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Table IV. Biological and Chemical Values of Pelletized Baits and Residues

	Total Dose of Sample, Mg./Rat	Av. Score per Rat	Total Dose of Warfarin, Mg./Rat		No. of Rats per Group
			Eble	Biological	
Warfarin	0.0625	0.25	...	...	8
	0.1250	0.75	...	...	8
	0.1875	1.37	...	...	8
	0.2500	2.37	...	...	8
LaClair residue A[a]	0.83	0.75	0.125	0.125	8
	1.25	2.37	0.1875	0.25	8
2B[a]	2.00	2.50	0.18	0.25[b]	4
4B[a]	3.60	2.50	0.18	0.25[b]	2
Eble residue	10.6	0.0	0.0	0.0	4
Pelletized bait	0.5	0.62	0.125	0.115	8
	0.75	2.00	0.1875	0.230	8

[a] Ether extraction conducted for 24 hours rather than 30 minutes.
[b] Values only approximations because of small number of animals used.

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MILK SOURING

Effect of 2-Methyl-1,4-naphthoquinone on the Rate of Souring of Milk

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As 2-methyl-1,4-naphthoquinone (menadione) is known to be bacteriostatic in very low concentrations, the effect of relatively low doses administered orally to lactating cows was investigated. Menadione, added at a concentration of 0.1γ per ml. of milk, significantly retarded the rate of souring of milk at 37° C. Milk from cows not fed menadione generally soured in about 12 hours when incubated at 37° C. Milk from cows that received 25 mg. of menadione per day remained sweet for 18 to 24 hours. During one period the feeding of menadione or its addition to milk did not retard the rate of souring. More research will be required to determine the cause for such variations; the type of pasture and fodder may be a factor. The nature of the compound secreted with the milk after feeding menadione has not been established. The milk from cows which received these relatively low doses of menadione had no detectable "off-flavor"; when it finally became sour, no hard curds were formed and the milk had a clean sour odor. Pasteurized milk from menadione-treated cows remained sweet about 20% longer. When milk was stored at 20°, 10°, or 5° C., the effect of menadione was more pronounced. The results of these preliminary experiments should stimulate research on an economical and practical method of reducing the spoilage of milk by feeding of menadione to milk cows.

2-METHYL - 1,4 - NAPHTHOQUINONE (MENADIONE), the easily available precursor of vitamin K, was reported in 1943 by Armstrong and coworkers (*6*) to be bacteriostatic against several Gram-positive pathogenic cocci. Earlier reports indicate the importance of the quinone structure to bacteriostatic and bactericidal properties of certain compounds (*5, 9, 11, 12*). Later some of these observations were confirmed and extended (*7, 8*).

Because the menadione is bacteriostatic in very low concentrations, and vitamin K is secreted in small amounts in milk (*7*), it seemed worth while to investigate whether relatively low doses of menadione administered orally

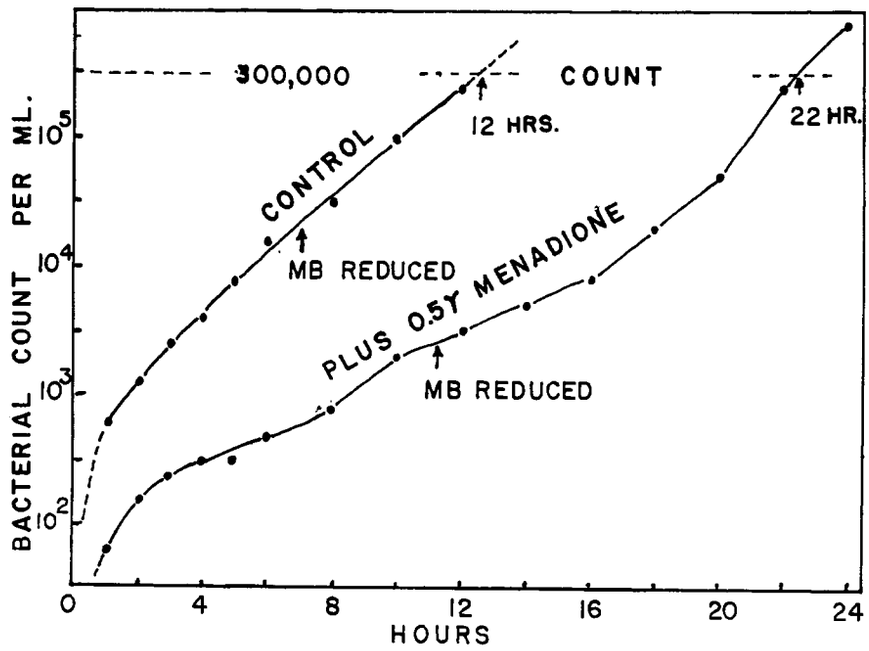


Figure 1. Rate of changes in bacterial count of normal milk and of milk to which 0.5 γ of menadione was added per 5.0 ml. of milk incubated at 37° C.

Methylene blue was reduced at 7 hours in the control milk and at 11 hours in milk treated with menadione. Control milk was sour in 12 hours; treated milk remained sweet for 22 hours.

to lactating cows would affect the rate of souring of milk. This paper reports preliminary results obtained in the retardation of the souring of milk by menadione added to milk in vitro and fed to lactating cows.

In Vitro Studies

Increasing amounts of menadione, ranging from 0.0010 to 1.0 γ dissolved in 0.5 ml. of water, were added to 5.0-ml. samples of fresh whole milk obtained from Guernsey cows. Each level of menadione was tested in triplicate, and three samples of milk received only 0.5 ml. of water to serve as the controls. All tests were made in sterile 18 × 150 mm. borosilicate glass test tubes. All samples of milk were incubated at 37° C., and the rate of milk souring was determined by three methods: (1) methylene blue reduction time (*3*), (2) detection of souring by odor and appearance, and (3) direct microscopic count of bacteria (*2*). In some instances the total acidity was determined, to check the other methods. The results of the in vitro experiments are summarized in Table I. In Figure 1 is plotted rate of the changes in bacterial counts per milliliter of normal milk and milk containing 0.5 γ of menadione per 5.0 ml. of milk plus 0.5 ml. of water when incubated at 37° C.

The data presented in Table I and Figure 1 indicate that menadione at a concentration of 0.1 γ per ml. or 1 part per 10^7 of milk significantly retards the rate of souring of milk.

In Vivo Studies

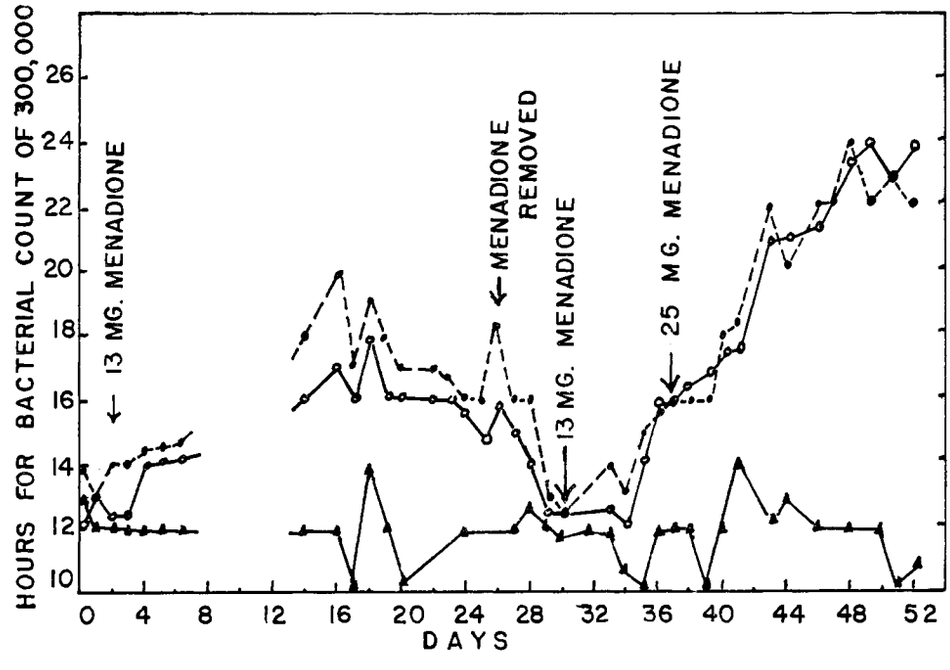
Two cows each weighing about 650 pounds were given 13 mg. of menadione per day in a gelatin capsule placed on top of the cows' daily feed supplement and ingested by the cow. [In addition to the green pasture, all cows received at the time of each milking several pounds of dairy feed which consisted of 2 parts of Florida's Hi-kwal-ity 20% dairy feed and 1 part of Bilgore's (Brand) citrus feed.] One other cow of equal size served as the control. Also serving as controls for the experiment were the periods of time before and after menadione was administered. Samples of raw milk were withdrawn from the milking machine container each morning, strained, and incubated at 37° C. Hourly observations in duplicate for each test were made with methylene blue, by direct count of the bacteria present, and by visual observation of the samples to detect curdling and any off-odors. In later experiments total acidity was used to follow the rate of souring (*4*).

The results of one long-range experiment are presented in Figure 2. The raw milk of these three cows before menadione was fed had to be incubated at 37° C. for 12 to 14 hours to attain a bacterial count of 300,000 per ml. (The time required to attain a bacterial count of 300,000 per ml. is designated here as the bacterial count time.) During a 90-day period the bacterial count time varied between 10 and 14 hours for any milk tested from any cow receiving no menadione. The methylene blue reduction time varied between 6 and 7.5 hours. On the third day of the experimental period presented in Figure 2, two cows (C and M) were given 13

Figure 2. Effect of menadione on time required for raw cow's milk incubated at 37° C. to attain a bacterial count of 300,000 per ml.

● Cow M
○ Cow C
▲ Cow R

Cows M and C received varying amounts of menadione. Cow R served as a control, even though M and C were their own controls when no menadione was fed.



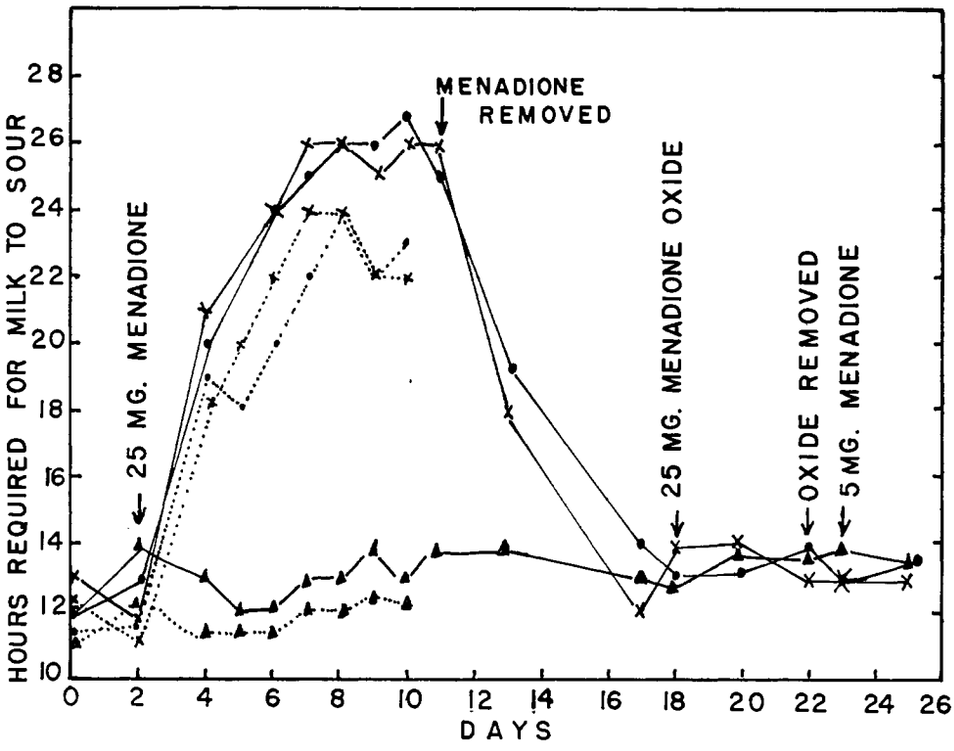


Figure 3. Effect of 25 mg. of menadione per day on time required for acidity to reach 0.25% lactic acid and bacterial counts to reach 300,000

× Cow A
● Cow B
▲ Third cow, control
Through 10th day both counts (dotted lines) were made and total acidity time (solid lines) was determined

mg. of menadione per cow per day. The milk of a third cow (R) was used as a control. After 3 days no significant change was observed in the bacterial count time nor in the methylene blue reduction time. (No milk tests were made from the 6th through the 14th day; however, the menadione was given daily.) On the 15th day—i.e., after 12 days of menadione feeding—the milk from the cows receiving menadione had a bacterial count time of 16 to 20 hours, whereas the control remained at 12 hours. The methylene blue reduction time increased from 6 to 7.5 hours to 10 to 11.5 hours. After 21 days the menadione was removed from the diet and in 3 days the bacterial count time and methylene blue reduction time returned to the control levels. After 2 days of tests at the control levels two cows (M and C) were again given 13 mg. per cow per day (this corresponds to the 30th day of the experiment reported in Figure 2). On the 4th day after the readministration of menadione a definite effect was obtained on the bacterial count time and methylene blue reduction time. The bacterial count time again reached 16 hours (controls 10 to 12 hours) and methylene blue reduction time increased to 10 hours (controls 6 hours).

On the 37th day the dose of menadione was increased to 25 mg. per cow per day. Within 5 days the bacterial count time increased to 20 hours and went as high as 24 hours (controls 10 to 12 hours).

Because direct bacterial counts were very time-consuming, it was decided to use total acidity expressed as per cent lactic acid (*10*) as a measure of the rate of souring of milk. In Table II, the results of the three methods used in this experiment are compared. There was good agreement between the methods and, as the measurement of total acidity was more convenient, further effects of menadione were evaluated on the basis of total acidity.

The results obtained by feeding 25 mg. of menadione to two other cows (A and B) are plotted in Figure 3. A sixth cow served as control during this experiment. Through the 10th day of this experiment, the time required for the total acidity to reach a level of 0.25% lactic acid was determined, in addition to the direct bacterial count. After the 11th day, only total acidity time was determined. In Figure 3 the bacterial count time is plotted in dotted lines and the time required to attain an acidity of "0.25% lactic acid time" is plotted in solid lines. The data presented in Figure 3 show that the feeding of 25 mg. of menadione retarded the souring of milk to 22 to 26 hours; controls soured after 12 to 14 hours. The removal of menadione from the diet resulted in the return to normal of the souring time.

The results of another experiment are plotted in Figure 4. The souring time of the milk of four cows without menadione varied between 10 and 13 hours.

Table II. Results Obtained with Three Methods for Determination of Rate of Souring of Milk

Cow	Menadione Administered, Mg./Cow/Day	Methylene Blue Reduction Time, Hours	Bacterial Count Time, Hours	0.25% Lactic Acid Time, Hours
R	0	6	10	12
Con	0	6	10	14
C	25	15	24	27
M	25	15	24	26
A	25	14	22	26
B	25	14	23	27

The administration of 13 mg. of menadione per cow per day did not affect the rate of souring over a period of 12 days, but when the dose of menadione was increased to 25 mg. per cow per day, a definite retardation of souring was noticed. In general, the milk from cows which received 25 or 50 mg. of menadione remained sweet for 6 to 8 hours longer than the milk from untreated cows.

The data presented in Figures 3 and 4 indicate that 5 mg. of menadione or 25 mg. of menadione oxide per cow per

Table I. Effect of Added Menadione on Souring Rate of Milk at 37° C.

Test Used	γ of Menadione Added in 0.5 Ml. of Water per 5.0 Ml. of Milk					
	0	0.001	0.01	0.1	0.5	1.0
Methylene blue reduction time, hours	6	6	6	7–10	11–12	12–14
Bacterial count[a] at MB reduction time	4×10^4	4×10^4	5×10^4	10^4	5×10^3	3×10^3
Time required[b] to obtain 3×10^5 bacterial count, hours	11	11	11	16	22	>22
Time required[b] for detectable sour odor, hours	12	12	12	16–17	23–24	24

[a] Bacterial counts made once an hour and reported as number of bacteria per ml.
[b] Obtained from data determined hourly.

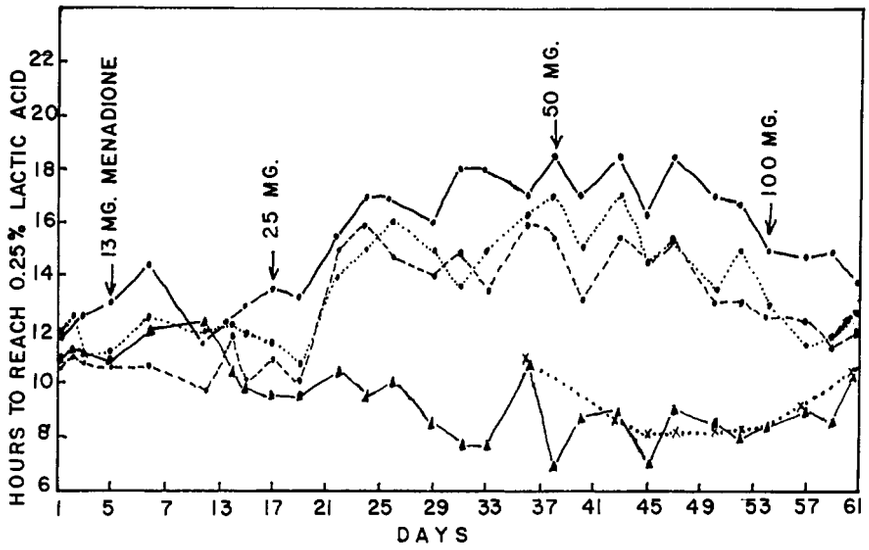


Figure 4. Effect of feeding menadione at four different levels on time required for acidity to reach 0.25% lactic acid

Rate of souring determined in raw cow's milk incubated at 37° C.
— · — · Cow A
– · – – · Cow B
. . • . . • Cow C
▲ Control throughout experiment
X. Another cow selected on 35th day at random from herd to give control rates of souring

day are without effect. Thirteen milligrams per cow per day seem to be at the threshold of producing an effect; in one experiment this dose retarded the souring, whereas in a second experiment it elicited no response. Increasing the dose to 50 mg. produced no greater effect than 25 mg. After 25 and 50 mg. of menadione had been fed to two cows for 20 days and to three cows for 27 days, the doses were increased to 100 and 200 mg. per day, but seemed to be less effective than 25 mg. These larger doses were administered only to cows which received menadione for over 30 days and experiments are needed to distinguish between a development of a resistance after a prolonged period of feeding and a decreased effect due to higher concentrations of menadione.

During one experiment, the milk from treated cows of one dairy herd soured as rapidly as the milk from untreated cows. During this time the milk did not respond to the in vitro treatment with menadione, whereas the milk from another dairy herd soured less rapidly when menadione was added in vitro. An explanation of this observation is lacking; however, during the refractory period the cows were on fresh red and white Dutch clover pasture, whereas during the time of all the other experiments except those plotted in Figure 4 the cows were on fresh winter oats pasture with corn ensilage added when the oats was low. The same herd again responded to the menadione treatment (data plotted in Figure 4) after a lapse of approximately 2 months, which coincided with a change of pasture from clover to native grass. These results indicate the need for further experimentation with careful control of the diet. Changes in bacterial flora during the periods of experimentation may also be responsible for the observed variations.

Table III. Effect of Feeding Menadione on Time Required for Pasteurized and Raw Milk to Attain a Bacterial Count of 300,000 per Ml.

Cow	Menadione Fed, Mg./Cow/Day	Bacterial Count Time, Hours: Raw milk	Bacterial Count Time, Hours: Pasteurized milk
R	0	7	11
		6	12
C	13	12	14
		11	16
M	13	12	15
		11	16

The effect of feeding menadione on the rate of souring of pasteurized milk was determined. From the data presented in Table III, it can be seen that feeding 13 mg. of menadione per cow per day retarded the souring of pasteurized milk 3 to 4 hours at 37° C., whereas raw milk was retarded 4 to 5 hours. Further work at higher doses of menadione is indicated.

Whether the milk from menadione-fed cows would sour more slowly than normal milk when kept at temperatures below 37° C. was also investigated. Preliminary results indicate that unpasteurized milk from cows receiving 25 mg. of menadione per cow per day when stored at 20° C. remained sweet up to 96 hours, whereas untreated milk soured in 24 hours. When stored at 10° or 5° C., milk from cows receiving 25 mg. of menadione remained sweet for as long as 24 days, whereas untreated milk soured in 5 to 14 days.

It is not known whether the effective material which was secreted with the milk and responsible for the effect was menadione or a derivative of it. The milk from cows treated with menadione had no detectable off-flavor. When milk from treated cows became sour, no hard ropy curd formed nor did the putrid odor appear, which was usually present in sour control milk. The vessels in which the milk soured were much more easily cleaned when the sour milk came from treated cows; this was again due to the absence of the hard ropy curd formed in the control milk.

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