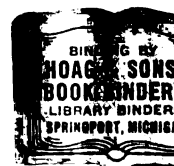




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A STUDY OF THE ORIGINAL ACID REACTION OF
MILK TO PHENOLPHTHALEIN.

by

M. B. Kurtz

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Submitted in Partial Fulfillment of the
Requirements for the Degree of
D. V. M.

in the

Michigan Agricultural College
1914.

The author takes great pleasure in thanking L. H. Cooledge, Research Assistant in Bacteriology at the Michigan Agricultural College, at whose suggestion this investigation was undertaken, for valuable assistance and encouragement in the pursuit of the same.

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A STUDY OF THE ORIGINAL ACID REACTION OF
MILK TO PHENOLPHTHALEIN.

Introduction.

Fresh normal milk while amphoteric to neutral indicators gives an acid reaction when titrated using phenolphthalein. The acidity of milk then, when spoken of in this thesis will refer to the reaction when phenolphthalein is used as an indicator. Some observations have been made upon the original acidity and these, while very limited seem to suggest to the investigators that the original acid reaction in milk may have some value in diagnosing inflammatory and gargety condition of the udder, as well as many other pathological changes brought about by disease or injury.

As very few observations have been made upon the original acidity of milk, and as most references to same are unaccompanied by substantiating evidences, it is thought that a study of the factors which influence the reaction of the milk at this period may be of interest and possibly have some practicable application.

The object of this investigation then will be to verify the statements already made as to the significance of the original acid reaction of milk; to determine any possible connection between the original acidity and pathological changes; to study the relation of the period

of lactation, the effect of standing, care with which drawn and any other factors that may enter in.

Unfortunately time and facilities will not permit of making a study of the chemical nature of the original acid reaction to phenolphthalein in milk.

Review of Literature.

Schorer* in an article "Experimental Studies on Milk", makes the following statements in regard to the original acidity of milk: "Milk when drawn from the healthy udder of the cow has an acidity which is hardly perceptible. After a very short time the acidity may be readily detected and determined.

The acidity of fresh milk as determined by phenolphthalein as indicator is due principally to the presence of carbonic acid, acid phosphates, ash and casein. The acidity that develops later is due principally to the change of lactose into lactic acid, and results from bacterial action, this being dependent on the kinds of bacteria present, their number and the temperature at which the milk is kept. The change known as coagulation is the result of precipitation of caseinogen by the combined action of the acid and calcium salts. The acid is usually lactic acid and can be titrated, though coagulation occurs as a result of the formation of amino acids and possibly also from other causes."

*Edwin H. Schorer, - Experimental Studies on Milk, Journal of Infectious Diseases, Nov. 11. 1912.

The original acidity of milk has been reported by different observers as varying between .11% - .18% figured as lactic acid. Milk is found to coagulate upon boiling if the acidity is over .225%. A sour taste is first detected at .3% and curdling occurs at .56%.

Shorer goes on to say, "Auzinger states that fresh milk from one cow has an acidity of .146 to .18% and that the percentage of acidity of fresh milk may give valuable information in regard to the cow from which it was drawn,

If the degree of acidity is less than .11% suspect mastitis.

If the degree of acidity is between .14 and .20% suspect first or second week after parturition.

If the degree of acidity is above .20% suspect colostrum or pathological milk.

Ruhn states that milk from a tuberculous udder has an acidity of less than .16%.

*Hoyberg has devised a method for determining inflammatory processes in the udder of the cow based on the alkaline reaction of serum exuded in inflammatory processes, using rosolic acid in alcoholic solution as an indicator".

*Metzger studied the value of the alcohol test in detecting the milk of diseased animals - "The value of

*Ztschr. f. Fl. v. Milch-Hyg., 1911, 21, p. 133.

*K. Metzger, Inaug. Disc. Univ. Stuttgart, 1912.
Abs. E. S. R. Vol. XXIX, No. 5.

the alcohol test was studied in connection with the acidity of milk coming from 18 cases of tuberculosis, 26 cases of indigestion, 17 cases in which the genital organs were affected and 9 cases of miscellaneous diseases. The results obtained varied, consequently the test is not deemed one which can be relied upon for general diagnostic purposes. An exception, however, is supposed to be made in diagnosing impending abortion. A negative finding in these cases points with certainty to the fact that abortion will not take place within a certain time."

An article by *Bordas upon the original acidity of milk has been abstracted in the Experiment Station Record as follows: "The lack of harmony in the various views concerning the reaction of milk is thought probably due to interpreting the results on the basis of different indicators. The author believes the best indicator to use is phenolphthalein. The total acidity of milk is chiefly due to free casein. Fresh milk never contains a free acid as lactic acid, citric acid or any acid salt. When there is an increase in the acidity as a result of decomposition of lactose, the calcium salt of casein is decomposed before the lactic acidity is manifest".

°Chretien found normal milk to contain .10 to .20% acidity reported as lactic acid. Colostrum milk had slightly less. The feeding of a liquid distillery residue did not

*Bordas. (Orig. Commun. 8 Internat. Cong. Appl. Chem.)
Abstract E. S. R. Vol. XXIX, No. 9.

°Chretien, A. (Hyg. Viande et Lait) 7, (1913) No. 8,
pp. 244-251. Abs. E.S.R. Vol. XXIX, No. 6.

materially affect the acid of milk. The acid content appeared to increase with the length of lactation period. Milk from a tuberculous udder showed a decrease in the acidity while that containing *Bacillus mastitis*, *B. coli*, staphylococci, and streptococci showed a greater acid content than normal milk.

*Bosworth and Prucha, while trying the method of Blau found fresh normal milk to contain .21% of citric acid. This they found to gradually disappear during souring.

°Richmond reports the original acidity of milk as being due to mono- and di-basic phosphates, and partly to dissolved carbonic acid.

Methods.

Taking samples. - Samples were taken at the barn from the whole milk, placed in sterile bottles and brought to the laboratory where acid determinations were immediately made. In most cases the samples were less than an hour old when titrated.

Method of determining acidity.- 40 c.c. of well mixed milk were placed in a clean white porcelain cup., 2 c.c. of 1% solution of phenolphthalein was added. Tenth-normal sodium hydroxide solution was then added from a graduated burette until the milk had a faint pink color.

*The fermentation of citric acid in milk. Alfred W. Bosworth and M.J.Prucha. Tech. Bulletin No. 14, N.Y. Agric. Exp't. Station.

°Richmond, H.D. Dairy Chemistry.

The following formula was used in calculating the results:

$$\frac{\text{c.c. of N/10 NaOH used} \times .009 \times 100}{\text{c.c. of Milk used}} = \% \text{ acidity as lactic acid.}$$

Experimental Part.

No literature was found showing the original acid reaction of milk from different species except from the cow.

In Table I, are given the results of titrations of fresh milk from different animals. Of the animals examined, sheep's milk had the highest average with a maximum of .198% and a minimum of .112. Cow's milk ranged between .114% and .243%, while mare's milk ranged between .063 and .07.

Table I.

Animal	No. of Samples Examined.	% Acid.
Sheep	16	.185
Cow	55	.177
Mare	3	.066
Woman	1	.031

Table II, shows the difference in acidity of different portions of the milking. For this experiment a sample of the fore milk was taken from the four quarters. Another sample was taken at the middle of the milking and a third at the end. The table shows the middle milk to have the highest average acidity with .159%, strippings next with .144% and fore milk lowest with .138%.

Table II.

Acidity to Phenolphthalein			
Cow No.	Fore Milk	Middle Milk	Strippings
1	.179	.207	.193
2	.139	.148	.135
3	.126	.148	.130
4	.126	.135	.121
5	.103	.125	.107
6	.144	.157	.144
7	.154	.162	.153
8	.112	.148	.121
9	.153	.198	.171
10	.144	.175	.157
11	.144	.166	.162
12	.140	.144	.135
Average	.138	.159	.144

Table III.

Cow No. 1.		Cow No. 2.	
Portion	Acidity	Portion	Acidity
1	.162	1	.144
2	.165	2	.180
3	.175	3	.177
4	.180	4	.166
5	.181	5	.157
6	.180	6	.157
7	.175	Cow No. 3.	
8	.175	1	.150
9	.177	2	.153
10	.166	3	.157
11	.166	4	.162
12	.166	5	.156
13	.166	6	.153
14	.161	7	.153
15	.155	8	.146
16	.155	9	.144

Table III shows the original acidity of different portions of the milking. As noted in the table, the first portion has a low acidity, reaching a maximum a little prior

to the middle of the milking, and falling again at the end. These results correspond closely with those obtained in Table II.

In Table IV the original acidity of all the normal samples of milk tested, are tabulated, also a record of the animals.

Table IV.

Cow.	Dairy.	Breed.	Age.	Period of Lactation.	% Acid.
1	College	Holstein	3 yrs.	9 Months	.206
2	"	"	4 "	6 "	.157
3	"	"	4 "	8 "	.180
4	"	"	4 "	2 "	.211
5	"	"	4 "	4 "	.166
6	"	"	5 "	3 "	.166
7	"	"	5 "	2 "	.180
8	"	"	5 "	4 "	.202
9	"	"	5 "	11 "	.202
10	"	"	6 "	6 "	.180
11	"	"	7 "	13 "	.175
12	"	"	7 "	2 "	.166
13	"	"	8 "	4 "	.139
14	"	"	8 "	3 "	.198
15	"	"	10 "	3 "	.198
16	"	Guernsey	7 "	7 "	.229
17	"	"	5 "	8 "	.211
18	"	"	3 "	4 "	.211
19	"	"	5 "	1 "	.229
20	"	"	7 "	9 "	.215
21	"	Jersey	3 "	4 "	.189
22	"	"	5 "	9 "	.180
23	"	"	3 "	8 "	.243
24	"	"	2 "	1 "	.234
25	"	"	2 "	9 "	.225
26	"	"	2 "	7 "	.206
27	"	"	3 "	7 "	.211
28	"	"	5 "	7 "	.211
29	"	"	5 "	7 "	.211
30	"	"	5 "	4 "	.202
31	"	"	6 "	3 "	.211
32	"	"	7 "	6 "	.229
33	"	"	8 "	3 "	.198
34	"	Brown Swiss	3 "	8 "	.198
35	"	"	5 "	2 "	.184
36	"	"	5 "	4 "	.184
37	"	"	13 "	19 "	.202
38	Spanore	Holstein	4.5 yrs.	12 "	.169
39	"	"	3.5 "	10 "	.154
40	"	"	5.5 "	7 "	.149

Table IV. (cont'd)

Cow.	Dairy.	Breed.	Age.	Period of Lactation.	% Acid
41	Espanore	Holstein	4.5yrs.	2 Months	.176
42	"	"	7 "	12 "	.125
43	"	"	5 "	12 "	.114
44	"	"	5 "	7 "	.153
45	"	"	5 "	6 "	.145
46	"	"	4 "	3 "	.131
47	"	"	4 "	4 "	.147
48	"	"	4.5 "	7 "	.122
49	"	"	9 "	10 "	.157
50	"	"	11 "	8 "	.129
51	"	"	7 "	6 "	.155
52	"	"	2.5 "	7 "	.138
53	"	"	2.5 "	7 "	.171
54	"	"	2.5 "	7 "	.152

Table V shows the influence of breed upon the original acid reaction of milk. The Guernsey is found to be the highest with an average acidity of .219%. The others ranking as follows; Jersey, Brown Swiss and Holstein.

Table V.

Breed	No. Tested	Highest	Lowest	Average
Guernsey	5	.229	.211	.219
Jersey	13	.243	.180	.212
Brown Swiss	4	.202	.184	.192
Holstein	32	.211	.114	.162

In Table VI all the normal animals tested are arranged in four groups according to their age. This table shows the milk of cows between 2 and 4 years to have an acid reaction above average for the breed, while cows between 4 and 10 years have a reaction which is close to the average for breed. Milk from cows that are over 10 years has a higher average acidity than the average for breed.

Table VI.

Age	No. tested	Average for Breed
2 - 4 yrs.	13	1.8 % above
4 - 6 "	25	1.2 % below
6 - 10 "	13	.71 % above
10 - 15 "	3	2.5 % above

Table VII shows the effect of the period of lactation upon the original acid reaction of milk. During the second to fourth month of the period of lactation, the acidity seems to be unusually high being 6.4% above the average for the breed. At all other times it is slightly below the average for the breed.

Table VII.

Period of Lactation.	No. tested.	Average for Breed.
Under 2 months	5	.48% below
2 to 4 "	12	6.4 % above
4 to 6 "	8	2.9 % below
10 months and over	24	1.7 % below
	8	1.3 % below

Table VIII shows the development of acid in samples of milk which have been carefully drawn and kept at room temperature. There seems to be no constant rule as to rate and time of development of acidity, probably depending upon the nature and amount of contamination.

Table VIII.

Monday								
Cow	4:00pm.	5:00pm.	6:00pm.	7:00pm.	8:00pm.	9:00pm.	10:00pm.	11:00pm.
1	.099	.112	.112	.112	.112	.111	.112	.112
2	.135	.155	.170	.172	.175	.170	.170	.165
3	.148	.157	.162	.153	.150	.150	.152	.150
4	.117	.112	.126	.121	.124	.123	.117	.125
5	.193	.180	.192	.193	.195	.202	.193	.185
6	.122	.121	.121	.126	.121	.125	.121	.121
7	.171	.189	.180	.180	.180	.175	.171	.175

Table VIII. (cont'd)

Monday								
Cow	4:00pm.	5:00pm.	6:00pm.	7:00pm.	8:00pm.	9:00pm.	10:00pm.	11:00pm.
8	.121	.121	.121	.121	.126	.121	.120	.118
9	.171	.162	.166	.171	.171	.171	.162	.157
10	.153	.153	.153	.157	.166	.153	.152	.157
11	.202	.202	.207	.202	.202	.205	.203	.210
12	.155	.156	.160	.155	.165	.165	.160	.160

Tuesday			Wednesday	
Cow	8:30am.	12:30pm.	5:30pm.	8:30am.
1	.110	.115	.115	.112
2	.170	.163	.165	.166
3	.150	.150	.148	.150
4	.124	.123	.123	.123
5	.190	.185	.193	.190
6	.125	.126	.126	.126
7	.178	.180	.185	.180
8	.121	.121	.121	.118
9	.171	.160	.175	.171
10	.160	.160	.160	.165
11	.210	.212	.216	.207
12	.163	.159	.160	.158

Table IX shows the difference in the original acidity of milk taken from the four quarters. This table shows a wide variation in the original acid reaction of the milk found in the different quarters.

Table IX.

Cow	Right Rear Quarter	Right Front Quarter	Left Rear Quarter	Left Front Quarter
1	.150	.202	.180	.191
2	.157	.126	.150	.130
3	.225	.202	.162	.252
4	.198	.229	.189	.238
5	.135	.161	.166	.207
6	.135	.152	.166	.148
7	.215	.229	.207	.211
8	.139	.148	.157	.135
9	.207	.202	.215	.202
10	.157	.157	.180	.152

Table X shows the original acid reaction of milk from day to day after calving. The first two or three days are seen to be usually high due to colostrum. It then reaches a point from which it varies slightly either up or down from day to day.

Table X.				
Day	Lassie Elzevere	College Goda	Bertha M.	Amis Fair
1	.405	.427	.210	.315
2	.150	.295	.135	.202
3	.137	.279	.162	.195
4	.103	.270	.210	.184
5	.121	.256	.184	.207
6	.101*	.246	.166	.177
7	.090*	.216	.175	.162
8	.101*	.202	.216	.171
9	.067*	.186	.207	.162
10	.067*	.211	.180	.159
11		.182		.150
12	*Gargety	.175		.135
13				.166
14				.117
15				.178

Table XI shows the relation of the health of the cow to the original acid reaction of milk. Unfortunately very few abnormal cows were available for this work. The samples obtained show a wide variation due to the different abnormal conditions.

Table XI.	
Condition of Milk or Udder	% Acid
Sick with indigestion	.072
Milk very bloody	.599
Teat injured externally	.157
Gargety milk	.063
" "	.090
" "	.050
" "	.069
" "	.090
" "	.065

Discussion of Results.

A study of the original acid reaction of the milk from the different species shows a wide variation. Of the species studied, sheep milk was the highest with others ranking as follows: Cow, mare, woman. The following table shows an interesting relation between the acid reaction and the percent of ash and casein present.

Species	Acidity	Casein	Ash
Sheep	.185	5.23	.97
Cow	.177	3.00	.75
Mare	.066	1.84	.30
Woman	.031	1.00	.20

The casein and ash are seen to decrease in exactly the same manner that the acid reaction does. This bears out the theory that the original acid reaction of milk is largely due to casein and ash.

Acid reaction of milk to phenolphthalein shows the fore milk to have the lowest acid reaction, while the middle milk is highest and the strippings having a reaction between the two. The reaction of the fore milk and strippings are again seen to correspond to the amount of casein and ash present in the respective portions as noted in relative % acid reaction to species. The author was unable to find a table showing the chemical composition of the middle milk. It is also thought that the presence of alkali producing organisms in the highly contaminated fore milk might influence the reaction.

A wide variation in reaction is found for breeds. There appears to be no constant relation between this reaction and the average amounts of casein or ash present.

The average reaction of milk to phenolphthalein for cows between 2 and 4 years of age is slightly above the average for the breed, cows between 4 and 10 years are about average while cows over 10 years of age have an average reaction which is 2.5% above that for their breeds. These differences are so small that this factor isn't thought to influence the reaction to any appreciable extent.

Between the second and fourth months of the period of lactation was the only time that the milk had an acid reaction which was above the average for the breeds. Except for this period the acid reaction gradually increased during the period of lactation as reported by Chretien.

Milk when very carefully drawn and stored at room temperature may sometimes be kept as long as 24 hours without appreciable change in reaction. In some cases, however, the reaction increases slightly after the first hour. Most titration in this work were made within one hour after having been drawn.

The milk from different quarters of the same cow sometimes has an acid reaction with a wide variation. This might be accounted for by some pathological condition of the quarter or by some bacterial invasion of the quarter in question.

Colostrum has an acid reaction which in some cases was found to be over .4%. Usually this reaction reaches normal in from three to four days. The high reaction of the colostrum is probably due to its extremely high percentage of casein and ash.

Very few samples of pathological milk were obtained for this work. Extreme cases of gargety milk and milk from a cow with indigestion gave unusually low titrations, while bloody milk gives a high acid reaction. Judging from the experiments the reaction of milk to phenolphthalein is of no practical importance in diagnosing abnormal conditions of the milk or udder for the following reasons: Too many other factors enter in and also a condition of the milk or udder, which could be detected by means of the reaction, could be much easier and more surely detected by other means.

SUMMARY.

The acid reaction of fresh milk to phenolphthalein is of no practical value in diagnosing abnormal conditions of the milk or general conditions of the cow.

Normal milk was found to have an original reaction to phenolphthalein varying between .114 and .243 percent as lactic acid. This according to Auzinger's table would include old milkers; milk from cows in first or second week after parturition, colostrum and pathological milk.

Bad cases of gargety milk and bloody milk were found to have reactions which were respectively below and above the limits for normal milk.

These conditions, however, could easily be detected by other means.

The different breeds were found to have varying acid reactions. The average reactions being: Guernsey .219%, Jersey .212%, Brown Swiss .192%, and Holstein .162%.

Middle milk has a higher titration than fore milk or strippings.

Age of cow apparently has very little influence upon the reaction.

The acid reaction of the milk ^{is} apparently gradually increases after the fourth month of the period of lactation.

Considerable variation is sometimes found between the reaction of the milk from different quarters.

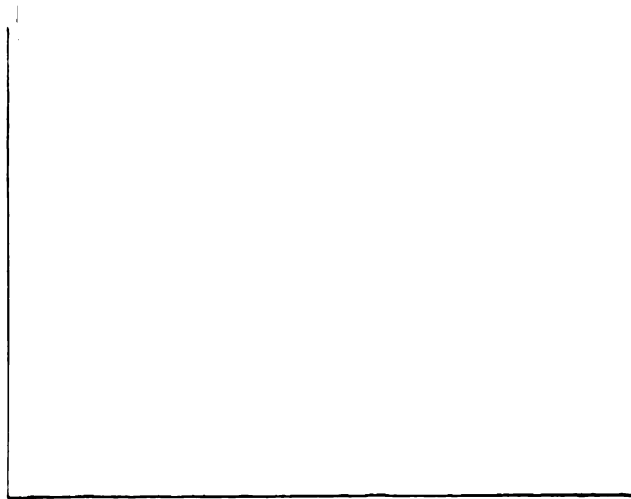
Colostrum has a high acid reaction.

There seems to be a relation between the acid reaction and the amount of casein and ash present.

1945

Hurtz

A study of the original
acid reaction of milk to
phenolphthalein



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