Reprinted from JOURNAL OF DAIRY SCIENCE, December, 1965, Vol. 48, No. 12, Pages 1585-1590

EFFECT OF SEASON ON THE WHEY PROTEIN NITROGEN DISTRIBUTION OF POOLED MILK

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ABSTRACT

Pasteurized homogenized milk was analyzed for 2 yr for variations in the noncasein proteins. Milk was chemically fractionated into the principal noncasein fractions, and the concentration of each fraction was determined by Kjeldahl nitrogen and correlated with season. Data were analyzed statistically, employing (as the regression model) the first two terms of a trigonometric series. Results indicate that all of the fractions but one varied with season and the variation was cyclic in character. The total albumin fraction did not vary. Also, with the exception of β-lactoglobulin, all of the fractions were highest in concentration during the summer and lowest in winter months. β-Lactoglobulin concentration followed a reverse variation.

The equation for the regression model and appropriate constants for each fraction are included. Also included are the predicted maximum and minimum concentration values for each fraction plus the date these values are expected.

In the continuous vacuum foam-drying process (1) developed for preparation of beverage-quality dry whole milk, the behavior of milk foam profoundly influences its drying characteristics. During the development of this process the foam behavior was observed to vary considerably with the season. Milk is known to contain several surface active components which, either alone or in combinations, can influence its foaming properties. Among these components are the milk proteins (5), especially the proteose-peptone fraction (2). However, data on the seasonal variation of the major whey proteins are not available. Thus, a detailed study of the effect of season on the concentration of the individual whey protein fractions was undertaken.

The purpose of this paper is to report the pertinent findings of a 2-yr study on the seasonal variation of the whey proteins. Other aspects of the over-all study, to elucidate the potential factors responsible for milk foam behavior, will be reported elsewhere (8).

MATERIALS AND METHODS

Pasteurized and homogenized, Grade A, whole milk, and pasteurized skimmilks, purchased from a local dairy on a weekly basis, were used in this study. In all, 76 lots of milk were tested. The whole milk was standardized to about 3.25% milk fat by diluting with skimmilk. Total solids content was determined by vacuum oven drying.

Received for publication August 23, 1965.

The relatively new fractionation procedure of Aschaffenburg and Drewry (3) was employed, because of its adaptability to routine analysis and its realistic estimation of the whey protein fractions. The nitrogen content of the various fractions was determined by the Kjeldahl method, as described by Ogg (12). Results are expressed as milligrams of nitrogen per 100 g of milk (corrected to a standard 12.00% total solids).

RESULTS

This study covers a period commencing in January, 1962, and continuing to December, 1963. Four fractions: noncasein, nonprotein, noncasein protein, and proteose-peptone, were studied for the entire 2-yr period; whereas, the four other fractions: total albumin, β-lactoglobulin, residual albumin, and globulin, were analyzed only during the second year. Figures 1 to 8 show nitrogen concentration of each fraction as a function of time. An initial statistical analysis of these data for each fraction was performed by first dividing the year into four seasons, pooling the 2-yr data according to season, and finally determining between-season variation by analysis of variance. The seasons were arbitrarily established as follows: Summer season included June, July, and August; fall, winter, and spring spanned the remaining nine months at three-month intervals. The analysis of variance indicated a highly significant ($P < 0.01$) seasonal variation for each fraction, with the exception of the total

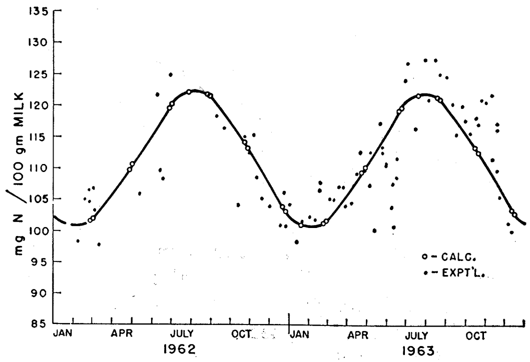

FIG. 1. Noncasein nitrogen concentration as a function of time.

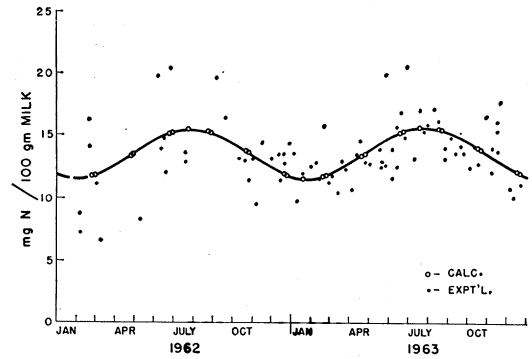

FIG. 4. Proteose-peptone nitrogen concentration as a function of time.

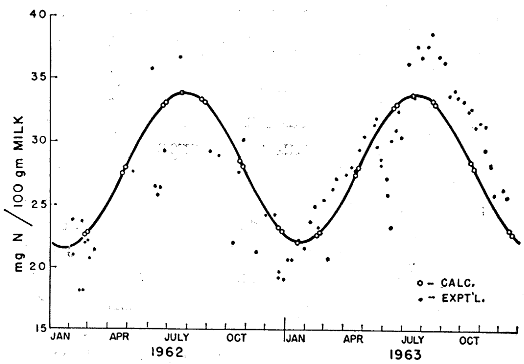

FIG. 2. Nonprotein nitrogen concentration as a function of time.

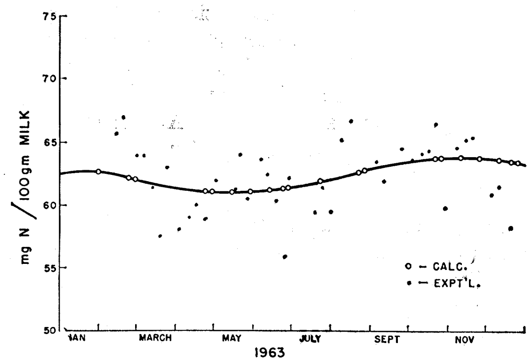

FIG. 5. Total albumin nitrogen concentration as a function of time.

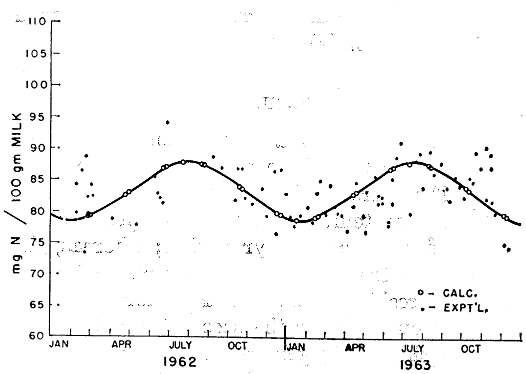

FIG. 3. Noncasein protein nitrogen concentration as a function of time.

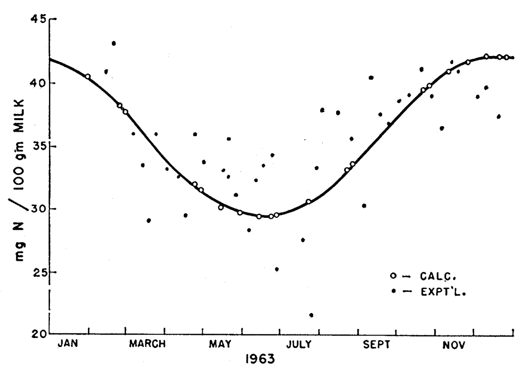

FIG. 6. β-Lactoglobulin nitrogen concentration as a function of time.

albumin fraction. These analyses, however, did not indicate where each fraction varied with season. To determine this, the Duncan's Multiple Range Test (4) was employed, again using the arbitrarily selected seasons. Results indicated that, for all fractions except the total albumin and β-lactoglobulin, the summer average value was significantly higher ($P = 0.05$) than the winter average. The β-lactoglobulin fraction was observed to be highest in concentration during the winter and significantly lower in the summer season. The spring and fall seasons appeared to be transitional, thus suggesting that the seasonal variations are cyclic.

To demonstrate the suspected periodicity of these data, a multiple regression of nitrogen concentration vs. time was performed. The regression model chosen comprised the first two terms of a trigonometric series and is of the form:

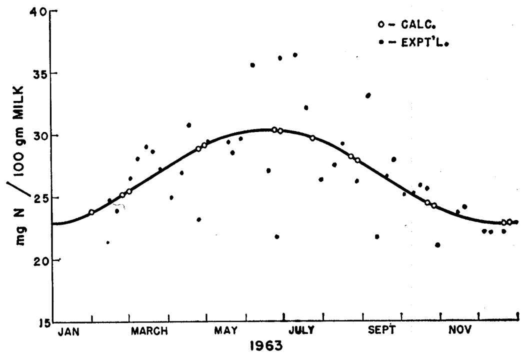

FIG. 7. Residual albumin nitrogen concentration as a function of time.

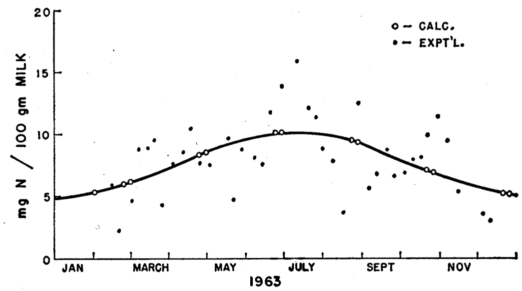

FIG. 8. Globulin nitrogen concentration as a function of time.

$$y = a_0 + a_1 \sin\left(\frac{2\,\pi\,D}{365}\right) + a_2 \cos\left(\frac{2\,\pi\,D}{365}\right)$$

where:

y is nitrogen concentration of a fraction
D is time in days (January $1 = 1$ or 366)
a_1, a_2 are regression coefficients
a_0 *is* $\bar{y}$ (mean nitrogen concentration of a fraction for the period covered).

Calculated values for a_0, a_1, and a_2 for each noncasein protein fraction are tabulated in Table 1, along with the F ratios obtained from analyses of variance on each regression. In all cases except that of the total albumin, the F ratios indicate greater than 95% probability that the above regression model describes the data to which it is applied. Thus, also shown in each Figure (1 to 8) is a curve calculated from the above equation, using the constants appropriate for each fraction.

Figures 1 to 4 show the concentration distribution of the four fractions: noncasein, nonprotein, noncasein protein, and proteose-peptone, studied for the full 2-yr period. Each figure graphically illustrates the periodicity of the data with the summer high point and the winter low level. The predicted maximum and minimum values for each fraction, calculated from the regression analysis, are listed in Table 2. It can be seen that the maximum level, for each of these four fractions, is attained at about the same time of year. Likewise, the winter low level was observed to occur almost simultaneously.

The total albumin fraction and the remaining three fractions were followed only during the second year of this study. With the total albumin fraction (Figure 5) no seasonal variation is illustrated. The Duncan's Multiple Range Test, as stated previously, likewise indicated this lack of a seasonal trend. Also, the F ratio (Table 1) obtained for this fraction was not significant at the 95% probability level.

With the fractionation procedure employed, the total albumin was separated to yield a β-lactoglobulin and a residual albumin fraction. β-Lactoglobulin (Figure 6) exhibited a highly significant seasonal trend, opposite to all other fractions. It was found to be highest in concentration during the winter season and lowest in the summer. Table 2 shows that the predicted maximum and minimum points occur somewhat earlier than with the first four fractions.

The residual albumin fraction, Figure 7, illustrates that the highest average concentration was once again obtained during the summer season. It is of interest to note (Figure 7 and

TABLE 1
Regression coefficients and F ratios for noncasein proteins

Fraction	a_0 (mg N/100 g milk)	a_1 (mg N/100 g milk)	a_2 (mg N/100 g milk)	F ratio
Noncasein	111.59	−6.002	−8.744	63.00
Nonprotein	27.98	−2.745	−5.310	71.99
Noncasein protein	83.46	−2.337	−3.976	39.68
Total albumin	62.42	−1.052	+0.916	3.53[a]
β-Lactoglobulin	35.86	−1.513	+6.223	80.28
Residual albumin	26.52	+0.881	−3.652	13.09
Proteose-peptone	13.58	−0.974	−1.731	13.72
Globulin	7.71	−0.366	−2.473	6.35

[a] Not significant at P = .05.

TABLE 2

Maximum and minimum concentrations for each noncasein protein fraction

	Maximum predicted value		Minimum predicted value	
Fraction	Concentration mg N/100 g milk	Date	Concentration mg N/100 g milk	Date
Noncasein N	122.20	Aug. 4	100.98	Feb. 4
Nonprotein N	33.96	Jul. 28	22.00	Jan. 28
Noncasein protein N	88.07	Jul. 31	78.85	Jan. 31
β-Lactoglobulin N	42.26	Dec. 16	29.46	June 17
Residual N	30.28	June 17	22.76	Dec. 16
Proteose-peptone N	15.57	July 30	11.60	Jan. 30
Globulin N	10.21	July 9	5.21	Jan. 9

Table 2) that these data for the residual albumin, the calculated difference between the total albumin and β-lactoglobulin fractions, are exactly 180 degrees out of phase with the β-lactoglobulin data (Figure 6). Such a phenomenon should occur if the total albumin does not vary with season, whereas the β-lactoglobulin does show considerable seasonal fluctuation.

The globulin fraction (Figure 8) represents the calculated nitrogen remaining in the noncasein fraction after subtraction of both the total albumin nitrogen and the proteose-peptone nitrogen. Thus, errors in any or all of these fractions would be reflected in the results obtained for this fraction. Nevertheless, these data were found to be significantly represented by the regression model (Table 1). Results show that the highest levels were obtained during the summer, with the predicted maximum level appearing early in July and the winter low at the beginning of January (Table 2).

DISCUSSION

In Table 3 the arithmetic averages, obtained from the Duncan's Multiple Range Test, are arranged according to the arbitrarily selected seasons. Over 99% of the noncasein protein nitrogen has been accounted for in all but the winter season, where 97% of the total nitrogen was recovered. Included in this table, for comparison purposes, are values obtained by the Rowland procedure (9) and by Larson and Gillespie (10).

The concentration of the noncasein nitrogen and nonprotein nitrogen fractions compares favorably with previously published data (6, 9, 11). The over-all average concentration of the proteose-peptone fraction agrees quite well with the Larson and Gillespie (10) electrophoretic value and is lower than the value reported by the Rowland procedure (9). This is in agreement with the findings of Aschaffenburg and Drewry (3).

The concentration range of the total albumin is somewhat lower than the electrophoretic value (Table 3), but considerably higher than the total albumin value obtained by the Rowland procedure. The β-lactoglobulin concentration is also somewhat lower than the electrophoretic value. The deviation of the present data from the electrophoretic values is an indication that some denaturation of β-lactoglobulin has occurred during pasteurization of the milk used in this study.

The significant seasonal differences in the β-lactoglobulin concentration of bulk milk may be an important factor in affecting the heat stability of milk during processing, e.g., in the preparation of evaporated milk. Tessier and Rose (13), from their work on the pH-heat stability relationship, recently reported that milk having no minimum heat stability point could be converted to milk having both a maximum and minimum heat stability by addition of β-lactoglobulin. The addition of κ-casein caused the reverse transformation. They suggest that the heat stability of milk is controlled by the proportions of micellar surface κ-casein and soluble β-lactoglobulin. They reported no consistent difference in caseins or β-lactoglobulin content of the two types of milk and, furthermore, all of the bulk milks they tested were found to be the type already possessing maximum-minimum heat stability characteristics. However, in light of the present findings, it would be of interest to know if the seasonal variation in β-lactoglobulin concentration would be sufficient to influence the heat stability behavior of bulk milk.

It is of interest to note that the proteose-peptone fraction, an effective foaming agent,[1] was found to be highest in concentration during the summer seasons. This coincides with the sea-

[1] Addition of a proteose-peptone (Fraction 5) preparation to milk produced a very stable foam, as measured by the static foam test of Holden (8).

TABLE 3

Seasonal average nitrogen values for noncasein protein fractions

Sample	Season (mg N/100 g milk)[a]				Larson and Gillespie	Rowland procedure[d]
	Summer	Fall	Winter	Spring		
Noncasein N	119.15	114.66	102.76	107.47	94[c]	110[c]
Nonprotein N	32.06	29.61	22.17	26.60		30
Noncasein protein N	87.09	85.01	80.85	80.88		80
Total albumin N	60.45 (69.4%)[b]	63.68 (74.91%)[b]	62.87 (77.76%)[b]	60.98 (75.39%)[b]	71	43
β-Lactoglobulin	30.49	38.50	39.91	33.04	47	
Residual albumin	29.96	25.18	22.96	27.94	24	
Proteose-peptone N	15.61 (17.92%)[b]	14.03 (16.50%)[b]	12.85 (15.89%)[b]	11.84 (14.63%)[b]	13	18
Globulin N	10.37 (11.90%)[b]	7.09 (8.34%)[b]	2.90 (3.58%)[b]	7.54 (9.32%)[b]	11	19

[a] Nitrogen values have been corrected to a standard milk containing 12% total solids.
[b] Percentage of the noncasein protein fraction.
[c] Mg N/100 ml milk (N factor = 6.38).
[d] From Jenness, R., and Patton, S. (9).

sonal period of maximum stability of milk foams in the vacuum dryer and probably contributes to the foaming behavior of milk concentrates (9).

CONCLUSION

The relatively new chemical fractionation procedure of Aschaffenburg and Drewry (3) is readily adaptable for the routine study of the principal noncasein protein fractions. All but one fraction studied has been found to vary significantly with time anl the variation was cyclic in nature, the exception being the total albumin fraction which had no seasonal trend. The season of highest concentration levels for all fractions but the β-lactoglobulin was during the summer months. The lowest concentration appeared during the winter season. β-Lactoglobulin showed a reverse trend.

ACKNOWLEDGMENT

The authors gratefully acknowledge the assistance of Joe N. Boyd and Mirita J. Smithey for the analysis of variance and Duncan's Multiple Range Test; Marie Porrecca and Maurice Snook for the numerous Kjeldahl nitrogen analyses; Dr. M. P. Thompson for supplying the proteose-peptone (Fraction 5) preparation; and R. K. Eskew for his encouragement and guidance.

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