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N-ACETYL-B-D-GLUCOSAMINIDASE AS A PREDICTOR
OF MILK LOSS AND RECOVERY
FOLLOWING CLINICAL MASTITIS

presented by

David John Wilson

has been accepted towards fulfillment
of the requirements for

Master of Science degree in Large Animal Clinical Sciences

Paul C Bartlett

Major professor

Date 2/22/90

N-ACETYL-B-D-GLUCOSAMINIDASE AS A PREDICTOR OF MILK LOSS AND RECOVERY FOLLOWING CLINICAL MASTITIS

By

David John Wilson

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Large Animal Clinical Sciences

1990

ABSTRACT

N-ACETYL-B-D-GLUCOSAMINIDASE AS A

PREDICTOR OF MILK LOSS AND RECOVERY

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N-acetyl-B-D-glucosaminidase (NAGase) is a lysosomal enzyme found in milk and other body fluids. Milk NAGase level has been reported to be increased in clinical and nonclinical mastitis, primarily due to leakage from damaged secretory epithelial cells.[1,2,8,10,13] This study evaluated milk NAGase at clinical onset as a predictor of severity of mastitis.

Milk samples were collected at clinical onset from 508 episodes of mastitis on a 1700 cow Michigan dairy farm. Daily milk production and disease events were recorded for all cows in the herd.

High milk NAGase levels were significantly associated with: 1) increased duration of treatment, 2) increased duration of clinical signs, 3) decreased daily milk production, and 4) increased risk of being culled because of mastitis. These associations were weak. Therefore, NAGase explained very little of the variability among cases, and was a poor prognostic test for clinical mastitis.

This thesis is dedicated to my wife, Lesa,

without whose support it could never have been completed.

ACKNOWLEDGEMENTS

I would like to express my appreciation to my graduate committee, Drs. Paul Bartlett, John Kirk, Roger Mellenberger, and Edward Mather for their assistance with this project. Indispensable help was also provided by Mr. Charles Green, Flow Labs, Inc., and Software City.

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INTRODUCTION

Increasing Importance of Clinical Mastitis:

Over the last twenty years, control measures have been developed and implemented for pathogenic gram-positive organisms associated with nonclinical mastitis. As their incidence has declined on well- managed farms, a greater percentage of mastitis cases are clinical in nature, caused by environmental pathogens such as the coliforms E. coli, Klebsiella, Enterobacter, and Citrobacter.[17,27] Signs may include swollen or painful quarters, clumps, flakes or watery appearance of milk, fever, hypothermia, inappetence, dehydration, depression, recumbency and death.[20]

Severe toxic mastitis comprises 10 to 23% of all cases of E. coli and Klebsiella mastitis.[16,17] Clinical signs include anorexia, depression and dehydration in addition to abnormal appearance of the milk and affected quarter.[16,17] Of these cows, seventy percent become agalactic.[17] Clinical cases without severe life-threatening signs may also result in decreased milk production for extended periods of time. Forty-two percent of coliform infections with mild clinical signs persisted for 1 to 22 months.[16] The phenomenon of chronic production loss following clinical signs is not limited to cows which have been affected by toxic mastitis.

Cows with less apparent clinical signs of mastitis caused by Staphylococcus aureus, E. coli, Klebsiella, environmental streps or other pathogens can also develop a chronic loss of milk production.[16,17,18,19,20]

Mastitis is the most costly disease in U.S. animal agriculture.[22,23] Total monetary loss in the United States due to clinical mastitis was estimated at 700 million dollars in 1977, equaling 4% of all total farm milk sales.[23] Cost of clinical mastitis includes decreased milk production (69% of total), milk discarded during treatment and antibiotic withdrawal periods (11%), loss of animals due to culling or death (13%), therapeutic costs (3%), veterinary fees (2%), and labor (2%).[22,23] There is substantial variation among cases of clinical mastitis in lost milk production, duration of therapy, duration of clinical signs, and risk of culling from the herd. At onset of clinical signs, it is difficult to predict which cases will be most severe. Accurate early prognosis for mastitis has economic value. Some cows suffer marked loss of production and are non-responsive despite prolonged therapy. Feed, labor, and medication would be saved if these cases could be quickly identified and culled. Other cases recover from clinical signs rapidly with minor production loss. If dairymen are uncertain that complete clinical recovery is imminent, they often treat even mild clinical mastitis for prolonged

periods. Perhaps the duration of antibiotic therapy and resultant discarding of milk could be reduced in these mild cases if they were identified early in the course of disease.

The main objective of this study was to evaluate N-acetyl-B-D-glucosaminidase (NAGase) as a prognostic test for clinical mastitis at onset of signs. Specifically, we investigated the relationship of NAGase to milk production change, duration of antibiotic therapy, time until return to the lactating herd, and risk of culling.

LITERATURE REVIEW:

NAGase is a lysosomal enzyme found in milk, serum, and other body fluids. NAGase level has been reported to increase in milk of cows with clinical and nonclinical mastitis. Magnitude of the increase may be as much as one hundred fold.[1,2,3,4,6,8,10,13] Part of the increase in NAGase in mastitic milk is due to damage to secretory epithelial cells which results in leakage of enzyme from the cellular compartment into milk.[1,2,8,10,13] This has led to speculation that NAGase can be used as a measure of severity of an episode of mastitis in contrast to tests such as Somatic Cell Count (SCC) which measures inflammatory response to mastitis.[2,3,4,6,9,10,13] There appears to be a

strong relationship between SCC and NAGase. Correlation between NAGase and SCC was reported higher in clinical cases (r=.97) than in nonclinical mastitis (r=.62).[14,32,33]

The percentage of NAGase increase in mastitis which is due to damaged secretory cells is a matter of some debate, with estimates ranging from 90% to about 45%.[1,2,8,10,13,24] This is an important question; increase in milk NAGase level as a measure of severity of mastitis would be most meaningful if it was highly specific for damage to milk producing tissue.

Age of the cow, stage of lactation, and parity affect the baseline level of NAGase in milk. Basal NAGase is highest during the first 30 days of lactation, declines sharply, and rises throughout lactation until days in milk exceeds 250 (Fig. 3).[4,5,25,33] NAGase baseline also increases with the lactation number of the cow, with the greatest increase occurring between the first and second lactations (Fig. 4).[4,5,10] There are considerable differences in baseline NAGase among cows.[4,5] These sources of variation must be taken into account when interpreting NAGase in mastitic quarters. To date, three methods of attempting to minimize the effects of basal NAGase variation have been described. The interquarter ratio is calculated by dividing the mastitic quarter NAGase by the lowest NAGase value of the other quarters from the same cow (the reference quarter).[4,5]

Combinations of the natural log of mastitic NAGase (Log NAGase) and the interquarter ratio have also been evaluated.[5,12] Difference in NAGase activity between the mastitic and reference quarter has also been used.[13] These transformations are reported to minimize background differences and facilitate comparison of NAGase levels among different cows.[4,5,12,13]

In comparison with other tests on mastitic milk, NAGase has been reported to be a superior predictor of associated lost milk production. Log Nagase was more negatively correlated with daily milk production (r^2=-.39, $p<.0001$) than milk antitrypsin or plasmin levels.[9] Difference in NAGase among infected and reference quarters was a better indicator of lost milk production (r^2=-.41, $p<.001$) than milk lactose, chloride, leukocyte percentages, bacterial status, or Log SCC.[13] These results are from study of nonclinical cases only.

Another important characteristic of NAGase is its rapid increase during clinical mastitis. Within 9 to 12 hours after quarters become infected, interquarter NAGase has already risen to a level at or near its peak value.[14,15] That is as soon as any signs such as swelling and hardness of quarters or abnormal appearances of milk are detectable. Peak level is maintained for 24 to 36 hours.[14,15] These

characteristics have led to speculation that the NAGase assay may be developed as a rapid test for predicting severity of clinical mastitis at the time of onset, yet to our knowledge no previous research has been conducted specifically addressing this question.[2,3,4,6,9,10,13]

MATERIALS AND METHODS

Sample Collection:

Milk samples were collected at clinical onset from every episode of clinical mastitis which occurred during a 6 month period in a 1,700-cow Holstein dairy herd in central Michigan. Milking personnel detected mastitis by presence of a hot, hard, or swollen quarter and/or abnormal flakes, color or consistency of the milk. Clinical mastitis was further confirmed with the California Mastitis Test (CMT). Any gel formation following addition of the CMT reagent (+1 or greater) was considered positive. All four quarters of clinical cows were stripped twice and then individually sampled for later NAGase determination and Wisconsin Mastitis Test (WMT). For clinical quarters only, teat ends were swabbed using 70% isopropyl alcohol and foremilk was aseptically collected for subsequent microbiological examination. All samples were frozen and returned to our laboratory.

Samples were obtained from 716 episodes of clinical mastitis between January 29 and July 27, 1988. Four milk samples leaked before they could be frozen. Another 42 samples were excluded because of visible dirt or manure contamination of the microbiology sample tubes. An additional 71 samples were not submitted due to inconsistent labelling of the mastitic quarter on the NAGase sampling tubes and the microbiology tubes. One hundred seventeen (117) samples were lost; 599 remained.

One episode of clinical mastitis occurred concurrently with a left displaced abomasum (LDA), and one cow had clinical mastitis in all 4 quarters, and therefore had no control quarter. These two episodes were excluded. Five episodes were lost to follow-up due to the cow moving to a show barn on another farm where no daily milk records are kept.

Case Definition:

Some cows had 2 clinical episodes in the same quarter within a few days of each other. Any such episode that occurred within 5 days of recovery (return from the hospital barn) following the earlier episode was not considered a new case of mastitis. Any such episode that occurred within 14 days of recovery from the earlier episode was not considered a new case of mastitis if the same etiologic agent was

isolated from both episodes. These exclusions eliminated 84 mastitis episodes from the study because they were judged to be a continuation of a previous case. This left 508 cases for inclusion in the study. Milk samples from each case were mailed to our microbiology laboratory, but 8 samples were spilled in shipment.

Microbiology:

Bovine blood agar and MacConkey agar were inoculated with .1 ml from each aseptic milk sample and incubated at 37 C for 48 hours. Colony morphology, hemolysis, and lactose fermentation were used for the first level of diagnosis and confirmatory tests were used for final identification of pathogens.[21]

Wisconsin Mastitis Test:

WMT was performed on all quarter samples. Two ml of milk from each sample were placed in a WMT test tube and mixed with 2 ml of WMT reagent. Brass caps with standard sized holes were placed on the tubes. After 10 seconds of mixing, the tubes were inverted for 15 seconds, placed upright for 1 minute of settling, and measured for height (mm) of column remaining in the tube.[29]

NAGase Measurement:

NAGase concentration was determined using a commercially available test kit.[7,12] Using an 8-channel micropipette, 10 ul of milk per sample were placed into each test well of a 96 well plate. Substrate (50 ul 4-Methylumbelliferyl-N-acetyl-B-D-glucosaminide) was added to each test well. Samples were incubated for 15 minutes at 25 C while being agitated by a plate shaker. Then 100 ul of stopping buffer were added to each well. The amount of NAGase in the milk sample is proportional to the amount of 4-methylumbelliferone (4MU) released from the substrate per unit time. Because 4MU is a fluorescent compound, the NAGase concentration in each sample test well was quantified by measuring the fluorescence using a fluorometer. The fluorometer was set at excitation wavelength 355 nm and emission wavelength 480 nm. Calibration of the fluorometer, including correction for room temperature, was performed before every test plate was submitted. NAGase activity was expressed as uMoles/min/L.

NAGase was measured for each clinical quarter and for one reference quarter from the same cow. The quarter used for reference NAGase was the quarter with the lowest WMT value. Interquarter ratio was calculated by dividing the mastitic

quarter NAGase by the NAGase value of the reference quarter from the same cow.

The following abbreviations were used for NAGase values: NAGM for NAGase in the mastitic quarter, NAGD for the difference between NAGM and reference quarter NAGase, and NAGR for reference quarter NAGase. NAGase results are reported as mean +/- standard deviation in units of uMoles/min/L.

Therapy:

Choice of therapy for clinical mastitis cases was based upon appearance of milk, palpation of affected quarters, and rectal temperature. If rectal temperature was greater than 39.7 C (103.5 F), 4 g oxytetracycline was administered intravenously (IV) once daily, while cephapirin was administered intramammary (IMM) twice daily. If rectal temperature was less than 39.7 C, but the quarter was swollen or milk was grossly abnormal, IMM cephapirin only was administered. If milk appeared normal and quarter swelling was absent, cows were returned to the milking herd following a 3-day antibiotic withdrawal period. Cases that were evaluated and not treated at all with antibiotics were immediately returned to the lactating herd.

Recording of Data:

Milk production was recorded every day for all cows at all milkings. All records of daily milk production were converted to twice daily milking (2X) basis using conversion factors according to the lactation number of the cows.[28] For conversion of three times daily (3X) milk weights to 2X, factors of .833, .855, and .869 were used for 1st lactation, 2nd lactation, and 3rd and greater lactation (3rd+) cows, respectively.

For all cows, records were kept of IV and IMM mastitis therapy, including total number of treatments and number of days until return to the milking herd. Reproductive events, diagnosis and therapy of diseases besides mastitis, deaths, culls, and reason for culling were also recorded. Stage of lactation was measured using the continuous variable DIM (days in milk at onset), and also using the categorical variable DGRP (days in milk group). Each case was placed into one of 5 DGRP categories: 1-30 DIM, 31-90 DIM, 91-150 DIM, 151-250 DIM, >250 DIM at onset. Cases were also classified according to parity (1,2, or 3+ lactations).

The study period was divided into three seasons: 1. Winter was defined as the period from January 29 (first day of sample collection) until March 31. 2. Spring was from April

1 until May 31. 3. Summer was from June 1 until July 27 (last day of sampling). Cases were categorized according to season of onset, so each case was assigned to one season using date of onset of clinical signs.

Data were entered into the D BASE III[30] data base program, and all statistical analyses were performed using the SAS PC program.[31]

Measures of Disease Severity:

Milk production change was used as a measure of severity of clinical mastitis. Mean daily production for the last 7 days before onset (BASE) was subtracted from mean daily milk production for the first 14 days following return to the milking herd from the hospital barn for each episode of clinical mastitis. This mean milk production change is called DIFF. DIFF is measured in kg., and is negative if production declines relative to BASE.

For a case to be included in calculation of DIFF, certain criteria had to be met. At least 4 complete daily milk weights had to be recorded during the 7 day BASE period, and no treatment or withholding from the lactating herd for any disease could be present during this period. The reason that some missing milk weights were allowed during BASE was

that some cows contracted mastitis early in lactation. They were only in the lactating herd long enough to have 4 daily milk weights before onset. For the first 14 days after recovery, complete daily milk weights were required for at least the first 7 days after return to the lactating herd. The two reasons for including cases with some missing post-recovery daily milk weights in calculation of DIFF were: 1) the cow was dried off after 7 days or 2) the cow's transponder was not read properly and therefore her production values were missing. Transponders randomly failed for 5% of all milkings each day. Records were excluded if any concurrent disease was present before or after the case. Therefore DIFF could not be calculated for 164 cases because: 10 were not fresh long enough to have a valid BASE before onset, 46 had mastitis during the 7 days before the case, 66 contracted mastitis again within 7 days after return from the mastitis barn, 9 were dried off, 32 were sold, and 1 had an LDA after clinical mastitis.

Another measure of clinical and economic severity of mastitis is the duration of antibiotic therapy before cessation of clinical signs or culling. The variable DUR represents the duration (in days) of antibiotic therapy for each clinical mastitis case. DUR was transformed for some statistical tests to the natural log(DUR +1) to achieve a more normal distribution.

The time a cow's milk was withheld from sale was also used as a measure of mastitis severity. The variable OUT is the number of days until return to the milking herd or until culling following each case of clinical mastitis. OUT was transformed to the natural log(OUT +1) for some statistical tests to achieve a more normal distribution.

Culling was used as another measure of mastitis severity. CULL and CULLM are categorical variables. The possible outcomes for CULL are "yes" or "no" regarding whether the cow left the herd before the end of lactation for any reason. CULLM (yes or no) refers specifically to culling due to mastitis.

General Linear Models:

Twenty nine potential predictor (independent) variables were evaluated (one-way ANOVA) for their relationship with each of the continuous outcome (dependent) variables DUR, OUT, and DIFF.

These same potential predictors were also compared to the categorical outcome (dependent) variables CULL and CULLM using one-way ANOVA or Chi-square analysis.

DUR, OUT, and DIFF were then each used as the dependent variable in separate general linear models. These models were constructed to use information obtainable at onset of a case of clinical mastitis to predict severity of that case. All significant predictors were identified and retained in each final linear model.

NAGase thresholds:

Previous reports have suggested that there may be thresholds of NAGase level useful in detecting mastitis.[3] It is uncertain whether NAGase thresholds can be used to group clinical cases according to severity. Therefore, various levels of NAGase were tested to see if cases above that level suffered worse outcomes than those below the level.

RESULTS:

Descriptive Epidemiology:

Etiologic agents isolated included S. aureus (13.8%), Staph. spp. (8.6%), Diphtheroids (A. pyogenes) (2.8%), Strep. non-ag's (7.0%), Coliforms (E. coli, Klebsiella, Citrobacter, and Enterobacter) (22.4%), Pseudomonads (5.6%), Serratia, Salmonella, Proteus and Yeast (2.8%), Contaminated (11.2%),

Bacillus (1.2%), Mixed Major Pathogens (4.8%), and No Growth (19.8%).

Mean NAGM and NAGD were significantly different among agents ($p<.01$, one-way ANOVA). As shown in Table 1, NAGM was lowest for Mixed Major Pathogens and highest for Strep. non-ag's. NAGD followed the same pattern (Table 1). NAGM mean for all 508 samples was 9.03 +/- 10.16 uM and mean NAGD was 7.85 +/- 10.03 uM (Table 1). NAGR did not differ significantly among different agents.

Table 1 also shows production change after clinical mastitis as measured by DIFF. Mean value of DIFF was -2.2 +/- 3.4 kg for 344 cases. DIFF mean varied among etiologic agents ($p <.05$, one-way ANOVA). Mixed Major Pathogens had the greatest loss of any agent group. Least milk loss was associated with Serratia, Salmonella, Proteus, and Yeast (Table 1).

Duration of therapy for clinical cases was measured as DUR (in days). For all 508 cases, the mean value of DUR was 2.0 +/- 3.5 days, ranging from 0 to 26 days of treatment. DUR was not significantly different among etiologic agents.

Time of milk withholding due to mastitis was measured as days OUT for each case. Mean value of OUT for 508 cases was

5.7 +/- 7.9 days with a range of 0 to 48 days. There were no significant differences among etiologic agents in days OUT.

Table 2 shows the rates of culling for all cases and for different etiologic agents. Culling for mastitis or for all reasons was not significantly different among agents.

NAGase was associated with parity ($p<.05$, one-way ANOVA). NAGM and NAGD were lowest in 2nd lactation cows and highest in 3rd plus lactation cows (Figure 1). NAGR was not associated with parity (Figure 1). Simple linear regression was used to test for effect of stage of lactation on NAGase. NAGM and NAGD were not significantly affected by stage of lactation (test for slope=0).

Analytical Epidemiology:

NAGase as a predictor of milk loss (DIFF):

Increased NAGM was associated with greater milk loss for the 344 cases that returned to the lactating herd. Table 3 shows the correlation of NAGM with DIFF for all cases and for different agents. NAGD was not significantly associated with DIFF (simple regression).

NAGase as a predictor of treatment response (DUR and OUT):

Increased NAGase was associated with longer treatment for mastitis, as shown by the correlation coefficients for NAGM and NAGD with DUR for all 508 cases ($r=.23$, $r=.21$, respectively, $p<.01$). Table 3 shows the relationship between NAGM and DUR for each pathogen. Higher NAGase was associated with longer milk withholding following mastitis. NAGM and NAGD were positively correlated with OUT for all 508 cases ($r=.24$, $r=.22$, respectively, $p<.01$). Table 3 shows the correlation between NAGM and OUT for each agent.

NAGase as a predictor of culling (CULL and CULIM):

Increases in NAGM and NAGD were associated with increased culling due to mastitis (CULIM) ($r=.13$, $r=.12$, respectively, $p<.01$). Risk of culling for all reasons (CULL) was not associated with differences in NAGase level.

Cows culled due to mastitis had a higher mean NAGase level at onset than those not culled ($p<.01$, Student's t test). NAGM did not exceed 1.0 uM for 67 (13.2%) of the cases, and none of those cases were culled. There were no NAGase levels above which a majority of cases were culled.

All cases were assigned to one of 4 NAGM groups, from the bottom 25% of the NAGM values to the top 25%. NAGM ranges and their respective mastitis culling rates were as follows: < 1.99, 1.5%, 1.99- 5.10, 6.4%, 5.11- 13.61, 7.1%, > 13.61, 12.5%. The increased risk of culling due to mastitis was statistically significant ($p<.01$, Chi-square).

Evaluation of NAGase Predictive Ability in Conjunction With Other Predictive Variables:

The best predictive general linear model for milk loss (DIFF) used 5 variables obtainable at day of onset. In order of statistical significance these were: BASE production, NAGM, DGRP (stage of lactation categories), laboratory batch of NAGase determination (see below), and NAGR. This model was highly significant ($p<.0001$) but 79% of the variation among cases in DIFF was not explained (R^2 =.21) (Table 4). As NAGM, NAGR, and BASE increased, there was a tendency toward greater production loss (lower DIFF). Cows from 91 to 150 days in milk lost the most production (DIFF -3.3 +/- 3.8 kg). Production was best maintained by cows in the first 30 days of lactation (DIFF -.05 +/- 4.1 kg) (Figure 2).

There appeared to be a consistent problem with the milk standards used with the commercial NAGase test kit.

Following their reconstitution, the NAGase activity of the milk standards increased steadily beginning within a few hours. Therefore, NAGase samples run on different test well plates could not be accurately compared by adjusting for NAGase activity of the milk standards. This raised the question of whether there were differences among laboratory batches in measurement of NAGase. Milk samples were tested for NAGase in chronological order in 7 batches, thus laboratory batch includes the effects of season of onset. A strong relationship between batch and season of onset is evidenced by the Chi-square test (X^2=732, p<.01). Ninety three percent of the variation in NAGase among samples was not due to batch. During construction of general linear models, season and batch were never statistically significant in the same model. Forced inclusion of both variables increased the type III p values for both. This shows that batch exerted upon NAGase the effects of seasonal as well as laboratory differences. As laboratory batch and season of onset progressed from winter to spring to summer, less milk production was lost (more positive DIFF).

The final linear model to predict DUR (duration of treatment) included in order of statistical significance: NAGM, DIM (days in milk at onset), and season. The entire model was statistically significant (p<.0001), but overall predictive ability was weak (R^2=.11) (Table 5). A trend was

observed that as NAGM increased, DUR was also greater. Mastitis earlier in lactation was treated longer. As season progressed from winter to spring to summer, DUR decreased.

The final linear model for predicting OUT (time until return to the lactating herd) used NAGM, DIM, and parity, in order of statistical significance. The overall relationship was statistically significant ($p<.0001$). However, like the other models, this model left much of the variability among cases unexplained ($R^2=.09$) (Table 6). There was a trend that as NAGM and parity increased, days OUT increased. Cases occurring earlier in lactation did not recover as quickly.

Evaluation of Using a NAGase Threshold for Prognosis:

The 21 cases above the NAGM threshold of 32 uM had greater DUR (4.9 +/- 4.7 days) and OUT (12.5 +/- 9.9 days) than cases below 32 uM ($p<.01$, one way ANOVA). Production dropped for all 21 cases. DIFF was -6.8 +/- 6.1 kg for cases above the threshold, a significantly greater drop in production than for cases below the 32 uM threshold ($p<.01$, one-way ANOVA). Cases above the threshold had a 14.3% CULLM rate compared to a 6.37% rate for those below it. This difference was not statistically significant ($p=.15$, Chi-Square), possibly due to small numbers. There was no

increased risk of culling for all reasons for the high NAGase cows.

Influence of Parity, Stage of Lactation, and Season on Mastitis:

DUR increased with parity ($p<.01$, one-way ANOVA). For cases in lactation 1, DUR mean was 1.1 +/- 1.6 days, lactation 2, 1.7 +/- 2.6 days, lactation 3+, 2.5 +/- 4.2 days. Table 7 shows that DUR varied with stage of lactation (DGRP) ($p<.05$, one-way ANOVA). Treatment was longest for cows from 91 to 150 days in milk, and shortest for cows greater than 250 days in milk. Parity did not significantly affect milk production loss following mastitis (one-way ANOVA).

CULLM rate was significantly associated with DGRP ($p<.01$, Chi-Square) and season ($p<.05$, Chi-Square). No cows greater than 250 days in milk were culled for mastitis, while 14.1% of those 31 to 90 days in milk were culled (Table 7). CULLM rate was highest for cases in the spring and lowest in the summer (Table 8).

The percentage of cases attributed to different agents was significantly different among seasons ($p<.01$, Chi-Square). Staph. spp. and diphtheroids comprised a higher percentage of cases in winter, while coliforms, Strep. non-ag's (Strep.

dysgalactiae and Strep. uberis), and pseudomonads were increased in spring and summer (Table 9). The overall rate of new clinical infections was not significantly affected by season (Chi-Square).

TABLE 1--Means for all cases and for etiologic agent groups

Agent	NAGM[a] **	NAGD[b] **	Days of Rx[c]	Days out[d]	Prod. dif[e]*
All cases	9.0	7.8	2.0	5.7	-2.2
S.aureus	7.9	6.8	1.6	4.5	-2.5
Staph. spp.	6.9	5.7	1.7	4.4	-1.6
No Growth	11.5	10.4	2.3	6.2	-1.9
Diphtheroids[f]	5.7	4.9	2.8	6.6	-1.2
S. non-ag's[g]	13.9	12.6	2.2	6.5	-3.4
Coliforms[h]	9.3	8.3	2.2	6.2	-2.6
Pseudomonads	7.5	6.0	1.3	3.8	-1.5
Serratia and Others[i]	10.7	9.9	2.9	6.4	-0.5
Contaminated	7.0	5.5	2.2	5.7	-1.8
Bacillus	9.0	7.0	0.8	3.3	-2.2
Mixed Major Pathogens[j]	5.6	4.1	2.6	9.3	-4.9

(a) NAGM = N-acetyl-B-D-glucosaminidase (NAGase) in the mastitic quarter expressed in uMoles/min./L.
(b) NAGD = Mastitic quarter NAGase minus reference quarter NAGase.
(c) Days of Rx = Days of antibiotic therapy for each case of mastitis.
(d) Days out = Days until return to the lactating herd for each case of mastitis.
(e) Prod. dif. = Change in daily milk production following mastitis expressed in kg.
(f) Formerly Corynebacterium pyogenes.
(g) Strep. dysgalactiae and Strep. uberis.
(h) E. coli, Klebsiella, Citrobacter, and Enterobacter.
(i) Serratia or Salmonella or Proteus, and Yeast.
(j) Two major pathogens isolated from sample.
* significantly different among agents, $p<.05$, one-way ANOVA.
** significantly different among agents, $p<.01$, one-way ANOVA.

TABLE 2--Culling among all cases and by agent groups

Agent	Culling for mastitis	Culling for all reasons
All cases	6.7% (34/508)	33.2% (169/508)
S.aureus	4.4% (3/69)	31.9% (22/69)
Staph. spp.	4.7% (2/43)	32.6% (14/43)
No Growth	6.1% (6/99)	30.3% (30/99)
Diphtheroids	7.1% (1/14)	21.4% (3/14)
S. non-ag's	2.9% (1/35)	28.6% (10/35)
Coliforms	9.8% (11/112)	33.0% (37/112)
Pseudomonads	0.0% (0/28)	28.6% (8/28)
Serratia and Others	21.4% (3/14)	57.1% (8/14)
Contaminated	5.4% (3/56)	33.9% (19/56)
Bacillus	16.7% (1/6)	83.3% (5/6)
Mixed Major Pathogens	12.5% (3/24)	41.7% (10/24)

See Table 1 for key.

TABLE 3--Correlation of NAGase with outcome variables

Agent	Correlation coefficients for NAGM with: Days of Rx	Days Out	Prod. Diff.
All cases	.23 **	.24 **	-.10 *
S.aureus	.20	.19	-.16
Staph. spp.	.35 *	.28	-.26
No Growth	.15	.22 *	.04
Diphtheroids	.06	.10	-.15
S. non-ag's	.27	.27	-.40 *
Coliforms	.13	.19 *	.19
Pseudomonads	.75 **	.74 **	-.55 **
Serratia and Others	.58 **	.48	-.18
Contaminated	.50 **	.33 *	-.22
Bacillus	.64	.30	.86
Mixed Major Pathogens	-.02	.07	.17

* statistically significant at p<.05.
** statistically significant at p<.01.
See Table 1 for key.

TABLE 4--Predictors of milk loss following mastitis

Source of variation	DF	Type III SS	Type III Prob.
Base production [a]	1	1750	.0001
NAGM	1	502	.0010
Stage of lactation	4	1684	.0001
Laboratory batch [b]	6	829	.0066
NAGR [c]	1	129	.0926
Error	330	14990	

(a) Daily milk production for 7 days before clinical onset.
(b) Laboratory batch for NAGase test (tested in chronological order so includes influence of season of onset).
(c) NAGR = Reference quarter NAGase.
R^2 = .21, p<.0001.
Grand mean for production loss = -2.2 kg.
See Table 1 for explanation of NAGM.

TABLE 5--Predictors of duration of mastitis therapy

Source of variation	DF	Type III SS	Type III Prob.
NAGM	1	19	.0001
DIM [a]	1	8	.0002
Season [b]	2	2	.0123
Error	503	272	

(a) DIM = Days in milk at onset of clinical mastitis.
(b) Season of onset of clinical mastitis.
R^2 = .11, p<.0001.
Grand mean for duration of therapy = 2.0 days.
See Table 1 for explanation of NAGM.

TABLE 6--Predictors of time milk withheld following mastitis

Source of variation	DF	Type III SS	Type III Prob.-
NAGM	1	34	.0001
DIM	1	8	.0062
Lactation [a]	2	6	.0588
Error	503	535	

(a) First, second, or third-plus lactation.
R^2 = .09, p<.0001.
Grand mean for time until recovery = 5.7 days.
See Table 1 for explanation of NAGM, Table 5 for explanation of DIM.

TABLE 7--Mastitis therapy and culling by stage of lactation

Days in milk at onset	Duration of Therapy [a] *	Culling for Mastitis **
1-30	2.3	6.1% (3/49)
31-90	2.2	14.2% (15/106)
91-150	2.9	7.9% (8/101)
151-250	1.8	4.9% (8/163)
>250	1.2	0.0% (0/89)

(a) Days of antibiotic therapy for each case of mastitis.
* Significantly different among stages of lactation, p<.05, one-way ANOVA.
** Significantly different among stages of lactation, p<.01, Chi-Square.

TABLE 8--Mastitis culling by season of onset

Season of Onset	Culling for Mastitis *
Winter [a]	6.6% (12/183)
Spring [b]	9.8% (18/184)
Summer [c]	2.8% (4/141)

(a) Jan. 29 until Mar. 31.
(b) April 1 until May 31.
(c) June 1 until July 27.
* Significantly different among seasons, $p<.05$, Chi-Square.

TABLE 9--Mastitis etiologic agents by season of onset

	Season			
Agent	Winter	Spring	Summer	All Cases
All cases	36.4%	35.8%	27.8%	100.0%
S.aureus	14.3%	14.5%	12.2%	13.8%
Staph. spp.	11.5%	6.7%	7.2%	8.6%
No Growth	20.9%	19.0%	19.4%	19.8%
Diphtheroids	5.0%	0.6%	2.9%	2.8%
S. non-ag's	5.0%	7.8%	8.6%	7.0%
Coliforms	20.9%	25.7%	20.1%	22.4%
Pseudomonads	4.4%	6.7%	5.8%	5.6%
Serratia and Others	2.8%	2.2%	3.6%	2.8%
Contaminated	4.4%	14.0%	16.6%	11.2%
Bacillus	1.7%	0.0%	2.2%	1.2%
Mixed Major Pathogens	9.3%	2.8%	1.4%	4.8%

Agents statistically differ among seasons, $p<.01$, Chi-Square.
See Table 1 for explanation of agents, Table 8 for explanation of seasons.

Figure 1. NAGase means by lactation number

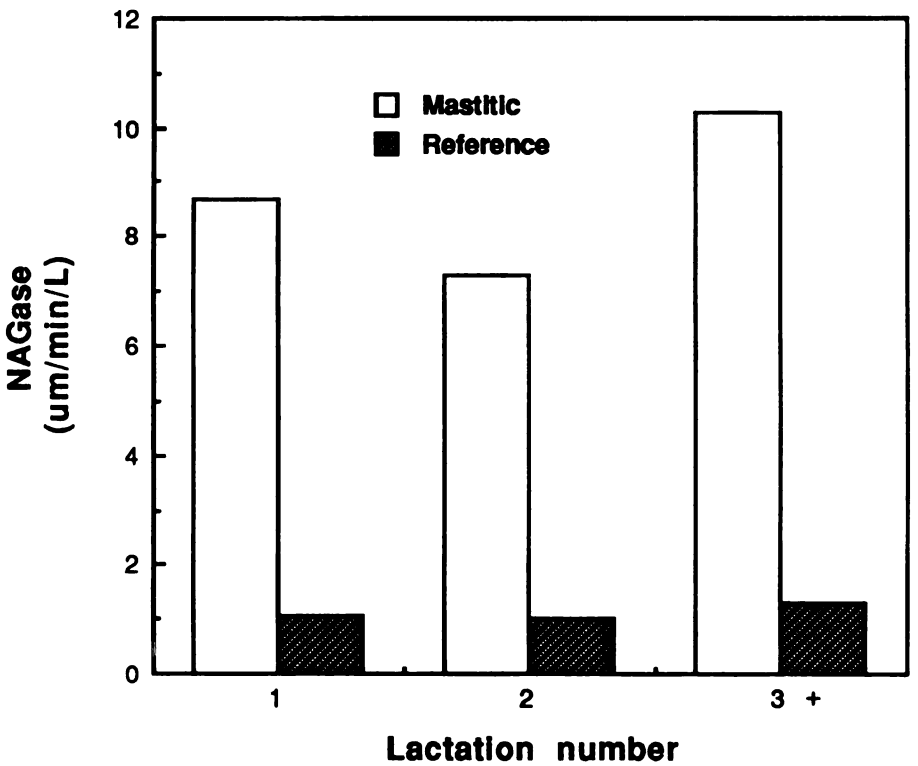
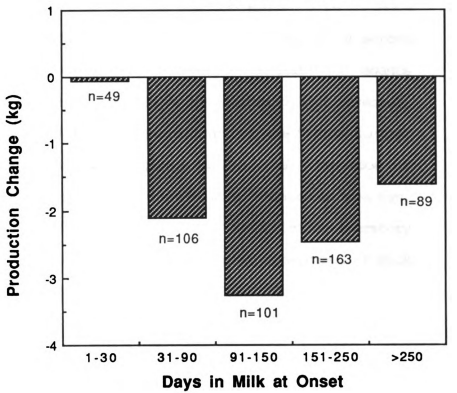


Figure 2 Production loss by stage of lactation



DISCUSSION:

Milk Production and NAGase:

Increased milk NAGase was associated with decreased milk production following clinical mastitis. This was as expected, but the correlation was too small to be of much biological significance. NAGase by itself was not a strong predictor of lost milk, and for some etiologic agents (Bacillus and coliforms), the relationship was reversed. Although statistically non-significant, these trends suggest that for coliform and Bacillus cases, the likelihood of return to production is greater when NAGase is greater. This suggests that NAGase may not be specific for secretory cell damage, or at least not for chronic impairment of milk secretion capability.

Duration of Mastitis and NAGase:

Mastitis cases with higher NAGase tended to be treated with antibiotics and withheld from the lactating herd for longer periods. As with production change, the relationship was weak. Used alone NAGase was a poor predictor of chronicity of clinical signs. Apparently, damage to quarters necessitating more extensive treatment and longer

convalescence before recovery from clinical signs is not specifically indicated by NAGase activity.

Culling and NAGase:

Cases with elevated NAGase were reported culled because of mastitis at a significantly greater rate, but the association was weak. For cases within higher ranges of NAGM values, mastitis culling percentage was greater. Despite this trend, there was no threshold of NAGase level above which a majority of the cases were culled due to mastitis. This may be further evidence that decreased capability for lactation is not specifically indicated by higher milk NAGase levels in clinical mastitis. Another reason for difficulty in predicting culling is that culling decisions are somewhat subjective and depend upon judgement by dairy farmers. While economically important, culling is not an objective measure of severity of disease.

NAGase Combined With Other Predictors:

With reliable predictive models, cows most likely to rebound rapidly from clinical mastitis, those expected to have moderate production loss, and those likely to suffer major production loss could be rapidly identified on day of onset. Management decisions could be made utilizing records of

previous production, reproductive status, and previous disease as well as the mastitis prognosis generated by the model. Cows whose previous performance and current prognosis (determined using a reliable model) were both poor could be treated to maximize survival for slaughter. Supportive therapy for clinical signs without use of antibiotics could be instituted so that they could be rapidly culled. Intramammary therapy or no therapy could be used for cases with a likelihood of minimal production loss. For highly valued cows that were predicted to have a severe case by the model, intense intravenous therapy with fluids and antibiotics would be indicated even if initial clinical signs were mild.

Even in combination with other information obtainable at onset of clinical signs, NAGase did not consistently identify cases that would ultimately have the greatest production losses. Nevertheless, NAGase was a better predictor of lost milk than milk culture or WMT. There was a trend that higher producing cows with higher milk NAGase at onset suffered the most post-mastitic production loss. This agrees with our hypothesis and previous speculation about NAGase and clinical severity [2,3,4,6,9,10,13], but the model in its entirety was a poor predictor of production change. Biological significance and practical usefulness of the model are doubtful.

NAGase combined with other information from day of onset could not accurately predict duration of therapy. NAGase was superior to WMT and milk culture for predicting DUR, however. A trend was observed that cases with higher NAGase were treated for a longer time, as was expected, but the predictive ability was weak. Persistence of clinical signs could not be reliably predicted using NAGase combined with other predictor variables. Again, NAGase was a better predictor of OUT than milk culture or WMT. Cases with higher NAGase tended to take longer to recover. This supported our hypothesis, but the model left much of the variability among cases unexplained and thus appears to have little practical use.

Effect of Parity and Stage of Lactation on NAGase:

Mastitic quarter NAGase was lowest in 2nd lactation cows and highest in cows in their 3rd or greater lactation. This differs from previous reports that NAGase elevation with mastitis increases with each lactation.[4,5,10] NAGase was not significantly affected by stage of lactation in the study cows, in contrast to previous reports.[4,5,25] It appears that the variability among cows in NAGase response to mastitis is influenced more by other factors than by days in milk and parity. Use of interquarter comparison of NAGase activity within cows may be preferable to attempting

to adjust for differences in parity and stage of lactation.

White blood cells and nonsecretory ductular epithelial cells have recently been postulated to be major sources of NAGase in mastitic milk.[24] The degree of white cell infiltration in the early stages of clinical mastitis as measured by SCC is highly variable between cows. This may be part of the reason why factors other than destruction of mammary cells appear to be important contributors to total NAGase level in clinical cases.

Clinical Mastitis and Factors in Addition to NAGase:

Treatment duration increased with parity. This may be due to more pronounced clinical signs or more chronicity of mastitis in older, high producing cows. It may also indicate greater perceived value of older cows such that they warrant longer treatment.

Cows greater than 250 days in milk at onset were treated for the shortest time for mastitis. No cows in this group were culled for mastitis. Cows in advanced lactation do not have the potential to lose as much daily milk production or become agalactic for as much of their lactation as cows with mastitis in earlier lactation. Advanced lactation cows usually recover rapidly with minimal mastitis treatment or

they are dried off earlier than originally planned. Clinical mastitis from 91 to 150 days of lactation resulted in the longest treatment. This is probably due to marked clinical signs and high value attributed to these cows. Most are producing well enough that they are not culled, and they are too early in the lactation and reproduction cycle to be dried off.

Fifteen of 106 cases from 31 to 90 days in milk resulted in culling for mastitis; this was the highest cull rate for any stage of lactation. If marked drop in milk production affects cows in this peak stage of the lactation curve, or they are perceived as chronic mastitis cows, they are at a stage of lactation where they represent a major economic loss. Many of them are not pregnant, and the expense of rebreeding and maintaining these cows is often not justified; therefore more of them are culled.

Staph. spp. and diphtheroids caused less mastitis in the spring and summer, while mastitis caused by Strep. dysgalactiae and Strep. uberis, coliforms, and pseudomonads increased in spring and summer. During the study period, there were management changes instituted on the farm as a result of increased awareness of mastitis. Washing and drying teats more thoroughly and segregation of mastitic cows (including milking them last) were instituted during

May (spring). The reduction in diphtheroid and Staph. spp. mastitis as the project progressed was probably a result of these changes. The agents which caused more mastitis during the spring and summer are environmental pathogens that primarily invade the mammary gland from cows' surroundings between milkings. Increased environmental mastitis is common during wet and muddy conditions of warmer seasons.

NAGase Threshold as a Prognostic Test:

We found a threshold of NAGM above which cases had a bad prognosis. Those cows with NAGM greater than 32 uM were more likely to suffer greater production loss and require a longer course of therapy. It should be remembered, however, that cases below 32 uM NAGM cannot be assumed to be mild cases. Perhaps thresholds could be useful in mastitis prognosis. Further study on other farms would be required before specific thresholds could be recommended for widespread use.

Milk Standards for NAGase Test:

The milk standards in the commercial NAGase test kit increased markedly in NAGase activity within a few hours of reconstitution. Failure of the milk NAGase standards was a

concern. Use of commercial milk standard NAGase activity to adjust for differences among test plates should be done with caution.

CONCLUSION:

Our results indicate that NAGase is a better predictor of several measures of clinical severity of mastitis than other information currently available at onset, such as age, stage of lactation, season of onset, level of milk production, milk culture, and WMT. But, NAGase alone or combined with other variables could not consistently predict the sequelae of clinical cases. Most of the variability in outcome among clinical mastitis cases remained unexplained.

APPENDIX

SAS-PC Programs Used in Analysis

This is the SAS-PC program used to convert the data set into its final form. Transformations of variables, daily milk weight adjustments, and mathematical calculations are performed by this program:

```
DATA SAV.TWO;
SET SAV.GRN;
LOU=LOG(OUT+1);
LDR=LOG(DUR+1);
DAT=FRESH+DIM;
IF DAT<=821 THEN SEAS=1; IF DAT>821 AND DAT<=882 THEN
SEAS=2;
IF DAT>882 THEN SEAS=3;
DIMSQ=DIM*DIM;
IF DIM LE 30 THEN DGRP=1;
IF DIM LE 90 AND DIM GT 30 THEN DGRP=2;
IF DIM LE 150 AND DIM GT 90 THEN DGRP=3;
IF DIM LE 250 AND DIM GT 150 THEN DGRP=4;
IF DIM GT 250 THEN DGRP=5;
IF NAGM EQ 0 THEN NAGM=1;
IF NAGR EQ 0 THEN NAGR=1;
NAGM=NAGM/176.4; NAGR=NAGR/176.4;
NAGMSQ=NAGM*NAGM; NAGMCU=NAGMSQ*NAGM;
NAGRSQ=NAGR*NAGR; NAGRCU=NAGRSQ*NAGR;
IF NAGM<=1.98 THEN NAGMC=1; IF NAGM>1.98 AND NAGM<=5.10 THEN
NAGMC=2; IF NAGM>5.10 AND NAGM<=13.61 THEN NAGMC=3;
IF NAGM>13.61 THEN NAGMC=4;
IF NAGM<=NAGR OR NAGM>=NAGR THEN IQR=NAGM/NAGR;
LNAGM=LOG(NAGM);
IF NAGM<=NAGR OR NAGM>=NAGR THEN NAGD=NAGM-NAGR;
NAGDSQ=NAGD*NAGD; NAGDCU=NAGDSQ*NAGD;
IF NAGD<=1.13 THEN NAGDC=1; IF NAGD>1.13 AND NAGD<=3.40 THEN
NAGDC=2; IF NAGD>3.40 AND NAGD<=10.77 THEN NAGDC=3;
IF NAGD>10.77 THEN NAGDC=4;
LNAGD=LOG(NAGD+10.87);
IF WMIM EQ 0 THEN WMIM=1;
IF WMIR EQ 0 THEN WMIR=1;
IF WMIM<=2 THEN WMIMC=1; IF WMIM>2 AND WMIM<=10 THEN
WMIMC=2;
IF WMIM>10 AND WMIM<=26 THEN WMIMC=3; IF WMIM>26 THEN
WMIMC=4;
IF WMIM<=WMIR OR WMIM>=WMIR THEN WMIRA=WMIM/WMIR;
LWMIM=LOG(WMIM);
IF WMIM<=WMIR OR WMIM>=WMIR THEN WMID=WMIM-WMIR;
```

```
IF WMTD<=0 THEN WMTDC=1; IF WMTD>0 AND WMTD<=5 THEN WMTDC=2;
IF  WMTD>5  AND  WMTD<=19  THEN  WMTDC=3;  IF  WMTD>19  THEN
WMTDC=4;
LWMTD=LOG(WMTD+35);
IF PMINUS7 GE 777 OR PMINUS7 EQ 0 THEN PMINUS7=.;
IF PMINUS6 GE 777 OR PMINUS6 EQ 0 THEN PMINUS6=.;
IF PMINUS5 GE 777 OR PMINUS5 EQ 0 THEN PMINUS5=.;
IF PMINUS4 GE 777 OR PMINUS4 EQ 0 THEN PMINUS4=.;
IF PMINUS3 GE 777 OR PMINUS3 EQ 0 THEN PMINUS3=.;
IF PMINUS2 GE 777 OR PMINUS2 EQ 0 THEN PMINUS2=.;
IF PMINUS1 GE 777 OR PMINUS1 EQ 0 THEN PMINUS1=.;
IF P1 GE 777 OR P1 EQ 0 THEN P1=.;
IF P2 GE 777 OR P2 EQ 0 THEN P2=.;
IF P3 GE 777 OR P3 EQ 0 THEN P3=.;
IF P4 GE 777 OR P4 EQ 0 THEN P4=.;
IF P5 GE 777 OR P5 EQ 0 THEN P5=.;
IF P6 GE 777 OR P6 EQ 0 THEN P6=.;
IF P7 GE 777 OR P7 EQ 0 THEN P7=.;
IF P8 GE 777 OR P8 EQ 0 THEN P8=.;
IF P9 GE 777 OR P9 EQ 0 THEN P9=.;
IF P10 GE 777 OR P10 EQ 0 THEN P10=.;
IF P11 GE 777 OR P11 EQ 0 THEN P11=.;
IF P12 GE 777 OR P12 EQ 0 THEN P12=.;
IF P13 GE 777 OR P13 EQ 0 THEN P13=.;
IF P14 GE 777 OR P14 EQ 0 THEN P14=.;
IF X3 EQ 1 AND LACT EQ 1 THEN PMINUS7=PMINUS7*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN PMINUS6=PMINUS6*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN PMINUS5=PMINUS5*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN PMINUS4=PMINUS4*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN PMINUS3=PMINUS3*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN PMINUS2=PMINUS2*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN PMINUS1=PMINUS1*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN P1=P1*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN P2=P2*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN P3=P3*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN P4=P4*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN P5=P5*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN P6=P6*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN P7=P7*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN P8=P8*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN P9=P9*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN P10=P10*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN P11=P11*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN P12=P12*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN P13=P13*.833;
IF X3 EQ 1 AND LACT EQ 1 THEN P14=P14*.833;
IF X3 EQ 1 AND LACT EQ 2 THEN PMINUS7=PMINUS7*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN PMINUS6=PMINUS6*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN PMINUS5=PMINUS5*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN PMINUS4=PMINUS4*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN PMINUS3=PMINUS3*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN PMINUS2=PMINUS2*.854;
```

```
IF X3 EQ 1 AND LACT EQ 2 THEN PMINUS1=PMINUS1*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN P1=P1*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN P2=P2*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN P3=P3*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN P4=P4*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN P5=P5*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN P6=P6*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN P7=P7*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN P8=P8*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN P9=P9*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN P10=P10*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN P11=P11*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN P12=P12*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN P13=P13*.854;
IF X3 EQ 1 AND LACT EQ 2 THEN P14=P14*.854;
IF X3 EQ 1 AND LACT EQ 3 THEN PMINUS7=PMINUS7*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN PMINUS6=PMINUS6*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN PMINUS5=PMINUS5*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN PMINUS4=PMINUS4*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN PMINUS3=PMINUS3*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN PMINUS2=PMINUS2*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN PMINUS1=PMINUS1*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN P1=P1*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN P2=P2*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN P3=P3*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN P4=P4*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN P5=P5*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN P6=P6*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN P7=P7*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN P8=P8*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN P9=P9*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN P10=P10*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN P11=P11*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN P12=P12*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN P13=P13*.869;
IF X3 EQ 1 AND LACT EQ 3 THEN P14=P14*.869;
BASE=MEAN(PMINUS7,PMINUS6,PMINUS5,PMINUS4,PMINUS3,PMINUS2,
PMINUS1);
RET1=MEAN(P1,P2,P3,P4,P5,P6,P7);
RET2=MEAN(P8,P9,P10,P11,P12,P13,P14);
RET=MEAN(P1,P2,P3,P4,P5,P6,P7,P8,P9,P10,P11,P12,P13,P14);
DIFF1=RET1-BASE;
DIFF2=RET2-BASE;
DIFF=RET-BASE;
IF AGENT=0 THEN AGENT=.;
IF AGENT=1 THEN MGRP=1; IF AGENT=2 THEN MGRP=2; IF AGENT=3
THEN
MGRP=3; IF AGENT=4 THEN MGRP=4; IF AGENT=5 THEN MGRP=5;
IF AGENT=6 OR AGENT=7 OR AGENT=8 OR AGENT=9 THEN MGRP=6;
IF AGENT=10 THEN MGRP=7; IF AGENT=11 OR AGENT=15
THEN  MGRP=8;IF  AGENT=12  THEN  MGRP=9;IF  AGENT=13  THEN
MGRP=10;
```

```
IF AGENT>15 THEN MGRP=11;
IF TIME>=600 AND TIME<=1400 THEN SHIFT=1;
IF TIME>1400 AND TIME<=2200 THEN SHIFT=2;
IF TIME>2200 OR TIME<600 THEN SHIFT=3;
IF SOLD>=1 THEN CULL=1; IF SOLD=0 THEN CULL=0;
IF CAR=1 THEN CULLM=1; IF CAR NE 1 THEN CULLM=0;
RUN;
```

This SAS-PC program constructs the final general linear model for production difference (DIFF):

```
PROC GLM DATA=SAV.TWO;
CLASS DGRP LABDAY;
MODEL DIFF=BASE NAGM DGRP LABDAY NAGR;
RUN;
```

This SAS-PC program constructs the final general linear model for log of duration of therapy (Log of DUR=LDR).

```
PROC GLM DATA=SAV.TWO;
CLASS SEAS;
MODEL LDR=NAGM DIM SEAS;
RUN;
```

This SAS-PC program constructs the final general linear model for log of time until return to lactating herd (Log of OUT=LOU).

```
PROC GLM DATA=SAV.TWO;
CLASS LACT;
MODEL LOU=NAGM DIM LACT;
RUN;
```

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