transportation of the vitamin from rumen to udder will probably be necessary before it would be possible to predict the vitamin B_{12} content of milk accurately or to produce milk of uniformly high vitamin B_{12} content.

Acknowledgment

The authors gratefully acknowledge the assistance of J. C. Johnson and R. D. Johnson of the Georgia Coastal Plain Experiment Station, and M. W. Neathery of the Georgia Mountain Experiment Station in planning this study and in collecting and preparing the samples for analysis.

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Received for review September 28, 1965. Accepted January 17, 1966. This is paper No. 487 in the Journal Series of the Georgia Experiment Station, Experiment, Ga.

IRRADIATION OF FATS

Effect of Ionizing Radiations on Antioxidants in Fats

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The effect of high energy radiations on several antioxidants dissolved in methyl myristate or methyl linoleate has been studied. When used at a concentration of 0.01% in methyl myristate and irradiated under vacuum, 27% of butylated hydroxyanisole, 50% of propyl gallate, and all of the tocopherol were destroyed with a dose of 5 megarads. In oxygen the same dose almost completely destroyed all antioxidants. Citric acid did not protect propyl gallate from destruction. No further changes occurred during storage of vacuum-irradiated samples. Destruction was greater in methyl myristate than in methyl linoleate.

Irradiation of fats produces free radicals which, in the presence of oxygen, form hydroperoxides. With unsaturated fats chain oxidation reactions are initiated and autoxidation proceeds rapidly. Antioxidants have been shown to have no effect on the formation of peroxides during irradiation (*5*) and are much less effective in preventing accumulation of peroxides during storage of irradiated materials than in simple autoxidation. This can be attributed either to the large number of chain reactions initiated by irradiation or to the destruction of antioxidant during irradiation.

Several investigators have reported that tocopherols are readily destroyed as a result of irradiation (*3, 8–12, 14*), but it has been suggested that other antioxidants such as propyl gallate and butylated hydroxyanisole can be added to fats prior to irradiation to prevent or minimize loss of stability (*7, 9*). However, little information was available on the effect of irradiation on common antioxidants. This paper reports the destruction of propyl gallate, butylated hydroxyanisole, and tocopherol when irradiated and stored under different conditions.

Experimental

Materials Used. The samples of irradiated methyl myristate and methyl linoleate containing the antioxidants studied were the same as those described earlier (*5*).

Determination of Antioxidants. The analytical procedure was a modification