California Mastitis Test Scores of Individual Quarters Compared With Composite Milk Samples and With Milk Leucocyte Counts

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CALIFORNIA MASTITIS TEST SCORES OF INDIVIDUAL QUARTERS COMPARED WITH COMPOSITE MILK SAMPLES AND WITH MILK LEUCOCYTE COUNTS

by

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TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Udder inflammation, whether caused by bacterial, physical, or neurological means, usually results in the secretion of milk that is abnormal in character.

When an inflammed condition exists there are several reactions of nature to restore the tissue to a normal state of health. One of these reactions is the increase of leucocytes at the site of inflammation. In the case of udder inflammation, the increase of leucocytes will result in an abnormal increase of these cells in the milk secreted by the udder. The milk may or may not be changed in the gross appearance from normal milk, depending upon the degree of change that takes place in the udder.

Mastitis is the term usually employed when referring to the inflammation of milk secreting tissue. The terms mastitis and udder irritation will be used interchangeably in this report to denote the same condition. Mastitis may be further defined as any udder infection, inflammation, irritation, injury or other abnormality that causes the milk which is secreted to be abnormal in its composition or properties. The causative agent in any one case of mastitis may be singular or there may be a complex of several factors working at the same time. Mastitis is not a simple disease but rather a disease complex resulting from an interplay between infectious agents and managerial practices. Mastitis may be acute, chronic, or subclinical.

Subclinical mastitis is probably the most common form of mastitis at the present time. This form is overlooked by dairymen because it is so mild it passes without recognition. In many cases, conditions causing subclinical mastitis are not corrected before considerable damage is done to the udder tissue.

The California Mastitis Test (CMT) and other similar tests such as the Milk Quality Test (MQT), Michigan Lye Test (MLT), and Wisconsin Mastitis Test (WMT) react to the Deoxyribonucleic Acid (DNA) of the leucocyte cells. Thus, any unusual increase of leucocytes in the milk could be detected by these tests. It has been suggested that the CMT and other similar tests could be practical screening tests to be made by Dairy Herd Improvement Association (DHIA) supervisors on a routine basis each month. In California and some other areas of the country, they are being made by DHIA supervisors on mixed milk samples in connection with each month's butterfat test. The popularity of such screening tests by DHIA supervisors is increasing.

An abnormal amount of udder irritation as shown by the test should be followed by other definitive tests under the supervision of a veterinarian. The function of the milking machine and certain management practices should be reviewed to determine causes of the irritation. Corrective measures should be taken before there is extensive permanent damage to udder tissue.

This study was designed to compare two ways of using the CMT as a screening test to aid dairymen in detecting udder irritation. The first method was to test the milk from each individual quarter at milking time. The second application was the less expensive one of testing only the composite milk sample taken by the DHIA supervisor. The results of the two methods were then compared.

Objectives

Objectives of this study were:

1. To evaluate the practicality of DHIA supervisors using the CMT to help dairymen detect mastitis in their cows.

2. To compare the CMT with direct leucocyte counts.

REVIEW OF LITERATURE

Prevalence

In noting the prevalence of mastitis, the National Mastitis Council (4, p.2) states,

> A reasonable estimate of the prevalence of mastitis today in the United States is 50% of the cows infected with pathogenic organisms in an average of 2 quarters per cow. At least one-fourth of the infected cows or approximately 12.5% of all cows show abnormal secretions at any one time.

Losses from mastitis are varied and difficult to estimate. Losses from chronic mastitis are especially elusive because the mild nature of the loss and damage are frequently overlooked.

The National Mastitis Council (4, p.2) said,

> To estimate the cost of this disease, it would be necessary to determine the loss that results from (1) reduction in milk secretion, (2) loss of milk produced that is unfit for human food, (3) extra labor costs, (4) loss involved in the death or culling of dairy cows because of mastitis, and (5) cost of treatment. However, accurate information on these losses is not available. Estimates of the annual loss to dairymen in the United States have ranged from $225,000,000 to $500,000,000, and it is reasonable to assume that the actual loss falls somewhere between these two figures.

The economic losses to the dairy industry because of mastitis are just beginning to be realized (12).

Barnard and Thomas (1) reported a USDA estimate of the annual cost to dairymen of $250,000,000, a cost of approximately $12.50 for every producing cow in the nation.

Gray and Schalm (9) found in comparing lactation yields between CMT-negative and CMT-positive groups, there was an average loss of milk production of 6.0 percent, 10.0 percent, 16.0 percent, and 24.5 percent for cows with CMT scores of trace, 1, 2, and 3, respectively. The differences were statistically significant.

Jasper (12, p.37) quoted from personal communication with Gray to point out some of the seriousness of the economic loss suffered by dairymen because of mastitis.

> Several workers have shown that SNF decrease in the presence of mastitis. This loss may become very substantial in a cow with a 3+ reaction to the CMT. Such a cow may be producing 25 percent less milk which in turn may contain less than 80 percent of the normal SNF. The total loss of nutrients in this case is about 40 percent of her potential production.

Noorlander (18) refers to a study of 3,785 cows in Sacramento County, California, in which CMT negative cows produced 2,178 pounds more milk than the CMT one's, 3,392 pounds more than the CMT two's, and 4,572 pounds more than the three's.

Huber (11) found a mean difference of .4727 pounds less yield per hundred pounds of milk when making cheddar cheese from abnormal milk and .527 pounds less per hundred pounds of skim when making cottage cheese. The abnormal milk selected in each case was normal in appearance but had a CMT score of two or three. He also reported a lower quality of finished product with both types of cheese. A loss of $0.02 to $0.04 per pound of finished cheese because of grade differences was noted at 1963 prices.

Morris and Huber (17) indicated a possible loss of $25.00 per 10,000 pound vat in making cheddar cheese if as much as 30 percent of the milk was abnormal. This included losses of both yield and quality of cheese.

Public health

From the consumer health standpoint, there seems to be little danger because most organisms that cause mastitis are killed when milk is pasteurized (4).

Some strains of staphlococci that cause mastitis can, however, under certain conditions, produce a toxin (enterotoxin) which causes vomiting and diarrhea. These micro-organisms constitute a potential problem when they are present in raw milk used for making cheese or dessert-type dishes that are not refrigerated. The enterotoxin develops in milk held at elevated temperatures and once formed, it is not destroyed by boiling or drying (4).

The wide spread use of antibiotics for treating mastitis has resulted in a problem of residues in milk. These residues are potential public health hazards. Steps should be taken to further reduce the antibiotic residue in the public milk supply (10).

Milking machines and mastitis

Whittlestone (24) said it is not possible to give a clear picture of the relationship between milking machines and the incidence of mastitis. The results of a very wide range of experiments are contradictory and no clear generalizations can be made from them. There are, however, a few points which seem to indicate trends. For example, leaving the teat cups on too long produces irritation, which in turn causes inflammation, and recent American experiments have shown that the leucocyte content of the milk from cows so treated tends to increase. A high leucocyte count indicates inflammation, which inflammation may be a predisposing factor in the production of mastitis or it may be caused by it.

The heavy molded or "hard" inflations cause congestion and swelling of the inner lining of the teat. This could be a predisposing factor (for mastitis). As hard inflations have no advantages from the milking point of view, there would seem to be no justification for their use (24).

Whittlestone (24) also cited a field case where several cows developed acute mastitis within a week or two following a change of claw pieces on the milking machine. The only fault found was an obstruction of milk flow in the claws, resulting in the incomplete milking out of some cows, which apparently had a short let down. Presumably, the increased intramammary pressure caused by incomplete milking precipitated a clinical form of what had been a subclinical infection.

Many cases of sub-clinical infection may be traced directly to milking machines that are improperly used or malfunctioning (16).

Where faulty milking machines are causing udder irritation, it is frequently noted that many CMT-positive quarters show no obvious signs of clinical mastitis and the milk may be devoid of bacteria. In such cases, correction of the mechanical problem is usually the only treatment necessary to effect recovery of the cows to normal production (16, 15, 18).

Stabilization of the vacuum at teat end is essential to prevent tissue damage (18).

Some authors (15, 18, 24) feel narrow bore liners are especially helpful in reducing injury. Others (16) think the usefulness of the narrow bore liner is doubtful and not essential if the milking machine is in good mechanical condition and is used properly.

Classification of leucocytes

Dukes (7) classifies leucocytes as follows:

Agranulocytes:

1. Lymphocytes.

2. Monocytes.

Granulocytes:

1. Neutrophiles.

2. Eosinophiles.

3. Basophiles.

The lymphocytes are formed in lymphoid tissue. They are believed to produce antibodies and to "fix" toxins. They are not phagocytic, although they show some ameboid motility.

Monocytes are the so-called transitional cells, otherwise known as large mononuclear leucocytes. Their motility is well developed. Being actively phagocytic, they are able to ingest almost any type of foreign particle. Their origin is probably the cells of the reticulo-endothelial system.

Neutrophiles are able to make ameboid movements, are actively phagocytic, and show rapid increases in numbers in pyogenic infections. Formed in red marrow of bones from extra-vascular neutrophilic myelocytes, they are probably the most important of the polymorphonuclear leucocytes.

Eosinophiles are large cells with acid staining granules. They have a polymorphic nucleus. It is said they are not ordinarily phagocytic. They are formed in the red bone marrow.

Basophiles constitute the smallest group of the leucocytes. They are formed in the red bone marrow also. Their phagocytic power is slight or absent and their function is not known.

<u>The significance of leucocytes in mastitis</u>

In general, a leucocytosis may be regarded as a response of the body to an increased demand for leucocytes.

A mild infection may call forth an insignificant response. If the infection is overwhelming, or if the reaction power of the body is seriously impaired, there may be no leucocytosis, but even a leucopenia. In an individual with adequate reacting power, the degree of leucocytosis is very roughly proportional to the severity of the infection. In interpreting the leucocytic response to an infection, however, changes in the differential count and qualitative alterations in the leucocytes are more significant than an increase in their total number. Earlier studies considered a rise in the percentage of neutrophiles as an indication of an individual's resistance. Later work seems to indicate the number of immature neutrophile cells is a more reliable index than the percent of neutrophiles (22, 24).

Whittlestone (24) said a high leucocyte count indicates inflammation.

Boyd (2) stated, it is believed a peptide-like substance called leukotaxine is liberated in the exudates in inflamed areas. This substance increases capillary permeability and thus enhances the migration of polymorphonuclear leucocytes through the capillary wall.

Not only is the mobilization of leucocytes one of the mechanisms of immunity, but there also seems to be an increased production of leucocytes (2).

After leucocytes have been lured to the scene of action by leukotaxine they accomplish their work by a process of engulfing bacteria and other particulate matter. This process is known as phagocytosis. The bacteria and particulate matter are digested by the cell.

In addition to leucocytes, other cells in the body have this phagocytic ability. The cells of the reticulo-endothelial system have this ability. The spleen, liver, lymph nodes, and bone marrow are all involved in this system.

Apparently there are substances in the serum of immune blood which have the property of increasing the degree of phagocytosis of the corresponding bacteria. Normal serum has some, but less activity. Opsonins are antibodies that facilitate the phagocytosis of bacteria (2).

An increase of leucocytes, particularly neutrophiles, seems to be a natural reaction of the body to an invasion of microorganisms as in the case of mammary gland infections. In mastitis caused by a virus, however, there may even be a leucopenia, a reduction in the number of leucocytes.

Schalm (20) noted an increase of the total number of leucocytes with increased grades of the CMT but there was a greater increase of the polymorphonuclear leucocytes than the mononuclear cells.

CMT and DHIA

Gray and Schalm (8) suggested that milk samples collected by DHIA supervisors could also be used for the CMT.

The Master Milkers Manual (15) indicates that the DHIA supervisor has the routine, the interest, and the capacity to test the cows using the CMT. Of utmost importance is the fact that he has a milk sample from each cow at monthly intervals. These same milk samples can be used for CMT evaluation, thus eliminating the costly task of sampling.

CMT testing has been included in the DHIA program in some California counties for over seven years (15). Five cents per cow provides a simple running account of the CMT score for each cow. Ten cents per cow provides an individual record chart for each cow with the CMT score and her milk production plotted for each month. When compared to an assumed normal curve, the amount and duration of production losses are clearly visible.

Noorlander (18) has suggested the use of the CMT or other similar tests in connection with a regular DHIA test each month. In fact, he goes so far as to suggest the possible future importance of the CMT type of test may outweigh the value of the DHIA test for butterfat and the butterfat testing may then be the test of incidental importance. Noorlander published a graph which may be used in connection with this test to enable dairymen to visualize more clearly the losses of an individual cow's production resulting from udder irritation.

Braund and Schultz (3) found individual quarter sampling with the CMT to be more accurate than bucket sampling but that either the CMT or the catalase test on bulk milk was a good reflection of the positive quarters of a herd. Since there were so many low reactions on the CMT on bulk milk, the catalase test was preferred because of ease of interpretation.

Schneider (21) indicates a much higher participation of DHIA associations in CMT testing by 1964 in California than was reported by the Master Milkers Manual in 1960. 75 percent of the DHIA organizations and from 30 to 100 percent of the herds within these organizations are now testing with the CMT. He also indicates studies are now in progress to determine the effectiveness of this testing program in the California herds.

Milking samples must be tested with 24 to 36 hours if reliable results are to be expected with the CMT. Schalm and Noorlander (19) reported that bacterial growth in milk will destroy the factor or factors that support a positive reaction with the CMT. Therefore, when bucket or bulk milk samples are tested, they should be refrigerated to control bacterial growth and the test should be conducted within 24 to 36 hours for best results.

The DNA in cells deteriorates upon standing (13). Refrigerated milk may be tested up to 48 hours but unrefrigerated milk cannot be tested accurately after about 12 hours.

The Wisconsin Mastitis Test (13) is a more objective test than the CMT and could be used by DHIA supervisors in laboratory testing. The WMT does not serve as well as the CMT for a cow side test.

PROCEDURE

Phase I

Milk samples from individual quarters of 578 Holstein cows from 12 herds were tested by the California Mastitis Test (CMT). The results of the individual quarter sample tests from both night and morning milkings were compared to a composite sample from all four quarters at night and also to a composite sample of the night and morning milk as normally taken by a DHIA supervisor.

A statistical study was then made to determine the correlation between individual quarter samples and the composite samples of night and morning milkings.

The CMT results were read as negative, trace, 1, 2, and 3 as suggested by the directions of the test. Individual quarters were considered to be positive when they were scored as 2 or 3. In order to compensate for the dilution factor, mixed and composite samples were considered to be positive with a score of 1, 2, or 3.

To eliminate possible bias of scoring, mixed and composite samples were evaluated at a sink with a good overhead light, while a scribe recorded the scores at a table a short distance away. The one evaluating the samples did not have access to the results of the individual quarters at the time of scoring the mixed and composite samples.

The period of June to September was chosen for this test because it is generally considered to be a period where the incidence of mastitis is relatively stable.

Several makes and models of milking machines and parlors were represented in the study, from floor-type milking machines in stanchion barns to herringbone milking parlors with pipeline milking machines and bulk tanks. No attempt was made to correlate the type of equipment or management with the CMT results in this study.

Phase II

Samples of milk were taken from individual quarters of five selected cows to study the relationship between the differential leucocyte counts and CMT scores.

Two cows were selected for this study that had a previous history of mastitis and also had a strong reaction to the CMT. One cow was selected which had no history of clinical mastitis but had a medium reaction to the CMT. Two cows were selected which had no history of mastitis and their milk had a negative CMT score.

The cow's udder and teats were washed thoroughly and dried. The ends of the teats were swabbed with alcohol and allowed to dry just before drawing the sample. Milk samples were drawn into Hotis tubes, using standard techniques.

The milk samples were held over night in a refrigerator. A slide was then prepared by taking a standard loop of milk from just below the cream layer and spreading it over a one-cm. square area on a glass slide. The smear was dried, fixed, and stained with methylene blue.

Leucocytes were counted under an oil immersion lens. 50 leucocytes per quarter were tabulated according to the CMT grade. The tests were repeated after a three week period.

One of the cows was then slaughtered, her udder stained by infusing formalin and methylene blue and the tissue of the udder was studied.

Unstained areas of tissue are non-secretory and may have been induced by mastitis.

RESULTS AND DISCUSSION

Phase I

The CMT results were tabulated for 578 cows on an individual quarter basis for an evening and morning milking as well as the results of the mixed bucket sample at night and the composite sample taken in the morning from the pail and added to the bucket sample of the previous milking.

For the 578 cows there were four blind quarters. A total of 2,308 milking quarters were tested night and morning. Of these quarters, 472, or 20 percent scored number 2 or number 3 at the evening milking. Of the 578 cows, 226 or 40 percent were included in the group with positive (score 2 or 3) CMT quarters. Of these 226 cows, 184 or 82 percent were detected by the CMT results on the mixed composite sample taken from the bucket milk when a score of 1, 2, or 3 was used as positive to compensate for the dilution factor.

The same number, 226, but not the same cows, were considered positive as a result of the CMT on the individual quarters in the morning milkings. Of these cows, 194 or 86 percent were detected by the CMT on composite samples of milk taken from the night and the morning milkings. This comparison was considered reliable, since the milk from cows reacting to the CMT at night represented half of the composite sample and half of the sample was taken from the morning milking.

The 280 cows that showed a positive reaction to the CMT at night and/or in the morning are shown in table 1. Of these, 223 or 80 percent were detected by the CMT on the two-milking composite samples.

Table 1. Number of cows showing a positive CMT reaction as indicated by individual quarter milk samples and by mixed bucket milk samples

Time	Total tested		Number positive cows[a]	Number detected by mixed sample[b]	Percent detected
	Cows	Quarters			
P.M.	578	2,308	226	184	81
A.M.	578	2,308	226	194	86
P.M. AND A.M.	578	4,616	280	223	80

[a] Cows were considered positive on individual quarter testing when one or more quarters had a CMT score of 2 or 3.

[b] Because of the dilution factor, cows were considered positive when the mixed milk samples had a CMT score of 1, 2, or 3.

In a similar study Braund and Schultz (3) found that 57 percent of 220 CMT-positive quarters were detected by using the test of the mixed sample of milk taken from the pail compared to the individual quarter tests. Braund indicated there were less than 220 cows involved because some cows had more than one positive quarter. The exact number of cows involved, however, was not available.

Of the 44 cows with four quarters scoring 2 and 3, 43 or 98 percent were detected using the CMT results from the mixed sample (table 2). 24 or 96 percent of the 25 cows with three positive quarters were detected. Of the 65 cows with only two positive quarters, 58 or 89 percent were detected. Only 65 percent of the cows with one positive quarter were detected as the mixed sample tests picked up 59 of the 91 cows in this grouping.

Table 2. Percentage of cows with positive CMT test scores using composite milk samples when individual quarter samples showed a positive CMT test.

	No. observed by individual quarter test[a]	No. detected by mixed sample test[b]	Percent detected
Cows with four positive quarters	44	43	98
Cows with three positive quarters	25	24	96
Cows with two positive quarters	65	58	89
Cows with one positive quarter	92	59	65

[a] Using CMT score of 2 or 3.
[b] Using CMT score of 1, 2, or 3.

Of the cows with a score of 1 in any quarter, some were still positive for the mixed sample (table 3).

Table 3. Percentage of cows with positive CMT test scores using composite milk samples when individual quarter samples showed a CMT reaction of 1 in one or more quarters.

Number of quarters per cow scoring one on CMT	Number of cows with quarters scoring one on CMT	Number of cows detected by mixed milk sample as positive[a]	Percent detected
Four quarters	13	10	77
Three quarters	8	5	63
Two quarters	23	14	61
One quarter	77	14	18

[a] Using CMT score of 1, 2, or 3.

Of the 13 cows with four quarters scoring number 1, 10 or 77 percent were detected. Five of the eight cows, or 63 percent with three quarters scored number 1 were detected. 14 or 61 percent of the cows scoring number 1 in two quarters were detected by the CMT on mixed samples. Only 18 percent of the 77 cows scoring number 1 in one quarter and the other three quarters scoring negative or trace were scored positive from the mixed sample. While the numbers of cows in the first two categories may be low, there is no reason to believe that further study with more cows would change the detected percentages materially from those found here.

There were seven cows with all quarters scoring negative or trace on the individual quarter sample, but scored 1 or 2 on the mixed composite milk sample. Two of these were negative at night on the quarter sample tests, but were positive at the morning milking. This might indicate the leucocyte numbers were few in the fore milk used for the quarter sample, but as the milking progressed the leucocytes came down with the milk and affected the mixed sample. The leucocytes were also present in the fore milk of the subsequent morning milking. This relationship might indicate the leucocytes were simply building up at the start of an infection.

For the other five cows indicating positive on the mixed sample, no logical explanation was apparent for their reactions other than the human error in scoring this subjective test.

While it was not a basic part of this experiment and the herds were in no way selected to reflect a cross section of herds in this area, the percent of cows with one or more positive quarters ranged from 25 percent to 75 percent in the individual herds. Herd size ranged from eight to 141 cows.

With one exception, which happened to be the largest herd tested, the herds had at least as many, if not more cows reacting in the morning than they had in the previous night milking. In fact, without the results of this herd in the study, there would have been nearly 9 percent more positive reactors in the morning milking than the evening milking. This is not evident in the data because the large herd had 28 more cows reacting at night (2:00 p.m.) than in the morning (2:30 a.m.) and this offset the smaller differences of the other herds. It was not determined what caused this difference or if the difference is real.

Phase II

California Mastitis Test results with the mean percentage occurance of the polymorphonuclear (PMN) leucocytes were compared (table 4).

Of the 40 quarters studied, 16 scored negative, 3 scored trace, six scored 1, and 15 scored 2 by the CMT. None of the quarters included in this study were scored as high as number 3. The data of the two tests of 20 quarters each, three weeks apart, were so similar the results were pooled in table 4.

Slides were stained with methylene blue and all the polymorphonuclear leucocytes were counted together. By using methylene blue as the only stain, no color differential existed to distinguish the different polymorphonuclear leucocytes. Lymphocytes and monocytes were counted together as a second group (table 4).

Because of the intimate relationship between the blood supply and milk manufactured in the udder, it would be reasonable to expect about the same proportion of the various types of leucocytes to be found in the milk as are in the blood (table 4).

Table 4. Differential leucocyte counts at the various CMT grades

CMT grade	No. of quarters	Lymphocytes and monocytes		Polymorphonuclear leucocytes (neutrophiles, eosinophiles, and basophiles)	
		Av. No. per quarter[a]	Percent	Av. No. per quarter	Percent
Negative	16	33	66	17	34
Trace	3	30	60	20	40
1	6	23	46	27	54
2	15	14	29	36	71
Normal bovine blood[b]	--	--	73	--	27

[a] 50 leucocytes per quarter were counted.
[b] As reported by Dukes (7).

The first statistical analysis was an overall Chi Square test comparing the total observed leucocytes in the milk compared to the reported averages of the leucocytes found in normal bovine blood as reported by Dukes (7) (73 percent Lymphocytes and Monocytes and 27 percent Polymorphonuclear Leucocytes -- the Neutrophiles, Baseophiles and Eosinophiles). The resulting Chi Square value was 259.04. With one degree of freedom, a Chi Square value of 3.84 or greater would be considered significant at the $P<.05$ level and a value of 6.63 or greater would be considered significant at the $P<.01$ level. The value of 259.04 was considered highly significant for this test.

A Chi Square value for between grades was 221.60. With three degrees of freedom, Chi Square values of 7.81 or greater at the P .05 level and 11.3 or greater at the $P<.01$ level would be significant.

Again, using the normal reported proportions of leucocytes in the blood as the base, a goodness of fit test was made for each of the individual CMT grades. Chi Square values were obtained of 71.28, 25.87, 169.40, and 1,061.0 for the CMT scores of negative, trace, 1, and 2 respectively. All of these were highly significant at the P<.01 level.

Since significant differences existed between the normal reported blood leucocyte content and the negative milk leucocyte count, the proportions found in the negative milk were used as the expected base instead of the blood values. Using this measure, Chi Square values of 1.88, 47.64, and 438.55 were obtained for the grades of trace, 1, and 2 respectively. In this analysis, the trace results were not significantly different from negative but the 1 and 2 grades were still highly significant at the P<.01 level.

At every grade level in this study, as the grade went up, it was observed the total number of leucocytes went up and, as shown in table 4, the proportion of polymorphonuclear leucocytes increased.

Boyd (2) indicated a normal reaction of body tissue to irritation was an immediate increase of total leucocytes. Furthermore, with irritation present, there is a greater increase in the polymorphonuclear leucocytes that have more phagocytic ability. This increase of leucocytes enables a body to more quickly overcome the irritation.

The percentages of polymorphonuclear leucocytes are listed in comparison to the various CMT grades in this study (table 5). These data agree to a large extent with those suggested by Schalm and Noorlander (19).

Table 5. Comparison of CMT scores with percentage of polymorphonuclear leucocytes

CMT grade	Percent of polymorphonuclear leucocytes found in this study	Estimated % of polymorphonuclear leucocytes[a]	Estimated total leucocytes. Cells per cc.[a]
Negative	34	0 to 25	0 to 200,000 cells
Trace	40	30 to 40	140,000 to 500,000 cells
1	54	40 to 60	400,000 to 1,500,000 cells
2	71	60 to 70	800,000 to 5,000,000 cells
3	None included	70 to 80	Generally over 5,000,000 cells

[a] From Schalm and Noorlander (19).

One of the cows included in this experiment had produced 19,262 pounds of milk testing 3.4 percent and 629 pounds of butterfat starting her record at seven years and two months of age, 305 day 2X milking. This record was made while she was being fed a normal hay plus grain ration.

At eight years and three months of age, she started another lactation. This time she was on an all hay ration. After starting the lactation she contracted mastitis. During the period of this test she had a CMT score of number 2 in each of her four quarters. Her completed 305 day 2X record this time was 8,468 pounds of milk testing 4.3 percent and 360 pounds of butterfat. This was a difference of 169 pounds of butterfat.

Lamb (14) indicates cows on all hay rations at the Utah station produced about 70 percent as much as comparable cows on a hay plus grain ration. At this rate she might have been expected to produce about 440 pounds of butterfat. Her actual production of 360 pounds butterfat was 18 percent lower than the expected production.

Gray and Schalm (9) reported an average loss of 16 percent production for cows with a number 2 CMT score.

It is possible that mastitis was responsible for most of the 18 percent lower production from that expected on the all hay ration.

As a further check on the damage of mastitis, after this cow was slaughtered, her udder was infused with a solution of methylene blue and formalin. Large areas of the udder tissue remained unstained after infusion. The unstained areas were non-secretory tissue and were considered to be an indication of extensive tissue damage due to mastitis.

SUMMARY AND CONCLUSIONS

Summary

A total of 578 Holstein cows was tested on an individual quarter basis using the CMT and the quarters were scored negative, trace, 1, 2, or 3. Scores of 2 or 3 were considered positive.

Samples of milk were taken from the pail or pipeline for each cow and tested. A score of 1, 2, or 3 was considered positive when scoring these samples because of the dilution factor.

About 80 percent of the cows considered positive by the individual quarter sample tests were detected by the mixed sample tests. The percent of the cows detected by the mixed sample tests were 98, 96, 89, and 65 percent of the cows with four, three, two, and one quarters respectively that scored positive on the individual quarter tests.

It should be practical for a DHIA supervisor to use the CMT on 24 hour composite milk samples for each cow instead of using the test on individual quarters.

When milk samples from 40 quarters were sampled and studied under a microscope, it was found that the polymorphonuclear leucocytes increased much more rapidly than did the lymphocytes and monocytes in the presense of inflammation as measured by the CMT. The percentages of polymorphonuclear leucocytes to the total leucocytes in this study were 34, 40, 54, and 71 percent for those quarters scoring negative, trace, 1, and 2 respectively.

Conclusions

Composite milk samples as taken by a DHIA supervisor were shown to be adequate for the CMT and such testing would detect about 80 percent of the cows detected by quarter samples. Of the cows with two or more positive quarters 89 percent or more would be detected when the composite sample was used.

As the CMT score increases, leucocyte numbers increase. The greatest increase was noted for polymorphonuclear leucocytes.

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