THE VARIATIONS IN MINERAL CONTENT OF MILK

by

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INTRODUCTION

The ash of milk constitues only about 0.7-0.8 percent of the total weight, yet it is one of the most important constituents from a nutritional standpoint as well as being of importance in many dairying problems. For instance the mineral balance, especially in respect to magnesium is of prime importance in coagulation by heat and consequently this balance is of value in the manufacture of condensed milk.

With these facts in mind, the problem of analyzing the milk of about sixty-five cows of Holstein, Ayrshire, Guernsey, and Jersey breeds was undertaken in conjunction with an experiment carried on jointly by the Dairy Department and the Chemistry Department of the Kansas Agricultural Experiment Station.

REVIEW OF LITERATURE

With the exception of the alkali metals, which have not been studied so carefully, a great deal of work has been done on this subject and therefore a review of the literature will be appropriate at this time.

Allen, 1931, (1) in an article prepared in the course of the review of the literature concerning the condensing and drying of milk states that sufficient data are available to demonstrate that minerals influence other properties of milk. He indicates, however, that much of the data are confusing, some of them contradictory and a great deal of them has been compiled with too little regard for experimental error. He goes on to show the partition of the minerals between the soluble and insoluble components, the effect of souring on this partition, and gives the range of variation of the mineral constituents. The approximate mean values (milligrams percent) given by Allen as taken from the works of various investigators are as follows: Na_2O 50; K_2O 180; Cl 106; P_2O_5 232; CaO 182; MgO 17; and total ash 740. Allen discusses a number of factors responsible for the variations found in the minerals, of which breed, stage of lactation, and feeding will be dealt with in this thesis.

Data on the subject of breed in its relation to the composition of milk are very few. Soxhlet (2), however, expresses the opinion that the more highly bred races give milk low in calcium. Katayama's experiments (3) do not support this view. No data are given for variations in the highly bred races.

Concerning the variation due to stage of lactation, Trunz (4) from the investigation of two cows makes the following observations.

(a) The total ash is higher in colostrum and towards the end of the lactation than in the intervening period.

(b) Chloride increases quite markedly towards the end of lactation.

(c) Potassium content falls towards the end of lactation while sodium follows the opposite rule.

(d) Both calcium and magnesium are higher in colostrum and toward the end of lactation.

(e) Phosphoric acid is higher in colostrum than subsequently.

(f) The CaO/P_2O_5 ration is definitely higher at the end of lactation.

Schrodt and Hansen (5) produced results from which it appeared that calcium and magnesium are high at the beginning of lactation and that potassium rather higher a few days after calving than at a later period--results which confirm the finding of Trunz.

Forbes and Beegle, et al, (6) give some evidence from cows that calcium shows a general increase with advance in state of lactation while the ash and magnesium appear to be lower during the earlier stages.

Meigs and Turner, et al, (7) working with two cows although finding some fluctuation in calcium and phosphorus, could reach no generalization with respect to stage of lactation.

Sommer and Hart (8) after studying the composition of the milk of three cows throughout lactation reached the conclusion that the calcium and magnesium are higher at the beginning and end of lactation than in the intermediate period and that the organic phosphorus decreased while the ration CaO/P_2O_5 increased toward the end of the period.

Much experimentation has been done concerning the effect of feeding on the mineral composition of milk and in general it may be said that the evidence available renders it doubtful whether the mineral composition is influenced to a measureable extent by changes in feeding. A few investigators claim, however, to have obtained positive results. Most of these experiments were carried out with various salts and therefore were not parallel with this study.

Davies and Provan (9) give details of experiments in which they found differences in the minerals in milk from cows on pasture and on winter feed respectively. Fourteen cows were divided into two groups: Group A being fed on control rations and group B on low protein rations. For analysis the milk of each animal was sampled just before grazing commenced and then a fortnight after. Twelve of the fourteen animals showed an increase in lime and inorganic phosphorus while an increase in total phosphorus occurred in thirteen animals. This result is outstanding among many negative results and it is disappointing to find that all results are apparently based upon one analysis of a composite sample in each case and that there is no evidence of consistency of duplicates to prove the accuracy of the methods employed. However the fact that changes occurred in twelve out of fourteen cases presents fairly strong evidence. In this experiment the increase of total phosphorus was greater for those animals which were fed a low protein ration previous to grazing from which the authors deduce that the differences depend on the nature of the winter feed.

Orr (10) investigated the relation of the mineral composition of grass to its feeding value and shows that there is an increase in ash content in the grass from June to September, a rise in CaO from May to September followed by a rapid fall, while the P_2O_5 remained fairly constant. The nutritive value would therefore appear higher in summer months than at other times. Whether this factor would be responsible for the increase in calcium and phosphorus content of milk as found by Davies and Provan is at present a matter of speculation.

Hess, Unger, and Supplee (11) give results showing a decided increase in phosphorus and calcium after pasture feeding but the fact that the cows had previously been fed on a diet devoid of vitamin C may introduce other factors.

The only comprehensive analysis correlating the composition of milk with the period of the year appear to be those of Crandfield, Griffiths, and Ling (12). According to their results the P_2O_5 content is influenced but little by the season of the year although there is a slight rise in May and June and fall in July. Lime shows a steady fall in late spring and summer reaching a low in August followed by a sharp rise. The ration CaO/P_2O_5 follows the changes in CaO.

This treatise is not primarily concerned with the variations due to disease, but such evidence as there is seems to indicate that in the course of udder infections there is a marked decrease in calcium, phosphorus, and potassium, and appreciable decrease in magnesium, and a considerable increase in sodium and chlorine.

Sharp and de Tomasi (13) in an article on the nonlactic acidity of milk in 1932 states that in milk from normal cows chlorides increase only slightly with advance of lactation but are high for colostrum, mastitis, and late lactation (stripper) milk. They also show that there is a definite relation between the chloride and lipase content of milk.

Notbohn and Phillipi (14) in 1933 deduced from their investigations that when the udder of the cow is infected as when a state of mastitis exists the chlorine and sodium content increase, potassium remains constant until a critical chlorine value of about two tenths percent is reached when the potassium falls and the ash content very nearly approximates that of the blood. The critical chlorine value is supposed to mark the breakdown of the selective secretory activity of alveolar cells with respect to blood constituents.

In an experiment conducted at the Florida Experiment Station, Becker, Neal, and Shealy (15) in 1932 fed grass forages and silages grown on acid sandy soils and which contained relatively small amounts of calcium to dairy cows and found that they withdrew minerals from their bones to such an extent that they were weakened and easily broken. Reproduction was found to be a far smaller drain on calcium and phosphorus than was lactation. These authors evidently did not determine whether or not the calcium and phosphorus content of the milk itself had been lowered by the calcium deficient feed.

Olson, (16) 1934, analyzed the milk of thirty-three cows through a complete lactation period and found that the calcium and phosphorus are relatively high in the beginning of the lactation period. The calcium drops to its lowest level within six to eight weeks and then remains constant until the drying up period when it reaches a level of from twenty to thirty percent above normal. The phosphorus drops to the normal level in about six weeks and decreases gradually until the end of the lactation period when it reaches its lowest level.

Jones and Davis, (17) 1935, determined the average amounts of chloride and sodium in milk as 113.4 and 76.8 milligrams per 100 cc. respectively and developed an empirical formula for the relation of chlorine to sodium.

In 1925, Sato and Murato (18) determined the magnesium in milk by a colorimetric method in which the color was developed by starch and tartar yellow and found a range of 7.7-9.12 milligrams per 100 cc. This is lower than the values found by most other investigators.

Caulfield and Riddell, (19) 1935, made a comprehensive study of the factors affecting the chloride content of milk over a period of nineteen months and reached the following conclusions:

(a) No statistically significant differences were observed in the chloride content of the milk from the different breeds.

(b) The individuality of the cow was found to have a marked influence on the chloride content of milk.

(c) The chloride content of milk was found to be highest at the outset of the lactation period, then declined rapidly for the first ten to twenty days, following which there was a general upward trend throughout the remainder of the lactation.

(d) Holstein milk was higher in chlorides than milk from the other breeds during the first half of the lactation period, following which there was no significant breed difference.

(e) Variations from month to month in the chloride content of the milk from the different breeds were not large. The period of lactation appeared to be the most important factor affecting the variation in chlorides from month to month.

(f) The average variations in milk chlorides from day to day and from milking to milking in a group of six cows were extremely small. Individual cows within the group did show variations ranging from 0.013 to 0.043 percent from one milking to the next over a ten day period.

METHODS OF INVESTIGATION

At three periods, Oct. 20-22, 1936; Nov. 20-Dec. 2, 1936; and Jan. 5-7, 1937, milk samples from approximately 65 cows were analysed for sodium, potassium, chlorine, calcium, magnesium, phosphorus, and ash. This group included ten cows of each of the four major dairy breeds and ten Holstein heifers which had not been pastured. The fifty animals included in these five groups were milked throughout the interval and were well matched as to stages of lactation. The sample from each cow was a composite of morning milkings over a period of three consecutive days thus obviating any abnormal daily fluctuations.

The analyses were carried on as follows:

Ten cubic centimeters of the sample was placed in a porcelain capsule and left in the drying oven (110 degrees C) overnight. The capsule was then placed in the muffle furnace and heated to a dull red heat until the sample was white. If some black carbon persisted, water was added after cooling to leach out the salts which were occluding the carbon and the heating was continued. There is some objection to the use of porcelain capsules for ashing, however any error will be common to all samples and the author holds that relative values are more valuable than absolute values. Moreover the average values for the several constituents agree closely with those listed by Allen (1). The ash was taken up with 15 ml. of 1-5 HCl and made up to volume in a 100 ml. volumetric flask.

Potassium was determined by the colorimetric chloroplatinic method of Cameron and Failyer (20) in which a pink color is developed in an acid solution of potassium chloroplatinate by potassium iodide.

The sodium values were obtained by difference from the values of total alkali and potassium. For total alkali the method of Barnett (21) was employed. In this method 10 ml. of the ash solution is wet ashed with concentrated sulfuric acid in a 50 ml. erlenmeyer flask, the sulfate precipitated by $Ba(OH)_2$, excess barium and metals other than the alkalis precipitated with ammonium carbonate, and excess of this reagent removed by boiling. The solution is then titrated to methyl red with N/50 HCl excluding CO_2. The use of the pyrex erlenmeyers for wet ashing may again be questionable but blanks were run, the error found to be almost constant and subtracted from the total. The amount lost during the procedure, which was considerable, probably because of adsorption on the filter papers (3 filtrations), was estimated by running a series of samples of known varying concentrations of sodium carbonate through the procedure and comparing their titre with the values obtained by direct titration of samples of corresponding concentrations. A curve was then plotted (ml. of N/50 HCl against percent error) and the error for any concentration obtained from the graph.

For the determination of phosphorus the method of Fiske and Subbarow as modified by Koch (22) was employed.

The sample was treated with molybdic reagent to form the phosphomolybdate ion which was then reduced with 1, 2, 4-aminonaphthol sulphonic acid to the dark blue phosphomolybdous ion and determined colorimetrically. Just enough acid is used in taking the milk ash into solution to give the correct acidity for this test.

Calcium was determined according to Koch (23). The calcium was precipitated as the oxalate, dissolved in sulfuric acid and titrated against standard potassium permanganate.

The method of Briggs (24) was used for the estimation of magnesium which was precipitated from the calcium filtrate by a two percent solution of ammonium hydrogen phosphate as the double phosphate of magnesium and ammonium. The concentration of magnesium was then obtained indirectly by measuring the phosphorus in this compound by the method outlined above.

The chloride content was obtained by direct titration of the milk sample with silver nitrate according to the method of Sharp and de Tomasi (13). Ten percent potassium chromate was used as an indicator.

RESULTS

The average milligrams percent of all the determinations of each constituent are as follows: Na_2O, 40; K_2O, 186; CaO, 174; MgO, 19.2; P_2O_5, 220; Cl, 126; and total ash, 760. With the exception of sodium which is somewhat low these values compare very closely with the average of those given by Allen in his review.

One important purpose of this experiment was to determine any difference in the mineral content due to the difference in feed. Table 1 gives the average values of the various constituents of the ash of the milk of the ten Holsteins pastured on wheat throughout the experiment and those of the ten heifers which had not been pastured but which had received prairie hay for roughage since they were six months of age. These data indicate that the type of feed is of very little significance in determining the ash content of the milk. The experimental animals were lower in sodium and had a somewhat higher total ash content than the controls but all other values were closely parallel. Phosphorus was nearly identical in the two groups while calcium was slightly higher for the prairie hay cows. This is contradictory to the findings of Davies and Provan (9) but the increase is not consistent enough to strengthen the conclusions of Crandfield, Griffith, and Ling (12).

To show the variation due to breed, table 2 has been compiled giving the average value of each ash constituent for each breed. Then from these data, table 3 has been prepared placing the breeds in their proper rank with respect to each other. Thus the breed having the highest average value was given a ranking of 1, next highest 2, and so on. If two breeds had identical values the sum of the two positions they occupied was divided between them. From this table it can be seen that Jerseys were high in ash, calcium, and phosphorus but low in potassium and chlorides; Guernseys were high in magnesium and low in sodium; Ayrshires were low in magnesium and ran slightly lower than average in the other constituents; the control Holsteins were high in sodium, potassium, and chlorides and low in calcium and ash. Except for the two deviations mentioned in the preceding paragraph the experimental Holsteins closely resembled the controls.

Table 3 also gives the standard deviation for the individuals within each breed. This value was obtained by taking the square root of the mean of the squares of the deviation of the individuals from the average. Row No. 12 gives the ratio $M_1 - M_p / \sqrt{D^2_{s1}/N_1 \text{ plus } D^2_{sp}/N_p}$ in which M_1 and M_p are the largest and smallest average values of any constituent among the breeds, D_{s1} and D_{sp} are their respective standard deviations, and N_1 and N_p are the number of respective individual cases. If this ratio is three or greater the chances are more than ninety-nine and a half in a hundred that the variation observed is significant. From the values shows it is apparent that all maximum breed variations are of significance excepting possibly that of sodium.

The effect of stage of lactation upon the mineral content of milk is shown in table 4. The first column represents the number of days the animals have been producing, the second the number of animals at that stage, and the succeeding columns give the percent of the amount present at the time indicated in the first column after forty days have elapsed. For example the table shows that the sample of colostrum obtained the first day of lactation contains almost four times as much sodium as milk taken from the same cows forty days later. This table shows that total ash is higher in colostrum than in the period immediately following, that this value remains fairly constant during the most of lactation and decreases slightly toward the end. The sodium content is comparatively high in colostrum, falls rapidly, fluctuates considerably throughout the period, and increases in advanced lactation although the data supporting the last part of this statement is rather meager. Potassium increases during the first stage of lactation, decreases gradually in the next five months, rises sharply and then decreases markedly in the final stage. Chlorides and phosphorus decrease rapidly to a fairly constant value and rise slowly throughout the remainder of the period. Calcium decreases rapidly to a constant value and rises slowly during the latter stages. Magnesium decreases gradually up until about the eighth month, rises and then falls off during the stripping period.

These findings support Trunz (4) in his conclusions that total ash is higher in colostrum, that chlorides increase in the latter part of lactation, that sodium increases in advanced lactation while potassium follows the opposite rule, that calcium and magnesium are higher in colostrum and toward the end of lactation and that phosphorus is higher in colostrum than subsequently. However, they differ from this author in that ash does not increase at the end of the period. They disagree with both him and Sommer and Hart (8) in that in this experiment the CaO/P_2O_5 ration remains constant throughout lactation whereas according to their results it increased toward the end of the period. The conclusion of Forbes and Beegle, et al, (6) concerning the increase of calcium is upheld but their findings concerning ash and magnesium are reversed. The results of this investigation in respect to chlorides very closely parallel the finding of Sharp and de Tomasi (13) and of Caulfield and Riddell (19) that the chloride content is higher for colostrum and late lactation milk. Phosphorus rise during latter part of lactation differs with Olson's (16) finding that it decreases gradually to the end of the period.

Table 5 gives the ratios K_2O/ Na_2O and CaO/ P_2O_5 by breed and by months of the year. Aside from a marked increase in the former ratio from October to November followed by an even more marked decrease from November to December no important changes can be seen although the CaO/ P_2O_5 values do decrease slightly from October to November. Also the K_2O/ Na_2O ratios for the experimental Holsteins rise somewhat higher than those of the controls.

SUMMARY

The average values obtained in this experiment for the various constituents agree very closely to the average of those obtained by other investigators.

Very little difference was found between the mineral content of milk from pastured cows and that from dry fed animals. The former showed a higher sodium content although the latter was higher in total ash.

Variations due to breed were quite small but were consistent from month to month.

In general the findings of other investigators in regard to stage of lactation were supported, however in contrast to the results of most workers the ash was found

to decrease slightly rather than increase in the final stage of lactation.

By far the greatest variations were found to be due to the individual, the standard deviation from the mean being of such magnitude as to lessen the significance of some of the variations due to other causes.

Table 1

Minerals in Milk
Holstein values using 2 rations.

	No.	a Na	a K	a Cl	a P	a Ca	b Mg	c Ash
Experi.	Sept.	24	136		88	121	101	74
	Oct.	39	158	141	88	120	113	77
	Nov.	16	184	137	89	123	113	76
	Dec.	28	164	139		118		75
	Ave.	27	160	139	88	120	109	75.5
Norm.	Sept.	33	160		88	125	78	72
	Oct.	37	142	133	92	124	119	74
	Nov.	32	172	135	90	107	104	73
	Dec.	36	165	151		100		73
	Ave.	36	160	140	90	114	100	73

a = %x1000.   b = %x10000.   c = %x100

Table 2

Variations of Breeds

	Na	K	Cl	Ca	Mg	P	Ash
Jerseys	30	139	106	149	12.4	109	.806
Guernseys	22	155	114	133	12.1	102	.767
Ayrshires	33	146	132	132	10.8	90	.759
N. Holsteins	34	160	140	114	10.0	90	.730
E. Holsteins	27	160	139	120	10.9	88	.750
Average	29	152	126	130	11.2	96	.763
D. of Jerseys	13.8	28.3	16.4	20.4	1.5	10.5	.065
D. of Guernseys	16.7	29.2	17.1	10.2	1.3	10.7	.039
D. of Ayrshires	24.6	41.0	30.0	11.7	1.3	7.0	.051
D. of N. Holsteins	21.6	26.2	28.6	13.2	1.4	14.9	.063
D. of E. Holsteins	16.8	30.8	18.7	12.9	1.2	7.9	.037
$M_1 - M_p \Big/ \sqrt{\frac{D^2_{s1}}{N_1} + \frac{D^2_{sp}}{N_p}}$	2.45	2.99	5.64	7.86	5.22	6.67	5.20

Values of Ash given in percent.

Other values in milligram percent.

D. = Standard deviation.

Table 3

Minerals in Milk
Ranks by breeds.

Breed	Na	K	Cl	P	Ca	Mg	Ash
Jersey	2	5	5	1	1	2	1
Guernsey	5	4	4	2	2	1	4
Ayrshire	3	3	3	3.5	3	5	2.5
E. Holstein	4	1.5	1.5	5	4	3	2.5
N. Holstein	1	1.5	1.5	3.5	5	4	5

Table 4

Stage of Lactation

		Percent of first test after 40 days.						
Days Start	No.	Na	K	Cl	P	Ca	Mg	Ash
1	3	20	130	69	76	76	85	85
30	14	371	87	95	97	100	76	99
100	18	168	94	102	105	97	95	98
160	6		90		106	106	121	105
220	6	100	112	104	102	109	109	101
340	3	53	114	102	98	87	96	99
380	3	252	71	93		105		97

Table 5

Minerals in Milk
Ratios by breeds and months

Breed	K_2O / Na_2O			CaO / P_2O_5	
	Oct.	Nov.	Dec.	Oct.	Nov.
Jersey	3.5	7.6	3.0	.90	.82
Guernsey	4.6	24.3	3.9	.82	.78
Ayrshire	4.2	10.2	2.0	.90	.91
E. Holstein	4.0	9.9	5.2	.83	.84
N. Holstein	3.4	4.8	4.1	.83	.73

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