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# COMPOSITIONAL AND METABOLIC EVALUATION OF COLOSTRUM PRESERVED BY FOUR METHODS DURING WARM AMBIENT TEMPERATURES

BY

SUSAN MARY ANNEXSTAD CARLSON

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science, Major in
Dairy Science, South Dakota
State University
1976

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# COMPOSITIONAL AND METABOLIC EVALUATION OF COLOSTRUM PRESERVED BY FOUR METHODS DURING WARM AMBIENT TEMPERATURES

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

_____ Thesis Adviser / Date

_____ Head, Dairy Science Dept. Date

ACKNOWLEDGEMENTS

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Thanks are also extended to all of the friends who have made my stay in South Dakota memorable and have made it difficult to leave.

To my husband, Garry, whom I love for his consideration and his needed support during my course of study and preparation of this thesis.

To Marlys Moberg for her patience and assistance in the preparation of this thesis.

To Mom and Dad for believing in me.

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TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

Figure Page

# LIST OF APPENDIX TABLES

## ABSTRACT

Colostrum from several cows was composited from the first 6 milkings postcalving and subdivided into the following treatments: frozen (F), naturally fermented (N), formaldehyde treated (FT) at .05% by volume, and propionic acid treated (P) at 1% by volume. All except F were stored at warm ambient temperatures. The above regime was replicated five times and colostrum was fed to 20 Holstein bull calves at 3.64 kg daily (3 parts colostrum to 1 part water) for three weeks. Composition of frozen colostrum was constant. For most components analyzed, N colostrum had significantly more nutrient breakdown during storage than did P with FT intermediate. Protein degradation as measured by nonprotein nitrogen content was significantly greater in N than in P and FT treatments. The most consistent pattern noticed for serum components was a higher serum urea level in calves fed N colostrum. The higher serum urea appears related ($r=.42$) to the nonprotein nitrogen content of the diet. Propionic acid, and to a lesser extent formaldehyde, were effective colostrum preservatives and produced a diet that was more acceptable by calves than naturally fermented colostrum during warm ambient temperatures.

# GENERAL INTRODUCTION

In the past few years, the practice of storing and feeding excess colostrum as a fermented product has been rapidly accepted by dairymen. Colostrum or colostral milk is the first drawn milk which provides immunoglobulins that are necessary for the survival of the newborn calf. While not marketable, the first 5 to 6 milkings of colostral milk are a rich source of nutrients, including protein, fat, lactose, vitamins, and minerals. Most cows produce colostrum in excess of the newborn calf's requirements. While the first colostrum has a beneficial effect on calf health, the excess can be an economical liquid diet because it can supply the nutrient requirements of a calf up to 3 to 4 weeks.

Interest in saving the excess colostral milk has increased substantially. A useful method for preserving colostrum has been by fermentation. By allowing the colostrum to ferment, considerable effort and time are saved because under practical dairy farm conditions, colostrum cannot be conveniently refrigerated or frozen. However, during the hot summer temperatures, the fermented product becomes putrid and unacceptable to the calf. Therefore, a more efficient way of preserving colostral milk must be found in order to obtain a more uniform product.

The purpose of this study was to find an acceptable preservative that would provide a means of convenient storage during warm ambient temperature. The study was initiated to compare: 1) the fermentation and composition of frozen, naturally fermented, formaldehyde treated,

and propionic acid treated colostrum and 2) the colostrum handled by these various methods in terms of metabolic utilization by Holstein calves.

# LITERATURE REVIEW

One of the keys to a profitable dairy operation is successful raising of replacement heifers. The first concern is for the calf to survive the first few weeks after birth. The mortality rate for this period is 6 to 17.7% for liveborn calves (19, 32, 46, 63, 68). Calf losses are related to factors such as feeding, management, and housing. Housing factors can be due to types of housing, temperature of calving facilities, ventilation, and supplemental heat in the calf barns (68). Other management practices resulting in healthier calves and lower mortality rates are related to the person caring for the calves (3). Dairymen exerting an effort to improve their dairy operations can reduce mortality, and thereby decrease economic losses and increase herd potential with healthier herd replacements.

## Importance of Colostrum

In the late forties and some years earlier, attention was focused on the effects of feeding colostrum to dairy calves. Smith and Little (65) were the first to recognize the importance and function of colostrum. They reported that all 10 calves permitted to consume colostrum survived while 8 of 12 calves deprived of colostrum died. Thus, colostrum was shown to be essential for protection against invading bacteria. In a later study, calves deprived of colostrum had a higher incidence of disease (23). Others (2, 70) realized that not only was colostrum important to the newborn calf for its immunizing agents, but colostrum was highly nutritious and wholesome. The composition of colostrum is presented in Table 1 (66).

Colostrum is not only high in total solids, protein, and fat but also high in carotenoids and fat soluble vitamins. Roy (62) reported that colostrum, the first 24 h after calving, has 24 to 25 ug carotenoids/g fat, 42 to 48 ug vitamin A/g fat, 0.9 to 1.8 IU vitamin D/g fat, and 100 to 150 ug vitamin E/g fat. In addition, Roy (62) reported colostrum with 14.3% protein of which casein, albumin, and immunoglobulins were 5.2%, 1.5%, and 5.5 to 6.8%, respectively.

TABLE 1. Composition of colostrum from Holstein cows.[a]

| Item | Total Solids | Protein | Fat | Lactose | Ash |
|---|---|---|---|---|---|
| | % | | | | |
| Colostrum | | | | | |
| At parturition | 27.42 | 13.97 | 8.45 | 3.63 | 1.37 |
| 12 hours | 15.63 | 4.77 | 5.68 | 4.29 | 0.89 |
| 24 " | 13.98 | 3.99 | 4.88 | 4.24 | 0.87 |
| 36 " | 13.54 | 3.85 | 4.08 | 4.75 | 0.86 |
| 48 " | 13.42 | 3.62 | 4.20 | 4.77 | 0.85 |
| 60 " | 14.22 | 3.70 | 5.02 | 4.66 | 0.84 |
| 72 " | 14.00 | 3.82 | 4.94 | 4.39 | 0.84 |
| Milk | | | | | |
| 11 days | 12.78 | 2.92 | 4.33 | 4.78 | 0.75 |

[a]Adapted from Smith (66).

In later years, Briggs and associates (7, 8) reported on the immunological aspects of the protective action of colostrum. Briggs (7) revealed that the protective mechanism of colostrum was largely an immunological one in that it was associated, in colibacillosis, with the possession of antibodies to the "K" antigens of infecting strains of Bacterium coli. Others (2, 3, 20, 44, 46, 56, 63) recognized that first colostrum was important for the passive immunity

obtained. First colostrum is much higher in immunoglobulins than any subsequent milkings and the globulin proteins are essential for the survival of the calf.

Oxender et al. (46) showed that the time of the first feeding of colostrum after birth and the duration of feeding colostrum significantly lowered calf mortality. Thus, one of the most important management practices for calf survival is early ingestion of colostrum before any other material enters the digestive tract. Early ingestion allows the newborn calf to absorb the ingested immunoglobulins while the intestines are permeable to the proteins (56) and prevents fatal septicemia and other disease hazards (3, 46). Appleman and Owen (3) indicated that absorption of the immunoglobulins is affected by amounts fed, time of feeding, age of calf, and birth weight. Absorption of ingested colostrum immunoglobulins declines rapidly the first 24 hours. Thus, feeding colostrum immediately after birth is particularly important.

Utilization of Surplus Colostrum

Calves do not consume or need the entire amount of colostrum produced the 3 or 4 days postpartum, therefore; surplus amounts are available. Many researchers (2, 14, 16, 24, 25, 26, 48, 49, 70, 81, 82) have studied methods for utilizing the entire colostrum supply. Wise and LaMaster (82) found that fresh colostrum in a 1:1 mixture with skim milk was a satisfactory substitute for milk with no physiological upsets to the calf. This allowed more milk to be sold through the fluid market. Allen (2) used frozen colostrum to replace the

marketable milk and repeatedly showed that colostrum could be stored by freezing without seriously affecting nutritional properties, and calves fed frozen colostrum had satisfactory gains. No digestive disturbances were found over the entire feeding period even when colostrum was employed as the sole source of liquid. Later researchers found that extended feeding of colostrum was not only beneficial and economical, but the calves fed colostrum had more rapid gains (25, 70). However, Kaeser and Sutton (25) realized freezing surplus colostrum was not practical for the average dairy farm, for few farms were equipped to refrigerate and freeze any quantity of colostrum. Considerable inconvenience was encountered in feeding, since extra time and effort were required. Therefore, Kaeser and Sutton (25) fed colostrum intermittently with milk for the duration of the milk feeding period. This proved to be one method of using colostrum to its greatest advantage during the first month of life. However, the practice of saving and feeding the excess colostrum was not readily accepted until later.

Further work (16, 24, 26) in the early fifties recognized the economics of substituting colostrum for whole milk in the diet of dairy calves in a limited whole milk feeding system. Jacobson et al. (24) indicated that when colostrum was fed on an equal dry matter basis as whole milk, it could effectively replace whole milk. Keyes et al. (26) agreed that when the total digestible nutrients consumed were comparable, no differences existed between whole milk and colostrum fed calves. In 1960, Wing (81) reported an alternative

for using surplus colostrum similar to that of Wise and LaMaster (82). A 1:1 mixture of colostrum and high solids skim milk was used to extend the colostral feeding period without causing digestive upsets.

In recent years, others (31, 39, 45) have compared frozen colostrum to whole milk. Muller et al. (39) reported improved weight gains for the first 3 weeks for calves fed colostrum compared to calves fed whole milk. In addition, colostrum fed calves were slightly more efficient in dry matter intake/kg gain than whole milk fed calves. Marshall and Smith (31) reported calves fed colostrum made satisfactory weight gains the first week. However, calves fed the whole milk and nonfat milk diets were more efficient than colostrum fed calves. Owen et al. (45) in two trials found that calves fed frozen colostrum had improved gains throughout a 12 week period over calves fed whole milk. Likewise, calves on colostrum showed improved starter consumption and reduced incidence of scours.

## Fermented Colostrum

Application of Fermented Colostrum. Swannack (72) reported in 1972 that an English woman farmer had been extending colostrum feeding since 1969 by allowing excess colostral milk to ferment. Since these initial reports (71, 72), the practice of fermenting or pickling the excess colostrum has become a rapidly accepted method of utilizing excess colostrum. In a recent poll, 28% of the dairymen responding now feed fermented colostrum (1). Allowing colostrum to ferment does not require the labor and cost of freezing the colostrum. Nevertheless, certain precautions must be taken in the fermentation

of colostrum. Cows need to be milked under sanitary conditions. Colostrum should not be taken from cows treated for mastitis because the residual antibiotics would kill the acid-producing organisms (20). The colostrum is normally allowed to ferment at moderate environmental temperatures. This results in a stable fermentation. The fermented colostrum can be stored for at least a month and should be diluted with water when fed (35). The product resulting from fermentation has a high nutritional value since colostrum is substantially higher in total nutrients than is milk (12, 13, 35, 42, 44, 51, 62, 66). Table 1 (66) shows that colostrum has higher concentrations of protein, fat, and minerals than milk. The end result of storing and feeding fermented colostrum has been a tremendous savings over milk and milk replacers because it fully utilizes unsaleable milk of fresh cows without the necessity of refrigeration (20, 35).

Fermentation Characteristics of Colostrum. Rapid lactic fermentation of colostrum is an effective way to preserve colostrum without refrigeration (35, 44). The low pH and high acid condition promotes growth of the lactic acid producing bacteria (36, 37, 38, 42, 47, 51, 52, 57, 59, 74, 78, 80). This rapid rise in acidity over the first days develops a stable pH thereafter, with the average composition of fermented product from the first 6 milkings postpartum being 16.8% total solids, 7.2% fat, and 5.2% protein (35). However, acidity and protein degradation increase with time (36, 37, 42, 51, 58, 59, 64) so that fermented colostrum must normally be fed within a month to efficiently utilize the product before putrefaction.

Additives to Control Fermentation. Naturally fermented colostrum has been unsatisfactory during warm temperatures because of protein degradation and because of the growth of yeasts and molds after a low pH is reached (11, 47, 74). Workers have used potassium sorbate (15), formaldehyde (36, 37, 58, 59), organic acids (18, 36, 37, 43, 51, 58, 59), and bacterial cultures (15, 18, 36) as additives for preserving colostrum and controlling fermentation.

In 1956, Barber et al. (5) used formalin, a 37% solution of formaldehyde, to increase storage time of skim milk and arrest bacterial growth. Then in 1974, Lindahl (29) used formaldehyde as a bacterial growth retardant in reconstituted artificial ewe milk. Lindahl (29) found formaldehyde to be effective in preventing souring of milk over an extended period. This replaced the need for refrigeration. Moreover, formaldehyde did not affect the initial pH of the milk. Other researchers (17, 54, 76) have used various levels of formaldehyde in milk and milk replacers to determine if formaldehyde was toxic. Gorill et al. (17) determined that formaldehyde had no adverse effect on utilization of nutrients, feed intake, or incidence of abomasal bloat in lambs. Furthermore, formaldehyde tended to improve growth of artificially reared lambs. Others (54, 76) found that formaldehyde treatment was an effective method for preserving milk and milk replacers and reducing bacterial growth.

With this background, researchers tried several levels of formaldehyde in colostrum (36, 37, 59). Formaldehyde was effective in maintaining a constant pH for at least 3 weeks, retarding microbial

growth, and keeping protein degradation minimal.

Other chemical additives used include organic acids. Propionic acid had been the major acid studied. Researchers (36, 37, 43, 51, 58, 59) used propionic acid to reduce pH, to prevent microbial growth, and to minimize protein degradation. Other researchers (18, 36, 37, 51) have used lactic, formic, and acetic acids to control fermentation and to preserve colostrum.

Muller and Syhre (36) and Hall and Daniels (18) used bacterial cultures to control the fermentation of colostrum. Muller and Syhre (36) used 3 bacterial cultures containing Streptococcus lactis, Streptococcus thermophilus, and Lactobacillus bulgaricus. Hall and Daniels (18) used lactic acid 253 culture. These cultures were not effective in preserving colostrum at high temperatures in that the colostrum became putrid and could not be held for long periods. However, Drevjany and others (15) used a culture of Streptococcus lactis. They reported this culture resulted in fair levels of residual lactose in the stored product.

Calf Performance

Naturally Fermented Colostrum. In recent years numerous calf trials comparing fermented colostrum with other liquid diets have been published (10, 18, 20, 34, 37, 38, 41, 50, 51, 52, 53, 57, 58, 71, 72, 78, 80, 84). Rindsig (57) compared treatments of 3.63 kg whole milk, 1.81 kg fermented colostrum plus 1.81 kg water fed once or twice daily in equal portions, and 2.72 kg fermented colostrum plus .9 kg water offered twice daily in two equal portions. Average

daily gains for calves fed 2.72 kg fermented colostrum were comparable to gains of whole milk fed calves. Others (38, 50, 52, 71, 78) have compared fermented colostrum diet to diets of whole milk or frozen colostrum. Muller et al. (38) fed diets of 3.64 kg whole milk, 2.73 kg fermented colostrum diluted with .91 kg water, and 1.82 kg fermented colostrum diluted with 1.82 kg water. They reported calves fed 2.73 kg fermented colostrum performed similar to calves fed whole milk. The 1.82 kg fermented colostrum diet was inadequate for satisfactory growth and health. In addition, Polzin et al. (52) reported calves fed whole milk or 2:1 dilution of fermented colostrum to water gained more weight than those fed undiluted or 1:1 dilution of fermented colostrum. Yu et al. (84) found growth responses identical between calves fed 1.64 kg whole milk or .9 kg fermented colostrum diluted with .9 kg water fed twice daily. Van den Broek and Shellenberger (78) recorded similar results. However, their dietary treatment prior to weaning had no effect on growth or feed intake of calves 6 to 12 wk of age. Plog et al. (50) detailed calf trials comparing whole milk with that of frozen or fermented colostrum. They found when liquid diets were equalized on total solids content, there was no relationship between treatments and weight gains. At the same time, Morrill et al. (34) reported higher total gains of young calves fed fermented colostrum than calves fed frozen colostrum.

Workers (10, 41, 53, 71) have used calf performance to compare fermented colostrum to that of milk replacers. Polzin et al. (53) reported calves fed fermented colostrum gained more from 0 to 4 wk

but at 12 wk there were no real differences between milk replacer and fermented colostrum. Chik et al. (10) observed better feed efficiency by calves fed milk replacers than calves fed fermented colostrum. Earlier, Swannack (71) had reported results from 5 different diets composed of milk replacers and/or fermented colostrum. Calves achieved the same liveweight gains at 84 days. Results from these trials indicate that the practice of feeding fermented colostrum produces equal calf performance to the feeding of whole milk and milk replacers for dairy calves.

Chemically Preserved Colostrum. Since naturally fermented colostrum does not ferment properly during warm ambient temperatures, several researchers (18, 37, 51, 58) have used chemical additives to control the fermentation of colostrum. In calf trials conducted by Muller et al. (37) colostrum with formaldehyde had low protein degradation and was readily accepted by calves, but gains and feed efficiency were lower than obtained with whole milk or colostrum with propionic acid. Weight gains on all 3 diets were greater than with naturally fermented colostrum. Hall and Daniels (18) reported calves fed acetic acid treated colostrum and naturally fermented colostrum grew faster than calves fed lactic acid 253 cultured colostrum or milk replacer. Further, calves fed colostrum diets had higher average daily gains and greater starter consumption than calves receiving milk replacer. Polzin et al. (51) found corresponding results in that calves fed fermented or acidified colostrum gained similarly to calves fed whole milk. Finally, Rindsig and Bodoh (58)

reported no beneficial effects on calf performance when chemically supplemented colostrums were fed during periods of moderate environmental temperatures. However, calves fed formaldehyde treated colostrum refused less liquid than calves fed fermented colostrum or propionic acid treated colostrum during periods of high environmental temperatures.

The use of fermented or preserved colostrum as a possible substitute for whole milk or milk replacers appears promising. Although several aspects of handling have been researched, better methods of preservation are needed to maintain a more uniform product and to obtain more efficient utilization of the colostrum by the calves.

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## EXPERIMENTAL PROCEDURES

Four Holstein bull calves born within a few days span were randomly assigned to one of 4 colostrum treatments: 1) frozen (F), 2) naturally fermented (N), 3) formaldehyde treated (FT) at .05% of volume, and 4) propionic acid treated (P) at 1% of volume. The design was replicated 5 times during the summer months (May to September) for a total of 20 calves.

Colostrum was collected from the first 6 milkings from each of 5 cows during each replication (Fig. 1). In order to ensure uniform colostrum composition and fermentation of each diet, colostrum was stored at 4C until the entire amount was collected, after which the colostrum was composited and then subdivided equally among the 4 treatments at which time 2 of 4 received their additives. Three of the diets were stored in metal containers with plastic liners and were stirred daily. These colostrum treatments were stored at ambient temperatures ranging from 16 to 37C. The frozen colostrum was stored at -32C in plastic-lined 9.5 liter containers and thawed when needed 24 h prior to feeding.

Calves ranged from 1 day to 1 wk of age when assigned to their respective diets. Calves were fed fresh colostrum for the first 24 h, then switched to their diets and fed once daily for 20 days. Calves were fed a mixture of 2.73 kg of colostrum and .91 kg of water (3:1 dilution). Calves were housed in metal digestion crates and were offered supplemental water free choice.

Colostrum samples were collected initially and at 5, 10, 15,

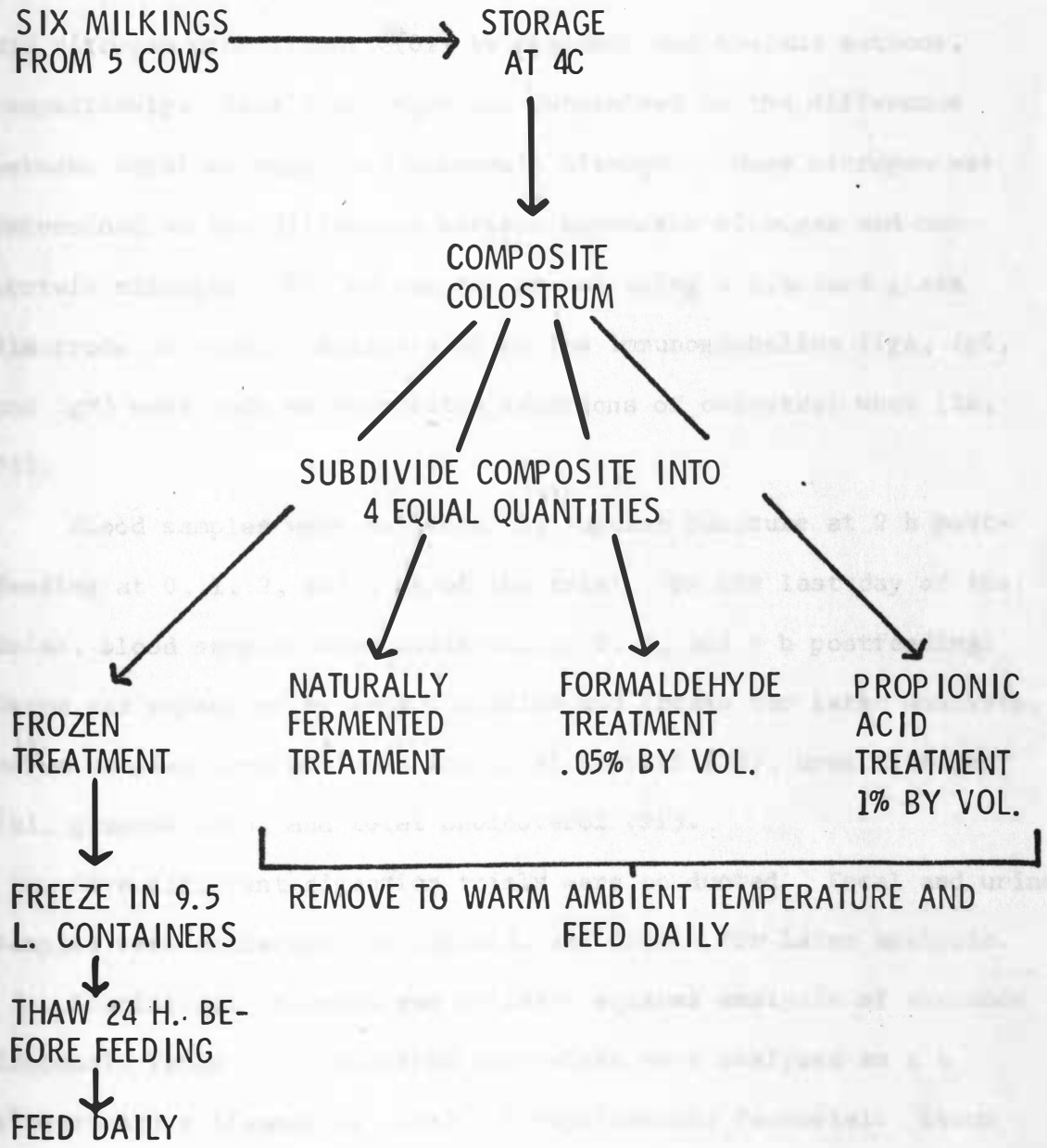
SIX MILKINGS FROM 5 COWS
STORAGE AT 4C
COMPOSITE COLOSTRUM
SUBDIVIDE COMPOSITE INTO 4 EQUAL QUANTITIES
FROZEN TREATMENT
NATURALLY FERMENTED TREATMENT
FORMALDEHYDE TREATMENT .05% BY VOL.
PROPIONIC ACID TREATMENT 1% BY VOL.
FREEZE IN 9.5 L. CONTAINERS
THAW 24 H. BEFORE FEEDING
FEED DAILY
REMOVE TO WARM AMBIENT TEMPERATURE AND FEED DAILY

Figure 1.

and 20 days of storage. Samples were initially frozen and later analyzed for titratable acidity (40), total solids by the Mojonnier method, milk fat according to the Babcock method, total nitrogen (4), and nitrogen constituents (61) by Kjeldahl and Rowland methods, respectively. Casein nitrogen was determined as the difference between total nitrogen and noncasein nitrogen. Whey nitrogen was determined as the difference between noncasein nitrogen and non-protein nitrogen. The pH was determined using a standard glass electrode pH meter. Analysis of bovine immunoglobulins (IgA, IgG, and IgM) were made on composited fractions of colostral whey (28, 33).

Blood samples were collected by jugular puncture at 2 h post-feeding at 0, 1, 2, and 3 wk of the trial. On the last day of the trial, blood samples were collected at 0, 2, and 4 h postfeeding. Serum was separated by centrifugation and frozen for later analysis. Serum samples were analyzed for total protein (22), urea nitrogen (9), glucose (83), and total cholesterol (21).

Five different digestion trials were conducted. Fecal and urine samples were collected, composited, and frozen for later analysis.

Statistical analysis was by least squares analysis of variance (Appendix Table 1). Colostrum parameters were analyzed as a 4 (treatment) x 5(sampling time) x 5(replication) factorial. Serum parameters were analyzed as a 4(treatment) x 4(sampling time) x 5 (replication) factorial except for the timed interval bleeding the final day of the trial which was a 4(treatment) x 3(sampling time)

x 5(replication) factorial (Appendix Tables 3 and 4). The colostrum treatment by day interaction as well as the serum by week and serum by hour interactions were analyzed by the Student-Newman-Keuls' multiple-range test (69). Further, overall colostrum treatment means and overall colostrum day means were analyzed by Student-Newman-Keuls' multiple-range test (69). Multiple regression and correlation techniques were used to predict serum components from colostrum composition.

# RESULTS AND DISCUSSION

## Colostrum Parameters

Colostrum compositional changes of greatest interest were the treatment by day of storage comparisons for components analyzed. Statistical analysis of specific colostrum components are provided in Appendix Table 1. The table shows mean square expectations and significant F test comparisons.

Frozen colostrum exhibited minor compositional changes as anticipated and will not be discussed in depth. The relatively small changes seen in some parameters were probably due to sampling errors.

The pH (Fig. 2) showed a rapid decrease in N at day 5. Colostrum with FT was higher at day 5 than P colostrum, while at day 10 to day 20 the two treatments were similar. The P treatment stayed at a constant reduced pH throughout the 20 days. Other researchers (38, 42, 57, 74, 80, 84) have found the pH of naturally fermented colostrum decreases with time, but it may increase after 10 to 12 days of fermentation under high ambient temperatures (36). In the FT and P treatments, the pH has been found to be reduced initially and remain low with time of storage (36, 37, 43, 51, 59).

Titratable acidity expressed as percent acidity (Fig. 3) increased for N and FT diets with time, with N higher ($P < .01$) at day 15 and 20. At day 20 the N treatment was higher ($P < .01$) than the FT and P treatments. This agrees with work by Otterby and Dutton (43) who reported an increase in titratable acidity over 28 days of natural fermentation. In addition, our data show that P treatment


NATURAL
FROZEN
0
5
10
15
20
DAYS

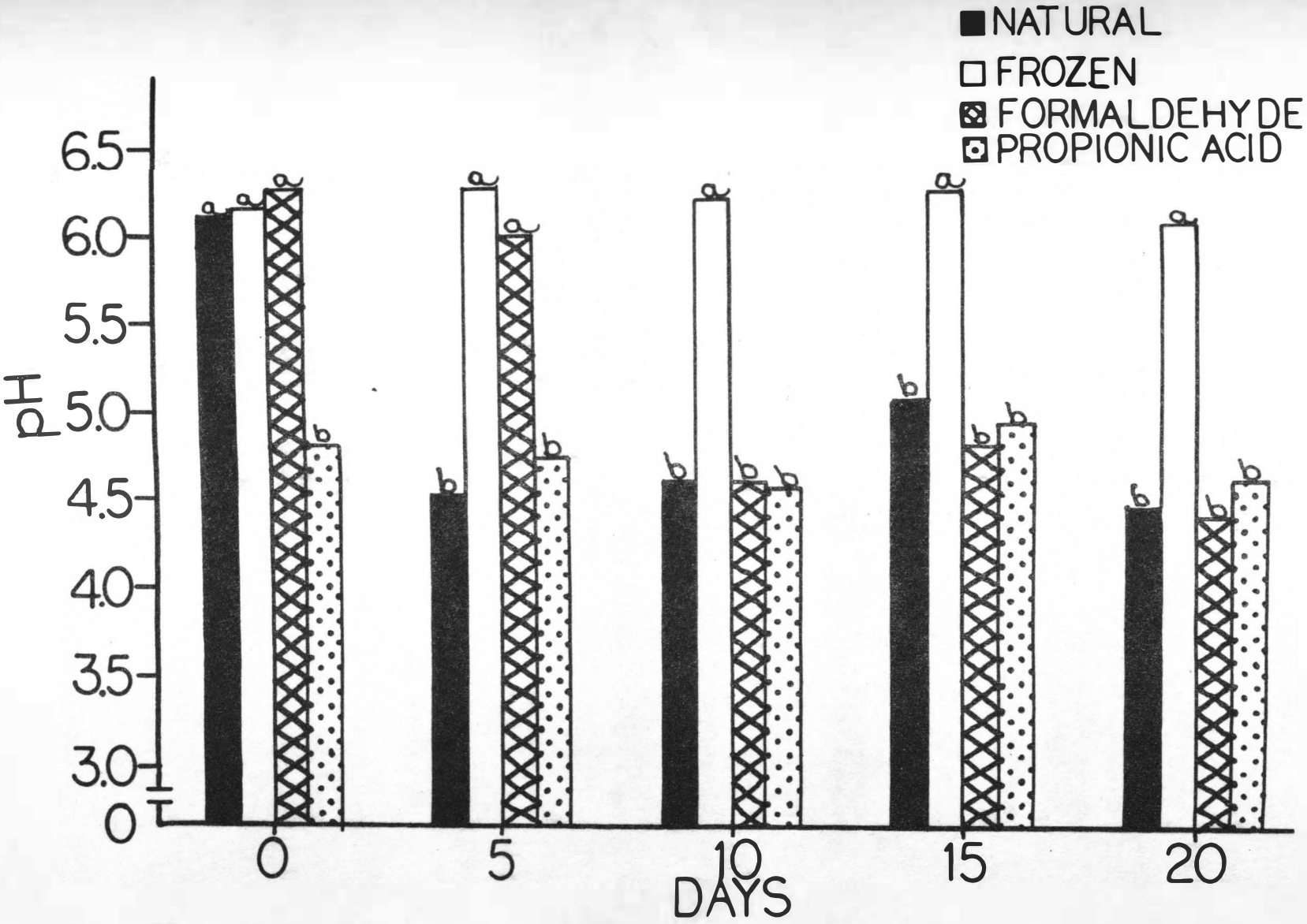
NATURAL
FROZEN
FORMALDEHYDE
PROPIONIC ACID
pH
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
0
0
5
10
15
20
DAYS

Figure 2.

FIG. 3. Influence of time on percent acidity of four colostrum treatments. Different superscripts within days indicate differences ($P < .01$).

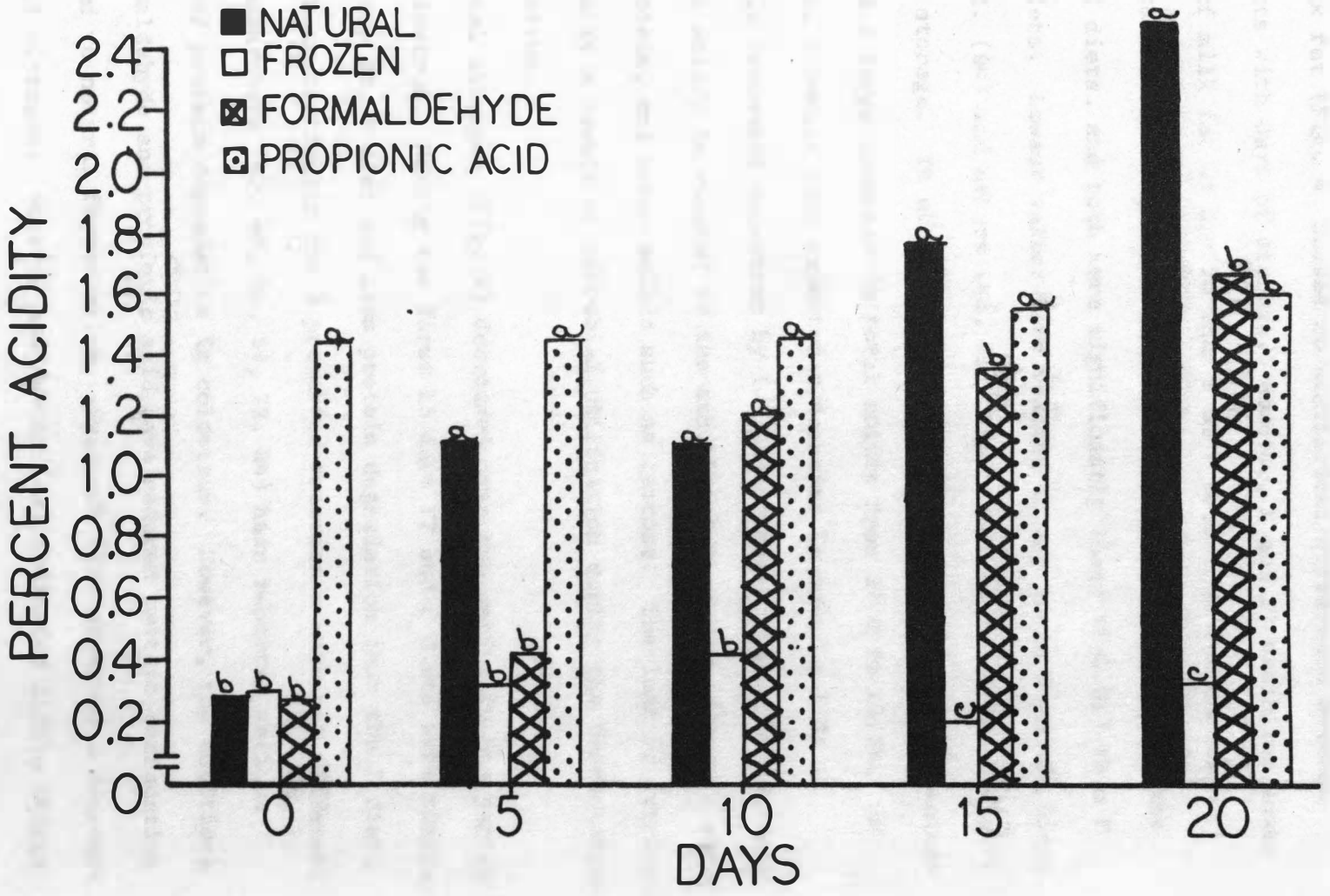
NATURAL
FROZEN
FORMALDEHYDE
PROPIONIC ACID
PERCENT ACIDITY
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0
5
10
15
20
DAYS

Figure 3.

held acidity constant at about 1.5% over days of storage.

Milk fat (Fig. 4) showed no statistical difference between treatments with days of storage. However, N and FT exhibited lower levels of milk fat at day 20 than F or P colostrum treatments.

Total solids (Fig. 5) decreased from day 10 to day 20 in the N and FT diets, and both were significantly lower ($P < .01$) than F and P diets. Lowest values were reached at day 20 in N and FT diets. Yu et al. (84) and others (43, 51) found total solids decreased with time of storage. Yu et al. (84) reported naturally fermented colostrum had a large decrease in total solids from 20.0 to 15.5%. In addition, Swannack (72) reported a decrease in total solids in naturally fermented colostrum by 1.5 percentage units. The decrease in total solids is related to the associated decreases found in milk fat, protein, and other solids such as lactose. The loss of nutrients is probably a result of microbial utilization during the fermentation of colostrum.

Total nitrogen (Fig. 6) decreased over the entire 20 day period in N colostrum. During the first 15 days FT and P diets were similar, but at day 20, P diet had less protein degradation than the F diet, although statistically the 3 preserved treatments were not different. Many researchers (42, 43, 51, 59, 72, 84) have reported various levels of protein degradation in colostrum. However, the additions of formaldehyde and propionic acid have reduced protein degradation compared to natural fermentation. Swannack (72) observed a decrease in total nitrogen. Otterby and Dutton (43) indicated little change

FIG. 4. Influence of time on milk fat of four colostrum treatments.

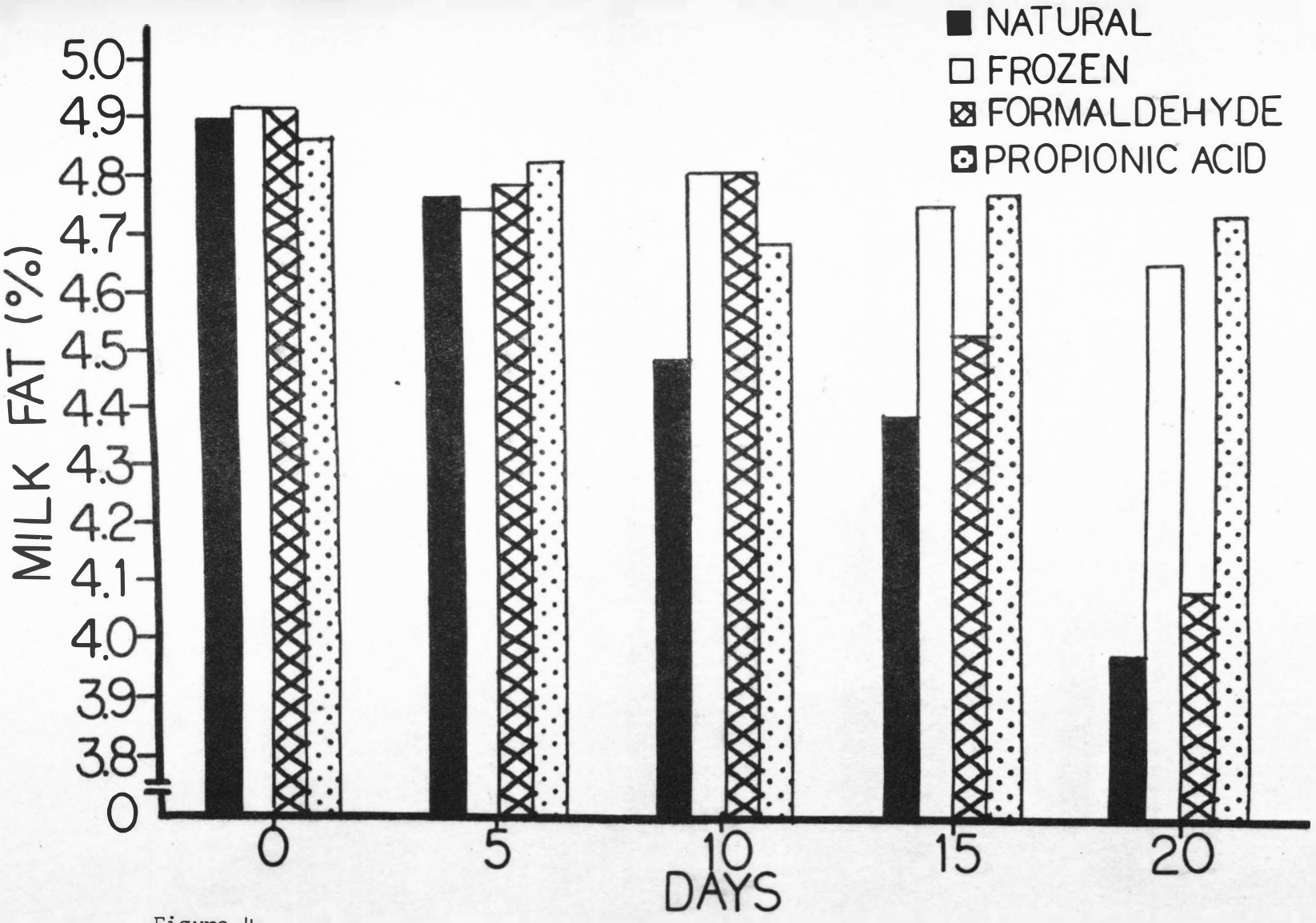
NATURAL
FROZEN
FORMALDEHYDE
PROPIONIC ACID
MILK FAT (%)
5.0
4.9
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4.0
3.9
3.8
0
0
5
10
15
20
DAYS

Figure 4.

FIG. 5. Influence of time on total solids of four colostrum treatments. Different superscripts within days indicate differences ($P<.01$).

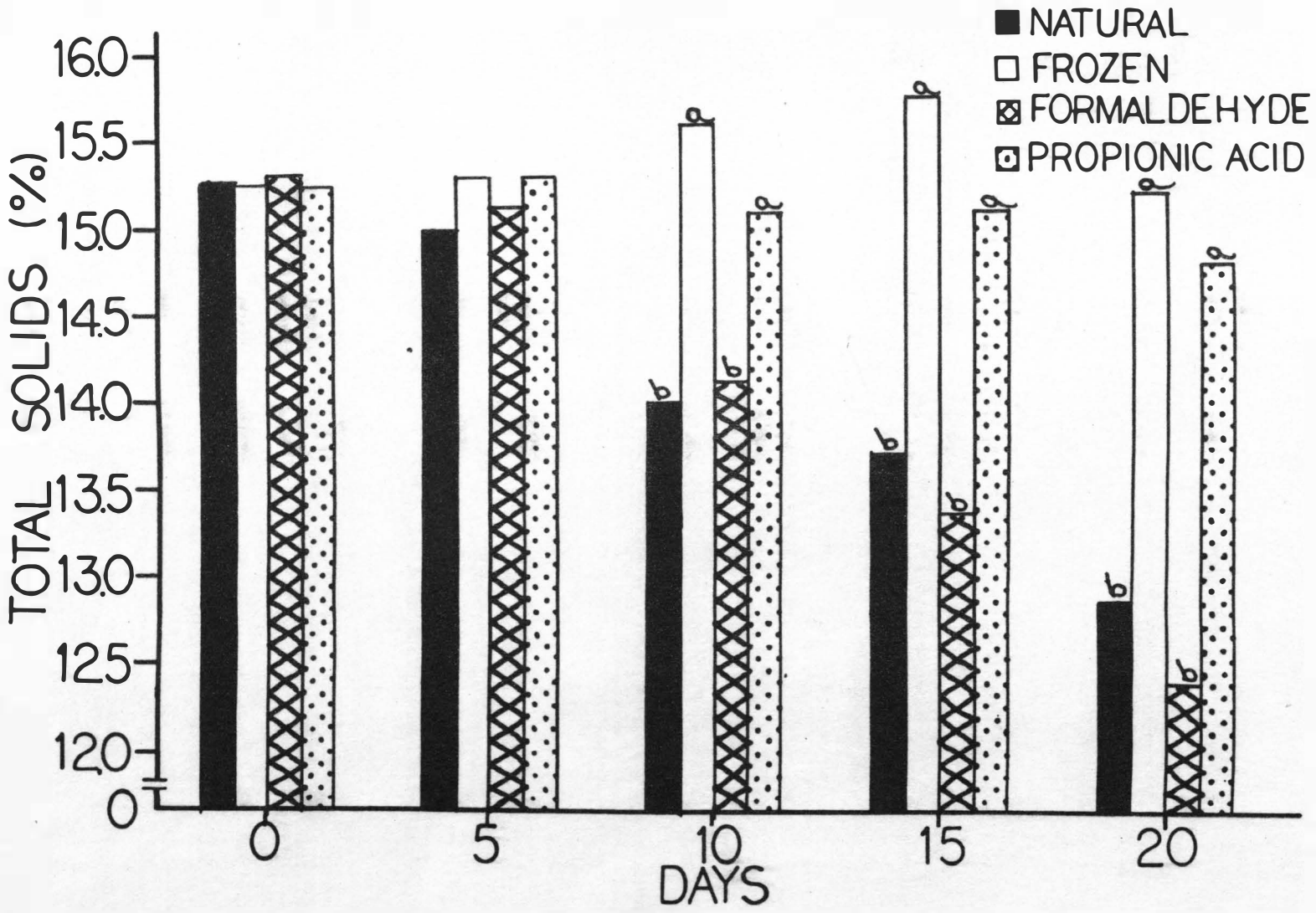
NATURAL
FROZEN
FORMALDEHYDE
PROPIONIC ACID
TOTAL SOLIDS (%)
16.0
15.5
15.0
14.5
14.0
13.5
13.0
12.5
12.0
0
0
5
10
15
20
DAYS

Figure 5.

FIG. 6. Influence of time on total nitrogen of four colostrum treatments. Different superscripts within days indicate differences ($P < .01$).

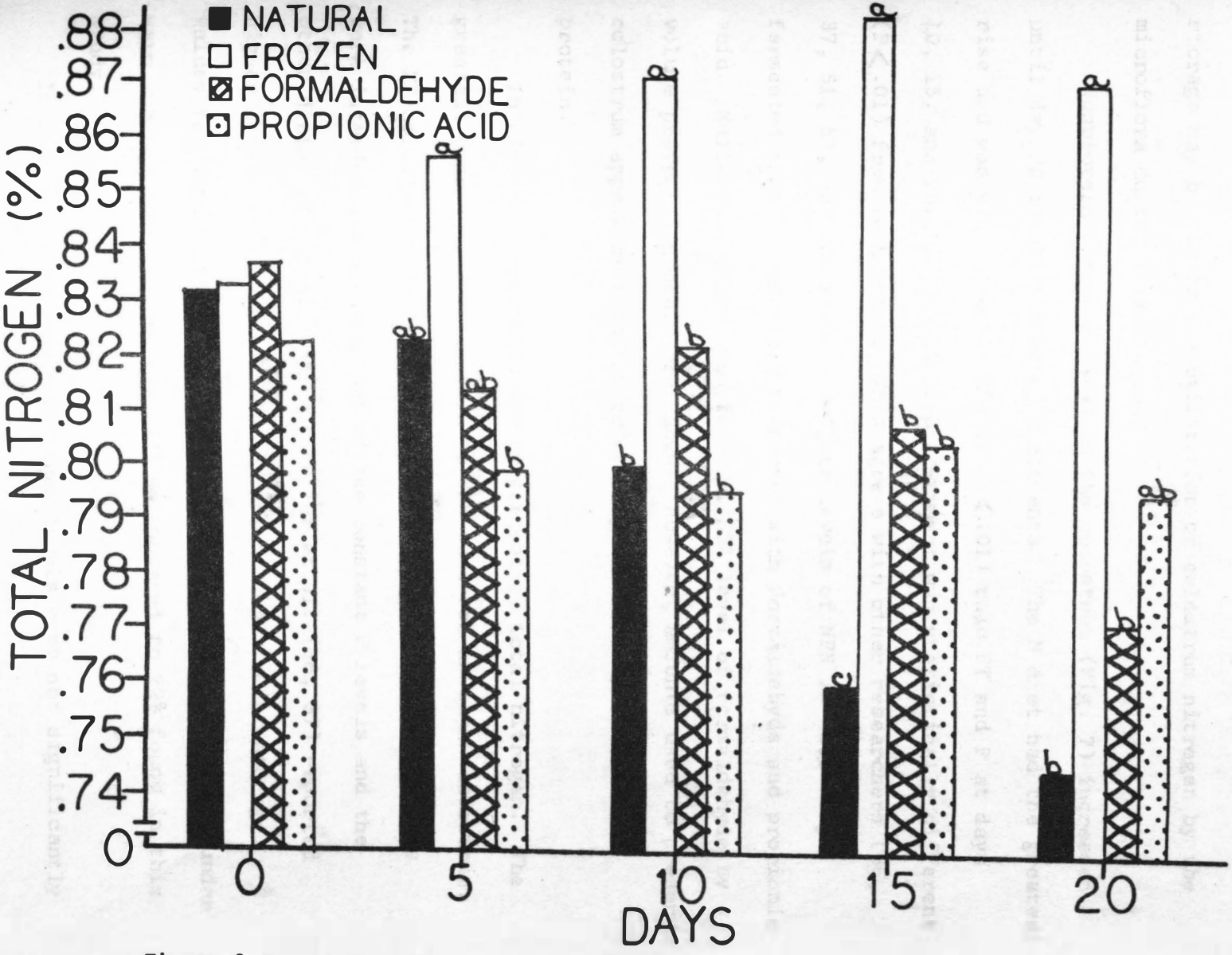
NATURAL
FROZEN
FORMALDEHYDE
PROPIONIC ACID
TOTAL NITROGEN (%)
.88
.87
.86
.85
.84
.83
.82
.81
.80
.79
.78
.77
.76
.75
.74
0
0
5
10
15
20
DAYS

Figure 6.

in total nitrogen with time, but considerable change was observed in the form of nitrogen. A decrease in total nitrogen with time of storage may be due to the utilization of colostrum nitrogen by the microflora during fermentation.

Nonprotein nitrogen (NPN) in the colostrum (Fig. 7) increased until day 20 in all preserved treatments. The N diet had the greatest rise and was significantly higher ($P < .01$) than FT and P at days 10, 15, and 20. At day 20 each treatment was statistically different ($P < .01$) from each other. This agrees with other researchers (36, 37, 51, 57, 59) who reported higher levels of NPN in naturally fermented than in colostrum preserved with formaldehyde and propionic acid. Muller and Syhre (36) found a .25% level of formaldehyde by volume prevented protein breakdown. However, amounts used to preserve colostrum appear critical in order to prevent overprotection of protein.

In Fig. 8 is presented NPN as percent of total nitrogen. The greatest degradation of protein to NPN occurred in the N colostrum. The FT colostrum paralleled these values while the P diet values were intermediate between that of the constant F levels and the other diets. Yu et al. (84) and Muller et al. (36, 37) reported higher levels of NPN as % TN in colostrum over time of storage. Values for naturally fermented colostrum ranged from 16 to 23% under warm ambient temperatures (36, 37) as compared to 22% found in this study.

In Fig. 9 the noncasein nitrogen levels were not significantly

FIG. 7. Influence of time on nonprotein nitrogen of four colostrum treatments. Different superscripts within days indicate differences ($P<.01$).

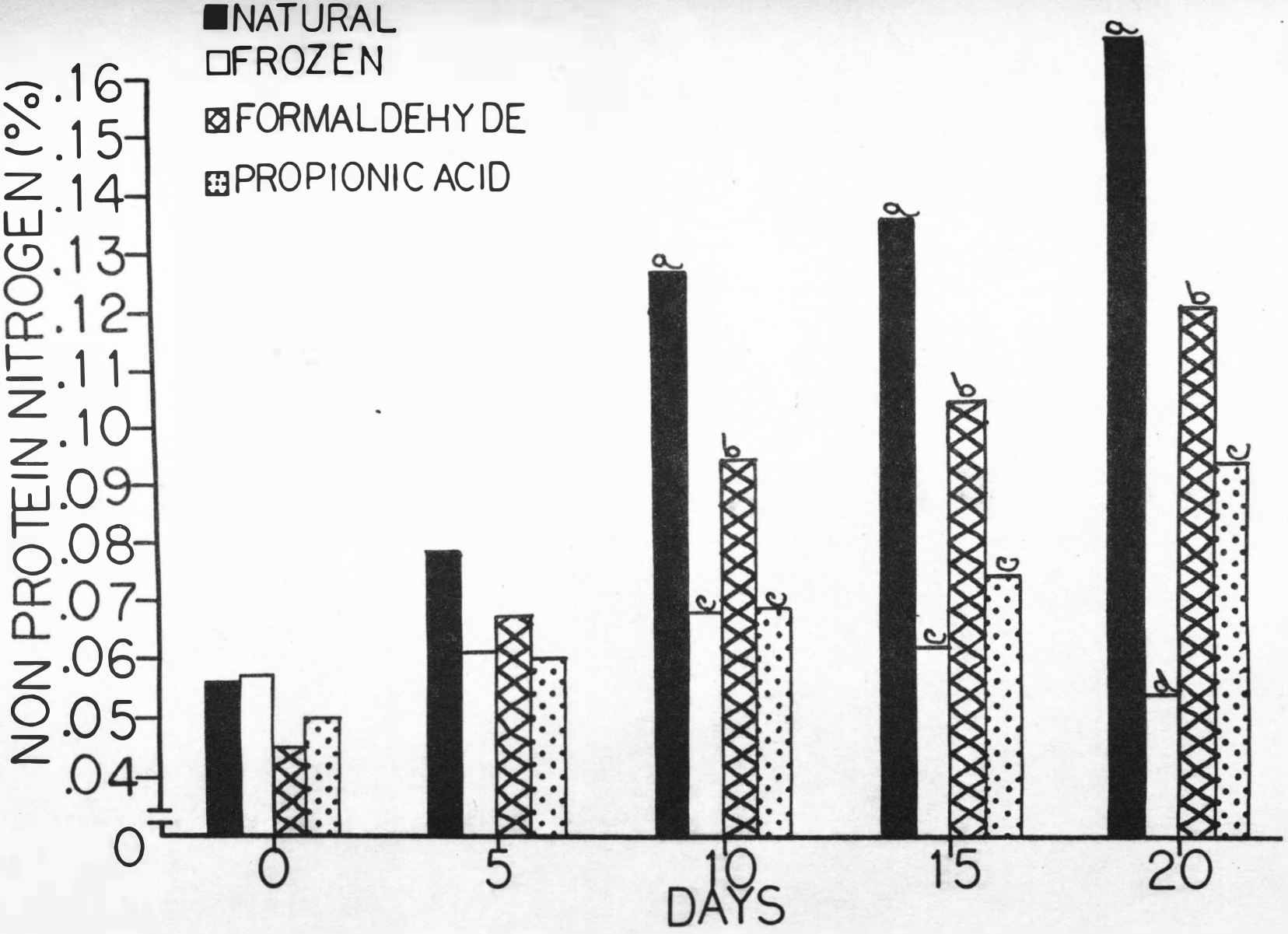
NATURAL
FROZEN
FORMALDEHYDE
PROPIONIC ACID
NON PROTEIN NITROGEN (%)
.16
.15
.14
.13
.12
.11
.10
.09
.08
.07
.06
.05
.04
0
0
5
10
15
20
DAYS
a
b
c
a
c
b
c
a
c
b
c
a
a
b
c

Figure 7.

FIG. 8. Influence of time on nonprotein nitrogen as a percent of total nitrogen of four colostrum treatments. Different superscripts within days indicate differences ($P < .01$).

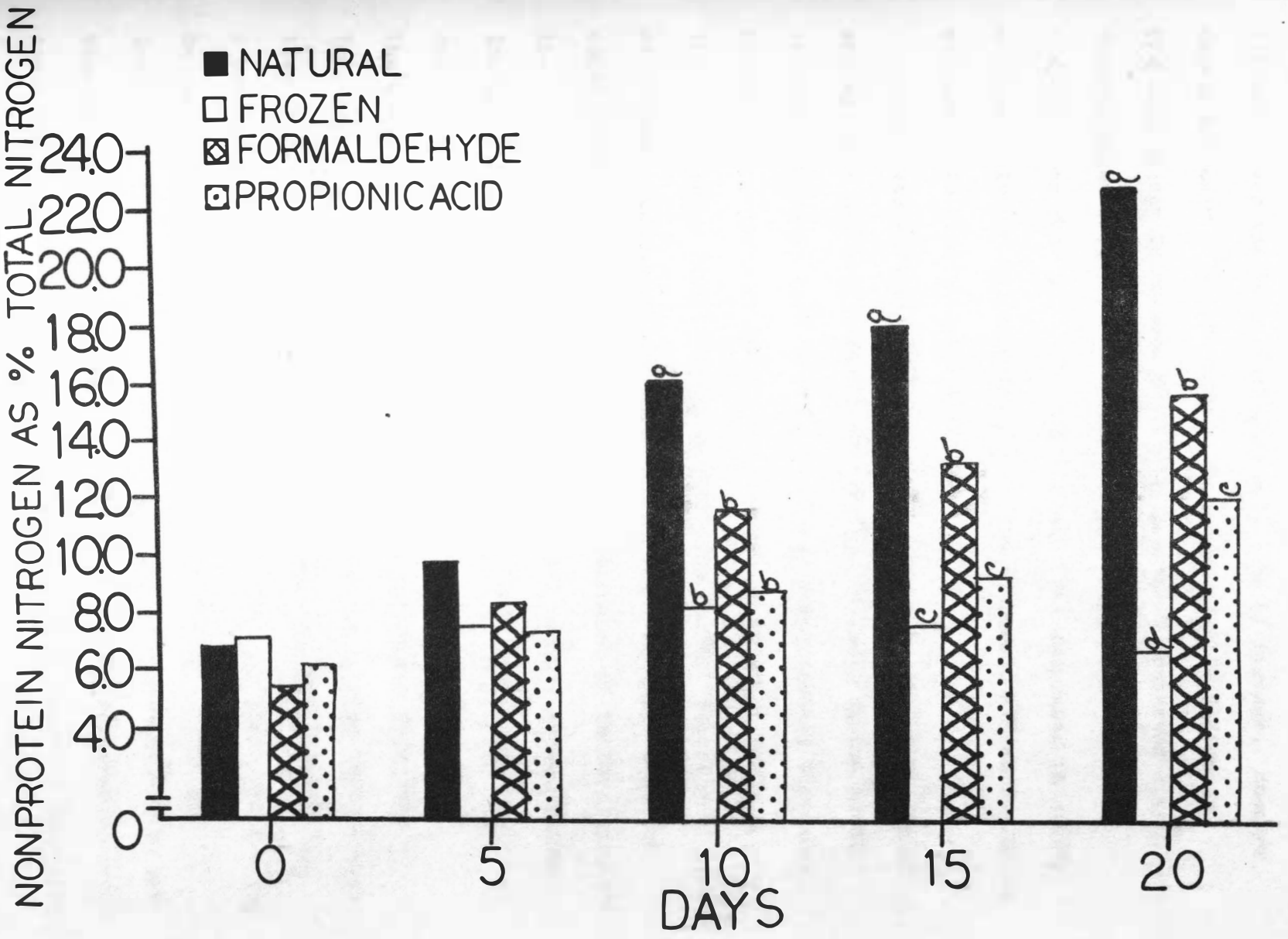
NONPROTEIN NITROGEN AS % TOTAL NITROGEN
NATURAL
FROZEN
FORMALDEHYDE
PROPIONIC ACID
24.0
22.0
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
0
0
5
10
15
20
DAYS
a
b
c

Figure 8.

different over the four treatments with time of storage. However, casein nitrogen (Fig. 10) values were statistically different ($P < .01$) at day 20 between the F diet and the 3 preserved diets. Whey nitrogen (Fig. 11) exhibited a significant decrease in N ($P < .01$) from that of F, FT, and P diets. The decreases in casein and whey nitrogen are associated with the increased NPN which can be attributed to microbial fermentation.

The average totals of individual immunoglobulins with time of storage were not statistically different. In Table 2, the total immunoglobulins in each treatment were not significantly different. Further comparisons of immunoglobulins are in Appendix Table 2. This table shows individual immunoglobulins concentrations over time of storage. Colostrum treatment effects are not separated. The nonsignificant differences may be due to a number of factors involved in colostral immunoglobulin concentrations. Some of these factors include different yields found in heifers' colostrum than cows' colostrum and circulating serum levels found in the cows (28). There was no apparent degradation of immunoglobulins with time. This result is not consistent with the decrease in whey protein with time (Fig. 10) and is not readily explained. The FT treatment had a nonsignificantly lower concentration. This lower concentration may be due to the protection of protein by formaldehyde which may have interfered with analysis. These results are not consistent with previously reported studies (6, 67). Plog et al. (50) reported no relationships between treatments of frozen colostrum, naturally fermented

FIG. 9. Influence of time on noncasein nitrogen of four colostrum treatments.

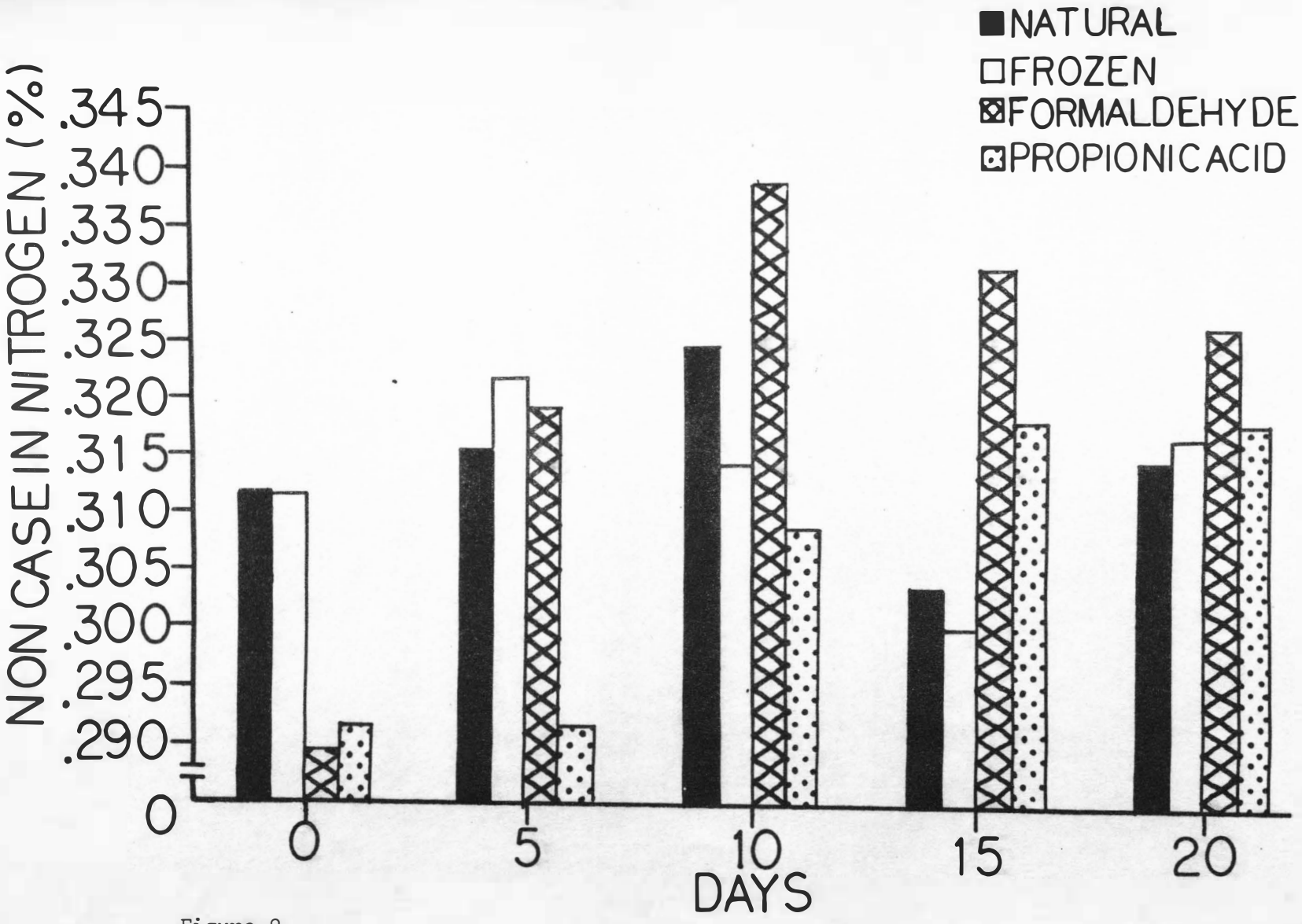
NATURAL
FROZEN
FORMALDEHYDE
PROPIONIC ACID
NON CASE IN NITROGEN (%)
.345
.340
.335
.330
.325
.320
.315
.310
.305
.300
.295
.290
0
0
5
10
15
20
DAYS

Figure 9.

FIG. 10. Influence of time on casein nitrogen of four colostrum treatments. Different superscripts within days indicate differences ($P < .01$).

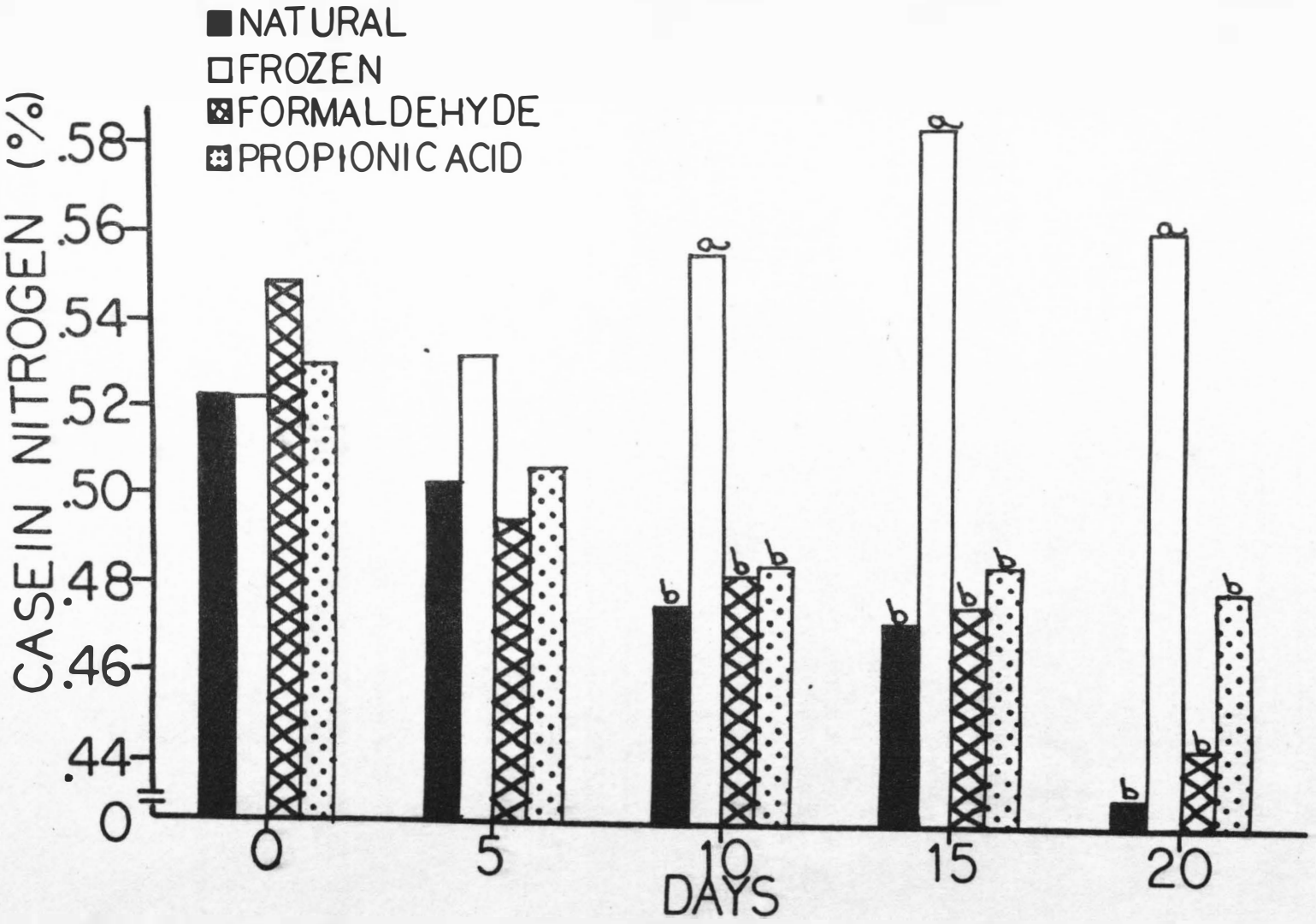
NATURAL
FROZEN
FORMALDEHYDE
PROPIONIC ACID
CASEIN NITROGEN (%)
.58
.56
.54
.52
.50
.48
.46
.44
0
0
5
10
15
20
DAYS
a
b

Figure 10.

FIG. 11. Influence of time on whey nitrogen of four colostrum treatments. Different superscripts within days indicate differences ($P < .01$).

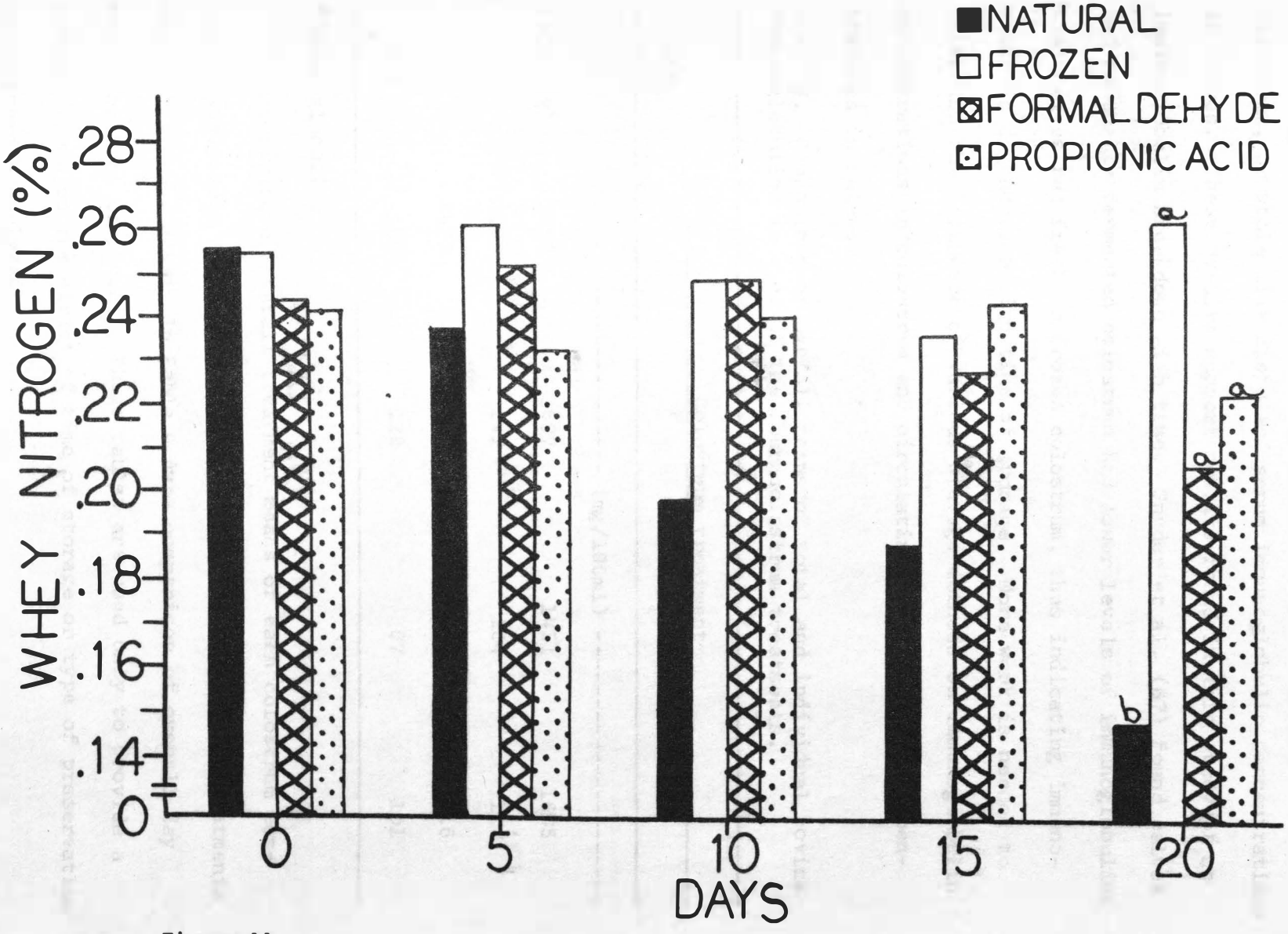
NATURAL
FROZEN
FORMALDEHYDE
PROPIONIC ACID
WHEY NITROGEN (%)
.28
.26
.24
.22
.20
.18
.16
.14
0
0
5
10
15
20
DAYS
a
b
a
a

Figure 11.

colostrum, or whole milk diets and serum immunoglobulin concentrations in calves. These results support results found in this study of no immunoglobulin breakdown with time. Snyder et al. (67) found calves fed naturally fermented colostrum had lower levels of immunoglobulins than calves fed fresh or frozen colostrum, thus indicating immunoglobulin degradation with time of storage. More work is needed to determine the influence of various storage methods on immunoglobulin concentrations in colostrum and circulating immunoglobulin concentrations in calves.

TABLE 2. Comparison of overall means of total and individual bovine immunoglobulins in whey of the four colostrum treatments.

| Item | Colostrum treatment | | | |
|---|---|---|---|---|
| | N | F | FT | P |
| | (mg/100ml) | | | |
| Total Ig[a] | 1659 | 1697 | 1451 | 1635 |
| IgA | 103 | 142 | 104 | 118 |
| IgG | 1452 | 1427 | 1260 | 1416 |
| IgM | 104 | 128 | 87 | 101 |

[a]Immunoglobulins

Comparisons of overall treatment means of each colostrum component analyzed are in Table 3. These means are values of treatments over all storage days. In Table 4 is a comparison of overall day means for each component. These tables are used only to provide a general trend on the effect of time of storage or type of preservation on components analyzed.

TABLE 3. Comparison of overall treatment means over storage time for each component.

| Treatment | pH | T.A. | Fat | T.S. | T.N. | NCN | NPN | C.N. | W.N. | NPN/TN |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | % | | | | | |
| Natural | $4.89^{bc}$ | $1.35^{ab}$ | 4.50 | 14.16 | $.7926^{B}$ | .3141 | .1128 | .4785 | .2053 | $14.60^{A}$ |
| Frozen | $6.18^{a}$ | $0.33^{b}$ | 4.77 | 15.46 | $.8622^{A}$ | .3128 | .0607 | .5515 | .2518 | $7.30^{B}$ |
| Formaldehyde | $5.14^{b}$ | $0.98^{ab}$ | 4.62 | 14.07 | $.8104^{B}$ | .3209 | .0863 | .4891 | .2350 | $10.80^{AB}$ |
| Propionic acid | $4.66^{c}$ | $1.51^{a}$ | 4.77 | 15.11 | $.8031^{B}$ | .3052 | .0694 | .4979 | .2358 | $8.74^{AB}$ |

$^{A,B}$Means with different superscripts are different ($P<.05$).
$^{a,b,c}$Means with different superscripts are different ($P<.01$).

TABLE 4. Comparison of overall day means over treatments for each component.

| | pH | T.A. | Fat | T.S. | T.N. | NCN | NPN | C.N. | W.N. | NPN/TN |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | % | | | | |
| Day 0 | $5.83^{A}$ | $0.59^{B}$ | $4.90^{a}$ | 15.28 | .8324 | .3015 | $.0525^{Cb}$ | .5309 | .2491 | $6.39^{Cb}$ |
| Day 5 | $5.35^{AB}$ | $0.83^{AB}$ | $4.77^{ab}$ | 15.18 | .8231 | .3120 | $.0667^{BCab}$ | .5110 | .2453 | $8.25^{ACab}$ |
| Day 10 | $5.04^{B}$ | $1.04^{AB}$ | $4.69^{ab}$ | 14.72 | .8216 | .3215 | $.0892^{ABab}$ | .4996 | .2326 | $11.10^{ABab}$ |
| Day 15 | $5.00^{B}$ | $1.23^{AB}$ | $4.60^{ab}$ | 14.49 | .8128 | .3131 | $.0942^{Aab}$ | .5007 | .2234 | $11.97^{Aab}$ |
| Day 20 | $4.88^{B}$ | $1.51^{A}$ | $4.35^{b}$ | 13.84 | .7954 | .3181 | $.1087^{Aa}$ | .4791 | .2094 | $14.09^{Aa}$ |

$^{A,B,C}$Means with different superscripts are different ($P<.05$).
$^{a,b}$Means with different superscripts are different ($P<.01$).

Calf Blood Parameters

Statistical analysis of specific serum components are provided in Appendix Tables 3 and 4. These tables show mean square expectations and significant F test comparisons.

Figures 12 through 19 contain data on serum constituents from calves fed the 4 diets. There were no significant statistical differences between treatments for any serum constituents analyzed. This was due to the large variations between individuals within treatments.

Weekly serum glucose concentrations (Fig. 12) ranged from 63 to 100 mg/100 ml of serum. The concentrations were within the normal range reported by Vagher et al.(77). Over the 3 wk period, calves fed N and FT diets tended toward an overall decrease in serum glucose which may be due to the decreased lactose and other major nutrients in these colostrums. These lower levels in colostrum may be caused by the loss of nutrients to the microbial population during fermentation. With time after feeding, serum glucose concentrations (Fig. 13) increased for all treatments. The increases in glucose followed a pattern similar to that found by Reece and Wahlstrom (55) where concentrations peaked at 4 h postfeeding and increased with time after feeding.

Weekly serum urea nitrogen (SUN) values (Fig. 14) ranged from 9 to 16 mg/100 ml of serum. Although concentrations were not significantly different between treatments fed, a correlation of .42 existed between the nonprotein nitrogen (NPN) levels in the colostrum diets and the associated SUN levels found in calves fed these

FIG. 12. Influence of time on serum glucose from calves fed four colostrum treatments.

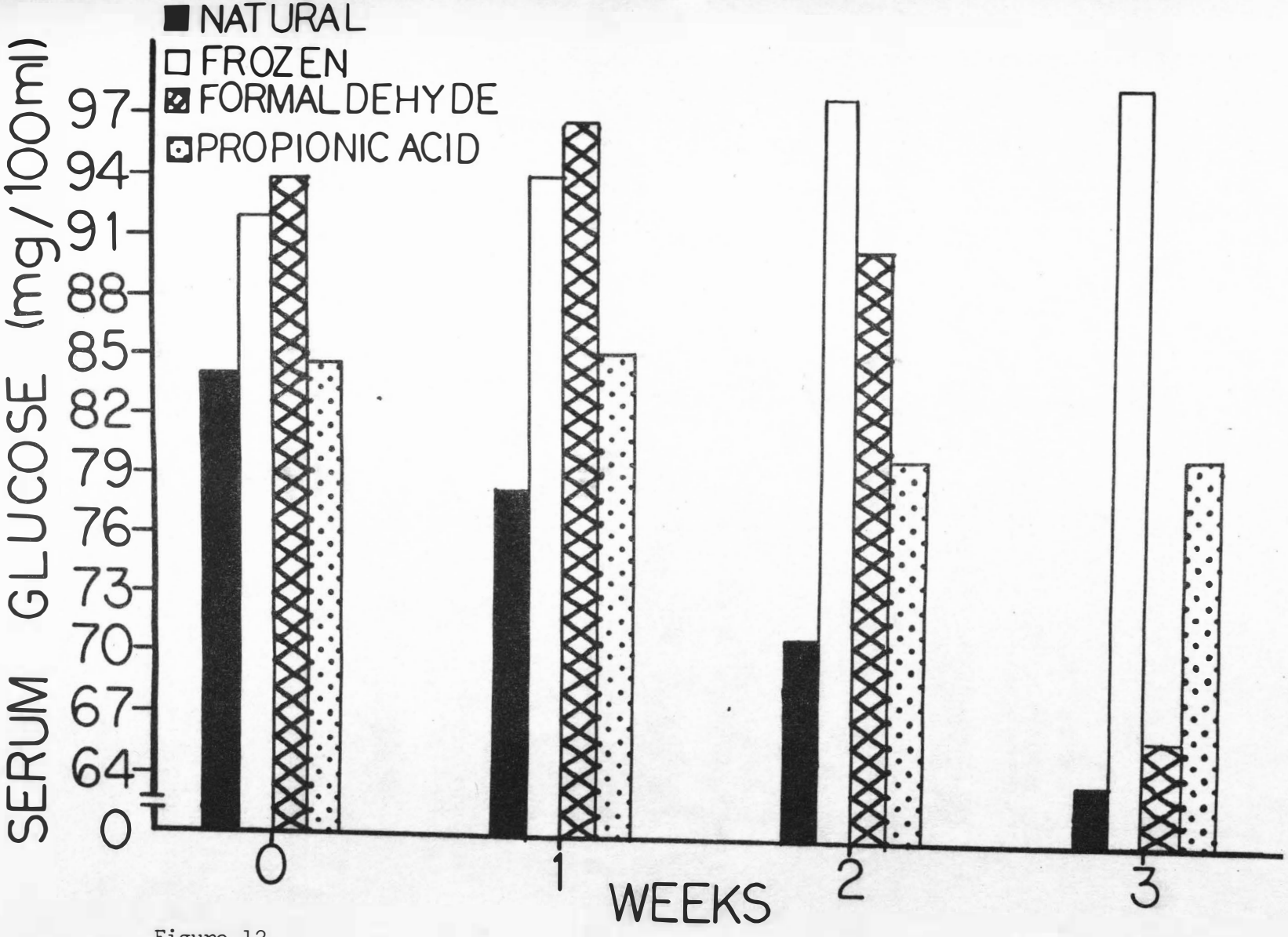
NATURAL
FROZEN
FORMALDEHYDE
PROPIONIC ACID
SERUM GLUCOSE (mg/100ml)
97
94
91
88
85
82
79
76
73
70
67
64
0
0
1
2
3
WEEKS

Figure 12.

FIG. 13. Influence of time on serum glucose from calves fed four colostrum treatments.

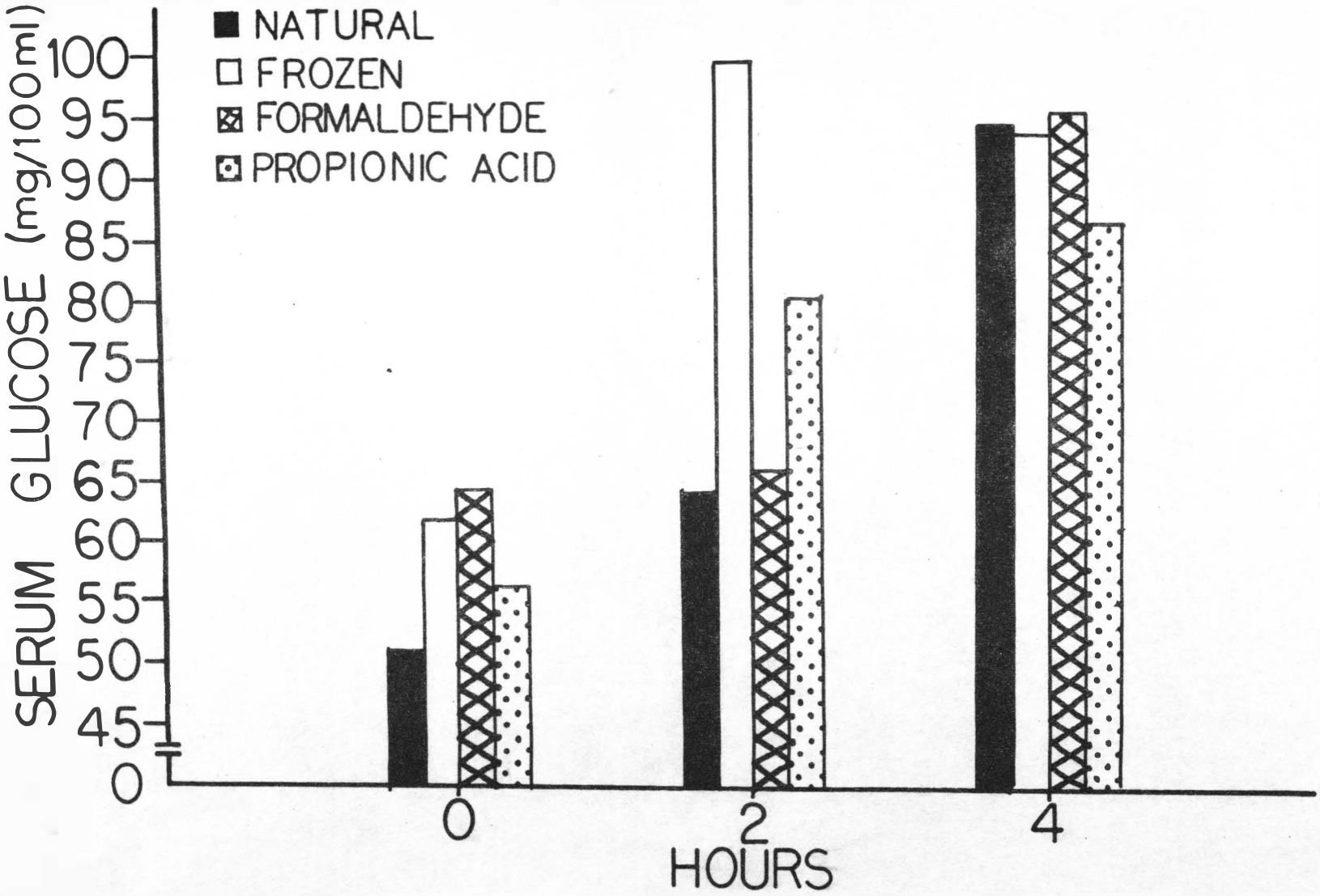
NATURAL
FROZEN
FORMALDEHYDE
PROPIONIC ACID
SERUM GLUCOSE (mg/100ml)
100
95
90
85
80
75
70
65
60
55
50
45
0
0
2
4
HOURS

Figure 13.

FIG. 14. Influence of time on serum urea nitrogen from calves fed four colostrum treatments.

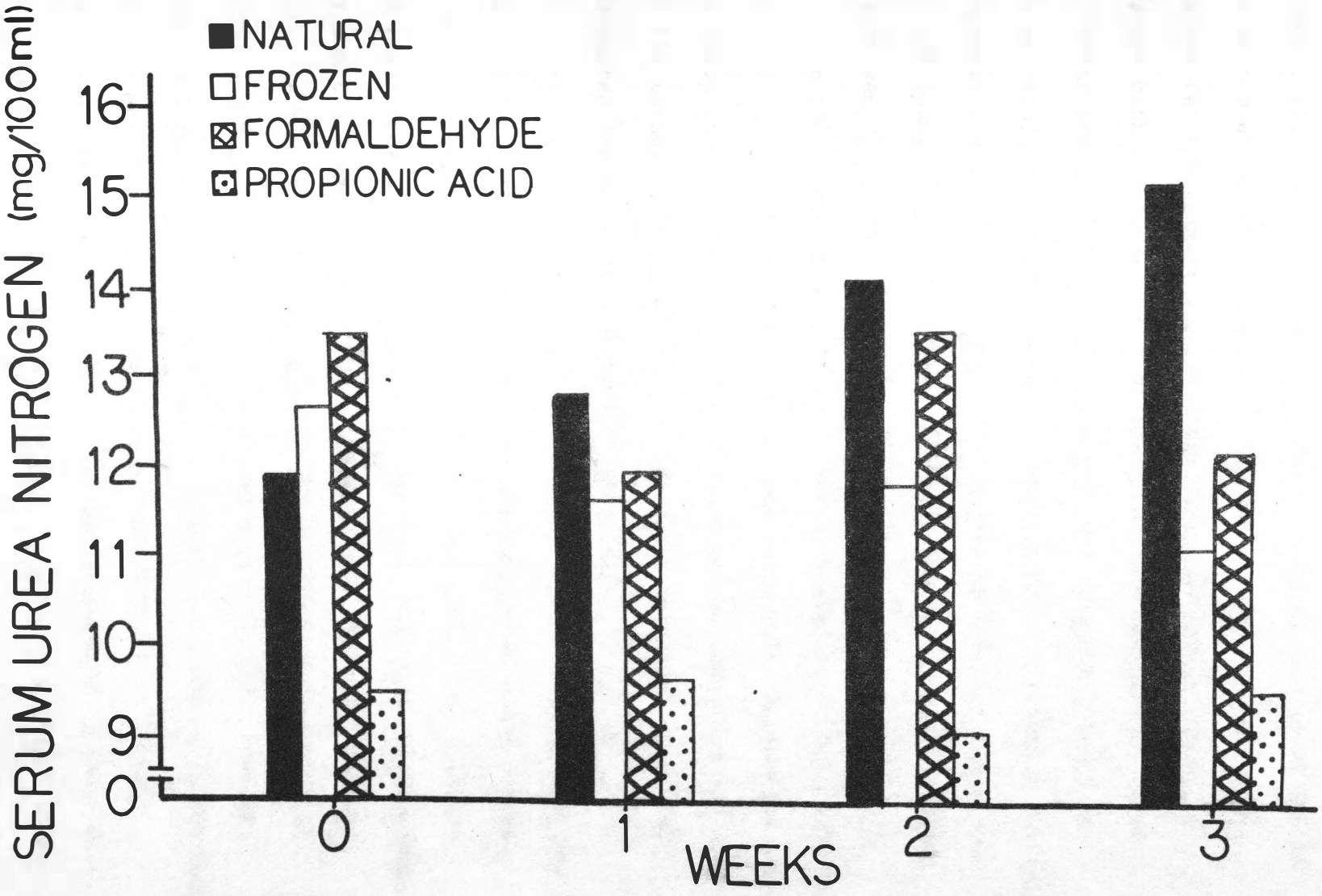
NATURAL
FROZEN
FORMALDEHYDE
PROPIONIC ACID
SERUM UREA NITROGEN (mg/100ml)
16
15
14
13
12
11
10
9
0
0
1
2
3
WEEKS

Figure 14.

treatments. The N treatment depicted the highest levels of NPN in colostrum while SUN levels were highest in calves fed this diet. Calves fed P treatment exhibited the lowest values in SUN which agrees with the lower levels of NPN found in P colostrum. These patterns are consistent with the higher SUN frequently found in mature ruminants fed high levels of dietary NPN. A stepwise multiple regression analysis was computed on factors influencing the levels of SUN found in calves. The regression equation was mg of SUN/100 ml of serum = 10.14 + 50.95(NPN) - 2.08(TA).

In 1948, Parrish et al. (49) reported a similar relationship in dairy cows. If the cows had an increase in protein consumption, the increase would raise the NPN levels found in the colostrum and also in the serum. Parrish et al. (49) indicated that the serum urea accounted for two-thirds or more of the NPN.

With time after feeding (Fig. 15) the N treatment depicted the highest levels of SUN while FT treatment paralleled these values. The P treatment showed the lowest levels although P had a larger increase from 2 to 4 h postfeeding than that of F which was constant at about 9.5 mg/100 ml. These SUN levels were within the normal range of 0 to 28 mg/100 ml reported by Watt (79), Thornton et al. (75), and Vagher et al. (77) for calves 0 to 4 wk old. However, Reece and Wahlstrom (55) reported no significant increase in SUN concentrations with time after feeding in calves fed milk or milk replacers. These diets and SUN values are comparable to the F diet.

Serum protein levels were unchanged (Fig. 16 and 17). Values

FIG. 15. Influence of time on serum urea nitrogen from calves fed four colostrum treatments.

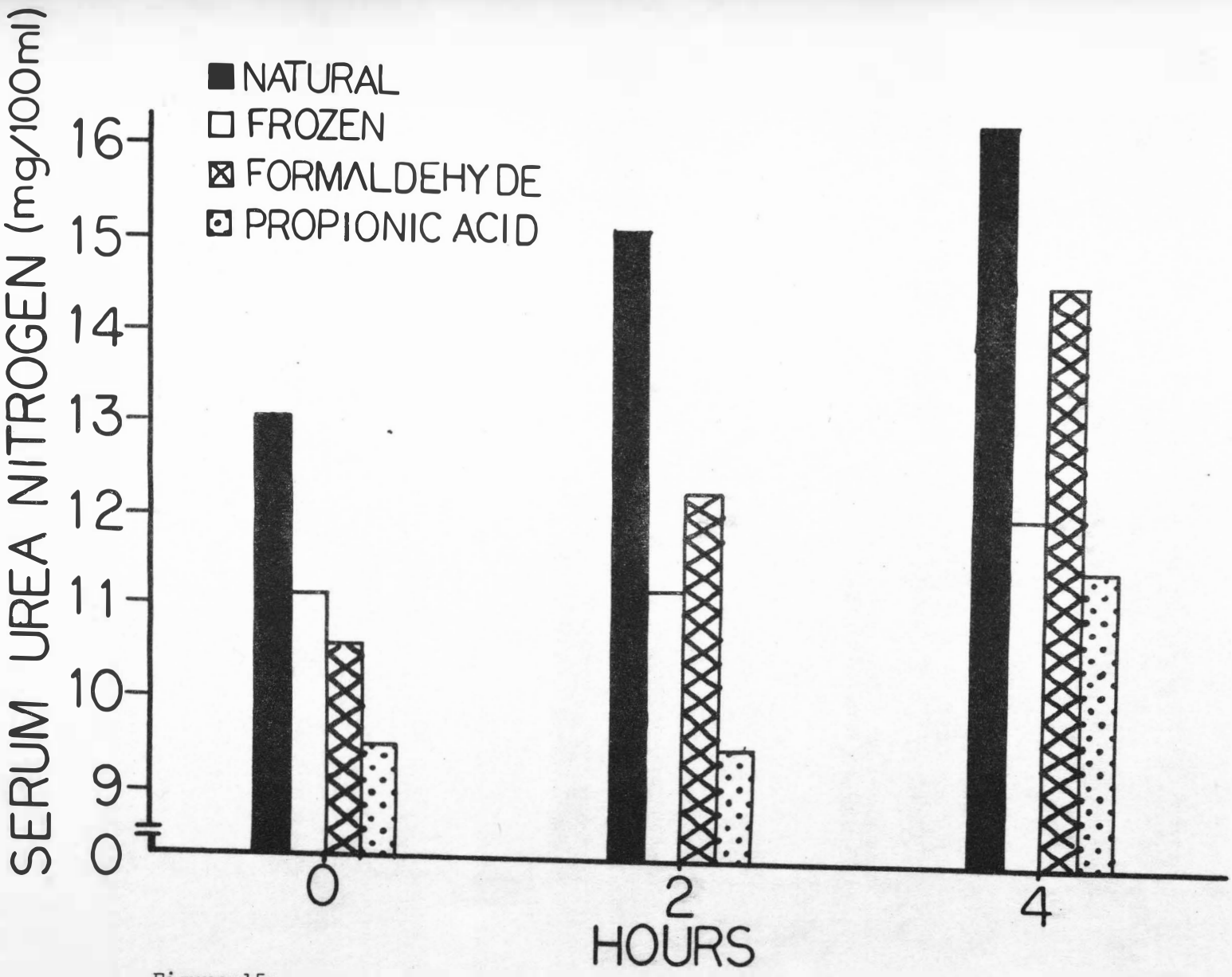
SERUM UREA NITROGEN (mg/100ml)
NATURAL
FROZEN
FORMALDEHYDE
PROPIONIC ACID
16
15
14
13
12
11
10
9
0
0
2
4
HOURS

Figure 15.

FIG. 16. Influence of time on serum protein from calves fed four colostrum treatments.

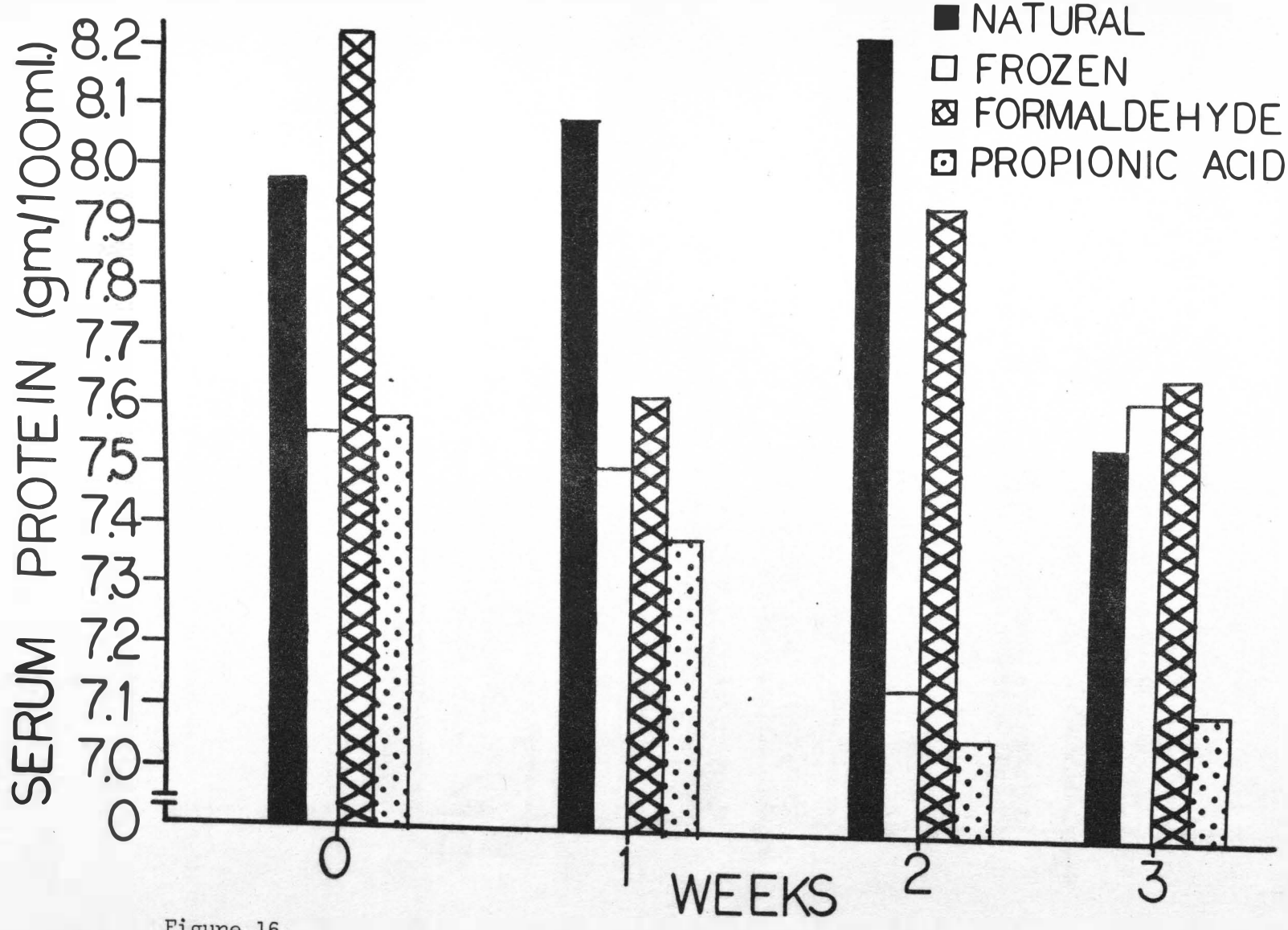
NATURAL
FROZEN
FORMALDEHYDE
PROPIONIC ACID
SERUM PROTEIN (gm/100ml.)
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
0
0
1
2
3
WEEKS

Figure 16.

FIG. 17. Influence of time on serum protein from calves fed four colostrum treatments.

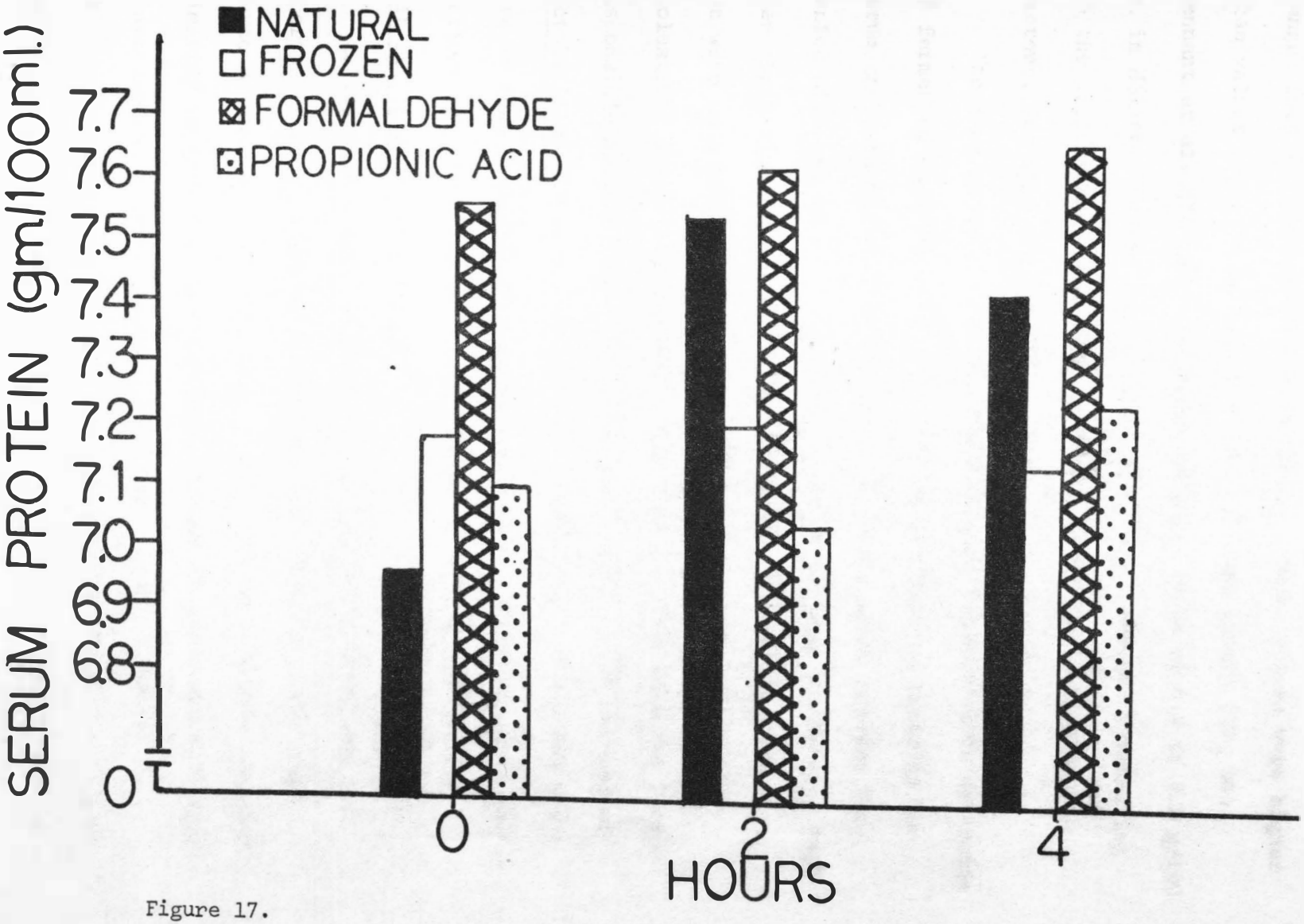
NATURAL
FROZEN
FORMALDEHYDE
PROPIONIC ACID
SERUM PROTEIN (gm/100ml.)
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
0
0
2
4
HOURS

Figure 17.

HOURS
4
2
0
SERUM PROTEIN (gm/100ml)
6
5
4
3
2
1
0
BARBITURIC ACID
ETHANOL
POLYHEMOGLOBIN
DEXTRAN
NEMOKID
ARGUTAN

ranged from 7.1 to 8.2 g/100 ml of serum. These values were higher than values reported by Vagher et al. (77) and others (27, 60). Tennant et al. (73) reported serum protein levels of 4.0 to 9.2 g/100 ml in Holstein calves from 1 to 35 days old. Factors contributing to the differences may be age, plane of nutrition, environmental factors, and time after feeding.

The last serum constituent analyzed was cholesterol to determine if fermented colostrum has any significant effect on lowering the serum cholesterol level in the calf. A relationship between fermented milk and serum cholesterol was found by Mann and Spoerry (30) when monitoring serum cholesterol levels in African men. The men were on a high milk diet and gained weight but their serum cholesterol levels were lowered. The milk may have been the factor responsible because the milk was fermented with a wild culture of Lactobacillus. However, in the calf trials (Fig. 18 and 19) there were no real trends found between the dietary treatments and concentrations of serum cholesterol by week or by hour after feeding.

Calf Performance Measurements

Calves were weighed each week. Average daily gains are not reported because calves were offered liquid diets as their only source of nutrients and thus lost weight. The trials were designed primarily to determine compositional changes in colostrum with time of storage and metabolic variations in calves fed different colostrum treatments, and not to evaluate calf performance. Digestion trials were conducted, but with the difficulty encountered in

FIG. 18. Influence of time on serum cholesterol from calves fed four colostrum treatments.

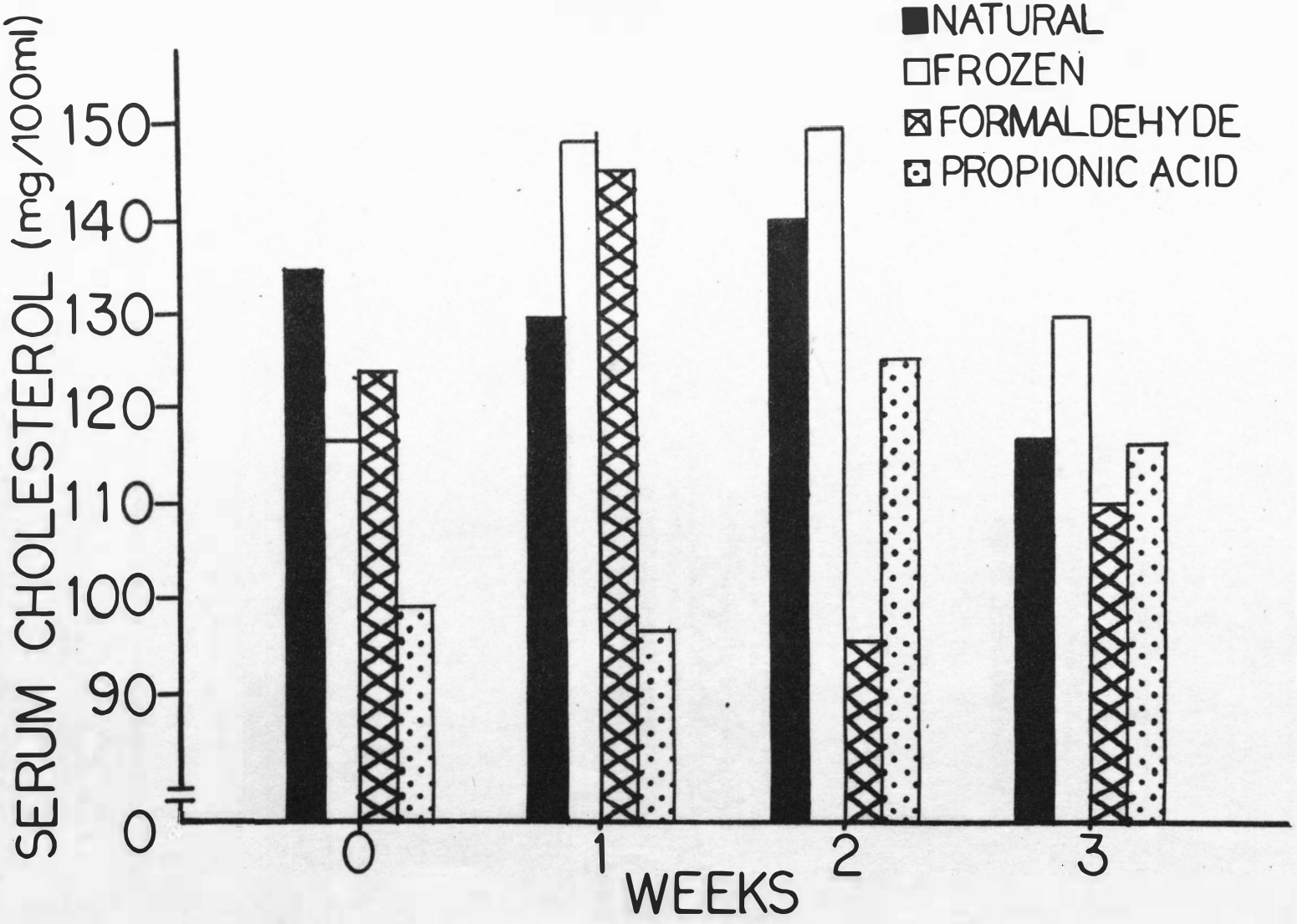
NATURAL
FROZEN
FORMALDEHYDE
PROPIONIC ACID
SERUM CHOLESTEROL (mg/100ml)
150
140
130
120
110
100
90
0
0
1
2
3
WEEKS

Figure 18.

FIG. 19. Influence of time on serum cholesterol from calves fed four colostrum treatments.

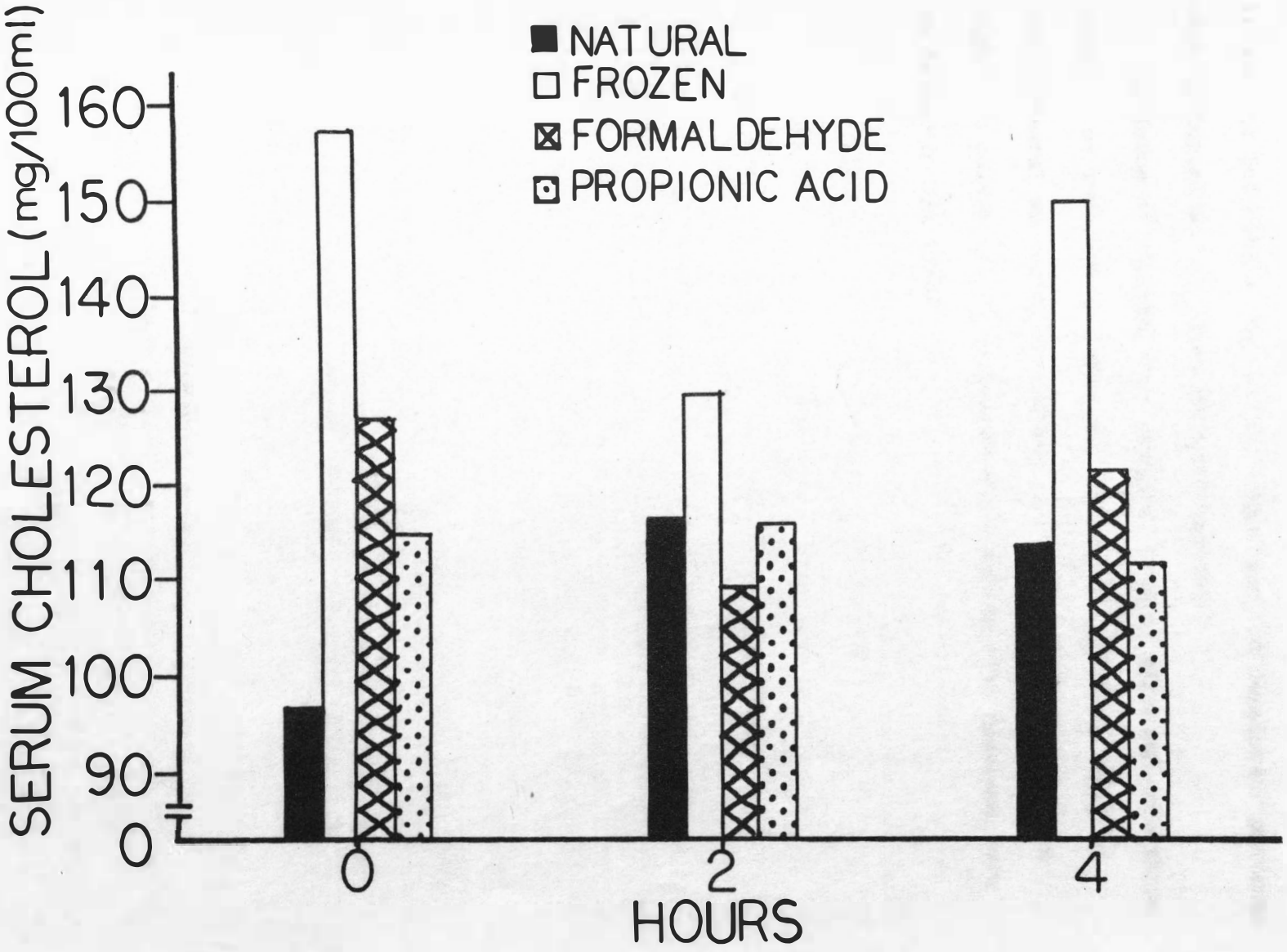
NATURAL
FROZEN
FORMALDEHYDE
PROPIONIC ACID
SERUM CHOLESTEROL (mg/100ml)
160
150
140
130
120
110
100
90
0
0
2
4
HOURS

Figure 19.

fecal and urine collection, considerable variation was found between treatments and within treatments. Therefore, no consistent patterns were indicated and the data are not reported.

Incidence of diarrhea was recorded. Calves affected were those bought from surrounding farms and may have been due to changing environmental surroundings. However, incidence of diarrhea was higher in calves fed FT treatment which agrees with previous work by Muller et al. (37).

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# APPENDIX

APPENDIX TABLE 1. Analysis of variance for 4 x 5 x 5 factorial.

| Source | Degrees of freedom | Estimated mean squares | pH | T.A. | Fat | T.S. | T.N. | NCN | NPN | C.N. | W.N. | NPN/TN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean squares | | | | | | | | | |
| Total | 95 | | | | | | | | | | | |
| Replication | 4 | $\sigma^2_e + td\sigma^2_R$ | 0.09 | 35.12 | 0.47 | 4.78 | 0.12 | 0.04 | 0.002 | 0.02 | 0.05 | 67.96 |
| Day [a] | 4 | $\sigma^2_e + t\sigma^2_{RD} + rt\sigma^2_D$ | 2.75** | 235.85** | 0.67** | 5.67** | 0.003* | 0.001 | 0.009** | 0.006* | 0.005* | 171.90** |
| Rep. x day | 16 | $\sigma^2_e + t\sigma^2_{RD}$ | 0.19 | 14.98 | 0.05 | 0.74 | 0.001 | 0.002 | 0.0004 | 0.002 | 0.002 | 6.96 |
| Treatment [a] | 3 | $\sigma^2_e + d\sigma^2_{TR} + rd\sigma^2_T$ | 9.28** | 575.48** | 0.34 | 9.54** | 0.02** | 0.001 | 0.01** | 0.02** | 0.008* | 230.74** |
| Rep. x trmt. | 11 | $\sigma^2_e + d\sigma^2_{TR}$ | 0.05 | 13.93 | 0.24 | 1.02 | 0.001 | 0.002 | 0.001 | 0.002 | 0.002 | 13.60 |
| Day x trmt. | 12 | $\sigma^2_e + r\sigma^2_{DT}$ | 1.13** | 82.81** | 0.12 | 1.64** | 0.002** | 0.0006 | 0.002** | 0.003** | 0.002* | 33.41** |
| Error(rep. x day x trmt.) | 44 | $\sigma^2_e$ | 0.04 | 5.85 | 0.07 | 0.34 | 0.0005 | 0.0009 | 0.0002 | 0.001 | 0.001 | 3.26 |

[a] fixed variables
* $P < .05$
** $P < .01$

APPENDIX TABLE 2. Comparison of overall means of individual bovine immunoglobulins with days of storage.

| | Days | | | | |
|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | 20 |
| | (mg/100ml) | | | | |
| IgA | 108.16 | 115.88 | 105.66 | 123.65 | 129.55 |
| IgG | 1225.75 | 1181.60 | 1673.20 | 1190.70 | 1672.15 |
| IgM | 121.12 | 129.48 | 114.85 | 74.53 | 85.13 |

APPENDIX TABLE 3. Analysis of variance for 4 x 4 x 5 factorial.

| Source | Degrees of freedom | Estimated mean squares | Serum glucose | Serum urea nitrogen | Serum protein |
|---|---|---|---|---|---|
| | | | ----------Mean squares---------- | | |
| Total | 80 | | | | |
| Replication | 4 | $\sigma^2_e + wt\sigma^2_R$ | 612.72 | 1.99 | 8.22 |
| Week[a] | 3 | $\sigma^2_e + t\sigma^2_{RW} + rt\sigma^2_W$ | 545.14 | 0.74 | 1.92 |
| Rep. x week | 12 | $\sigma^2_e + t\sigma^2_{RW}$ | 745.52 | 0.23 | 4.93 |
| Treatment[a] | 3 | $\sigma^2_e + w\sigma^2_{RT} + wr\sigma^2_T$ | 1625.01 | 2.39 | 63.76** |
| Rep. x trmt. | 12 | $\sigma^2_e + w\sigma^2_{RT}$ | 1031.88 | 6.04 | 11.69 |
| Week x trmt. | 9 | $\sigma^2_e + r\sigma^2_{WT}$ | 305.87 | 0.23 | 5.00 |
| Error(rep. x week x trmt.) | 36 | $\sigma^2_e$ | 640.50 | 0.27 | 3.86 |

[a]Fixed variables
*$P < .05$

APPENDIX TABLE 4. Analysis of variance for 4 x 3 x 5 factorial.

| Source | Degrees of freedom | Estimated mean squares | Serum glucose | Serum urea nitrogen | Serum protein |
|---|---|---|---|---|---|
| | | | ----------Mean squares---------- | | |
| Total | 60 | | | | |
| Replication | 4 | $\sigma_e^2 + ht\sigma_R^2$ | 1649.96 | 13.99 | 2.46 |
| Hour[a] | 2 | $\sigma_e^2 + t\sigma_{RH}^2 + rt\sigma_H^2$ | 5885.67 | 33.70** | 0.19 |
| Rep. x hour | 8 | $\sigma_e^2 + t\sigma_{RH}^2$ | 1043.07 | 3.64 | 0.13 |
| Treatment[a] | 3 | $\sigma_e^2 + h\sigma_{RT}^2 + rh\sigma_T^2$ | 613.13 | 56.97 | 0.66 |
| Rep. x trmt. | 12 | $\sigma_e^2 + h\sigma_{RT}^2$ | 1016.50 | 28.43 | 4.34 |
| Hour x trmt. | 6 | $\sigma_e^2 + r\sigma_{HT}^2$ | 499.63 | 2.95 | 0.11 |
| Error(rep. x hour x trmt.) | 24 | $\sigma_e^2$ | 510.05 | 1.74 | 0.08 |

[a]Fixed variables
*P<.05
**P<.01