COMPARISON OF ORANGE G DYE, FORMOL TITRATION, AND KJELDAHL METHODS FOR MILK PROTEIN DETERMINATIONS

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SUMMARY

A highly significant correlation of +0.975 between Orange G dye and Kjeldahl methods of protein determinations demonstrated that for most practical purposes the protein content of milk can be determined as effectively by the use of the rapid Orange G dye binding method as by the time-consuming Kjeldahl method. The correlation of +0.839 between the percentage of total protein determined by formol titration and the Kjeldahl method indicated that the Orange G dye method has an advantage in accuracy compared to formol titration as a rapid method for determination of protein in milk. This is, in part, due to effects from stage of lactation and month of the year on the formol factors for total protein.

Protein, the most important of the two major constituents of solids-not-fat, is receiving more attention from both the consumer and dairyman. This is due in part to the high nutritional value of milk protein, to the changing food habits of consumers, and to the declining importance of milk fat. Also, there is an increasing trend toward including protein, in addition to fat content, in determining the payment for milk (6, 11, 14). From a practical standpoint, the recent development of a rapid test for total protein, comparable in accuracy to the Babcock method for fat determination, is of great value to dairymen.

The objectives of this research were: to compare the Orange G dye and formol titration methods with the Kjeldahl method for testing the protein content of milk.

MATERIALS AND METHODS

Guernsey cows on Cornell’s McDonald Farms near Cortland, New York, were used. Samples were taken at monthly intervals. Unweighted samples were taken from the evening and morning milk from each cow.

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2 Research presented was used for a thesis submitted by the senior author to the Faculty of the Graduate School of Cornell University in partial fulfillment of the requirements of the Ph.D. degree.
3 Present address: College of Agriculture, University of the Philippines.
are not presented, but Buffalo Black gave a clear solution after centrifuging, so that filtering was not necessary.

**Formol titration.** The modified method of Pyne (19) was used to determine the protein content of milk by formol titration. A saturated potassium oxalate solution was made. To dissolve the crystals, the solution was heated but not boiled. A 37% formaldehyde and 0.1 N sodium hydroxide solution and 0.5% phenolphthalein indicator were used. Acidity of the formaldehyde solution was corrected. A color standard was made by adding 0.1 ml of a 0.01% solution of basic fuchsin to 10 ml of milk and 0.4 ml of potassium oxalate in a test tube. This procedure was dispensed with later, when sufficient skill in determining the end point was acquired. Formol titrations on samples were run in conjunction with the analyses for total protein and casein, to determine the conversion factors for both total protein and casein.

**Casein.** The method of Rowland (20) was used in the determination for casein. However, after the analyses of 183 samples, the amount of 10% acetic acid was increased from 1 to 1 1/2 ml to conform with the procedure of the A.O.A.C. (4). The former amount was found insufficient to precipitate all casein from 10 ml of milk, because Guernsey milk has a high buffer value. The method of Rowland uses the filtrate and corrects for the volume occupied by the precipitate, whereas that of the A.O.A.C. measures casein directly. There was a close agreement between the two methods.

**Whey proteins.** This was calculated by difference, substracting casein from total protein.

**Errors in analyses.** Duplicates were not run for all Kjeldahl analyses, but it was a practice to check on the analyses by running blanks and duplicates on at least six samples taken at random, two in each of the three digestion set-ups of 12 flasks each. Errors in determining total protein and casein were estimated by the method in which the difference between duplicates is divided by the smallest value and the quotient is multiplied by 100 to get the percentage error. The error for 85 duplicates in total protein determination was 0.9%; for 40 duplicates in casein determination, it was 1.9%. This is considerably less than the per cent error suggested as permissible for the method.

**RESULTS AND DISCUSSION**

**Methods of analyses for total protein and casein.** A comparison of Kjeldahl, Orange G dye, formol factor, and linear regression equation based on formol titration is given in Table 1. The results from 61 samples were compared.

<table>
<thead>
<tr>
<th>Method of estimation</th>
<th>Mean, total protein</th>
<th>Mean, casein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kjeldahl</td>
<td>3.65 ± 0.048</td>
<td></td>
</tr>
<tr>
<td>Orange G dye</td>
<td>3.61 ± 0.046 *</td>
<td></td>
</tr>
<tr>
<td>Formol factor (1.77)</td>
<td>3.75 ± 0.048 *</td>
<td></td>
</tr>
<tr>
<td>Linear regression equation based on formol titration</td>
<td>3.65 ± 0.045</td>
<td></td>
</tr>
</tbody>
</table>

*Tukey's test of significance of difference between means was used with D = 0.12. (Snedecor, Statistical Methods.) Differences were significant between the two extremes, but not for comparisons of the different methods with the standard Kjeldahl method.

The means for protein from the Kjeldahl and Orange G dye methods were 3.65 ± 0.048 and 3.61 ± 0.046, respectively. There was a highly significant correlation of +0.975 between the Orange G dye and Kjeldahl methods of protein determination. Thus, it appears that the protein content of milk can be determined as effectively by the use of the rapid Orange G dye binding method as by the long and tedious laboratory procedure required for the Kjeldahl method.

Analysis of variance of the data shows that there was no significant difference between the formol titration and Kjeldahl methods for the determination of total protein content of milk. This is shown in Tables 2 and 3, and confirms results reported in other research (2, 5, 9–12, 14, 15, 17). Standard errors reported were larger for individual cows than for herd milk; however, the deviations for both total protein and casein from the Kjeldahl method were usually of the order ±5% for both.

The average formol factor of 1.77 for total protein was obtained by dividing percentage of total protein by milliliters of N/10 sodium hydroxide, and was based on 274 samples. The factor of 1.35 for casein was based on 360 samples. There was a highly significant correlation

<table>
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<th>Mean, casein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kjeldahl</td>
<td>3.80 ± 0.016</td>
<td>2.93 ± 0.015</td>
</tr>
<tr>
<td>Formol titration</td>
<td>3.82 ± 0.015</td>
<td>2.93 ± 0.014</td>
</tr>
</tbody>
</table>

*A total of 650 and 536 samples from 142 cows was compared with the Kjeldahl method for protein and casein, respectively.
TABLE 3

<table>
<thead>
<tr>
<th>Stage of lactation</th>
<th>No. of cows</th>
<th>Casein/total protein</th>
<th>Formal factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total protein</td>
</tr>
<tr>
<td>1</td>
<td>32</td>
<td>0.789</td>
<td>1.73</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>0.780</td>
<td>1.75</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>0.781</td>
<td>1.74</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>0.778</td>
<td>1.76</td>
</tr>
<tr>
<td>5</td>
<td>53</td>
<td>0.772</td>
<td>1.74</td>
</tr>
<tr>
<td>6</td>
<td>49</td>
<td>0.773</td>
<td>1.80</td>
</tr>
<tr>
<td>7</td>
<td>51</td>
<td>0.758</td>
<td>1.78</td>
</tr>
<tr>
<td>8</td>
<td>43</td>
<td>0.756</td>
<td>1.75</td>
</tr>
<tr>
<td>9</td>
<td>44</td>
<td>0.745</td>
<td>1.77</td>
</tr>
<tr>
<td>10</td>
<td>33</td>
<td>0.737</td>
<td>1.84</td>
</tr>
</tbody>
</table>

* A total of 453 samples from 124 cows. The factor for total protein and casein was based on 274 cows and 360 tests, respectively.

of ±0.839 between percentage of total protein and milliliters of N/10 sodium hydroxide, and a correlation of +0.729 between percentage of casein and milliliters of N/10 sodium hydroxide. These are lower than those reported by Bannenberg and van den Hoek (5) for mixed herd milk. Budslawski et al. (7) and Comberg (8) report on the relationship of different milk constituents during lactation and the composition of bulk milk. The lower correlation for casein may be attributed to errors in the separation of casein from whey, especially during the first two months of the experiment. It is evident that the correlation of +0.839 between the percentage of total protein determined by formol titration and the Kjeldahl method indicated that the Orange G dye binding method, with a correlation of 0.975, is more accurate than the formol titration method.

The estimation of total protein by the use of linear regression equation gave the same results as did the Kjeldahl method, thus confirming the observation of Solberg et al. (21). Using the Kjeldahl method as the standard for comparison, none of the different methods gave significantly different results. However, since the correlation between the Orange G dye and the Kjeldahl method was much higher than between the formol factor and the Kjeldahl, it indicates an advantage in accuracy for the Orange G dye method for rapid determination of protein in milk. Amido Black was found equally as good as the Kjeldahl (1, 26) and has the advantage that filtering is not necessary after centrifuging. This has been used in Holland for routine testing of 1,400,000 milk samples from 80,000 individual cows in herd tests, and for pricing and selling milk at 30 dairies.

The use of the formol factor overestimated total protein by 0.10 from the Kjeldahl (Table 1). Gilmore and Price (13) reported a significantly higher value of 0.045 with formol titration than with Kjeldahl, but did not consider it important in practice. Since stage of lactation and month of the year may exert an influence on the results obtained, both of these were tested to determine their effect on the formol factor.

The effects of stage of lactation on the formol factors for total protein and casein are presented in Table 3. A total of 453 samples from 124 cows are represented in the table. Total protein formol factors showed a trend upward as the lactation period advanced. Casein formol factors exhibited a tendency to decrease, but the trend was not as consistent as for total protein. These opposite trends can be attributed to the increase in whey protein as the lactation period advances, which may also contribute largely to the increase in total protein percentage. This is shown in Table 3 by a decreasing ratio of casein to total protein, which is 0.789 and 0.737 for the first and tenth months, respectively.

Results on 536 samples from 124 cows presented in Table 4 indicated that months of the year do have some effect on the formol factors for total protein and casein. However, if an average formol factor is used, based on determinations and calculations of representative months during the different seasons, sizable errors will not be introduced.

Both the dye binding and formol titration methods have some advantages for routine laboratory analyses. The dye binding method is more accurate, especially for individual cow samples. The formol titration has an advantage from the standpoint of time required and simplicity of apparatus. However, the continuous
presence of formaldehyde gas, even with good ventilation, may prove a hardship for the laboratory technician when a large number of samples are analyzed. Kay (16) reviewed recent methods for estimating milk protein, and concluded that formol titration could be very suitable for routine analytical work. Mogensen (18) reviewed published data on formol titration and concluded that the method is not sufficiently accurate to form the basis for payment for milk proteins. The dye method is rapidly gaining acceptance for accurate use on large-scale analyses of milk samples for protein content.

REFERENCES


