Citrus pulp is an important feed for dairy cows in California and other citrus-growing regions.

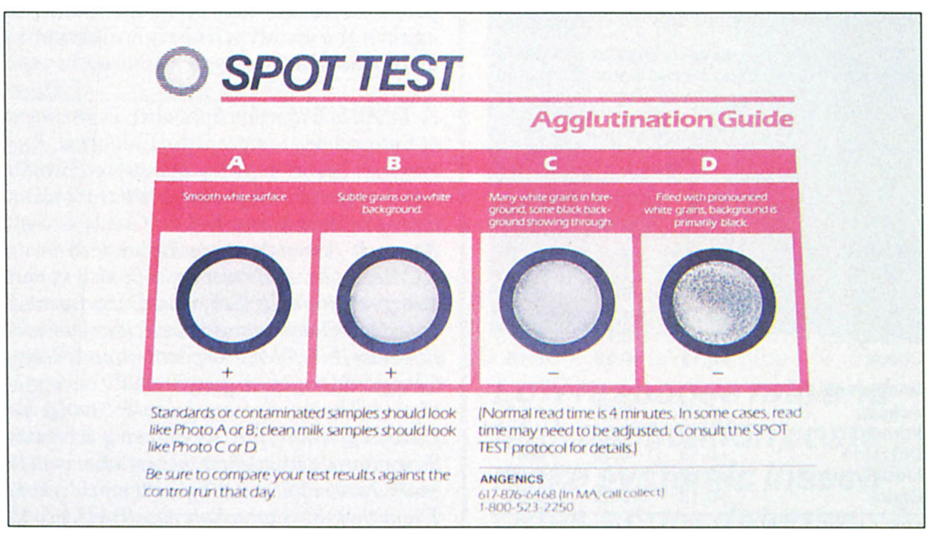
# False positive tests for penicillin in milk

Carol Collar ❑ Donald L. Bath

## *False readings are linked to feeding citrus pulp to lactating dairy cows*

There are more than 260,000 acres of lemons and oranges in California and Arizona. Much of this acreage is in areas heavily populated with commercial dairies.

In addition to the fresh fruit market, a portion of the citrus crop is processed into concentrated juice, generating as a by-product about half a million tons of wet citrus pulp each year. This residue is an important feed for dairy cattle, in dried (90 percent dry matter), pressed (30 percent dry matter), or wet (15 percent dry matter) form. Dried pulp is similar in feeding value to dried beet pulp. It is relatively high in energy and fiber, but low in protein. The pulp must be introduced gradually into a ration, but once cows are accustomed to the taste, concentrate mixes with 40 percent or more citrus are very palatable to cattle.




Antibiotic-coated latex beads of Angenics Spot Test react with antibodies in contaminated milk to form a smooth white texture (two discs at left of test guide). Uncontaminated milk results in clumping or agglutination of the milk sample and a grainy, textured appearance (discs at right).

In 1985, a milk processor in northern California began experiencing false positive tests for penicillin contamination in milk from dairy farms where citrus pulp was fed. Apprehensive that the possibly contaminated milk could pose a public health threat, or might reduce cheese yield, the processor refused to accept milk from dairies that fed citrus pulp to milking cows.

We undertook the following experiment to determine if the false positive tests for antibiotics, particularly penicillin, were related to feeding citrus pulp and, if so, at what level of pulp in dairy cows' rations the false readings began to appear. We also wanted to learn if the condition causing the false positive tests had a detrimental effect on cheese yield and, if possible, to learn what causes false positive tests.

## Background

Milk received at processing plants is routinely tested for antibiotics and other inhibitors to ensure that these substances do not get into the human food chain. The *B. stearothermophilus* disc assay is the official bacteriological test recognized by the U.S. Food and Drug Administration and the California Department of Food and Agriculture to detect antibiotics and other inhibiting substances. It is used by all milk handlers in California as the basis for accepting or rejecting raw milk for processing. The test involves incubating milk samples with bacteria grown in a culture plate. Antibiotics and other inhibitors in the milk will inhibit growth of the bacteria. A zone of growth inhibition greater than 16 mm is considered positive for antibiotics and other inhibitors.

Even in the most rapid version of the assay, it takes 90 minutes to detect antibiotic contamination of raw milk. This is too long for most handlers, because contaminated milk may already have been pumped into receiving tanks and perhaps processed before the inhibitors are detected.

Commercial tests have been developed that detect antibiotics in raw milk in 4 to 10 minutes. Milk handlers often use one of these rapid tests as a quick screening method to predict the outcome of the official disc assay. Milk that tests positive for

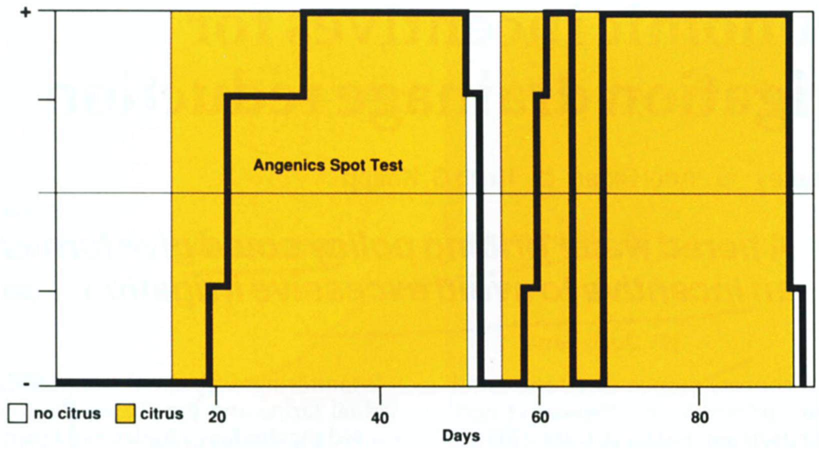



Fig. 1. Angenics Spot Test for penicillin showed false positive readings shortly after citrus pulp feeding began and returned to normal after citrus feeding ended. Other quick assay tests and the official Food and Drug Administration disc assay showed negative readings throughout the trial.

antibiotics with the rapid screening test is then subjected to the official disc assay test for confirmation.

The most widely used rapid test for penicillin is the Charm Test developed by Dr. Stanley Charm of Tufts University. This test, approved by the Association of Analytical Chemists, is based on the fact that certain receptor sites in microorganisms selectively bind penicillin. When carbon-14-labeled penicillin is added to a milk sample containing the test bacteria, it will compete with any contaminating penicillin in the milk for binding sites in the bacteria. The degree of penicillin contamination is inversely proportional to the amount of carbon-14 penicillin that is bound, and it can be accurately measured.

Another rapid test for detecting antibiotics in raw milk is the Angenics Spot Test, which requires less expensive equipment than is needed for the Charm Test. The Spot Test makes use of a monoclonal antibody to detect penicillin, cephapirin, and cloxacillin at levels that will cause a positive *B. stearothermophilus* disc assay. It is based on interaction of antibiotic-coated latex beads and an antibody with a specific attraction for these three antibiotics. The antibodies bond with the antibiotic molecules on the latex beads, causing them to link together in clusters and chains, a process called agglutination. When the coated latex beads and antibody are mixed in a milk sample, antibiotics in the milk will bind to some of the antibodies, reducing agglutination. Presence or absence of antibiotics is determined by evaluating the extent of agglutination. Contaminated samples have a smooth white surface; uncontaminated milk samples appear grainy and textured.

## Feeding trial

For baseline values, bulk tank milk samples from a large commercial dairy (580 cows) in Kings County were tested daily for antibiotics with the Charm and Angenics tests for two weeks before citrus pulp feeding began.

Pressed citrus pulp from Valencia oranges (30 percent dry matter) was then added to a complete mixed ration at the rate of 7 pounds per cow per day (4.4 percent of total ration dry matter intake). After one week at this level, the citrus pulp was raised to 14 pounds per cow per day (8.8 percent of total ration dry matter intake) for one week, and finally to a maximum of 21 pounds per cow per day (13.2 percent of total ration dry matter intake). The maximum level was maintained for four days, and then the citrus in the ration was reduced by 7 pounds each week until eliminated.

Bulk tank milk samples were tested daily by both tests throughout the entire study. When the samples returned to baseline values for several days, citrus pulp from navel oranges was introduced to the ration in the same amounts for a second feeding period. After 14 days at the maximum level (21 pounds per cow per day), citrus pulp was gradually eliminated from the ration. During this second citrus pulp feeding period, bulk tank milk samples were tested for antibiotics and inhibitors using the *B. stearothermophilus* disc assay in addition to the Angenics and Charm tests.

**TABLE 1. Ration composition for three levels of citrus pulp feeding (weighted average for 580 cows in three milk production groups)**

| | Citrus at: | | |
|---|---|---|---|
| Ingredient | 7 lb | 14 lb | 21 lb |
| | *lb/cow/day (as fed)* | | |
| Rolled barley | 9.96 | 8.24 | 7.68 |
| Alfalfa hay, chopped | 7.00 | 7.00 | 9.56 |
| Alfalfa hay, baled | 3.00 | 3.00 | 3.00 |
| Alfalfa haylage | 20.24 | 20.24 | 15.12 |
| Corn silage | 16.16 | 16.16 | 14.47 |
| Whole cottonseed | 6.24 | 6.24 | 6.24 |
| Wet citrus pulp | 7.00 | 14.00 | 21.00 |
| Cottonseed hulls | 3.50 | 3.50 | 3.50 |
| Liquid fat supplement | 2.78 | 2.78 | 2.78 |
| Wheat mill run | 4.00 | 3.12 | 2.24 |
| Alkaten buffer | 0.20 | 0.20 | 0.20 |
| Total as fed | 79.48 | 84.48 | 85.79 |
| Total ration, % DM | 60.00 | 56.50 | 55.70 |

Cows were housed in a freestall barn, and grouped and fed by milk production. A complete mixed ration (table 1) was formulated for three levels of fat-corrected milk production: high (88 pounds), medium (69 pounds), and low (50 pounds). Citrus pulp replaced wheat mill run in the ration. At 21 pounds citrus per cow per day, some alfalfa haylage was replaced by alfalfa hay to keep the total ration dry matter percentage at desired levels.

## Results and discussion

All tests for penicillin using the Charm and the Angenics Spot Test were negative before citrus pulp was fed (fig. 1). In the first feeding period, bulk tank milk tested positive for penicillin with the Spot Test within 72 hours after citrus pulp was introduced into the ration at 7 pounds per cow per day. Milk continued to test positive with the Spot Test at the 14- and 21-pound citrus-feeding levels. The same milk tested negative for penicillin throughout the citrus feeding period when the Charm test was used. Within 24 hours of eliminating citrus from the ration, milk tested negative for penicillin with the Spot Test and remained negative until citrus pulp was reintroduced to the ration.

In the second feeding period, the Spot Test gave a positive result for penicillin in milk within 48 hours after citrus pulp was introduced. The test remained positive throughout the citrus feeding period with the exception of one day. On November 5, after three days at the 14-pound citrus level, a negative test for penicillin occurred with the Spot Test. (Explanations for this are complicated by the fact that the previous day's sample was missing. Bulk tank samples are collected by haulers who typically pick up milk from 10 to 12 dairies on a route. It is possible that sample bottles were switched by the driver or by a technician in the laboratory.)

All milk tested negative for penicillin by both the Charm test and the *B. stearothermophilus* disc assay throughout the second feeding period. As in the first feeding trial, milk tested negative for penicillin using the Spot Test within 24 hours of eliminating citrus from the ration. Tests for cephapirin and cloxacillin with the Spot Test were negative throughout the entire feeding period.

Cows readily ate the rations containing 7 and 14 pounds of citrus. The cooperating dairyman had been concerned that feed intake would be reduced at the 21-pound citrus level, so this high amount was maintained for only 4 days during the first trial. During the second feeding period, intake at the high citrus level was not a problem. The maximum amount of 21 pounds per cow per day was maintained for 14 days with no adverse effects.

## Conclusions

These results indicate that feeding citrus pulp to lactating dairy cows can lead to false or unconfirmed positive tests for penicillin when bulk tank milk is screened for antibiotics with the Angenics Spot Test. Our data demonstrate that false positives occur within 48 to 72 hours of initiating a relatively low level of citrus feeding (7 pounds as fed or 2.1 pounds of dry matter per cow per day).

False positives occurred with the feeding of both navel and Valencia oranges. Other field observations have indicated that false positives occur with both dry and wet citrus pulp. Little information is available on lemon pulp. Feeding dried lemon peel did not result in false positives for penicillin with the Spot Test in bulk tank milk samples taken recently from a large commercial dairy in Kings County.

The Charm Test and *B. stearothermophilus* disc assay produce absolute values above or below which a milk sample is considered positive or negative for antibiotics. The Angenics Spot Test requires subjective visual interpretation by a trained technician. This may cause inconsistencies in results of milk tests by different milk processing plants or by different technicians in the same plant. The 12 days of weak positive response to citrus pulp feeding in the first feeding period of this study reflects the subjective nature of the test. It took that long for the technician conducting the assay to decide that the test looked absolutely positive.

It is possible that the Angenics Spot Test detects a substance in milk that is in some way chemically related to penicillin, although negative disc assay tests indicate no antibiotic activity. The substance appears to occur in milk as a result of citrus feeding. It is also possible that the Spot Test is not specific for penicillin and that the antigen used in the test reacts with a natural constituent in citrus or a metabolite of the natural constituent in milk. Another University of California experiment is in progress to determine whether the condition causing false positive tests for penicillin could have a detrimental effect on cheese yield.

*Carol Collar is Farm Advisor, Cooperative Extension, Kings County, and Donald L. Bath is Extension Dairy Nutrition Specialist, University of California, Davis.* *The authors thank the Barreto and Silveira Dairy of Hanford for their cooperation in this trial; the Dairyman's Cooperative Creamery, Tulare, for assistance with laboratory analyses; Dr. Lee Larson, Dairy Nutritionist, Fresno, for ration formulation; and Sunkist Growers, Inc., Ontario, for supporting the experiment.*

# Economic incentives for irrigation drainage reduction

John Letey ❑ Ariel Dinar ❑ Keith C. Knapp

### *A tiered water pricing policy could give farmers an incentive to avoid excessive irrigation*

Public concern about environmental problems related to the disposal of agricultural drainage waters in California led to the adoption of Order WQ85-1 by the State Water Resources Control Board. One outcome of the order was a recommended interim water quality objective that would limit selenium to 5 parts per billion (ppb) in the San Joaquin River. The technical committee making the recommendation also stated that the interim water quality objective could be achieved without treatment of agricultural drainage water if subsurface drainage from existing tile-drained areas in the drainage study area were reduced from the existing 0.7 acre-foot per acre (8.4 inches) to 0.45 acre-foot per acre (5.4 inches).

Reports by a University of California Committee of Consultants and the Agricultural Water Management subcommittee of the San Joaquin Valley Drainage Program concluded that the proposed reduction of drainage flows was feasible and suggested adjustments in water management leading to the reduction. Actual drainage flow reduction, however, will only be achieved after farmers adopt the proposed management practices. As with any business, agricultural management decisions are driven by economic considerations. We conducted a research project analyzing economic incentives that might lead to adoption of the recommended practices.

## Incentives

Reduction of drainage flows to avoid costly treatment processes represents an economic incentive to the agricultural community as a whole, but would not necessarily translate into individual farmer incentive. For example, a few farmers could reduce their drainage volumes to the target value and still have to contribute to costly treatment processes if the group as a whole did not reach the target value. Conversely, a few farmers who did nothing to reduce drainage volumes could benefit if the majority reduced volumes sufficiently to meet the overall goal. The drainage discharge goal is more likely to be achieved if the incentive is directed towards individual farmers.

Monitoring drainage flows from individual farms and penalizing those who exceed the discharge limit would provide a direct incentive. Such monitoring would be costly, however, and possibly unfair because of subsurface lateral water flows making it difficult to identify the source of the discharge.

Since drainage waters are generated by irrigation, placing a surcharge on irrigation water might indirectly provide an incentive for individual farmers to reduce drainage volumes. Increasing the price of irrigation water could also provide revenue for drainage water disposal. Our research considered both aspects of a surcharge, but this report addresses only the incentive to reduce drainage volumes into the San Joaquin River. Discharge would be free if the standards were met.

Drainage volumes are not directly related to irrigation volumes over the entire range of water application. Irrigation equal to or less than crop evapotranspiration (ET) results in very low drainage flows, but irrigation in excess of ET contributes significantly to drainage flows. A tiered irrigation water pricing policy in which water amounts greater than ET are priced higher than those less than ET might be appropriate to induce reduced drainage volumes. A flat fee increase on all irrigation water is an alternative policy. We compared both policies, which we refer to hereafter as tiered and flat fee.

## Analysis

We selected cotton for analysis, because it is grown on more acreage than any other crop in the area. Crop-water production functions were computed from experimental data reported by scientists from the U.S. Department of Agriculture Water Management Laboratory in Fresno, California. Maximum crop ET was 28.7 inches, and we used this value as the cutoff point for imposing the tiered water pricing policy; that is, the first 28.7 inches of irrigation water would be provided at the usual base irrigation water price, and a higher premium price would be imposed for greater quantities.

The water quantity and drainage volume that would maximize farmer profits