LACTIC STARTER CULTURE ACTIVITY IN MILK FROM COWS ON PASTURE AND IN MILK FROM COWS ON DRY FEED[1]

T. J. CLAYDON AND H. C. FRYER

Department of Dairy Husbandry and Statistical Laboratory

Kansas State University, Manhattan

(Received for publication August 19, 1959)

Lactic starter culture activity in milk from cows on rye pasture and in milk from cows on dry feed was compared, using two cultures widely distributed by commercial laboratories. The milk was pasteurized at 62.5°C. for 30 min. In 16 and 24 hr. at 21°C. one culture developed slightly higher acidities in milk from the pasture cows than in milk from the dry-feed cows, as shown by the means of 8 trials. The differences were statistically significant. On the other hand, the second culture, which showed lower activity in the test milks, developed a higher acidity in the milk from cows on dry feed. The differences at the 16-hr. period were statistically significant.

The total solids content of milk averaged 13.70% from the cows on pasture and 13.20% from those on dry feed. The proteose-peptone content varied but the mean value was about the same for milk from the pasture cows as for that from the dry-feed cows: 2.4 and 2.3 mg./10 ml. milk, respectively. Non-protein nitrogen averaged 4.5 mg./10 ml. milk from cows on pasture and 2.8 mg./10 ml. milk from cows on dry feed.

Although the differences in milk composition might have influenced lactic culture activity, it is considered that the effect of milk from cows on pasture depended on the characteristic of the lactic culture. Such effect appears to be of doubtful practical significance.

Cheese manufactured during spring and summer seasons sometimes has been considered superior to that made during winter. Van Slyke and Price (*11*) surmise that summer milk, as opposed to "fodder" milk, may contain factors that stimulate the growth and activity of microorganisms in cheese starter and during cheesemaking and ripening processes. Ritter (*9*) concluded that slow acid development in cheesemaking was not due to inherent differences in milk and that no significant effect was produced by feeding cows winter-type or summer-type feed.

Riel and Sommer (*8*) noted that milk from pasture-fed cows showed an increase in total nitrogen content and the proteose-peptone fraction. Anderson *et al.* (*1*) reported that milk with relatively high peptide content was usually more stimulatory to lactic culture development than milk low in these constituents. However, responses of different cultures varied. Milk from two cows on a low carotene diet supported poor growth, although the protein content of the milk was normal.

Variations in cheese starter activity associated with season or weather have been noted (*2*, *5*, *6*) but were attributed to causes other than the type of feed. At the Kansas Station, duing a period when some cows were on spring pasture and others still remained on winter feed, exploratory tests suggested greater activity of lactic cultures in milk from cows on pasture than from cows on dry feed. Since further information on the effect of pasture feeding of cattle on the milk as a growth medium for lactic culture organisms seemed desirable, additional study was undertaken.

METHODS

The activity of lactic starter cultures usually varies somewhat over a period of time, even when the cultures are propagated under uniform conditions. Pasture conditions also change with time. Therefore, comparisons were made simultaneously with different groups of cows rather than successively with the same group. Such procedure permitted inoculation of milk samples from the same culture transfers in each comparison. However, it limited the number of cultures that could be tested at one time.

In early spring, while the College dairy herd was still on dry feed, 20 cows were selected for the experiment. Those picked were free from mastitis and had received no recent antibiotic treatment. Cows at extreme ends of their lactation periods were excluded. Eight Holsteins and four each of Ayrshires, Jerseys, and Guernseys were included.

Balancing of cow groups

In an effort to divide the cows into balanced groups so that milk from each group might be equally suitable for culture development, culture activity tests were made on the milk from each cow before starting the main experiment. It was considered that such activity tests would provide the best information for equalizing the cow groups.

Two trials were conducted with a 2-day interval between. From the results on individual samples, cows were placed in four groups so that the developed acidity in the milk averaged the same for each group. The cow groups, designated I, II, III, and IV were also arranged to include two Holsteins

[1]Contribution No. 269, Department of Dairy Husbandry, and No. 44 Statistical Laboratory, Kansas Agricultural Experiment Station, Manhattan.

and one each of the Ayrshire, Jersey, and Guernsey breeds.

Following the group balancing procedure and using a table of random numbers, cow groups I and II were placed on rye pasture with a grain supplement. During the first 3 days, they were on pasture only 3 hr. daily and received some alfalfa hay each day. After 3 days, groups I and II were on pasture day and night except for about 1 hour before and during milking and received no hay. During much of the experiment the weather was wet and cool, the pasture muddy, and cows grazed relatively short periods. Groups III and IV remained on dry feed consisting of Atlas sorgo silage, grain, and alfalfa hay. Comparisons of lactic culture activity were started after groups I and II had been on pasture 4 days. The investigation was continued for 4 weeks to allow adequate time for possible cumulative effects of pasture feeding. A longer period was not used because of increased maturity and decreased productivity of the pasture.

Sampling and testing procedures

In trials comparing milk from cows on pasture with milk from cows on dry feed, representative milk samples were obtained from each cow during morning and evening milkings. Night portions were held refrigerated and morning portions were added to the same containers. The samples were taken to the laboratory promptly after the morning milking and composite samples were prepared for each cow group (I, II, III and IV) in accordance with the production of the different cows.

To check culture activity and permit some comparison between trials, control milk samples were used as a standard. Control milk was made from reconstituted nonfat milk solids, previously tested to insure absence of inhibitory substances. The same lot of powder was used throughout the study. Reconstituted control milk was prepared to contain 9.0% solids. Each cow group sample and the control milk was dispensed in 100-ml. lots into four 6-ozs. prescription bottles. Portions were also taken for initial titratable acidity, pH, total solids, proteose peptone and non-protein nitrogen determinations. Total solids determinations were made gravimetrically and proteose peptone and non-protein nitrogen fractions were measured by methods of Shahani and Sommer (*10*).

The 100-ml. milk samples were pasteurized promptly in a water bath at 62.5°C. ± 0.5° for 30 minutes. After cooling in water they were held in the refrigerator 3 to 4 hr. until inoculated. At that time samples were tempered to 21°C., inoculated with 1% lactic culture, and incubated at 21°C. The pasteurizing treatments, amounts of inoculum, and incubation conditions were selected as approximating methods followed in the overnight set for cottage cheese. Also, the relatively low incubation temperature and inoculation rate would be more likely to show differences in acid production than would higher temperatures, heavier inoculations and shorter incubation periods.

Although it would have been advantageous to use a number of different lactic cultures, the desirability of making all comparisons in each trial at the same time limited the number of cultures that could be used satisfactorily. Since the procedure described required 10 samples for acid development with each culture, the number of cultures tested was limited to two. These (A and B) were widely-used, mixed strain commercial cultures. One culture (A) was the same as previously used in the preliminary balancing trials. The cultures were propagated in reconstituted, pretested, nonfat milk, made to 9% solids. Transfers were made three times weekly with incubation at 21°C. for 18 hr. followed by refrigeration. Inoculations of milk samples for activity tests were made from cultures transferred the previous day. Titratable acidity and pH determinations were made on the samples at 16 and 24 hr. After adjusting for initial titratable acidity and pH, results were reported as developed acidity and pH changes. All samples were tested in duplicate with each culture. Trials were conducted twice weekly during the 4-wk. period, making eight trials in all. The data were studied by analyses of variance and t-tests. Unless otherwise stated, the 5% level of significance is used throughout this manuscript.

RESULTS

With culture A and a 16-hr. incubation period, the mean developed acidity for eight trials was somewhat higher in milk from cow groups on pasture than in milk from cow groups on dry feed (Table 1). Differences varied with trials but were statistically significant for the 4-wk. period. Differences between reconstituted control milk and milk from pasture groups were not statistically significant.

Results with culture B at 16 hr. differed from those obtained with culture A and, in general, the developed acidity was lower. Milk from the pasture groups developed significantly less acid than milk from the dry-feed groups, as shown by the means for the eight trials. Milk from each cow group was significantly lower in acid production than was the control. Milk from group II rather consistently supported poor growth of culture B.

At 24 hr. with culture A, milk from the pasture groups remained significantly higher in developed

TABLE 1—ACID DEVELOPED BY TWO LACTIC CULTURES IN 16 HOURS AT 21°C. IN RECONSTITUTED, PASTURE, AND DRY-FEED MILKS

Trial	Sample	Culture A					Culture B				
		Reconstituted milk	Pasture milk Cow groups I	II	Dry-feed milk Cow groups III	IV	Reconstituted milk	Pasture milk Cow groups I	II	Dry-feed milk Cow groups III	IV
		Per cent acid developed[a]									
1	1	.45	.45	.46	.39	.41	.50	.37	.39	.42	.41
	2	.47	.46	.49	.39	.42	.50	.36	.38	.42	.51
2	1	.47	.47	.49	.44	.41	.48	.35	.31	.27	.35
	2	.50	.49	.47	.42	.41	.45	.37	.27	.28	.35
3	1	.50	.49	.52	.49	.51	.50	.35	.30	.38	.39
	2	.55	.50	.54	.48	.52	.50	.36	.32	.39	.40
4	1	.50	.46	.45	.39	.43	.44	.31	.22	.30	.30
	2	.53	.47	.44	.41	.41	.43	.30	.22	.29	.31
5	1	.38	.39	.38	.40	.40	.41	.32	.25	.40	.37
	2	.38	.39	.39	.40	.40	.43	.33	.25	.39	.37
6	1	.57	.51	.50	.48	.55	.54	.40	.37	.41	.44
	2	.57	.52	.52	.49	.54	.56	.41	.37	.44	.46
7	1	.51	.54	.57	.46	.48	.46	.33	.22	.28	.32
	2	.54	.56	.54	.47	.49	.46	.34	.22	.29	.33
8	1	.42	.35	.36	.37	.37	.37	.25	.12	.23	.25
	2	.40	.34	.38	.38	.38	.35	.27	.12	.23	.25
	Means	.484	.465		.437		.461	.304		.348	
			ns		*		*		*		
			*				*				

[a]Final titratable acidity minus initial; ns = Statistically nonsignificant; * = Statistically significant at or beyond the .05 level.

acidity than milk from dry-feed groups (Table 2). Also, with all groups the developed acidity was significantly higher than for the reconstituted control milk. With culture B, at 24 hr., differences between pasture and dry-feed groups were not statistically significant, but developed acidities were significantly higher in milk from all cow groups than in the control milk. Unlike the situation after 16 hr. of incubation, the level of developed acidity was the same for cultures A and B after 24 hr. of incubation.

Changes in pH values generally corresponded to changes in titratable acidity, but there were variations in this relationship. Data on pH changes from the eight trials are summarized in Table 3. With culture A at 16 hr. the mean pH changes for milk from pasture groups and from dry feed groups were similar, but each was significantly less than the reconstituted control. At 24 hr. the mean pH change was greater statistically in milk from the dry-feed groups than in milk from the pasture groups, with the latter being similar to the control. With the B culture, mean pH changes were statistically in accordance with changes in titratable acidity except after 24 hr. incubation, at which time there was not an important difference between any two groups.

Data on total solids content of the samples are presented in Table 4. Milk from the groups on pasture averaged 0.5 of a percentage point higher in solids than milk from the groups on dry feed, and the difference was statistically significant. The reconstituted control milk, which was prepared to contain 9.0% total solids, deviated somewhat from this figure in the different trials. However, there appeared to be no close relation between the total solids in control samples and the corresponding developed acidities. Also, all linear correlations between developed acidity and total solids computed within each of the five sources of milk were insignificant, well above the 10% level.

In non-protein nitrogen content, the milks from Groups I and II (on pasture) were similar and quite uniform throughout the experiment (Table 5). The same was true with Groups III and IV (on dry feed). However, the level was 61% higher for the pasture groups than for the dry-feed groups. This difference was significant even at the 0.1% level. The proteose-peptone nitrogen fraction in the milk varied with groups and trials. Group III, on dry feed, averaged the lowest in this fraction, while Group IV, also on dry feed, averaged the highest. However,

TABLE 2—ACID DEVELOPED BY TWO LACTIC CULTURES IN 24 HOURS AT 21°C. IN RECONSTITUTED, PASTURE, AND DRY-FEED MILKS

Trial	Sample	Culture A					Culture B				
		Reconstituted milk	Pasture milk Cow groups I	II	Dry-feed milk Cow groups III	IV	Reconstituted milk	Pasture milk Cow groups I	II	Dry-feed milk Cow groups III	IV
					Per cent acid developed[a]						
1	1	.56	.65	.66	.66	.65	.65	.64	.65	.66	.64
	2	.55	.65	.66	.58	.65	.65	.65	.65	.66	.63
2	1	.61	.67	.66	.67	.66	.61	.65	.64	.64	.63
	2	.63	.67	.66	.67	.63	.63	.65	.65	.66	.63
3	1	.60	.61	.64	.65	.56	.68	.68	.69	.69	.64
	2	.63	.64	.62	.62	.59	.66	.67	.70	.67	.63
4	1	.57	.66	.67	.63	.64	.67	.67	.64	.67	.63
	2	.56	.64	.66	.64	.65	.66	.66	.66	.65	.65
5	1	.61	.67	.68	.63	.64	.64	.68	.67	.67	.63
	2	.61	.67	.69	.61	.64	.62	.67	.68	.65	.65
6	1	.65	.65	.67	.65	.66	.62	.68	.66	.69	.66
	2	.66	.66	.68	.66	.66	.65	.68	.68	.67	.68
7	1	.60	.63	.62	.62	.59	.50	.65	.67	.63	.62
	2	.61	.64	.63	.61	.60	.51	.66	.66	.63	.63
8	1	.60	.68	.70	.66	.65	.69	.65	.59	.65	.60
	2	.60	.68	.69	.67	.65	.60	.66	.60	.64	.62
	Means	.603	.658		.639		.621	.645		.659	
		*	*				*	ns			
		*									

[a]Final titratable acidity minus initial; ns = Statistically nonsignificant; * = Statistically significant at or beyond the 0.5 level.

TABLE 3—SUMMARY OF DECREASES IN pH VALUES PRODUCED BY TWO LACTIC CULTURES AT 21°C. IN RECONSTITUTED, PASTURE, AND DRY-FEED MILKS[a]

Incubation Period	Culture A					Culture B				
	Reconstituted milk		Pasture[b] milk		Dry-feed[b] milk	Reconstituted milk		Pasture[b] milk		Dry-feed[b] milk
					pH changes[c]					
16 hrs.	-1.83	*	-1.74	ns	-1.73	-1.66	*	-1.22	*	-1.46
24 hrs.	-1.95	ns	-1.97	*	-2.05	-2.06	ns	-2.01	ns	-2.05

[a]Mean values from 8 trials in duplicate with each culture; [b]Includes 2 cow group; [c]Final pH minus initial pH = ms Statistically nonsignificant; * = Statistically significant at or beyond the .05 level.

the difference between the mean for the pasture groups and the mean for the dry feed groups was not significant.

DISCUSSION

Whole milk was used in the investigation, partly as a matter of convenience and partly because some differences in milk from cows on pasture and from cows on dry feed are known to be associated with the butterfat. Although it has been reported (3, 7) that differences in acid production in whole milk and skim milk are negligible, the milk used in the studies was heated to a relatively high temperature and one which would reduce subsequent creaming. Since creaming of milk is considered by Wright and Tramer (*13*) to affect culture development, it may account for some of the variations occurring in this experiment with milk heated to only 62.5°C. for 30 min.

The generally lower activity in the pasture and dry-feed milks than in the reconstituted control milk at 16 hr. may arise from the relatively low heat treatment. Although the control milk was similarly pasteurized, its previous heat treatment during pow-

TABLE 4—TOTAL SOLIDS IN RECONSTITUTED, PASTURE, AND DRY-FEED MILKS[a]

Trial	Reconstituted milk	Pasture milk, Cow groups I	Pasture milk, Cow groups II	Dry-feed milk, Cow groups III	Dry-feed milk, Cow groups IV
	Per cent total solids				
1	9.02	13.60	13.63	12.60	12.74
2	8.90	13.57	14.26	13.43	13.50
3	9.09	13.82	13.66	13.57	13.08
4	9.11	13.52	13.66	12.85	13.24
5	9.06	13.93	13.89	13.26	13.24
6	9.22	14.10	13.66	13.35	13.36
7	8.91	13.55	14.00	13.58	13.26
8	8.78	13.16	13.13	12.89	13.20
Means	9.01	* 13.70		* 13.20	

[a]Av. of duplicate samples; * = Statistically significant at or beyond the .05 level.

TABLE 5—NON-PROTEIN NITROGEN AND PROTEOSE-PEPTONE NITROGEN IN PASTURE, AND DRY-FEED MILKS

Trial	NPN, Pasture milk, Cow groups I	NPN, Pasture milk, Cow groups II	NPN, Dry-feed milk, Cow groups III	NPN, Dry-feed milk, Cow groups IV	PPN, Pasture milk, Cow groups I	PPN, Pasture milk, Cow groups II	PPN, Dry-feed milk, Cow groups III	PPN, Dry-feed milk, Cow groups IV
	Mg./10 ml. milk							
1[a]	—	—	—	—	—	—	—	—
2	4.7	4.7	2.7	2.7	2.0	2.8	1.9	2.3
3	4.4	4.6	2.7	2.9	2.2	2.5	1.9	2.6
4	4.4	4.6	2.7	3.0	2.0	2.1	1.4	2.7
5	4.3	4.4	2.7	2.9	2.1	2.5	2.3	2.3
6	4.4	4.7	2.7	2.9	2.3	2.5	2.1	2.7
7	4.9	4.6	3.1	3.1	2.6	2.4	1.9	2.8
8	4.5	4.4	2.8	2.8	3.0	2.6	2.2	3.1
Means	4.5	***	2.8		2.4	ns	2.3	

[a]Data not obtained in trial 1; ns = Statistically not significant; *** = Statistically significant at or beyond the .001 level.

der manufacture, undoubtedly contributed to a greater total heat effect. While higher heat treatment of the pasture and dry feed milks probably would have resulted in higher developed acidity, it was preferred to keep the treatments in accordance with cheesemaking processes.

The fact that cultures A and B reacted differently to milk from different cow groups is not unusual in view of recognized variations in culture characteristics. Since both cultures developed about equally well in reconstituted milk, the differences presumably arose from response of the cultures to certain characteristics of the pasture and dry feed milks. The generally lower activity of culture B in pasture and dry feed milks at 16 hr. indicates that it was more fastidious than culture A.

Although the mean developed acidity with culture A at 16 hr. in milk from cow groups on pasture was significantly greater statistically than that developed in milk from cow groups on dry feed, the difference is of doubtful practical significance. The slightly higher mean total solids content of the milk from pasture groups may have contributed to the somewhat higher acidity, although analyses of the data showed no significant linear correlation with total solids variations within groups. Also, it has been previously reported (*4*) that the correlation between acid development and total solids is not a close one.

The uniformly greater non-protein nitrogen content of milk from pasture cows might partially account for the slightly greater activity of culture A in this milk. However, Walker reported (*12*) that these fractions were not generally utilized by lactic acid bacteria, although proteose-peptone was stimulatory. In this investigation the average values for proteose-peptone nitrogen fractions were generally the same in milk from cows on pasture as in milk from cows on dry feed. Differences were not correlated with differences in culture activity.

At 24 hr. differences in developed acidity in milk from pasture groups and dry feed groups with either culture A or B would be of little practical significance.

REFERENCES

1. Anderson, A. W., Parker R. B., and Elliker, P. R. The Nutritional Requirements of Lactic Streptococci from Starter Cultures. III. Variation in Growth Promoting Properties of Fresh Whole Milk. J. Dairy Sci., **38**: 1083. 1955.

2. Czulak, J., and Meanwell, L. J. Seasonal Variations in Cheese Starter Activity. Pro. Soc. Applied Bact., **14**: 1. 1951.

3. Foster, E. M. The Effect of Heat on Milk as a Culture Medium for Lactic Acid Bacteria. J. Dairy Sci., **35**: 988. 1952.

4. Hammer, B. W., and Babel, F. J. *Dairy Bacteriology* 4th Ed. John Wiley and Sons, Inc. New York. P. 398. 1957.

5. Hunter, G. J. Growth Requirements of Lactic Streptococci: Differences within this Group. J. Dairy Res., **16**: 152. 1949.

6. Jago, G. R. Factors Influencing the Lactic Acid Producing Properties of Streptococci Used in the Manufacture of Cheddar Cheese. I. Observations Relating Inhibitory and Stimulatory Phenomena. J. Dairy Res., **21**: 111. 1954.

7. Olson, H. C., Beachboard, R. F., and Cohenour, F. D. The Influence of Propagation Procedures on the Activity of Lactic Cultures. Okla. Agr. Expt. Sta. Bull. B-446. 1955.

8. Riel, R. R., and Sommer, H. H. Oxidized Flavor in Milk. II. Nitrogen and Sulphur Distribution in Milk of Vari-

ous Susceptibilities to Oxidized Flavor Development. Contr. Inst. Oka. 11-11a. 1955 (Dairy Sci. Abstr. **20**: 441. 1958).

9. Ritter, P. Einfluss den Fütterung und Einiger Anderer Faktoren auf das Wachstum Kasereitechnisch Wichtiger Bakterien in Milch. 12th Intern. Dairy Congr. Stockholm 1: 140. 1949.

10. Shahani, K. M., and Sommer, H. H. The Protein and Nitrogen Fractions in Milk. I. Methods of Analysis. J. Dairy Sci., **34**: 1003. 1951.

11. Van Slyke, L. L., and Price, W. V. *Cheese.* 2nd Ed. Orange Judd Pub. Co., Inc. New York. P. 14. 1949.

12. Walker, H. W. Utilization of Nitrogenous Compounds in Milk by Lactic Acid Bacteria. Dairy Sci. Abstr., **19**: 318. 1957.

13. Wright, R. C., and Tramer, J. The Influence of Cream Rising upon the Activity of Bacteria in Heat Treated Milk J. Dairy Res., **24**: 174. 1957.

SANITATION PROBLEMS IN THE NEW PRESSURIZED FOODS[1]

HAROLD WAINESS

Harold Wainess and Associates

Chicago, Illinois

The amazing acceptance by the consumer of both the pressurized whipped cream container and non-food aerosols is finally leading to the development of many other pressurized foods.

Special public health precautions must be taken, including sanitary design of equipment, temperature controls, aseptic gassing, mechanical valve insertion, and sanitizing of valve and can.

The consumer is convenience-package-minded, but the industry must not neglect to continuously follow basic public health requirements for these new products.

At a recent meeting of manufacturers and suppliers for the pressurized packaging field, a manufacturer of pressurized foods issued the following warning:

" . . . The custom filler *must* provide complete services along with active sales campaigns to demonstrate to merchandising food people the facts and potential of this field. He must have the facilities and personnel to perform and a willingness to cooperate with suppliers and food companies alike, for the problems can easily be too vast for one to handle. He must be prepared to meet high production demands on short notice with positive and unwavering quality control. Costs must likewise be carefully controlled to avoid pricing above the volume market (2)."

It was further pointed out that bacteriological control was important and descriptive tests for the final product were given, but no mention was made of the need for the application of sanitation procedures and the importance of using equipment designed for sanitation.

The sanitarian is familiar with pressurized whipped cream and its phenominal growth to over 80 million cans in 1958. It has been the forerunner in the convenience packages that are steadily becoming an important part of the food industry. The list of products capable of being marketed in the pressurized package continues to grow. Available today are milk and milk products, cream, dairy dressings, horseradish whip, barbecue sauce, catsup, coffee, chocolate and other syrups, tea, toppings, batter, cheeses, sweeteners, butter, and mustard.

This is only a partial list and food technologists are rapidly developing new products. Many of them have one common feature. They are capable of supporting microbiological organisms. This is why the sanitarian must play a vital role in the metamorphosis of pressurized foods. No attempt will be made in this discussion to delve into the technological problems that beset the industry, other than in their relation to public health.

Pressurized foods properly prepared can be a boon to the restaurant sanitation program. Their very nature makes them single service and some day may eliminate the insanitary cream pitcher, the open sugar bowl, the unsightly mustard and catsup container, the open and unrefrigerated bowls of salad dressings, the finger print on a butter patty, and the mold-coated syrup containers.

A short review of the processing problems and techniques will serve to introduce the role the sanitarian must play in this field.

There are a number of reasons, each in itself contributing a small but significant part, why pressurized foods have taken so long to reach the consumer. Some of these are:

1. The slow speeds of the present whipped cream fillers.

2. The reluctance on the part of governmental officials to approve certain of the newer gases for food products.

3. The need for more basic research into formulae and types of food to be pressurized.

[1]Presented at the 46th Annual Meeting of the INTERNATIONAL ASSOCIATION OF MILK AND FOOD SANITARIANS, INC., at Glenwood Springs, Colorado, August 26-29, 1959.