5-1985

Dietary Sodium Bicarbonate and Magnesium Oxide for Early Postpartum Lactating Dairy Cows: Effects Upon Milk Coagulation Parameters

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DIETARY SODIUM BICARBONATE AND MAGNESIUM OXIDE FOR EARLY POSTPARTUM LACTATING DAIRY COWS: EFFECTS UPON MILK COAGULATION PARAMETERS.

by

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A thesis submitted in partial fulfillment
of the requirements for the degree
of
MASTER OF SCIENCE
in
Nutrition and Food Sciences

UTAH STATE UNIVERSITY
Logan, Utah
1985

ACKNOWLEDGMENTS

I would like to express my appreciation to Dr. G.H. Richardson for his tremendous aid and guidance towards the fulfillment of this work. I am also greatly thankful to Dr. R.C. Lamb for his great assistance. Thanks Dr. J.L. Walters for his help with statistics analysis. I would like to thank Dr. C.A. Ernstrom for serving as member of my graduate committee and Dr. R.L. Boman for giving me the lactation and mastitis data of the cows and the explantion of basic dairy farm management. I would like to express my sincere appreciation to my parents for helping me advance in my academic pursuits. Finally, I would like to thank all the students and the staff of the Department of Nutrition and Food Sciences , especially Steve Winkel and Leslie Okigbo, for their encouragement and support during this study.

Shu Chuan Lee

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ABSTRACT

Dietary Sodium Bicarbonate and Magnesium Oxide for Early Postpartum Lactating Dairy Cows: Effect upon Milk Coagulation Parameters.

by

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Utah State University, 1985

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Forty-eight Holstein cows at Utah State Dairy Farm were blocked statistically according to date of calving, previous milk production, and numbers of lactation at parturition. The cattle were assigned randomly to one of four treatments within blocks. The four treatments included a base ration (control, treatment #1), base ration plus .8% of sodium bicarbonate (treatment #2), base ration plus .4% of magnesium oxide (treatment #3), and base ration plus both .8% of sodium bicarbonate and .4% of magnesium oxide (treatment #4). The research was conducted from February 1983 to November 1984. A formagraph was used to measure milk coagulation parameters and pH was determined.

There was no significant difference in milk coagulation parameters or pH between the control and the buffer treatments. Milk parameters were significantly different in individual cow, week, and milk pH. Milk parameters did not

appear to be dependent upon season. Curd firmness was significant in interaction of season and treatment. Significant variations in milk pH were observed in relation to week, season, and individual cow.

Overall treatments, the clotting time, K20, and pH value increased each week, and A30 decreased each week. The milk parameters and pH in each treatment were significant between weeks except K20 and A30 in treatment #3, and A30 in treatment #2 ($p>0.05$). The clotting time and K20 were negatively correlated with firmness, and there was positive correlation between Ct and K20 as expected.

Somatic cell count was positively correlated with clotting time, K20, and pH and negatively correlated with A30. Milk pH was the most significant and had positive correlation coefficient with clotting time and firming rate and negative correlation coefficient with curd firmness.

(65 pages)

INTRODUCTION

Cheese is one of the most important milk products in the world. Milk quality can affect the yield and the quality of the cheese(45). Yield is based on milk composition (% fat, % protein, % lactose, % moisture) which will influence the milk coagulation time (Ct), curd firming rate (K20) and curd firmness (A30) (10). Other factors include milk pH (35), season (12,46), mastitis (2), feed, age of cow, stage of lactation, weather, temperature history (21), and natural variation among the individual animals (46). Milk quality is important in the cheese industry, for good coagulating milk (GCM) has better curd firmness and cheese yield than poor coagulating milk (PCM) (2,49).

High-protein and high-energy rations were fed to cows in peak production. Buffers in dairy rations are recommended primarily when milk fat depression is a problem (43). In recent years, researchers have evaluated the effects of feeding buffers to cows on weight, rumen pH, and milk yield (24,33,34,55). Stoke and Bull (55) reported that supplement with sodium bicarbonate had no effect on milk yield or composition of milk in lactating cows. Erdman, Hemken, and Bull (24) reported that milk yield was unaffected by feeding cows 1.0% sodium bicarbonate, .8% magnesium oxide, or both simultaneously. Milk fat was increased (24), indicating buffers could prevent "low-milk

fat syndrome", which generally resulted in increased rumen levels of acetate and decreased levels of propionate.

The purpose of this experiment was to determine the effects of feeding magnesium oxide and sodium bicarbonate to early postpartum dairy cows upon milk coagulation parameters and pH and thereby determine how the addition of these buffers affect cheese manufacture.

REVIEW OF LITERATURE

Coagulation

The coagulation of milk by proteolytic enzymes is the result of two reactions (15,22,23). First, the enzyme causes proteolysis of the milk protein K-casein to form para-K-casein and a macropeptide. If the enzyme used is chymosin (calf rennin), then K-casein is split between a specific (phe(105)-met(106)) peptide bond in the K-casein chain via a Michaelis-Menten rate mechanism (15,22). Splitting of K-casein destroys the stability of the milk system. Second, casein aggregrate by a Von Smoluchowski mechanism, forming curd (15,22,27).

Though the K-casein is the only initiator of the milk coagulation process in the micelle, other factors like milk composition, temperature, somatic cell count, storage conditions, genetics, pH, calcium concentration, and coagulant concentration will influence milk coagulation (1,3,27,35,47,49). Hossain and Gravert (30) quantitated the kappa-casein content of milk by a method based on the estimation of macropeptide released by rennet action. They indicated that kappa-casein content varies from breed to breed and with season, being the lowest in November-January and in May.

The influence of concentration of rennet and calcium has been reported (1,27,47). Green and Morant (27) reported that the rate at which curd forms at 23-35^{o} C is

proportional to concentration of rennet added. This suggests that the rate of micellar aggregation depends of the rennet concentration, even after K-casein hydrolysis. Low content of calcium disfavors casein aggregation during milk coagulation. Non micellar (soluble) caseins do not participate in coagulation but are expressed in the whey protein fraction (1). Olson and Bottazzi (47) stated that a greater interaction between casein micelles resulted from a greater retention of calcium phosphate on the micelle.

Kowalchyck and Olson (35) observed that when temperature is increased, the higher the firming rate in milk coagulation. The previous temperature history of milk affects its coagulation properties, therefore samples should be kept at the same temperature to ensure uniformity results (21). Ali et al. (3) reported that storage at 4 or 7°C was accompanied by dissociation of micellar casein into the soluble phase during the first 48 h. Losses of fat and curd fines in whey were greater when soluble phase increased, casein and clotting time were prolonged (3). To minimize losses of curd forming properties during collection and coagulation of milk, Richardson (49) suggested that every effort be made to minimize protease (plasmin) and psychrotrophic bacterial activity in the milk.

Coagulation properties have been measured by many researchers (7,47,50,51,54). The method of observing the clotting point and clotting time of milk were determing by Sommer and Matsen (54) and Berridge (7). Richardson and

coworkers measured curd tension by Brookfield viscometer in 1971 and developed a "Vatimer" to measure milk coagulation in cheese vat in 1985 (50,51). Olson and Bottazzi (47) used a thrombelastograph to find that longer clotting times resulted in slower rate of increased curd rigidity.

Genetic factor effecting milk coagulation

Milk composition is an inherited trait (28,38,52,57) and is divided into three sources: breed differences, sire differences, and individual differences (38,57). Legates (38) gives the following ranges in heritability estimates for the contents of the various milk components for all breeds: fat, 0.33-0.75; protein, 0.45-0.76; lactose, 0.36-0.70; SNF, 0.35-0.83. The percentage of each of these components in milk is strongly influenced by inheritance and the degree of influence is similar for each of the components.

When milk from individual cows is coagulated with rennin or pepsin, the toughness and adhesiveness of the coagulum varies wildy with animals (16). Some milk forms a soft, friable type of curd known as soft curd milk which measures less than 30g of curd tension, while other milk exhibits an extremely tough rubbery curd and is known as hard curd milk (16). Soft curd milk is not different kind of milk but merely a milk low in solids or low quality protein. The ingredient of milk most closely related to curd tension is casein (16). Soft curd milk is lower than hard curd milk

in titrable acidity, buffer capacity, energy value, calcium and phosphorous (16), because of its dilute composition.

Natural soft curd milk is found in all breeds of dairy cattle but predominates in the Holsteins, followed in order by the Ayrshires, Brown Swiss, Guernseys and Jerseys (16,29). Since time of initial clot to cutting of cheese curd decreases as percent fat and percent protein increases, genetic factors which influence milk composition influence coagulation indirectly.

pH

The variation of pH will influence the soluble casein (1,44), coagulation time (10,41,44,45,46), and curd firmness (10,16,45,46). The normal pH of milk is 6.63 (1), but the pH of colostrum is lower down to 6.0 (42).

Ali et al. (1) reported that both soluble calcium and phosphate showed a steady pH dependence, being lowest at highest pH used. Reducing pH causes a shift of calcium, and presumably casein, into the soluble phase (41,46) and therefore, might be expected to disrupt the complex present in micellar phase between Ca and casein leading to dissociation (1,9). Ali et al (1) observed that solubilization of casein was least at pH 6.6 and increased with either decreasing or increasing pH.

Milk pH was the most significant factor that affected coagulation time and curd firmness (44,46). Reducing milk pH to 6.3 caused a significant decrease in coagulation time

(41). As pH of milk is reduced from the normal (pH 6.4 to 6.7) a toughening of enzyme curd is observed until the pH drops under approximately 5.9 (16). From this point to the isoelectric point (pH=4.6) the curd tension decreases (16). Proteolytic activity of rennin increases with a lowering pH (40) and variation of pH in the milk caused increasing of soluble casein and curd tension decreased (1).

Rations and adding buffer

The feeding of dairy cows is not only a science, but also an art (14). The primary concern in feeding lactating cow is to provide a ration adequate in energy, protein, fiber, salt, calcium, phosphorous, and vitamin A (or carotene) (20). Additional considerations in feeding lactating cows include palatability of ration; physical form; protein and mineral content of concentrates; ratio of concentrate to roughage; relative prices of ingredients; voluntary feed intake; and frequency and regularity of feeding (20).

When high production cows are in peak production, they are fed rations containing a minimun of roughage and a maximum of high-energy and high-protein feeds (19). The ration can produce declining rumen pH (13,31,43), digestive problems and can result in milk fat depression (11,19,23).

One means to reduce these disorders is to add buffers to the feed (19). Sodium bicarbonate is the most commonly used buffer in rations of lactating cows (19). Bunn and

Matrone (13) reported that the animals had better weight gains, higher rumen pH, a tendency towards a lower acetate/butyrate ratio, and a decreased level of lactic acid in the rumen with the addition of sodium and potassium bicarbonates to animals fed a complete ration. The addition of buffers for the prevenation of "low-fat syndrome" has generally resulted in increased rumen levels of acetate, precursor of short-chain fatty acid in milk, and decreased levels of propionate (58). Milk fat was increased (24). However, there was no significant effect of feeding sodium bicarbonate and magensium oxide on milk fat (18,23). The addition of buffers to rations will not affect the milk yield (18,55), but may have certain disadvantages, such as adverse effects on the mineral balance of milk (19).

Stage of lactation

The average lactation period of a cow is about 305 days followed by a dry period of 30-60 da (63). The milk yield (63) and milk composition change during lactation (5,8,25,32,36,38,52). The secretion of the mammary gland for the first few days of lactation is known as colostrum which is richer in practically all milk components. The amount of solids-not-fat (SNF) drops to a lactation low at two to three months, increases slowly to six months, and then increases rapidly to the end of lactation (32). Total protein (32) and percentage of fat (8,32) vary during lactation following the same trend as SNF. The main changes

in milk components occur in the first, third, and tenth month of lactation (28). Milk yield is increased in early lactation from calving to about the 12th week. From the 12th to the 30th week the milk yield gradually declines. From the 30th to the 44th week the milk yield declines steeply, then goes into the dry period (63).

The average lactation production of all constituents was higher for cows calving in the winter than for the cows calving in the summer (8). Cows calving in August and September had the highest lactation average percentage, and those calving in the early spring had the lowest (8).

The curd tension of rennet coagulated milk is high during the colostral period, drops to a low point in the second or the third month of lactation, then slowly increases with duration of lactation, reaching a high level when the milk flow becomes small, and finally drops to zero when properties of milk become abnormal at the extreme end of the period (16).

The effect of stage of lactation on curd character is dependent upon milk yield and on gross composition of milk (% fat, % protein, % minerals, % lactose, and % water) which vary as the lactation period progresses. Colostrum has high solids, hence hard curd is obtained with colostrum (16,29,32). The low or zero curd tension at the late stage of lactation, when total solid is extremely high, might be associated with mineral inbalance and milk pH (16,40). Stage

of lactation effect milk composition and effect the coagulation inversely.

Number of lactation

Bailey (5) stated that the highest quality milk is produced by cows after their first calf which was confirmed by Waite et al. (61). The decline in SNF percentage with advancing age or lactation number (25,36,38) is approximately twice the magnitude of the decline in lactation fat percentage (38). The milk produced by old cows frequently contains a lower concentration of SNF than that of young cows, mainly because of a low concentration of lactose (38,60). Crude protein does not change much with age, the casein percentage declines during the first seven lactations as much as lactose (52), hence the whey protein and nonprotein nitrogen increase with advancing lactation number (38). Calcium, phosphorous, and potassium decline with advancing age (59). The part played by age per se is further confused by the possible influence by udder deterioration through normal usage, and the increasing incidence of mastitis with advancing age (38).

Season

Since Overman (48) reported that milk composition varied monthly, many studies (8,12,37,39,40,56) have been made on seasonal variations in composition of milk. The highest milk yield was obtained during March, April, and

May. Protein content of the milk was lower in summer than it was in winter, and other components were also affected by season (8,32,37). Bruhn and Franke (12) said differences between months were very highly significant. Fat and protein concentrations for all herds were lower from May through August and higher from November through February than during other months. The concentration of lactose was lower in November and higher in April than in the other months (12). There were significant differences in amounts of casein, whey proteins, and non-protein nitrogen between milks from different areas and between seasons (56). The renneting time generally increased throughout the season with small maximum in mid-December followed by a trough towards the end of January which was followed by an increasing rise until mid-May (40). Okigbo et al. (46) reported milk coagulation time and curd firmness are related to season of the year. Lyall reported that there is a seasonal pattern of high moisture (autumn and winter) and low fat in the dry matter (later winter and early spring) of cheddar cheese in Queensland, Australia. Late autumn and winter were the periods when composition is least satisfactory and improvement is required (39).

Somatic cells

There are three types of somatic cells in milk which are lymphocytes, neutrophils, and epithelial cells. In normal milk, these somatic cells will be mostly the epithelial

type. When there is a bacterial infection, tissue damage, or other inflammation of the mammary tissue, the number of somatic cells in the milk may increase dramatically, especially, the neutrophils (6).

Onset of mastitis causes significant alterations in the composition of milk (2,4,17,32,53,62) and cheese yield (2,6). Ali et al. (2) and Doan and Welch (17) reported that mastitis would cause the pH value to increase. Ali et al. implicated mastitis as causing a significant reduction in alpha-$_s$ and beta-caseins in mastitic milk. They also indicated a reduction in kappa-casein. The percentage of fat, solids-not-fat, and lactose are reduced (32). Following an increase of somatic cell count, the rennet clotting time was prolonged, whey volume increased, the curds became significantly wetter and softer, and cheese yield was decreased (2).

Decreased casein is the result of proteolytic damage to milk casein which results in a loss of enzymatically damaged casein to the whey (6). A lower amount of casein for curd formation may also lead to higher fat loss in whey (6).

METHODS AND PROCEDURES

Design

Forty-eight Holstein cows were fed the same dry-cow ration. At parturition, cows were blocked statistically according to date of calving, previous milk production, and lactation number (37). Within blocks the cattle were assigned randomly to one of four treatments. The four treatments included a base ration (control) without added mineral buffers (Treat #1), base ration plus .8% of sodium bicarbonate (Treat #2), base ration plus .4% of magnesium oxide (Treat #3), and base ration with both .8% of sodium bicarbonate and .4% of magnesium oxide (Treat #4). The components of a total mixed ration fed for each cow in this experiment are listed in Appendix 2. The buffer was fed at calving time. There were twelve cows in the first and fourth treatments, fifteen cows in the second treatment and nine cows in the third treatment. The buffers were fed 3-7 days before calving, and buffer feeding continued for twelve weeks of lactation. The research was carried out from February, 1983 to November, 1984.

Sampling

Milk was weighed each milking and samples from each cow were collected weekly. The samples used for this study were from the evening milking each Wednesday. The first sample was received 3-7 days after calving. The samples were

refrigerated immediately with no preservatives and were not pasteurized. The samples (15-20 ml each) were sent to our laboratory every Thursday afternoon.

Information on data from individual cow milking to remove due to mastitis was supplied by Dr. Ron Boman, manager of the Utah State University Dairy Farm.

Description of formagraph

The formagraph is a multi-channel modular instrument system designed for the recording of coagulation properties in milk.

A recorder module records the results of up to 10 samples simultaneously over a period of 30 min or longer. Results can be obtained 2 min after the actual recording. A normal measurment cycle is completed within 30 min and the capacity for a recorder module is then 20 samples/h. Modules can be combined to form an instrument which records up to 100 samples/h.

A service module necessary for the operation can be used in conjunction with up to 5 recording modules. The service module is responsible for maintaining a constant temperature in the recorder module and also tempering the cuvette block to temperature. After simultaneous renneting of the samples they are transferred to the recording module. Small stainless steel loop pendulae are lowered into the samples and these detectors pick up the tiny forces induced

when the gel of coagulating milk is exposed to linear movement. The stroke-sample displacement is ± 0.70 mm, and the frequency is 4/min. A 10-channel optical system and a strobe flash unit transmits the amplitude for the movement of the 10 pendulae simultaneously to the recording section. Registered results are displayed on a "self-developing" photographic strip chart.

Firmness/time dots are recorded under strict temperature control. Besides grading of milk samples, the instrument can also be used for testing rennet activity and for "real-time" simulation of full scale cheese vat processes.

Milk sample preparation

The milk was stored at 0-4.4° C for 18 h. Samples were tempered at 37° C in a water bath for 90 min before being run on the Formagraph for uniformity of results (21).

Enzyme preparation

The enzyme used was a mixture of rennin and adult bovine enzyme coagulant(HANSEN'S). A 1/50 dilution was prepared using distilled water (5 ml in 250 ml) for an enzyme concentration of 2 RU/ml of solution.

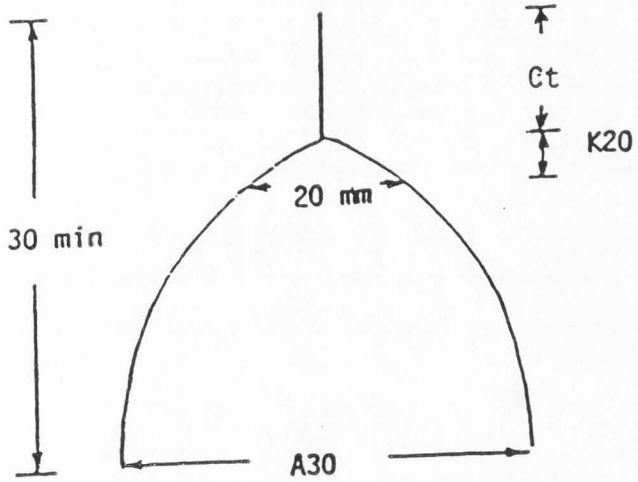
Enzyme activity test

A solution of 10% reconstituted Non-fat dry milk (RNDM) was prepared by adding 5.000g of non-fat dry milk (NDM) to 45g of deionized water.

Formagraph tracings were compared from day to day to detect any activity change during the experimenal period. The enzyme activity only very slightly decreased (about 0.5%), thus it should have little effect upon the results of this research.

Milk curd parameters

Ten milliliters of milk sample were transferred to the Formagraph cuvettes (26,42,46) which had been preheated at 37^{o}C for >30 min (26). Two hundred microliters (200 ul) of enzyme dilution were simultaneously delivered into each well of cuvette using a set of ten silver-plated small teaspoons and result in .04 RU/ml of milk. The formagraph was run for 30 min (46). Regressions of curd parameters Ct (clotting time after enzyme addition), K20 (time from Ct to a firmness of 20 mm), and A30 (the firmness reached 30 min after rennet addition) (Fig.1) vs. week for each treatment were performed. If the samples didn't reach a firmness of 20 mm during the 30 min run time, the data were pulled out of thedata set. Statistical analyses were made using SAS (SAS Institute Inc. Cary, N.C.). Table of the number of samples without K20 is found in the Appendix 4, Table 18 & 19.



Ct: Clot time after enzyme addition.

K20: Time from r to a firmness of 20mm.

A30: The firmness reached after 30 min.

Fig. 1: The illustration of coagulation of milk in formagraph.

Lactation data

The lactation number was used as a coding number. Each cow had a lactation number and the lactation number was confounded with individual cows in the statistical analysis. Other data included in the statistical analysis were the cow identification number and milk yield for each cow.

Season data

Okigbo reported milk coagulation time and curd firmness are related to season of the year (46). Therefore, the milk samples from October to March, cold weather, were coded "1" and the milk samples from April to September, hot weather, were coded "2" in the analysis of variance.

Somatic cell count (SCC) data

The SCC was measured by the DHIA center lab. (Dairy Herd Improvement Association, Logan, Utah), using the FOSSMATIC. The correlation between SCC, coagulation parameters and pH were determined.

Missing data

Missing data were caused by cows mistakenly not sampled, milk already sour when received in the laboratory, or insufficient quantity of milk to do the experiment. The number of missing data for each treatment is shown in Table 1.

pH measurement

The pH of each sample was measured using a pH meter (ALTEX 60, Beckman) with an Orion Ross elecrtode, afer they had been tempered 120 min at 37^{o} C (10).

Table1: The number of missing data during the 12 week study in each treatment.

Treat #1	Treat #2	Treat #3	Treat #4	Total
10	19	9	8	46

Statistical procedure

From the formagraph tracing, r, K20, and A30 were measured. Split-plot and least square analysis of unbalanced data on SAS were used to evaluate treatment differences in curd parameters and pH.

1. Mathematical model

a. The mathematical model used with pH as the dependent variable was:

$$Y_{ijkl} = U + T_i + C{:}T_{ij} + W_k + S_l + T{*}W_{ik} + T{*}S_{il} + E_{ijkl}$$

where U = overall mean of coagulation parameters and pH values

T = effect of treatments.

C:T = effect of cow in the treatments.

W = effect of week of sampling.

S = effect of season of sampling.

T*W = interaction of treatments and week.

T*S = interaction of treatments and seasons.

E = errors; remainders.

b. The mathematical model used with pH as the independent variable was:.

$$Y_{ijkl} = U + T_i + C{:}T_{ij} + W_k + S_l + T{*}W_{ik} + T{*}S_{il} + BX_{ijkl} + E_{ijkl}$$

where B = slope of line.

X = pH reading on deviation of Y_{ijkl}.

2. Modified mathematical Model

a. The modified mathematical model used with pH as the dependent variable was:

$$Y_{ijk} = U + T_i + C{:}T_{ij} + W_k + E_{ijk}$$

b. The modified mathematical model with pH as the independent variable.

$$Y_{ijk} = U + T_i + C{:}T_{ij} + W_k + BX_{ijk} + E_{ijk}$$

3. Curve regression

Equations for the relationships between milk parameters and week were obtained from the SAS output. The relationships between treatments and milk parameters with week can be seen clearly from those curves.

RESULTS AND DISCUSSION

Analysis on treatments

The data of milk parameters and pH for each treatment (included noncoagulating milk samples, but not mastitic milk) were analyzed on SAS. The mathematical model in Statistical Procedure 1.b. was used to analyze the milk parameters, and model 1.a. was used to analyze pH. The results are in Appendix 1, table 13-16. The clotting time (Ct) and firming rate (K20) were not significantly different in season, interactions of treatment * week, and treatment * season (Table 12 and 13). Curd firminess (A30) was insignificant in season and interaction of treatment * week, and pH was insignificant in interaction of treatment * week and interaction of season * treatment. Statistically, since above factors were not significant, they were elimiated in the analysis of variance. The mathematical models were changed to 2.b. for the milk parameters and 2.a. for pH (Tables 2-5). From these tables, it can be seen that the milk parameters and pH are not significant in treatment, and highly significant in individual cows, milk pH and week.

Okigbo et al. (46) reported that significant variation in clotting time and curd firmness were observed in relation to period of season, lactation, individual cow difference, and milk pH. The season was not significant in clotting time, K20, and A30 in this research, but it was significant in pH (Appendix 1). One reason for this difference in milk

Table 2: The analysis of variance table of Ct (clotting time).

Source	df	MS	F	Sig.
Treatment	3	42.817882	0.782	NS
Week	11	62.524332	10.232	S
Linear	1	612.248383	100.191	S
Quad	1	41.502485	6.792	S
Cubic	1	6.539081	1.070	NS
Quard	1	0.001997	0.000	NS
Quint	1	6.208327	1.016	NS
Residual	6	3.544563	0.580	
Cow (in Treat))	44	54.723923	8.955	S
pH B linear	1	703.638180	115.146	S
Remainder	454	6.110810		
Total	513			

S= significant and NS= not significant with $p<0.05$.

Table 3: The analysis of variance table of K20:

Source	df	MS	F	Sig.
Treatment	3	39.271033	0.693	NS
Week	11	36.947218	6.502	S
Linear	1	312.124910	54.931	S
Quad	1	36.783879	6.474	S
Cubic	1	5.681929	1.000	NS
Quard	1	7.749068	1.364	NS
Quint	1	10.749440	1.892	NS
Residual	6	5.555029	0.978	
Cow (in Treat)	44	56.681254	9.975	S
pH B Linear	1	243.925938	42.928	S
Remainder	396	5.682178		
Total	455			

S= significant and NS= not significant with $p<0.05$.

Table 4: The analysis of variance table of A30 (curd firmness).

Source	df	MS	F	Sig.
Treatment	3	690.058593	1.312	NS
Week	11	234.409543	4.203	S
Linear	1	1832.018783	32.847	S
Quad	1	143.201218	2.568	NS
Cubic	1	269.408832	4.830	S
Quard	1	88.093893	1.579	NS
Quint	1	105.077740	1.884	NS
Residual	6	23.450751	0.420	
Seasons	1	0.302721	0.005	NS
Cow (in Treat)	44	525.755810	9.426	S
Trt x Seas	3	197.306830	3.538	S
pH B Linear	1	1209.136735	21.679	S
Remainder	450	55.774274		
Total	513			

S= significant and NS= not significant with $p<0.05$

Table 5: The analysis of variance table of pH.

Source	df	MS	F	Sig.
Treatment	3	0.003366	0.111	NS
Week	11	0.121589	21.777	S
Linear	1	0.667220	119.504	S
Quad	1	0.259851	46.451	S
Cubic	1	0.224923	40.286	S
Quard	1	0.099043	17.739	S
Quint	1	0.034316	6.146	S
Residual	6	0.008687	1.556	
Seasons	1	0.048167	8.627	S
Cow (in 1treat)	44	0.030223	5.411	S
Remainder	454	0.005583		
Total	513			

S= significant and NS= not significant with $p<0.05$.

coagulation results is that each cow in this experiment was used for only the first 12 weeks of its postpartum lactation and each had a different calving time during the year. The second reason was limited number of observations used.

Because milk coagulation parameters would be affected by pH (see Table 13-16), this has been approved by previous researchers (10,16,45,46), it was used as the independent variable in the analysis of variance. Highly significant variations were obtained when pH was linearly regressed with milk parameters (Tables 2-4). Individual cow variations were also very significant as previous work has shown (46).

Salt balance in the milk would also effect milk coagulation (45). Erdman et al. (24) reported that there was no significant differences of salt balance in the blood for early postpartum lactating cows. Thus, the salt balance in the milk will not be influenced by feeding buffer during the first 12 weeks lactation.

In this research, we found no significant influences on coagulation time and curd firmness at Logan, Utah, when farmers fed buffer to cows in the early lactation, but we cannot conclude what influences will exist in mid- or late-lactation.

The difference in degrees of freedom between K20 and the other three parameters was because 24 samples in treatment #1, 13 samples in treatment #2, 9 samples in treatment #3, and 12 samples in treatment #4 weren't coagulated in 30 min. From Appendix 4, it can be seen that

the ratio of samples without K20 during 30 min in each treatment coagulation was 2:1:1:1. This means the percentage of control samples (treatment #1) that had no K20 during 30 min coagulation was twice as high as the cows which had been given buffer treatment. No conclusion can be drawn from this result, since there were other factors such as genetics, stage of lactation, age of cow, week etc.

Weekly influence in milk parameters

Tables 2-5 show that milk parameters and pH were highly significant in week. Plots of milk parameters and pH vs. week were made in Figure 2 through 5. The quadratic relationship between clotting time and week (r^2=.975) is shown in Figure 2. The slope increases with each week. A linear rate equation, y=0.058x-0.026, was obtained, after differentiation of the quadratic equation.

A quadratic relationship (r^2=.927) also exists with firming rate (K20) vs. week (Fig. 3), but the slope decreaes with week. Differentiating the equation gives y = -0.058x+0.658. Clotting time and firming rate increased each week.

A cubic relationship (r^2=.903) was obtained for firmness vs week. Curd firmness decreased each week. The rate of decrease in curd firmness decreased then increased. The equation of rate is $y = -0.072x^2+1.084x-4.114$. The intersection of the plots for the two linear rate equations

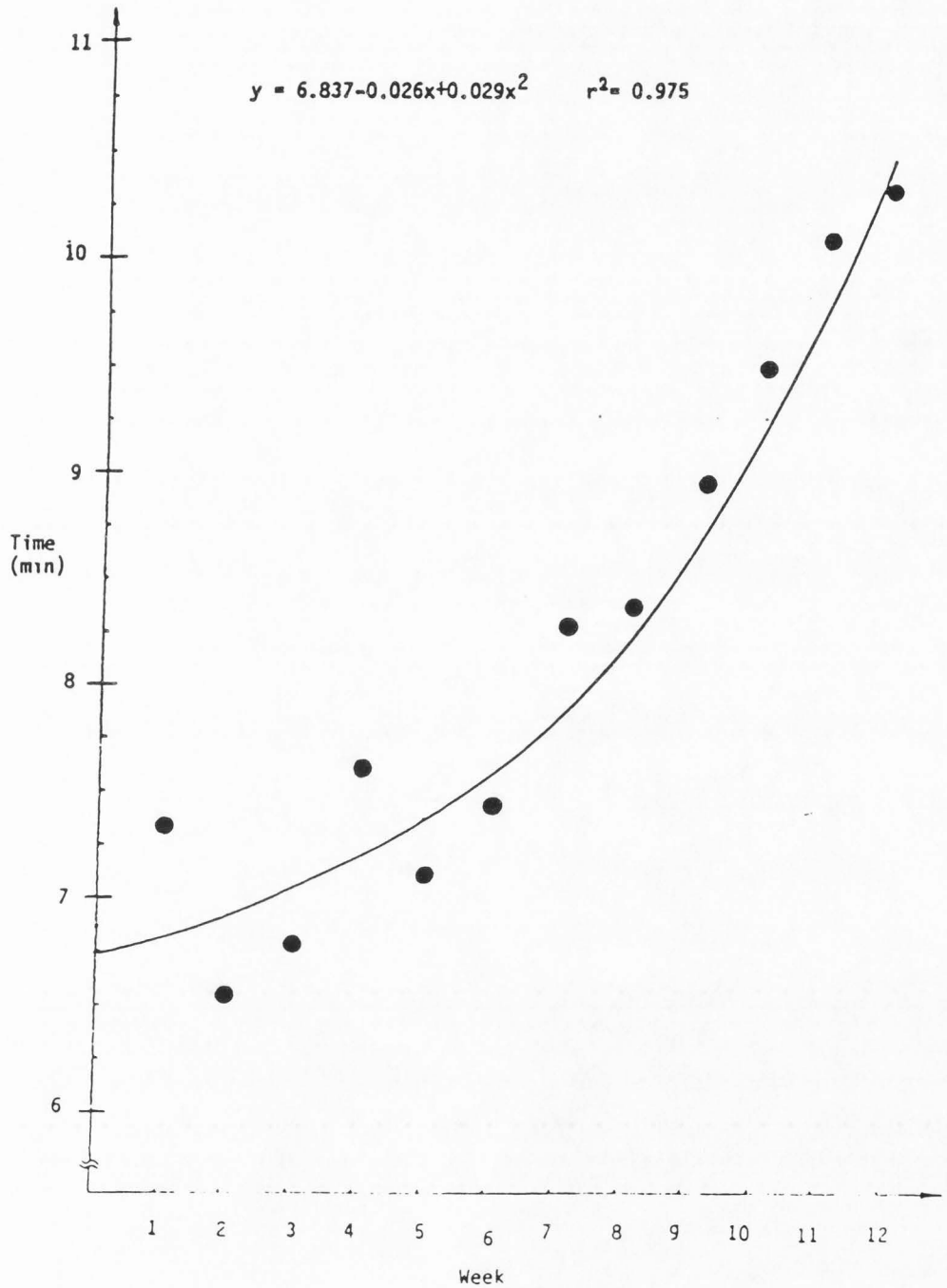


Fig. 2: The relationship between clot time (Ct) and week.

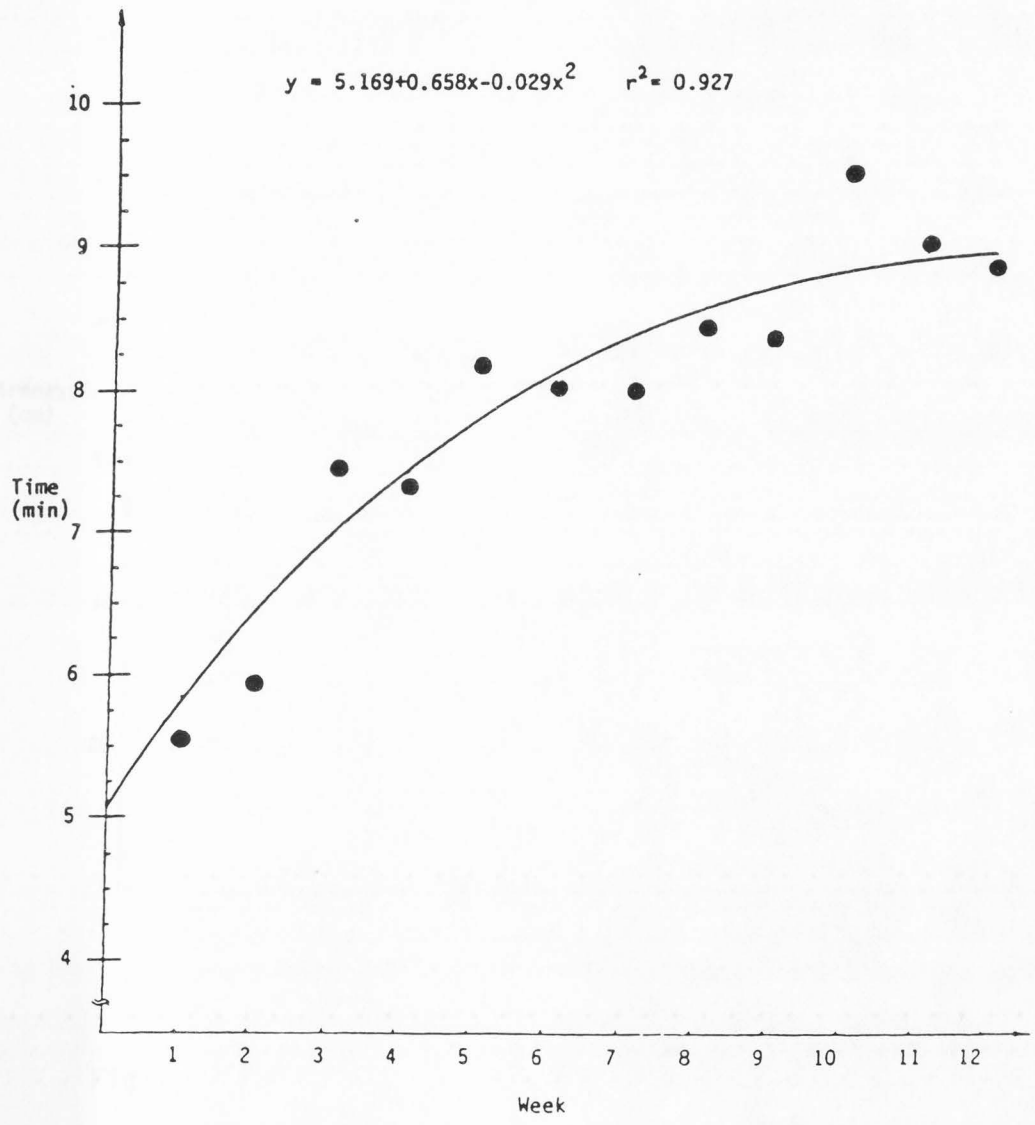


Fig. 3: The relationship between K20 and week.

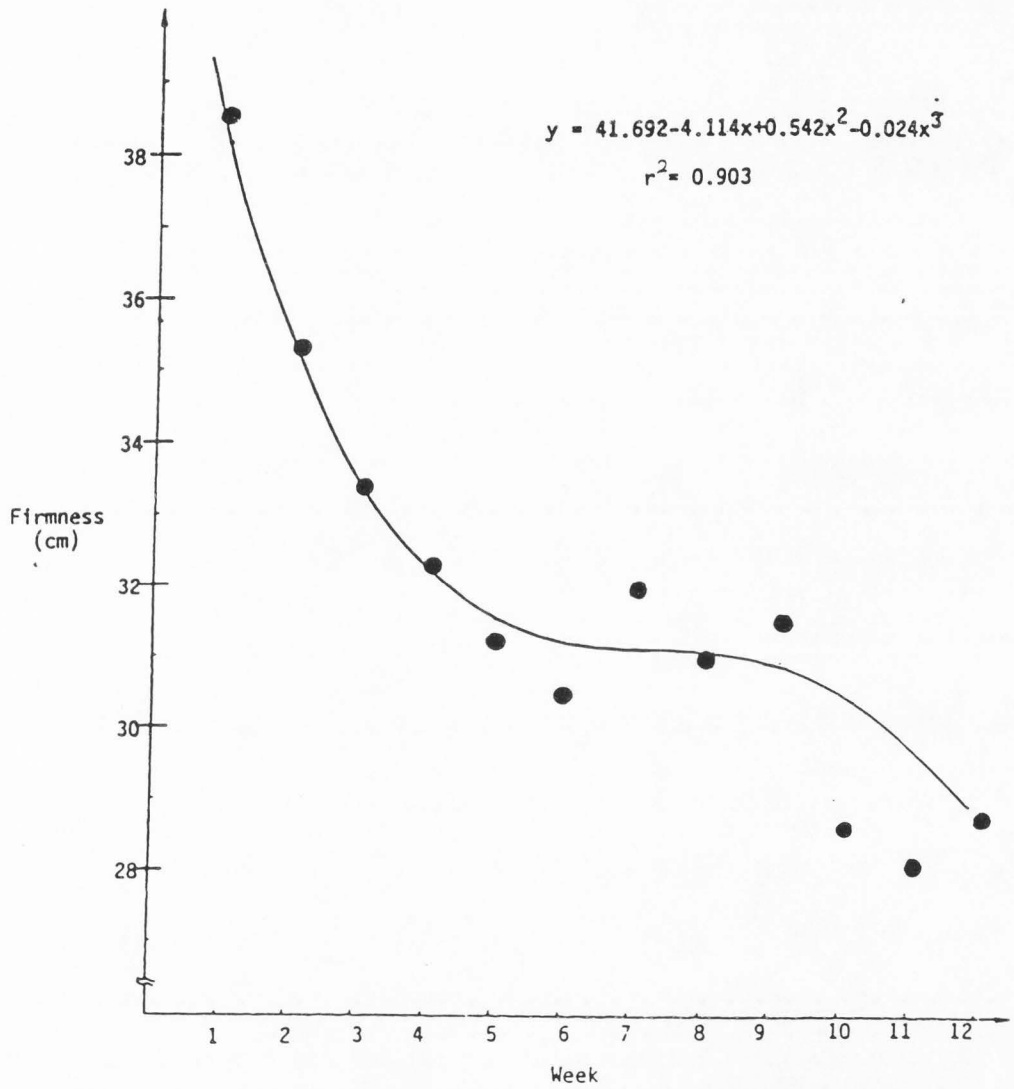


Fig. 4: The relationship between firmness (A30) and week.

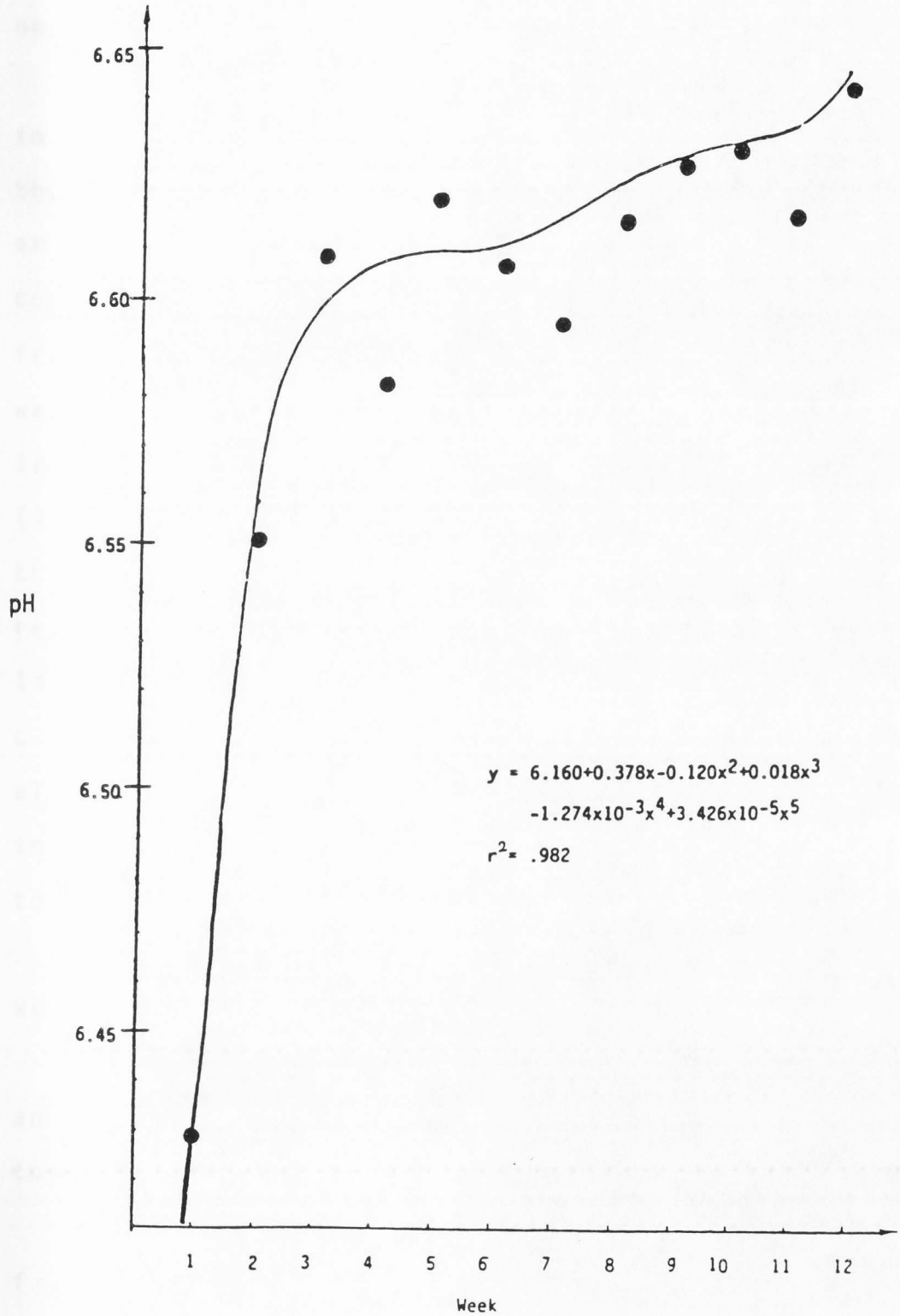


Fig. 5: The relationship between pH and week.

(at 6th week) is very near the week in which the rate of decrease in curd firmness reaches a minimum (at 7th week).

The relationship (r^2=.982) between pH and week is shown in Figure 5. Okigbo et al. (46) reported that milk pH was the most significant factor that affected coagulation time and curd firmness. Variation of pH would change milk composition (1). From this plot, we see that the pH raised from 6.43 to 6.65 during the 12 week period. This increase was mainly during the first three weeks of the experiment. In general the pH is lower (down to pH 6.0) in colostrum (41) and milk components are higher (32). This explains why the pH increased so fast in the first three weeks. Doan (16) reported that milk composition would affect curd firmness. If Figure 2-5 are superimposed, it can be seen that the clotting time increased as pH increased and firming rate also increased. The decrease in A30 and Ct, as well as the increase in K20, during early postpartum lactation are due to milk composition and pH.

Weekly influence in each treatment

Since the week was important to milk parameters and pH, an analysis of variance was done for each treatment in order to obtain relationship between each treatment and week.

For clotting time linear relationships were obtained for treatments #2, #3, and #4, while a quadratic relationship was obtained for treatment #1. Figure 6 showed that clotting time was increasing each week (Appendix 5,

Table 20). The slopes of treatments #1, #2, #3, and #4 were 0.1346x-0.5721, 0.2517, 0.4353, and 0.4753 respectively. The coefficients of variation were .822, .791, .848, and .956, respectively.

For K20, quadratic relationships were obtained for treatment #2 and #4 and linear relationships were obtained for treatments #1 and #3. The slopes were 0.1722, 0.8255+0.0852x, 0.2108, and 1.0130-0.0828x respectively. Since week was insignificant in treatment #3 (see Appendix 6), a dash line was used in Figure 7. The coefficients of variation in each treatment were .469, .844, .676, and .922.

For A30, linear relationships were obtained for treatments #1 and #2, quartic relationship was for treatment #4, no significant relationship for treatment #3 (Appendix 7). Treatments #2 and #3 were insignificant in weeks which means the curd firmness did not significantly change from the first week until the twelfth week. A30 in treatment #4 decreased more drastically than the other three. Though A30 is highly related with pH (Table 4), treatment #1 and #4 were not significant when regressed linearly with pH. The relationship is probably non-linear.

As was discussed before, pH increased each week (Fig.9). Treatment #1 had quartic relationship with week, treatment #2 had a quintic relationship with week, and treatment #3 and #4 had cubic relationships with week (Appendix 8). All of them had steep slopes in the first three weeks. The pH was significant for all four treatments.

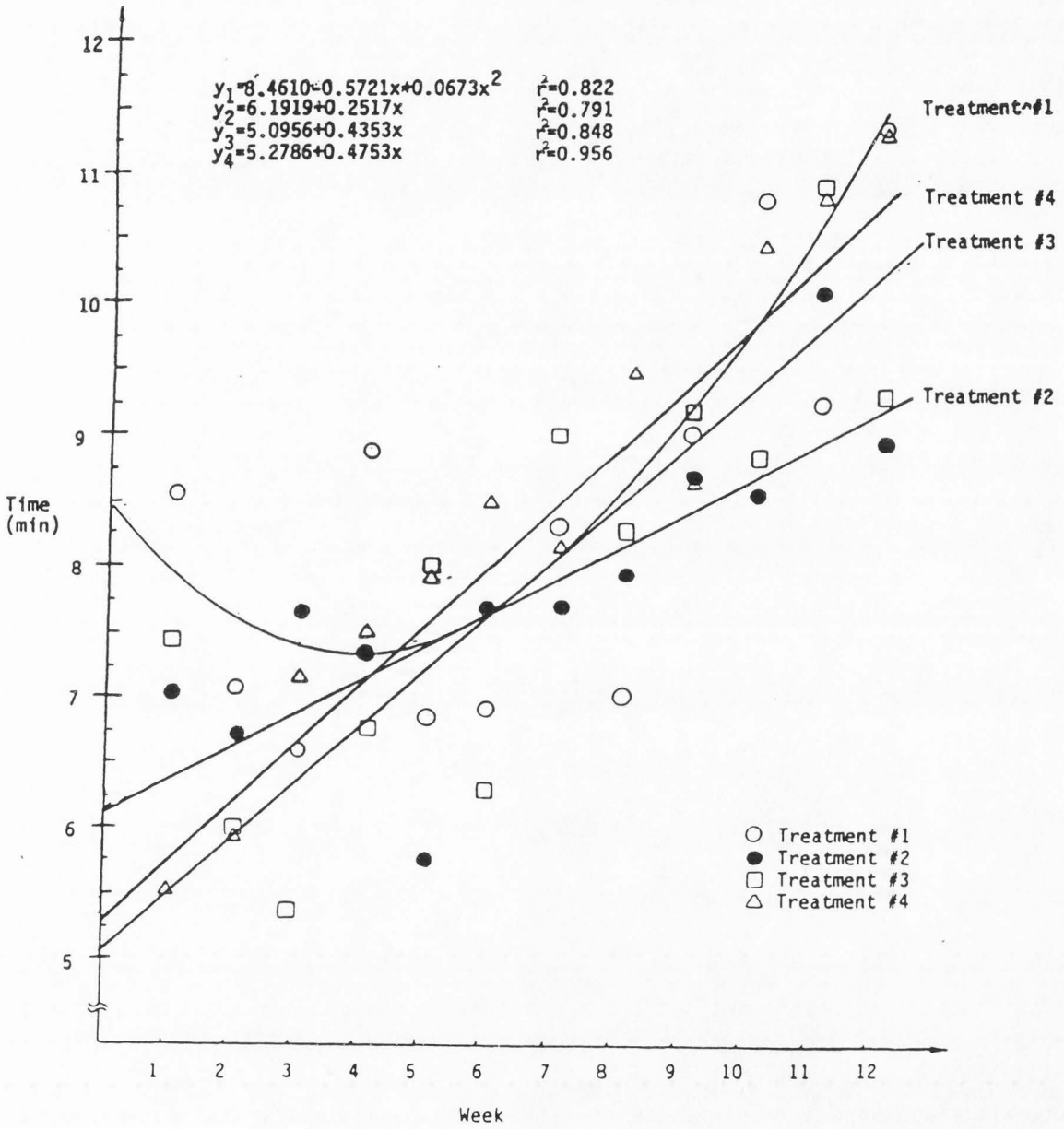


Fig. 6: The relationship between Ct and week for each treatment.

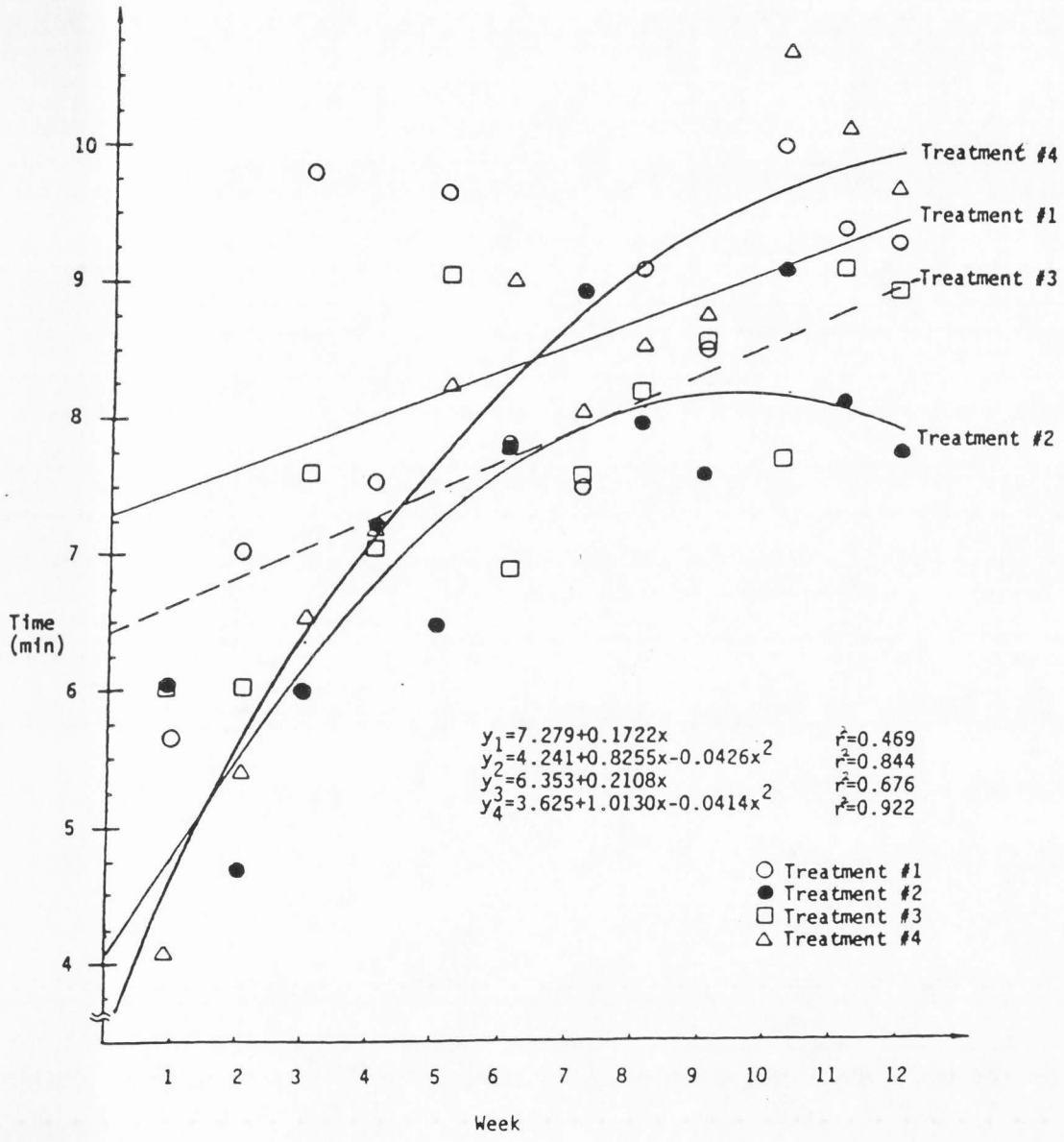


Fig. 7: The relationship between K20 and week for each treatment.

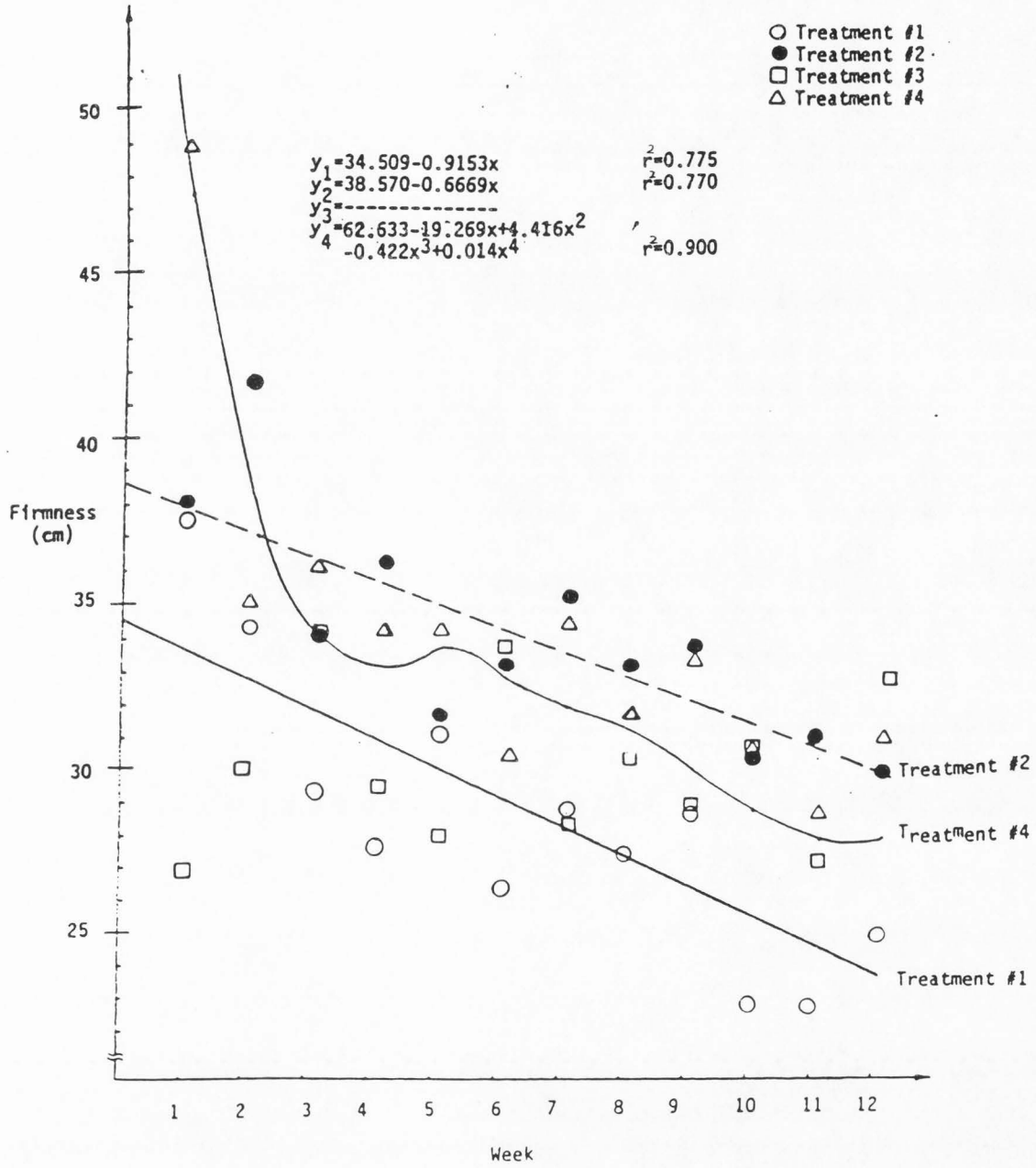


Fig. 8: The relationship between A30 and week for each treatment.

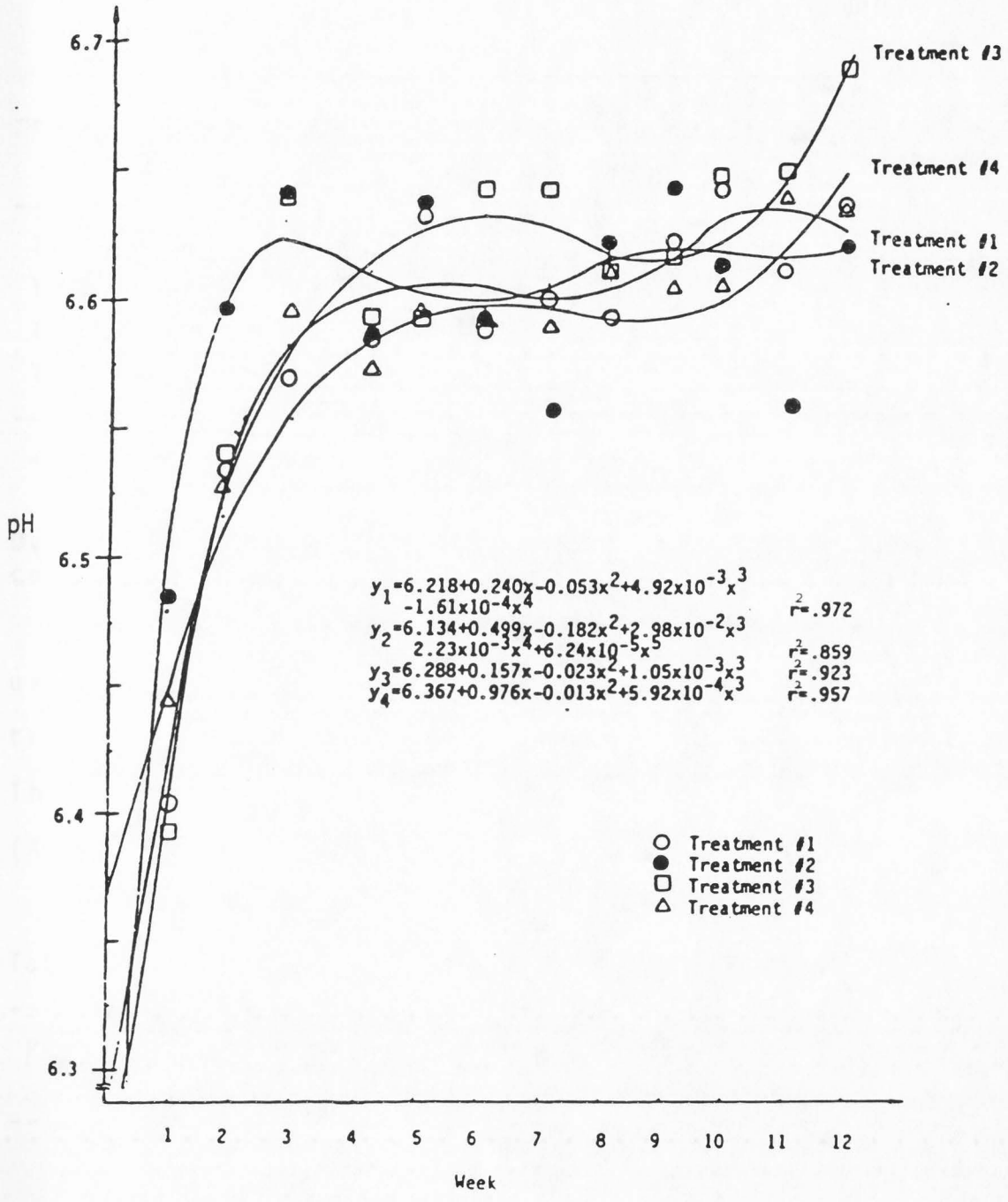


Fig. 9: The relationship between pH and week for each treatment.

The relationships for the milk coagulation parameters for each treatment with week are listed in table 6.

Table 6: The relationship between milk coagulation parameters and week for each treatment.

	Ct	K20	A30	pH
Treatment #1	+	+	+	+
Treatment #2	+	+	-	+
Treatment #3	+	-	-	+
Treatment #4	+	+	+	+

\+ significant - nonsignificant

Overall mean and correlation coefficient

An unbalanced adjusted least square mean procedure was used to obtain the overall means, the week means and treatment means are in table 7, 10, and 11, respectively. There are 46 missing data (Table 1), 16 due to mastitis (Appendix 3) and 58 without a K20 (Appendix 4).

Table 7: The overall means of milk parameters and pH.

Parameter	Adj. L. S. Mean	S.D.	Lowest Data	Highest Data	Number of Data
Ct (min)	8.21	0.112	1.5	30.0	514
K20 (min)	7.90	0.121	1.5	23.6	456
A30 (mm)	31.78	0.367	0	62.3	514
pH	6.60	0.004	6.09	6.88	514

The correlation coefficient for milk coagulation parameters, when pH is independent variable, is listed in Table 8. Table 9 shows the correlation coefficient between pH and each milk coagulation parameters when pH was a dependent variable.

Table 8: The correlation coefficients among variables.

	Ct	K20	A30
Ct	1	0.414	-0.393
K20	0.414	1	-0.472
A30	-0.393	-0.472	1

Table 9: The correlation coefficient between pH and milk parameter (pH is independent variable).

	Ct	K20	A30
pH	0.450	0.354	-0.224

Somatic cells

The somatic cell count (SCC) was highly correlated ($p<0.001$) with the milk coagulation parameters and pH in linear regression (Appendix 9). The equation for the linear relationship between clotting time and SCC was $Ct=7.7080+0.0038SCC$, and the standard error of slope was 0.0007.

Table 10: Overall means of each week.

Week	Ct (min)	K20 (min)	A30 (mm)	pH
1	7.32	5.53	38.50	6.43
2	6.58	5.91	35.33	6.55
3	6.80	7.50	33.21	6.61
4	7.62	7.28	32.45	6.58
5	7.15	8.24	31.21	6.62
6	7.46	8.02	30.52	6.61
7	8.29	8.02	31.95	6.60
8	8.35	8.45	31.04	6.62
9	8.98	8.35	31.76	6.63
10	9.58	9.51	28.68	6.63
11	10.11	9.04	27.95	6.62
12	10.33	8.94	28.73	6.64

Table 11: Overall means of each treatment.

	Ct (min)	K20 (min)	A30 (mm)	pH
Treatment #1	8.39	8.40	28.56	6.58
Treatment #2	7.83	7.30	34.24	6.60
Treatment #3	7.93	7.72	30.10	6.60
Treatment #4	8.37	7.97	33.99	6.58

For K20, the equation was K20=9.3363+0.0054SCC, and the standard error of slope was 0.0014. The relationship between SCC and A30 was A30=32.4550-0.0062SCC, the standard error of slop was 0.0019. Following an increase of somatic cell count, the rennet clotting time was prolonged, whey volume increased, the curd became significantly softer and wetter (2).

Doan and Welch (17) proved that mastitis would cause pH to increase. In this research, the relationship of SCC and pH can be represented by equation of pH=6.5791+0.000069SCC, and the standard error of slop was 0.000039. The r^2 for each equation are listed in Table 12.

The reason of these equation are highly significant in analysis of variance, but low in r^2 is because of other factors which influence milk parameters such as week, individual cow, milk pH.

Table 12: The coefficient of determination (r^2) of somatic cell count (SCC) with coagulation parameters and pH.

	Ct	K20	A30	pH
Somatic cell count (SCC)	0.107	0.057	0.044	0.058

CONCLUSIONS

1. There were no significant differences in clotting time, K20, A30, and pH between the control and the buffer treatment (.8% sodium bicarbonate and/or .4% magnesium oxide) in the early postpartum lactation. Thus, they won't creat differences in making cheese. There were highly significant difference among week, individual cow, and milk pH in analysis of variance of milk parameters.
2. Over all treatments, the clotting time, K20 and pH values increased each week, and A30 decreased each week since concentration of milk components decrease during this time. The clotting time and K20 were negatively correlated with firmness, and there was positive correlation between clotting time and K20 as expected.
3. Superimposed Figures 2-4, the intersection of Ct and K20 rate increasing equation (6th week) was near the minimum of A30 decreasing rate equation (7th week).
4. The milk parameters and pH in each treatment were significant between weeks except K20 or A30 for treatment #3, or A30 in treatment #2 ($p>0.05$).
5. Somatic cell count was highly correlated ($p<0.001$) in a positive, linear relationship with clotting time, K20, and pH, and in a negative, linear relationship with A30.
6. Season was not significant in the analysis of the milk coagulation parameters, but it was significant in pH

(Mean of pH is 6.61 in cold weather, 6.67 in hot weather.).

7. Milk pH was the most significant and independent source of variation affecting clotting time, firming rate, and curd firmness. Milk pH was negatively correlated with curd firmness, and positively correlated with clotting time and firming rate.

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APPENDIXES

Appendix 1

Table 13: The analysis of variance table of Ct with interaction.

Source	df	MS	F	Sig.
Treatment	3	32.421639	0.636	NS
Week	11	59.201819	9.821	S
Linear	1	569.883389	94.538	S
Quad	1	37.032675	6.143	S
Cubic	1	8.221984	1.362	NS
Quard	1	0.227305	0.038	NS
Quint	1	8.003670	1.328	NS
Residual	6	4.643499	0.770	
Seasons	1	0.433067	0.072	NS
Cow (in Treat)	44	50.978639	8.457	S
Treat x Week	33	7.690898	1.276	NS
Treat x Seasons	3	2.655867	0.441	NS
pH B Linear	1	624.511867	103.601	S
Remainder	417	6.028065		
Total	513			

S= significant and NS= not significant with $p<0.05$.

Table 14: The analysis of variance table of K20 with interaction.

Source	df	MS	F	Sig.
Treatment	3	26.719278	0.501	NS
Week	11	28.355357	5.020	S
Linear	1	223.525773	39.574	S
Quad	1	22.016581	3.898	S
Cubic	1	6.988769	1.237	NS
Quard	1	10.169179	1.800	NS
Quint	1	12.962327	2.295	NS
Residual	6	6.041049	1.070	
Seasons	1	2.275379	0.403	NS
Cow (in Treat)	44	53.382493	9.451	S
Treat x Week	33	5.305776	0.939	NS
Treat x Seasons	3	10.856072	1.922	NS
pH B Linear	1	174.097236	42.274	S
Remainder	359	5.648253		
Total	455			

S=significant and NS= not significant with $p<0.05$.

Table 15: The analysis of variance of A30 with interaction.

Source	df	MS	F	Sig.
Treatment	3	701.146746	1.354	NS
Week	11	208.107594	3.639	S
Linear	1	1630.597737	28.517	S
Quad	1	161.644712	2.827	NS
Cubic	1	216.333125	3.783	NS
Quard	1	83.905828	1.467	NS
Quint	1	101.348354	1.772	NS
Residual	6	15.892296	0.278	
Seasons	1	0.395097	0.007	NS
Cow (in Treat)	44	517.942696	9.058	S
Treat x Week	33	38.005735	0.665	NS
Treat x Seasons	3	185.074741	3.237	S
pH B Linear	1	1215.580935	21.259	S
Remainder	417	57.180417		
Total	513			

S= significant and NS= not significant with $p<0.05$.

Table 16: The analysis of variance table of pH with interaction.

Source	df	MS	F	Sig.
Treatment	3	0.005863	0.209	NS
Week	11	0.123174	22.422	S
Linear	1	0.667663	121.538	S
Quad	1	0.264272	48.107	S
Cubic	1	0.250251	45.554	S
Quard	1	0.090587	16.490	S
Quint	1	0.035963	6.547	S
Residual	6	0.007697	1.401	
Seasons	1	0.059329	10.800	S
Cow (in Treat)	44	0.028090	5.114	S
Treat x Week	33	0.006479	1.179	NS
Treat x Seasons	3	0.004358	0.793	NS
Remainder	418	0.005493		
Total	513			

S= significant and NS= not significant with $p<0.05$.

Appendix 2

The basic ration during experiment was a total mixed ration composed of feeds in the following proporations:

Silage	3.2 kg
Hay	4.1 kg
Grain	7.7 kg
Cotton seed	1.2 kg
Wheat bran	1.2 kg
Rolled barley	4.1 kg
Beet pulp	1.2 kg
Trace mineral salt	0.04 kg
Dicalcium phosphate	0.02 kg
Vitamin A	0.002 kg
Molasses	0.02 kg

The cows were fed twice a day. The amount of total mixed ration fed each cow each day was based on body size, milk production, and milk composition.

Appendix 3

Table 17: Number of cows with mastitis during experiment.

Week	Treat #1	Treat #2	Treat #3	Treat #4
1	0	1	1	1
2	0	2	0	1
3	0	0	0	1
4	1	0	0	0
5	0	0	0	0
6	0	1	0	0
7	0	0	0	0
8	2	0	0	0
9	0	1	0	0
10	0	1	0	0
11	0	1	0	0
12	1	1	0	0
Total	4	8	1	3

Appendix 4

Table 18: Samples where K20 was not measureable.

Week	Treat #1	Treat #2	Treat #3	Treat #4
1	0	0	0	0
2	0	1	1	1
3	1	1	1	0
4	3	2	0	1
5	1	1	1	2
6	3	1	1	1
7	3	0	1	1
8	1	0	0	1
9	2	1	1	1
10	5	1	1	1
11	2	3	2	1
12	3	2	0	2
Total	24	13	9	12

The percent of samples without a K20 during 30 min per cow per week

$$= \frac{\#\text{ of data in each treatment without a K20 in 30min}}{\#\text{ of week x \# of cow}} \times 100$$

The treatment #1 : 24/12x12 x 100=16.67%

The treatment #2 : 13/15x12 x100=7.22%

The treatment #3 : 9/9x12 x100=8.33%

The treatment #4 : 12/12x12 x100=8.33%

The ratio of samples without K20 during 30min coagulation was 16.67%:7.22%:8,33%:8.33% =2:1:1:1.

Table 19: Number of samples without K20 in each cow in each treatment.

Treatment #1

5214	5410	5216	5488	5458	5394
4	4	3	6	1	6

Treatment #2

5270	5292	5452	94	5448
1	2	1	6	3

Treatment #3

5366	5454	4884	5018
1	1	2	5

Treatment #4

5156	5506	5138	92	5538
1	8	1	1	1

Appendix 5

Table 20: Four analysis of variance tables of clotting time in each treatment.

Treatment #1

Source	df	MS	F	Sig.
Cow	11	75.166264	10.541	S
Week	11	24.715608	3.466	S
Linear	1	127.491408	17.879	S
Quad	1	56.225001	7.885	S
Cubic	1	3.067163	0.430	NS
Quard	1	0.000379	0.000	NS
Quint	1	2.843788	0.399	NS
Residual	6	13.707325	1.922	
pH B Linear	1	314.905821	44.161	S
Remainder	106	7.130828		
Total	129			

Treatment #2

Source	df	MS	F	Sig.
Cow	14	32.330499	5.740	S
Week	11	14.719816	2.613	S
Linear	1	101.288079	17.892	S
Quad	1	7.784074	1.382	NS
Cubic	1	3.194962	0.567	NS
Quard	1	8.984821	1.595	NS
Quint	1	0.048006	0.009	NS
Residual	6	6.769672	1.202	
pH B Linear	1	230.252315	40.878	S
Remainder	126	5.632658		
Total	152			

Treatment #3

Source	df	MS	F	Sig.
Cow	8	49.823627	7.345	S
Week	11	21.085468	3.109	S
Linear	1	166.791341	24.589	S
Quad	1	0.516215	0.076	NS
Cubic	1	9.476263	1.397	NS
Quard	1	1.380067	0.203	NS
Quint	1	12.644561	1.864	NS
Residual	1	6.855283	1.011	
pH B Linear	1	96.683415	14.254	S
Remainder	77	6.783072		
Total	97			

Treatment #4

Source	df	MS	F	Sig.
Cow	11	19.610123	5.412	S
Week	11	21.232089	5.860	S
Linear	1	213.630824	58.960	S
Quad	1	0.061973	0.017	S
Cubic	1	4.441992	1.226	NS
Quard	1	0.0001647	0.000	NS
Quint	1	1.414440	0.390	NS
Residual	6	2.333684	0.644	
pH B Linear	1	27.770173	7.664	S
Remainder	97	3.623346		
Total	120			

S= significant and NS= not significant with $p<0.05$.

Appendix 6

Table 21: Four analysis of variance tables of firming rate (K20) in each treatment.

Treatment #1

Source	df	MS	F	Sig.
Cow	11	66.611571	11.517	S
Week	11	11.274807	1.949	S
Linear	1	27.217933	4.706	S
Quad	1	4.445925	0.769	NS
Cubic	1	14.259768	2.465	NS
Quard	1	21.496798	3.717	NS
Quint	1	1.649883	0.285	NS
Residual	6	9.158762	1.584	
pH B Linear	1	30.607674	13.832	S
Remainder	82	2.212799		
Total	105			

Treatment #2

Source	df	MS	F	Sig.
Cow	14	69.690162	10.654	S
Week	11	15.725421	2.404	S
Linear	1	97.293765	14.875	S
Quad	1	25.988530	3.973	S
Cubic	1	7.163053	1.095	NS
Quard	1	1.903201	0.291	NS
Quint	1	5.722563	0.875	NS
Residual	6	4.387286	1.014	NS
pH B Linear	1	98.442980	22.750	S
Remainder	113	4.327137		
Total	139			

Treatment #3

Source	df	MS	F	Sig.
Cow	8	29.098550	5.391	S
Week	11	6.702221	1.242	NS
Linear	1	33.704183	6.245	S
Quad	1	2.169351	0.402	NS
Cubic	1	3.826870	0.709	NS
Quard	1	0.100997	0.019	NS
Quint	1	1.288724	0.239	NS
Residual	6	5.439050	1.008	
pH B Linear	1	52.310440	9.692	S
Remainder	68	5.397248		
Total	88			

Treatment #4

Source	df	MS	F	Sig.
Cow	11	43.996652	8.927	S
Week	11	22.443200	4.554	S
Linear	1	190.001854	38.553	S
Quad	1	19.717998	4.001	S
Cubic	1	4.709183	0.956	NS
Quard	1	3.835895	0.778	NS
Quint	1	11.696293	2.373	NS
Residual	6	2.818996	0.572	
pH B Linear	1	20.796773	4.220	S
Remainder	97	4.928296		
Total	120			

S= significant and NS= not significant with $p<0.05$.

Appendix 7

Table 22: Four analysis of variance tables of firmness in each treatment.

Treatment #1

Source	df	MS	F	Sig.
Cow	11	666.508874	13.815	S
Week	11	137.051847	2.841	S
Linear	1	942.223614	19.530	S
Quad	1	93.889047	1.946	NS
Cubic	1	71.219080	1.476	NS
Quard	1	109.477424	2.269	NS
Quint	1	45.559873	0.944	NS
Residual	6	40.866879	0.847	
pH B Linear	1	62.946810	1.305	NS
Remainder	106	48.244931		
Total	129			

Treatment #2

Source	df	MS	F	Sig.
Cow	14	680.366516	11.001	
Week	11	109.126984	1.764	NS
Linear	1	711.344864	11.502	S
Quad	1	10.806857	0.175	NS
Cubic	1	32.252742	0.521	NS
Quard	1	0.006398	0.000	NS
Quint	1	156.662302	2.533	NS
Residual	6	48.220610	0.780	
pH B Linear	1	568.271485	9.188	S
Remainder	126	61.846371		
Total	154			

Treatment #3

Source	df	MS	F	Sig.
Cow	8	300.608690	4.961	S
Week	11	43.481218	0.718	NS
Linear	1	13.853440	0.229	NS
Quad	1	0.955155	0.016	NS
Cubic	1	63.046017	1.040	NS
Quard	1	0.118342	0.002	NS
Quint	1	89.572752	1.478	NS
Residual	6	51.791282	0.855	NS
pH B Linear	1	1856.594152	30.638	S
Remainder	77	60.598353		
Total	97			

Treatment #4

Source	df	MS	F	Sig.
Cow	11	216.323625	5.897	S
Week	11	160.737115	4.382	S
Linear	1	624.542156	17.026	S
Quad	1	313.863191	8.556	S
Cubic	1	318.574887	8.685	S
Quard	1	175.140785	4.775	S
Quint	1	7.224411	0.197	NS
Residual	6	54.793807	1.494	
pH B Linear	1	2.318729	0.063	NS
Remainder	97	36.681987		
Total	120			

S= significant and NS= not significant with $p<0.05$.

Appendix 8

Table 23: Four analysis of variance tables of pH in each treatment.

Treatment #1

Source	df	MS	F	Sig.
Cow	11	0.032712	5.322	S
Week	11	0.044909	7.306	S
Linear	1	0.269247	43.805	S
Quad	1	0.105634	17.186	S
Cubic	1	0.065226	10.612	S
Quard	1	0.026578	4.324	S
Quint	1	0.001700	0.277	NS
Residual	6	0.004270	0.695	
Remainder	107	0.006147		
Tatal	129			

Treatment #2

Source	df	MS	F	Sig.
Cow	14	0.032312	5.676	S
Week	11	0.025167	4.421	S
Linear	1	0.050399	8.853	S
Quad	1	0.038764	6.809	S
Cubic	1	0.039140	6.875	S
Quard	1	0.043910	7.713	S
Quint	1	0.032361	5.684	S
Residual	6	0.012045	2.116	
Remainder	127	0.005693		
Total	153			

Treatment #3

Source	df	MS	F	Sig.
Cow	8	0.061217	9.723	S
Week	11	0.041761	6.633	S
Linear	1	0.236800	37.661	S
Quad	1	0.052738	8.376	S
Cubic	1	0.102098	16.216	S
Quard	1	0.021908	3.480	NS
Quint	1	0.014852	2.359	NS
Residual	6	0.005163	0.820	
Remainder	78	0.006296		
Total	97			

Treatment #4

Source	df	MS	F	Sig.
Cow	11	0.038510	8.714	S
Week	11	0.028617	6.475	S
Linear	1	0.202568	45.836	S
Quad	1	0.041103	9.301	S
Cubic	1	0.045029	10.189	S
Quard	1	0.012064	2.730	NS
Quint	1	0.002248	0.509	NS
Residual	6	0.001963	0.444	
Remainder	110	0.004419		
Total	132			

S= significant and NS= not significant with $p<0.05$.

Appendix 9

Table 24: The analysis of variance table of SCC to milk parameter in linear regression.

Ct

Source	df	MS	F	Sig.
Regression	1	476.658239	29.25	S
Error	243	16.297751		
Total	244			

K20

Source	df	MS	F	Sig.
Regression	1	932.342308	14.67	S
Error	243	63.566179		
Total	244			

A30

Source	df	MS	F	Sig.
Regression	1	1242.246420	11.25	S
Error	243	110.389908		
Total	244			

pH

Source	df	MS	F	Sig.
Regression	1	0.151611	15.00	S
Error	243	0.010108		
Total	244			

S= significant and NS= not significant with $p<0.001$.

VITA

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