Folate Concentration and Pattern in Bovine Milk

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The purpose of this study was to identify factor(s) that may influence the concentration and/or pattern of folates in milk. Fresh milk samples were collected from 18 dairy herds in California over a period of eight months. Folate was measured with *Lactobacillus casei*, *Streptococcus faecalis*, and *Pediococcus cerevisiae*, with and without conjugase. The range of *L. casei* activity was equivalent to 27-98 μg of folate per liter before and 49-118 μg per liter after conjugase. The range of *S. faecalis* and *P. cerevisiae* activity was equivalent to 1-4 and 2-5 μg of folate per liter, before and after conjugase, respectively. Folate concentration prior to conjugase digestion was highly predictive of that after conjugase for all assay organisms; "free" folate averaged 75% of total folate. High folate concentrations were measured in milk from herds grazed on green pastures; low concentrations were found in milk from herds fed dry feeds. There were no breed differences.

The folic acid content of bovine milk is of particular importance to human infants who depend upon milk as the principal source of nutrients. Failure to ingest sufficient folate results in megaloblastic anemia and malabsorption. Early reports (Hodson, 1949; Collins et al., 1951) indicated that milk contained only 1-2 μg of folate activity per liter, which would not satisfy the infant requirement of about 20 μg of folate per day (Velez et al., 1963; Sullivan et al., 1966). Current nutrition textbooks (Bogert et al., 1973; Davidson et al., 1972) also classify milk as a poor source of folate, even though recent investigations (Naiman and Oski, 1963, 1964; Luhby and Cooperman, 1963; Ghitis, 1966) using improved assay procedures find up to 100 μg of folate per liter of milk. However, these data are variable and it is difficult to determine if the variation is due to differences in methodology or to an actual wide range of folate concentration in milk. If the latter, it is important to determine the factors that influence folate concentration in milk. Furthermore, in view of the multiplicity of chemical forms of folates in foods it is of interest to establish if various types of folates vary proportionately or independently.

In order to establish base-line data upon which to evaluate the effects of processing on milk folate, we undertook to determine the amount, variability, and pattern of folates in fresh bovine milk produced in California. Samples were selected so as also to examine the influence of breed, seasonal variation, and geographical location.

MATERIALS AND METHODS

Assay Procedure. The double-strength assay medium designed by Baker et al. (1959) was used for assay of folate activity in milk with three microorganisms—*Lactobacillus casei* (ATCC no. 7469), *Streptococcus faecalis* (ATCC no. 8043), and *Pediococcus cerevisiae* (ATCC no. 8081). Minor modifications of the assay medium and details of the maintenance of the microorganisms were reported by Dong and Oace (1973). The phosphate-ascorbate diluting buffer (0.05 *M* sodium phosphate containing 0.2% of sodium ascorbate) which was used with samples and standard solutions was adjusted to pH 6.1 for *L. casei* and *S. faecalis* assays and to pH 5.3 for *P. cerevisiae* assays. Folic acid (pteroylmonoglutamic acid, PGA) was used as the reference standard with *L. casei* and *S. faecalis*; calcium leucovorin (formyltetrahydrofolate, a gift from Lederle Laboratories) was the folate standard used with *P. cerevisiae*. The release of monoglutamyl from polyglutamyl folate was achieved by digesting the milk samples with hog kidney conjugase, prepared according to the method of Eigen and Shockman (1963).

All samples were assayed at least three times. Each assay consists of triplicate tubes at three aliquot levels. Only data from assays where at least two of the three aliquot levels were in reasonable agreement (within 10%) were utilized in calculation of the final folate concentration value for each sample. Uninoculated milk blanks were prepared as suggested by Naiman and Oski (1964) at each aliquot level and were used to zero the spectrophotometer to account for turbidity due to the milk itself. This precaution does not correct for turbidity due to protein precipitated by acid produced by the assay organism. However, in such cases, aliquot levels do not agree within 10% and the data are not considered.

Sample Collection and Preparation. In all, 40 samples were taken of fresh, raw milk from 18 dairy herds—three in each of six counties in California. Some herds were sampled as many as four times during the period of July, 1972, to February, 1973. Only herds that were made up entirely of one breed (Jersey, Guernsey, or Holstein) were chosen for this study so that a breed difference with respect to folacin content could be examined. The complete sampling scheme is presented in Table I.

Samples were refrigerated for 1-3 days during transfer to the laboratory. After gentle shaking, these samples were poured into thin-walled plastic test tubes and were allowed to separate during refrigeration at 6°. Cream-free milk was drained through a hole in the bottom of the tube. One-milliliter aliquots were diluted with phosphate-ascorbate buffer to 10 ml for *L. casei* assays, to 5 ml for *P. cerevisiae* assays, and to 2 ml for *S. faecalis* assays. Diluted samples were frozen for 24 hr, thawed, and centrifuged. The supernate was heated in a boiling water bath for 10 min, cooled, and centrifuged. This supernate was stored frozen at −20° until the day of assay.

Eighteen samples (one from each herd) were subjected to a complete differential microbiological assay, i.e. *L. casei*, *S. faecalis*, and *P. cerevisiae* each with and without conjugase treatment, in order to determine the pattern of folic acid active compounds in milk.

Another 22 samples, taken from 10 of the herds at two or three additional collection periods, were assayed with *P. cerevisiae* and with *L. casei* without conjugase treatment in order to determine seasonal influence on the folate pattern. We use the term "free" folate to describe the results of these assays though Tamura et al. (1972) have demonstrated that *L. casei* responds to some extent to polyglutamyl forms of folate, prior to conjugase treatment.

The folate pattern can be estimated by taking into consideration the different specificities of the three assay or-

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Table I. Description of Dairy Herds Sampled during Each Collection Period

County	Location[a]	Collection period I, July–Aug 1972	II, Sep–Oct 1972	III, Nov–Dec 1972	IV, Jan–Feb 1973
Humboldt	N	H, J, J[b]	H, J, J	H, J	H, J
Tehama	N	H, J, J			
Yuba-Sutter	N	G, H, J	G, H, J		G, H, J
San Diego	S		H, H, H	H, H	H, H
San Luis Obispo	S	H, H, H			
Tulare	S		G, H, J	G, H, J	G, H, J

[a] N = Northern California; S = Southern California. [b] H = Holstein; J = Jersey; G = Guernsey.

Table II. Differential Microbiological Assay for Folate Activity of Milk from 18 Different Herds

Assay organism	"Free" folate (without conjugase), μg/l.[b]	Total folate (with conjugase), μg/l.	("Free"/total) × 100[a]
L. casei	59 ± 4.3[c] (30.8)[d]	77 ± 5.1 (27.7)	76 ± 2.2 (12.4)
S. faecalis	2.1 ± 0.2 (39.8)	2.8 ± 0.3 (45.1)	75 ± 2.5 (14.2)
P. cerevisiae	2.3 ± 0.2 (37.3)	3.0 ± 0.2 (31.7)	78 ± 2.2 (12.1)

[a] (Without conjugase/with conjugase) × 100 (calculated individually). [b] Folate activity data expressed as equivalent weight of PGA on a molar basis. [c] Mean ± standard error of the mean. [d] Coefficient of variation (percent).

ganisms. These have been summarized by Blakley (1969). *L. casei* fully utilizes oxidized and reduced folates, regardless of single carbon attachment, with up to three glutamates. *S. faecalis* utilizes oxidized and reduced folates except methyl derivatives and also responds to pteroic acid. *P. cerevisiae* utilizes only nonmethyl tetrahydrofolates. Conjugase hydrolysis releases additional activity for each organism from polyglutamyl folates. Therefore, comparison of folate activity before with that after conjugase treatment gives a rough estimate of the distribution of the glutamic acid chain lengths in the sample. The difference between *L. casei* and *S. faecalis* activity represents the amount of methyl folate present. If *S. faecalis* activity is greater than *P. cerevisiae* activity, the presence of incompletely reduced folates and/or pteroic acid is indicated.

PRELIMINARY EXPERIMENTS

Prior to adoption of the procedures outlined above, several aspects were tested to assure validity of the final data. These experiments are briefly summarized.

Extraction Procedure. Several different extraction procedures were tested with the objective of minimizing interfering substances (lipid and protein) without destroying folate activity. Milk was assayed without heating or freezing, with heating alone, with freezing alone, and with both heating and freezing as outlined. When appropriate uninoculated blanks were employed, no significant differences in folate values were detected among these treatments. However, the least turbid supernate was obtained with the combination of heating and freezing. Rennin treatment of undiluted milk significantly reduced folate activity, presumably due to trapping of folate in the coagulum. Rennin treatment of diluted milk did not reduce folate activity, but neither did it result in clearer supernate, so this treatment was not used.

Storage. Several milk samples prepared as outlined were assayed at intervals between 1 and 12 weeks of frozen storage. One group of nine samples averaged 75 ± 17 (standard deviation) and 72 ± 15 ng of folate per ml after 3 and 10 weeks, respectively, at −20°. Another group of nine samples averaged 44 ± 8, 40 ± 10, and 44 ± 18 ng of folate per ml after 1, 8, and 12 weeks of storage, respectively. It was concluded that folate activity was stable under our storage conditions.

Recovery. Two milk samples were assayed with and without synthetic PGA added to the diluting buffer. Both sets of diluted milk samples were extracted and were assayed with *L. casei*. Recovery of added folate was 100 and 106% in the two samples, indicating that our procedures did not destroy or remove folate activity.

Incubation Time. Streeter and O'Neil (1969) found that the growth response of *L. casei* to folate was more rapid in serum than in standard solutions, resulting in quantitative differences in calculated serum folate depending on length of incubation time. In order to determine whether or not this occurred with milk folate, several milk samples were assayed against standard curves incubated for from 22 to 30 hr. No significant change in calculated folate concentration in milk was observed during this time interval.

Reproducibility. The average coefficient of variation for 40 milk samples assayed at least three times was 10%. In no case did this statistic exceed 20%. This is similar to the reproducibility of repeated assays of serum folate (Streeter and O'Neil, 1969).

RESULTS

Folate Concentration and Pattern in Milk. The results of the complete differential microbiological assay of folate activity in milk from each of the 18 herds in the sample are presented in Table II. *L. casei* activity was variable, ranging from 40 to 98 μg/l. before conjugase treatment and from 40 to 118 μg/l. after conjugase. Nonmethyl folates, measured by *S. faecalis* and *P. cerevisiae,* ranged from 1.3 to 4.2 μg/l. before conjugase and from 1.6 to 4.8 μg/l. after conjugase. *S. faecalis* and *P. cerevisiae* values were nearly identical, indicating that the milk samples contained negligible amounts of incompletely reduced folates and pteroic acid. The coefficients of variation of "free" folate (31–40%) and of total folate (28–45%) were very high for all three assay organisms, reflecting the wide range in these parameters among the samples measured. However, the proportion of total folate that was available to the assay organisms prior to conjugase treatment (approximately 3/4) was similar for each organism. Coefficients of variation for these proportions were low (12–14%), indicating uniformity among samples. Therefore, the pattern of folate (short vs. long glutamate chains) appears to be fairly constant though the total amount of folate varies considerably. Nonmethyl (*P. cerevisiae* active) forms constituted from 2.2 to 6.3% of folate before conjugase and from 2.2 to 7.5% of folates after conjugase. The average folate activity available to *P. cerevisiae* was 4% of that available to *L. casei* both before and after conjugase digestion, again demonstrating the uniformity of the pattern of folates in milk.

Further definition of the relationships among components of the folate pattern was derived by regression analysis by the method of least squares. The linear regression equations, correlation coefficients, and levels of significance are presented in Table III. *L. casei* activity before conjugase digestion correlated very highly with that after conjugase; the same was true of free and total *P. cerev-*

Table III. Correlations among Components of the Folate Pattern in Milk

	y parameter		
x parameter	Total folates[a]	Free nonmethyl folates[b]	Total nonmethyl folates[c]
Free folates[d]	$y = 1.1x + 12.6$[e] $r = 0.934$[f] (**)[g]	$y = 0.025x + 0.85$ $r = 0.525$ (*)	$y = 0.026x + 1.43$ $r = 0.508$ (*)
Total folates		$y = 0.021x + 0.68$ $r = 0.526$ (*)	$y = 0.021x + 1.33$ $r = 0.484$ (*)
Free nonmethyl folates			$y = 1.0x + 0.62$ $r = 0.929$ (**)

[a] *L. casei* activity after conjugase. [b] *P. cerevisiae* activity before conjugase. [c] *P. cerevisiae* activity after conjugase. [d] *L. casei* activity before conjugase. [e] Linear regression equation (micrograms per liter). [f] Correlation coefficient. [g] Probability that relationship is not linear (student's *t* test); * = $P < 0.05$; ** = $P < 0.001$.

Table IV. Concentration and Pattern of "Free" Folate in Milk from California Dairy Herds. Relationship to Season, Breed, and Geographical Location

	Period I (Jul–Aug, 1972)	Period II (Sept–Oct, 1972)	Period III (Nov–Dec, 1972)	Period IV (Jan–Feb, 1973)	Totals
All herds	(12)[a]	(11)	(7)	(10)	(40)
L.C.[b] (μg/l.)	67 ± 5.0[c]	44 ± 1.7	42 ± 2.3	39 ± 2.6	49 ± 2.5
P.C.[d] (μg/l.)	2.7 ± 0.2	2.1 ± 0.2	2.4 ± 0.3	2.6 ± 0.2	2.5 ± 0.1
Methyl folate[e] (%)	96 ± 0.4	95 ± 0.6	94 ± 0.7	93 ± 0.9	94 ± 0.4
Jersey–Guernsey herds	(6)	(5)	(3)	(5)	(19)
L.C. (μg/l.)	70 ± 6.3	44 ± 2.8	42 ± 0.3	39 ± 4.6	50 ± 3.9
P.C. (μg/l.)	3.2 ± 0.4	2.5 ± 0.4	2.4 ± 0.2	2.6 ± 0.3	2.9 ± 0.2
Methyl folate (%)	95 ± 0.7	94 ± 1.2	94 ± 0.6	92 ± 1.7	94 ± 0.6
Holstein herds	(6)	(6)	(4)	(5)	(21)
L.C. (μg/l.)	64 ± 8.1	44 ± 2.4	43 ± 4.2	39 ± 3.2	48 ± 3.4
P.C. (μg/l.)	2.1 ± 0.1	1.8 ± 0.2	2.4 ± 0.5	2.6 ± 0.3	2.2 ± 0.1
Methyl folate (%)	96 ± 0.3	96 ± 0.5	94 ± 1.2	93 ± 1.0	95 ± 0.4
Northern herds	(9)	(5)	(2)	(5)	(21)
L.C. (μg/l.)	73 ± 5.2	45 ± 3.3	38 ± 4.5	41 ± 4.4	55 ± 4.2
P.C. (μg/l.)	2.9 ± 0.3	2.8 ± 0.3	2.4 ± 0.2	2.7 ± 0.3	2.8 ± 0.2
Methyl folate (%)	96 ± 0.6	94 ± 1.0	93 ± 0.2	93 ± 1.6	94 ± 0.6
Southern herds	(3)	(6)	(5)	(5)	(19)
L.C. (μg/l.)	50 ± 5.5	43 ± 1.8	44 ± 2.4	37 ± 2.9	43 ± 1.6
P.C. (μg/l.)	2.0 ± 0.1	1.7 ± 0.2	2.4 ± 0.4	2.6 ± 0.3	2.1 ± 0.2
Methyl folate (%)	96 ± 0.2	96 ± 0.4	94 ± 1.0	93 ± 1.2	95 ± 0.5

[a] Number of herds in group. [b] *L. casei* activity without conjugase, expressed as equivalent weight of PGA on a molar basis. [c] Mean ± standard error of the mean. [d] *P. cerevisiae* activity without conjugase, expressed as equivalent weight of PGA on a molar basis. [e] (L.C. − P.C.)/L.C. × 100.

isiae active folates. Free and total *L. casei* activities were also linearly related to *P. cerevisiae* active (nonmethyl) folates, but the levels of significance of these correlations were much lower. However, the ratio of *P. cerevisiae* active to *L. casei* active folates before conjugase digestion was highly correlated with that after conjugase digestion ($r = 0.763$; $P < 0.001$). It was therefore decided that further investigations of the impact of seasonal, breed, and location influences on folate concentration and pattern could be carried out conveniently by measuring only *L. casei* and *P. cerevisiae* activity without conjugase treatment. *S. faecalis* assays added little information since they were similar to *P. cerevisiae* assays; *L. casei* and *P. cerevisiae* activity after conjugase digestion, as well as the ratio of these two activities, could be predicted from the same analyses before conjugase digestion.

Factors Influencing Folate Pattern. The range of free folate concentration measured by *L. casei* in 40 raw milk samples taken from 18 herds from 6 counties in California over an 8 month period of 1972-1973 was 27 to 98 μg/l. The average data, grouped according to season, breed, and geographical location, are presented in Table IV. Considering all samples analyzed during each collection period, there was a highly significant decline ($P < 0.001$, student's *t* test) in *L. casei* active folate concentration between period I (July-August) and periods II, III, and IV, but no significant differences among periods II, III, and IV. For purposes of comparing breed differences, data from Guernsey and Jersey herds were combined, since the sample included only two Guernsey herds. There were no significant differences in *L. casei* active folate concentration between Holstein milk and Jersey-Guernsey milk at any collection time. Both groups exhibited similar significantly ($P < 0.025$) higher values during period I than in periods II, III, and IV. When milk samples were grouped according to geographical location in California it is apparent that milk produced by Northern California herds during period I (July-August) is at least 50% higher in folate than is milk from Southern California herds or Northern California herds at other times of the year. Milk from Northern California herds was highly significantly greater ($P < 0.001$) in folate concentration in period I than it was in periods II, III, and IV; Southern California herds produced milk with significantly less ($P < 0.05$) folate in period IV than in periods I, II, and III. The high concentration of folate in Northern herds during period I accounts for a large part of the variability in the 40 samples. The remaining 31 samples range from 27 to 61 μg of *L. casei*

Table V. Literature Values of Folate Concentration in Bovine Milk

	L. casei "Free" Activity (μg/liter)	% methyl folate	Location	Reference
Fresh bovine milk	100[a]	*b*	New York	Luhby and Cooperman (1963)
	38 (17–63)[c]	66	Israel	Matoth et al. (1965)
	42 (38–46)	*b*	Columbia, S.A.	Ghitis and Canosa (1965)
	55 (48–71)[d]	44[e]	Columbia, S.A.	Ghitis (1966)
	89 (62–100)	*b*	Massachusetts	Naiman and Oski (1964)
	49 (27–98)	95	California	Present study
Pasteurized bovine milk	52	*b*	Western Australia	Nicol and Davis (1967)
	54 (43–70)	82[e]	Columbia, S.A.	Ghitis (1966)

[a] Mean value. [b] Not determined. [c] Range. [d] Values from a single herd sampled seven times. [e] Calculated by authors from stability data, not microbial specificity.

active folate per liter and average 44 ± 7.6 μg/l. The coefficient of variation for the 40 samples is 32%; excluding the nine samples from Northern herds in the summer, the coefficient of variation is only 17%.

Reduced, nonmethyl folates (*P. cerevisiae* activity, Table IV) were present at a concentration of 2.5 μg/l. of milk overall. Minor variations occurred with breed, season, and location. Milk from Jersey and Guernsey herds contained higher concentrations of these folates during periods I and II than did that from Holstein herds. Northern milk was higher in *P. cerevisiae* activity than was Southern milk during these two periods as well. Though these differences achieved statistical significance ($P < 0.05$) they probably are not of practical importance. By and large non-methyl-reduced folates comprised 4–7% of the *L. casei* values and were fairly consistent over the seasons.

Percent methyl folate, calculated from the difference between *L. casei* and *P. cerevisiae* activity divided by *L. casei* activity, was highest (96%) during period I and declined steadily to 93% in period IV. This decline was largely due to the decrease in methyl folates; *P. cerevisiae* activity remained fairly constant, while *L. casei* activity declined.

DISCUSSION

The data presented herein corroborate reports of folate in milk as measured by methods similar to our own (Table V) and help to explain some of the differences among these reports. Mean values from the literature range from a low of 38 μg of "free" folate per liter from herds in Israel to 89 and 100 μg per liter in Eastern United States. Considerable variability is evident even within investigations. This is of particular interest in the study of Ghitis (1966) where concentrations of 48 to 71 μg of folate per liter were measured in milk from a single South American herd sampled on seven different occasions. There appears to be a real variation in folate concentration of bovine milk that cannot be explained solely by differences in methodology. Data on breed, season, and feeding practices were not presented in the other reports, so inferences as to explanation for the variation cannot be drawn.

In our sample of 40 herds, a wide range of folate concentration was found. However, high and low values were not random, but were concentrated in certain groups. All of the seven highest values (63–98 μg/l.) were from milk collected from Northern herds during period I (July–August, 1972); five of the seven lowest values (27–37 μg/l.) were in milk collected during period IV (January–February, 1973).

Holstein herds predominated in the South and Jersey cattle comprised a disproportionate part of the Northern sample. However, the strikingly high folate concentration of Northern milk in the summer could not be attributed to breed (Table IV). Stage of lactation (Karlin, 1967) and prepartum milking (Ford et al., 1972), both known to influence folate concentration in milk, were not peculiar characteristics of the herds with high or low folate milk. However, feeding practices did differ coincidentally with the differences in folate concentration in the milk. Herds in the north grazed on green fields and irrigated pastures during the summer. In the late fall and during the entire winter, due to the abundance of rain, dry feeds are given. On the other hand, in the hot and dry climate of Southern California, the herds are fed alfalfa hay and other dry feeds throughout the entire year (Bruhn, 1973).

Feed could influence milk folate concentration either through its folate content or by stimulation of folate production by rumen microorganisms. This information should not be construed as conclusive evidence that the above differences in feeding practices are responsible for the variation in folate content of milk. However, we were unable to discover any other consistent factors. The variation among the folate concentrations in milk reported by investigators from many parts of the world (Table V) tends to support our contention that differences in feeding practices and/or other environmental influences are implicated.

Where the calculation can be made, there is general agreement that methyl folate is a predominant chemical form of folate in bovine milk, but others do not agree with the very high percentage (95%) we found. The low value, 44%, reported by Ghitis (1966) was calculated as that which was labile to heating prior to dilution with ascorbate buffer. We have found that heating or rennin coagulation of undiluted milk lowers folate concentration, but that this loss appears to be due to trapping of folate in the coagulum, not specific destruction of a particular form of folate. On the other hand, our calculation of percent methyl folate may be high. Although ascorbate was present whenever samples were heated, some labile tetrahydrofolates may have been destroyed. This would result in low *P. cerevisiae* activity and an elevated calculation of methyl folates. Chromatographic separation and aseptic microbiological assay of milk folates would be necessary to settle this point.

The emphasis we have placed on "free" folate concentration in this paper requires some justification. The measurement that is of practical importance, of course, is one that would indicate the amount of folate available to man. "Free" folate measured by *L. casei* before conjugase digestion represents not only monoglutamyl derivatives, but also di- and triglutamyl folates, and proportions of the higher conjugates as well (Tamura et al., 1972). All but monoglutamyl folate require conjugase digestion prior to absorption across the intestinal mucosa (Butterworth et al., 1969). The extent to which conjugated folates are absorbed by man has not yet been established. If conjugated

folates were efficiently digested and absorbed, then total folate (*L. casei* assay after conjugase) would be the measurement of choice. However, Tamura and Stokstad (1973) found that the mean availability to man of total folate from several food items ranged from 25 to 96%, with considerable variation among subjects. Milk folate was not investigated in this study. Ghitis and Tripathy (1970) have shown that "free" folate in milk is equal to PGA in its ability to overcome megaloblastic anemia. This is in agreement with earlier suggestions (Herbert, 1963; Spray, 1956) that, in foods, *L. casei* activity without conjugase digestion approximates folate available to man. It appears therefore that "free" folate in milk, as reported in the present study, represents the minimum available folate in the product. If it is subsequently demonstrated that higher conjugates of folate in milk are also efficiently utilized, our data can be readily converted to total folate. In the subsample of 18 herds from which milk samples were assayed with and without conjugase, a highly significant ($P < 0.001$) correlation was found between "free" and total folate. Seventy-five percent of total folate was available to *L. casei* before conjugase; also, 75% of total nonmethyl folate was available to *P. cerevisiae* before conjugase. These calculations and statistics indicate that the pattern of folates in milk, insofar as the proportion of lower and higher degree of conjugation with glutamic acid is concerned, is fairly constant.

Among our 40 samples the "free" (presumably available) folate concentration ranged from 27 to 98 μg/l. of farm fresh milk. According to Ghitis (1966), pasteurization does not alter the concentration of "free" folate in milk. Since bovine milk is usually diluted with folate free components for use as infant formula (Willis, 1964) samples at the lower end of the range would provide insufficient folate and those at the upper end of the range would provide adequate folate for infant nutrition. From this standpoint it would be desirable if the minimum "free" folate concentration in bovine milk were at least 50 μg/l., an amount well within the normal range. Our information suggests that this could be achieved by manipulation of the feed offered to dairy cattle. Further research is necessary in order to determine the appropriate methods and the economic feasibility of such intervention.

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