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Effects of Aureomycin in Milk Used for the Manufacture of Cheese

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EFFECTS OF AUREOMYCIN IN MILK USED FOR THE MANUFACTURE OF CHEESE

by

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INTRODUCTION

Importance of project

In recent years many antibiotics have come to the foreground as a treatment for mastitis. Aureomycin is one of the more recent antibiotics that has been used for this purpose.

Aureomycin has been reported to be successful in curing some types of mastitis, but milk produced by cows that have been treated with Aureomycin does not act normal in the cheese manufacturing process. The most noticeable effect in milk from cows treated with Aureomycin has been slow or complete cessation of acid production by bacteria in cultured dairy products; this has been especially true in the cheese manufacturing process.

In the event of slow acid production, or complete cessation of acid production, the cheese produced is either of lower quality than would normally be expected, or the entire vat of cheese may be lost. In either case there is a definite adverse effect on the dairy industry.

Purpose of investigation

The purpose of this problem is to determine the percentage of Aureomycin necessary in milk to cause slow or complete cessation of acid production in the cheese manufacturing process, and to find a chemical or heat treatment that can be used to inactivate the aureomycin so that there will be no harmful effect in milk used to manufacture cheese.

REVIEW OF LITERATURE

Antibiotics as a mastitis cure

Spenser et al. (17) warns that workers have reported a remarkable high efficiency for most of the antibiotic drugs used in mastitis therapy. Such reports are misleading and may cause dissatisfaction among laymen who anticipate better results than can be obtained with clinical cases of mastitis treated in the average dairy cattle practice. Treatment with antibiotics must be used only as an aid to control mastitis.

Aureomycin has been found by Paine et al. (14) to be active against many organisms, including some penicillin resistant and streptomycin resistant bacteria. The number of organisms present will influence the concentration of antibiotic required for complete inhibition of bacterial growth.

Price et al. (15) has found that gram-positive organisms are, in general, affected by much lower concentrations of aureomycin than are the gram-negative organisms. The most sensitive group appears to be the aerobic spore-bearing microorganisms. Inhibition in 24 hours may be brought about b y small concentrations, but continued incubation results in growth of the bacteria. This could be due to the survival of resistant strains or to the destruction of the antibacterial agent during the incubation period, or both.

Spenser et al. (17) found a definite therapeutic activity by aureomycin against streptococcus mastitis.

Aureomycin

Dudley et al. (6) defines aureomycin as an antibiotic produced by Streptomyces aureofaciens and which has the advantage that it can

be taken orally. It has a molecular weight of about 508.

Broschard et al. (3) reported a new antibiotic principle active against both gram-negative and gram-positive microorganisms. Aureomycin has been named such from the parent actinomycete, and from the golden color of the crystalline antibiotic.

Aureomycin forms a hydrochloride which decomposes above 210°C., has an approximate solubility in water of 14 mg./ml. at 25°C., and has a pH of aqueous solution of 2.8-2.9. Chemical analysis shows it to contain C, 51.84%; H, 5.24%; N, 5.46%; total Cl, 13.27%; ionic Cl, 6.69%; and O, 24.18% (by difference).

Loomis (11) reports the toxic effect of aureomycin on animals is derived from its ability to inhibit aerobic phosphorylation. Microorganisms might be differently susceptible to any lowering of the level of phosphate bond energy beyond that of the mature and undivided host cell.

Source of antibiotics in milk

During the past two years numerous complaints have been registered by dairy plant operators concerning certain difficulties in getting milk to set properly. Many of these complaints come from experienced operators who have successfully produced starters, buttermilk, and cheese.

In some cases there is no difficulty in carrying the mother culture; the trouble begins when they try to set a batch of milk for buttermilk or cheese.

Experimental evidence obtained by Ruehe (16) shows that appreciable amounts of penicillin were present in milk drawn from

cows treated with penicillin, and that penicillin, depending on its concentration has a strong inhibitory effect upon the growth of lactic starters.

Doan (5) reported such antibiotics as penicillin, aureomycin, and streptomycin used for the treatment of mastitis in udders of producing dairy cows have been found in milk from such animals for several milkings after treatment.

Hunter (7) suggests there is a tendency to use the "blitz" method of treatment and, therefore, it is conceivable that milk delivered to a cheese factory may, on occasions, contain a high level of antibiotics. In some cases antibiotics may be found in sufficient quantity in mixed herd milk to cause arrested acid development when such milk is used in the manufacture of certain cultured dairy products.

Katznelson and Hood (9) recommend that careful consideration be given by dairy plant operators to the acceptance of milk from aureomycin treated cows for 5 to 6 days following treatment.

Amounts of antibiotics in milk that inhibit acid production

Doan (5) reported very serious inhibition of starter activity was caused by the presence of 0.1 units penicillin per ml. Partial arrest, or slow acid development, results from 0.05 units penicillin per ml. Katznelson and Hood (9) in experiments with various antibiotics found penicillin the most active substance, with aureomycin and subtilin equal in regards to dilutions giving complete inhibition of growth. The results obtained by Katznelson and Hood (9) are shown in table 1.

Table 1. Influence of six antibiotics on acid production in milk by a mixed strain starter culture.

Antibiotic	Reciprocal of dilution necessary for: Complete inhibition	No inhibition
Penicillin	3,300,000	166,000,000
Streptomycin	500,000	20,000,000
Aureomycin	1,000,000	20,000,000
Chloromycetin	100,000	5,000,000
Subtilin	1,000,000	100,000,000
Bacitracin	100,000	20,000,000

The same two investigators found that one percent of milk from treated quarters had a restrictive action for the first two milkings after treatment, and after the third milking 50 percent of milk from treated quarters was necessary to restrict acid production.

Krienke (10) found that at incubation temperatures of 70°F. and 95°F. there was practically no acid production at the end of 18 hours and 7 hours respectively, when the milk contained .0005 mg. of aureomycin hydrochloride per ml. of milk, and the milk containing the aureomycin had been pasteurized at 143°F. for 30 minutes. When the concentration of aureomycin was reduced to one tenth the previous amount at both incubation temperatures acid production was nearly normal as compared to that of the control samples. The milk of 3 cows treated with aureomycin contained sufficient aureomycin twelve milkings after treatment to retard acid production considerably. There was 0.45 percent acid developed in the treated milk as compared with 0.60 percent acid in the control when incubated 6 hours at 95°F.

In most instances milk mixtures containing 10 percent from treated cows and 90 percent drug free milk did not favor acid development approaching that of the control samples until the sixth milking after treatment. Milk from 3 cows treated with aureomycin did not favor acid production approaching that of the control when mixed with 75 percent "drug free" milk until the tenth milking after the aureomycin treatment. Aureomycin in varying amounts was visible on the surface of the milk from each cow until the fifth milking, after which time none could be detected.

Table 2. Concentrations of antibiotics showing starter inhibition as reported by different investigators.

Investigator	Product observed	Antibiotic	Concentration
Katznelson and Hood (9)	Starter	Penicillin	0.05 unit
Hunter (8)	Starter		
	S. cremoris K13	Penicillin	0.07 unit
	S. cremoris R1	Penicillin	0.10 unit
	S. lactis	Penicillin	0.17 unit
Krienke (10)	Starter	Aureomycin	0.0005 mg.
Doan (5)	Starter	Penicillin	0.05 unit

Destruction of antibiotics

Many investigators (1, 5, 8, 9) have found that ordinary pasteurization has little or no effect on any of the antibiotics studied. It has been reported by Hanson et al. (1) that when 1 part of skim milk from cows treated with aureomycin was mixed with 99 parts of milk from untreated cows, and the milk dried then recombined, there was a stimulating effect on acid production.

Doan (5) found that autoclaving reduces the potency of penicillin to a detectable degree.

It has been reported by many investigators (5, 9, 16) a positive antidote for penicillin is the enzyme penicillinase now available from Schenly Laboratories and Difco Laboratories, however, Doan (5) states that enough enzyme to treat milk containing 0.005 unit per ml. of penicillin would cost more than the milk itself. Katznelson and Hood (9) found the enzyme penicillinase permits almost a normal acid production at a concentration of 0.05 unit penicillin per ml. which otherwise stopped fermentation completely, and 50 percent of the total acid produced in the control at 5 hours with 1.0 unit penicillin per ml.

Doan (5) used oxidizing agents such as copper, reducing agents such as cysteine and ascorbic acid, and surface active agents such as quadrafos with no effect on the antibiotics.

In preliminary studies Paine et al. (14) found no inhibition of aureomycin by aerobioses, or with 3.5 percent CO_2 in a decreased oxygen tension, however, greater anaerobiosis may interfere with the antibiotic action of aureomycin. No aureomycin-inhibiting substance, such as penicillinase, could be produced by the usual methods of obtaining such substances.

The percentage of starter used can be increased, but Hunter (8) found the strict regularity of acid production desired in the cheese making process cannot be guaranteed.

Detection of antibiotics in milk

Stoltz and Hankinson (18) describe a method for the detection of antibiotics in milk using a modification of the Scharer field test for phosphatase. The principle modification is the reduction of the amount of milk used from 0.5 ml. to 0.05 ml. It is claimed the test will detect the presence of several antibiotics including penicillin and aureomycin if present in quantities sufficient to cause reduced activity in cultured dairy products. A summary of the results by Stoltz and Hankinson (18) is given in table 3.

Table 3. Results of modified phosphatase test to detect antibiotics in milk.

Cow No.	Dosage	No. of milkings after treatment	Results
1 Aureomycin	200 mgm.	4	Gray Complete inactivation
2 Streptomycin	1 gm.	2	Gray Complete inactivation
3 Tyrothricin	40 mgm.	2	Pale green Partial inactivation
4 Penicillin	25000 units	1	Gray Complete inactivation
5 Penicillin	25000 units	1	Green Partial inactivation

Churchill et al. (4) investigating the modified phosphatase test described by Stoltz and Hankinson (18) for the detection of antibiotics in milk found that the test cannot be depended upon to indicate the presence of antibiotic substances in raw milk; rather it indicates the variation of phosphatase content of milk from different cows. The presence of antibiotics do not interfer with the regular

phosphatase test as now employed by health agencies. The antibiotics tested by Churchill et al. (4) were; penicillin, neomycin, streptomycin, aureomycin, terramycin, and chloromycin. All of the antibiotics were tested by direct addition of the drug into the milk, and by milk coming from cows treated with the drug.

Ruehe (16) found no simple method to determine antibiotics in milk, however, suggested that suspected milk can be checked for starter making qualities by pasteurizing 10 ml. samples in test tubes, heating to at least 175°F., and inoculating with 1 ml. of starter. If no antibiotic is present a satisfactory coagulum should form in 10 hours or less.

Hunter (7) found that penicillin in milk has a definite effect on the reductase time, prolonging the reductase time more in fresh milk than in aged milk. The proportions of Streptococcus which are sensitive and Coliform organisms which are non-sensitive will exert a marked effect on the results. Although the presence of penicillin in milk retards somewhat the decolorization of methylene blue when the reductase test is run Ruehe (16) found it's influence is not significant, and does not change the classification of the milk.

Hunter (8) found in cheese made from milk containing penicillin in amounts sufficient to affect the rate of acid production had curds which tended to be "short" and "chippy" varying to harsh with the larger amounts of penicillin. Cheese 3 months old made from milk containing 0.10 penicillin units per ml. had a pasty body, and cheese made with milk containing 0.15 units per ml. gave a weak pasty and fermented cheese.

Hunter (8) suggests the possibility that organisms growing in the presence of an antibiotic may become more susceptible to the influence of phage action.

PROCEDURE

The manufacture of cheddar cheese

Flav-O-Lac culture obtained from Dairy Laboratories Inc. containing the organisms Streptococcus lactis, Leuconostoc dextranicum, and Leuconostoc citrovorum was used as the source of starter in this experiment. The mother culture was carried in grade A skim milk which had been heated in a water bath to 190°F. for 1 hour. Good activity was maintained by transferring the mother culture in duplicate daily, and selecting the best of the duplicates for the inoculum. One percent inoculum was used for the transfer, and the cultures were incubated at 70° F. for 16 hours. Bulk starters were prepared in one quart milk bottles using the same quality of milk, method of sterilization, percent inoculum, and incubation temperature. The cultures developed an acidity of about 0.70 percent and possessed a smooth texture and pleasing flavor and aroma typical of good lactic starter.

The aureomycin used in this experiment was crystalline aureomycin hydrochloride supplied by Lederle Laboratories Division, American Cyanamid Company.

The milk used was grade A milk from the regular supply of Colorado A and M College, and was from cows which did not have mastitis, and had never been treated with any of the antibiotics. The milk was pasteurized at 143° F. for 30 minutes then cooled to 41° F. and stored overnight in clean 10 gallon milk cans. The following morning the milk was dumped into two cheese vats, each vat holding 387 pounds of milk, and heated to 86° F.

Uniformity in the cheese making procedure was maintained by the use of Wilson's (19) clock method of cheese manufacture as outlined in figure 1.

Hansens cheese rennet was used in all batches at the rate of 4 ounces per 1000 pounds of milk.

The cheese was manufactured in two vats using 1 percent starter in both vats. The first vat of milk was used as a control, and the cheese making procedure outlined above was followed as closely as possible. To the second vat aureomycin was added at the rate of 5.6 parts per million. Subsequent trials were run in which 2.8, 1.4, 0.7, and 0.35 p.p.m. were added to vat number 2.

During the manufacture of the cheese the rate of acid development was carefully noted. The titratable acidity was measured before adding the starter, at the time of adding rennet, when the curd was cut, before draining the whey, and at 15 minute intervals during the matting and cheddaring process. The titratable acidity was measured using 0.10 N sodium hydroxide with 1.0 percent phenolphthalein being used as the indicator. Nine ml. samples were titrated to a faint pink color which remained for at least 15 seconds. Any difference in titratable acidity between the two vats was carefully noted and recorded.

Inactivation of aureomycin in milk

In studying the effect of pasteurization, sterilization, and hydrogen peroxide treatment of milk upon inhibiting the effects of aureomycin on starters, Hansen's lactic ferment culture containing the organism Streptococcus lactis was used as the source of starter.

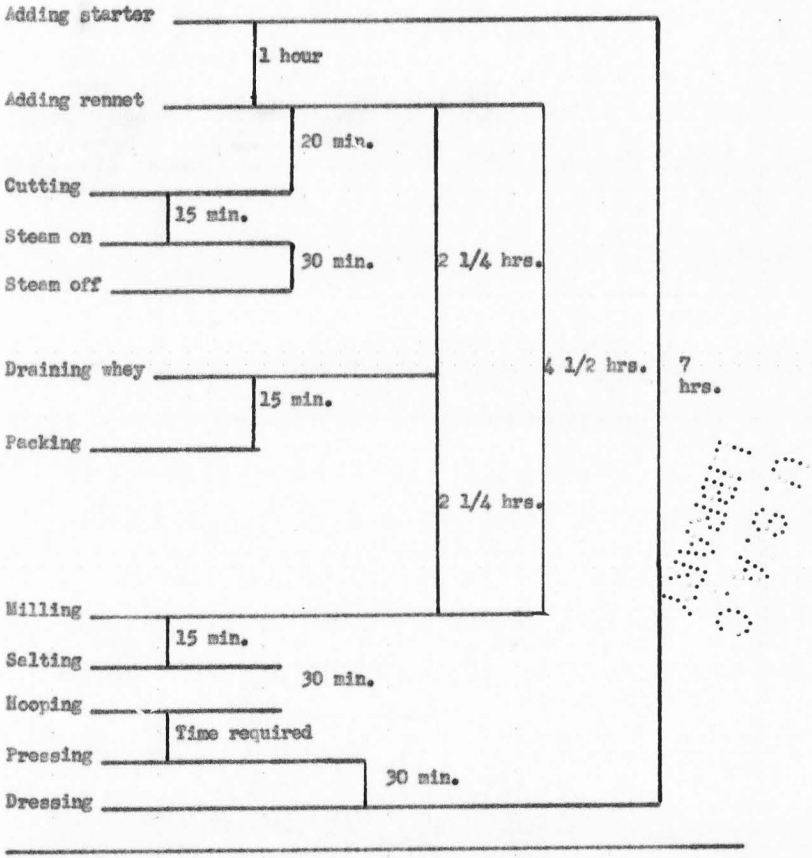

Figure 1. Time intervals between steps in the manufacture of cheddar cheese.

The mother culture was carried in grade A whole milk which had been treated in an autoclave to a temperature of 240° F. for 20 minutes. Good activity was maintained by transferring the culture daily using a one percent inoculum. The cultures were incubated at a temperature of 70° F. for 14 to 15 hours.

The milk used in this experiment was grade A milk from the regular supply of Utah State Agricultural College creamery. Part of the milk used was grade A pasteurized milk, the remainder was grade A raw milk.

The aureomycin used was the same as that described in the manufacture of cheddar cheese.

Edible hydrogen peroxide known as "perone" was used in this experiment. "Perone" contains 35 percent hydrogen peroxide and is made by Du Pont E. I. Nemours, Electro-Chemical Division, Elmonte, California.

The catalase used was a highly purified, dry, nonhygroscopic powder known as "Catalase 30" produced by Armour and Company, Chicago, Illinois.

Six lots of milk with five 700 gram samples per lot were used in this experiment. The samples were prepared as shown in table 4. Aureomycin was added to the 5 samples in each lot at the rate of 1.0, 0.5, 0.25, and 0.12 parts per million of aureomycin, with the fifth sample being used as the control.

Two additional lots of milk consisting of six 700 gram samples were prepared to test the effects of increased amounts of hydrogen peroxide on aureomycin. The samples were prepared as shown in table 4a. Aureomycin was added to 5 of the samples in each lot at the rate of 0.25 parts per million, with the sixth sample used as the control.

Table 4. Preparation of samples for testing the inactivation of aureomycin in milk.

Treatment [1]	Milk used	Time of adding aureomycin
1	Past.	Before sterilization at 240° F. for 20 min.
2	Raw	Before hydrogen peroxide treatment.
3	Past.	Before hydrogen peroxide treatment.
4	Raw	Before pasteurization (143° F. for 30 min.)
5	Past.	No treatment after addition.
6	Raw	No treatment after addition.

1. Each treatment represents one lot of five samples.

Table 4a. Preparation of samples for testing the effects of high concentrations of hydrogen peroxide on aureomycin.

Milk used	Time of adding aureomycin
Raw	Before pasteurization at 143° F. for 30 min.
Raw	No treatment after addition.

Hydrogen peroxide was added to 5 of the samples in each lot at the rate of 1.0, 0.8, 0.6, 0.4, and 0.2 percent hydrogen peroxide, with the sixth sample used as the control.

The aureomycin was added to the samples by preparing a 1/10,000 dilution of the crystalline aureomycin hydrochloride in distilled water, and measuring the dilution into the samples with the aid of a 1 ml. pipette graduated in 0.10 ml. divisions.

The hydrogen peroxide treatment used was the same as outlined by Morris (13) using 0.2 percent of 35 percent hydrogen peroxide added to milk at 120° F. and holding the samples at that temperature for 30 minutes. The samples were then cooled to 100° F. and the catalase enzyme added at the rate of one drop of a 1/100 dilution delivered from a 2 ml. pipette to convert the remaining hydrogen peroxide into water and oxygen. The same procedure was followed when increased amounts of hydrogen peroxide were used, with the exception of the percentage of hydrogen peroxide.

Ten ml. portions of the hydrogen peroxide treated milk were tested with 5 ml. of a 30 percent potassium iodide solution to determine when all of the hydrogen peroxide had been converted into water and oxygen. A yellow color indicates the presence of hydrogen peroxide.

After all lots had been treated in the manner described above, each sample was adjusted to 70° F. and inoculated with 1 percent of the culture. The samples were incubated at 70° F. for 16 hours, and titrated in the same manner as previously described. Any differences in the titratable acidity caused by the aureomycin were carefully noted and recorded.

RESULTS AND DISCUSSION

The manufacture of cheese

The acid development during the manufacturing process of cheese as determined by the titratable acidity of the milk at the time of adding starter, and the titratable acidity of the whey at cutting, dipping, and during the cheddaring process is shown in table 5.

The results show that there was practically no difference in titratable acidity due to aureomycin until the time of dipping. At the time of dipping there was still very little variation in acidity due to the presence of aureomycin, however, in all cases there was a slightly lower acidity in the vats containing aureomycin indicating that acid development had ceased.

The control vats in all cases showed normal acid development from the time of dipping until the time of milling, with an average increase of 0.56 percent acid. No acid development was noted in the vats containing aureomycin until the last trial in which there was a 0.04 percent increase in titratable acidity from the time of dipping until the time of milling.

Figure 2 shows graphically the acid development obtained with the different amounts of aureomycin compared with the control samples.

During the cheddaring process the curd made from the milk containing aureomycin showed marked differences in texture and matting properties from the curd made from drug free milk.

The curd made from milk containing aureomycin did not expel moisture as rapidly as did the curd from drug free milk, and it did not mat and produce the same degree of elasticity in the time allowed

Table 5. Titratable acidity developed during the cheddar cheese manufacturing process using milk containing aureomycin.

Time	Control[1]	Aureomycin parts per million				
		5.6	2.8	1.4	0.7	0.35
Adding starter	.16	.16	.18	.16	.16	.17
Cutting	.10	.09	.09	.10	.10	.10
Dipping	.14	.09	.09	.10	.10	.10
Cheddaring[2]	.64	.10	.09	.10	.10	.13
Milling	.72	.10	.09	.10	.10	.14

1. Average of five vats.
2. Average of determinations taken at 15 min. intervals.

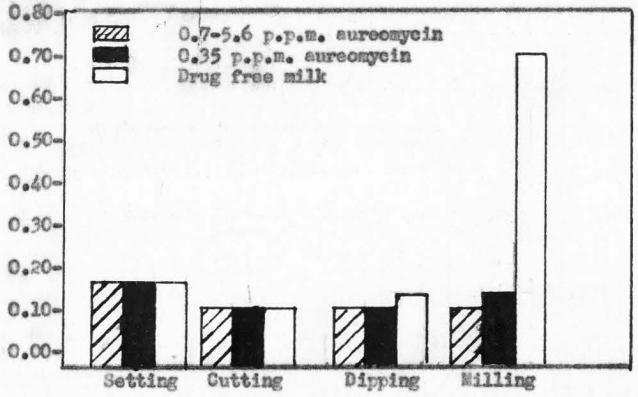

Figure 2. A comparison of the titratable acidity at 4 stages in the manufacture of cheddar cheese using milk containing aureomycin.

for cheddaring. In the milling process the curd had a tendency to crumble and break up. When an attempt was made to press the curd the particles failed to adhere to each other and it was impossible to produce a cheese that could be pressed into suitable condition for saleable purposes.

The differences in the appearance of the curd during the packing and cheddaring stages were probably due to the variation in acid production between dipping and milling. The development of good matting and pliability in cheddar cheese is dependent upon the rate and amount of acid production which changes the chemical character of the casein.

Inactivation of aureomycin in milk

The effects of milk treatments and concentrations of aureomycin on titratable acidity developed in milk are shown in table 6.

The results show that heat treatment was the most effective means used in this experiment to inactivate aureomycin. The most effective heat treatment was to heat the milk to 240° F. for 20 minutes. Samples containing aureomycin treated at this temperature showed a titratable acidity comparable to that of the control after 16 hours.

Pasteurizing milk at 143° F. for 30 minutes had some effect on the inactivation of aureomycin, but the acidity developed did not approach that of the control at 16 hours. The most noticeable effects of inactivation by pasteurization were at the lower concentrations of the antibiotic, and as the concentration of antibiotic was increased the inactivation effect decreased. When the aureomycin was added to

Table 6. Effects of milk treatments and concentrations of aureomycin on the titratable acidity developed in milk.

Treatment No[1]	Aureomycin parts per million 1.00	0.50	0.25	0.12	0.00
1	.69	.69	.72	.72	.70
2	.21	.24	.23	.32	.39
3	.20	.22	.25	.31	.57
4	.23	.24	.50	.49	.77
5	.21	.21	.22	.29	.67
6	.21	.21	.24	.32	.63

1. From table 4.

Table 7. Effects of hydrogen peroxide on acid development in milk containing 0.25 parts per million aureomycin. Average of 5 trials.

Milk used	Control	Percentage hydrogen peroxide 1.0	0.8	0.6	0.4	0.2
Raw	.80	.39	.37	.37	.40	.25
Pasteurized[1]	.80	.33	.36	.34	.35	.30

1. Aureomycin added before pasteurization.

the milk after pasteurization the antibiotic effect of the aureomycin was not changed as compared to the effect the aureomycin had in raw milk. This would indicate that it was the heat treatment that destroyed some of the aureomycin in the pasteurization process.

Treating milk with 0.2 percent hydrogen peroxide caused a slight decrease in the antibiotic effects of aureomycin. The titratable acidity developed in milk containing aureomycin and treated with 0.2 percent hydrogen peroxide did not approach that of the control, or that of milk which was pasteurized or sterilized, but did show a greater increase than was obtained in raw milk or milk which had aureomycin added after pasteurization. The inactivation of aureomycin by hydrogen peroxide found in this experiment was so slight that it is conceivable the increase in titratable acidity may have been due to another factor such as greater starter activity. Further trials would have to be run before final conclusions should be drawn.

The effects of increased amounts of hydrogen peroxide in milk containing 0.25 parts per million aureomycin are shown in table 7.

The results show that by increasing the percentage of hydrogen peroxide used to 0.4 percent there is a slight increase in the titratable acidity obtained. Increasing the amount of hydrogen peroxide above 0.4 percent had no noticeable effect on inactivation of aureomycin, and in some cases a decrease in titrable acidity was noted .

CONCLUSIONS

Aureomycin, when present in milk used for the manufacture of cheddar cheese, may be responsible for complete cessation of acid production when the concentration of the antibiotic exceeds 0.35 parts per million. When the concentration of the antibiotic was between 0.35 p.p.m. and 0.12 p.p.m. acid production proceeded at a reduced rate, and the cheese produced was of a lower quality than is desirable. It would seem probable that milk from cows treated with aureomycin when shipped to a cheese factory, could contain the concentrations of aureomycin found in the experiment to inhibit acid production in the manufacture of cheese. The treatment commonly used, as suggested by the manufacture, is 200 mg. of aureomycin hydrochloride in ointment injected into each infected quarter of the cow. If this amount was present in 1700 pounds of milk the concentration would be 0.25 p.p.m., or enough to cause serious difficulties in the cheese manufacturing process. Where several producers is a community are treating with aureomycin it is entirely in the realm of possibility that, on occasions, sufficient aureomycin may be present in the milk to cause slow or complete cessation of acid production in the cheese manufacturing process.

Sterilization of milk by heating to 240° F. for 20 minutes in an autoclave will inactivate aureomycin, and acid production will be normal. Sterilization in this manner can be used to treat milk that is to be used to propogate mother cultures, but is not practical for milk to be used in the manufacture of cheddar cheese. The mechanism

by which aureomycin is inactivated by sterilization is not known.

Pasteurization at 143° F. for 30 minutes did not completely inactivate aureomycin, but appeared to be the best practical method studied to aid in the inactivation of the antibiotic. It may be that pasteurization, and increasing the percentage of starter, as suggested by other investigators, is the best answer to the problem of increasing the rate of acid production in cultured dairy products when the milk is known to contain aureomycin.

It was suggested that hydrogen peroxide might inactivate aureomycin. When the hydrogen peroxide was used at the rate of 0.2 percent there was very little effect found on the inactivation of aureomycin, and very little increase in acid development due to the treatment with hydrogen peroxide. Increasing the amount of hydrogen peroxide to 0.4 percent resulted in some decrease in the inhibitory effect of aureomycin, but increasing the hydrogen peroxide beyond 0.4 percent did not have any beneficial effect.

SUMMARY

Aureomycin when present in milk in concentration greater than 0.35 parts per million will cause complete cessation of acid production when the milk is to be used to manufacture cheddar cheese. When the concentration of aureomycin is between 0.25 and 0.12 parts per million acid production will be slow, and the milk is not satisfactory for cheese making purposes.

Sterilization of milk, containing aureomycin in concentrations that will inhibit acid production, will destroy the antibiotic action of aureomycin and the titratable acidity developed in 16 hours will be normal.

Pasteurization temperatures will partially inactivate the antibiotic action of aureomycin, but the rate of acid development will be slow, and the total acid developed will be below normal at any given time in the cheese making process .

Hydrogen peroxide treatment of milk, containing aureomycin in amounts that inhibit acid production, cannot be depended upon to destroy the antibiotic action of aureomycin.

The sterilization temperatures used in this experiment could be used to prepare milk for mother cultures, but could not be used to prepare milk for other cultured dairy products.

A solution to the problem of antibiotics in milk lies in a good producer distributor relationship, wherein the distributor agrees to accept the milk containing the antibiotic, if the producer will label all milk coming from cows with antibiotics for a period of

seven days after the last treatment. In this way the distributor could use the milk containing the antibiotic in channels that do not require the growth of bacteria for acid development.

To this date no detrimental effect other than the inhibitory action of aureomycin on the growth of lactic acid producing organisms can be traced to the antibiotic.

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APPENDIX

Table 8. Titratable acidity developed in 5 lots of milk containing 4 different concentrations of aureomycin and sterilized at 240° F. for 20 minutes.

Trial	Aureomycin p.p.m.					
	1.00	0.50	0.25	0.12	0.00	A.S.[1]
1	.71	.72	.74	.72	.73	.22
2	.58	.61	.64	.62	.60	.19
3	.70	.73	.73	.75	.69	.23
4	.72	.66	.71	.73	.72	.21
5	.72	.75	.77	.78	.76	.20
Average	.69	.69	.72	.72	.70	.21

1. Titratable acidity at time of adding starter.

Table 8a. Titratable acidity developed in 5 lots of milk containing 4 different concentrations of aureomycin added after pasteurization.

Trial	Aureomycin p.p.m.					
	1.00	0.50	0.25	0.12	0.00	A.S.[1]
1	.20	.20	.22	.29	.57	.20
2	.21	.25	.23	.30	.60	.18
3	.21	.20	.22	.31	.75	.18
4	.21	.20	.22	.28	.69	.19
5	.22	.21	.21	.27	.73	.19
Average	.21	.21	.22	.29	.67	.19

1. Titratable acidity at time of adding starter.

Table 8b. Titratable acidity developed in 5 lots of raw milk containing 4 different concentrations of aureomycin.

Trial	Aureomycin p. p. m.					
	1.00	0.50	0.25	0.12	0.00	A.S.[1]
1	.23	.21	.23	.29	.55	.21
2	.20	.20	.29	.32	.41	.17
3	.22	.22	.25	.37	.66	.19
4	.22	.22	.22	.30	.76	.20
5	.20	.22	.23	.33	.75	.19
Average	.21	.21	.24	.32	.63	.19

1. Titratable acidity at time of adding starter.

Table 8c. Titratable acidity developed in 5 lots of milk containing 4 different concentrations of aureomycin added after pasteurization and before treatment with hydrogen peroxide.

Trial	Aureomycin p. p. m.					
	1.00	0.50	0.25	0.12	0.00	A.S[1]
1	.20	.21	.23	.28	.30	.17
2	.20	.20	.23	.31	.45	.17
3	.21	.21	.26	.29	.70	.17
4	.18	.24	.25	.24	.74	.18
5	.22	.23	.28	.44	.63	.19
Average	.20	.22	.25	.31	.57	.18

1. Titratable acidity at time of adding starter.

Table 8d. Titratable acidity developed in 5 lots of milk containing 4 different concentrations of aureomycin added to raw milk before treatment with hydrogen peroxide.

Trial	Aureomycin p. p. m. 1.00	0.50	0.25	0.12	0.00	A. S.[1]
1	.22	.23	.23	.29	.28	.18
2	.20	.27	.24	.32	.30	.17
3	.21	.26	.22	.30	.25	.20
4	.21	.20	.22	.36	.55	.19
5	.20	.22	.26	.33	.57	.19
Average	.21	.24	.23	.32	.39	.19

1. Titratable acidity at time of adding starter.

Table 8e. Titratable acidity developed in 5 lots of milk containing 4 different concentrations of aureomycin added before pasteurization.

Trial	Aureomycin p. p. m. 1.00	0.50	0.25	0.12	0.00	A.S.[1]
1	.22	.23	.27	.55	.76	.20
2	.23	.27	.37	.60	.77	.21
3	.22	.26	.29	.31	.74	.20
4	.23	.20	.29	.50	.80	.20
5	.23	.25	.30	.48	.76	.20
Average	.23	.24	.50	.49	.77	.20

1. Titratable acidity at time of adding starter.

Table 9. Titratable acidity developed in 5 lots of raw milk containing 0.25 p.p.m. aureomycin added before treatment with 4 different concentrations of hydrogen peroxide.

Trial	Percentage Hydrogen Peroxide 1.00	0.80	0.60	0.40	0.00[2]	A.S.[1]
1	.39	.36	.30	.30	.80	.20
2	.72	.41	.53	.63	.79	.21
3	.41	.40	.36	.32	.81	.20
4	.20	.47	.46	.43	.81	.20
5	.22	.20	.20	.33	.78	.20
Average	.39	.37	.37	.40	.80	.20

1. Titratable acidity at time of adding starter.
2. Sample did not contain aureomycin.

Table 9a. Titratable acidity developed in 5 lots of milk containing 0.25 p.p.m. aureomycin added before pasteurization and before treatment with 4 different concentrations of hydrogen peroxide.

Trial	Percentage Hydrogen Peroxide 1.00	0.80	0.60	0.40	0.00[2]	A.S.[1]
1	.39	.37	.32	.34	.79	.20
2	.36	.44	.39	.39	.87	.21
3	.39	.41	.42	.34	.76	.20
4	.33	.35	.24	.27	.79	.20
5	.20	.21	.33	.42	.81	.20
Average	.33	.36	.34	.35	.80	.20

1. Titratable acidity at time of adding starter.
2. Sample did not contain aureomycin.