

JOURNAL OF DAIRY SCIENCE

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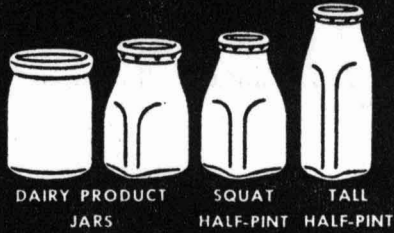


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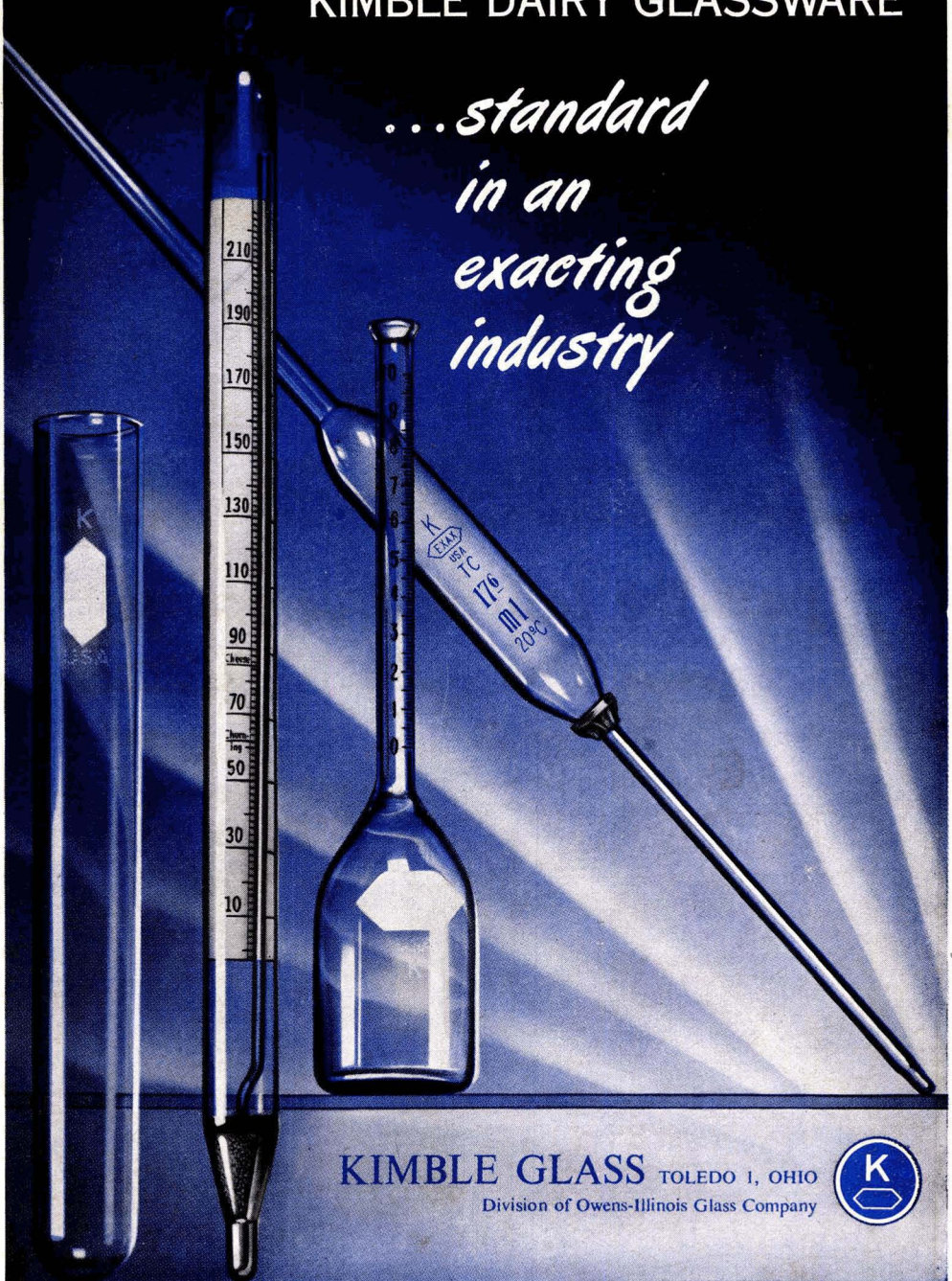
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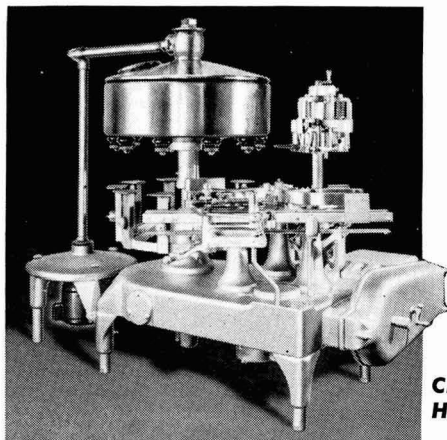
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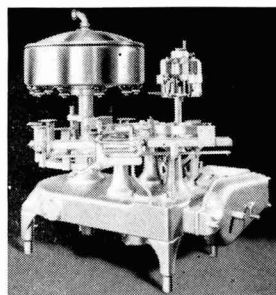
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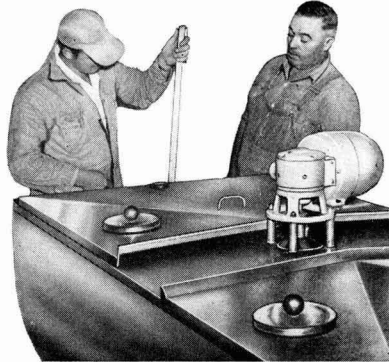
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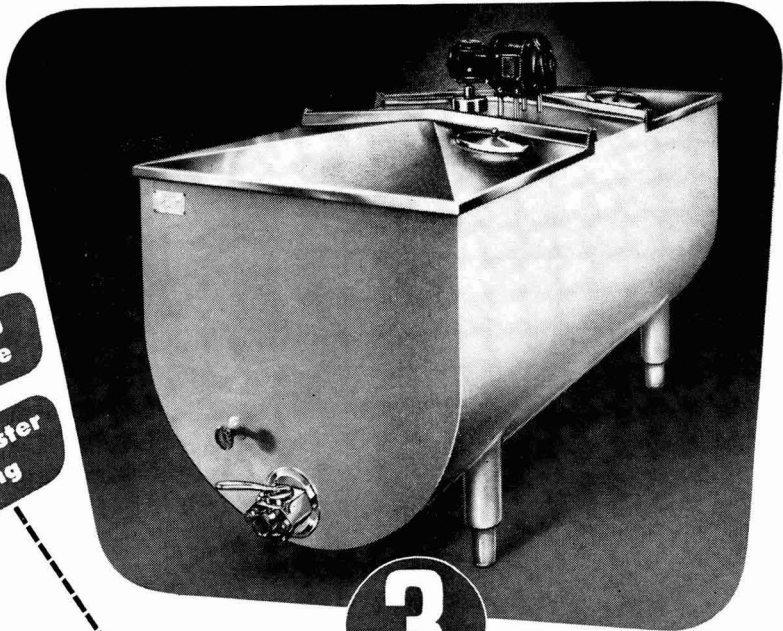
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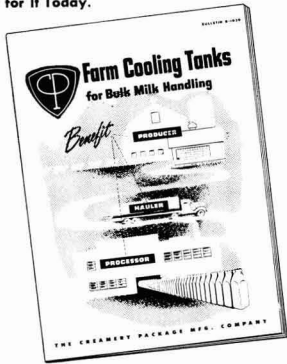
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ABSTRACTS OF PAPERS PRESENTED AT THE FORTY-SEVENTH ANNUAL MEETING

University of California
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June 24-26, 1952

MANUFACTURING SECTION

M1. The occurrence and survival of *Brucella abortus* in Italian cheese curd made from raw and pasteurized milk.¹ H. L. GILMAN, New York State Vet. College, and J. C. MARQUARDT, Dept. of Agr. and Mkts. Albany, N. Y.

A progress report on Italian cheese curd studies was made at the Forty-fifth Annual Meeting of the American Dairy Science Association held at Cornell University at Ithaca in 1950. This report included methods for making the curd from properly pasteurized milk. The procedures have been advanced by the use of D.K. cultures developed at Cornell University.

The making of Italian curd in 1 plant and forming it in another is confined mainly to the New England and Middle Atlantic States. Most of this curd used to make Mozzarella or other fresh types is manufactured in New York State, where the annual production was 18 million lb. in 1951, an increase from 10 million lb. in 1945 and 5 million in 1940.

In 1950, the New York City Health Department started enforcing a regulation regarding the pasteurization of milk for cheeses consumed fresh.

It was for this reason that milk before and after pasteurization and the resulting curd and raw milk and its curd were studied.

When milk was received at the brucellosis laboratory, it was placed in the refrigerator overnight to permit creaming. The next morning each of 2 guinea pigs was injected subcutaneously with 5 ml. of the gravity cream. The cheese was prepared by placing 25 g. of the curd in a sterilized Waring blender with 100 ml. of sterile physiological saline solution and agitated for 10 min. to produce a smooth creamy mixture. The mixture then was filtered through a layer of cheesecloth, and 2-ml. amounts were injected subcutaneously into each of 2 guinea pigs. Positive tests were those in which the guinea pigs, 5 wk. after injection, gave positive blood agglutination reactions, characteristic lesions in the spleen and recovery of *Br. abortus* from the spleen.

Br. abortus was recovered from 5 out of 6 Italian cheese curds made from raw milk.

Br. abortus was recovered from 3 out of 4

samples of batch milk from a plant making the curd. After proper pasteurization of this same milk, the organism could not be recovered from either the milk after pasteurization or from the curd made from this milk.

Inasmuch as viable *Brucella* have been found to survive the manufacturing process when raw milk is used, it seems advisable from the public health standpoint that all such milk should be pasteurized before manufacture into cheese consumed in the fresh state.

¹ Approved for Publication by Cornell University and the New York State Dept. of Agr. and Mkts.

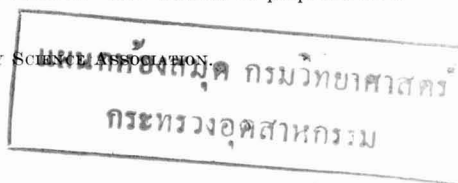
M2. Paper partition chromatography of the free amino acids of foreign-type cheeses. H. J. FAGEN AND R. V. HUSSONG, Kraft Foods Co., Research Lab. Glenview, Ill.

By means of 1-dimensional filter paper chromatography the free amino acids of 13 foreign-type cheeses are reported. An attempt is made to correlate "make" and "ripening" characteristics with the amino acid patterns. Data are presented for the following cheeses: Aged Cheddar, Emmentaler, Gouda, Munster, Roquefort, Blue, Gorgonzola, Camembert, Limburger, Lager Kase, Chantelle, Romano and Parmesan. Results indicate large differences in the amino acid patterns between types of cheeses. To a small extent differences were noted between varieties of the same types.

M3. A direct chromatographic method for the determination of the lower fatty acids in cheese.¹ W. J. HARPER, Dept. of Dairy Technol. The Ohio State Univ., Columbus.

A chromatographic method has been developed to separate the lower fatty acids directly from cheese without the necessity of prior distillation or extraction of the fatty acids. The method is a modification of the methods of Moyle *et al.* (1948) and Bulen *et al.* (1952).

The method depends upon the combination of a buffered and acidified column to permit separation of the acids from the cheese added directly to the column. The column is prepared in 3



steps: (a) 10 g. silicic acid and 6 ml. of 2 *M* pH 6.5 phosphate buffer are mixed in a mortar and then slurried with chloroform into a 35-mm. glass tube closed at the bottom with a perforated porcelain plate. (b) 3 g. silicic acid are acidified with 10% H₂SO₄ and slurried into the column with chloroform. (c) 5 g. cheese are acidified to pH 2.0 with 20% H₂SO₄ and ground in a mortar with 10 g. silicic acid. The resulting dry powder is slurried into the column with 75 ml. chloroform. The chromatogram is developed using the following concentration of mixtures of *n*-butanol added to chloroform: (a) 25 ml. of 0.5%, (b) 50 ml. of 1.0%, (c) 75 ml. of 5.0% and (d) 100 ml. of 20.0%. Using a siphon device, 5-ml. fractions are collected and titrated with 0.01 *N* alcoholic NaOH to phenol red end point. A curve showing representative peaks for each acid is obtained by plotting the titration value against the fraction number. The acid is identified by its threshold volume.

The method provides for comparative results of good accuracy for acetic, propionic and butyric acids. Caproic and higher fatty acids are separated as a group. Other organic acids, such as lactic and pyruvic, do not interfere with the method.

¹ Cooperative project with the Bureau of Dairy Industry, U.S.D.A. Fund provided in part by the Research and Marketing Act of 1946, and by the Ohio Dairy Products Research Fund.

M4. A preliminary report on the relationship of the lower fatty acids to the ripening of Provolone cheese¹. W. J. HARPER, Dept. of Dairy Technol. The Ohio State Univ. Columbus.

The relationship of the lower fatty acids to the ripening of Provolone cheese was studied by applying the chromatographic technique described by Harper (M-3). Acetic, propionic and butyric acids were determined in a number of commercial Provolone cheese which were the same age but varied in quality. Good-flavored Provolone cheese contained relatively large concentrations of butyric acid, while cheese lacking flavor contained only small amounts. The butyric acid content of good Provolone was found to be from 4–10 times that reported by Krett (1950) for Cheddar cheese of the same age. Acetic and propionic acids were present in nearly equal concentrations, and generally were not related to quality. However, the acetic acid content of cheese with an acid flavor defect was found to be abnormally high.

A definite relationship was established between the relative amounts of the lower fatty acids and the type of rennet paste or related product used in the manufacture of the cheese. Cheese made with rennet extract was found to lack flavor and also contained a relatively small amount of butyric acid. On the other hand, cheese made with a goat rennet paste developed flavor normally and contained relatively high concentrations of butyric acid.

M5. The development of the lower fatty acids in Swiss cheese. O. J. KRETT, A. LEBOYER, H. E.

¹ Cooperative project with the Bureau of Dairy Industry, U.S.D.A. Fund provided in part by the Research and Marketing Act of 1946, and by the Ohio Dairy Products Research Fund.

DAUME AND J. B. STINE. Kraft Foods Co., Research Lab., Glenview, Ill.

The relationship between the lower fatty acids and the quality of Swiss cheese was reported in a previous paper. In the present investigation the development of these fatty acids was studied throughout the normal curing time of Swiss cheese. At short intervals during the life of the cheese, the fatty acid content was determined using a method which involved the removal of the fatty acids from the cheese by steam distillation and their separation by 2-phase chromatography.

Acetic acid is the only fatty acid present in any appreciable quantity at the time the cheese is removed from the press and until the cheese goes into the "hot room" at about 75° F. After a short time at the higher temperature the other acids begin to develop. The propionic acid increases greatly in amount after about 20–30 d. in the hot room. This rapid increase in propionic acid coincides in time with the appearance and rapid development of the eyes. A typical piece of Swiss going into the hot room at 15 d. of age had about 46 mg. of acetic acid/100 g. of cheese, and only traces of propionic and higher fatty acids. Coming out of the hot room, the fatty acids content of the cheese in mg./100 g. of cheese was 167 acetic, 344 propionic, 18 butyric and 30 higher volatile fatty acids calculated as caproic. When the cheese was 140-d.-old, the content of fatty acids/100 g. of cheese was 290 mg. of acetic, 630 mg. of propionic, 38 mg. of butyric and 50 mg. of higher volatile acids calculated as caproic.

M6. The use of white mutants of *Penicillium roqueforti* in cheese making. H. A. MORRIS, J. J. JEZESKI AND W. B. COMBS, University of Minnesota, St. Paul.

Two colorless mutants of *P. roqueforti* (Knight *et al.*, *J. Dairy Sci.*, **33**: 929–933, 1950) have been compared with the colored parent strain in the manufacture of Roquefort-type cheese. Data have been obtained which included pH, acid number and total volatile acidity determinations and the cheese were graded for flavor, body, texture and appearance.

In general, cheese inoculated with white mutant strain UW12 had a higher acid number in the fat, a greater total volatile acidity, higher pH in addition to a milder flavor and softer more creamy body than did cheese inoculated with the parent strain or the other white mutant.

Excellent cheese can be made with a white mutant of *P. roqueforti*. Its color, body, texture and comparatively milder flavor are enough different from Blue cheese to constitute a new cheese which may be classed within the semi-soft, mold-ripened group of cheese.

M7. A measurement of cheese body. D. M. IRVINE AND W. B. PRICE, University of Wisconsin, Madison.

The usual method of manipulating cheese between the thumb and fingers to determine the resistance to breakdown does not give finite values needed to evaluate firmness of curd. A device has been developed which offers 1 approach to this difficult problem. This device consists of a fine wire which is pulled through a cube of cheese of standard size. The force required to pull the wire through the cheese is provided by a reservoir of mercury. The mercury flows into the reservoir at a constant rate. The instant the wire has been forced through the cheese, a solenoid switch cuts off the flow of mercury. The body characteristic is recorded as the weight of mercury needed to pull the wire through the cheese.

The device has been used on semi-soft cheese, brick and cheddar. There is a highly significant correlation between this measurement and the judgments made by manipulation.

The device has been used in experimental work to study results of curd-making procedures. It has been used successfully to study variations in salt and acidity.

Suggested commercial applications indicated include: standardization of slicing properties of process cheese; judgment of firmness of fresh cheese; evaluation of the rate of body breakdown during curing; selection of cheese for specific market requirements.

It is applicable over a wide range of body characteristics from those of the typical cheese spreads to those of Romano. The movement of the wire through the cheese is sensitive to variations of 0.25 g. of stress on the cutting wire. The results are reproducible.

M8. The influence of feeding corn, pea vine or alfalfa silage to dairy cows on pH and titratable acidity, per cent butterfat and total solids, and milk flavor and production. H. C. HANSEN, E. D. McGLASSON, R. H. ROSS, W. R. HARVEY AND D. L. FOURT, Idaho Agr. Expt. Sta., Moscow.

Six Holstein and 6 Jersey cows were used to determine the influence of corn, pea vine or alfalfa silage on pH and titratable acidity, per cent butterfat and total solids in milk and milk production. There was no appreciable difference in titratable acidity or pH, per cent butterfat or total solids of fresh whole milk or total milk production when cows were fed the three silages. The milk was separated and the cream and skimmilk were inoculated with starter organisms. There was no difference in the acidity developed in the cream that could be attributed to the kind of silage. Skimmilk from cows fed pea vine or alfalfa silage developed significantly more acidity than when fed corn silage.

M9. Antioxidant properties of milk as influenced by the type of pasture fed to the cow. V. N. KRUKOVSKY, Cornell Univ., Ithaca, N. Y.

The susceptibility of milk to oxidized flavors is affected not only by the antioxidant activity of the fat (tocopherols), but also by the presence and availability of readily oxidizable non-flavor-forming compounds in the plasma phase of the milk. These properties of milk are influenced by

the type and quality of the roughages fed to the cow. Holstein cows on predominantly orchard grass with some ladino clover, green oats and alfalfa-brom-ladino clover pastures, each yielding about 200 γ tocopherols/g. of lyophilized plant, produced milk with fat having approximately the same tocopherol content: -3044, 3449 and 3411 γ /100 g., and of 41.07, 36.30 and 39.96 Iodine Nos., respectively, but of different susceptibilities to oxidized flavors, especially in the presence of 0.1, 0.2, 0.4, 0.5, 1.0 and 5.0 mg. copper/l., and of 20-mg. portions of ascorbic acid re-added subsequently to milk at the point of its depletion. Orchard grass milk was found to be exceptionally stable. It resisted oxidized flavors through the 10th day of trial in the presence of 0.5 mg. Cu and 3 portions of added ascorbic acid. One mg. of Cu and 2 portions of ascorbic acid were required to induce flavors at the end of 7-d. period. Milk produced on alfalfa-brom-ladino pasture was the least stable. It developed flavors at the end of 7, 4, 2 and 1 d. with an increase in Cu content alone from 0 to 0.4 mg., respectively, without re-added ascorbic acid. Feeding of oats pasture resulted in deferment of oxidized flavors for 9, 8, 7 and 3 d. with an increase in Cu alone from 0.2-1.0 mg., and together with re-added ascorbic acid for 4, 3, 3 and 1 d., respectively. All samples of milk containing 5.0 mg. Cu developed oxidized flavors during the first 0.5-2 d. of trial.

M10. Two phenomena related to casein precipitation in frozen storage of milk. G. CHRISTIANSON, S. T. COULTER AND R. JENNESS, Univ. of Minnesota, St. Paul.

In a study on protein stability of frozen concentrated milk it was found that (a) dialysis rendered the product stable to extend frozen storage and (b) that the otherwise undispersible casein precipitate could be dispersed by acidification to pH 2.5.

Concentrated skimmilk dialyzed for 30 hr. against distilled water at 40° F., remained for 15 mo. in 15° F. storage with no apparent change in its physical state. An undialyzed control, concentrated to the same degree (3:1), was destabilized within 1 mo. The milk salts and lactose were shown to be factors in the protein destabilization.

Concentrated skimmilk stored frozen for 1 yr. yielded a casein precipitate that could not be dispersed with heat, agitation or addition of alkali. However, the addition of HCl to bring the pH to 2.5 followed by NaOH to bring the pH to 7.0 completely dispersed the precipitate. This redispersion was accomplished both on destabilized concentrated skimmilk and on the isolated protein precipitate.

M11. A method for measuring the wettability of milk powders. U. S. ASHWORTH, State College of Washington, Pullman.

Spray-dried milk powders are nearly completely soluble under the conditions employed in making solubility tests, yet these powders may not disperse readily when stirred into water. The difference probably is due to variable wetting characteristics

—the initial phase of the dispersion process. A simple wettability test has been developed which, after being in use for over 1 yr. has proved valuable in evaluating milk powders for their ability to disperse readily.

Steps in the method are: (a) Cover the bottom of a 250-ml. beaker with filter paper. Place 20 mesh tea strainer in the beaker mouth. (b) Force 2.5 g. of milk powder through the sieve to form a uniform layer on the filter paper below. (c) Allow 17.6 ml. (Babcock pipette) of water at 30°C. to flow down the edge of the beaker and form a layer under the milk powder. (d) Without disturbance, the powder and water are allowed to stand 5 min. at room temperature. The beaker then is carefully picked up and the contents, strained through an empty gooch crucible allowing 10 sec. for the beaker to drain. (e) Total solids are determined on a 5-ml. aliquot of the strained milk. This value multiplied by the factor 160 gives "per cent wettability".

Factors affecting the test will be discussed.

M12. Some observation of the use of surface-active agents as a means of improving the dispersibility of whole milk powder. H. A. HOLLENDER, Purdue Univ., Lafayette.

Various surface-active compounds and/or emulsifiers were studied as to their effectiveness in improving the dispersibility of whole milk powder.

The compound or compounds were added to the concentrated milk before homogenization at a total concentration of 0.1% by weight (3.4% fat, fluid basis).

Quiescent solubilities were determined by a method developed by the Quartermaster Food and Container Institute Laboratories, Chicago, Ill. As much as a 20-fold increase in the quiescent solubility, as determined by the above method, has been made.

In general, the use of a combination of compounds to give a good hydrophilic-lipophilic balance was found to be more effective than 1 compound alone.

The partial esters of both oleic and stearic acids were effective in improving the initial dispersibility but stearic acid esters maintained their effectiveness better during storage.

Dispersibility or wettability decreases materially during storage with or without the use of additives. This decrease in dispersibility is accelerated with increase in storage temperature. Certain additives lose their effectiveness more readily during storage than others.

Flavor observations on milk reconstituted from the powder containing the additives indicate that there is no adverse effect on the keeping quality. Very slight off-flavors are present but the use of additives in most cases improves the over-all flavor of the reconstituted milk.

Churning of the butter fat took place when the powder was reconstituted using a high-speed mixer, but this property was lost during storage.

M13. A study of the particle size of nonfat dry

milk solids. J. J. JANZEN AND A. M. SWANSON, Univ. of Wisconsin, Madison.

The fractionation of nonfat dry milk solids has been attempted by using the air elutriation principle. Two instruments have been used successfully, namely, the Infracizer and the Roller Particle Size Analyzer. In both instruments analysis is accomplished by means of an air current carefully regulated as to its velocity. The powder thus is sized according to the settling rate of the particles in this air stream. Theoretically, any number of particle size fractions may be obtained with the Roller Particle Size Analyzer, simply by making a suitable combination of the variables, such as air pressure, nozzle size and settling chamber.

Nonfat dry milk solids have been separated into 5 fractions. The size distribution has been determined by microscopic measurement using a calibrated Whipple disc. The main objective of this study was to determine the existence, if any, of a relationship between the particle size of nonfat dry milk solids and solubility. A typical fractionation run on a sample of nonfat dry milk solids having an average size of 16.7 μ yielded the following sized fractions: 6.8, 26.3, 45.1, 59.3 and 67.6 μ . Solubility determinations indicated that the 26.3 μ fraction was considerably more soluble than either the finer or coarser fractions.

M14. The effect of various milk serum proteins and sulfhydryl groups on bread quality. B. L. LARSON, Univ. of Illinois, Urbana AND R. JENNESS AND W. F. GEDDES, Univ. of Minnesota, St. Paul.

Baking studies with purified milk serum protein fractions have revealed that β -lactoglobulin, which can account quantitatively for most of the sulfhydryl-reducing capacity of milk and which makes up 50-60% of the total serum proteins, is not deleterious when incorporated into doughs. Furthermore, bovine blood serum albumin and colostrum immune euglobulin and pseudoglobulin, each of which comprise about 5% of the total serum proteins of milk, are not deleterious. Heat treatment of the serum proteins and of β -lactoglobulin results in an activation of the very unreactive sulfhydryl groups present in the native protein, and these do have a somewhat deleterious action on baking quality. Egg albumin behaves similarly to β -lactoglobulin.

These findings imply that all of the methods based on measurement of reducing capacity or amount of undenatured serum proteins are fundamentally unsound as absolute indices of the baking quality of nonfat dry milk solids, since they measure materials and properties which in themselves are not deleterious. Sulfhydryl groups of milk apparently are not the factor responsible for poor baking quality of milk which has not received an adequate treatment.

M15. Effect of spoilage bacteria on flavor component of cottage cheese. R. B. PARKER AND P. R. ELLIKER, Oregon Agr. Expt. Sta., Corvallis.

The study employed species which previously had been identified as responsible for a gelatinous

defect in cottage cheese (Parker *et al.*, J. Dairy Sci., **34**, 887-893, 1951). The importance of biacetyl to flavor of cottage cheese was demonstrated by classifying various samples of the product according to aromatic flavor and subjecting the same samples to a chemical analysis for biacetyl. Samples with high biacetyl content received high flavor scores, with the occasional exception that some samples were criticized for high acid. Lower-scoring samples were correspondingly lower in biacetyl values.

If pure cultures of *Pseudomonas viscosa* and *Pseudomonas fragi* were added to cottage cheese, the biacetyl present would be destroyed within a 48-96 hr. period. Cultures of *Alcaligenes metalcaligenes* had relatively minor effects on biacetyl. Experimental samples inoculated with the *Pseudomonas* species lost almost all aroma before the slightest suggestion of physical spoilage was apparent. An occasional uninoculated commercial sample underwent rapid biacetyl loss several days before appearance of the slimy defect.

Studies on destruction of biacetyl in milk indicate that biacetyl is converted primarily to acetylmethylcarbinol by the *Pseudomonas* species but only partially so by *A. metalcaligenes*.

M16. Isolation of *Streptococcus thermophilus* bacteriophage from Swiss cheese whey. D. D. DEANE, F. E. NELSON AND F. C. RYSER, Iowa Agr. Expt. Sta., Ames.

Eighty-one samples of Swiss cheese whey and milk were obtained from 25 Swiss cheese plants in 3 states. A number of plants were having trouble with slow acid formation in the cheese kettle. The majority of the samples were taken from the kettle after the curd had been dipped and many were approximately 1-wk.-old when received through the mail.

Bacteria-free filtrates were prepared from all samples and tested for presence of bacteriophage active against *Streptococcus thermophilus*. Several filtrates inhibited slightly the growth of 1 or more of the 4 test strains of *S. thermophilus* in fortified (10% V-8 juice and 0.5% peptone) litmus milk at 36° C. Only 1 filtrate which had caused retarded acid development gave plaques when tested by the two-layer plate technic, using modified trypticase soy agar. Plaques were formed in 11 hr. at 36° C. and in 6 hr. at 45° C. The original whey filtrate, as well as bacteriophage purified by single-plaque isolation, lysed only strain Mc of the four strains of *S. thermophilus* tested. None of 21 selected strains of *S. lactis* and *S. cremoris* were lysed. It has not been possible to prepare, by the usual laboratory procedures, filtrates with a titer higher than 1×10^7 particles of bacteriophage/milliliter. Additional characterization is in progress.

Some of those cheese plants that experienced slow acid formation in their cheese kettles have been able to minimize this difficulty by using a nonsusceptible strain of *S. thermophilus*.

M17. Some factors affecting the action of benzoyl peroxide in the bleaching of milk for cheese

manufacture. S. KURATO AND J. J. JEZESKI, Univ. of Minnesota, St. Paul.

Although the use of benzoyl peroxide is permitted for the bleaching of milk in the manufacture of certain types of cheese, little or no information is available in the literature as to the optimum conditions to promote bleaching and the possible defects that might appear in milk as a result of the process.

Experiments were conducted to determine the time, temperature and concentration of bleaching agent necessary to effect a 50% decrease in total carotenoids without causing an appreciable fat oxidation or objectionable flavor defects. Vitamin A, carotenoid and peroxide values and flavor scores were determined on cream bleached with concentrations of benzoyl peroxide varying from 0.001-0.004% (on a whole milk basis) and over a temperature range of 125-185° F.

Milk of a flavor quality acceptable for cheesemaking resulted when cream was bleached at temperatures from 125-145° F. using 0.002% benzoyl peroxide. A loss of 50% of the total carotenoids was obtained after 1.5-2 hr. without the appearance of objectionable tallowy flavors or increased peroxide values. The vitamin A content was not lowered significantly and in some instances a small increase was noted. Faster bleaching was observed as the fat percentage of the cream was increased. The percentage of carotenoid loss was independent of the initial carotenoid content, provided the concentration of reagent was the same in each case. Carotenoid losses were dependent primarily on the concentration of benzoyl peroxide used. Temperature affected the rate of bleaching rather than the total amount of carotenoids lost. Higher temperatures and higher concentrations of bleaching agent produced objectionable flavors and fat oxidation.

M18. Acetaldehyde production by *Streptococcus lactis* and *Streptococcus lactis* var. *multigenes*. E. ZURAW AND M. E. MORGAN, Univ. of Connecticut, Storrs.

Virtanen and Nikkilä (J. Dairy Research, **15**: 89-93, 1947.) have indicated that the malty aroma produced in milk cultures by their "malt coccus" was due to the liberation of acetaldehyde by the organism and that the characteristic malty aroma could be duplicated by addition of acetaldehyde to milk. Attempts in our laboratory to simulate the malty defect caused by *Streptococcus lactis* var. *multigenes* by addition of acetaldehyde to milk have been unsuccessful.

Mild aeration or nitrogen sweeping of 2-1. skim-milk cultures of strains of *S. lactis* var. *multigenes* and passage of the exhaust gases through saturated aqueous solutions of dimedon resulted in precipitation of a dimedon derivative. Melting points of the recrystallized derivative and its anhydride agreed closely with observed and published melting points for dimedon derivatives of acetaldehyde. More significant was the fact that during and after development of the cultures, the characteristic malty aroma was detected in the exhaust gases

after passage through the efficient aldehyde trapping agent.

Free acetaldehyde produced by 9 strains of *S. lactis* var. *maltigenes* and 10 strains of *S. lactis* in 400-ml. skimmilk cultures containing CaCO_3 was swept into bisulfite traps and determined colorimetrically as the derivative of p-hydroxydiphenyl (Stotz, J. Biol. Chem., **148**: 585-591, 1943). The non-malty strains yielded acetaldehyde values equal to or greater than those of the malty strains during a 48-hr. incubation period. The maximum aldehyde values obtained were 223 γ /100 ml. of culture for the non-malty strains and 99 γ for the malty strains. It seems improbable that the characteristic aroma of cultures of *S. lactis* var. *maltigenes* is related to liberation of acetaldehyde.

M19. Starter activity tests using non-fat-dry milk solids. N. S. GOLDING AND L. McCORKLE, State College of Washington, Pullman.

Activity tests were made using commercial starters with commercial and laboratory non-fat-dry milk solids reconstituted at a 10% level in distilled water. It is shown that with both types of milk (12 samples of commercial and 12 samples of laboratory-prepared milk) there was a wide variation in production of acidity with identical starters. Some of the milks consistently yielded high acid production, others low. There was no indication that this was a seasonal effect. As far as can be proved, the dried milks did not change in their suitability as a medium over long periods of storage at 35-40° F. It is suggested that a proven non-fat-dry milk could be used as a standard for activity tests, since the activity test is as much dependent on the milk as the starter.

M20. Preliminary studies on the thermal resistance of micrococci in milk. A. N. MYHR AND J. C. OLSON, JR., Univ. of Minnesota St. Paul.

Individual quarter samples of milk from 25 cows were drawn aseptically, composited and laboratory-pasteurized at 143° F. for 30 min. Micrococci predominated before pasteurization; none was observed after pasteurization. The above was repeated on the same samples after storage at 5° C. for 30 hr. with no change in results.

In 3 trials the z values of thermal death time curves of a thermophilic *Sarcina* species isolated from pasteurized milk were 16.8, 17.5 and 18.2. Similar observations were made from results of preliminary studies on each of 3 additional cultures of thermophilic micrococci.

Of 30 cultures of micrococci isolated from plates prepared after routine laboratory pasteurization of patrons' milk, only 5 cultures survived 143° F. for 30 min. (population of suspension approx. 100,000/ml.) following subculture in milk.

M21. A study of methods for evaluating the keeping quality of sweet cream butter. B. M. ZAKARIASEN, L. ECKBERG AND R. W. MYKLEBY, Land O'Lakes Creameries, Inc., Minneapolis, Minn.

A large number of commercial churnings of butter was analyzed by a number of methods in an effort to secure information on predicting the keeping quality of sweet cream butter. Churnings of butter were selected from creameries, some having histories of poor keeping quality and others having histories of good keeping quality insofar as the putrid or cheesy type of flavor defects were concerned.

Incubation tests were made at 45, 55, 72 and 98° F. for varying periods of time to determine the relationship between short-time incubation tests and incubation tests simulating retail store and household refrigeration temperatures.

Various bacteriological tests were made during the course of this experiment to record this quality feature. Chemical tests included moisture, salt, peroxidase and pH determinations.

Little correlation was found between short-time incubation tests for keeping quality and tests simulating retail store and household refrigeration temperatures of printed butter.

M22. The behavior of the lipase from Pseudomonas fragi in cream and butter. S. A. NASHIF AND F. E. NELSON, Iowa Agr. Expt. Sta., Ames.

The lipase of *Ps. fragi* is active in experimental cream between pH 5.2 and pH 8.2, with an optimum at the normal pH of sweet cream. Extensive growth of *Streptococcus lactis* in cream with the accompanying drop in pH does not cause any appreciable inhibition of growth of *Ps. fragi*, but it markedly reduces lipase production.

An appreciable portion of lipase produced by several cultures of *Ps. fragi* in cream remains active after pasteurization of the cream at 71.5° C. for 30 min. Even though growth of the organism and lipase production in sterile cream at 15° C. for 3 d. is rather slow, yet enough enzyme is produced to cause extensive fat breakdown in the cream and resultant butter.

Residual lipase was demonstrated in butter churned from cream in which *Ps. fragi* grew at 15° C. for 3 d. and then pasteurized at 71.5° C. for 30 min. Organoleptic and chemical tests on butter samples held at 36, 21, 5 and -10° C. for various periods showed that considerable fat degradation had taken place in the inoculated and subsequently pasteurized samples at all 4 temperatures, while the control samples were unaffected.

M23. The effect of aging of cream upon the distribution pattern of free C₄, C₅ and C₆ fatty acids. I. I. PETERS, Texas Agr. Expt. Sta., College Sta.

Samples of fresh 30% fat cream with and without added formaldehyde (1:500) were examined immediately and after 7 d. incubation at 85° F. for the presence of free C₄, C₅ and C₆ fatty acids.

The method of Ramsey and Patterson (J.A.O.A.C., **28**: 644, 1945) was used in preparing the dried distillate containing the sodium salts of the free volatile fatty acids for each cream, whereas the method of Moyle *et al.*

(Biochem. J., **43**: 308. 1948) was used in separating the C₄, C₅ and C₆ fractions of free fatty acids by means of partition chromatography from the above dried distillates. The method of Kline (Biochem. Ztschr., **296**: 202. 1938) was used in identifying butyric and isobutyric acids in the C₄ fraction.

All cream samples showed the presence of C₆ and C₅ fractions; the fractions were low and similar for all creams. The values for C₄ free fatty acids increased upon incubation, the increase being greatest in the cream samples with no added formaldehyde. Examination of the C₄ fraction showed the presence of *n*-butyric acid only.

M24. The role of ascorbic acid and tocopherol in the development of oxidized flavor in cream and butter. A. C. SMITH, M. LOEWENSTEIN, R. E. ANDERSON AND H. C. OLSON, Okla. A. & M. College, Stillwater.

The anti-oxidant value of ascorbic acid in fresh pasteurized milk is well established and the use of α -tocopherol in combination with ascorbic acid as an anti-oxidant in vegetable oils has proved beneficial. However, no mention has been made of the role of ascorbic acid and α -tocopherol as an anti-oxidant in cream and butter.

The addition of 100 mg. of ascorbic acid/kg. of cream prevented development of oxidized flavor in frozen cream after 9-mo. storage at -10° F., and in "whipping" cream and "coffee" cream stored for 5 d. at 40° F. Higher levels of ascorbic acid fortification are necessary for Cu-contaminated cream. Low levels of ascorbic acid fortification tend to increase the degree of oxidized flavor in bottled cream. Placing cream in frozen storage immediately after adding ascorbic acid and prompt use of defrosted cream are necessary to keep oxidized flavor at a minimum.

Butter fortified at levels up to 200 mg. of ascorbic acid/kg. of butter showed more pronounced oxidized flavor than the controls.

There appears to be no advantage in adding α -tocopherol at the 10 mg./kg. level by conventional methods, either separately from or concurrently with ascorbic acid, to summer cream and butter as a means of preventing oxidized flavor development during frozen storage.

M25. "Skunkweed" flavor in cream and butter. H. C. OLSON, R. F. BEACHBOARD AND R. E. ANDERSON, Oklahoma A. & M. College, Stillwater and L. F. EDMONDSON AND C. LACEY, Oklahoma State Dept. of Agr.

A defect commonly called "skunkweed" flavor develops in butter churned from cream produced in the sandy and hilly areas in southwestern Oklahoma during the spring of the year. The butter develops an odor resembling that of the common skunk. The defect is not present in the fresh milk, cream or freshly churned butter but develops within 1 or 2 d. after the butter is churned.

By feeding cows kept on dry lot freshly cut weeds of various kinds, it was established that the defect is caused by the consumption of a combination of 2 weeds: wild carrot (*Spermo lepis*

echinata) and mule's tail (*Erigeron canadensis*). When the wild carrot was fed in combination with freshly cut grass or weeds other than mule's tail, the defect was reproduced occasionally to a slight degree, indicating that the wild carrot is the key weed. The defect appeared in butter churned from cream produced 12 hr. after feeding the causative weeds. The defect persisted in the cream produced as long as 48 hr. after removal of the weeds from the cows' diet. Cheese made from milk produced by cows feeding on weedy pastures developed a skunky odor during ripening. The development of the defect appeared to involve an oxidative reaction, but anti-oxidants added to cream before churning failed to prevent development of the defect.

Various levels of neutralization, various pasteurization exposures and vacuum treatment of the cream did not minimize the intensity of the defect in butter. Cream containing the "skunkweed" defect can be detected by mixing 1 part of nitric acid with 4 parts of cream and observing the odor of the mixture.

M26. Observations on the keeping quality of pasteurized milk with and without added aureomycin, which was obtained from various sources and stored at 45° F. J. C. OLSON, JR., D. S. WILLOUGHBY, E. L. THOMAS AND H. A. MORRIS, Univ. of Minnesota, St. Paul.

The objectives were (a) to obtain information about keeping quality of pasteurized milk from commercial milk plants which employ similar methods of processing, but whose past records show marked differences in bacterial counts on finished products; and (b) to obtain information regarding keeping quality of pasteurized milk as affected by the presence of aureomycin.

Samples of homogenized, pasteurized milk were obtained from 2 plants. Products from plant A had a rather long record of generally high coliform counts, while those from plant B had a history of uniformly low counts and often complete absence of coliform bacteria in 2-ml. quantities of finished products.

Standard plate counts (35° C.), coliform counts, psychrophile counts (45° F.), and flavor scores were determined initially, after 4 d. and after 7 d. of storage at 45° F. Samples from plant A were divided. To 1 lot, crystalline aureomycin was added to yield a concentration of 0.2 γ /ml.

Initial flavor scores on samples from plant A ranged from 35.5-37; those from plant B were uniform at 37. After 4 d., samples from plant A had dropped from 2-9 points, while samples from plant B showed little or no change. After 7 d. all plant A samples scored 0, while plant B samples still showed little or no change.

Psychrophile counts on plant A samples initially were excessive and increased greatly with storage. In contrast, plant B samples initially showed almost complete absence of psychrophiles and counts after storage were low.

Standard plate counts showed essentially the same trends as those observed from psychro-

phile counts, except that such counts on plant A samples were always lower than the psychrophile counts after 4 and 7 d. of storage.

Coliform counts on samples from both plants initially were low or 0 in 2 ml. quantities. Coliforms increased greatly in plant A samples during storage; yet, remained low or absent in plant B samples.

The preserving effect of aureomycin was nil. Evidence of suppression of specific bacterial types was obtained, but this was nullified by growth of other types with accompanying deterioration to the same extent as that which occurred in samples containing no aureomycin.

M27. The glyceride structure and polymorphism of butterfat. G. R. GREENBANK, Bureau of Dairy Ind., Agricultural Research Admin., U. S. D. A.

The fractionation of the glycerides of fats and oils requires very low temperatures when the usual solvents are employed. To obviate use of such low temperatures, 2 methods of fractionation have been developed, especially for the fractionation of butterfat. They are as follows: (a) Absolute alcohol is used as a solvent; (b) acetone containing different concentrations of water is used to separate the different fractions. With absolute alcohol, it is possible to separate 11 fractions at temperatures not below 0° C. The acetone-water method is especially useful in fractionating the lower melting fractions. Butterfat from mixed herd milk was resolved into 13 fractions by the absolute alcohol method. The fractions varied from white hard wax-like glycerides, having a melting point of 54.5° C., to an orange-colored oil with a melting point of -12° C. The chemical properties of the different fractions were determined. These data were used in calculating the glyceride structure. The volatile acid content of the glyceride fractions varied from 0-8.9%, calculated as butyric acid. The unsaturated fat acids calculated as oleic acid varied from 6.6-54.4%. The most unsaturated glycerides were highest in volatile acids. The glyceride structure shows a remarkably uniform variation in the fatty acid constituents of the different fractions. The calculations show that some trisaturated but no triunsaturated glycerides are present. Most of the glycerides are the monosaturated and disaturated type. Data are presented to show that butterfat does exist in different crystalline forms.

M28. Isolation and compositional characterization of the fat-membrane proteins of nonhomogenized and homogenized milk. J. R. BRUNNER, C. W. DUNCAN AND G. M. TROUT, Michigan State College, East Lansing.

Fat-membrane materials were obtained from nonhomogenized and homogenized milk of common origin by the conventional washing and churning technique. The buttermilk-like sera were condensed 10:1 at 45° C. and dialyzed for 72 hr. against running distilled water. Lipoid materials were extracted by an ethanol-ether washing procedure. The residual membrane proteins were dispersed in distilled water and lyophilized to an anhydrous powder.

The lyophilized membrane proteins had a nitrogen content of approximately 13.5% on the anhydrous, lipid-free basis. Microbiological analyses for arginine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine and valine made on fat-membrane proteins from nonhomogenized and homogenized milk revealed only slight differences in the concentration of these amino acids between the two membrane-protein preparations. A similar determination for lysine, glutamic acid, tryptophan, cystine and glycine showed a significant increase in the concentration of lysine and glutamic acid and a decrease in the concentration of tryptophan, cystine and glycine in the membrane preparations from homogenized milk. The amino acid composition of the membrane-protein preparations from nonhomogenized and homogenized milk differed markedly from any of the recognized milk proteins.

M29. Electrophoretic characterization of the fat-membrane proteins of nonhomogenized and homogenized milk. J. R. BRUNNER, H. A. LILLEVIK, G. M. TROUT AND C. W. DUNCAN, Michigan State College, East Lansing.

Lyophilized membrane-protein preparations from nonhomogenized and homogenized milk were dispersed for electrophoretic examination in the following buffer systems: Glycine-HCl, pH 1.5; phosphate, pH 6.5; veronal-citrate, pH 8.0; ammonia-HCl, pH 9.0 and veronal-ethylamine, pH 10.8. These buffer systems were made up to an ionic strength of approximately 0.1.

Although no definite conclusion could be made from an examination of the electrophoretic patterns concerning the nature of the protein components of the fat-membrane preparations, there was evidence that differences in protein composition did exist. Generally, 2 or 3 major components were observed in the nonhomogenized milk membrane-proteins, whereas 3 to 4 components of different characteristics were observed in the homogenized milk membrane-proteins. The identification of the individual components was not made. The temperature of the ethanol-ether treatment and the concentration of protein in the electrophorized buffer solution influenced the mobility patterns of the protein components.

M30. Preparation of samples of skimmilk for electrophoretic studies. J. TOBIAS, R. McL. WHITNEY AND P. H. TRACY, Univ. of Illinois, Urbana.

Skimmilk prepared by a single separation in a De Laval airtight separator tested between 0.05-0.08% fat, which was satisfactory for electrophoresis. Higher concentrations of fat gave rise to light absorption, as evidenced by uneven exposure of the Schlieren-Scanning photograph.

While good quality raw skimmilk stored at ice bath temperature remained electrophoretically unchanged for 3 d., skimmilk heated to 300° F. with a Mallory heat exchanger could be stored for 14 d. under the same conditions without change. Therefore, the time required to reach dialysis equilibrium was determined with the later skimmilk.

A 1:3 dilution of skimmilk was dialyzed in a stirrer-type dialyzer at ice bath temperature for varying periods of time against Michaelis buffer of pH 6.8 and 0.173 μ . The progress of dialysis was followed by measuring the light transmission (375 $m\mu$), specific conductance, electrophoretic mobility with the Perkin-Elmer Tiselius electrophoresis apparatus, and the area under the ϵ boundary. Dialysis equilibrium was reached between 15–19 hr.; however, a 24-hr. dialysis period is more convenient.

To confirm that this time of dialysis also is satisfactory at other pH's, this phenomenon was investigated with a 1:4 dilution of skimmilk at pH 2.2 with glycine HCl buffer of 0.1 μ , at pH 5.6 with sodium acetate buffer of 0.1 μ and at pH 8.7 with veronal buffer of 0.1 μ . In all cases, a 24-hr. dialysis period was found adequate.

A comparison of electrophoretic properties of a sample of skimmilk dialyzed for 24 hr. and a sample of the same skimmilk dialyzed for 48 hr. with a change of buffer after 24 hr. showed that the latter procedure gave rise to slightly higher mobilities.

M31. Effect of heating to 300° F. by means of the Mallory small-tube heat exchanger on the electrophoretic properties of skimmilk. J. TOBIAS, R. MCL. WHITNEY AND P. H. TRACY, Univ. of Illinois, Urbana.

Preliminary studies, in which the electrophoretic properties of skimmilk were determined in the range of pH 2–9, indicated that the electrophoretic patterns differed most markedly at pH 2.2, 5.6 and 8.7. Therefore, 1:4 dilutions of skimmilk in glycine HCl buffer of pH 2.2, sodium acetate buffer of pH 5.6 and veronal buffer of pH 8.7, all having an ionic strength of 0.1, were selected for this study.

By comparison of mobilities and relative areas of the unheated samples at pH 8.7 to those reported by other investigators, the components detected in skimmilk may be postulated as α -casein, β -lactoglobulin, β -casein and γ -casein in the order of decreasing mobilities. At pH 5.6, 6 components were observed in the ascending, and 4 in the descending patterns. The additional components may be explained by complex formation between β -casein and γ -casein, and between α -casein and β -lactoglobulin at this pH. No association need be postulated between α - and β -casein to account for all components, indicating that the association observed by Warner possibly was due to the treatment in preparation of casein. At pH 2.2, 5 components were observed but only 1 could be identified with any degree of certainty. This was β -lactoglobulin. The casein fractions have apparently redistributed themselves to form a new set of components in line with the observations of other investigators, who found that α -, β - and γ -caseins were not homogeneous on the acid side of their isoelectric points.

The effect of heating to 300° F. by means of the Mallory heater was to lower the mobilities of the components tentatively identified as β -lactoglobulin and α -casein, and to make the boundary

of the latter more symmetrical at pH 8.7. At pH 5.6, 1 of the postulated complexes of β - and γ -casein and the β -lactoglobulin- α -casein complex apparently have associated with α -casein to form a more or less electrophoretically homogeneous component with a mobility lower than that of the original α -casein. At pH 2.2, β -lactoglobulin and 1 of the casein components were redistributed among the other casein fractions and the resulting components had a lower mobility than their counterparts in the unheated samples.

M32. Limitations of the use of serum protein determinations in evaluating heat of milk. H. A. HARLAND, S. T. COULTER AND R. JENNESS, Univ. of Minnesota, St. Paul.

Protein denaturation is used as an index of the extent of heat treatment of milk and its dry products. Since there are methods for determining only the undenatured serum protein, the amount of denatured protein can be calculated only as the difference between the original and the final values. Except where used in control procedures in manufacture, the actual serum protein content of the original milk seldom is known. To determine the variability in the serum protein content of milk, samples of raw whole milk were obtained on each of 3 successive wk. during the winter, spring and fall seasons from each of 10 widely separated milk sheds throughout the United States. Following separation of the fat, raw, heated (165° F., 30 min.) and boiled samples were analyzed for nitrogen distribution (Rowland) and serum proteins by a turbidimetric method (Harland and Ashworth).

Individual samples of milk were found to vary not only in serum protein content on a solid-not-fat basis but also in the extent to which the serum proteins were denatured by a given heat treatment. Variability due to individuality of the samples exceeded that due to season or geographic origin. The variability in serum protein content as well as in response of heat treatment seriously limits the usefulness of undenatured serum protein measurements as an index of heat treatment in low-heat powders. This fact, however, does not detract from the value of the method for control of processing during manufacture.

Serum protein estimation by turbidimetric methods show that considerable protein may be denatured when nonfat dry milk solids is held at 170° F., such as may occur in certain types of spray driers. This apparent serum protein denaturation in the "dry" state is much more pronounced when the "cloud" turbidimetric method is used than with the Harland and Ashworth procedure.

M33. The phosphatase inactivation curve in the HTST pasteurization range.¹ S. A. HANSEN, F. W. WOOD AND H. R. THORNTON, Univ. of Alberta, Edmonton, Canada.

For the study of phosphatase inactivation in the HTST region, the small commercial plate-type heat exchanger offers certain advantages over laboratory-size pasteurizers in that precise temperature, time and pressure measurements may

be made directly at any desired point in the process. The experimental pasteurizer was of this type with a capacity of 1000 lb./hr.; temperatures were measured by indicating thermometers and thermocouples calibrated against an indicating thermometer graduated to 0.1° C. and certified by the National Research Council of Canada to 0.01° C.; milk times were calculated from water times measured by the salt-conductivity method; the thermal effect of heating-up and cooling was evaluated by Ball's method; and the Sanders and Sager phosphatase test was the criterion of inactivation.

Assuming instantaneous heating-up and cooling, phosphatase was found to be inactivated at 160° F. in 16.8 sec. and the Z value (the temperature range in degrees during one logarithmic cycle of time) of the semi-logarithmic inactivation curve between 153.3 and 160.7° F is 9.7° F.

¹ Based on a thesis presented by the senior author to the University of Alberta in partial fulfillment of the requirements for the degree of Master of Science. The study was conducted as National Research Council of Canada P.R. Project no. 29.

M34. Estimation of calcium ion activity in milk and other biological fluids. G. CHRISTIANSON, R. JENNESS AND S. T. COULTER, Univ. of Minnesota, St. Paul.

Calcium ion activity in milk has been estimated from the level of Ca "adsorbed" on a cationic exchanger brought to equilibrium with a relatively large amount of milk. Ca adsorbed on an exchanger is a function of the activities of all the cations present. In the case of milk it was assumed that the concentrations of the non-Ca cations (Na and K) were relatively constant and that they were completely ionized.

A standard curve was set up in which a solution containing constant levels of Na and K salts and varying levels of Ca salts (as chlorides) were equilibrated against a cationic exchanger. The level of Ca "adsorbed" was determined for each of several ionic concentrations of Ca and it then was possible to graph the Ca "adsorbed" vs. the Ca ion activity (*pCa*). In determining the *pCa* of milk it was necessary to (1) equilibrate a known amount of exchanger with milk, (2) wash the exchanger free of adhering milk, (3) elute the Ca with strong acid and (4) determine the level of Ca in the eluate. From this value for Ca adsorbed, the *pCa* was determined from the standard curve.

Several samples of skimmilk examined had apparent levels of ionized Ca of 4.0-4.5 mg./100 ml.

M35. Observations on the color of evaporated milk. S. PATON, Pennsylvania State College, State College.

Eleven commercially produced evaporated milks were studied with regard to discoloration. All samples were sterilized by the retort method. Sterilization treatment ranged from 241-247° F. for 12-16 min. Color was rendered soluble in the serum by digesting a 25-ml. sample of reconstituted milk with 2 ml. of a 10% suspension of

trypsin for 1 hr. at 43° C. Before reading in a colorimeter at 520 m μ , the digested sample was treated with 2 ml. of 50% trichloroacetic acid and filtered to remove residual protein. Color values were determined immediately on receipt of samples from the manufacturer and at monthly intervals for 1 yr. of storage at room temperature. All samples showed the same trend in color development. Rather rapid increases in amount of color were noted during the 1st 3 mo. of storage. Color values leveled off during the 4th month, after which time very gradual additional discoloration was observed. In general, the extent of discoloration in the samples was seen to be positively correlated with the amount of heat treatment the sample received during sterilization. The initial color of the samples correlated well with their values throughout storage.

Color development at 4, 22 and 43° C. was determined for 1 vacuum-packed (lacquered can) sample and 1 representative air-packed sample. During 12 mo. storage at 4° C., extremely little increase in color was noted. Greatest increase was at 43° C. The trend in color development was the same at all temperatures for both samples, with rapid increases occurring during the 1st 3 mo. followed by very gradual increases thereafter. The vacuum-packed sample developed more discoloration at all storage temperatures, the difference between the 2 types of packs being greatest at 22 and 43° C.

M36. A spectrographic method for the determination of tin, copper, iron, and lead in evaporated milk and the effects of storage on evaporated milk. C. W. GEHRKE AND C. VAN RUNYON, Univ. of Missouri, Columbia.

A quantitative spectrographic procedure was developed for the analysis of Sn, Cu, Fe and Pb in evaporated milk. Cd and Ni were used as internal standards. Working curves were prepared by plotting on log-log paper the ratios of the intensities of the lines of desired elements to those of the lines of internal standard elements vs. concentration.

Evaporated milk in electrolytic-plated tin cans stored 50 d. at 37° C. contained 97 ppm. of tin, and 45 ppm. when stored at room temperature. Milk stored in hot-dipped plated cans 50 d. at 37° C. contained 79 ppm. of tin, and 50 ppm. when stored at room temperature. Evaporated milk stored in electrolytic and hot-dipped plated cans contained 210 ppm. of tin after 340 d. at 37° C., and approximately 115 ppm. at room temperature. Evaporated milk as received contained about 20 ppm.

No significant increase was noted in the concentration of Fe, Cu and Pb when the milk was stored in either electrolytic or hot-dipped cans at the 2 temperatures. The pH decreased with storage and changed more rapidly for milk held at 37° C.

The milk gradually became darker and separation occurred as storage time increased. The can bodies showed increased pitting, general detinning, discoloration and staining with storage.

PRODUCTION SECTION

P1. Relationship of maximum daily air temperature, mean daily air temperature and humidity to physiological reactions of dairy bulls.

J. E. JOHNSTON AND C. BRANTON, Louisiana State Univ., Baton Rouge.

Fourteen dairy bulls of the Guernsey, Holstein and Jersey breeds were studied for a 53-wk. period. Daily observations of air temperature, water vapor pressure, body temperature, respiration rate and pulse rate were made between 2:00 and 4:00 p.m. Semen samples were collected weekly. When the data were grouped at maximum temperature intervals of 40-60, 60-70, 70-80, 80-85, 85-90 and 90-95° F., no significant breed differences in animal responses could be shown. Body temperature was found to be correlated significantly with maximum temperature only within the 40-60° F. and 70-80° F. intervals with vapor pressure within the 40-60, 85-90 and 90-95° F. intervals. Coefficients of correlation between respiration rate and all environmental measurements were highly significant except for those with maximum temperature within the 80-85 and 85-90° F. intervals, which did not attain significance. Pulse rate was significantly correlated with vapor pressure within the 80-85, 85-90 and 90-95° F. intervals and with mean daily temperature within the 85-90° F. interval. Principal changes in semen characteristics noted were an increase in % morphologically abnormal spermatozoa and a decrease in % motility with increasing temperatures. Fertility data also will be presented.

P2. Uniformity and nutritional studies with monozygotic bulls.

H. H. OLSON, Univ. of Minnesota, St. Paul.

Two sets of monozygotic twins and 1 set of triplet bulls were subjected to a uniform environment. The quantity and quality of all semen samples were measured by volume, concentration, total number of sperm/ejaculate, motility, methylene blue reduction, abnormal counts and percentage of live spermatozoa. A number of similarities in these semen characteristics were found within each set. Behavior patterns were alike within sets.

Members of each of 2 sets of monozygotic triplet bulls were placed respectively on 70, 100 and 130% levels of T.D.N. intake based on Morrison's standards for feeding bulls. One set was placed on these rations at 8 mo. of age and continued to 36 mo. of age. The other set was on this study from 27-64 mo. of age. All semen samples were subjected to the tests listed above. Measurements of quantity and quality of semen generally favored the bulls fed 100 and 130% of the normal ration. During the 1st part of the trial, the well fed bulls exhibited the greatest libido. Toward the end of the trial, the ability of the overweight bull to serve decreased.

P3. The effect of frequency of ejaculation on the semen characteristics and libido of young bulls.

F. N. BAKER AND N. L. VANDEMARK, Univ. of Illinois, Urbana.

A study was made on 9 Holstein male calves to determine the age at the 1st expression of sexual interest, the age at which an ejaculate first could be obtained and the effect of frequency of ejaculation upon semen characteristics and libido. The calves were assigned at random to collection frequencies of once, twice, or three times/week. Sexual interest was checked each week with a teaser female. When a bull became able to ejaculate he was started immediately on his preassigned collection schedule.

Sexual interest was first exhibited at 25-45 wk. of age. Desire to mount was shown in most instances before the ability to produce an erection was attained. First ejaculates were produced at 36-46 wk. of age. Semen characteristics were highly variable, but total spermatozoa, % motile spermatozoa, % normal spermatozoa and rate of spermatozoa motility generally increased in all groups during the first 8 wk. of collection. However, 2 bulls in the thrice weekly collections group have, by the 16th week of collection, shown a reduction in semen quality and libido.

P4. The influence of gonadotropic hormones on semen quality.

J. H. BYERS, G. McCURLEY AND M. VONKROSIGK, Oregon State College, Corvallis.

Four bulls in the Oregon State College herd were divided into 2 groups. Group I received chorionic gonadotropin and group II received anterior pituitary gonadotropin. The anterior pituitary gonadotropin was given in the form of Gonadovet¹ at the rate of 25 rat units 3 times/week, and the chorionic gonadotropin, given as Antuitrin-S², at the rate of 1,000 units/injection 3 times/week. Each bull served as his own control. Semen was collected artificially and the usual laboratory quality tests were run. In addition, the oxygen consumption of the semen was determined on the Warburg respirometer.

The intramuscular injection of the follicle-luteinizing principle and the chorionic gonadotropin increased the number of spermatozoa/ml. of semen and the % of live spermatozoa in the semen. The injection of the hormone did not influence the % abnormal forms of spermatozoa. The oxygen consumption/hr./ml. of semen was increased by both the anterior pituitary and chorionic gonadotropins. With 1 bull, the chorionic gonadotropin reduced the oxygen consumption/100 million live sperm, while, with the 2nd bull, the oxygen consumption/100 million live sperm was increased. These differences were not statistically significant. The anterior pituitary gonadotropin significantly increased the oxygen consumption/hour/100 million live sperm with both group II bulls.

¹ The Gonadovet supplied through the courtesy of the Jensen-Salsbury Lab., Inc.

² The Antuitrin-S supplied through the courtesy of the Parke, Davis and Co.

P5. The use of bacitracin and terramycin in semen diluters and the storage of semen at -15° C. in a glycerin-citrate-yolk diluter.

O. T. STALLCUP AND H. K. McCARTNEY, Univ. of Arkansas, Fayetteville.

The effect of terramycin, bacitracin, aureomycin, penicillin + streptomycin + sulfanilamide, and a combination of all the aforementioned bactericidal agents on the motility of bovine spermatozoa and upon the control of bacterial growth in bovine semen diluted with citrate-buffered yolk and stored at 5 and 30° C. was investigated. There was no significant difference in motility of the spermatozoa after 48-hr. storage or in duration of a 30% motility rating that could be attributed to differences in the action of diluters. A significant difference was found in the % motility after 48-hr. storage and the duration of motility between samples stored at 5 and 30° C. There was a significant interaction of diluter \times temperature insofar as motility after 48-hr. storage is concerned. All antibacterial agents studied were highly bacteriostatic and/or bactericidal at both temperatures, with the exception of bacitracin in samples stored at 30° C.

Toxicity studies with terramycin hydrochloride indicate that it is toxic at levels above 20 mg./100 ml. of diluted semen.

Studies are in progress on storage of bull semen at -15° C. using a glycerin-citrate-yolk diluter. Results of this study will be reported.

P6. The relationship between dilution rate of bull semen or the number of motile spermatozoa and fertility. C. BRANTON, H. C. KELLGREN AND T. E. PATRICK, Louisiana State Univ., Baton Rouge.

Continued expansion of artificial breeding makes it necessary that high fertility be maintained with as few spermatozoa used/insemination as possible. Therefore, a study was conducted during the months of Nov. and Dec., 1951, and Jan., 1952, to relate 2, 6 and 12 million motile spermatozoa/ml. of diluted semen or/insemination to the fertility of 4 dairy bulls. The split-ejaculate technique was employed in 3 \times 3 Latin squares for each bull. In all cases the diluter was egg yolk-citrate-sulfanilamide-streptomycin-penicillin. Only semen samples meeting the following minimum quality standards were used: spermatozoa concentration of 500 million or more/ml., at least 50% progressive initial motility and a modified methylene blue reduction time of less than 9 min.

Preliminary fertility results based on percentages of 30- to 60-d. non-returns to a total of 791 first services for 3 of the 4 bulls during Nov., 1951, were 66, 70 and 77% for 2, 6, and 12 million motile spermatozoa/ml. of diluted semen, respectively. Results on a 60- to 90-d. basis and the statistical analyses of the data for the entire experiment will be presented later.

P7. The reducing components of bull semen as determined by an iodimetric titration. B. L. LARSON AND G. W. SALISBURY, Univ. of Illinois, Urbana.

An *o*-iodosobenzoate-iodine amperometric titration has been used to determine reducing capacity of various components of a pooled sample of freshly collected bull semen. Ascorbic acid was determined independently, using 2,6-

dichlorophenolindophenol. The semen was separated by centrifugation into the seminal fluid and the sperm cells, which were washed several times by resuspension in buffer and centrifugation. The seminal fluid was further separated by dialysis into the seminal proteins (non-dialyzable material) and the dialyzable material.

Of the total *o*-iodosobenzoate-iodine reducing titer of fresh semen, 50% is due to the sperm cells (25% of which is due to reactive sulfhydryl compounds), and 50% is due to the seminal fluid (33% of which is accounted for by the seminal proteins and 67% by the dialyzable reducing substances, only half of which is accounted for by ascorbic acid).

The unknown dialyzable reducing substance(s) is present in semen to the extent of about 9 mg. % calculated as ascorbic acid or 30 mg. % calculated as glutathione. The reducing substance was shown not to be glutathione or any other active sulfhydryl compound by virtue of a negative nitroprusside test, failure to react with iodoacetamide and chromatographic analysis.

Further work is contemplated to determine the absolute identity of this reducing material and its importance in semen. Such material is not present in blood, milk or other common biological fluids.

P8. The effect of spermatozoa concentration and dilution on the respiratory activity of bull semen. M. W. H. BISHOP AND G. W. SALISBURY, Univ. of Illinois, Urbana.

Investigations have been carried out to determine the effects of cell concentration and dilution on the respiratory activity of bull semen as expressed by O₂ consumption/unit number of spermatozoa at 37° C. Individual semen samples vary considerably in their respiratory activity, which tends to be inversely related to cell concentration, and in their response to dilution. The greatest dilution effects appear to occur with semen samples originally showing the highest rates of respiration; these effects are not related to pH changes. Dilution with seminal plasma maintains or increases respiratory activity. Dilution with egg-yolk diluents maintains or increases initial respiratory activity and this effect persists with dialyzed egg yolk or in the presence of catalase. Dilution with phosphate buffer, isotonic saline-phosphate or saline-phosphate with fructose depresses respiratory activity. Dilution of washed spermatozoa with Ca-free Ringer phosphate buffer containing glucose indicates that maximal sustained respiratory activity is obtained, under these conditions, at a final concentration of 500 million spermatozoa/ml. and that respiration decreases progressively at higher or lower concentrations.

P9. Oxygen consumption of semen from dairy bulls on three levels of carotene and vitamin A. M. VONKROSIK, Oregon State College, Corvallis.

The O₂ consumption of semen from 5 Holstein bulls and 7 Jersey bulls on 3 levels of carotene intake was determined by use of the Warburg respirometer. Two Holstein and 2 Jersey bulls

were on a ration of a grain mix, dried molasses beet pulp and alta fescue refuse straw. The daily carotene intake of these low-carotene bulls was limited to 50 γ /kg. body weight. Four Jersey control bulls on a normal ration of grain and good quality alfalfa hay were obtaining approximately 360 γ /kg. body weight, although, at times, they may have consumed more than this. One Jersey and 3 Holstein bulls were on the same ration as the controls, and, in addition, received a daily supplement of 40,000 I.U. of vitamin A.

Little difference in O_2 consumption/ml. of semen was obtained between the low-carotene bulls and the controls, between the low-carotene bulls and the vitamin-supplemented group and between controls and the vitamin-supplemented group. Greater differences in O_2 consumed were obtained between the groups when calculated on the basis of 100 million live spermatozoa. Data will be presented on the O_2 consumption of fresh semen from the 3 groups and on the diluted semen after storage at 4° C. for 24 and 72 hr.

P10. Reconstituted skimmilk as a diluent for bovine semen. G. B. MARION AND H. H. OLSON, Univ. of Minnesota, St. Paul.

Preliminary studies were conducted using boiled and unboiled reconstituted dry skimmilk as a semen diluent. Boiling of reconstituted low-heat skimmilk powders proved to be necessary before they would serve as a suitable diluter; however, boiling did not increase the desirability of high-heat skimmilk powders. Sperm livability was maintained in unboiled reconstituted high-heat skimmilk equally as well as in yolk citrate, boiled whole milk or boiled reconstituted skimmilk. Sperm livability was based on motility during 16-d. storage.

Using the split-sample technique, a preliminary field trial is being conducted at 3 Minnesota artificial breeding cooperatives to compare reconstituted high-heat skimmilk with yolk citrate diluter.

P11. Impedance change frequency in bull semen. M. W. H. BISHOP, R. C. CAMPBELL AND J. L. HANCOCK, Agricultural Research Council, Animal Research Sta., Cambridge, England.

Active samples of bull semen exhibit well-defined periodic changes of electrical impedance due to changes in orientation of large numbers of spermatozoa between and with respect to the measuring electrodes. The frequency of such changes has been used to evaluate the quality of semen samples from bulls being used for artificial insemination purposes. This frequency is found to be highly significantly related to visual assessments of spermatozoan motility, concentration of living spermatozoa, proportion of dead spermatozoa, proportion of morphologically abnormal spermatozoa, fructolytic activity, dehydrogenase activity and respiratory activity. Fructolysis was the variable with the greatest explaining effect. No satisfactory relationship has yet been established between impedance change frequency and fertility.

P12. Simplified calf starter containing corn, oats and expeller or solvent soybean oil meal. K. E. GARDNER, Univ. of Illinois, Urbana.

Two simple experimental starters, containing either 49 parts corn, 20 oats and 28 expeller soybean oil meal, or 52 corn, 20 oats, and 25 solvent soybean oil meal, plus 1 salt, 1.8 steamed bone meal and 0.2 of vitamin A-D supplement were compared with a control starter composed of 35 parts corn, 30 oats, 15 linseed oil meal, 6 soybean oil meal, 2 bran and 10 dried skimmilk, plus minerals and vitamins.

Heifer calves of the 5 breeds received 370 lb. whole milk over an 8- to 10-wk. period with starter limited to 4.5 lb. daily and either alfalfa or clover-timothy hay fed *ad lib* over the 16-wk. study. Calves were individually fed.

Weight gains averaged 114% Ragsdale's normal for 28 heifers on the expeller starter, 115% for 25 on solvent, and 110% for 28 on the control starter. T.D.N. requirements/lb. gain averaged 2.1, 2.0 and 2.1 lb. T.D.N. for expeller, solvent and control starters, respectively.

P13. Slacked lime as a preventive of scours for whey-fed calves. D. C. BROWN, J. READ AND H. S. WILLARD, Wyoming Agr. Expt. Sta., Laramie.

Studies involving 30 Holstein calves were carried out to formulate the best system of starting and continuing calves on reconstituted whey (9 lb. water and 1 lb. dried whey) to 60 d. of age.

The calves were not all on experiment at the same time but were placed on test as space became available. Seven groups were provided: Group 1: Whole milk discontinued at 10 d. of age. Whey introduced on 4th day of age. Calves on all-whey diet on 10th day of age. Group 2: Whole milk discontinued at 3 wk. of age. Whey introduced on 15th day of age. Calves on all-whey diet on 20th day of age. Group 3: Whole milk discontinued at 2 wk. of age and slacked lime added at start of scouring. Whey introduced on 8th day of age. Calves on all-whey diet on 15th day of age. Group 4: Whole milk discontinued at 3 wk. of age and slacked lime added at start of scouring. Whey introduced on 15th day of age. Calves on all-whey diet on 21st day of age. Group 5: Whole milk to 60 d. of age. No whey or lime fed. Group 6: Whole milk to 60 d. of age. No whey fed. Lime added at start of scouring. Group 7: Whole milk to 30 d. of age. Whey introduced on 15th day of age. Calves on all-whey diet at 30 d. of age.

The results indicate that reconstituted whey should not be fed until the calf is at least 2 wk. of age and that the change to an all-whey liquid diet should be gradual. In the groups where no lime was fed, the calves stopped scouring in 2-4 d. after whey was eliminated from the ration. When scouring was once stopped by feeding lime, there was no recurrence of scouring. Remade whey always was palatable and the whey calves seemed to eat grain sooner than the calves on whole milk.

Approximately normal rates of growth were

obtained when whole milk was fed for 2 wk., then a gradual change made to reconstituted whey, provided scours were stopped with 0.25 tsp. of slacked lime stirred into the remade whey at each feeding whenever scouring occurred.

P14. Effect of dietary lipids on the polyunsaturated fatty acids in blood plasma of young dairy calves. R. S. ALLEN AND J. H. ZALETEL, Iowa Agr. Expt. Sta., Ames.

During the age period from 2-6 wk. young dairy calves were fed various types of lipids to determine the effect on the plasma polyunsaturated fatty acids. The calves were restricted to whole or reconstituted milk diets (10% of body weight daily) which contained the respective lipids at a 3% level. At weekly intervals venous blood samples were drawn and the plasma polyunsaturated fatty acids were determined by the alkali conjugation method.

Approximately 2 wk. were required for maximum expression of the effects of the dietary lipids. The subsequent mean dienoic (linoleic), trienoic (linolenic) and tetraenoic acid values (mg./100 ml. plasma), respectively, for the calves receiving the various dietary lipids were: milk fat (whole milk)—71, 6 and 12; milk fat (butter oil)—35, 8 and 10; lard—65, 2 and 4; crude soybean oil—111, 5 and 5; and hydrogenated soybean oil—27, 1 and 5.

P15. The effect of vitamin supplements on carotene utilization from hay by dairy heifers. S. R. SKAGGS, New Mexico Agr. Expt. Sta., State College.

Calves on this experiment all received colostrum from their dams for 3 d. From the 4th-30th days they were fed limited amounts of whole milk, then weaned from milk entirely.

After 4 d. of age, all calves had free access to leafy, green alfalfa hay and a dry, home-mixed starter containing only ground grain sorghum, wheat bran and cottonseed meal+1% each of salt and steamed bone meal.

Daily vitamin supplements fed from 2nd through 60th days as follows: Group I: (Control) 1 capsule, minimum potency fish liver oil (less than 250 I.U. vitamin A per capsule). Group II: 1 capsule fish liver oil (25,000 I.U. vitamin A). Group III: Same as group II+50 mg. niacin. Group IV: 1 capsule fish liver oil (30,000 I.U. vitamin A and 25,000 I.U. vitamin D) plus 50 mg. niacin.

Vitamin A blood levels showed no significant differences between groups. Rates of growth averaged near normal on all groups with no significant differences. Blood plasma carotene levels rose faster in the control group and remained consistently higher for several weeks after the vitamin supplements were discontinued than in the other 3 groups.

These results indicate that carotene from the roughage is not utilized well when calves receive high levels of vitamin supplements. Leafy, green alfalfa hay appears as good or better than vitamin capsules as a source of vitamin A for growing calves.

P16. The value of arsonic acid derivatives as a growth stimulant when fed to calves. G. C. GRAF AND C. W. HOLDAWAY, V. P. I., Blacksburg, Va.

Para-amino-phenyl arsonic acid at the levels of 60, 120 and 240 g./ton was incorporated into a commercial milk replacement and calf starter. Sixteen calves were divided into 4 groups: Group A served as the control; Group B received the feeds containing the lower level of arsonic acid derivative; Group C, the intermediate level; and Group D, the highest level. All calves were taken from their dams 24 hr. after birth and given whole milk at the rate of 1 lb./10 lb. of body weight the 1st week. At the start of the 2nd week, a milk replacement was fed in addition to a reduced amount of whole milk. No milk was fed after the 2nd week. The calf starter was placed before the calves during the 2nd week and consumption up to 6 lb. daily allowed. A high-quality 2nd cutting clover hay was fed from the 2nd week on. Records of consumption of the milk replacement, calf starter and hay were kept. Mineralized block salt was before the calves at all times. The milk replacement was discontinued at the end of the 8th week. The experiment continued for 16 wk.

Body weights, chest circumference and height at withers were obtained at weekly intervals. Preliminary results indicate a lowered incidence of scours and more rapid gains during the 1st 4 wk. after birth when fed the arsonic acid derivatives. Levels of 60 and 120 g. per ton gave slightly better results than the 240-g. level for increased gains. All calves showed satisfactory gains and excellent bloom and condition regardless of the levels of para-amino-phenyl arsonic acid fed.

P17. Effect of ration upon riboflavin levels in calf tissues. E. G. MOODY, S. M. HAUGE AND N. S. LUNDQUIST, Purdue Univ., Lafayette, Ind.

Levels of riboflavin in muscle, heart, kidney and liver were determined in 20 Holstein, Guernsey and Jersey calves maintained for up to 36 wk. exclusively on 1 of the following dietary regimens: Lot I, whole milk chromatographed through Florisil (0.12 mg. riboflavin/1.); Lot II, chromatographed whole milk supplemented with riboflavin (0.40 mg. riboflavin/1.); Lot III, untreated milk (1.5 mg. riboflavin/1.); and Lot IV, untreated milk plus hay and grain *ad lib.* For each lot the average riboflavin levels for muscle, heart, kidney and liver, respectively, were: Lot I—1.58, 16.35, 23.35 and 20.57; Lot II—2.72, 17.99, 20.54 and 16.30; Lot III—2.64, 16.63, 21.15 and 29.06; and Lot IV—2.50, 15.65, 26.49 and 41.58 γ /g. wet tissue. In this experiment the level of riboflavin intake did not uniformly influence the level of this factor in these tissues.

P18. Effect of type of dietary lipid upon the blood plasma lipids of young dairy calves. N. L. JACOBSON, J. H. ZALETEL AND R. S. ALLEN, Iowa Agr. Expt. Sta., Ames.

Fifteen calves were allowed colostrum *ad libitum* for 3 d. following birth and during the

next 2 wk. were fed whole milk (3% fat). The animals then were divided into 5 comparable groups and during the subsequent 4-wk. period were fed various types of milk. One group continued to receive whole milk. The other groups received homogenized reconstituted milks containing 3% butter oil, lard, crude soybean oil and hydrogenated soybean oil, respectively. All milks were fed at daily rate of 10 lb./100 lb. body weight. No other feeds were allowed.

Weekly determinations of the blood plasma lipids by oxidative procedures revealed major dietary effects. The values for total lipids, ester cholesterol, free cholesterol, phospholipids and total fatty acids were highest for the groups fed whole milk and crude soybean oil, lowest for the group fed hydrogenated soybean oil and intermediate for the groups fed butter oil and lard. Differences in plasma neutral fats among the various dietary groups were not as great as those observed for the other plasma lipids. Similarly, no major differences were observed in "free" fatty acids, although values for the group fed lard were highest.

P19. The value of various levels of aureomycin in milk replacements for dairy calves¹. C. B. KNOTT AND EARL B. ROSS, Pennsylvania Agr. Expt. Sta., State College.

Thirty-six Holstein male calves have been divided into 6 similar groups in a study of the value of higher levels of aureomycin supplementation of a milk replacement formula developed at this station. This formula has been supplemented with 2, 4, 6, 8 and 10 g. of aureomycin/100 lb., respectively, for each of the 5 experimental groups. In addition, a control group which is being fed the same milk replacement formula but without aureomycin supplementation is being used. The calves also are being fed a suitable calf starter and a good quality 2nd cutting grass-legume hay.

Preliminary results with these calves through 6 wk. of age indicate no harmful effects of the higher intake of aureomycin and possibly some beneficial effects in terms of growth and control of scours. These results are, however, preliminary and inconclusive at this time of reporting but complete data on all calves through 12 wk. of age will be presented.

¹ This work was supported in part by a grant from the Lederle Lab., Pearl River, N. Y.

P20. The influence of aureomycin and cud inoculation on the growth of dairy calves. A. D. MCGILLIARD, M. RONNING, E. R. BEROUSEK AND C. L. NORTON, Oklahoma A & M College Stillwater.

Earlier investigations with the administration of crystalline aureomycin HCl to dairy calves indicate that calves utilize feed to a lesser extent after aureomycin administration is discontinued than do control calves. Since this may be due to a lag in the reestablishment of the normal rumen flora, it appeared desirable to determine the value of rumen inoculations with cud mate-

rial from an adult animal following aureomycin feeding.

Eighteen Holstein, Ayrshire and Guernsey newborn calves were divided into 3 groups. Group I (controls) received a daily basal ration consisting of 6 lb. of whole milk, a maximum of 4 lb. of calf starter, and prairie hay *ad libitum*. Group II received the same basal ration + 70 mg. of aureomycin by capsule daily for 35 d. and were inoculated with cud material the 36th and 41st days. Group III received the basal ration + 70 mg. aureomycin by capsule daily for 35 d. and no inoculations. Milk was eliminated from the diet of all calves at the age of 10 wk.

Completed data for 3 calves from each group through 16 wk. indicate that calves receiving aureomycin made better gains the 1st 35 d. than the controls. Upon discontinuation of aureomycin feeding both the control group and the group receiving cud inoculations made better gains through the 2nd 35 d. than group III which received aureomycin and no inoculum. In all cases periods of decreased growth apparently were followed by periods of acceleration so that growth in all groups was approximately the same at the end of 16 wk. of age. Scours were not a problem in any of the groups.

P21. Effect of type of protein on the response of young dairy calves to aureomycin with data on the microflora of the feces. L. L. RUSOFF, J. A. ALFORD AND C. E. HYDE, Louisiana, Agr. Expt. Sta., Baton Rouge.

A study was made of aureomycin supplementation to calves receiving a 27-37% level of soybean meal, cottonseed meal or gossypol-free cottonseed meal in the calf starter. Thirty-six purebred male calves (18 Holsteins and 18 Jerseys) were divided into 3 groups. One-half of each received the antibiotic starting at 3 d. of age. Fifty mg. of aureomycin were added daily in the milk for 28 d.; the calf starters which contained a 1% level of an aureomycin supplement were fed at 8 d. of age. The remaining animals in each group served as controls. At 90 d. of age, aureomycin supplementation stimulated the growth of calves about equally on either the soybean or cottonseed meal starters.

Fecal samples were obtained biweekly from representative calves on all rations until they were 9 wk. of age. The following bacterial counts were made: Aerobic, anaerobic, carrot-liver shake tubes (lactics?), coliforms, enterococci and H₂S-producing anaerobes. All counts were considerably higher while the calves were receiving milk than after weaning. The bacteria in the carrot-liver medium were not typical lactics after the calves were weaned. There were wide variations between samples from different calves on the same ration. No correlation between the numbers of any group of bacteria and the presence of aureomycin in the diet was found.

P22. Effect of aureomycin supplementation on changes in weight and body measurements of dairy calves. N. L. JACOBSON, J. G. KAFETZA-

KIS AND P. G. HOMEYER, Iowa Agr. Expt. Sta. Ames.

Thirty-two calves at 16 wk. of age were divided into four groups which were comparable in breed and sex distribution. Calves in groups I and II previously had received crystalline aureomycin HCl (80 mg./calf daily from 4 d. of age), whereas calves in groups III and IV had been fed no antibiotic. During the subsequent 12-wk. period each calf in groups I and III received daily 80 mg. aureomycin (Aurofac A) in the concentrate mixture and the calves in groups II and IV were fed no aureomycin. The concentrate mixture was limited to 4 lb./calf daily; hay was fed *ad libitum*.

During the 12-wk. experimental period, gains in body weight and increases in circumference of chest and of barrel were significantly greater ($P=0.01$) for calves fed aureomycin (groups I and III) than for calves receiving no antibiotic (groups II and IV). Increases in height at withers and in chest depth also were greater in the supplemented than in the non-supplemented groups, but the differences were not significant at $P=0.05$.

Although previous treatment (groups I and II *vs.* groups III and IV) had less effect than experimental treatment on changes in weight and in the various body measurements, the mean values in each instance were greater for calves not previously fed aureomycin.

Total weight increases (birth to 28 wk.) were greatest for calves in group I, followed in decreasing order by those in groups III, II and IV. Hay and water consumptions were greater for calves fed aureomycin.

P23. The effect of stress conditions on dairy cattle. G. C. GRAF¹, Univ. of Minnesota, St. Paul.

The effects of low ambient temperature, exercise, dehorning, adrenaline injection, parturition and intermittent electrical stimuli on dairy cattle were determined. The changes in the respiration and heart rates, rectal temperatures, plasma lactic acid levels and plasma creatinine levels were used to indicate the degree of stress. Nine pairs of identical twin heifers and 2 sets of identical triplets were used as experimental subjects.

All the stress conditions, with the exception of dehorning, caused large increases ranging from 36-272% in the plasma lactic acid levels. These increases were highly significant. Heart rates also were affected materially during exercise and dehorning, when intermittent electrical stimuli were applied and as a result of adrenaline injection. Adrenaline injection resulted 1st in an increase followed by a decrease in heart rates. Rectal temperatures showed a slight increase when the animals were exposed for 1 hr. in -16 to -16° F. ambient temperatures.

Exercise and adrenaline injection appeared to produce the greatest amount of stress. Dehorning and parturition (except just after calving) exhibited slight changes in the measures used to evaluate stress.

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P24. Factors influencing the production of high-quality milk. J. L. COVINGTON, W. L. GRIEBELER, J. B. RODGERS, I. R. JONES, P. M. BRANT AND L. W. BONNICKSEN, Oregon State College, Corvallis.

A study was conducted on 171 farms to determine the influence of various factors related to production of high-quality milk. All farms were rated with a total possible score of 1,000. The items making up the score card can be classified into 3 general sections, namely, methods and sanitation, buildings, and equipment, with totals of 640, 190 and 170 points, respectively.

The average bacteria count on 12 monthly samples of raw milk ranged from a low of 2,600 bacteria/ml. to a high of 1,083,000/ml., with an average of 66,300/ml. for all producers. 69% of the producers supplied milk with less than the average of bacteria for all herds.

Preliminary statistical analyses indicate that the factors concerned with methods and sanitation are of more significance in production of high-quality milk than are the factors related to buildings or equipment.

P25. Methods of milking and milk-handling as factors affecting the quality and economy of milk produced. II. A further study of the effect of permanent pipelines in the dairy barn on milk quality. M. H. ALEXANDER, W. O. NELSON AND E. E. ORMISTON, Univ. of Illinois, Urbana.

A previous study has shown that milk of acceptable quality may be produced with use of permanent pipelines in the dairy barn. This study was designed to measure further their effect under varying conditions of climatic changes. A conventional system of milking was compared directly with a permanent stainless-steel pipeline and with a permanent Pyrex glass pipeline, the pipelines being cleaned without dismantling throughout the study.

The study was divided into 4 stages in order to measure the possible climatic effects throughout the 4 seasons of the year. Bacteriological tests were made throughout each of the 4 periods for each night milking and for each composite night and morning milking. Results confirm previous findings, namely, that milk of acceptable quality can be produced when handled through permanent pipelines in the dairy barn.

P26. Growth uniformity trials with identical twin dairy heifers—estimates of heritability and twin efficiency. H. W. THOLE AND M. C. HERVEY, Univ. of Minnesota, St. Paul.

Growth of 21 sets of identical twin dairy heifers kept under similar conditions of feeding, housing and management was studied. Included were 8 sets of grade Holsteins, 8 sets of grade Guernseys and 6 sets of twins of mixed breeding. Measurements for height at withers and body weight taken at 6, 12 and 15 mo. of age were treated collectively and by breeds.

Heritability estimates of body weight at the ages designated were found to vary from 86.3–96.1%. Furthermore, twin efficiency studies indicated that 1 set of identical twins will replace from 6–24 pairs of unrelated cattle for experiments in which results are measured in terms of body weight. The heritability of height at withers for the different ages and for the various breeds varied from 93.5–97.8% with the exception of the 1 group of 5 sets of twins of mixed breeding. Moreover, 1 set of identical twins would replace from 14–43 pairs of unrelated animals in experiments in which results are measured in terms of height at withers.

P27. Progress report on the production records of crossbred dairy cattle. J. P. LAMASTER, G. W. BRANT AND C. C. BRANNON, S. C. Agr. Expt. Sta., AND M. H. FOHRMAN, Bureau of Dairy Industry, U. S. D. A.

The breeding plan being followed to obtain crossbred dairy cattle and the environmental conditions which are provided for the crossbreeds and their purebred relatives at this station were discussed by LaMaster *et al.* (J. Dairy Sci., **33**: 375–376. 1950).

Since the above report, more females have completed their 1st records. Production comparisons will be given for 15 two-breed crossbreeds and their dams. Nine of these 2-breed females are from reciprocal crosses of animals of the Guernsey and Holstein breeds. Five cows sired by Guernsey bulls and from Holstein dams average 10,460 lb. of milk, which is 172 lb. of milk less than their dams. Four cows by Holstein sires and from Guernsey cows average 10,265 lb. of milk, which is an increase of 2,437 lb. of milk over their dams. The records used in these comparisons were converted to a 2 × 305-d., mature-equivalent basis before calculating the 4% milk. Seventeen heifers from reciprocal crosses of the Guernsey and Holstein breeds are now in the herd and will be tested as they freshen.

Comparisons also will be given for 9 two-breed females and their purebred maternal sisters; 23 three-breed and 12 four-breed crossbreeds and their dams. All sires, except 1, have been or are in the process of being proved in the station herd on purebred daughters. These proofs will be used in giving the results obtained to date.

P28. The partition of evaporative cooling between the respiratory and outer surfaces in European and Indian cattle¹. H. H. KIBLER AND S. BRODY, Missouri Agr. Expt. Sta., Columbia.

A study was made of the influence of temperature (5–105° F.) on the levels and proportions of heat and moisture dissipation by evaporative cooling from the outer body surface and respiratory tract in lactating Jersey, Holstein, Brown Swiss and Brahman cows and in non-lactating Brahman cows and Brown Swiss and Brahman heifers. All breeds attained the same maximal outer surface evaporative or "sweating" rate of 150 g. (or 87 Cal.)/sq. m./hr. but the rise to this level occurred between 80 and 90° F. in the European cattle and between 95 and 105° F.

in Indian cattle. The increase in respiratory surface vaporization with increasing temperature was greater in the European than Indian cattle; the maximal respiratory vaporization rates were 50 g. (or 29 Cal.)/sq. m.²/hr. in the European cattle and 30 g. (or 17 Cal.)/sq. m.²/hr. in the Indian cattle. The percentage of metabolic heat dissipated by vaporization varied with temperature but, while that from the outer surface was very similar for all breeds, that from the respiratory surface was higher for European than Indian cattle. There was no evidence that Brahman cattle "sweat" more per unit surface area than European cattle, but their 12% greater surface area/unit weight gives them an advantage in outer surface evaporative and non-evaporative cooling (limited to the range, 60 to about 105° F.) and a corresponding disadvantage in heat conservation below 40° F.

¹ Contribution from the Department of Dairy Husbandry as Journal Series Paper no. 1302, approved by the Director of the Missouri Agr. Expt. Sta.

^a Refers to outer surface area.

P29. Mammary development and heart girth relationships and changes with age. V. L. BALDWIN AND M. C. HERVEY, Univ. of Minnesota, St. Paul.

A total of 528 Holstein calves ranging from 11–25 wk. of age and in 156 herds doing D.H.I.A. testing was examined. Among data recorded on these calves were the mammary evaluation grade, according to method developed by Swett, and the heart girth.

The change of heart girth measurements with age will be presented. There was a tendency for the mammary evaluation grade to decrease with age. Correlations between heart girth and mammary evaluation grade were low and ranged from –0.08 to 0.59 at the various ages but showed no consistent trend. While few of the correlations at the different ages between heart girth and mammary development grade were statistically significant, they were, with 1 exception, all positive. Data from these field studies indicate that body size has a small influence on mammary evaluation grade.

P30. A summary of a ten-year comparison between loose run and stanchion barn housing for dairy cattle. E. E. HEIZER, C. E. ZEHNER AND V. R. SMITH, Univ. of Wisconsin, Madison.

The herds in these experiments produced equally as well in an uninsulated loose-run barn as in an insulated stanchion barn. There was little difference in general health of the animals housed under these 2 systems. However, there were considerably more cases of stepped-on teats, swollen hocks and stiffness and lameness in herds housed in the stanchion barn than in herds housed in the loose-run barns. The herds housed in the loose-run barns consumed more roughage, but they also had a greater average gain in weight than herds in the stanchion barn. Bedding requirements for the loose-run barns were con-

sistently greater than for the stanchion barn. The average plate counts of the milk from the stanchion barn were slightly lower than from the loose-run barns.

P31. A study of some causative mechanisms in bovine streptococcal mastitis¹. C. P. MERILAN AND H. A. HERMAN, Univ. of Missouri, Columbia.

The effect of *Streptococcus agalactiae* filtrates upon the *in vitro* anaerobic glycolysis of bovine mammary tissue slices has been studied by means of the Warburg technique using sodium acetate as the metabolic substrate.

The β -hemolytic, Lancefield group B, streptococci were isolated from acute cases of *S. agalactiae* mastitis and cultured in the laboratory on a semi-synthetic casein-acetate-lactose broth medium. Eighteen-hr. cultures were rendered sterile by means of Selas #03 porcelain filters and the comparative effect of these filtrates and uninoculated media determined manometrically. Addition of the streptococcal filtrate to reaction flasks containing the metabolizing tissue caused a marked decrease in the rate of anaerobic glycolysis compared to the slight inhibitory effect of the uninoculated media.

Intramammary infusions of the streptococcal filtrates produced hardness and swelling of the infused quarters within 1-2 hr., followed by rectal temperature increases of 3-5° F., reaching a peak 6-9 hr. after infusion. The rectal temperatures returned to approximately normal at 12 hr. although some induration of the quarters and production of physically abnormal milk persisted for 2-3 d. Subsequent daily infusions of the filtrate in the same quarter resulted in continued production of physically abnormal milk; however, the response of the quarter in the form of swelling and induration gradually decreased with each consecutive infusion. The rectal temperature response to these continued filtrate infusions varied from no effect to a sharp decline in temperature of 3-5° F. Subsequent infusions in different quarters of the same cow caused the marked physical and temperature responses characteristic of the first infusion.

Infusions of the uninoculated media produced only minor symptoms of irritation in the infused quarter, although the milk obtained at the first post-infusion milking period was physically abnormal.

¹Contribution from the Dept. of Dairy Husbandry, Missouri College of Agriculture, Expt. Sta. Journal Series no. 1301.

P32. The role of plasma cells in the production of globulins within the mammary gland and time studies on antibody response from experimentally induced inflammation of the udder¹. R. M. PORTER, New Mexico A. & M. College.

This work was carried out to determine if antibodies produced by plasma cells could be a possible explanation for the high protein concentration of colostrum milk and for the increase in protein concentration during other inflammatory conditions of the udder. The presence of the plasma cell in numerous inflammatory

conditions indicates its important role in antibody production.

From the agglutination and histological studies carried out it could be concluded that: (a) Plasmacytosis occurs in the developing udder prepartum and may be associated with the high production of immune lactoglobulin in colostrum. (b) Plasma cells are present in the udder during the inflammatory condition brought about by the cessation of milking. (c) The time of antibody response is not correlated to the degree of inflammatory response upon injection of the antigen, but seems to be correlated to the sensitivity of the tissues to the antigen. (d) Antigens will readily pass into the blood from ducts of the mammary gland, but there is a wide variation in the ability of the antibody to pass from the blood into the milk. (e) The comparative concentrations of antibody and antigen in the udder may explain the exacerbations which occur in cases of mastitis.

¹Based on a thesis presented by R. M. Porter in the graduate school of the Univ. of Minn. in partial fulfillment of the requirements for a Ph.D. degree.

P33. Histological evidence of a hyalin-fibrin complex in the bovine mammary gland. J. R. KUIKEN, D. L. HILL AND N. S. LUNDQUIST, Purdue Univ., Lafayette, Ind.

Histological examination has revealed the presence of a hyalin-fibrin body in many of the alveoli of some dairy cow udders. This body was found free in the lumen of the alveolus and is somewhat similar in appearance to corpora amyloacea of the prostate. Bodies somewhat similar to corpora amyloacea were observed in the alveoli by McFadyean (1930). He questioned whether or not they actually are amyloid as reported by Ottolenghi (1901) and others, but did not form a firm opinion as to any other possible composition. In this study the bodies resisted solution in varying strengths of HCl, HNO₃, NaOH and alcohol. Jenkins decalcifying agent had no effect. All sections 1st were stained with hematoxylin-eosin and Mallory's triple stain. Differential staining methods for amyloid, mucin, calcium, hyalin and fibrin then were used to characterize the body and it was found to be a hyalin-fibrin complex. Microscopically this complex is circular in all cases but varies in size and structure within the same section. Some are small and dense, others are an intermeshed network and others have stratified or concentric layers surrounding a nucleus. They may occur in groups or isolated alveoli. Efforts are being made to correlate the conditions under which they are found.

P34. Histological and chemical studies of fat metabolism in the mammary glands of cows and goats. M. L. Y. SMITH, B. C. HATZIOLOS AND S. KUMAR, Univ. of Maryland, College Park.

Mammary gland tissues were excised from 3 lactating cows, 1 non-lactating cow and 2 lactating goats, and fixed at various time intervals in preservatives such as 10% formalin. The tissues were cut, for the most part, by the frozen-section technique and stained with scarlet red, Nile blue sulfate, oil red O, osmic acid, brilliant cresyl

blue, neutral red and Fishler's stain, all of which are fat stains and some of which are specific for fatty acids. Little or no evidence was obtained of the presence of free fatty acids in the epithelial cells. The findings are in disagreement with the earlier interpretation placed on the shaded areas depicted in the basal part of the alveolar cells. These areas are believed to be artifactual pictures of coagulated protoplasm. The test for cholesterol proved positive, demonstrating a distribution of pale blue-green cholesterol droplets in the free end of the cell. The neutral fat droplets in the vicinity of the nucleus are small; those in the distal end are larger. The incubation of lactating mammary gland tissue for 12-24 hr. resulted in a positive reaction in the small fatty droplets to the Fishler staining method for free fatty acids, the color deepening with time of incubation, suggesting that the free fatty acids in incubated tissue are due to lipase activity. Chemical tests corroborated the histologic findings that free fatty acids are not present in any appreciable amount in fresh tissue but increase markedly on incubation. The very small amount found in fresh tissue consisted almost entirely of water-soluble, steam-volatile acids.

P35. Blood levels and urinary excretion of certain constituents in ketotic cows.¹ P. J. VAN SOEST, T. H. BLOSSER, G. M. WARD, J. B. CRILLY AND M. F. ADAMS, Washington State College, Pullman.

Studies were made in Wahkiakum County, Wash. on 6 cows diagnosed by a competent veterinarian as having ketosis and on 6 normal cows of the same breed and same stage of lactation from the same herds. Blood samples were taken on the farm from the ketotic cows 24 hr. prior and immediately prior to treatment and from a corresponding normal cow in the herd at the same time. Samples also were taken from the same cows 15 d. post treatment. Twenty-four hr. urine collections were made on 3 of the ketotic cows and on all of the normal cows.

Blood constituents determined were ketones, glucose, pyruvic acid and lactic acid. Blood hematocrit also was followed. Urine was analyzed for K, Na and chlorides. Urinary collections were made at 10, 15 and 30 d. postpartum on 13 cows of 3 breeds in the college herd to compare urinary excretion of K, Na and chlorides under controlled conditions with that occurring under field conditions.

The levels of the blood constituents were as follows for the ketotic samples and normal samples, respectively: Whole-blood ketones, 23.7 and 5.0 mg. %; whole-blood glucose, 33.0 and 41.6 mg. %; whole-blood pyruvic acid, 1.59 and 1.06 mg. %; whole-blood lactic acid, 24.4 and 28.8 mg. %; hematocrit, 42.2 and 40.4%. Other than the values for ketones and glucose, the only blood values consistently varying from normal in the ketotic cows were for the whole-blood pyruvic acid.

Daily urinary excretions were as follows on samples from the ketotic cows, normal cows in

the field and normal cows under controlled conditions, respectively: Urinary volume, 9.5, 7.8 and 9.8; K, 105.0, 101.4 and 135.0 g.; Na, 10.7, 11.0 and 6.8 g.; chlorides, 4.4, 3.0 and 12.0 g.

Studies of this type are being continued, and further experimental data will be presented.

¹ This investigation was supported in part by funds provided for biological and medical research by the State of Washington Initiative Measure no. 171.

P36. The effects of sodium acetate given orally upon cows with ketosis. W. J. MILLER AND N. N. ALLEN, Univ. of Wisconsin, Madison.

At the 1951 meetings of this Association, it was reported that cows with clinical symptoms of ketosis had responded favorably to the oral administration of sodium acetate. Since that time 23 cows showing definite symptoms have been treated with amounts varying from 0.25-1 lb. daily, and chemical and clinical changes were observed. Of these, 12 recovered without other treatment. The pattern of the recovery in relation to the acetate feeding indicated beneficial effects from the treatment. With 5 others the response to the acetate was questionable, due to very slow recovery or to other treatment given before recovery could be expected. The other 6 failed to give any response to acetate feeding. Of these, 3 recovered when given glucose intravenously; 3 failed to respond to glucose. Two of these were known to have uterine infections and continued to show symptoms of ketosis until the infections cleared up.

In a few cases the response to acetate was almost immediate, while in most it was not as rapid as is usually observed when glucose is given intravenously. Reports from veterinarians suggest that the acetate might be of value when used as a follow-up for glucose or other treatment.

Observations on 8 normal cows on varied roughage diets showed no change in blood glucose level when 1 lb. of sodium acetate was fed daily. A more carefully controlled experiment is in progress to study the effect of feeding 1 pound of sodium acetate daily upon the milk fat and blood glucose and ketone levels of normal cows. The results will be reported.

P37. Pituitary-adrenal cortical syndrome in ketosis of dairy cows as evidenced by the adrenaline test, eosinophil levels, and replacement therapy. J. C. SHAW, B. C. HATZIOLOS, E. C. LEFFEL, W. M. GILL AND A. C. CHUNG, Univ. of Maryland, College Park.

Seven cows with ketosis and 13 normal cows in the early postpartal period were injected subcutaneously with 5 ml. of adrenaline (1-1,000). After 4 hr. the ketotic cows exhibited only slight changes in eosinophil count, 6 of the cows varying from -3 to +20% and one -9%. The eosinophil count of the normal cows decreased markedly, varying from -25 to -46%. This is considered a further indication of a hypofunction of the pituitary-adrenal cortical system in ketosis. The eosinophil levels in ketotic cows vary from normal to high, this is preceded by a low eosinophil level.

In a study of 16 normal cows the high-producing cows had low eosinophil levels for several weeks postpartum, whereas the lower producers maintained rather normal levels. The maximum fall in eosinophils was noted on the day of parturition. Forty-three cows with ketosis were treated with varying doses of cortisone, 22 with ACTH, 4 with compound F, 3 with growth hormone, 1 with a coenzyme concentrate, 1 each with sodium acetate and sodium propionate intravenously, 2 with a solution of amino acids intravenously and 11 with sodium acetate *per os*. Clinical observations + blood chemistry and hematologic studies were used in evaluating the results. Cortisone, ACTH and compound F all produced rapid recovery. The optimum dose of cortisone acetate appeared to be 1.5 g., followed by an additional gram within 48 hr., although 1 g. was often sufficient. Smaller doses (0.5–0.75 g.) also were effective, although additional injections usually were required. 300 mg. of cortisone (free alcohol) in saline gave a quick but transient response. 300 I.U. of a highly purified preparation of ACTH had about the same potency as 1.5 g. of cortisone. A combination of glucose with cortisone acetate or ACTH did not appear to be more effective than cortisone or ACTH alone. Compound F appeared to be from 2 to 3 times as potent as cortisone. Ten cows showed little or no clinical response to the administration of 1 lb. of sodium acetate, although the blood glucose increased to some extent and 1 cow recovered within a few days. In 1 case a cow was continued on 1.5 lb. of sodium acetate/day for 8 d. with no clinical improvement, but recovered rapidly following the injection of 1.5 g. of cortisone, showing complete remission of clinical symptoms within 24 hr. None of the other substances produced a favorable response.

P38. The incidence of a sterility syndrome in the rabbit fed soybean hay and the failure of certain supplements to alleviate the symptoms. K. A. KENDALL AND R. L. HAYS, Univ. of Illinois, Urbana.

In an earlier report impaired reproduction in the rabbit was shown to be associated with the feeding of soybean hay. Of the 34 mated control females fed alfalfa, lespedeza or timothy hay in combination with wheat, 32 females were known to be pregnant and produced an average of 8.3 young/litter. Of the 71 mated females fed soybean hay and wheat, 48 known pregnancies resulted in an average of 4.71 young/litter carried to term.

Further investigation has revealed through a series of sacrifice-autopsy experiments terminated on the 28th day following mating, that 56 females out of a total of 70 mated and fed soybean hay and wheat had an average of 8.54 corpora lutea with an average litter size of 4.45 living and 2.14 dead young/litter. The remaining 14 females failed to show evidence of having been pregnant. Laparotomy observations at 17 d. after mating revealed that numerous resorptions occur during the early part of the gestation period.

The administration of DL-methionine, 2-methyl-1,4-naphthoquinone, β -carotene, α -tocopherol, vitamin A, vitamin B₁₂ or progesterone have failed to alleviate the sterility symptoms.

P39. A study of the birth weights of purebred and crossbred calves. R. W. TOUCHBERRY, Univ. of Illinois, Urbana, and K. A. TABLER, Bureau of Dairy Industry, U. S. D. A.

In the fall of 1949, 10 Holstein and 10 Guernsey cows were mated to a Holstein bull and 10 of each breed to a Guernsey bull. Late in 1950 similar matings were begun again, except that the groups of females were reversed with respect to the 2 bulls. From these matings a total of 79 calves have been dropped and 2 cows are yet to calve as a result of the 2nd matings. Two of the 40 cows aborted during the 8th month of pregnancy and two sets of twins were born. Only the weights of the 73 full-term, single-birth calves (37 females and 36 males) were used in the analysis. By an analysis of variance the main effects for bull and breed of dam and the interaction, bull by breed of dam, or crossbreds *vs.* purebreds, were found to be significant at less than the 0.01 level of probability. The mean square for sex of calf was significant at the 0.05 level of probability. The components of variance for bulls, breed of dam, sex of calf and crossbreds *vs.* purebreds accounted for 0.9, 50.6, 9.5 and 18.0% of the variance, respectively, while error accounted for 20.9%.

P40. Thiouracil-induced hypothyroidism in sexually mature dairy bulls. E. W. SWANSON AND J. P. BOATMAN, Univ. of Tennessee, Knoxville.

Thiouracil was fed to 1 aging 7-yr.-old and to 2 normal 2-yr.-old Jersey bulls for the purpose of producing recognizable symptoms of hypothyroidism in behavior and seminal characteristics. The young bulls received step-wise doses of 5–30 g. thiouracil daily covering a period of 131 d. Semen collections (2 ejaculates/week) showed no harmful effect of the hypothyroidism, as evidenced by insignificant changes in volume, concentration, livability, percentage of motile, live and abnormal sperm. Libido was only slightly affected, although spontaneous activity and alertness were noticeably decreased. Low concentrations of thiouracil (0.1–1.0 γ /ml. of diluted semen) did not affect motility or livability of normal sperm. Sperm from the hypothyroid bulls were not benefited by adding 0.1 γ thyroxine/ml. of diluted semen. No significant changes were observed in testis histology of these bulls at 34 d. after cessation of thiouracil feeding. The aged bull was fed 20 g./day of methyl thiouracil for 3 mo. The bull became more sluggish in activity and rough in appearance. Service remained satisfactory at 5–6 ejaculates/month, with normal volume and concentration. After 1 mo. the percentage of dead, abnormal and immotile sperm increased and sperm livability decreased. This study indicates that the effects of hypothyroidism upon seminal characteristics are more pronounced in old than in young bulls.

P41. The intracrypt space in the placentome of the cow¹. H. W. WEETH AND H. A. HERMAN, Univ. of Missouri, Columbia.

The definitive placentome or cotyledon is a mushroom-shaped structure about 6.0 cm. in diameter. It consists of many branching chorionic villi surrounded by the caruncular crypts of the uterus. When microscopic sections are prepared by the usual paraffin technique, involving dehydration, a space is seen to separate the fetal villus from the uterine crypt. The space normally varies from 2.5–10.0 μ in width. That this space is a constant artifact created by the technique is indicated by the following evidence: (a) Frayed edges of the cells bordering the space indicate a pulling apart of these tissues. (b) Morphologically, the connective tissue of the villus appears looser than that of the uterine crypt, thus it shrinks more during dehydration. (c) Analytically, the villi have 6.6% less dry matter, therefore, the villi shrink away from the crypt wall.

Since the outer or trophoblastic cells of the villi in the functioning placentome are in direct contact with the uterine crypt wall, the exchange of metabolites between the 2 tissues does not involve passage through a space. An appreciation of the intracrypt space artifact also casts some doubt upon the general assumption that the discrete living cells on the crypt walls are of uterine epithelial origin.

¹Contribution from the Dept. of Dairy Husbandry, Missouri College of Agriculture, Expt. Sta. Journal Series no. 1300.

P42. The effects of relaxin¹ on the cow's cervix. E. F. GRAHAM AND A. E. DRACY, So. Dakota Agr. Expt. Sta., Brookings.

Three cows were injected, 5 d. post estrus, with 3 different levels of relaxin after being primed with 20 mg. of diethylstilbesterol on the 2nd, 3rd and 4th days post estrus. Since previous work by Hisaw and others on small animals seems to indicate that relaxin acts only after the tissue has been sensitized by one of the estrogens, 60 mg. of stilbesterol were injected as a sensitizing agent.

The 3 levels of relaxin injected were 250, 1500 and 8500 guinea pig units (G.P.U.). All 3 levels exhibited signs of cervical relaxation. The cervix was dilated 0.93 in. when 250 G.P.U. were administered, 1.27 in. when 1,500 G.P.U. were injected and 1.31 in. when 8,500 G.P.U. were injected. Histologically none of these levels showed any signs of detrimental effects upon the cervical or uterine tissue. The cervixes of the 3 control cows and of the 3 experimental cows could be dilated during heat, but the 3 control cows could not be dilated 5 d. post estrus. Two cows receiving diethylstilbesterol could not be dilated; thus the apparent effective factor was relaxin.

¹The relaxin for these experiments was furnished by the Chilcote Lab., Morris Plains, N. J.

P43. The sampling of the endometrium of the bovine using a biopsy technique. J. D. DONKER, Univ. of Minnesota, St. Paul.

Endometrial biopsies should be a valuable adjunct to other means of obtaining information for studies in reproductive physiology. An instrument designed to secure uterine samples was constructed of stainless steel, except for a blade of case-hardened steel. It is tubular in construction with over-all dimensions of 57 cm. \times 6 mm. There are 2 parts to this instrument: an outside tube with a rounded tip and an opening on one side adjacent to this tip and an inside shaft connected to a circular knife which seats against the tip and fits against the inside perimeter of the tube. When the blade is seated the opening is occluded; otherwise, it is open.

The tube is manipulated through the cervical canal as in intrauterine insemination. The blade is retracted to admit uterine tissue which is gently pressed into the opening by rectal manipulation. The blade then is pressed forward, the instrument is withdrawn, and the sample removed to the fixative.

From 6 animals, one of which was ovariectomized, 23 samples have been obtained at various stages of the cycle or after different treatments. The samples are comparable to timed slaughter materials obtained by several workers.

P44. A dairy cattle pregnancy test. J. H. BYERS, Dept. of Dairy Husbandry, Oregon State College, Corvallis.

In the Oregon State College dairy herd, 136 cows were checked for pregnancy, using an oxidation-reduction indicator with urine. The test consisted of taking a 3-ml. sample of urine that had cooled to room temperature in approximately 30 min. and adding 0.6 ml. of sodium benzone-indophenol indicator. The tubes were inverted for mixing, allowed to stand for 30 sec. and then read. The mixture of urine and indicator gives a green color. Pregnant cow's urine does not return to colorless in 10 min., while that of the non-pregnant cow returns to the original urine color within 30 sec. The intensity of the color varies with length of gestation. Greatest density of color on the pregnant cow shows at approximately 60 and 210 d. gestation.

Of the 136 cows tested, 82 showed positive as being pregnant. Seventy-five of these cows were pronounced pregnant at 60- to 90-d. gestation by rectal palpation. Two of 82 cows had retained corpus lutei, 5 had calved recently and continued to show positive for 3 wk. post-partum, and 2 unbred yearlings showed positive. Of the 54 cows showing negative, 3 were pronounced pregnant on rectal palpation. Two cows known to have cystic ovaries showed negative to the test. Taking all cows into consideration, 91.1% efficiency in predicting pregnancy was obtained.

Other oxidation-reduction indicators were used but were not as efficient as the sodium benzone-indophenol. These studies are being continued.

P45. Effect of hormones on uterine motility and sperm transport in the perfused genital tract of the cow. R. L. HAYS AND N. L. VANDEMARK, Univ. of Illinois, Urbana.

In further studies on sperm transport in the

reproductive tract of the cow, motility of perfused uteri was recorded by the balloon technique. Oxytocin, added to the perfusate, caused a tetanic contraction of the uterus lasting approximately 1 min. and followed by a series of contractions with the tone gradually decreasing toward normal. The amplitude and duration of the contractions varied with the amount of oxytocin given. Epinephrine, given 30 sec. prior to oxytocin, almost completely inhibited the above responses. When epinephrine was given alone, it frequently caused 1 sharp contraction followed by an inhibition of activity and a reduction in tone. The inhibition of activity and the reduction in tone also occurred when there was no sharp contraction. When semen was deposited mid-way into the cervix at the same time oxytocin was injected into the perfusate, sperm were transported to the ovarian portion of the oviduct in a matter of minutes. When no oxytocin was given, no evidence of sperm transport was found even after 30 min.

P46. Chromatographic separation of the neutral steroids of cows' urine. J. P. MIXNER AND H. L. SAUNDERS, JR., New Jersey Agr. Expt. Sta., Sussex, in cooperation with Bureau of Dairy Ind., U. S. D. A.¹

A flowing chromatogram has been developed to separate what are presumed to be the neutral steroids of cows' urine into 6 separate fractions. The chromatographic column was prepared with alumina, the neutral extract was added to the column as a benzene solution and elution of the column was carried out with benzene, benzene-ethyl alcohol mixtures, ethyl alcohol, ethyl alcohol-acetic acid mixture and acetic acid. Forty fractions were collected from the column and each fraction was assayed by the Zimmerman, Pincus and Kober reactions, respectively. Since the extraction procedures resulted in production of colored extracts, a determination of extract color density also was made on each fraction. Plotting of these various assay data showed the presence of 6 separate steroid peaks among the fractions and also revealed a high degree of correlation among the measures of steroid potency. Coefficients of correlation calculated among these various measures of steroid potency were as follows: Zimmerman \times Pincus, 0.75; Zimmerman \times Kober, 0.64; Pincus \times Kober, 0.91; Extract density \times Zimmerman, 0.75; Extract density \times Pincus, 0.93; Extract density \times Kober, 0.90. This chromatographic procedure was applied to a comparison of extracts prepared from pooled pregnant and non-pregnant lactating cows' urine. Assay of the fractions was by the Kober method only and a very high correlation ($r=0.92$) was found between the steroid content of the 2 extract fraction series. Figures will be presented to illustrate these various relationships.

¹ Supported in part by Research and Marketing funds (Sec. 10b). This research was conducted as part of Regional Project N.E.1, "Studies on causes and prevention of reproductive failures in cattle."

P47. Comparison of neutral steroids from cows'

urine after various hydrolytic procedures. J. P. MIXNER AND H. L. SAUNDERS, JR. New Jersey Agr. Expt. Sta., Sussex, in cooperation with Bureau of Dairy Ind., U. S. D. A.¹

Three rather different hydrolytic procedures were applied to cows' urine preliminary to extraction of the urine for neutral steroids. In the 1st procedure, HCl and boiling were the hydrolytic agents and resulted in reddish brown neutral ether extract upon extraction. In the second procedure, zinc dust was added in addition to HCl and boiling, resulting in a colorless extract. A colorless extract also resulted when β -glucuronidase enzyme of bacterial origin (optimum pH about 6.5) was used as the hydrolytic agent. Absorption spectra analysis were performed on these 3 types of extracts employing the Zimmerman, Pincus and Kober color reactions. This analysis showed similar absorption spectra for the extracts by the various color reactions. This indicates that similar steroids are obtained by each hydrolysis procedure and also indicates that steroid transformations were not caused by the procedures. The absorption spectra obtained do not correspond to those secured with pure 17-ketosteroids such as androsterone by these color reactions. A chromatographic analysis of extracts prepared by the HCl and the zinc-HCl procedures showed the extracts to be extremely similar in nature when the assays were performed by the Kober reaction.

¹ Supported in part by Research and Marketing funds (Sec. 10b). This research was conducted as part of Regional Project N.E.1, "Studies on causes and prevention of reproductive failures in cattle."

P48. Preliminary studies of irregular breeding in dairy cattle. E. G. MOODY, W. A. SMITH, J. W. CRUMBAKER AND G. B. MCLEROY. Arizona State Coll., Tempe.

The purebred Holstein and Jersey herd (averaging 48 head yearly) used in this study was located in the desert climate of the Salt River Valley, Arizona. Since 1937, 142 of the cows milked have left the herd, the primary reason being irregular breeding (28.2%) with a combination of irregular breeding, health and/or production accounting for 9.1%. Other reasons for disposal were: miscellaneous accidents and diseases, 19.0%; dairy purposes, 18.6%; low production, 7.7%; old age, 6.3%; "hardware", 4.2%; undesignated, 4.2%; and mastitis, 2.8%.

Examination of the last 12 cows slaughtered primarily for irregular breeding revealed 5 cases of abnormal liver, 2 cases of occluded oviducts, in every instance at least 1 apparently functional ovary, and no clear-cut indications of inflammation and/or infection in the uterus, cervix or vagina. One 42-d. old embryo was recovered. Microbiological examinations were made of the blood and reproductive tracts. Blood was further analyzed clinically yielding the following average and certain S. D. values: 4,300,000 erythrocytes and 6,900 leucocytes/mm.³, 12.2 \pm 1.6 g.% hemoglobin, 45.8 \pm 5.0% hematocrit, 7.0 \pm 1.4 mg.% plasma P and 65.4 \pm 10.4% prothrombin times

using the method of Quick with "Standardized Solu-Plastin", 15 sec. control. Tissues were fixed for histological examination.

Irregular breeding, though a serious problem, was found to be attributable to no definite symptom(s), thus emphasizing its over-all complexity. Further investigations are in progress.

P49. Three year's studies using sulfur dioxide as a preservative for forage crops.¹ C. B. KNODT, S. R. SKAGGS AND P. S. WILLIAMS, Pennsylvania Agr. Expt. Sta., State College

During the past 3 yr. studies have been conducted of the value of SO₂ in preserving various forage crops using 50-gal. barrels as well as 4-, 40- and 80-ton silos. Forage crops such as orchard grass, brome grass, alfalfa, orchard grass-Ladino clover and corn have been used. When palatability, cost and preservation of the crop are considered, 5 lb. of SO₂/ton of green material has proved most satisfactory. The use of this preservative appears to decrease fermentation losses as shown by decreased production of lactic acid, volatile acids and ammonia, as well as decreased losses of reducing substances (primarily sugar), carotene and dry matter. Cows have been fed SO₂ preserved forage crops for 3 yr. during the normal silage feeding periods without any apparent harmful effects. Feeding forage crops preserved with SO₂ has resulted in higher carotene levels of the milk produced. On the basis of these experimental studies and the experience of over 1,200 farmers, SO₂ appears to be a valuable preservative for forage crops.

¹ This research was supported in part by the Virginia Smelting Co., West Norfolk, Va., Ansul Chemical, Marinette, Wis., and Tennessee Corp., College Park, Ga.

P50. Grass silage vs. hay as the supplementary roughage for milking cows on good pasture. (Preliminary Report). S. H. MORRISON AND J. F. DEAL, Univ. of Georgia, Athens.

It is well known that grass silage is a satisfactory roughage for cows being barn-fed. However, its merits as a roughage for cows grazing good pasture are not well established. Many farmers state that cows on such grazing should have a supplemental roughage in the form of hay, since they believe that silage will cause extremely loose feces, loss in weight and lowered milk production. This experiment was undertaken to answer some of these questions.

A field of mixed alfalfa and Johnson grass (50% approximately of each) was cut in randomized blocks. Half of the plots were put up as grass silage without added preservatives, the remaining half being put up as barn-dried hay. Sixteen cows (8 Jersey and 8 Guernsey) were divided into 2 equalized groups on basis of milk production, weight, age and previous record. All cows had access to the same pasture (rated as good fescue-orchard grass pasture) and were fed either silage or hay on an equalized dry matter content basis.

The feeding experiment was a single change-over-design with 4 wk. experimental periods and

a 1 wk. transition period. No significant differences were observed in physical condition of cows or feces, 4% F.C.M. production, dry matter consumption or weight changes.

P51. Self-feeding a ground hay and grain ration to dairy cows. K. E. HARSHBARGER, Univ. of Illinois, Urbana.

The performance of dairy cows housed in pens and self-fed a mixed ration containing coarsely ground hay and grain has been studied. In trial I, 6 Holstein cows housed by pairs in 3 pens were fed 3 rations for 3 5-wk. periods, using a Latin square experimental design. The rations were (a) completely self-fed mixture, (b) self-fed mixture plus corn silage and (c) self-fed mixture plus long hay. The proportions of hay and grain remained constant in the self-fed mixtures. In trial II, 5 Holstein and 3 Jersey cows were completely self-fed a mixed ration of coarsely ground hay and grain for 20 wk. The proportion of grain in the ration was adjusted according to the level of milk production for each group of cows. The coarsely ground hay was mixed with the grain to prevent the cows from selecting concentrates in preference to hay.

In trial I the average decline in milk production (F.C.M.) during 15 wk. was approximately 2%/week. The average gain in live weight was 0.75 lb./day. The rations containing long hay and silage were not superior to the basic self-fed ration. In trial II the average decline in milk production (F.C.M.) during 20 wk. was approximately 1.7%/week. The average gain in live weight was 0.82 lb./day. The fat percentage in milk tended to increase slightly throughout each trial, indicating that the feeding of coarsely ground hay did not result in lower fat tests.

P52. Alfalfa hay vs. prairie hay for dairy calves. J. B. WILLIAMS, S. D. MUSGRAVE, C. L. NORTON AND W. D. GALLUP, Oklahoma A & M College, Stillwater.

A 16-wk. feeding trial was initiated to compare the relative merits of alfalfa and prairie hay for dairy calves under 2 different systems of management, *ad lib* hay feeding beginning at birth and deferred hay feeding until after the 8th week. Body weight change, heart girth, height at withers, plasma carotene and plasma vitamin A values were used to evaluate results obtained. Twenty Holstein and Jersey calves were divided into 4 groups. Group I received alfalfa hay from birth, group II—alfalfa hay after the 8th week, group III—prairie hay from birth and group IV—prairie hay after the 8th week. An average of 375 lb. of whole milk was fed to all calves. Calf starter was fed *ad lib* up to 4 lb. daily. All feed offered and refused was weighed daily. Preliminary results show no differences in rate of gain or body measurements taken that are due to treatment. Plasma carotene and vitamin A values show no benefit for alfalfa hay over prairie hay, nor does feeding hay from birth hold an advantage over deferred hay feeding until after the 8th week. In terms of current feed prices, the T.D.N. consumed, when hay was fed from the time of birth,

proved to be slightly less expensive per pound of gain.

P53. Effect of kind of pasture and the feeding of supplements on persistency of milk production in summer. D. M. SEATH, Univ. of Kentucky, Lexington.

Much variation in persistency of milk production was found among groups of dairy cows grazing various kinds of pasture during the relatively dry summer of 1951. Orchard grass and ladino clover apparently furnished the highest quality all-season pasture, for cows grazing it ranked highest in persistency of milk production, with bluegrass and ladino outranking it prior to July 1, and rating 2nd for the whole grazing season. Ky. 31 fescue and ladino ranked next in order and about the same as an older established pasture consisting of orchard grass, bluegrass and white Dutch clover. Cows grazing a straight stand of Ky. 31 fescue showed a decline in milk production which averaged 50-100% greater than for other pastures tested.

During the months of July, Aug., Sept. and Oct., tests were made as to the effect on milk production of feeding alfalfa silage or an extra amount of grain to dairy cows on pasture. The silage was fed at the rate of approximately 1.25 lb./100 lb. of liveweight, and an amount of grain equivalent to this in T.D.N. was fed over and above the amount usually fed cows on pasture. The results of this program varied with the season and with the kind of pasture the cows were grazing.

In general, neither supplement produced significant changes in level of milk production while cows were grazing bluegrass and ladino, orchard grass and ladino, or an old established pasture consisting of orchard grass, bluegrass and ladino. Some improvement in production was noted when silage was fed to cows while grazing Ky. 31 fescue and ladino and when extra grain was fed to cows grazing straight Ky. 31 fescue.

P54. Different grazing intervals on ladino clover-fescue pasture as affecting milk production and flavor of milk. (Preliminary Report) S. H. MORRISON, J. J. SHEURING, R. A. MARDEN AND J. F. DEAL, Univ. of Georgia, Athens.

Four replicated randomized trios of milking cows (3 Holstein and 9 Jersey) had access to rotated ladino clover-fescue pasture, for grazing periods totaling either 4, 8 or 20 hr. daily, immediately after each milking. The 4- and 8-hr. groups had free access to U. S. no. 2 Alfalfa Light Grass Mixed Hay when not on pasture. The 20-hr. group had free access to the hay at all times.

The experiment consisted of 3 5-wk. experimental periods, with 2-wk. preliminary and transitional periods. A double-changeover-design was used so that each member of each trio was pastured at the various intervals during some phase of the work. All animals received the same grain mixture fed according to Morrison's (1948) standard for cows on good pasture.

The cows on the various grazing periods main-

tained their weights equally well and produced almost the same amount of 4% F.C.M. daily. (4 hr. total grazing—22.5 lb.; 8 hr.—23.0 lb.; 20 hr.—23.1 lb.). The average daily hay consumption/cow was 7.8 lb., 7.6 lb. and 5.9 lb. for the 3 groups, respectively. In the same order, the grain consumption was: 8.5, 8.2 and 8.3 lb.

A flavor panel of 6 members scored bi-weekly samples of the milk and concluded that grazing intervals had no effect on flavor of milk, but rather appeared to be an individual cow problem. Further trials are being conducted.

P55. Soil fertilization and plant development as factors relating to nitrogen digestibility. K. A. KENDALL, R. W. TOUCHBERRY AND W. B. NEVENS, Univ. of Illinois, Urbana.

In an earlier investigation it was shown that limestone treatment of soil was the factor responsible for an improved lactation response in rabbits fed lespedeza hay and wheat. Further investigation in 2 digestion trials has revealed that the addition of limestone to a PK-treated soil, as well as harvesting lespedeza forage at an early stage of plant development, resulted in a highly significant increase in the apparent digestibility of the nitrogen in the rations fed.

In trial I the mean coefficients of apparent digestibility of nitrogen in rations as associated with soil treatment were 55.6% (CaPK) and 40.6% (PK) or an increase of 36.9% favoring Ca fertilization. In trial II nitrogen digestion coefficients, as related to soil treatment and stage of development of lespedeza when harvested for hay, were 42.6% (CaPK early), 37.2% (PK early), 36.6% (CaPK late) and 32.5% (PK late) or respective increases of 14.5% and 11.3% in favor of the added limestone.

A small but significant increase in the apparent digestibility of dry matter ingested was observed; however, no significant differences in food intake could be associated with either stage of forage development or with soil fertilization.

P56. Relationship between TDN and energy values of feeds. L. A. MOORE, Bureau of Dairy Ind., U. S. D. A. AND H. M. IRWIN AND J. C. SHAW, Univ. of Maryland, College Park.

There is considerable difference of opinion about methods of evaluating feeds and expressing their value. In the United States, total digestible nutrient content (TDN) has become the common method of expressing the value of feeds. In Europe, the net energy (NE) system or some modification thereof has been in vogue.

In order to study the relationship between these 2 systems, the estimated net energy (ENE) values of Morrison, the revised net energy (NE) values of Forbes, and the calculated productive energy (PE) value of Fraps were plotted arithmetically against their respective TDN values for various feeds. In all 3 comparisons, a definite linear relationship was noted. The following regression equations were obtained by the method of least squares: $ENE = 1.393 \text{ TDN} - 34.63$; $NE = 1.45 \text{ TDN} - 38.82$; $PE = 1.776 \text{ TDN} - 53.69$. The

respective correlation coefficients, 0.977, 0.929 and 0.926, were all highly significant.

From the equations, it is apparent that the difference between the TDN values and the energy value becomes progressively greater as we go from feeds high in TDN value to feeds lower in TDN value. For instance, 1 lb. of TDN from a feed like corn would be equal to 1.0 therm; 1 lb. of TDN from the better hays would be worth about 0.75 therm, while 1 lb. of TDN from poor roughages would be worth only 0.5 therm. In other words, there can be up to 100% difference in the energy value of 1 lb. of TDN, depending on the feed.

P57. The relation of sulfur compounds to lactation in ruminants. I. R. JONES, J. R. HAAG AND P. H. WESWIG, Oregon State College, Corvallis.

Two studies have been conducted to determine the importance of sulfur supplementation with low S rations (0.1% total S) for milking cows. Results are available in the 1st study for 2 groups of 7 paired cows fed continuously for 17 mid-lactation weeks, the S supplement of the experimental group being 1% of Na_2SO_4 in the grain mixture. In the 2nd study, two groups of 5 paired cows were used. One group was fed a low S ration for 10 wk. followed by a 10-wk. period when the grain mix was supplemented with 0.5% methionine. The 2nd group was started on the methionine-supplemented ration.

From the feed and milk production data, it was found that there was no difference in the efficiency of feed utilization when low S rations were supplemented with either an organic or inorganic source of S. Apparently, the ruminant possesses a remarkable S economy for the efficient nitrogen utilization for lactation.

P58. The effect of thyroprotein feeding on the level of the protein-bound and inorganic serum iodine in the bovine. J. F. LONG, L. O. GILMORE, J. W. HIBBS AND F. ELY, Ohio State Univ., Columbus, and Agr. Expt. Sta., Sussex.

Thyroprotein ("Protamone"¹) was fed to 3 Ayrshire cows (10, 15 and 20 g., respectively/cow/day) for a period of 17 d. Serum protein-bound iodine (P.B.I.) and inorganic iodine determinations were made prior to the thyroprotein feeding period, approximately every 2nd day during the experimental feeding period and for 13 d. thereafter.

In a later experiment 15, 20 or 25 g. of thyroprotein/1,000 lb. of body weight/day were fed, respectively to 1 of 3 Jerseys and 1 of 3 Holstein cows for a 49-d. period. Serum P.B.I. and inorganic iodine determinations were made prior to and at approximately weekly intervals during and after the period of thyroprotein feeding.

In every case initiation of thyroprotein-feeding produced a sudden elevation in the protein-bound iodine to about 10-20 times the normal value. Cows fed the larger amounts of thyroprotein had higher P.B.I. values than cows receiving small amounts. Upon termination of the thyroprotein feeding, the P.B.I. values fell sharply to a level

about 3 times the normal value within 2 d. and then gradually declined over the next several weeks to about 2 times normal value. The serum inorganic iodine rose sharply after the beginning of thyroprotein to from 100-400 times the normal level, depending on the amount of thyroprotein fed. The inorganic iodine remained at a high level throughout the thyroprotein feeding period and upon termination of the thyroprotein feeding returned to normal within 7 d. It is suggested that the P.B.I. test may be of practical value in detecting animals which are being fed thyroprotein, or which have recently been fed this substance.

¹ Kindly supplied by Cerophyl Laboratories, Kansas City, Mo.

P59. The utilization of pear-cannery waste as a feed for dairy cows. F. R. MURDOCK, A. S. HODGSON, T. H. BLOSSER AND A. O. SHAW, State College of Washington, Pullman.

Three lots of pear-waste silage have been prepared successfully by combining poor quality roughage and fresh pear waste in the proportions of 1 part roughage to from 3-4 parts of pear waste.

The relative nutritive values of pear waste and grass silages have been determined by feeding trials with dairy cows and by digestion trials with sheep. In the feeding trials with cows, the small differences in average daily production of 4% milk and in changes in body weight indicated the 2 silages were of near equal feeding value for dairy cows. In digestion trials with sheep, the grass silages were found to be appreciably higher in T.D.N. than the pear-waste silages. The T.D.N. values of the 2 lots of pear-waste silage were 52.8 and 53.5% on the dry-matter basis. One lot of grass silage contained 63.9% T.D.N. and another of molasses-grass silage contained 68.1% T.D.N., both on the dry-matter basis.

One lot of dehydrated pear waste and 1 lot of fermented and dehydrated pear waste were found to contain 80.4 and 57.2% T.D.N., respectively, on the dry-matter basis.

P60. Hormones in lactation: Administration of hormones in declining phases of lactation. J. D. DONKER AND W. E. PETERSEN, Univ. of Minn., St. Paul.

Single 30-35 mg. equivalent injections of growth hormone resulted in a 10.4% average increase in milk production in 7 animals when comparing 4-d. post-injection milk production to a like base period pre-injection. In 3 animals, 35 mg. of thyrotropic hormone resulted in no change during the post-injection period at the time the increase was noted from growth hormone, or later. There were no changes in milk composition due to treatment.

Prolactin (50 mg. daily), over the same comparison periods, depressed production by 50 and 35% in 2 cows.

Stillbrestol injected at the rate of 10 mg. each 2nd day produced varying results. The 1st animal's production rose from 3-30 lb. daily during 1-mo. treatment. Increasing the dosage to 10 mg.

daily depressed daily production from 32–20 lb. A second animal increased from 3–9 lb. daily over 40-d. treatment but the increased production was noted to arise entirely from 1 quarter which had previously given the least. In a 3rd cow at a 10-lb./day level, the animal dried up completely within 2 wk.

Additional data from work in progress will be presented.

P61. Induction of lactation in dairy cattle by diethylstilbestrol-progesterone implants. J. METES, E. P. REINEKE AND C. F. CAIRY, Michigan State Coll., East Lansing.

Studies have been in progress during the past 2.5 yr. to determine in which levels of diethylstilbestrol and progesterone are optimal for inducing udder growth and lactation in dairy cattle. Thus far, the best results have been obtained by first implanting 3 g. of progesterone and 100 mg. diethylstilbestrol, followed 90 d. later by a second implant of 1.5 g. diethylstilbestrol. The 1st implant is intended to promote udder growth and the 2nd to initiate lactation by stimulating prolactin secretion. At the end of 4 mo. the residues from both implants are removed and milking is begun.

The best lactations to date by the above procedure were attained by 3 Holstein cows, each of which had calved once previously but could not be settled again. Each gained between 200–300 lb. in body weight during the implantation period, and reached peak daily milk yields of 45, 52 and 80 lb., respectively. All other cattle treated with the hormones also have shown significant gains in body weight. Perhaps the outstanding problem encountered thus far has been the variable growth of fibrous connective tissue around the implanted hormone tablets, preventing maximum hormone absorption and frequently resulting in relatively low milk production.

P62. Experimental development of the mammary gland of the bovine. D. L. HILL,¹ Univ. of Minnesota, St. Paul.

The hormonal control of udder development was studied in a freemartin, 2 virgin heifers, and 5 multiparous cows. Stilbestrol in corn oil was injected subcutaneously as a primary treatment for an arbitrary length of time and was followed with progesterone. The stilbestrol treatment induced udder development and a lactation in the freemartin which showed a leveling-off at 10.8 lb. milk daily. After treatment with 85 mg. progesterone given over 10 d., the average daily milk production increased to 14.4 lb. A multiparous cow responded to stilbestrol with a 295-d. lactation which yielded 5,268 lb. milk, but udder development in this cow could not be induced a 2nd time with stilbestrol until progesterone was included in the treatment. The udder was extremely involuted after injection of 410 mg. stilbestrol over a 36-d. period. A growth response was observed within 24 hr. after injecting 20 mg. progesterone. The rear quarters which were milked continued to grow while a 10-d. delay in milking the forequarters appeared to nullify the

effects of the hormone treatment. Three of the multiparous cows showed no response to the treatments used and udder growth was obtained without any lactation in a virgin heifer.

¹ Present address: Purdue Univ., Lafayette, Ind.

P63. The metabolism of lactose. W. J. RUTTER, E. M. CRAINE AND R. G. HANSEN, Univ. of Illinois, Urbana.

Utilization of lactose by a strain of *Lactobacillus bulgaricus* is of interest because its requirements for growth can be met by lactose but not by galactose or glucose. Cell-free extracts of *L. bulgaricus* have been obtained which actively utilize lactose. Under some conditions, glucose and galactose may be produced in equimolar amounts. However, glucose-6-phosphate is formed by these extracts when galactose-1-phosphate is incubated in the presence of uridine-diphospho-glucose and phosphoglucomutase. Glucose-6-phosphate also may be produced from galactose itself by this system when A.T.P. is added.

The presence of a similar system in lactating mammary gland and liver from nursing animals also is indicated.

P64. The utilization of carboxyl labelled C¹⁴ acetate by the perfused bovine mammary gland. L. S. MIX, W. E. PETERSEN AND H. E. STRUSS, University of Minnesota, St. Paul.

Three perfusions with bovine lactating mammary glands were performed in 1951. The right half of each gland was premilked using pitocin. One millicurie of carboxyl-labelled C¹⁴ acetate in 50 ml. of physiological saline was added to each arterial blood after premilking the gland. Ten ml. of 40% dextrose was added at 0.5-hr. intervals. The duration of the 3 perfusions was 3 hr., 5 hr. 50 min. and 5 hr. 15 min., respectively. All 3 glands were milked 2 hr. after the activity was added and again before the perfusion was discontinued.

The ether extract and the glycogen from the gland tissue were labelled in all 3 experiments, but no activity was present in any fraction of the milk in experiment I. The experiments II and III, cholesterol was labelled as well as the volatile soluble, volatile insoluble and non-volatile acids. In addition, the following milk constituents were found radioactive: casein, non-casein protein, non-protein nitrogen fraction, lactose, urea, citric acid and free CO₂.

Venous blood samples contained radioactive CO₂. Little or no residual activity was present in CO₂ free blood.

An acetate pool in equilibrium with other milk precursors in the mammary gland is postulated.

P65. Early development and function of the bovine stomach. R. B. BECKER, P. T. DIX ARNOLD, S. P. MARSHALL AND JAMES WING, Florida Agr. Expt. Sta., Gainesville.

Jersey and Guernsey calves were sacrificed to study stomach anatomy and early function.

Omasum. The omasum is situated vertically, its greater curvature to the right side and lesser

curvature medially. The reticulo-omasal orifice ("neck") high on the medial face, connects with the rumino-reticular cavity. The omaso-abomasal orifice connects with the abomasum lower on the same face. The inlet is a 3rd or 4th the size of the outlet.

Laminae of 5 orders insert on the inner faces and greater curvature, extending more or less toward the medial wall. Upper insertions of the 8-16 first-order laminae are close into the neck, preventing entrance of coarse feed. The free border is thicker than the body of each first-order lamina, giving a pedunculated appearance and diverting fine feed between the laminae. Second, 3rd- and 4th-order laminae insert at variable distances from the neck. Between, many small ridges (5th order) extend along the inside wall, and sometimes on lateral faces of larger laminae. Many laminae change in width sufficient to change their order toward the lower orifice, and some insert terminally on another lamina. Large laminae increased in numbers, while 3rd-, 4th-, and 5th-order laminae decreased, toward the outlet. There were 94 to 192 laminae of the 5 orders/omasum.

Papillae on the free margin and sides of laminae were large and prominent near the neck, with smaller papillae in increased numbers toward the outlet. One or more papillae with horny tips were on the proximal edge of several 1st order laminae, or margin of the esophageal groove. Their tips pointed into the omasum. They ranged from 6-25, and averaged 12.1 horny papillae/animal.

Feed in the stomach. On nursing, milk normally goes to the abomasum. One calf had 3 blades of grass when not 1 d. old. The rumens of 5 calves 3- to 5-d.-old contained some hay and excelsior bedding. Calves (19) observed at 3- to 24-d. of age had feed in the rumen. Feed was between the laminae in a 3-d.-old calf; in 5 others between 7- and 10-d.-old, and 2 animals 15- and 16-d.-old. Omasums of 12 others 1-18-d.-old contained no feed. Calves observed at 20 d. or older had feed in the omasum. A 24-d.-old calf regurgitated and chewed its cud and it was suspected but not verified with some younger one.

Feed samples from between selected laminae were partially air-dried, separated through sieves and weighed. With 17 groups of 1-5 calves (46 animals), 68.6% of the air-dry feed from the upper half of the omasum passed through a 1-mm. sieve, as compared with 71.4% from the lower half, indicating that some reduction had taken place therein. Corn fragments showed little change in the rumen, reticulum and omasum. In all but 1 young calf with an abnormal rumen aroma, omasal contents were less moist than those of other compartments of the stomach.

P66. Effects on ruminating calves of changing to omasal-abomasal feeding through a rumen fistula. H. J. LARSEN AND G. E. STODDARD, Iowa Agr. Expt. Sta., Ames.

Two Milking Shorthorn yearling males provided with permanent rumen fistulas were fed a

complete hog ration directly into the omasal-abomasal cavities. The rumen was left intact to facilitate converting the animals, at will and in principle, from a monogastric to a polygastric state.

Three trials were conducted and were terminated at 10, 6 and 9 d., respectively, when health became critically endangered. During the short omasal-abomasal feeding period, the animals scoured, became weak and unthrifty and lost weight; blood fat levels were depressed; blood glucose levels did not change appreciably; corn particles appeared in the feces in much the same form as in the feed, indicating little or no digestion.

When the experimental feeding was increased from 3-8 times daily, the animal still showed the symptoms described above to about the same degree and in about the same length of time. When water, instead of the experimental ration, was fed to the omasal-abomasal region of 1 animal, no detrimental effects were apparent. Feeding the experimental ration directly to the rumen through a fistula apparently had no adverse effect on either animal.

P67. Rumen synthesis of protein and amino acids in the bovine on natural and purified rations. C. W. DUNCAN, C. F. HUFFMAN AND I. P. AGRAWALA, Michigan Agr. Expt. Sta., East Lansing.

Quantitative studies were made concerning the synthesis of protein and amino acids in the rumen of fistulated calves maintained on a natural ration and on a ration in which urea furnished the only source of dietary nitrogen. From 51-63% of the dry matter disappeared from the rumen within 6 hr. after feeding either ration. The amount of protein in the rumen of the experimental calves 6 hr. after feeding varied from 19-190% more than was present before feeding. The increase in protein was due to the utilization of urea nitrogen by the rumen microorganisms and was considered to be of microbial origin. The increase on the natural ration was 78%, which could be accounted for by the ingested protein.

When the amino acid composition of the rumen protein was expressed as the per cent of the sample, no consistent difference was observed between the values obtained before or 6 hr. after feeding, but when the total amounts in the rumen were determined, a from 1- to 15-fold increase was observed over that ingested in the ration. The amino acid content of the rumen protein was the same both before and 6 hr. after feeding and also on both the purified and natural rations when the values are expressed as grams/100 g. of protein. Minimum molecular weight determinations indicate that regardless of whether the nitrogen intake is from organic or inorganic sources, the proteins that are elaborated in the rumen appear to be characteristic of the naturally occurring microflora.

P68. Some chemical and nutritional properties of the rumen contents of dairy cows. B. M.

PATEL AND N. N. ALLEN, Univ. of Wisconsin, Madison.

Fluid material aspirated from the rumens of 10 dairy cows was found to contain from 1.24–2.48% dry matter, of which approximately one-half was ash. Na and K compounds, largely bicarbonates, accounted for all except a very small portion of the minerals, and presumably were derived largely from the saliva.

Representative portions of the entire rumen contents were taken from 14 freshly slaughtered cows. Of these, 6 were from the University herd where the normal feeding routine had not been disturbed, while 8 were from a packing plant where fasting routinely precedes slaughter. The liquid was pressed from a part of this material. Proximate analyses and Vitamin B₁₂ values were run on the complete raw material and on the liquid and solid portions. A portion of the material was autoclaved before separation and analysis. The rumen contents of the packing-house cows were lower in dry matter and much lower in protein and B₁₂ than of the University cows. When autoclaved before pressing, the dry matter of the liquid portion was increased by about 30% for the packing-house and 50% for the University material. The protein was increased by about 25 and 50%, and the B₁₂ by 200 and 180%, respectively. These increases were due primarily to materials liberated from the solid portion by autoclaving. A substantial part of this probably was derived from the microorganisms adhering to the fibrous parts of the rumen contents.

Feeding tests with the various materials were run with rats and chicks. Detailed data will be distributed.

P69. The effect on milk production, body weight and feed digestibility of feeding hay and grain in various ratios. G. E. STODDARD AND T. G. MARTIN, Iowa Agr. Expt. Sta., Ames.

Four levels of hay were fed at rates ranging from 0.5–2.5% of body weight to 4 groups of cows in 2 separate trials. A concentrate mix was fed to each group at levels to supply TDN at 100% of Morrison's recommended requirements. Hay and concentrate samples were analyzed and appropriate digestibility values were selected from Morrison's tables. The first trial was designed as a 4-way Latin square with 4 paired cows and 4 3-wk. trial periods. Decline in milk production for the 3-wk. periods averaged 0.1, 2.9, 5.8 and 6.1 lb., respectively, for the low to high levels of hay feeding. No consistent effect on body weight could be determined.

The second trial included 4 groups of 4 cows each, maintained on the experimental ration for 16 wk. Drop in average daily milk production from the 1st to the last week was 14.0, 7.9, 13.1 and 18.1 lb. on the low to high levels of hay feeding, respectively. Body weight increased 48, 19, 21 and -4 lb., respectively, during the same period.

Digestibility data for both trials as determined by the chromogen technique will be presented.

FILLED MILKS FOR DAIRY CALVES. III. COMPARATIVE VALUE
OF VARIOUS SOYBEAN OILS AND BUTTER OIL IN A
PRACTICAL DIETARY REGIME¹

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Investigations of the effects of feeding soybean oil-filled milks to young dairy calves have shown that crude soybean oil is markedly inferior to milk fat (6, 8) but that hydrogenated soybean oil is almost equal to milk fat (8, 12). Studies of the dietary value of cottonseed oils (9) also revealed that growth and health of calves fed a purified diet containing hydrogenated oil were better than those of calves fed a similar diet containing nonhydrogenated refined oil.

Since in the previous work with hydrogenated vegetable oils (8, 9, 12), the calves were restricted to purified diets or reconstituted "milks" supplemented with vitamins and minerals, it seemed desirable to determine the effects of feeding various soybean oil-filled milks to young calves raised under conditions generally encountered in dairy herds. The trial reported herein was designed to ascertain the practicability of using soybean oil-filled milks with hay and grain as a diet for young calves.

PROCEDURE

The experimental subjects were young dairy calves of the Ayrshire, Brown Swiss, Holstein and Jersey breeds. The calves, after receiving colostrum from their respective dams for 3 days following birth, were divided into three comparable dietary groups of ten animals each. The principal difference in the diets was the type of fat used in the reconstituted milk: Group I—milk fat (butter oil), Group II—hydrogenated soybean oil (melting point approximately 102° F.),⁴ and Group III—crude soybean oil.⁵

Throughout the trial, from 4 to 88 days of age, the calves were fed *ad libitum* good quality alfalfa hay and a concentrate mixture (50 parts ground yellow corn, 50 parts crushed oats, 2 parts steamed bone meal and 2 parts iodized salt). In addition, approximately 10,000 U.S.P. units of vitamin A and 2,000 U.S.P. units of vitamin D were administered in capsules to each calf daily. During the first 56 days of the experiment, reconstituted milks containing 3 per cent oil or fat, 10 per cent non-fat dry milk solids and 87 per cent water were fed, whereas during the terminal 28 days, a reconstituted milk containing only 10 per cent non-fat dry milk solids was fed.

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⁴ The hydrogenated oil fed to the first five calves in this group was obtained from Swift and Co., Chicago, Ill.; that fed to the other animals was supplied through the courtesy of the Buckeye Cotton Oil Co., Cincinnati, O.

⁵ Produced by Swift and Co. Soybean Mill, Des Moines, Ia.

Prior to each feeding, the ingredients for the respective filled milks were mixed and subsequently homogenized at 3,000 lb. pressure. These milks, maintained at approximately 37° C., were fed twice daily *via* nipple pails at the rate of 1 lb. per day per 10 lb. body weight of calf. When diarrhea occurred, therapeutic doses of sulfathalidine⁶ were administered and the quantity of milk fed was decreased until improvement was noted. Subsequently, the level of milk feeding was increased gradually to the pre-treatment amount. The quantity of milk fed was adjusted weekly in accordance with changes in body weight.

The effects of including the various types of soybean oils in the diets of the calves were determined by health and general appearance, changes in body weight and in height at withers, efficiency of feed utilization and concentrations of fat in blood plasma and of hemoglobin in blood. Intakes of all feeds were recorded. At the beginning of the experimental period and at weekly intervals thereafter, weight and height at withers of each calf were determined and venous blood samples were collected. For hemoglobin determinations 0.04 ml. of oxalated blood was used (13). The remainder of the blood sample was centrifuged to obtain the plasma for fat determinations (1).

RESULTS

Analysis of the data was complicated by the high mortality rate among the calves receiving crude soybean oil in reconstituted milk (group III). Thus, statistical interpretation (4, 14) was limited to group I (butter oil) and Group II (hydrogenated soybean oil). Averages for the various objective measurements used in comparative estimations of the responses to the crude soybean oil feeding were computed on the basis of the four animals that survived the entire experimental period.

Health and general appearance. In the initial stage of this study several calves fed the crude soybean oil ration died within 3 days after the first feeding. These subjects exhibited mild diarrhea but no other apparent ante-mortem symptoms of health impairment. These sudden deaths suggested that the crude soybean oil-filled milk might have produced a toxic effect. Post-mortem lesions also indicated toxemia. Since the crude soybean oil used during this first phase of the experiment had been stored under refrigeration for approximately 18 mo., a supply of freshly processed oil was obtained to exclude complications possibly due to oil deterioration during storage. Animals fed the aged soybean oil were replaced; thus, all the calves listed in table 1 were fed the fresh oil. Although these animals responded unfavorably to the fresh crude soybean oil, they survived for a greater length of time (all deaths occurring between the second and sixth weeks of the experiment) than those fed the oil that had been stored. Ante-mortem symptoms of the calves that received newly processed crude soybean oil were emaciation, listlessness, rough hair-coat, dry skin and severe diarrhea. Autopsies revealed numerous petechial and ecchymotic hemorrhages in the intestinal mucosa and cloudy swellings and evidence of fatty infiltration in the liver.

Calves surviving the crude soybean oil diet maintained the unthrifty appear-

⁶ Provided through the courtesy of S. F. Scheidy, Sharp and Dohme, Glenolden, Pa.

ance developed during the early stages, whereas calves fed butter oil as the principal source of fat either maintained or developed a smooth glossy coat of hair. Though calves receiving hydrogenated soybean oil were somewhat rough in hair-coat during the first phase of the experiment, during the second stage, when all

TABLE 1
Incidence of scouring among calves on the various dietary regimes

Group	Calf no.	No. of days each week that diarrhea was observed												Total
		1	2	3	4	5	6	7	8	9	10	11	12	
I (Butter oil)	3088	4	1	3										8
	3095					1								1
	3099	2												2
	3100		1											1
	3103	2	2											4
	3109							1			1			2
	3113						1							1
	3114	5	1		2									8
	3116													0
	3128	1	2											3
Av.	1.4	0.7	0.3	0.2	0.1	0.1	0.1			0.1			3.0	
II (Hydrogenated soybean oil)	3081a	1	1	2	2	2	1							9
	3083	1	7	6	1	1						1		17
	3085	1	5	6	1	1								14
	3093	2	2	7	7	2		1				2	2	26
	3094	5	4	3	2	3					1			18
	3101b		1				1			1				3
	3110		1		1	1					1			4
	3111				1	1								2
	3117	2												2
	3129	2		2										4
Av.	1.4	2.1	2.6	1.5	1.1	0.2	0.1		0.1	0.3	0.2	0.2	9.9	
III (Crude soybean oil)	3084	2	5	7	1	2	4							21
	3096	4	6	2	5	1	5							23
	3097	3	5	2	Died (24th d.)									
	3098	3	5	3	3	1			Died (64th d.)					
	3102	3	4	5	4	Died (31st d.)								
	3104	2	4		1	2		2	1					12
	3106		1			1			3	3				8
	3107	2	1		1	2	Off expt. (38th d.) to save							
3112	2	6	Died (15th d.)											
3125	1	1	Off expt. (8th d.) to save											
Av.	2.2	3.8											16.0	

a 3081-3094 received oil stored under refrigeration for approximately 8 mo.
b 3101-3129 received freshly processed oil.

calves received the same diet, the appearance was similar to that of calves previously fed butter oil.

The order of increasing incidence of scouring among the calves of the various dietary groups was butter oil, hydrogenated soybean oil and crude soybean oil (table 1). The incidence of diarrhea among the first five calves in Group II, which received stored hydrogenated soybean oil, is strikingly high in comparison with that among the last five animals which received the freshly processed oil.

Growth. The mean weight changes (as percentages of initial weights) for each group of calves are shown in fig. 1. Calves fed butter oil (group I) gained most rapidly throughout the experiment, followed in order by calves fed hydrogenated soybean oil (group II) and those fed crude soybean oil (group III). The differences between the two former groups (I and II) were significant statistically at the 1 per cent probability level.

The growth of the calves fed the freshly processed, hydrogenated soybean oil was similar to that of the calves fed butter oil, whereas the animals fed the hydrogenated soybean oil that had been stored gained weight at a slower rate. A comparison of the weights and of general appearance of four heifers in Group I (butter oil) with those of a similar number in Group II (hydrogenated soybean oil) revealed no differences at approximately 185 days of age, thus indicating no difference in residual effects from the two fats given in the early phase of the trial.

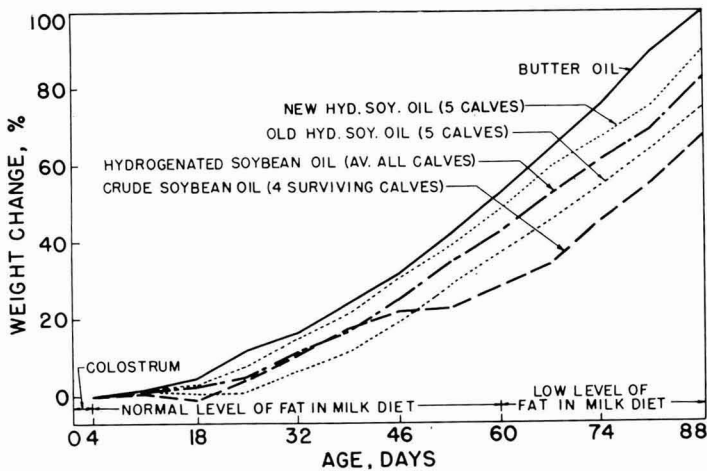


FIG. 1. Mean weight changes in calves fed various oils in reconstituted milks.

No significant differences in changes in height at withers were apparent among the various groups.

Apparent efficiency of feed utilization. The calculated T.D.N. intakes per pound of gain in body weight for the various groups during the initial 8 wk. (normal fat level in milk) and the terminal 4 wk. (low fat level in milk), respectively, were: butter oil, 2.1 lb. and 1.6 lb.; hydrogenated soybean oil, 2.5 lb. and 1.9 lb.; and crude soybean oil, 3.2 and 1.6 lb.

Blood constituents. a. Hemoglobin. The mean concentrations of hemoglobin in the blood from calves in the various groups (fig. 2) were approximately the same initially. Subsequently, the level in all groups gradually declined until an equilibrium was established during the fourth week. Though anemia did not appear to be a complicating factor, there were individuals in each group that showed sub-normal hemoglobin levels at various times during the course of the experiment.

b. Plasma fat. The mean plasma fat level for each group (fig. 3) decreased during the first week of the experiment, this decrease being less pronounced in calves fed a reconstituted milk containing crude soybean oil than in the other dietary groups. After the general initial decline in the plasma fat levels, there was an upward trend. Plasma fat values during the first 8 wk., when fat was incorporated in the milks fed, were highest in the group fed crude soybean oil, followed in order by the groups fed, respectively, butter oil and hydrogenated soybean oil. Comparison of the levels in the two latter groups revealed differences significant statistically at the 1 per cent probability level. After the fat was

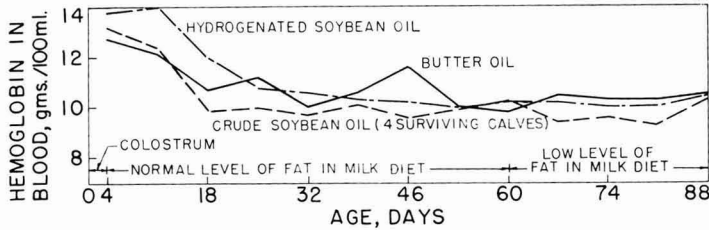


FIG. 2. Mean concentrations of hemoglobin in the blood of calves fed various oils in reconstituted milks.

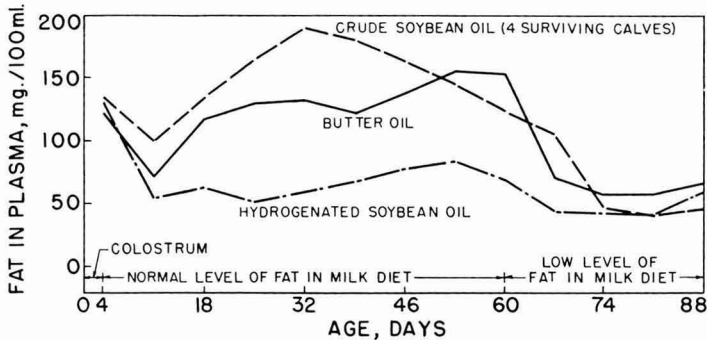


FIG. 3. Mean concentrations of fat in the blood plasma of calves fed various oils in reconstituted milks.

omitted from the milk, the plasma fat levels of all groups declined rapidly to comparatively low values, which were not significantly different.

DISCUSSION

Retarded growth, frequent occurrences of diarrhea and morbidity, and high mortality among calves fed crude soybean oil are in accord with previous findings (6, 8). The physiological effects observed in the present investigation were more severe, however, than those reported by Murley *et al.* (12). Since various batches of crude soybean oil differ in their physical and chemical composition, depending on the locality in which the beans are grown (7), the method of expressing the oil from the bean (11) and the method and length of storage (7), some of the differences in physiological responses of the experimental subjects fed the crude

oil may be attributed to one or more of the aforementioned factors. The severe reactions of calves to the "aged" crude soybean oil fed during the initial phase of the current study as compared to the less acute responses to the freshly processed product suggest that during storage crude soybean oil may undergo chemical and/or physical changes that augment its toxic effects on the young calf. Results (10) indicating that calves react more favorably to cottonseed oil following addition of lecithin and subsequent thorough homogenization suggest the possibility that the degeneration of crude soybean oil may be associated with changes in the phosphatide fraction.

Hydrogenated soybean oil which had been stored also was less satisfactory as a dietary component for young calves than hydrogenated oil fed soon after processing. It is possible that some of the adverse physiological reactions from this product may be due to changes similar to those occurring in the crude oil during storage. Inasmuch as most of the phospholipids are removed during refining prior to hydrogenation, it seems doubtful that changes in this fraction could be responsible for the differences observed. Since the sources of the two hydrogenated oils were not the same, other factors possibly may have caused the difference in physiological reactions. The batch of oil from which the stored hydrogenated oil was obtained, however, produced satisfactory results when fed shortly after processing (12).

Results of the present investigation indicate that the differences in responses of calves to crude and hydrogenated soybean oils noted previously on restricted diets (8, 12) also are manifest when hay and grain constitute a part of the diet. After removal of fat from the milk diets, however, the growth of calves in the crude soybean oil group improved. The extent to which hydrogenation *per se* contributed to the differences in effects is unknown, but Graham and Cupps (5) noted that herring oil containing a high percentage of unsaturated fatty acids was highly toxic when fed to goats, whereas a similar oil that had been hydrogenated did not produce the adverse reactions.

The hemoglobin values observed were in accord with those reported for calves receiving a normal diet (15) and for calves fed restricted diets (12), rather sharp decreases being noted during the first 2 to 3 wk. following birth.

The high blood plasma fat levels accompanying ingestion of crude soybean oil and the correspondingly low plasma fat levels in calves fed hydrogenated soybean oil are similar to observations made by Murley *et al.* (12) on calves restricted to similar filled milks supplemented only with vitamins and minerals. Since there were no extended residual dietary effects on plasma fat values in the second phase of the trial, the differences in concentration noted during the first phase apparently were due partially to absorption. Although blood samples were collected 9 hr. after the a.m. feeding, the differences in concentrations of fat in plasma at this time were characteristic of those at other periods during the day (2). The specific effects of the various dietary fats on plasma fat levels remain obscure, but differences in the metabolism of the fats also may be involved (3).

In accordance with previous observations (6) crude soybean oil was found

to be an unsatisfactory substitute for milk fat in the diet of the young calf, even when hay and grain were fed *ad libitum*. Freshly processed, hydrogenated soybean oil, when fed under similar conditions, was satisfactory. This dietary fat apparently should be used with caution, since there are indications of deterioration during storage.

SUMMARY

Three groups of dairy calves maintained under a practical herd regime were used to compare the nutritive values of crude and of hydrogenated soybean oils with butter oil. Alfalfa hay and a concentrate mixture were accessible to the calves from 4 to 88 days of age. Milk, reconstituted from non-fat milk solids plus the various oils at a 3 per cent concentration, was fed the first 56 days of the experiment, but the fats were omitted from the milk rations during the subsequent 28 days, thus making all the diets qualitatively identical the latter period.

The incidence of scouring was highest among the calves fed crude soybean oil, followed in order by those receiving the hydrogenated soybean and the butter oils. Poor growth, rough haircoats, excessive morbidity and a high rate of mortality also were observed among calves fed crude soybean oil.

No significant differences in the hemoglobin levels among the various groups were apparent.

When milks containing oils were fed, there were significant group differences in the blood plasma fat levels; the effects of the dietary oils in order from highest to lowest were crude soybean oil, butter oil and hydrogenated soybean oil. After the oils were omitted from the milk diet, differences in plasma fat values among the various groups became insignificant.

Although butter oil produced slightly greater weight gains and somewhat better general appearance than the other oils, freshly processed hydrogenated soybean oil was satisfactory as the principal source of dietary fat for young calves, whereas crude soybean oil was unsuitable.

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THE USE OF DEHYDRATED FORAGES IN DAIRY CATTLE RATIONS.
II. COMPARATIVE VALUES OF FINELY GROUND, CHOPPED
AND PELLETED DEHYDRATED ALFALFA AS GRAIN
REPLACEMENTS FOR LACTATING DAIRY COWS¹

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Most dairymen know that finely ground feeds are unpalatable to dairy cows. Olson (10) demonstrated that finely ground grains also were no more digestible than medium ground grains and, in addition, fine grinding increased the cost of preparing the ration.

In an earlier study in this series, Hope *et al.* (5) found that milk production declined when 15, 30 or 45 per cent of the grain ration was replaced with a finely ground, dehydrated, cereal grass-legume mixture. In this experiment, cows went off-feed occasionally at the higher levels of grass consumption. The grass was fed in a finely ground form principally because it appears on the market in that way and because dairymen have purchased it in this form as a grain replacement for cows.

Some studies have been reported in which the value of roughage in finely ground, chopped and long forms has been compared. Forbes *et al.* (3) found that the crude protein, dry matter and crude fiber all were more completely digested when a steer was fed long hay than when fed the same material in a finely divided form as the sole roughage. Kiek *et al.* (7) found that steers fed finely ground hay spent less time in rumination and mastication than steers fed long hay. Hayden *et al.* (4) reported that some cows refused to eat any of a finely ground roughage.

There is some evidence in the scientific literature that pelleting improves the value of fibrous feed for certain classes of livestock. Eaton *et al.* (2) have reported that calves fed artificially dried and pelleted hay gained more weight and ate more hay than calves fed field-cured field-baled hay or artificially dried and ground hay. Jensen and McGinnis (6) have demonstrated an advantage in pelleting laying diets containing high levels of alfalfa. Schneider and Brugman (12) pelleted a ration containing 10 per cent alfalfa for growing fattening pigs and found that this ration produced significantly greater gains than the same ration unpelleted. Murdock *et al.* (9) determined the value of third cutting dehydrated alfalfa in finely ground, chopped and pelleted forms in digestion trials with sheep and found that the total digestible nutrient (TDN) content of the finely ground material was significantly lower than was the TDN content of either the chopped or pelleted alfalfa.

The purpose of the experiment reported herein was to compare the value of

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finely ground, chopped and pelleted alfalfa when fed at a 30 per cent grain-replacement level to lactating dairy cows.

EXPERIMENTAL PROCEDURE

Twenty-one high producing cows (18 Holsteins, 3 Jerseys) were divided into three lots of seven each and were paired as evenly as possible as regards level of production, body weight and stage of lactation and gestation. Information on the experimental cows is presented in table 1. One cow (237) had to be with-

TABLE 1
Age, breed, stage of lactation, stage of gestation and level of production of experimental cows

Group no.	Herd no.	Breed	Age	Days in lactation at start of experiment	Av. 4% FCM daily for preliminary week	Days in gestation at start of experiment
			(<i>yr.</i>)		(<i>lb.</i>)	
	2106	J	8	191	35.4	5
	280	H	4.5	152	45.8	24
	305	H	3.0	104	35.9
1	297	H	3.5	36	47.5
	229	H	8.5	125	57.5	2
	232	H	8.5	327	34.6	102
	259	H	6.5	192	35.4	27
Av.			6.0	161	41.7	23
	2116	J	5.5	163	32.2	46
	271	H	5	139	53.5	10
	306	H	2.5	124	41.3
2	903	H	4	81	42.3
	245	H	7.5	145	45.8
	237	H	8	389	29.5	72
	267	H	5.5	163	41.7	54
Av.			5.4	172	40.9	26
	2118	J	4.5	196	21.3	59
	290	H	4	150	45.9	19
	299	H	3.5	149	52.7	21
3	285	H	4	163	40.3
	270	H	5.5	58	62.8
	274	H	5	595	25.2	30
	278	H	4.5	173	35.0	35
Av.			4.4	212	40.5	23

drawn from the experiment because of a foreign body in her reticulum. The trial was set up as a complete change-over type of experiment during which time each group of cows was on each treatment for 6 wk. following a 1-wk. transition period to allow them to become accustomed to their new ration. The experiment, therefore, lasted a total of 21 wk.

Feeds fed. Cows on this experiment were fed medium quality, chopped alfalfa hay, grass silage (grass-legume mixture), grain and dehydrated alfalfa. For the first two experimental periods, the following grain ration was fed: (Grain ration "a," table 2) 150 lb. ground barley, 200 lb. ground cull peas, 100 lb. ground corn, 300 lb. ground oats, 150 lb. linseed meal, 100 lb. mill run (wheat mixed

feed), 10 lb. bone meal, 10 lb. iodized salt, 2 oz. $MnSO_4$, feeding grade, and 4 oz. type 9F irradiated yeast. At the beginning of the preliminary week of the third experimental period, the grain ration was changed slightly for all cows because of changes in availability and prices of certain of the ration constituents. This ration (Grain ration "b," table 2) was the same as that previously fed except the linseed meal was omitted, 50 lb. of soybean oil meal were used, and the amount of barley was increased to 250 lb.

All the dehydrated forage used was harvested from the same field on the same day. It was cut shortly before it had started to bloom, was chopped in the field and then was dried in an Arnold-type dehydrator with the ingoing gas temperature at 1,600° F. and the outgoing temperature at 250° F. Two-thirds of the material which had been put aside for use in the feeding trial then was finely ground in a hammer mill. One-half of this was pelleted into small pellets 0.25 in.

TABLE 2
Gross chemical analyses of feeds used in experiment on as-fed basis

	Dry matter	Crude protein	Ether extract	Crude fiber	Ash	Nitrogen free extract	T.D.N.
	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Corn silage	17.6	1.0	0.3	5.9	1.3	9.1	10.8
Grass silage, preserved with wood molasses ..	23.1	1.6	0.9	10.5	2.7	7.4	12.6
Grass silage	29.4	3.8	0.9	9.6	3.0	12.1	17.5
Alfalfa hay	91.8	13.9	1.6	33.8	7.4	35.1	49.4
Grain mix "a"	90.1	15.9	3.2	8.1	5.4	57.5	72.3
Grain mix "b"	90.6	15.0	3.4	7.3	5.3	59.6	74.2
Dehydrated alfalfa, finely ground	92.3	18.5	2.8	24.1	9.4	37.5	54.9
Dehydrated alfalfa, chopped	91.8	18.2	2.6	25.5	9.2	36.3	54.6
Dehydrated alfalfa, pelleted	92.9	19.0	3.3	24.3	9.7	36.6	54.8

in diameter and 0.25 in. long. The chopped material used was fed in the same physical state as it came from the field chopper, *i.e.*, 0.25- to 2-in. lengths.

Methods of feeding. The alfalfa hay was fed *ad libitum*. Silage was fed during a given week at the rate of 3 lb. per 100 lb. of body weight, based on body weights taken at the end of the preceding week. Grain was fed for a given week at the rate of 1 lb. per 3 lb. of 4 per cent fat-corrected milk (FCM) produced during the preceding week. After the necessary grain was calculated, 30 per cent was replaced with one of the three physical forms of first cutting, dehydrated alfalfa. The dehydrated alfalfa was fed along with the grain.

Data collected. The cows were weighed on 2 consecutive days at the end of each experimental period. Milk samples were composited and tested for percentage butterfat at the end of each experimental week. Feed weighbacks were made routinely and were chemically analyzed. Results of chemical analyses of the feeds fed are presented in table 2. The TDN content of the feeds was calculated by using appropriate digestion coefficients as listed in Morrison (7) for the

TABLE 3
Average chemical analyses and standard errors of weighbacks by rations, as-fed basis

Material	Treatment	No. of analyses	Dry matter (%)	Crude protein (%)	Ether extract (%)	Crude fiber (%)	Ash (%)	NFE (%)	TDN (%)
Hay weighbacks	Finely ground, alfalfa	20	93.0 ± 0.27	9.8 ± 0.42	1.1 ± 0.07	40.5 ± 0.98	6.6 ± 0.14	34.1 ± 0.52	47.4 ± 0.22
Hay weighbacks	Chopped, alfalfa	20	92.9 ± 0.28	10.0 ± 0.41	1.1 ± 0.19	41.1 ± 0.80	6.7 ± 0.16	33.9 ± 0.19	47.2 ± 0.21
Hay weighbacks	Pelleted, alfalfa	20	93.1 ± 0.19	9.5 ± 0.39	1.1 ± 0.05	41.8 ± 0.86	6.6 ± 0.14	34.2 ± 0.42	47.5 ± 0.17

hay, grain and silage. For the dehydrated alfalfa, the TDN content was determined by means of digestion trials with sheep. Procedures used in conducting these trials were those described by Murdock *et al.* (8).

Chemical analyses and standard error of the weighbacks are given in table 3. There were no grain or dehydrated alfalfa weighbacks. The composition of the small amount of silage weighbacks was much like that of the silage fed, except for dry matter. Appropriate corrections were made for dry matter in calculation of the TDN consumed by the cow. The statistical analyses of the data were made essentially as outlined in Snedecor (13).

RESULTS AND DISCUSSION

The performance of the cows in this experiment is summarized in table 4.

TABLE 4
Milk produced, body weight changes and per cent butterfat

		Treatment		
		Finely ground	Chopped	Pelleted
Av. lb. 4% FCM produced/cow/day	Group 1	38.4	27.3	36.3
	Group 2	36.4	38.4	28.5
	Group 3	26.8	36.2	39.4
Av. of all groups		33.7	34.2	35.4
Av. lb. gain in body weight/cow/week	Group 1	2.8	6.8	8.5
	Group 2	5.4	1.8	4.5
	Group 3	6.2	6.0	2.2
Av. of all groups		4.8	4.8	5.0
Av. % butterfat	Group 1	3.73	3.87	3.63
	Group 2	3.65	3.66	3.77
	Group 3	3.93	3.83	3.87
Av. of all groups		3.77	3.79	3.76

The cows fed pelleted alfalfa produced significantly more ($P = < 0.05$) 4 per cent FCM than cows fed finely ground alfalfa. The difference in favor of the pelleted material was 1.7 lb. of 4 per cent FCM per cow per day. There was no significant difference between the pelleted and chopped alfalfa or between the chopped and finely ground material in 4 per cent FCM produced.

As can be seen from figure 1, the decline in lactation was more rapid on finely ground and chopped than on pelleted alfalfa.

Table 4 also shows the gains in body weight by treatments. Obviously body weight gains on the three materials were not greatly different. A statistical analysis showed no significant difference in the rate of gain on the three physical forms of the forage. There were no marked differences between the cows on finely ground forage and those on chopped forage in number of times off feed. However, there were fewer cases of off feed on pelleted alfalfa than on either of the other physical forms.

In some of the experiments reported on the value of pelleting, *e.g.*, Eaton *et al.* (2), the difference in favor of the pelleted material was due largely to the fact that animals consumed more of the pelleted material. This, of course, increased TDN intake. In the experiment reported herein, it seemed unlikely that there would be any marked differences between treatments in TDN intake over minimum requirements, for the amount of grain (and dehydrated alfalfa) fed was based on the previous week's production and the amount of silage fed was

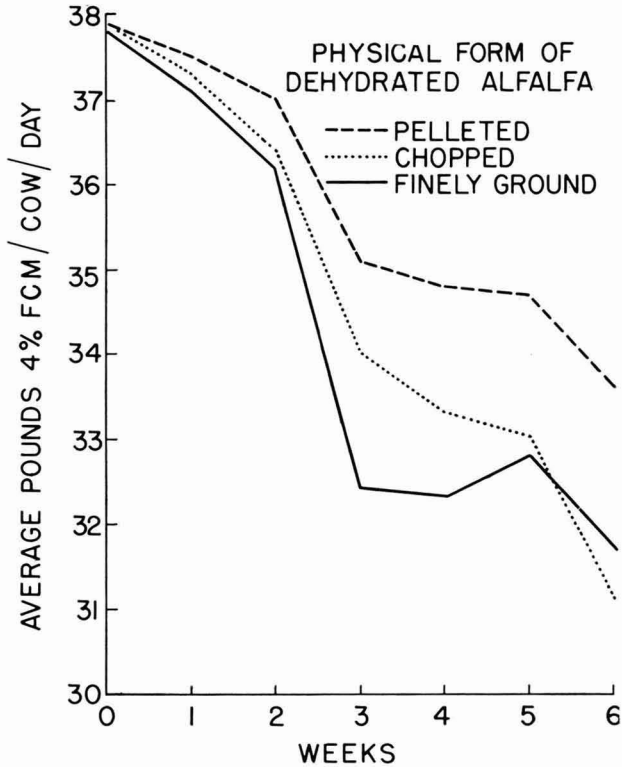


FIG. 1. Average lactation decline by rations for 6-wk. experimental periods when 30% of the grain was replaced with dehydrated alfalfa.

based on body weight. Only if a difference in the *ad libitum* hay consumption existed, could a difference in TDN intake over requirements be expected. There were no differences between treatments in average percentage butterfat. While Powell (11) has reported a lowered fat test when cows were fed finely ground alfalfa as their only roughage, in the experiment reported herein, a normal amount of chopped roughage was fed and the dehydrated alfalfa merely served to replace part of the grain.

Table 5 shows the TDN consumption and utilization. This table shows that the TDN consumed over requirements was nearly the same for all three treat-

ments. The amount of TDN consumed over requirements also was subjected to an analysis of variance, and there was no significant difference between treatments. Furthermore, there was little difference between the three treatments in efficiency of TDN utilization for milk production after maintenance requirements were subtracted.

The advantage shown for pelleted alfalfa in this experiment is difficult to explain. Certainly the explanation does not seem to lie in TDN consumption, for amounts consumed and TDN levels were nearly the same for all treatments. However, one source of error might have been that the TDN of the dehydrated forages was determined with sheep, while in the experiment reported herein the

TABLE 5

Feed consumed, TDN intake and requirements, TDN consumed over minimum requirements and TDN used/100 lb. of 4% FCM produced

		Treatment					
		Finely ground alfalfa ration		Chopped alfalfa ration		Pelleted alfalfa ration	
		Feed consumed	TDN	Feed consumed	TDN	Feed consumed	TDN
		(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
Av. feed and TDN consumed/cow/day	Hay	14.1	6.9	14.3	7.0	14.4	7.0
	Silage	37.0	6.0	37.4	6.2	37.3	6.1
	Grain	8.0	5.9	8.2	6.0	8.4	6.2
	Alfalfa	3.4	1.9	3.5	1.9	3.6	2.0
Total		20.7		21.1		21.3	
TDN required/cow/day ^a	Maintenance	8.5		8.6		8.5	
	Milk production	10.5		10.6		11.0	
Total		19.0		19.2		19.5	
TDN consumed over minimum requirements/cow/day		1.7		1.9		1.8	
Av. TDN required/100 lb. 4% FCM (after subtracting maintenance requirements)		36.1		36.5		36.0	

^a Morrison's (7) minimum requirements.

forage was fed to dairy cattle.

It is interesting that, contrary to the findings of Murdock *et al.* (9), the TDN content of the three physical states of alfalfa used in the trial reported herein was nearly the same on a dry basis. The only difference between the alfalfa used in this trial and that cited above was the size of pellet and the cutting of alfalfa. The forage used by Murdock *et al.* was third cutting (compared with first cutting used in this trial) and the pellets were larger than those used in the trial reported herein.

In searching for a possible reason for the superiority of the pelleted material, the only actual difference in forage treatment prior to feeding which one can find

between the pelleted and other physical forms of forage fed was the actual act of pelleting. During this process, some steam is added and the material being pelleted becomes quite hot, but the heat treatment to which the forage is subjected in pelleting is not nearly so rigorous as that which it undergoes in the process of dehydration. In the pelleting process, the material being pelleted is also subjected to considerable pressure, but it is difficult to see why this should alter nutritive value.

Although experiments have been conducted by Balch (1) showing that different feeds pass through the digestive tract at different rates and that subsequent changes in digestion may result, it is difficult to see why, on this basis, pellets should prove superior to the finely ground forage. For it can be shown by simply dropping several alfalfa pellets into a beaker of water that they disintegrate almost completely in 2 min., even without the constant churning which occurs in the rumen. In view of this, it is surprising that more cases of off feed occurred when the finely ground alfalfa was fed than occurred on pelleted alfalfa. If, on the other hand, some of the finely ground material passed directly to the abomasum, thereby escaping action of rumen microorganisms, then the advantage in favor of the other physical forms would be understandable. The fact that more cases of off feed did occur on finely ground and chopped than on pelleted alfalfa would seem to be the most logical explanation for the differences in favor of the latter material. While cases of off feed were not numerous, cows going off feed during a given week were penalized by virtue of the experimental procedure which based the amount of grain to be fed during a given week on the preceding week's production. This procedure would tend to under-feed cows which had been off feed and therefore would serve to overemphasize real nutritive differences between the forages. On the other hand, if finely ground forage does cause digestive disturbances when used as a grain replacement, as seems apparent from the studies of Hope *et al.* (5) and those reported herein, it is not a good feed for dairy cows, even if its nutrient content is essentially equal to that of the same material in pelleted form.

SUMMARY

Twenty cows were used in a complete change-over type experiment to compare the value of dehydrated alfalfa prepared in finely ground, chopped and pelleted forms when fed at a 30 per cent grain replacement level. Cows produced significantly more 4 per cent FCM on pelleted than on finely ground alfalfa, but chopped alfalfa did not differ significantly from either of the other two forms. Average production per cow per day on the three materials was: pelleted, 35.4 lb.; chopped, 34.2 lb.; and finely ground, 33.7 lb. That the advantage for the pelleted material was not related to the amount of TDN consumed is evident from the fact that there was no significant difference between treatments in TDN consumed over minimum requirements. Neither were there any differences in body weight gains nor in percentage fat. It is concluded that pelleted dehydrated alfalfa is superior to finely ground alfalfa as a grain replacement for dairy cows.

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THE KEEPING QUALITY OF WHOLE MILK POWDER. IV. THE
ANTIOXIDANT PROPERTIES OF NDGA AND CERTAIN
EMULSIFYING AGENTS^{1, 2, 3}

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Lundberg *et al.* (14) first reported the antioxidant properties of nordihydroguaiaretic acid (NDGA) in fats in 1944. It since has been used successfully alone and with added synergists as an antioxidant with a number of fats and oils and also has been used successfully in dairy products as an antioxidant for milk fat.

Coulter (3) tried NDGA in whole milk powders and found it to be effective but to cause a fruity flavor. Stull *et al.* (15) found it would retard development of oxidized flavor in spray-dried whole milk powder and ice cream mix, while methionine, ascorbic acid and citric acid acted synergistically with NDGA in retarding oxidized flavor development.

Stull *et al.* also tried NDGA in milk, unsweetened frozen cream and sweetened frozen cream (15), and found that it would retard development of oxidized flavor successfully in each of these products. Krukovsky *et al.* (10, 11) found NDGA effective as an antioxidant in cream and as a stabilizer of fat-soluble vitamins during storage of cream. The concentration of NDGA used by the various authors varied from 0.00125 to 0.1 per cent on a fat basis, depending on the product to which it was added.

Certain specific polyhydric alcohol esters of stearic acid were used by Hibbs and Ashworth (7) in a solubility study of whole milk powder. These emulsifiers had marked value in preventing development of oxidized flavor in the resultant whole milk powders. However, the quantities used in the milk were in excess of those needed, and three of the four substances used in the whole milk powder caused churning during reconstitution with distilled water.

The present study was developed to determine the antioxidant properties of NDGA in whole milk powder and to study how these properties are manifested when NDGA is used alone and with the added synergists, ascorbic acid and citric acid. Since the emulsifiers have not been reported previously in the literature as having specific antioxidant properties in dairy products, the present study was inaugurated to test the degree as well as the mechanism of antioxidant activity of these esters in whole milk powder, the esters being employed at the highest levels which would not cause churning during reconstitution.

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³ Data in part from a thesis submitted in partial fulfillment for the degree of Master of Science for the senior author.

EXPERIMENTAL PROCEDURE

Detailed processing and drying methods were given in a previous paper (2). For the first part of this study, mixed milk from the College herd was preheated at 170° F. for 20 min. and concentrated. Each of six portions of this concentrate then was treated differently as follows: (a) Control, no additions; (b) NDGA added at the rate of 0.05 per cent of the butterfat; (c) NDGA and citric acid added, each at the rate of 0.05 per cent of the butterfat; (d) ascorbic acid added at the rate of 0.005 per cent (50 mg. per liter) of the reconstituted milk; (e) NDGA and ascorbic acid added, each at the above rate; and (f) NDGA, ascorbic acid and citric acid added, each at the above rates. After these additions, each lot of concentrated milk was homogenized twice at 2,000 and 500 lb. pressure in a Manton-Gaulin two-stage homogenizer of 25-gal. per hour capacity. The concentrate so treated was cooled and dried the same day in an experimental drier based on the design given by Coulter (4). The powder was air-packed in no. 2 flat tins (about 120 g. to the can) and part of the cans stored at 45° F., while the remaining cans were stored at 85° F. The experiment was repeated five times for each of the six treatments, making a total of 30 powders all prepared within a month. After the first month of storage, the samples were removed at 2-mo. intervals for chemical and flavor analysis.

Flavor analysis was done by a panel of six experienced judges using a scoring system ranging from 0 to 10. A score of 9 to 10 indicated the equivalent of good fresh pasteurized milk; 8 to 9, no defect except a very slight heated flavor; 7 to 8, a slight defect not readily definable; 6 to 7, a slightly oxidized or stale milk; and 0 to 6, a milk progressively more oxidized or stale, foreign, rancid, etc. Scoring was done in half points above 5 and in full points below 5. A statistical analysis was made of the significance of the difference between means according to a method used by Hening (6).

Chemical tests were made to determine the way in which the antioxidant activity was exhibited in whole milk powder. Reduced ascorbic acid was determined by the method of Hochberg *et al.* (8), peroxide development by the method of Loftus-Hills and Thiel (13), the acid ferricyanide-reducing groups by the method of Chapman and McFarlane (1) as modified by Crowe *et al.* (5) and the oxidation-reduction potential (Eh) with a platinum electrode using a Model G Beckman potentiometer. The moisture content of the powder was determined by the vacuum oven method.

For the second part of the study the following four emulsifiers were used: Sorbitan monostearate (Span 62), 0.025 per cent; polyoxyethylene sorbitan monostearate (Tween 60), 0.020 per cent; polyoxyethylene stearate (G2152), 0.010 per cent; and glycerol monostearate (Myverol 18-00), 0.040 per cent, all percentages being based on the weight of the reconstituted milk. As determined by preliminary trials, these levels were the highest usable in the whole milk powder if churning on reconstitution was to be avoided.

During a 1-mo. period, five lots of mixed-herd milk were preheated at 170° F. for 30 min. and concentrated to approximately 40 per cent total solids. Each lot was divided into five portions and one of the emulsifiers added to each of four

portions. The fifth portion was used as a control. These substances were added at a temperature of 145° F. The mixtures then were agitated to aid solution, homogenized and cooled. Each concentrate was dried the same day in an experimental drier. Within 1 hr. after drying, each powder was air-packed in no. 2 flat tins and some of the cans were double nitrogen-packed. A total of 25 powders was made in this manner.

The cans of milk powder all were stored at 85° F. The air-packed samples were examined after 0, 1, 3, 5, 7 and 9 mo., and the nitrogen-packed samples were examined after 3 and 7 mo. Chemical and tasting studies were made and averages of the resulting values taken of the five replicates for each of the five treatments. This was done to average out possible variations due to slight differences in the milk and drying procedures (*e.g.*, moisture contents of the powders) which might have occurred in the five trials carried out in a month. Chemical tests were similar to those employed in the first part of this study.

The stearic acid esters or polyoxyethylene derivatives also were added to pasteurized and pasteurized homogenized fluid milk, the substances being added to the milk at pasteurizing temperatures. Copper was added at the rate of 0.3 ppm. in the form of CuSO_4 . The resulting milk samples were cooled in ice water and held in the refrigerator for tasting studies. The same flavor panel scoring system was used as for powdered whole milk. Five trials were conducted over a period of 3 mo.

RESULTS

Part one. Average results of the chemical analyses of five replicate powder samples for each differently treated lot of milk appear in table 1.

The addition to whole milk powders of NDGA, alone and with citric acid as a synergist, resulted in a definite lowering of peroxide values and an increase in ferricyanide-reducing values. There appeared to be only a slight lowering of the oxidation-reduction potentials and little or no influence on ascorbic acid values. The use of NDGA prevented the peroxide values from increasing, but the effect of storage temperature on peroxide development was not marked. There seemed to be an inverse relation between the loss of flavor (table 2) and the peroxide value (table 1) for the powders stored at 85° F. Such a relation did not extend to the powders stored at 45° F. Where ascorbic acid was added alone or as a synergist with NDGA, no peroxide values were obtained due to its reducing action on the ferric ion used in the test. However, as previously found by several investigators, an increase in the ferricyanide-reducing value was noted. This was particularly true where NDGA and ascorbic acid or NDGA, citric and ascorbic acid were used.

Average flavor scores for the five replicate powder samples of each treatment appear in table 2. Statistical treatment showed the significance of the difference between the mean of the control and that of each of the five treatments after storage. After 5 mo. at 85° F. storage all samples containing NDGA, alone or with synergists, were significantly better than the control samples. It was only after 9 and 11 mo. of storage that the addition of a combination of citric acid and ascorbic acid to NDGA exhibited synergistic activity. This is shown by the

TABLE 1

Average analysis values^a of whole milk powders with and without NDGA and added synergists stored air-packed at 45° F. and 85° F.

Treatment	Moisture content	Age	Ascorbic acid (mg./l.)		Eh (volts)		Ferrieyanides (Mx10-6/g. Pd.)		Peroxide values (MeO ₂ /kg. Pd.)	
			45° F.	85° F.	45° F.	85° F.	45° F.	85° F.	45° F.	85° F.
			(mo.)							
Control	2.00	0	10.5	8.0	0.354	0.354	7.9	7.9	0.025	0.025
		1	4.9	0.357	9.2	0.043
		3	6.4	4.0	0.350	0.366	9.7	8.9	0.130	0.111
		5	4.8	2.9	0.362	0.367	9.1	10.4	0.181	0.177
		7	4.8	2.8	0.341	0.345	9.8	11.6	0.255	0.257
		9	2.6	0.369	11.2	0.263
N.D.G.A.	2.00	11	6.1	3.1	0.340	0.350	9.4	11.3	0.312	0.403
		0	10.4	7.9	0.350	0.351	13.7	13.7	0.018	0.018
		1	5.4	0.355	14.1	0.022
		3	7.4	4.8	0.346	0.373	15.1	13.4	0.084	0.067
		5	6.9	3.1	0.357	0.362	14.1	15.1	0.116	0.100
		7	5.8	3.4	0.333	0.344	15.7	16.6	0.169	0.145
N.D.G.A. + Citric	2.33	9	3.1	0.364	16.1	0.146
		11	6.5	3.4	0.339	0.355	14.9	16.2	0.179	0.193
		0	6.9	6.9	0.352	0.352	13.2	13.2	0.018	0.018
		1	4.0	0.358	14.3	0.029
		3	6.0	3.5	0.350	0.365	14.9	14.1	0.079	0.068
		5	4.4	2.0	0.354	0.356	14.3	15.2	0.127	0.096
Ascorbic	2.21	7	4.6	2.3	0.335	0.347	15.1	16.7	0.174	0.120
		9	1.9	0.368	16.2	0.109
		11	5.6	2.1	0.343	0.359	15.3	16.8	0.178	0.169
		0	70.5	70.5	0.314	0.314	12.4	12.4	b
		1	40.8	0.318	13.9
		3	44.5	35.0	0.305	0.316	15.7	14.3
N.D.G.A. + Ascorbic	2.03	5	38.5	27.8	0.315	0.315	15.0	14.5
		7	35.9	24.0	0.294	0.299	15.8	15.7
		9	24.1	0.317	14.6
		11	38.1	22.3	0.287	0.295	14.5	15.0
		0	70.0	70.0	0.311	0.311	17.1	17.0
		1	42.5	0.317	19.0
N.D.G.A. + Ascorbic + Citric	2.50	3	45.4	36.3	0.302	0.313	20.8	19.7
		5	39.6	27.9	0.310	0.314	19.6	18.7
		7	36.0	25.5	0.290	0.297	20.6	20.0
		9	25.5	0.317	20.1
		11	39.3	23.0	0.284	0.294	20.5	20.7
		0	70.0	70.0	0.311	0.310	17.4	17.5
1	41.3	0.315	19.6		
3	45.8	35.3	0.302	0.316	21.0	19.1		
5	38.5	27.0	0.314	0.318	20.1	20.0		
7	36.9	23.1	0.290	0.304	21.7	21.4		
9	21.5	0.319	20.5		
11	38.5	19.1	0.286	0.299	21.0	21.0		

^a Each value in the table represents the average of 5 replicate samples.

^b The presence of added ascorbic acid interferes with the peroxide test of Loftus-Hills and Thiel.

greater spread between means of the flavor scores in table 2. Samples with ascorbic acid alone did not differ significantly from the control samples at any time during the 11-mo. storage. Storage of the samples at 45° F. altered the flavor picture to some extent. Powder samples containing NDGA or NDGA and added

synergists differed significantly from the control in flavor score only at 7 mo. storage and not at any other time during storage.

Part two. Table 3 gives the average results of all chemical tests and of the flavor scores of the emulsifier-treated powders air-packed and nitrogen-packed over a period of 11 mo. of storage at 85° F. Since each of the four emulsifying agents used showed about the same antioxidant activity, the results were averaged. The controls showed a loss of one point in flavor score (7.0 or lower) between the third and fifth months, while all the powder samples made with stearic acid esters showed a flavor score loss of one point between the fifth and seventh months. This means approximately doubling the storage life. There appears to be little difference in the antioxidant activity of the various stearic acid esters or polyoxyethylene derivatives used. Throughout the entire storage period, the

TABLE 2
Average flavor scores of milk powders during storage in air at 45° F. and 85° F.

Age	Storage temp.	Control	NDGA	NDGA + citric acid	Ascorbic acid	NDGA + ascorbic acid	NDGA + ascorbic + citric acid
(mo.)	(° F.)						
0		8.2	8.2	8.1	8.1	8.2	8.1
3		7.9	8.0	8.0	8.0	8.0	8.0
5	45	7.7	7.7	7.8	7.9	7.8	7.9
7		7.7	7.9	8.0	8.0	8.0	7.9
11		7.4	7.6	7.6	7.5	7.6	7.5
0		8.2	8.2	8.1	8.1	8.2	8.1
1		7.8	7.8	7.9	7.9	7.9	8.0
3		7.2	7.5	7.5	7.6	7.6	7.7
5	85	6.5	7.2	7.1	7.0	7.1	7.2
7		6.1	7.0	6.7	6.5	7.0	6.8
9		5.7	6.3	7.0	6.3	6.6	7.0
11		5.2	6.3	6.1	5.1	6.3	6.4

powder samples containing the stearic acid esters or polyoxyethylene derivatives exhibited high acid ferricyanide-reducing values and lower peroxide values than the control samples, but none of the treatments employed appeared to affect markedly the oxidation-reduction potentials (Eh) or the ascorbic acid retention.

It was thought that if the emulsifiers were effective in preventing oxidized flavor in whole milk powder, they might be effective in fluid whole milk. Table 4 shows the results of adding stearic acid esters or derivatives to pasteurized and pasteurized, homogenized whole milk having 0.3 ppm. added Cu. As a group the esters in fluid pasteurized milk did not prevent off-flavor due to Cu-induced oxidized flavor. However, homogenization of the milk containing 0.3 ppm. Cu was sufficient to prevent the off-flavor. This was true for milk with or without the added stearic acid esters or polyoxyethylene derivatives.

DISCUSSION

The mechanism of the antioxidant activity of the stearic acid esters or derivatives may involve surface phenomena in addition to changes in chemical struc-

TABLE 3
Average analytical values for all emulsifiers compared with controls

Months of storage	0		1		3		5		7		9		
	Control ^a	Treated ^b	Control ^a	Treated ^b	Control ^a	Treated ^b	Control ^a	Treated ^b	Control ^a	Treated ^b	Control ^a	Treated ^b	
Flavor	Air	8.3	8.3	7.6	8.1	7.2	7.6	7.0	7.7	6.2	7.1	5.8	6.9
	N	8.3	8.3	7.5	7.8	7.3	7.6
Peroxide values	Air	0.0	0.0	0.079	0.054	0.189	0.083	0.237	0.169	0.325	0.243
	N	0.0	0.0	0.091	0.045	0.174	0.080
Ascorbic acid	Air	11.0	10.9	9.2	9.7	6.8	6.8	7.5	7.9	6.6	7.0	4.9	5.9
	N	11.0	10.9	8.2	8.1	8.5	9.1
Ferrieyanide-reduction values	Air	7.8	8.9	8.3	9.4	9.2	10.2	9.8	10.9	10.0	11.8	11.8	12.6
	N	7.8	8.9	8.9	10.2	10.0	11.8
Eh	Air	0.351	0.351	0.355	0.345	0.357	0.354	0.357	0.352	0.348	0.336	0.366	0.359
	N	0.346	0.346	0.347	0.346	0.335	0.326

^a Control values are averages of 5 replications.
^b Treated powder values are averages of 5 replications for each of the 4 emulsifiers used making a total of 20 replications.

ture. If action is due to surface activity, it may be that a film of the substance involved is absorbed on the exposed fat surfaces of the powder particle, including those of the "free" fat that results from de-emulsification during drying. Lampitt and Bushill (12) have shown that the free fat varies from 3 to 14 per cent in commercial spray-dried powders, and it was demonstrated microscopically on the surface of particles of reconstituted spray-dried milk powder by King (9). If the substance is surface absorbed, homogenization and drying also might be involved in the formation of the absorption film and its function could be one of simple mechanical prevention of oxygen contact with the butterfat or an adsorbed oxidizable substance such as lecithin.

TABLE 4

Average flavor scores^a of fresh milk, both pasteurized and homogenized with added copper to which different amounts of stearic acid esters had been added and samples held at 40° F.

	Amount Added Cu	Flavor score at 40° F. storage								
			Pasteurized Days				Pasteurized homogenized Days			
			1		4		1		4	
			1	4	1	4	1	4	1	4
	(%)	(ppm.)	<i>Trial 1</i>		<i>Trial 2</i>		<i>Trial 3</i>			
Control	None	None	6.5		5.3		8.04			
Control plus Cu	None	0.3	6.5		5.8		7.90			
Polyoxyethylene sorbitan monostearate	0.02	0.3	7.2	5.8	5.8		7.90			
Sorbitan monostearate	0.02	0.3	6.5	5.8	5.8		7.90			
Glycerol monostearate	0.04	0.3	6.5	5.0	5.0		7.90			
Polyoxyethylene stearate	0.01	0.3	7.6	5.2	5.2		8.04			
			<i>Trial 4</i>		<i>Trial 5</i>		<i>Trial 4</i>		<i>Trial 5</i>	
Control	None	None	8.1	5.8	8.1	5.6	8.3	7.8	7.9	7.8
Control + Cu	None	0.3	5.8	5.6	4.2	5.8	8.2	8.0	8.1	8.1
Polyoxyethylene sorbitan monostearate	0.04	0.3	7.8	6.5	5.1	5.8	8.4	8.2	7.8	8.2
Glycerol monostearate	0.05	0.3	6.3	5.8	6.6	6.5	8.4	8.1	7.7	7.8
Polyoxyethylene stearate	0.04	0.3	7.8	6.8	5.4	5.8	8.3	8.3	8.0	8.3

^a Average of 5 judges.

There are several clues as a result of the present work which may throw some light on the mechanism involved. The different stearic acid esters and polyoxyethylene derivatives all were about equally effective in preventing off-flavor development. The one thing they have in common is their surface activity. This is a point in favor of the theory that they are adsorbed as protective films.

Results of the chemical tests show that the whole milk powders which contained the stearic acid esters and polyoxyethylene derivatives had greater acid ferricyanide-reducing values and lower peroxide values throughout the storage period than did the control samples. Such a picture might be the result of an adsorption film only being involved. However, for this to be true, the anti-oxidant substances would have to have either the ability to reduce potassium ferricyanide themselves or to render the protein more available for the formation of reducing groups, since high acid ferricyanide-reducing values result when these substances are added to whole milk powder.

Results of the first part of this study showed that increased ferricyanide-reducing values and decreased peroxide values also characterized whole milk powder to which NDGA have been added. Thus, it might be concluded that stearic acid esters have definite antioxidant properties because of their chemical structure. Judging from the results of two separate studies, the stearic acid esters and polyoxyethylene derivatives are at least equal to if not superior to NDGA in preventing off-flavor development, even if synergists are added to the NDGA.

The use of these esters resulted in a flavor slightly different from the control in the reconstituted whole milk powder. There is less heated flavor in the reconstituted milk, the taste being slightly richer. This may be compared with the improvement that takes place when pasteurized fluid whole milk is homogenized.

Because of the danger of churning on reconstitution, the amount of these substances which may be used in milk to be dried is limited for three of the four substances tried. The fact that the amount of these substances which may be used is limited is indicative that they are surface active. Compared with the other substances used, glycerol monostearate (Myverol 18-00) could be used in larger amounts and the amounts apparently were not so critical from the standpoint of causing churning. The level of the other three substances, though limited, may vary with different plant processing and drying methods. Sorbitan monostearate (Span 62) is the least soluble and is not recommended for this reason.

The stearic acid esters or polyoxyethylene derivatives tried in this experiment have been those used most successfully in ice cream for emulsifying purposes. Although their efficacy as antioxidants in ice cream was not tested, their effectiveness might be doubted because of their questionable value in fluid whole pasteurized milk.

SUMMARY

NDGA, alone and with added synergists, exhibited antioxidant activity when used in whole milk powder. The synergistic effect of ascorbic acid and citric acid with NDGA did not become noticeable until after about 9 mo. of storage at 85° F.

The antioxidant activity of NDGA, alone and with citric acid as a synergist, appears to be manifested by a decrease in peroxide formation and an increase in ferricyanide-reducing values.

Ascorbic acid added alone to whole milk powder at the rate of 50 mg. per liter of reconstituted milk did not exhibit any antioxidant activity.

Certain polyhydric alcohol esters and polyoxyethylene derivatives of stearic acid, when used in whole milk powder, appear to have antioxidant properties, as judged by results of organoleptic tests. The mechanism involved apparently is that of increasing the acid ferricyanide-reducing groups in the powder and decreasing peroxide formation. A surface adsorption film on the exposed fat surfaces may be indicated. They were not effective in preventing copper-induced oxidized flavor in pasteurized fluid whole milk.

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THE INCIDENCE OF ANTIBIOTICS, SULFA DRUGS AND QUATERNARY AMMONIUM COMPOUNDS IN THE FLUID MILK SUPPLY OF NEW YORK STATE

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The use of antibiotics, such as penicillin, and other drugs, including sulfa drugs, as a treatment for mastitis in cows has become popular in recent years. Sanitization of dairy equipment with quaternary ammonium compounds now is being practiced on a wide scale. As it is possible for these chemicals to find their way into the milk, some question has been raised regarding ultimate side effects resulting from milks containing antibiotics, sulfa drugs or quaternary ammonium compounds. In particular, the manufacturer of fermented dairy products is concerned with the possible deleterious effects of such a milk on his product. A number of investigators (3, 4, 5, 6) have reported that milk containing 0.1 unit penicillin per milliliter will exhibit definite inhibitory properties toward lactic starter organisms. Any condition which adversely affects lactic acid production will either seriously handicap or prevent production of fermented dairy products.

Interested medical and public health groups also are giving serious consideration to the possibility that milks in general may contain enough antibiotics to become a factor in the sensitization of children to these drugs.

Little or no information is available to indicate the presence of these chemicals in blended pasteurized fluid milk and without it the relative importance of the over-all problem and the questions which have been raised cannot be fully evaluated or answered. This study of the antibiotics, sulfa drugs and quaternary ammonium compounds in the commercial fluid milk of New York State was initiated as an aid in this direction.

METHODS

A total of 1,796 samples of fresh blended pasteurized whole milk was obtained either from dairy plants or from route wagons prior to delivery. The area canvassed covered 36 New York State counties and the milk samples were obtained by representatives of the state, city and county health departments. Two test periods, April-May, 1951, and July-August, 1951, were employed. All milk samples were shipped under refrigeration to the University for analysis.

Four methods of analysis as outlined in detail in an earlier paper (10) were employed.

Starter activity test to indicate total inhibitory substances in milk. This method, similar in principle to methods suggested by Horral and Elliker (2) and Krienke (7), measures the acid increase of a known control milk to that of milk with unknown history under comparable incubation and testing conditions. If

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any differences are noted they are expressed as per cent of activity of the control milk. Milk showing total activity values of 80 per cent or higher are considered not inhibitory, whereas milk with values below 80 per cent are considered as being of inhibitory nature toward lactic starter organisms. Ten-ml. quantities of milk were used in this test with a 4.5 per cent starter inoculation. Incubation was for 4 hr. at 95° F. Two types of control milks were run with each set of 20 samples, one was a reconstituted 10 per cent, spray-dried skimmilk powder in which acid was produced at an excellent rate with commercial starter organisms; the other was mixed milk from the untreated Cornell University Dairy herd.

Disc assay method for penicillin and other antibiotics. This method adapted to milk by Difeo Laboratories is based upon the observation that if a paper disc containing milk contaminated with antibiotic is placed on a hardened agar layer previously seeded with bacteria sensitive to penicillin or other antibiotics, the antibiotic will diffuse from the disc radially and where inhibition occurs a circular clear zone is formed, indicating no bacterial growth, whereas the rest of the agar layer in the petri dish is of turbid nature. The size of the diameter of the clear zone is directly related to the antibiotic concentration, up to a critical level. In this survey *B. subtilis* was used as the test organism and was grown on whey agar. *B. subtilis* and lactic acid organisms exhibit approximately the same sensitivity toward penicillin and many other antibiotics. *S. lactis* is not used directly because of its inability to produce rapid and dense growth. The quantity of milk used was 0.017 ml., but in addition to this, duplicate samples of milk were concentrated under vacuum to bring about increased sensitivity which was approximately at the level of 0.05 unit of penicillin per milliliter of milk. Penicillin may be identified in milk by using penicillinase discs whenever positive results are obtained with the penicillin disc assay method.

Free sulfa drugs. The presence and concentration of free sulfa drugs in milk were obtained by slightly modifying the chemical method of Bratton and Marshall (1) which is an accepted method for blood and urine. A sensitivity of 0.005 per cent sulfa drug could be obtained in milk. One ml. of milk was the test sample.

Quaternary ammonium compounds. A method recently presented by Miller and Elliker (8), involving the use of eosin yellowish dye and citric acid buffer, was employed. This method was sensitive to 3 ppm. in milk, but to achieve such sensitivity fresh reagents must be used. One ml. of milk was used as test sample.

In analyzing for total inhibitory substances and antibiotics in milk, the pasteurized milk was reheated to 180° F. for 5 min. and cooled to 40° F., although for a small percentage the disc assay test was carried out directly on the pasteurized milk. For sulfa drugs and quaternary ammonium compounds the tests were applied directly to pasteurized milk.

RESULTS

Total inhibitory characteristics of milk. The starter activity test tends to show the general inhibitory properties of a milk. On the other hand, due to the high heat treatment required for the milks, it appears unlikely that initial bac-

terial contamination, bacteriophage or natural heat-labile milk inhibitors can exert any appreciable influence on this test. Consequently, the results probably show a more representative picture of the presence of such heat-stable inhibitory compounds as the antibiotics, sulfa drugs and quaternary ammonium compounds and heat-stable natural milk inhibitors.

In this study the great majority of the milks examined were without any significant inhibitory qualities. Of 1,794 pasteurized milk samples analyzed, 139 were considered of inhibitory nature against lactic starter organisms and of this group only 22, or about one per cent, exhibited strong inhibitory qualities.

Approximately 6 per cent of the milks showed activities over 120 per cent, which indicates that they were able to set up an environment in which lactic acid bacteria could produce acid more rapidly than the bacteria in the control milks.

TABLE 1

The inhibitory characteristics of 1,794 pasteurized whole milks from New York as shown by the starter activity test^a

Range of activity ^b	April-May, 1951 (920 milks)		July-August, 1951 (874 milks)		Classification
	(%)	(no.)	(no.)	(%)	
130-150	18	2.0	18	2.1	No significant inhibition, 92.25%
120-130	33	3.6	37	4.2	
110-120	96	10.4	135	15.4	
100-110	296	32.1	301	34.4	
90-100	219	23.8	272	31.1	
80-90	136	14.8	94	10.8	
70-80	78	8.5	14	1.7	Mild to medium inhibition, 6.52%
60-70	23	2.5	2	0.2	
50-60	15	1.6	0	0.0	Strong inhibition, 1.23%
40-50	1	0.1	0	0.0	
30-40	1	0.1	0	0.0	
10-30	4	0.4	1	0.1	

^a Analyses of approximately 200 milk samples from individual cows of the entire untreated dairy herd at Cornell U. during the same test period indicated a possible extreme variation of ± 20 per cent activity from the control samples.

^b Acid activity of test milk compared to control milk under comparable test conditions.

Av. titratable acidity increase of all control milk samples after 4 hr. incubation with 4.5% starter at 95° F. was 0.35% expressed as lactic acid.

Season of the year had an effect upon the incidence of milks displaying inhibitory characteristics. During the July-August period a much lower percentage of inhibitory milks was obtained than during the first test period in April-May (table 1).

Antibiotics. The number of milks producing zones of inhibition was quite low. Of 1,794 milk samples, 107 or 6.0 per cent exhibited zones which showed that the milks were inhibitory against the test organism, *B. subtilis*. This is nearly on the same order as the results obtained with the starter activity test. Of these inhibitory zones, 98 occurred during the April-May test period, whereas only nine were found among the milks collected during July-August. Penicillin probably was present in the majority of positive cases, as indicated by penicillinase discs which were run on many but not all positive milks. Most of the diam-

eters of zones were of such size as to indicate the presence of very small quantities of penicillin or associated antibiotic ranging between 0.05 and 0.1 unit per milliliter of milk. Only five samples of milk had concentrations greater than 0.1 unit per milliliter; the greatest being 0.5 unit per milliliter. When active starter was incubated in the latter milk, an almost total inhibition of the lactic acid organisms resulted.

Incidence and concentration of sulfa drugs. Not one sample of the 920 pasteurized milks obtained during the spring test period (April–May) was found to contain sulfa drugs. Because of the conclusive nature of these earlier data, sulfa drug testing was abandoned on the second set of samples obtained during July–August.

Quaternary ammonium compounds. Among the samples obtained during April–May, 832 milks were tested for quaternary ammonium compounds. Of this number, 33 milks or 4 per cent gave a positive test. However, 26 of these 33 samples showed presence of only small amounts of quaternary ammonium compounds, ranging from a trace to 3 ppm. Apparently, these positive cases of quaternary ammonium compounds exerted little influence on starter organisms, as samples of milk shown to contain only quaternary ammonium compounds ranged from 76 to 113 per cent activity as regards starter activity test and the average for this group was 100.4 per cent activity. No testing of milks for quaternary ammonium compounds were conducted on samples from the second test period.

DISCUSSION

A general survey has been made to obtain basic information necessary for an evaluation of the problem of inhibitory substances such as antibiotics, sulfa drugs and quaternary ammonium compounds in fluid milk supplies. However, any attempt to interpret these results on a nation-wide scale should be taken with caution as sampling was restricted to New York State. Although this region appears typical of many others throughout the country, clearly a need exists for a broader nation-wide survey possibly of similar nature to achieve a more comprehensive view of any existing problem.

In so far as incidence of antibiotics in fluid milk is concerned, naturally the sensitivity of the analytical method would limit the numbers of milk showing positive cases. The limit of the disc assay method was 0.05 unit per milliliter. Based on this sensitivity, the number of milks showing antibiotics in the New York State milk supply was not high. Yet the authors feel that many milks probably contained extremely small amounts of antibiotics in levels too low to be detected or to cause any inhibitory effects. With large storage and pasteurization tanks used in modern dairy plants, it would appear very likely that at least milk from one treated producer's cow, within a 3-day period, was present to contaminate this large supply. Though apparently not of critical nature at the present time, such concentrations in many milks might conceivably increase to a critical level at some future date if treatments become more common and if contaminated milk is not withheld. Even in light of the low incidence of milks over 0.05 unit per milliliter, this potential threat is an excellent reason in New York

State for educational programs to be carried to milk producers and for tests to be used to detect penicillin or antibiotics in the milk supply.

It must be recognized that even before the introduction of penicillin as an antibiotic agent, difficulties with "slow" milk were encountered. Solving problems associated with inhibitory milks does not appear to hinge on one or even two simple factors. Penicillin can be a factor, but as clearly pointed out by Nelson *et al.* (9) other factors such as bacterial contaminants, bacteriophage and natural milk inhibitors exert a decided influence under certain conditions. The relative incidence of each of these inhibitory agents in this overall problem is not known and in many respects basic information concerning their character and mode of action must first be obtained.

Comparison of data obtained during the spring and summer months points to the fact that during the test periods in 1951, milk producers of New York State did not deliberately add antibiotics or other bacteriostatic substances to their milk in order to control the bacterial count. It appears that more antibiotics are present in the milk during early spring because producers have greater opportunities for observation and treatments or possibly that the dilution factor becomes significant during the period of higher individual milk production by the cows in the summer months when they are on grass.

The small but definite percentage of milks showing high activity qualities, over 120 per cent, is of interest. Apparently, these data are significant, as analyses of approximately 200 milk samples from individual cows of the untreated herd at Cornell University during the same test period indicated a possible extreme variation of only ± 20 per cent from the control samples. At certain very small but critical concentrations, antibiotics are known to stimulate bacterial growth. This can be observed easily by noting the much denser growth which surrounds the boundary of the halo where concentration of the antibiotic is lower than any other area on the penicillin disc assay plate. As a number of these highly active milks produced zones, most of them extremely small, the possibility might be considered that these qualities of this milk can be explained by the presence of low concentrations of antibiotics. Further study should be undertaken to prove this interesting point, however.

Starter activity values and zone diameters on samples obtained during this survey did not show as good a relationship to each other as milks containing known, varying amounts of penicillin, when the latter were tested in the laboratory.

That a relationship between the starter activity results and the disc assay results does exist is indicated by the observation that the average activity value of all milks (1,794) was 110 per cent, whereas with the 107 milks exhibiting significant zones it was 84 per cent. On the other hand, a number of milks with definite zones had normal activity, while in a few instances milks with very low activities did not exhibit any zones. For example, 44 of the 798 milks above 80 per cent activity produced zones, whereas in the 21 milks showing activity below 60 per cent eight showed no zones. Though this does not invalidate these methods, it shows other factors exert an influence in the field and they must be

considered from a practical point of view. The experiences of the authors indicate that the disc assay test will prove more valuable for testing individual producer's samples or suspected blended samples than for the average blended pasteurized milk sample, as levels of antibiotics usually encountered in the latter are below or near the lower limits of sensitivity of the method. This should not be as true for positive individual producer's samples or for milks suspected of containing antibiotics.

SUMMARY

An analysis of 1,794 samples of fresh blended pasteurized milks obtained throughout New York State during the early spring and late summer months indicated the presence of about 7 per cent inhibitory milks of varying degree of intensity. Approximately 1 per cent were strongly inhibitory toward commercial lactic starter.

Antibiotics in these milks were indicated in relatively few cases and, where present, the amounts generally were between 0.05 and 0.1 unit per milliliter of milk.

It was apparent from the results at the time this study was made that the incidence of inhibitory milks or antibiotics milks was not high enough to create any grave general problem for the New York dairy industry insofar as fermented dairy products are concerned. However, the few instances where antibiotics were found in significant quantities would create a serious individual problem for the manufacturer of fermented dairy products.

No sulfa drugs were found in 922 samples of milk tested specifically for this chemical.

Quaternary ammonium compounds were indicated in about 4 per cent of 832 milks analyzed and in most of these positive cases they existed from a trace to 3 ppm. Milks containing this compound apparently did not show on the average any greater inhibitory properties.

Milks obtained during the hot summer months showed a significantly smaller incidence of inhibitory samples than milks obtained during the early spring months.

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HERD DATA MEASURES OF THE EFFECT OF CERTAIN ENVIRONMENTAL INFLUENCES ON DAIRY CATTLE PRODUCTION¹

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In the development of an environmental index (3) for dairy cattle production records, the present authors studied the relationships of nine environmental influences with milk and butterfat production and the relationships of the environmental influences with each other. Results were similar to those reported by other workers for four of these influences: age at calving (8, 10, 13, 19, 21, 22, 23, 26, 28, 31, 35, 51, 43, 45); number of days carrying calf while milking (17, 20, 23, 37); length of preceding dry period (1, 7, 11, 18, 23, 29, 36); and month of freshening (4, 6, 14, 24, 32, 34, 41).

The other five influences studied were selection rating, pounds of total digestible nutrients fed per 1,000 lb. of body weight, nutritive ratio, condition before calving and herd size. Four of these utilized different measures of selection and feeding than have been reported previously (2, 10, 12, 25, 27, 30, 36, 39, 42). There have been no reports of efforts to measure the effect of herd size on production.

The purpose of this paper is to describe the methods used and results obtained in the measurement of the effects of the above five influences.

PROCEDURE

During the period from September, 1947, to May, 1949, data for this study were collected by personal interviews with herd operators and by examination of their records and cattle. Information was obtained on 967 cows in 47 grade and purebred Holstein-Friesian herds in Wisconsin. At the start of the study two qualifications were established for selection of the herds to be included: (a) Their continuous enrollment in Holstein-Friesian HIR should be for at least 7 yr. (b) Their production, breeding and identification records should be complete. Later, in order to increase the scope of the study, a few herds were added which met the second qualification but they had been enrolled in HIR or DHIA for only 3 or more yr. The herds finally included had been tested continuously for an average of 16 yr.

The most recent complete lactation record for all cows bred, born, raised and still living in the herds visited was copied from the herdbooks. The length of the lactation was standardized by recording the milk and butterfat produced in

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² The data are taken from a thesis submitted by this author in partial fulfillment for the Doctor of Philosophy, 1950.

the first 305 days of the lactation. Actual records without correction for age were used. Records were omitted which followed abortions earlier than 150 days and in which severe ailments or injuries had seriously affected the production. The study was confined to animals milked twice daily.

The five environmental influences under discussion in this paper, as well as the other four mentioned in the introduction were selected for study from the results of a preliminary survey which involved twenty-one possible influences. Twelve of the twenty-one influences studied in the preliminary survey were not included in the later study because their effects did not appear important or because the measures used in evaluating their effects appeared inadequate.

The first of the five influences, selection rating, was devised to evaluate the methods of selection used in the herds and also the carefulness with which these methods were carried out. The details of the scoring system are presented in table 1. The increase in rating as the number of lactation records increased was based on the relative heritability of one record, of the average of two records and of the average of three records (5). Credit for the increasing numbers of progeny was approximated from the results of Davidson (9) and Edwards (15).

Both of the feeding measures were based on the rations fed during the winter months in the barn. The feeding information obtained was: (a) the amount and kind of silage fed; (b) the amount, kind and quality of hay fed; (c) the constituents and proportions of the grain ration; (d) the average amount of grain fed daily to each cow; (e) the amount fed and kind and quality of feeds used, other than those mentioned above.

The average amount of grain fed daily was based on the "rule of thumb" followed by the dairyman or on direct estimates by him. When considered necessary, checks were made of the estimates by examining the feeding equipment or observing the procedures. In herds where the operators reported that cows were fed all the hay they wanted, the amount of hay eaten was estimated at 1.5 lb. per 1,000 lb. body weight. Corn silage was fed in all the herds studied. Some operators stated that they fed silage according to the size of the cow. In the herds where this was practiced, the cows were classified into three groups according to body weight: 1,450 lb. or above, 1,000 to 1,449 lb. and less than 1,000 lb. The heavier cows received the larger amounts indicated by the operator and the lighter cows, the smaller amounts. Body weight was estimated from heart girth measurements taken at the time of the visit to the herd.

From these data the pounds of total digestible nutrients fed daily per 1,000 lb. of body weight and the nutritive ratio of the ration were computed. Seventy per cent was used as the proportion of T.D.N. in all grain rations. Other values, including protein content, were based on Morrison's tables of feed composition (33).

The condition of the dry cows and heifers was graded according to the four classifications shown in table 2.

Herd size was measured as the number of cows of milking age in the herd at the time of the visit.

A general description of the data is presented in table 3. The data were

TABLE 1
The scoring system for the selection rating

For each cow:	Answers and points awarded	
	Yes	No
Selection of the cow's sire was based on:		
Pedigree?	1	0
Type?	1	0
Progeny test? If so, number of progeny considered.		
Were records of progeny considered?	—	—
	If records considered award points equal to half number of progeny up to 12.	
Was the type of progeny considered?	1	0
His being a key animal in the breeding program?	1	0
Selection of cow's dam was based on:		
Pedigree?	1	0
Type?	1	0
Individual performance? If so, how many records were available at time this mating was made?		
	—	
	4 points for 1 record 6 points for 2 records 7 points for 3 or more records	
Her being a key animal in the breeding program	1	0
This cow was not culled as a heifer because of:		
Pedigree?	1	0
Type?	1	0
Her being a key animal in the breeding program?	1	0
This cow was kept in the herd since 1st lactation because of:		
Individual performance? If so, how many records were available at the beginning of this lactation?		
	—	
	8 for 1 record 12 for 2 records 14 for 3 or more records	
Type?	2	0
Pedigree?	2	0
Her being a key animal in the breeding program?	2	0
Add 1 point if herd has been tested continuously for 5 or more yr.		
Add 1 point if herd has been officially classified for type.		

TABLE 2
Description of classifications used for grading condition at calving

	Excellent	Good	Fair	Poor
Heifers	Well grown out and moderately fat to fat as revealed by fleshing over shoulders, ribs and rump.	Moderately developed with indications of fleshing but some "hookiness."	Thin and sometimes showing signs of underdevelopment.	Obvious evidence of under-nourishment, serawny appearance, skin dry and tight.
Dry cows	Plentiful amount of fleshing over the shoulders, rump and ribs; hooks and pins lacking sharpness.	More fleshing than the milking cows in the herd, but no indications of being particularly fat.	Thin	Same appearance as heifers. Cows without any dry period were automatically rated in this class.

analyzed according to the multiple regression techniques described by Snedecor (40), Wallace and Snedecor (44), Ezekiel (16) and Wright (46). Computations were carried out by means of tabulating card equipment.

RESULTS AND DISCUSSION

None of the correlation coefficients calculated from records of first lactations differed significantly from the coefficients obtained with records of later lactations. Therefore, the results presented are based on the data for all 967 cows. The correlation coefficients, standard partial regression coefficients and group averages are shown in tables 4 and 5. The standard partial regression coefficients were statistically significant for the regression of milk and fat yield on

TABLE 3
General description of data

	Average	Range	Standard deviation
No. of years herds have been on continuous testing under present management	16	3-39	
Production in first 305 d. (lb.)			
Milk	11,402	4,569-19,857	2,567.8
Butterfat	413	160-740	96.2
% Butterfat	3.6	2.6-6.0	0.3
Age at first freshening (mo.)	28	17-44	3.7
Length of preceding dry period (wk.)	9.0	0-30	4.3
Age at calving for last lactation (mo.)	56	21-163	28.1
Days with calf during last lactation	178	0-283	62.3
Pounds TDN daily/1,000 lb. body weight during barn feeding period of last lactation	17.3	12.4-27.0	2.0
Body weight (lb.)	1,354		
Nutritive ratio of ration during barn feeding period of last lactation	6.6	5.2-9.2	0.8
Selection rating	20.0	1-45	11.2
No. of milking cows in the herds	61	12-165	47.2
No. of cows included in the study from a single herd ^a	21	3-135	

^a The qualifications for cows included in the study were that they be bred, born, raised and still living in the herd and that they be milked only twice daily during their most recent complete lactation.

selection rating, pounds of T.D.N. fed daily per 1,000 lb. body weight, nutritive ratio and herd size. Statistically significant regressions were found for fat test on selection rating, pounds T.D.N. per 1,000 lb. body weight and herd size. The standard partial regression coefficient of fat test on nutritive ratio was not significant.

The regression of milk and fat yield on the selection rating showed that for a five point increase in the selection rating, there was, on the average, a 305-lb. increase in milk and a 14-lb. increase in butterfat. The regression of fat test on selection rating was curvilinear. Test increased at a decreasing rate when the selection rating was higher than 7 and no further increases were found above a rating of 31. The maximum effect of this rating on test was 0.19 per cent. Selection is not an influence for which records should be corrected. However, a measure of its effects was necessary in order to get independent evaluations of other factors which are associated with it.

TABLE 4

The correlation (r)^a and standard partial regression coefficients (b') of selection rating, feeding measures and herd size with production, and the correlations with each other and with age at calving, days carried calf, and length of dry period

	Production in 1st 305 d.				Selection rating	Pounds TDN daily/1,000 lb. body weight	Nutritive ratio	Herd size	Age at calving	Days carried calf during last lactation	Length of preceding dry period
	Milk	Fat	Test	Test							
Selection rating	r	b'	r	b'	r	r	r	r	r	r	r
Pounds TDN daily/1,000 lb. body weight	+0.45	+0.27	+0.48	+0.33	+0.12 ^b	+0.10	+0.30	+0.20	+0.35	-0.10	-0.02
Nutritive ratio of ration	+0.38	+0.44	+0.35	+0.38	-0.11	+0.10	+0.03	+0.30	-0.08	-0.06	-0.08
Herd size	-0.31	-0.22	-0.31	-0.20	-0.07	-0.30	0.00	0.00	-0.11	+0.04	+0.03
	0.02 ^b	0.02 ^b	0.01 ^b	+0.20	0.00	-0.03	-0.13	+0.08

^a $r = 0.063$ is significant at 5% level.

^b The regression was found to be curvilinear and thus r underestimates magnitude of correlation.

TABLE 5
The association of condition at calving of the heifers and cows with production and other environmental influences

Condition of heifers:	No. of cows	Average production		Production residuals		Selection rating	Pounds TDN/1,000 lb. body weight	Nutritive ratio	Herd size	Age at calving (mo.)	Days carried calf	Length of preceding dry period (wk.)
		Milk (lb.)	Fat (%)	Milk (lb.)	Fat (%)							
Excellent	123	10,776	3.7	1,182	49.0	16	17.4	6.5	82	29	168
Good	106	9,812	3.6	332	12.8	9	17.2	6.6	37	27	181
Fair	41	8,119	2.93	296	15.2	7	16.4	7.5	48	28	198
Poor	0
Condition of cows during dry period:												
Excellent	367	12,476	4.58	153	10.3	27	17.9	6.5	78	68	169	9.0
Good	230	12,192	4.33	174	-1.8	22	17.0	6.3	41	67	189	9.3
Fair	95	9,386	3.31	-428	-17.9	10	16.1	7.3	46	68	187	8.4
Poor	5	10,255	3.50	1,383	28.0	18	16.4	7.8	55	108	218	2.0

For an increase of 1 lb. of T.D.N. daily per 1,000 lb. body weight, there was an average increase of 551 lb. of milk and 18 lb. of fat. An increase of 1 lb. in T.D.N. rate was accompanied by a decrease of 0.02 per cent in test. Inasmuch as more than nine-tenths of the observed T.D.N. rates were within the range 14.0 to 21.9, the greatest expected effect of T.D.N. rate on fat test would be approximately 0.16 per cent.

The range of the nutritive ratios in these data was from 9.2 to 5.2. A change of this amount in nutritive ratio was accompanied by a change of 2,952 lb. of milk and 100 lb. of fat.

The analysis of the influence of condition at calving, a non-quantitative influence, was carried out with two methods. The first of these was to compare the average production found for each condition classification. These averages are shown in table 5. The second method was to determine the residual influence of condition at calving after the total influence of the quantitatively measured variables had been considered. This is shown in table 5 as the average difference between observed production records and their values predicted from the multiple regression of the quantitatively measured factors. The residual influence is in addition to the influence of the quantitative influences, rather than being independent of them (16).

The effect of condition of the heifers, according to the residuals, was an increase of 46 lb. of milk from "fair" to "good" grades and an increase of 850 lb. from "good" to "excellent." The respective increases for fat were -2 and 36 lb. The effect of the condition of the dry cows was an increase in milk of 600 lb. between the grades "fair" and "good." The increase in fat between these two ratings was 20 lb. No increase in milk was indicated between the grades "good" "excellent," but there was an increase of 12 lb. of fat. There were no heifers rated as in "poor condition." The cows considered as in "poor" condition were mostly those which had no dry period. The results indicated that putting cows with no dry period in this class may be misleading. Fat test changes accompanying changes in condition were slight.

The maintenance of high production in large herds often is more difficult than in small herds. The operators of large herds are dependent on hired help to attend to the individual needs of the cows. Disease control often is more difficult in large herds. In this study significant decreases were found in milk yield, fat test and fat yield as herd size increased. A total decline of 775 lb. of milk was associated with an increase in herd size from 20 cows to 49. The decline was 521 lb. from 50 cows to 79 and only a slight decrease could be predicted when herds contained 80 cows or more. A similar association was determined regarding fat; a decline of 35 lb. with an increase from 20 to 49 cows, 23 lb. with an increase from 50 to 79, and only a minor decline as herd size increased beyond that limit. Fat test dropped 0.05 per cent as herd size increased from 20 to 50 and dropped another 0.03 per cent between herd sizes of 50 and 100 cows.

The effects of T.D.N. feeding rate, nutritive ratio, condition at calving, herd size and also length of the dry period and days carrying calf while milking have been combined in the environment index. The index expresses the number of

pounds of butterfat a cow should produce above or below a 350-lb. level because of the influence of the environmental factors. Tables have been prepared for use in computation and are available upon request.

The measures of the effects of the influences discussed in this paper were devised for use on herd data in order to obtain results which would be directly applicable for the index. Herd data and multiple regression techniques were used in evaluating the measures because they offered opportunity to analyze concomitant observations. They also permitted the use of a wider range of observations than would have been possible with the use of data limited to within-herd observations or experimental trials. Environmental differences between herds including disease control, milking practices, pasture yields, etc., contribute to the magnitude of the effects obtained in this study. They are of particular importance in regard to the effects of condition of calving, nutritive ratio and herd size. Experimental trials which exclude differences between herds may yield different evaluations of some of the effects. Knowledge of the extent to which the experimental trial results are comparable to herd data results would be useful in the interpretation and intelligent use of the index. However, the evaluations obtained from the trials might not be as readily applied for use in the index as the results from herd data.

The herds included in the study cannot be considered representative of all Holstein-Friesian herds in Wisconsin. The criteria for selecting them would automatically make them herds in which management often will be above average. However, they probably are reasonably representative of herds from which many proved sires are being purchased. Adaptability of the results to other breeds of dairy cattle needs yet to be determined. The management practices and climatic conditions involved in the data of this study probably are similar to those found in most of the North Central and Northeastern parts of the United States.

Further work now is in progress checking the efficacy of the index in predicting production differences of experimental trials. Other studies are being planned to simplify and make more accurate the measures used, to devise methods for routinely collecting environment data and to check the index in a field test with a large group of data.

SUMMARY

Herd data information on 967 cows in 47 Holstein-Friesian herds of Wisconsin was analyzed by multiple regression techniques to determine the effect of nine environmental influences on production. Results were similar to those reported by other workers for age at calving, number of days carrying calf while milking, length of preceding dry period and month of freshening.

Statistically significant regressions were obtained for milk and fat yield on selection rating, pounds T.D.N. fed daily per 1,000 lb. body weight, nutritive ratio, condition at calving and herd size. The regressions of fat test on selection rating, T.D.N. feeding rate, condition at calving and herd size were statistically significant but unimportant. The regression of test on nutritive ratio was not significant.

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A PROPOSED METHOD AND APPARATUS FOR THE DETERMINATION
OF EXTRANEOUS MATTER IN ROLLER-DRIED MILK POWDER
FOR USE IN CHEESE FOOD PRODUCTS

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With the industrial usage of dried milk powder increasing daily, industry long has felt the need for a suitable method for the detection of extraneous matter in roller-dried milk powder used in cheese food products. One of the common color defects of dried milk powder made by roller or drum process gives the appearance of dark brown specks of burnt milk. Such a condition often indicates unclean conditions in the plant or improper technique used in the drying process. These burnt specks often show up in the finished product and should be guarded against in the making of any cheese food.

In 1946, a filtration method involving the use of pepsin-HCl solution was worked out and submitted to the American Dry Milk Institute. The method, now appearing in the American Dry Milk Institute, Inc., (1) Bulletin 911, page 28, is essentially the method we originally submitted to them.

The present recommended procedure as proposed by Strobel & Babcock (2) in a recent publication is much the same method as appears in this American Dry Milk Institute bulletin. Strobel & Babcock reported the pepsin-HCl solution method to be satisfactory for determining the total sediment content of roller-process nonfat milk solids by filtration through a standard lintine disc.

In our proposed method, it never has been found necessary to use a defoaming agent or to heat samples to boiling prior to filtering. A constant-speed motor operating at approximately 1,500 r.p.m. also has been employed. The proposed method is believed to be simple, accurate and time saving.

Although the work was completed a few years back, it was not possible at that time to write a report covering the findings. Because it was reasoned that this work might be of general interest, this paper was prepared for publication.

PROPOSED EXTRANEOUS MATTER APPARATUS

Because extraneous matter apparatus to carry on the work was not fabricated by any of the laboratory supply houses contacted, it became necessary to design equipment for use in this study. The unit designed is a multiple heating and stirring unit which it was believed would meet a great need in the agricultural field.

The apparatus is made up of six individual testing units on a stainless steel framework. Each unit is composed of a 4.25-in. hot plate, stainless steel beaker, electric motor and stainless steel stirring rods with special-type blade. The framework is braced by angle irons so designed as to prevent vibration during

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the operation. Special construction features are the spring steel clamps lined with asbestos cord holding the beakers rigid, the stainless steel beaker covers containing a rubber gasket which prevents vibration and the stainless steel agitators. The support rod on which the motors are attached is so designed as to allow free movement of stirring rod by lifting motor up without detaching from rod. In this way, it is possible to remove an agitator from the solution and rinse agitator with water so the rinsings drain into the rest of the sample in the beaker. A specially constructed funnel and filtering apparatus designed to speed up the filtering work also is shown in figure 1.

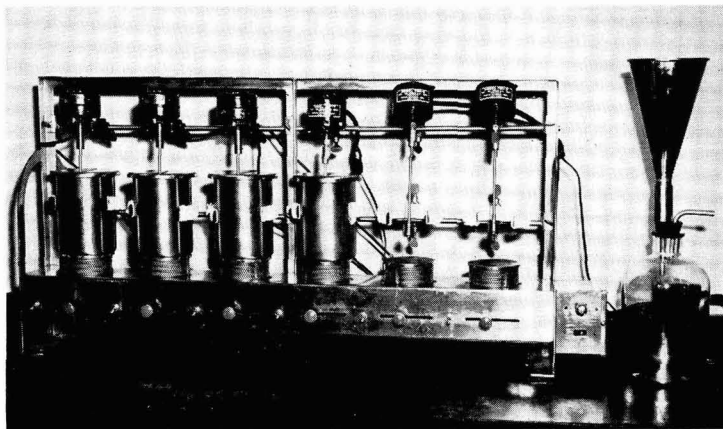


FIG. 1. Extraneous Matter Heating and Stirring Unit and Filtering Apparatus.

EXPERIMENTAL PROCEDURE

The proposed method is based upon the peptic digestion in an acid medium of the roller-dried nonfat milk. This action renders the protein molecule soluble and allows easy filtering of the sample through a cotton lintine disc. The primary concern is hydrolysis of the protein (casein) found in the milk product. Dilute acid and alkali hydrolysis first were used until enzymes were discovered that had the peculiar properties of catalyzing the hydrolysis of proteins. In preliminary experimental work, pepsin was found the most suitable enzyme. Preliminary investigations of various acids, HCl, H₂SO₄, H₃PO₄ and lactic acid revealed that HCl was the most suitable, with H₃PO₄ running a close second.

The method used in arriving at optimum conditions for filtration was as follows: Samples were weighed on a Torsion balance and transferred into 2-l. stainless steel Griffin-type beakers containing varied concentrations of solvent solution. (In order to prevent charring of the milk powder samples, it was necessary to add the milk powder to the solution rather than add the solution to the powder.) The stainless steel beakers containing the samples then were transferred to the specially designed extraneous matter heating and stirring unit and the samples were digested at varied temperatures using constant agitation for different lengths of time. Next, the samples were filtered with the aid of suction (water aspira-

tor) through a cotton lintine disc, using a specially constructed filtering apparatus as shown in figure 1. Notes were taken on unfilterable samples and discs then were thrown away. Filterable samples were dried, using a specially constructed infra-red drying oven. After drying, discs were mounted on an index card and filed for comparative study of extraneous matter.

RESULTS

Approximately 500 samples of milk powder were used in the various trials to produce a filterable substrate of roller-dried milk powder. In all trials, 50 or 100 g. of sample and 750 or 1,000 ml. of solvent solution were used. Solvent solutions were made using combinations from 5 to 25 g. of pepsin and from 10 to 25 ml. of HCl. In all trials, temperature of sample solution was varied from 30 to 60° C. and the time of agitation was for either 0.5 or 1.0 hr. Optimum conditions for obtaining a suitable filterable substrate were obtained when using 10 g. of pepsin and 25 ml. of HCl made up to 1,000 ml. with distilled water and heated at $40 \pm 5^\circ$ C. The samples treated in the above manner all filtered fast and no trouble was encountered with the filter disc clogging. Additional samples were run at the optimum conditions using 100 g. instead of 50 g. of sample and again all samples filtered fast. Data showing optimum conditions for producing filterable substrate are tabulated in table 1.

TABLE 1

Trials showing optimum condition found necessary for producing a suitable filterable substrate (all samples were agitated 0.5 hr.)

Wt. of sample	No. of analyses	Amt. of ^a solvent used	Temperature	pH of solvent solution	pH of substrate	Filterability
(g.)		(ml.)	(° C.)			
50	20	500	35	0.71	0.88	fast
	50	500	40	0.71	0.88	fast
	25	500	45	0.71	0.88	medium fast
	20	500	50	0.71	0.88	slow
			50	0.71	0.88	slow
100	20	1,000	35	0.71	1.0	medium fast
	75	1,000	40	0.72	1.1	fast
	40	1,000	45	0.71	1.1	medium fast

^a 10 g. of pepsin and 25 ml. of HCl were made up to 1,000 ml. with distilled water.

PROPOSED METHOD

Weigh a 100-g. sample of powder on a Torsion balance and transfer to a 2-l. beaker containing 1,000 ml. of solvent solution (10 g. pepsin and 25 ml. of HCl diluted to 1,000 ml. with distilled water). Mechanically agitate while digesting the sample for 0.5 hr. at a temperature of $40 \pm 5^\circ$ C. (the ideal temperature is 40° C.). Do not heat the sample over 46° C. as this temperature will cause the protein material to coagulate and produce slime which clogs the filter pad. A cover of some kind should be placed on the beakers during agitation to prevent contamination of sample from outside sources. After 0.5 hr. remove agitator from solution and hold above the solution in the beaker. Rinse agitator with

filtered distilled water so the rinsings drain into the rest of the sample in the beaker. Filter the solution using medium suction through an extraneous-matter disc. This solution should filter within 2 min. After all the solution has passed through the disc, rinse beaker with about 150 ml. of filtered, distilled water and filter rinsings through the same disc. Remove disc from filtering apparatus and dry under infra-red lamp for 5 min. The disc should be covered while drying to prevent contamination. Enclose dry disc in cellophane bag and mount on an index card bearing its identity. The disc then is examined under a binocular microscope and graded according to existing standards for dried milk powder.

SUMMARY AND CONCLUSIONS

In working with roller-dried nonfat milk powder, the optimum conditions for hydrolysis for obtaining a suitable substrate which can be filtered satisfactorily through a lintine disc have been found. Practically all extraneous matter, including scorched particles, can be recovered on lintine disc. A filterable substrate can be obtained without the use of a defoaming agent, a high-speed stirring motor and subjecting samples to boiling temperatures prior to filtering.

A newly designed piece of equipment for use in extraneous matter testing has been introduced. This unit is so constructed as to allow tests requiring different time and temperatures to be run at the same time; more sanitary operation also is possible by a compact unit with covered containers. Greater economy of manpower working space and equipment is achieved.

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GAS PRODUCTION IN CHEDDAR CHEESE CAUSED BY *LEUCONOSTOC CITROVORUM*¹

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The occurrence of openness in Cheddar cheese at various seasons of the year has become of some concern to the cheese industry. This openness, generally referred to as "slits," occurred in some lots of experimental cheese at this Station to the extent of causing noticeable bulging of the surfaces of the cheese. The history of the milk and methods of its handling did not lead one to believe the usual gas-formers of Cheddar cheese were involved. Also, the cheese did not show the typical gas holes of a gassy cheese, but rather irregular openings more or less following the curd particles, thus giving the appearance of a mechanical opening that had split the curd (fissures). In severe cases of slit openness, the opening had the shiny or glazed appearance of typical gas holes in Swiss cheese.

Sherwood (5) reports a defect of this type in New Zealand cheese and states that gas-producing lactobacilli are the most common organisms causing slit openness. However, he also stated that the presence of *Leuconostoc citrovorum* in sufficient numbers may be a cause of the defect. In the study of the flora of New Zealand cheese, Sherwood (6) did not isolate *L. citrovorum* from the cheese examined, even though these organisms were common in starters. Galesloot (2) studied the early gas defect in Cheddar cheese and concluded that *Betacoccus arabinosaceus* (*L. mesenteroides*) and *Betacoccus bovis* (*L. dextranicum*) were able to produce the "slits." However, he also felt that if the lactic acid bacteria of the starter were very active the defect would be minimized. Prouty and Golding (4) studied the action of the associate starter organisms during the ripening process of vacuum-packed Cheddar cheese and found that a greater loss of vacuum occurred when *Streptococcus citrovorus* (*L. citrovorum*) and *Streptococcus paracitrovorus* (*L. dextranicum*) were used in conjunction with *Streptococcus lactis* than when *Lactobacillus bulgaricus* was used. From the nature of this defect it was felt that the associate organisms might be involved in this case.

METHODS

No attempt was made to isolate coliform organisms from the cheese since the defect did not indicate gas in the quantities produced by the coliforms and also the defect ordinarily shows up in 3 to 4 wk. of ripening at 50° F. A slightly longer period was required for the defect to occur at 40° F. A modification of the technique used by Abdel-Malek and Gibson (1) for the detection of gas by the *Leuconostoc* species was used and gas-producing organisms were easily isolated. The modification consisted of adding 0.25 per cent sodium citrate to the

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glucose milk. The agar plug then was poured on the solidified glucose milk and about 5 ml. of sterile water or litmus milk were placed on top of the agar plug. This prevented drying of the agar and escape of any gas produced along the side of the tube. The glucose milk then was prepared with the following ingredients: 800 ml. fresh skim milk, 200 ml. filtered tomato juice neutralized with NaOH to pH 6.5, 5 per cent glucose, 20 per cent gelatin, 0.25 per cent yeast extract and 0.25 per cent sodium citrate. The medium was heated to bring about suspension of the gelatin, tubed to a depth of 5-6 cm. and sterilized by intermittent steaming for 1 hr. on 3 successive days.

Small quantities of cheese (1 to 3 g.) were taken aseptically and emulsified in 9 ml. of sterile 2 per cent sodium citrate solution, using a sterile mortar and pestle for each sample. The glucose milk was melted and cooled at 45° C. and then inoculated with 0.1 to 1 ml. of the emulsified cheese sample. Incubation was at 21° C. for 7 to 10 days.

Various dilutions of the glucose milk from tubes showing evidence of gas were plated on tomato juice agar with the following compositions: 200 ml. filtered tomato juice, 5 g. each of tryptone, tryptose and yeast extract, 15 g. apart and 800 ml. distilled water. The pH of the medium was adjusted to 6.8-6.9. These plates were incubated at 21° C. for 5 days and individual isolated colonies were picked into litmus milk. Invariably this procedure gave a large majority of gram positive cocci occurring singly, in pairs and in long chains and producing little or no change in litmus milk. When these purified cultures were reinoculated into the modified glucose milk, an abundant amount of gas was formed in 3 to 5 days.

Eight lots of cheese were made in 50-gal. vats from pasteurized mixed herd milk heated at 144° F. for 30 min. The cheese was made into 9 to 10 lb. young Americans with three or more from each lot. A cheese from each lot was ripened at 40, 50 and 58° F. Three of these lots were made using a single-strain *S. lactis* (712) starter. Three lots were made with commercial cheese starters characteristically possessing high flavor. One lot was made with *S. lactis* (712) plus 100 ml. of a 5-day-old sterile milk culture of one of the isolated gas-producing cocci. The remaining lot was made with commercial starter plus 100 ml. of a 5-day-old sterile milk culture of one of the gas-forming cocci. At the end of 30 days' ripening, the cheese were examined for the defect.

RESULTS

Six cheese, varying in age from 1 to 3 mo. and showing various degrees of slit openness, were examined for gas-producing organisms. Twelve tubes of glucose milk were inoculated from each of the six cheese. Table 1 shows the age of the cheese and the per cent of tubes of glucose milk that showed gas production after 10 days incubation at 21° C. From these six cheese, 11 cultures were selected and purified for identification and further study. These organisms were all gram positive cocci occurring singly, in pairs and in short-to-long chains. They produce little or no change in litmus milk when incubated at 21° C. Gas was produced in glucose milk and acid was produced from glucose, galactose, lactose and sucrose, but no acid was produced from mannose, mannitol or salicin. All cul-

tures showed an increase in volatile acidity of milk containing citric acid as compared with milk alone (Hammer, 3). From this information, these organisms were assumed to be *L. citrovorum*. The fourth (4) isolate was selected more or less at random from these 11 cultures for use in this work. Its characteristics were typical of those indicated above.

TABLE 1
The extent of gas production in glucose milk tubes from 6 lots of cheese

Cheese	Age (d.)	% of tubes showing gas
1	59	25.0
2	59	41.6
3	102	8.3
4	54	41.6
5	94	16.6
6	94	25.0

Table 2 gives the results of cheese made with various culture organisms and the degree of the defect after ripening for 30 days at various temperatures. The single strain culture of *S. lactis* (712) showed practically no defect (cheese 1, 3 and 5) while the same culture plus *L. citrovorum* (4) showed evidence of the slits at 40° F. with pronounced slits at temperatures of 50 and 58° F. (cheese 6). Both the commercial cheese cultures (cheese 2, 4 and 7) showed about the same degree of slit openness as did the *S. lactis* (712) plus *L. citrovorum* (4). How-

TABLE 2
The degree of slit openness in cheese made with various cultures and ripened for 30 d. at 40, 50 and 58° F.

Cheese	Culture used	Degree ^a of slit openness at ripening temperature of		
		40° F.	50° F.	58° F.
1	<i>S. lactis</i> (712)	±	±	±
2	Commercial cheese culture #I	+++	+++	++++
3	<i>S. lactis</i> (712)	-	-	±
4	Commercial cheese culture #I	++	++	+++
5	<i>S. lactis</i> (712)	-	±	-
6	<i>S. lactis</i> (712) + <i>L. citrovorum</i> (4)	++	+++	++++
7	Commercial cheese culture #II	++	+++	++++
8	Commercial cheese culture #II + <i>L. citrovorum</i> (4)	++++	++++	++++

^a - = no evidence of slits; + = very slight evidence of slits; ++ = slight evidence of slits; +++ = moderate evidence of slits; ++++ = distinct slits.

ever, when *L. citrovorum* (4) was added to the commercial culture distinct slits occurred even at 40° F. and at the higher temperatures the cheese was bulged on top with a spongy body.

DISCUSSION

The slit openness in Cheddar cheese in this laboratory to the extent of giving the appearance of a gassy cheese occurred quite suddenly. In previous years

the defect, if present, was not serious. However, when ripening studies at elevated temperatures were initiated, openness of texture was more prevalent. In the early stages of this work it was felt that the cheese possessed some inherent weakness toward openness, and this concept has not been entirely abandoned. Oftentimes the defect in commercial plants of the area has been seasonal. This would seem to point towards milk composition and its possible relation to a weakness in the cheese toward openness.

When the first severe cases of the defect were examined and the slit openings showed the shiny or glazed appearance of gas holes, the possibility of gas-producing organisms was considered. Since the milk was handled in such a manner as to eliminate likely contaminants, the hetero-fermentative *Leuconostoc* species suggested a possibility. The *Leuconostoc* species *dextranicum* and *citrovorum* are common in commercial cheese cultures and the data (table 2) indicate that the defect occurred when two different commercial starters were used. Of course, there is a large amount of cheese made with commercial cultures which does not develop the openness. This would tend to bear out the assumption that the curd possesses some inherent weakness toward openness.

It would seem possible under certain conditions that *L. dextranicum* and/or *L. citrovorum* could produce enough gas to build up considerable pressure within the cheese. The fact that CO₂ is more soluble at the lower temperatures and the rate of CO₂ production at the lower temperatures is slower may account for the less frequent occurrence of the defect at ripening temperatures of 40° F. But with elevated temperatures, the CO₂ becomes less soluble and is produced more rapidly; it is not able to diffuse out of the cheese rapidly and thereby could create enough pressure to produce the slits. These assumptions seemed to be verified by the more pronounced defect at the high temperatures (table 2) and the occurrence of the defect when *L. citrovorum* was used in conjunction with a single strain *S. lactis* starter. Galeslout (2) felt that *L. citrovorum* was not involved in the slit openness but that other species of this genus were the probable cause. However, since the classification of this group is open to controversy there may be a relationship between the *L. citrovorum* isolate in this laboratory and the species that were responsible for the defect as noted by Galeslout (2).

The data presented here indicate that in areas where this difficulty occurs, the use of starters without the associate organisms would be of value. It may be that carrying mother cultures at temperatures of 80 to 85° F. and using an incubation period of 6 to 8 hr. may cut down the number of associate organisms and still not affect the *S. lactis* or *S. cremoris* strains now used.

SUMMARY

Several cultures of *L. citrovorum* were isolated from Cheddar cheese showing slit openness. Two separate commercial cheese cultures of different origin produced the defect quite distinctly at ripening temperatures of 50 and 58° F. With a single-strain *S. lactis* (712) culture, it was possible to make cheese with little or no evidence of the defect. However, when *S. lactis* (712) was used in

conjunction with an isolated strain of *L. citrovorum* (4) the defect was very pronounced.

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FEEDING VALUE FOR MILK PRODUCTION OF U. S. NO. 3 AND SAMPLE GRADE KOREAN LESPEDEZA HAY¹

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Hay is one of the most important feed crops on American dairy farms. More than one-third of the nutrients furnished dairy cows comes from hay. In the States of Missouri, Tennessee, Kentucky, Arkansas, Virginia and North Carolina annual lespedeza has become one of the important hay crops. Because many weeds start to grow in the spring before the lespedeza plants are large enough to compete and because of the grain straw left on the field if the lespedeza is grown in a grain crop, the hay often contains large quantities of foreign material. Standards of quality for lespedeza hay (1), based on certain physical factors, have been issued by the United States Department of Agriculture.

Recognizing the small amount of information on relation of official grade of hay, especially lespedeza hay, to its feeding value for milking cows, the Bureau of Dairy Industry, in cooperation with the Tennessee Agricultural Experiment Station and the Grain Branch, Production and Marketing Administration, U.S.D.A., undertook investigations at the Dairy Experiment Station, Lewisburg, Tenn., to determine the comparative feeding value of some grades of lespedeza hay. In this work, grades of lespedeza hay were used which appeared most closely to represent the greater part of the lespedeza hay produced. It was recognized that much of the hay now produced is high in foreign material, so hays selected for the investigations contained a considerable mixture of foreign materials. The investigations continued over 2 yr. Each year a feeding test was conducted comparing the value of U. S. no. 3 Leafy Green Lespedeza hay with U. S. Sample Grade Extra Leafy Lespedeza hay.

EXPERIMENTAL METHODS

The hays: The Lespedeza hays used both years were obtained from farms located in Franklin County, Tenn. At the time the hays were obtained, selection was made on the basis of grade classifications made by an official hay grader, who examined the lots before they were purchased. However, the final grade given for each lot of hay was made by averaging the grade classifications given to samples taken at 10-day intervals during the time the hay was fed in the feeding experiment. The average grade of each lot of hay used is shown in table 1.

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Both hays used in the first experiment (1948-49) apparently were harvested in the late-bloom stage. The hays were baled in the field, and a few of the bales contained a small amount of mold when they were fed to the cows in the experiment. Data relative to the two lots of hay are shown in table 1. It should be pointed out that leafiness determinations are based on the total weight of the lespedeza in the hay and not on the sample as a whole. The foreign material in lot 1 was largely grain stubble and weeds (mainly dodder). The hay also contained a small amount of grass. The foreign material in lot 2 was mainly ragweed, with a lesser amount of grain stubble. It also contained a small amount of grass, but less than the 10 per cent which is allowed in the Class Lespedeza hay.

The hays used in the second experiment likewise contained a high enough leaf content in the lespedeza hay and a high enough color to qualify as no. 1 or no. 1 Extra Leafy U. S. grade, but the amount of foreign material was so high that hay lot 3 qualified for U. S. Grade 3 hay and lot 4 for U. S. Sample grade hay.

TABLE 1
Official U. S. grade of hays used in feeding experiments

Expt.	Lot	Leaf content of lespedeza	Foreign material in hay	Color in hay	Official grades
(no.)	(no.)	(%)	(%)		
1	1	53.1	17.6	Green to greenish-brown	U. S. #3 leafy green
1	2	57.7	31.9	Green to greenish-brown	U. S. sample grade, green, extra leafy
2	3	53.4	19.0	Green to greenish-brown	U. S. #3 leafy green
2	4	58.3	34.8	Greenish-brown to brown	U. S. sample grade, extra leafy
Av. 1 and 3		53.2	18.3	Green to greenish-brown	U. S. #3 leafy green
Av. 2 and 4		58.0	33.3	Green to greenish-brown	U. S. sample grade, green, extra leafy

These hays were cut in late August in the late-bloom stage, and were baled in the field. They were in good condition but as the hays were fed there was evidence of mold in a few bales of each kind of hay. The foreign material in lot 3, averaging 19.0 per cent, consisted mainly of grain stubble, a small amount of brushy material and dodder; it also contained some Johnson grass and Dalliss grass. The foreign material in lot 4 (34.8 per cent of the hay) was largely grain stubble, ragweed and a few corn stalks.

During the course of the 150-day feeding experiment that was conducted during the winter of 1948-49 with hay lots 1 and 2, and during the winter of 1949-50 with lots 3 and 4, each hay was sampled three times on the second, fifth and eighth days of each 10-day interval. The samples for each hay were composited at the end of 10 days and were representative of the hays fed during that period. Likewise, the hay that was refused by the cows was removed from the mangers and carefully sampled on the second and eighth day of each 10-day interval to obtain a representative portion of refused feed. These 10-day composite samples of the hays and refused feed were used for determination of the leaf content of the lespedeza, the amount of foreign material and color from which the average grade determinations shown in table 1 were made (no grade was given to the re-

fused feed). Samples of the hays and refused feed were similarly obtained for dry matter determination and for chemical analysis.

The feeding trials: In both experiments, 16 milking Jersey cows were used. The 16 cows were divided into two groups of eight animals as nearly comparable as possible, considering their ages, weight, daily milk production and number of days in milk. The grouping of the cows, together with the data on which the allotments were based, are shown in table 2.

The 150-day experimental period was divided into three 50-day periods. In the first experiment, one group of cows was fed hay lot 1 for 50 days, lot 2 for the next 50 days and lot 1 again for the last 50 days. The other group of cows

TABLE 2
Data used for selecting the cows into groups in the feeding tests with the lespedeza hays

	Days in milk	Age (<i>mo.</i>)	Daily milk production (<i>lb.</i>)	Body weight (<i>lb.</i>)
<i>Experiment 1</i>				
Group A				
Extremes	165-85	97-28	29-16	977-697
Av.	113	57	23	852
Group B				
Extremes	168-45	71-29	32-19	925-743
Av.	104	50	24	793
<i>Experiment 2</i>				
Group A				
Extremes	168-24	91-34	31-14	1000-720
Av.	89	66	24	895
Group B				
Extremes	168-21	89-39	30-15	991-717
Av.	90	63	23	863

was fed lot 2 the first 50 days, lot 1 the next 50 days and lot 2 the third 50 days. The same procedure was used in the second experiment with lots 3 and 4. The first 10 days of each 50-day period were considered preliminary for the cows to become accustomed to the new feed, and while all data were taken during these 10-day intervals, they were not included in summarizing the results.

In addition to the hays fed in both experiments, the cows received a grain mixture at the rate of 1 lb. daily for each 4 lb. of 4 per cent fat-corrected milk produced. Adjustment in the feeding rate of the grain mixture was made each 10 days. The grain mixture consisted of 50 per cent barley, 25 per cent oats and 25 per cent wheat bran. This mixture was palatable and, when fed at the rather limited rate indicated above, was readily consumed, there being no grain refused. Each time a batch of the grain mixture was prepared, a representative sample was obtained from which the dry matter and chemical analysis were determined.

The hays, refused feed and grain mixtures were analyzed for dry matter, protein, ether extract, ash, fiber and nitrogen-free extract according to the official methods of the Association of Official Agricultural Chemists (2).

During the feeding trials, the cows were housed in a stanchion barn on bedded concrete floors. The daily portions of hay and grain mixture fed to each cow were weighed at each feeding and the hay refused was weighed once each day. The amount of milk produced by each cow was weighed at each milking. A 1-day composite of the milk produced by each cow was tested for butterfat content each 10-day interval. The cows were weighed on 3 consecutive days at the beginning and end of each 50-day period and on the first and last day of each intermediate 10-day interval.

The data were analyzed by 40-day periods (without the first 10 days preliminary), but the results obtained for each kind of hay fed in both experiments are presented in summarized form as follows: The data for the first and third 40-day test periods, experiment 1, for hay lot 1 that was fed to one group of cows were summarized and averaged; the data for the second 40-day period for hay lot 1 that was fed to the other group of eight cows were added to their average and then averaged to give the average results obtained for hay lot 1. The data for hay lot 2 fed to the second group of cows in periods 1 and 3 and to the first group of cows in period 2 were similarly treated to obtain the results for hay lot 2. Similar analysis was given the data in experiment 2 to obtain the results for hay lots 3 and 4.

RESULTS AND DISCUSSION

The average U. S. grades given the hays based on leaf content of the lespedeza, the color content and the amount of foreign material the hay contained have been given in table 1. The data showing the dry matter content and the chemical composition of the dry matter of the feeds fed and the feeds refused are shown in table 3. The only difference in the official grades of the hays used in the two experiments is that in the U. S. Sample grades, in experiment 1 the designation "green" was carried, while that in experiment 2 did not carry this designation. Since the color of the U. S. Sample grade hay in experiment 2 is relatively high, it is unlikely that this would be a factor influencing the outcome of the experiment. Both U. S. no. 3 grade hays contained a higher protein content and a lower fiber content than their corresponding U. S. Sample grade hays. This was about the only difference in the composition of the two grades of hay as represented by these samples. Even though the leaf content of the lespedeza was higher in the U. S. Sample grade hays (in both experiments), the amount of foreign material contained in these hays apparently was great enough to overcome this advantage, resulting in hays of lower protein content than the corresponding hays in experiment 2. This seemed to be the only real difference in their composition, even though there was a higher content of foreign material in the hays used in experiment 2.

The composition of the dry matter of material refused by the cows was in all cases lower in all constituents determined except fiber, which was higher. This would indicate that the refusal contained relatively much more foreign material than the original hays and that the cows were picking out lespedeza for consump-

TABLE 3

Average dry matter content and chemical composition of the dry matter of the hays and grain mixture fed and the refused feed

Material	Dry matter	Protein	Ether extract	Fiber	N-free extract	Ash
	(%)	(%)	(%)	(%)	(%)	(%)
<i>Experiment 1</i>						
Lot 1, U. S. #3 hay fed	89.4	12.8	2.3	36.4	44.4	4.1
U. S. #3 refusal	87.7	7.8	1.2	47.7	39.8	3.5
Lot 2, U. S. sample grade hay fed	89.5	11.0	2.4	37.5	44.3	4.5
U. S. sample grade refusal	87.4	6.8	1.3	46.0	42.2	3.7
Grain mixture	87.9	12.4	3.3	8.8	70.8	4.8
<i>Experiment 2</i>						
Lot 3, U. S. #3 hay fed	91.7	11.0	2.5	34.9	47.5	4.1
U. S. #3 refusal	88.5	6.9	1.4	47.4	40.7	3.6
Lot 4, U. S. sample grade hay fed	91.1	8.8	2.1	37.5	47.2	4.4
U. S. sample grade refusal	89.0	4.9	1.2	47.1	43.3	3.5
Grain mixture	88.6	12.1	3.0	9.1	69.7	6.1
<i>Av. experiments 1 and 2</i>						
U. S. #3 hay fed	90.5	11.9	2.4	35.6	46.0	4.1
U. S. #3 refusal	88.1	7.4	1.3	47.6	40.2	3.6
U. S. sample grade hay fed	90.3	9.9	2.2	37.5	45.8	4.4
U. S. sample grade refusal	88.2	5.8	1.2	46.6	42.8	3.6
Grain mixture	88.3	12.2	3.2	9.0	70.2	5.4

TABLE 4

Feed consumption and production of milking cows fed the experimental lespedeza hays (per cow per day basis)^a

Lot no.	No. of cows	Feed consumption			Dry matter consumption			Liveweight		Milk prod. ^b per lb. milk produced ^b	Dry matter consumed per lb. milk produced ^b
		Hay fed	Hay re-fused	Grain fed	From hay	From grain	Total	Total	Daily change		
		(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
<i>Experiment 1</i>											
1 ^c	8	26.5	4.9	4.7	19.2	4.4	23.6	820	-30	18.2	1.30
2 ^d	8	32.6	10.2	4.7	20.3	4.3	24.6	820	-28	19.0	1.30
<i>Experiment 2</i>											
3 ^e	8	28.6	4.0	5.6	22.7	5.0	27.7	873	-.07	21.7	1.28
4 ^f	8	34.6	8.9	5.5	23.7	4.8	28.5	862	+0.4	20.7	1.38
Av. U. S. no. 3 Leafy Green Lespedeza Hay (Expt. 1, lot 1 and Expt. 2, lot 3)											
1 & 3	8	27.6	4.4	5.2	21.0	4.7	25.6	846	-18	20.0	1.29
Av. U. S. sample Grade Green Extra Leafy Lespedeza Hay (Expt. 1, lot 2 and Expt. 2, lot 4)											
2 & 4	8	33.6	9.6	5.1	22.0	4.6	26.6	841	-12	19.8	1.34

^a In expt. 2, cows no. 168 and 345, both in lot 2, had attacks of mastitis at the beginning of the preliminary interval of period 1. Cow no. 272 of lot 1 aborted on the 12th day before the end of the experiment. Since her performance apparently was normal up to that time, her feed consumption, body weight and milk production for the last 12 d. were estimated on the basis of her previous performance.

^b Milk production expressed in terms of 4% F.C.M.

^c U. S. 3 Leafy Green Lespedeza Hay.

^d U. S. Sample grade Green Extra Leafy Lespedeza Hay.

^e U. S. 3 Leafy Green Lespedeza Hay.

^f U. S. Sample grade Extra Leafy Lespedeza Hay.

tion. This is substantiated by the analysis of leaf content and foreign material in the refusal.

The amount of feed consumed and refused and the production of the cows fed the experimental rations are given in table 4. In experiment 1, the cows, when fed on the two different hays, produced about the same amount of 4 per cent F.C.M. daily, 18.2 lb. when on lot 1 and 19.0 lb. on lot 2. This is 0.8 lb. per cow per day in favor of the U. S. Sample grade hay. The live weight changes were about the same, as was the average weight for the cows when receiving the two different hays. The consumption of dry matter was a pound per cow per day greater for the cows when fed lot 2, although the amount of dry matter consumed per pound of milk produced was about the same. The difference came mostly from a greater dry matter consumption from hay, since that from grain was about the same. However, the amount of hay fed was considerably greater (6.1 lb. per cow per day) in the case of lot 2, the U. S. Sample grade hay. The amount of hay refused by the cows was over twice that when the lot 1 hay (U. S. grade 3) was fed. The high rate of refusal apparently is associated with a high content of foreign material in the hay. It soon was observed that the cows were picking out the lespedeza and leaving the foreign material. In order to have the cows consume about as much dry matter in one hay as the other, it was necessary to feed lot 2 at a heavier rate and then weigh back that part which they would not eat, consisting largely of foreign material.

The second experiment yielded much the same information. In this experiment, milk production was slightly higher when the cows received lot 3, the U. S. 3 grade Leafy Green hay, but as in experiment 1, the differences were small. Consumption of dry matter per cow per day was again greater for the cows fed U. S. Sample grade Green Extra Leafy hay (0.8 lb. per day), but the dry matter consumed per pound of milk was higher for this hay, being 1.38 lb. compared to 1.28 lb. when the cows received U. S. 3 Leafy Green hay. It was necessary in this experiment to again feed lot 4, containing a higher content of foreign material, at a considerably heavier rate in order to have the cows consume about as much dry matter as they did when receiving lot 3 of lower foreign material content. The amount of hay refused was again twice as much for U. S. Sample grade Green Extra Leafy hay as for U. S. 3 Leafy Green hay.

When the two experiments are combined and averaged, the production per cow per day was found to be almost the same. There was little or no difference in the average live weight losses of the cows on the two kinds of hays. The dry matter consumed in grain averaged the same, but because of the heavier rate of feeding of the U. S. Sample grade Green Extra Leafy hay in order to obtain consumption commensurate with that of the U. S. 3 Leafy Green grade hay, the cows fed this hay consumed an average of 1 lb. dry matter daily more than they did when receiving the U. S. 3 Leafy Green grade hay. Because of this, the amount of dry matter consumed per pound of milk produced was a little greater for the U. S. Sample grade Green Extra Leafy hay, being 1.34 lb. compared to 1.29 lb.

In terms of the dry matter consumed by cows maintained on the rations under consideration, the hays apparently were equally effective in providing nutrients for milk production and the maintenance of body weight. It would have been

desirable to have had cows in the stages of lactation represented by these experimental cows make sizeable increases in live weight, whereas these cows on the average just failed to maintain their weights. The consumption of dry matter from hay per cow per day was 2.5 lb. and 2.6 lb. per hundredweight, respectively, for the cows when receiving the U. S. 3 grade and U. S. Sample grade hays. Had the hays been more palatable, consumption might have been somewhat greater, resulting in weight gains and increased milk production.

The important fact revealed in these experiments is that the U. S. Sample grade hays, apparently because of their higher content of foreign material, were less palatable than the U. S. grade 3 hays and therefore considerably more of the Sample grade hays had to be fed to obtain the same results that were obtained from feeding U. S. 3 grade hay. Considering both experiments, 6 lb. more U. S. Sample grade hay than U. S. 3 grade hay were fed per day to produce essentially the same result. On the basis of the amount of hay fed, it took 33.6 lb. of U. S. Sample grade hay per day to produce 19.8 lb. of milk, or 170 lb. of hay per 100 lb. of milk produced. For the U. S. 3 grade hay, it required 27.6 lb. of hay to produce 20.0 lb. of milk, or 138 lb. of hay per 100 lb. of milk. Using this comparison, it would require about 23 per cent more U. S. Sample grade hay to produce the same amount of milk. From the results of these studies, it appears that this is the difference that may be ascribed to the two U. S. grades of lespedeza hay used under practical conditions. This would mean that about 5 tons of U. S. Sample grade lespedeza hay would be needed to equal 4 tons of U. S. 3 grade lespedeza hay.

SUMMARY AND CONCLUSIONS

Two feeding experiments with milking cows have been conducted to compare the feeding value of U. S. no. 3 Leafy Green Lespedeza hay with U. S. Sample grade Green Extra Leafy Lespedeza hay. The main difference in the two grades of hay was in the amount of foreign material which they contained, 18.3 per cent in the U. S. no. 3 grade and 33.3 per cent in the U. S. Sample grade.

The U. S. Sample grade hay was less palatable since it required 33.6 lb. per day to obtain a dry matter consumption from hay of 22.0 lb., compared to 27.6 lb. for a dry matter consumption from hay of 21.0 lb. for U. S. 3 grade hay.

On the basis of 4 per cent F.C.M. produced, it required about 23 per cent more of the Sample grade than of the no. 3 lespedeza hay to produce the same amount of milk. Thus, about 4 tons of U. S. 3 Lespedeza hay was equal to 5 tons of U. S. Sample grade lespedeza hay.

The official grades placed on the hays used indicated differences of this magnitude in the feeding value of the graded hays. The results indicate that it would prove beneficial to farmers to reduce the amount of foreign material in the lespedeza hay which they produce.

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REFERENCE JOURNALS USED BY DAIRY SCIENTISTS

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The basic training needs of dairy scientists best calculated to serve the industry and the common welfare of the future should be of concern to those who have the responsibility of directing that training now. Current problems require the application of basic science in their solution and considered speculation suggests that problems of the future will require for solution no less talent, training and understanding than the problems of today.

Believing that what dairy scientists themselves do in the solution of current problems might serve as a guiding principle in the training of advanced students in the field, the author, with the aid of his graduate students, carefully studied selected volumes of the *Journal of Dairy Science* and the reference citations for a lead as to basic needs of students in this field. Only one other study of a bibliographical nature on the source of the references used in the *Journal of Dairy Science* has come to the attention of the author (2). The results of the present study appear to be of interest to teachers in this field. It is the purpose of this paper to give the major observations made. The inferences to be drawn from the results are clear.

METHODS

Volumes 1, 5, 9, 13, 17, 21, 24, 27, 30 and 33 of this *Journal* were selected for study of the source of references cited in articles dealing with original research and review articles of interest to the industry. A graduate student was assigned a single volume and was instructed to determine the number of papers in the volume and the number of literature citations, to list each journal cited and to record the frequency of such citations. Bulletin citations and their frequency were recorded, but the references to textbooks were not. The data then were colligated and summarized by the author.

No attempt was made in the summary to distinguish between citations from original articles dealing with dairy production or dairy manufacturing subject matter. Except for the use of trade journals and reference to some field-specific journals, such as those dealing with genetics for a relatively limited number of production papers, there appears to be little difference between the two fields of specialization in the kind of reference journals used, though the individual articles cited may have been quite different.

RESULTS

In the ten selected volumes, 724 original and review articles were published. Reference was made a total of 5,023 times to 351 journals reviewed by *Chemical*

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Abstracts and a total of 307 times to 180 journals not covered by *Chemical Abstracts*. Reference was made a total of 118 times to bulletins of the United States Department of Agriculture and 864 times to bulletins of the Agricultural Experiment Stations of the states and territories.

As was to be expected, this *Journal* led all others in the number of references made to it and did so in each volume studied except the first. The number of references made to this *Journal* in each of the ten volumes studied and to the ten other leading journals is shown in table 1. A striking fact is that four of the first five other journals are in the field of chemistry. Three of the others are in the field of biology in which chemistry is a basic tool. The other three are in the fields of application.

TABLE 1

Number of citations of this JOURNAL and of the 10 leading journals in 10 selected volumes of the JOURNAL OF DAIRY SCIENCE

	No. of citations in volumes:										Total in the 10 volumes
	1	5	9	13	17	21	24	27	30	33	
J. Dairy Sci.	1	17	49	35	79	147	245	211	275	386	1,445
J. Biol. Chem.	4	13	46	76	73	27	50	55	82	426
Ind. Eng. Chem.	9	19	7	16	32	30	24	137
J. Nutrition	2	8	4	25	35	45	17	136	136
Biochem. J.	1	7	7	9	15	28	29	10	24	130	130
J. Am. Chem. Soc.	3	6	6	6	34	8	6	14	24	107
J. Animal Sci. and Proc. Am. Soc. Animal Prod.	2	1	13	10	20	47	93
Proc. Soc. Exptl. Biol. Med.	1	9	4	3	13	7	21	18	76
J. Dairy Research	6	18	22	15	9	70	70
J. Am. Vet. Med. Assoc.	2	6	2	1	14	8	7	20	60
Am. J. Physiol.	2	10	6	2	4	11	3	11	9	58	58

The total of 351 journals reviewed by *Chemical Abstracts* and cited in the ten volumes of this *Journal* may be classified roughly into the general subject matter areas given in table 2.

Here, the emphasis on use of chemistry and basic biology, including the application in the general field of medicine in its many branches, is overwhelming. A review of the remaining 180 journals mentioned indicates that few of this group would be included under chemistry, but would increase the proportion listed under medicine, general biology and general science, and at least 30 of the list would be classified under dairy trade journals. However, it is not possible to classify this list under the several fields given above, for it contains some references to journals not known to the writer and contains all the errors inherent in such work, including citations where standardized abbreviation forms have not been used and where name changes in journals have occurred. In spite of re-checking the original citations in the *Journal*, it was impossible to classify the original source of some citations. As the total of these is only 307 references, it seemed unprofitable to pursue the matter further.

Languages used. While the country of origin of the work mentioned in the citations would be of interest, of far greater importance to the student is the language in which the journal in question normally appears. Some foreign journals may publish separate articles in a single issue in different languages. Others include summaries in more than one language. No attempt was made to determine for the individual citations the language used. However, considering the country in which the journal was published and the evidence available on publication policy as given in *Chemical Abstracts* (1), 231 of the 351 journals were printed in English, 64 in German, 20 in French, 9 in Italian, 6 in Dutch, 4 in Swedish,

TABLE 2

General subject matter area covered by 351 reference journals cited in ten selected volumes of the JOURNAL OF DAIRY SCIENCE and reviewed by CHEMICAL ABSTRACTS

Area of subject matter	Total no. of journals	Specialization	No. of journals
Medicine	102	General	48
		Pathology, parasitology, pharmacology and immunology	19
		Veterinary medicine	18
		Public health and hygiene	17
Agriculture	69	General	34
		Dairy science	9
		Dairy trade journals	15
		Food technology	7
		Agronomy	4
Biology	60	General	30
		Physiology	13
		Bacteriology	8
		Botany	4
		Zoology	2
		Anatomy	3
Chemistry and physics	60		
Nutrition and pediatrics	15		
Engineering	10		
Genetics and animal breeding	8		
Mathematics and statistics	2		
General science and miscellaneous	25		
Total	351		

4 in Danish, 2 in Norwegian, 6 in Russian, 2 in Japanese and one each in Finnish, Chinese and Yugoslav. Thus, approximately one-third (120) of the different journals cited were published in a language other than English. When to these 120 journals are added the 70 journals, using the English language, which are published in the British Commonwealth of Nations and in India, and 11 journals published in English but in other countries, it is seen that more than one-half of the journals used by dairy scientists in the United States are published outside of the United States.

Approximately one-half of the foreign language journals were published in German. On the other hand, when the ten volumes of the *Journal of Dairy*

Science were grouped into three groups as volumes 1, 5, 9 and 13 in group 1; volumes 17, 21 and 24 in group 2; and volumes 27, 30 and 33 in group 3, it was found that there were 144 references to journals published in German in group 1, 170 in group 2, and only 61 references to journals published in German in group 3, which represents the more recent years. This fact suggests a trend away from the use of the German language by dairy scientists, but does not suggest a reason for this fact. Of the 6,312 references cited, exclusive of textbooks, a total of but 655, roughly 10 per cent, were to journals published in a language other than English.

Although an analysis of the use of textbooks as reference material by dairy scientists was not made in this study, an impression was gained in carrying out its details that textbooks were used mainly in reference to standard methods. Frequent use of textbooks was made in referring to the mathematical or statistical techniques employed. Thus, more emphasis was placed on mathematics and on the analytical methods of statistics in the original articles than is suggested by the number of different journals referred to in that field.

In a study of this sort one is impressed not only by the emphasis placed by dairy scientists in their writings on the fundamental sciences, especially chemistry and physics, but by the enormity of the scientific literature having a bearing on the field. It would appear that, to be properly informed in his own field and in the sciences related to it, the dairy scientist, as perhaps are all applied scientists, is faced with a task of reading a wider variety of journals than is the worker in the basic fields.

With the rapid advances now current in science, especially in chemistry and physics, and the shifts apparent in specialized agricultural science towards a closer union with chemistry, mathematics, physics and biology in its several branches, the question may be pertinent as to whether the training currently received by students in the dairy field will prove sufficient to form the background for comparable progress.

SUMMARY

A survey was made of the source of literature citations in ten volumes of the *Journal of Dairy Science*. While the *Journal* provided nearly 25 per cent of the total references, citation of chemical journals occurred more frequently than did those from any other field. Citations from journals in the biological and medical sciences followed in order. German was the foreign language most frequently used in the citations, followed by French, Italian and the Scandinavian languages. However, only slightly over 10 per cent of the reference citations, exclusive of textbooks, were to journals published in a language other than English; of these somewhat over one-half were published in German.

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- (2) VOIGT, M. J. Scientific Periodicals as a Basic Requirement for Engineering and Agricultural Research. College and Research Libraries, 8 (3): 354-359, 375. July, 1947. Am. Library Assoc., 50 E. Huron St., Chicago 11.

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

BOOK REVIEW

314. The Mammary Gland I. The Anatomy of the Udder of Cattle and Domestic Animals. C. W. TURNER. Lucas Bros., Columbia, Mo. 389 pp., 72 tables and 205 illustrations. Case bound in maroon Dupont Fabrikoid. \$10.00. 1952.

This is a welcome revision of the 1939 "Multi-lith" publication. The author has done an excellent job and again makes available the most complete information on the subject and presents it in a comprehensive manner.

The subject matter consists of an introduction in which the skeletal structure and conformation of the dairy cow are considered. Part I consists of 7 chapters and, while the emphasis is on the anatomy of the udder of cattle, due consideration is given to the nervous, vascular and lymphatic systems. An entirely new chapter on the "Surgery of the Udder and Teats" has been added. There are 7 chapters in Part II which deal with the microscopic anatomy of the udder of cattle. Three chapters appear for the first time; 1 on the pathologic anatomy of the udder, 1 on histochemistry and 1 on the enzyme systems of the mammary gland. The chapters on histochemistry and enzyme systems are important additions since they summarize a field where there is a great deal of activity. Part III consists of 6 chapters on the mammary glands of hoofed animals. Although the mammary glands of these animals contribute little to the dairy industry, nevertheless they secrete the milk that enables the females to raise their young and hence are of importance to successful husbandry. This book is primarily an anatomy book, yet the author has included sufficient physiology to whet one's interest.

The text is replete with excellent illustrations and there is an adequate index. Desirable features from the standpoint of a textbook are the inclusion of review questions and suggestions for laboratory exercises. Complete references at the end of each chapter are a most desirable feature for the research worker.

R. P. Reece

314a. Sanitation for the Food Preservation Industries. The Association of Food Industry Sanitarians, Inc. in cooperation with The National Canners Association. McGraw-Hill Book Com-

pany, Inc., New York. 284 pp. \$5.00. 1952.

This book contains much detailed information of interest to sanitarians generally but particularly to those in the canning, freezing and dehydrating fields of food processing. For those interested in dairy products sanitation there is information concerning relation of management to sanitation, inspection technics, plant house-keeping, animal and insect pests, water supply, toilet facilities and formulation of detergents.

The material is well organized and is presented in a detailed and easily understood manner. Many practical suggestions concerning food plant sanitation are included.

M. P. Baker

CHEESE

A. C. DAHLBERG, SECTION EDITOR

315. Controlling bacterial spoilage of cottage cheese. P. R. ELLIKER, V. N. SMITH AND R. B. PARKER, Oregon State College, Corvallis. *Am. Dairy Prod. Mfg. Rev.*, **14**, 1: 32-35. Jan., 1952; *Milk Dealer*, **41**, 4: 86-90. Jan., 1952.
See *J. Dairy Sci.*, **34**: 887-893. 1951.

316. Device for cutting a slab from a block of soft material such as butter or cheese. M. J. WEINBERG. U. S. Patent 2,589,911. 7 claims. Mar. 18, 1952. *Official Gaz. U. S. Pat. Office*, **656**, 3: 842. 1952.

A hand operated cutter for slicing cheese and butter is described.

R. Whitaker

317. Apparatus for making cheese. C. COHEN AND M. L. COHEN. U. S. Patent 2,590,510. 8 claims. Mar. 25, 1952. *Official Gaz. U. S. Pat. Office*, **656**, 4: 1100. 1952.

A slightly tapered conical-type cheese hoop is described.

R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

318. Drying centrally controlled. A. ANDERSON, Rochester Dairy Co-op., Rochester, Minn. *Food Eng.*, **24**, 2: 73. Feb., 1952.

Controlling details of a high-volume drying operation required an abnormal amount of supervision and effort. To meet the situation, a multiple-recording potentiometer was installed to bring all vital temperature readings from the

drying floor to 1 central point. The recorder charts records for 4 dryers. It provides a quick check on operating conditions for the product being run and has increased efficiency of spray-dryer operations.
T. J. Claydon

319. Noncrystallizing lactose syrup and method of making. R. J. BLOCK (assignor to the Borden Co.). U. S. Patent 2,592,509. 5 claims. April 8, 1952. Official Gaz. U. S. Pat. Office, 657, 2: 608. 1952.

Lactose in syrup form and at a concentration sufficiently great to cause crystallization is kept from crystallizing by having an equi-molar mixture of dextrose and galactose present at the rate of 40% of the lactose.
R. Whitaker

320. Food product from whey. J. P. MALKAMES, JR. (assignor to the United States of America as represented by the Secy. of Agr.). U. S. Patent 2,585,951. 2 claims. Feb. 19, 1952. Official Gaz. U. S. Pat. Office, 655, 3: 664. 1952.

A palatable food product is prepared from the precipitated protein of whey by action of a protease of fungal origin. The protease, with 1 or 2% of salt based on the curd, is allowed to act at 20–50° C. for 15 min.–24 hr. until the formol titration is between 0.1–0.2% amino nitrogen. The enzyme finally is inactivated.
R. Whitaker

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

321. Coliform bacteria in pasteurized milk. W. H. HASKELL, Klenzade Prod., Inc., Beloit, Wis. Milk Dealer, 41, 6: 48, 76–77. Mar., 1952.

The presence of coliform bacteria in milk usually involves 1 or more of the following sanitation failures: (a) Underpasteurization; (b) adding raw milk to vats during the periods of pasteurization; (c) rinsing equipment, after sanitation, with contaminated water; (d) exposure of milk equipment to dust and fly contamination; (e) hand contamination of equipment; (f) inadequate cleaning and sanitizing.
C. J. Babcock

322. Inhibition of bacterial growth by bacteriophage as distinct from lytic action. H. R. WHITEHEAD, G. J. E. HUNTER AND G. A. COX, Dairy Research Inst., Palmerston North, New Zealand. J. Gen. Microbiol., 6, 1–2: 21–29. 1952.

In experiments with cultures of *Streptococcus cremoris* used in cheese manufacture, it was demonstrated that bacteriophage capable of lysing certain strains of streptococci would adsorb on and inhibit growth of other strains without causing lysis. In addition, no multiplication of phage was observed. This inhibition was of a temporary nature as compared with that observed in the variants arising as a result of lytic action. Cultures recovered after temporary inhibition contained strains of organisms which were resistant both to inhibition of growth and lysis by phage specific for the original culture.
J. J. Jezeski

323. The mechanism of propionic acid formation by *Clostridium propionicum*. A. T. JOHNS, Grasslands Div., D.S.I.R., Palmerston North, New Zealand. J. Gen. Microbiol., 6, 1–2: 123–127. 1952.

The mechanism of production of propionate from lactate in *Clostridium propionicum* apparently is different than in *Veillonella gazogenes* and certain propionibacteria in the following reactions: Decarboxylation of succinic acid, inhibition of pyruvate and lactate metabolism by NaF, and CO₂ fixation in propionic acid. The suggested scheme of propionic acid formation by *Cl. propionicum* is lactate→acrylate→propionate; but no evidence for the formation of acrylate is presented.
J. J. Jezeski

324. Stérélization par flambages des récipients et utensils utilisés en laiterie (Sterilization by flame of receptacles and utensils employed in the dairy). G. RAY. Lait, 32: 30–35. Jan.-Feb., 1952.

Conditions which prevail on the farm frequently make sterilization of dairy equipment and utensils by conventional methods difficult. Flame sterilization is proposed as a practical solution to the problem. It is recommended that surfaces to be flame-sterilized should be clean and dry, that the burner employed produce a blue flame leaving no residue and the flame should be easily guided and regulated in intensity. The entire surface should be brought systematically in contact with the flame. Combustible liquids such as gasoline, fuel oil and ethyl alcohol, as well as bottle butane and other gases may be used to produce the flame. The design of a burner to use in conjunction with bottle gas is illustrated. Procedures for flaming various utensils and equipment are given.
S. Patton

325. Pour la pasteurization obligatoire du lait de consommation (Toward compulsory pasteurization of market milk). J. PIEN. Lait, 32: 1–19. Jan.-Feb., 1952.

In defense of compulsory pasteurization of milk, statistics on raw milk-borne diseases and epidemics are presented. Typhoid fever, scarlet fever, septic sore throat, gastroenteritis, diphtheria, poliomyelitis and other epidemic cases histories are given. Non-epidemic diseases which occur more sporadically, such as bovine tuberculosis and undulant fever, are discussed, including information relative to numbers of cases, per cent of tuberculosis cases due to the bovine bacillus, foci of infection in children and adults and incidence of various types of tuberculosis in several countries.
S. Patton.

326. Some observations on bacterial growth activity when milk cooling is delayed. G. M. TROUT, A. L. BORTREE, H. M. DELAYA AND P. S. MEDORA, Mich. State College, East Lansing. Am. Dairy Prod. Mfg. Rev., 14, 1: 16–18. Jan., 1952.

The "germicidal property" of milk was rapidly lost at a temperature of 80° F. Milk cooled promptly to 40° F. and held 10 hr. retained its inhibitory property. When the holding temperature was 60° F., little of the retarding effect on growth remained after 10 hr. From the prac-

tical standpoint, if producers are to obtain much benefit from the "germicidal action" during transportation to the receiving plant, milk must be promptly and adequately cooled on the farm.

T. J. Claydon

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

327. Automatic temperature control on rail shipments of milk. Anonymous. *Milk Dealer*, **41**, 6: 45, 60-61. Mar., 1952.

The Bellows Falls Co-operative Creamery, Bellows Falls, Vt., reduced refrigeration costs at least 80% by converting their leased train cars to giant portable refrigerators, by using a self-contained mechanical refrigerating unit similar to the smaller units installed on trucks. A saving of 16% was made in cargo space as the refrigerating mechanism occupied half the space required by ice bunkers.

C. J. Babcock

328. Sheet aluminum reflective insulation for milk storage rooms. Anonymous. *Milk Dealer*, **41**, 5: 46, 54-55. Feb., 1952.

Heat is transmitted by radiation, convection and conduction. Refrigeration insulation should prevent to a substantial degree heat transmission in all 3 categories. Commercial sheet aluminum-reflective insulations are based upon principles entirely different from those involved in insulating by mass insulation materials. Besides being fire-, moisture- and vermin-proof, sheet aluminum is 95-97% efficient in reflecting radiant heat. Aluminum also is a good conductor of heat. To be effective as a refrigeration insulation, more than 1 layer of sheet aluminum must be used. The number of layers is determined by the degree of coldness that is to be maintained in the unit. The aluminum layers are separated by an air space, which itself is a good insulator, and minimizes the transfer of heat by convection and conduction. Aluminum insulation also acts as a vapor barrier which is necessary if an insulated structure is to maintain its efficiency. Some experiences with this type of insulation are discussed.

C. J. Babcock

329. New developments in ice cream hardening rooms. G. ANDERSON, The King Co., Owatonna, Minn. *Ice Cream Trade J.*, **48**, 2: 28-30, 104. Feb., 1952.

In addition to cork board, foam glass, rubbatex, stereform and alumiseal now are available for ice cream hardening room insulation. Although somewhat more expensive than cork, these materials are used in combination with cork to improve the moisture barrier properties of the wall. The trend today is away from multiple rooms to 1 large room, resulting in a saving in labor costs, simplified handling of products in the room, improved house keeping and better inventory control.

Rules to follow in low-temperature room construction are: (a) Provide the best moisture barrier possible on the warm side of the room walls, ceiling and floor. (b) Provide adequate, well-ventilated crawl space for inspection and

repair of ceiling, or if no crawl space is possible, the ceiling should be treated with the best possible moisture barrier. (c) Reduce infiltration to a minimum by having a minimum of openings into the room, tight-closing doors and properly maintained double-batten, double-swing doors. (d) House conveyers in an insulated tunnel with doors on the cold end of the tunnel fitted with 2 sets of soft rubber strip curtains or well-maintained, self-closing flapper doors. (e) Provide removable prefabricated ante-room to prevent direct air and moisture infiltration. (f) Ventilate or insulate the fill under ground-floor rooms to protect against freezing. (g) Use floating room construction which provides for expansion and contraction with temperature changes. (h) Provide ceiling heights of 10-12 ft. for good distribution of cold air. (i) Provide for a maximum storage space of 12 gal./ft.². (j) Equip room with an alarm system, axe and wrecking bar.

Frost-freeze rooms, with blower units, carried at -15 to -30° F. should be provided. Storage capacity should be 14-16 gal./ft.² for bulk, 10-14 gal./ft.² for packages and 8-12 gal. for novelties and special items. The trend is toward floor stacking of bulk and packages. Conveyers are labor savers and an intercommunication system is a good safety device.

Several types of blower systems are available including water-defrosting units, continuous defrosting units, air defrosting units, hot-gas defrosting units and electrical heating elements.

All low-side refrigeration systems should include sufficient coil surface, trouble-free controls, ducts for air distribution, air change every 35 sec., or 20 sec. if rooms are heavily loaded, air distributed at the ceiling and returned to the floor and the air supply deflected downward at an angle and diffused. Oil drainage from the system is important. Maintenance and defrosting should be simple, checked and maintained regularly. The units should be located with their return air openings at low pressure points as far removed from the walk-in-door and conveyers as convenient and take up as little floor space as possible. Booster compressors reduce power costs and increase flexibility and temperature range available for operators.

W. H. Martin

330. Consider the conveyor line. R. M. CARTER AND A. BRADFIELD, Vt. Agr. Expt. Sta., Burlington. *Milk Dealer*, **41**, 4: 48, 49, 56-57. Jan., 1952.

Observations made in milk plants in the Northeast point to the conveyor line as a major regulator of the rate of intake in a receiving station. The maximum efficiency which may be expected in dumping milk even in plants equipped with an automatic dump and all other customary labor-saving devices is about 720 cans/hr. for each receiving unit. Where hand dumping is used, the top limit is about 500 cans. Under almost identical conditions with optimum loads of 50 cans, workman are able to place about 600 cans/hr. onto a conveyor line. In other words, 12 trucks with 50 cans each could drive up, unload and drive away in 60 min. During 52 min.

of every hour, cans could be placed on the conveyor line; and during the remaining 8 min., or $\frac{2}{3}$ min./truck for moving in and out, no cans would be unloaded. This means 12 breaks in can reception of about 40 sec. each due to truck movement, or about time enough to have unloaded 10 cans. Accepting 600 cans/hr. as about maximum unloading speed for truckers, 500 cans is a reasonable expectation at plants where variability in load size ranges from 6–160 cans/load. This number of cans may be delivered at the dump every working hr., by using either gravity or power conveyors. Graphs are presented showing the relative merits of various types of conveyor systems as applied to 5 possible types of conveyor arrangements. Data are presented showing that the conveyor line should be long enough to handle all cans from the maximum load delivered to a receiving station and as much of that conveyor as possible should be at the receiving end of the can washer.

C. J. Babcock

331. Set-up for safety. D. K. MacDONALD, D. K. MacDonald & Co., Seattle, Wash. *Am. Dairy Prod. Mfg. Rev.*, **14**, 2: 22, 23, 28. Jan., 1952.

Installation of a properly managed safety program by a dairy products manufacturer will not only help to prevent accidents to his trucks on the highways, but will contribute other important advantages. The requirements of such a program are: (a) See your insurance company representative; (b) determine the incentive which will be offered to enlist cooperation of your driver-salesmen; (c) present the program to your driver-salesmen in a clear persuasive manner; (d) require physical examinations of drivers; (e) keep records of conditions and factors causing accidents; (f) remove accident-prone drivers from the payroll; (g) establish controls for proper maintenance of vehicles. Explanations, with examples, are given for the requirements.

T. J. Claydon

332. Single or multiple floor plant design. G. R. JOHNSON, Pace Associates, Chicago, Ill. *Milk Dealer*, **41**, 6: 44, 90–92. Mar., 1952.

Important questions that must be answered when starting a plant improvement program are: (a) Can results be achieved by installing new equipment, must the plant be remodeled or should an addition be added? (b) Is a new plant necessary or economically advantageous? (c) What products and how much of each should be processed and distributed? (d) How much expansion should be provided for? (e) What techniques of reducing labor can be adopted? and (f) Is a 1- or 2-story building more desirable? Important questions that can influence the selection of a single or multiple-story building are discussed. Other factors which need study are flexibility of planning and operating, provision for expansion, engineering of trucking facilities and distribution techniques, site selection and subsurface soil conditions.

C. J. Babcock

333. Flow line sampler. R. M. LANGSENKAMP and J. M. TRIMBLE (assignors to The Langsenkamp-Wheeler Brass Works, Inc.). U. S. Pat-

ent 2,589,712. 2 claims. Mar. 18, 1952. Official Gaz. U. S. Pat. Office, **626**, 3: 786. 1952.

A device for continuously sampling a fluid, such as milk, as it flows through a pipeline under positive pressure is described.

R. Whitaker

334. Sanitary milk bottle capping head. R. E. BODENDOERFER, D. C. FEE AND H. C. FRENTZEL (assignors to Federal Mfg. Co.). U. S. Patent 2,591,501. 4 claims. April 1, 1952. Official Gaz. U. S. Pat. Office, **657**, 1: 266. 1952.

Details are given for a device for inserting disc caps on milk bottles.

R. Whitaker

335. Special heat transfer problems of the food industry. Engineering and process development. J. P. BOLANOWSKI AND D. D. LINEBERRY, The Girbler Corp., Rotator Div., Louisville, Ky. *Ind. Eng. Chem.*, **44**, 3: 657–660. Mar., 1952.

A special, continuous, agitating heat exchanger suitable for crystallization, emulsification, aeration, pasteurization and sterilization of food is described. The exchanger consists chiefly of a long cylinder through which the product is forced by agitating scraper blades. The blades scrape the heat exchange surface and prevent accumulation of crystallizing or burned material on this surface. The product remains in the heating or chilling zone for 6–20 sec., being propelled by the rapidly revolving blades. The exchanger is widely used for plasticizing margarine. It has been used for sterilizing homogenized milk at a rate of 214 lb./hr., raising the temperature from 50–290°F. Cream, concentrated, evaporated and chocolate milk also have been sterilized and aseptically packaged.

B. H. Webb

336. Liquid industrial wastes. Dairy industry. H. G. HARDING, Natl. Dairy Research Labs., Inc., Oakdale, L. I., N. Y. *Ind. Eng. Chem.*, **44**, 3: 487–491. Mar., 1952.

The biochemical oxygen demand in dairy waste waters should be reduced by waste prevention and waste saving. It is best to segregate the various liquids wastes in the plant for separate treatment or disposal. Domestic wastes should be discharged to city sewers or septic tanks. Uncontaminated water should be segregated and reused if possible. Spoiled or excess products should be processed for animal feed. The dairy waste should be combined and treated with city waste whenever possible. In small dairy plants, aerated flow equalization may provide sufficient treatment or a valuable pretreatment. Present trends are toward modifications of standard biochemical oxidation treatments in trickle filters or activated sludge systems. There is no satisfactory method for disposal of sludge from trickle filters and aeration units.

B. H. Webb

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

337. Streamlining receiving operations. C. E. FRENCH, Purdue Univ., Lafayette, Ind. *Food Eng.*, **24**, 1: 99–102, 160. 1952.

Studies in 70 milk receiving plants showed

wasted time varied from 28-76% in different plants, with larger crews wasting more time than smaller ones. Adoption of work simplification practices and changes in installations resulted in reduction in labor costs. In some instances labor costs were cut 50% by proper arrangement of the right equipment and effective utilization of time. In 2 plants where equipment changes were made, receiving labor was reduced one-third in 1, and one-fourth in the other. The costs of making the changes were more than offset by savings achieved in a few months. T. J. Claydon

338. The trend of milk packaging and distribution. G. NEELEY, Nat'l. Assn. of Sanitary Milk Bottle Closure Mfrs., Philadelphia, Penna. Milk Dealer, 41, 5: 41, 102-105. Feb., 1952.

The mortality of dairies in the last 15 yr. has been around 50%. This has been due to various factors—mergers, consolidations and bankruptcies. Profits of milk distributors are measured in fractions of a cent. According to the Penn. Milk Commission, 565 dairies in that state submitted financial statements in 1949 and the average net profit per dollar of sales after taxes was only 3%. This means a net profit of only 3/5¢/qt. 127 dairies in this group, or 1 out of every 4.5, actually showed a net loss for their operations for the year. Another survey made by the Indiana Univ. Bureau of Business Research on a nation wide scale covering 313 companies having over a billion dollar sales in 1949, showed an average net profit of only about 2/5¢/qt. Some of the major problems confronting independent dealers are discussed. It is recommended that strong emphasis be placed on the retail market, superiority of products and packages, modern merchandising techniques, cultivation of the customer, operating efficiencies and education. C. J. Babcock

339. Reducing your delivery costs. Anonymous. Milk Dealer, 41, 6: 70-72. Mar., 1952.

In the field of traffic management, milk dealers do not have the same analysis and control which exists in other parts of their business. Some of the faults are: (a) Delivery costs are not figured on a route basis; (b) failure to cost each individual vehicle; (c) rivalry between sales and transportation, working to the disadvantage of both; (d) subordination of delivery considerations to sales considerations with inadequate knowledge of the cost factors involved; (e) vehicle maintenance, and even decisions as to vehicle replacements are influenced to an undue degree by sales. Five recommendations made to bring improved fleet operation, lower operating and maintenance expense and extended economical vehicle life are to install maintenance records, install preventive maintenance, install adequate maintenance facilities, replace inefficient vehicles and continue to watch fleet costs. C. J. Babcock

340. Is this the end of an era? E. L. GENARO, Nat'l. Sales Council, Hazelton, Pa. Milk Dealer, 41, 6: 46-56. Mar., 1952.

The following reasons are advanced to show

that door-step delivery is coming to an end: (a) With delivery costs reaching an all time high of 11¢/qt., in some markets, there seems to be cause for concern. (b) Contrary to belief, the modern housewife does not appreciate the so-called convenience of door-step delivery. (c) The trend is toward store sales throughout the country, despite stubborn resistance of some milkmen. New York City already sells nearly 80% of its milk through stores, Los Angeles nearly 75%. (d) The easy switch made from daily, early morning delivery to every-other-day, daylight delivery, is a "tip-off" that the housewife really was and is not concerned about the "wonderful service" supposedly inherent in door-step delivery. (f) Elimination of door-step delivery and its added cost of collections and credit losses would make it possible to reduce the resale price of milk and increase the volume of sales. On the opposite side the following points are made: (a) Retail door-step delivery provides a controlled, steady and wholly predictable market for the average milk company which makes for a more efficient dairy operation. (b) Door-step delivery provides a wonderful opportunity for aggressive selling on a personal basis, which is impossible in store sales. (c) Milk is in a class by itself and personal, door-step delivery is appreciated and desired by the housewife. (d) Several well known milk companies, originally established as 100% store distributors of paper bottled milk, have found it advantageous and desirable to add retail delivery routes to reach a market they were losing by exclusive store sale. (e) Excessive delivery costs can be offset by carrying a larger, more versatile line of dairy products to the door, thereby making each stop more efficient sales-wise.

C. J. Babcock

341. Prices for ice cream and their relation to profits. K. C. FISHER, Gen. Ice Cream Co., Schenectady, N. Y. Ice Cream Trade J., 48, 2: 46, 47, 92-96. Feb., 1952.

On a gallon basis, a net profit of 12¢/gal., after taxes, a few years ago required a net profit before taxes of 20¢/gal. compared to 35¢/gal. today. Increased efficiency and price increases to cover the increased cost of doing business will be required for an industry to maintain its profit. About the only way for the ice cream industry to get any relief from increased costs is to take advantage of the parity adjustment provision of the General Ceiling Regulation. The base period to be used in making calculations is Dec. 19, 1950-Jan. 25, 1951. The 2 principal products are butterfat and solids-not-fat. The regulation provides that if "the cost to you of a current purchase of the listed agricultural commodities exceeds the highest price you paid during the base period, you may increase your ceiling price for the commodity by the dollars and cents difference per unit between the highest price paid by you for a customary purchase during the base period and the cost to you of the most recent customary purchase", you may increase your ceiling prices of ice cream by an amount equal to the

increased ingredient cost of the fat and solids-not-fat contained therein. Ceilings may be increased by filing of a proper notification, even though you may or may not actually put new prices into effect. A letter must go to the Director of Price Stabilization, Washington 25, D. C., by Registered Mail giving existing ceiling and description of the commodities, the highest price you paid for a customary purchase of the pertinent commodity during the base period, the new cost of the pertinent commodity and increased price. It now appears that ingredient costs will be about 12.5¢/lb. of fat and 2¢/lb. of nonfat solids higher than last year, depreciation will be up about 13-14%, labor up about 10%, containers up 13.5%. Any decrease in volume will increase expenses. W. H. Martin

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

342. Syndactylism in Holstein-Friesian cattle. F. E. ELDRIDGE, W. H. SMITH AND W. M. MCLEOD, Kansas State College, Manhattan. *J. Heredity*, **42**: 241-250. 1951.

Failure of the common digital extensor to be divided into 2 branches at the fetlock joint and insertion of this extensor into the proximal end of the 1st phalanx characterizes this syndrome found in Holstein as well as Indian cattle. Associated flexors (medial, lateral, superficial and deep-digited flexors) accommodated this abnormality, as did the ligaments and other tissues. In distinction to 1-hoofed ungulates which have an enlarged digit, bovine syndactylism results from a fusion of the 2 distal digits. The dew claws were located normally but the lateral dew claw was larger than the medial one. Fore limbs are affected more often than rear limbs.

Seventeen cattle of both sexes from several farms have been observed. Autosomal recessive inheritance was indicated from pedigree analysis. A low frequency of the responsible gene was suggested. Difficulties of eliminating recessive genes from a breed are discussed. L. O. Gilmore

343. Device for artificially inseminating animals. D. W. EDWARDS. U. S. Patent 2,587,984. 2 claims. March 4, 1952. Official Gaz. U. S. Pat. Office, **656**, 1: 169. 1952.

Structural features are given for a piston-type injector for introducing the semen into the vaginal passage. Provision is made for first withdrawing a portion of fluid from the uterus and then forcing it back again after release of the semen. R. Whitaker

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

344. Branding and dehorning chute for cattle. A. OSMETTI. U. S. Patent 2,591,191. 8 claims. April 1, 1952. Official Gaz. U. S. Pat. Office, **657**, 1: 183. 1952.

A device is described which holds an animal while it is branded or dehorned. R. Whitaker

345. Cattle guard. G. C. WELTER. U. S. Patent 2,591,220. 4 claims. April 1, 1952. Official Gaz. U. S. Pat. Office, **657**, 1: 191. 1952.

A rail-type of guard for keeping cattle from crossing ditches, culverts and roadways, etc. is described. R. Whitaker

346. Fertilizer separator for barns and like structures. J. VOLMER. U. S. Patent 2,591,643. 1 claim. April 1, 1952. Official Gaz. U. S. Pat. Office, **657**, 1: 302. 1952.

A conveyor, operating in the gutter, moves the litter from behind a row of cows to a trough at a lower level. R. Whitaker

347. Stanchion. A. E. ANDERSON. U. S. Patent 2,591,766. 3 claims. April 8, 1952. Official Gaz. U. S. Pat. Office, **657**, 2: 410. 1952.

A stanchion for cows is attached to the floor and ceiling with chain and is so counterbalanced that the frame may be adjusted easily either to the open or closed position. R. Whitaker

348. Anti-kicking device for cows. E. L. MCCLEES. U. S. Patent 2,591,958. 6 claims. April 8, 1952. Official Gaz. U. S. Pat. Office, **657**, 2: 464. 1952.

To prevent cows from kicking, a stiff bar is clipped by spring-controlled clamps to the cows' legs above and below the knees. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

349. Bananas, from the plantation to the ice cream plant. E. R. JAGENBURG, JR., Pres., Brooklyn, N. Y. *Ice Cream Trade J.*, **48**, 2: 52, 107-108. Feb., 1952.

Vast improvements have been made in the production of frozen banana puree. Stainless steel equipment is used to insure the making of a frozen, fresh puree that is uniform in flavor, color and consistency. New production-line concepts coupled with sanitation standards and techniques, comparable to those found in the ice cream plant have been developed to enable the making of an almost sterile product. Today's banana puree is ideal for sherbets, low-fat and other frozen dairy foods made by the ice cream industry. New flavors combining banana puree with pineapple, orange sherbet with banana rippled through and sundry other combinations offer interesting variations. W. H. Martin

350. Ice cream composition. J. LOKEMA (as signor to Naamlouze Vennootschap: W. A. Scholten's Chemische Fabrieken). U. S. Patent 2,590,077. 3 claims. Mar. 25, 1952. Official Gaz. U. S. Pat. Office, **656**, 4: 984. 1952.

A stabilizer for ice cream is described consisting of water-soluble starch ethers and esters. R. Whitaker

351. Ice cream freezer support. W. W. VARNADO. U. S. Patent 2,592,495. 6 claims. April 8, 1952. Official Gaz. U. S. Pat. Office, **657**, 2: 605. 1952.

An ice cream cabinet is so modified that con-

tainers of ice cream are held in a sleeve-like frame, supported from the top of the cabinet; containers can be placed in the cabinet with the top closed. R. Whitaker

352. Carton for making ice cream sandwiches. P. I. HENDERSON. U. S. Patent 2,591,812. 2 claims. April 8, 1952. Official Gaz. U. S. Pat. Office, **657**, 2: 423. 1952.

Ice cream from the freezer is introduced between 2 thin wafers in a folded cardboard frame. R. Whitaker

353. Mixing machine. A. F. KIPPER (assignor to Arden Farms Co.). U. S. Patent 2,589,615. 21 claims. Mar. 18, 1952. Official Gaz. U. S. Pat. Office, **656**, 3: 761. 1952.

This machine is designed for use at soda fountains to produce individual servings of very heavy-bodied milk drinks from milk, ice cream, ice milk and flavoring materials. R. Whitaker

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

354. Tank truck pick-up of farm tank bulk milk. E. THOM, Ed., Milk Dealer. Milk Dealer, **41**, 4: 38, 39, 100-107. Jan., 1952.

Tank truck pick-up of farm tank bulk milk originated in California 13 yr. ago. The system also is now used in Columbia, So. Carolina, Hadonfield, N. J., Hartford, Conn., Iowa City, Ia., Washington, D. C., Pocatello, Idaho, Lynden, Wash. and LaGrange, Ill. There are 4 types of tanks in use: (a) The plain insulated tank where the milk is cooled over a surface cooler and then stored in the tank; (b) a cold wall tank, which has refrigeration coils or a liquid refrigerant in the jacket between the outer shell and the inner stainless steel shell; (c) an integrated tank with submersible surface cooler and condensing unit built into it; (d) an enclosed case, originally designed for cooling cans, but which has been converted or can be converted into a bulk cooler. This has 1 or 2 compartments with 1 or 2 tanks. The 2-tank-system permits holding morning milk in 1 tank and evening milk in the other. The size of the tank trucks range from 1,000- to 3,000-gal. capacity with 1,500 being the most generally used outside of California. Milk dealers and producers regard the advantages of the system as far outweighing its disadvantages. The disadvantages discussed relate mainly to the problem of sanitation. C. J. Babcock

355. Half-gallon containers show increased popularity in many areas. Anon. Milk Dealer, **41**, 4: 52-55. Jan., 1952.

Sales of milk in 1- and 0.5-gal. containers in several marketing areas in the United States have shown varied and interesting changes in the last 2 yr. In Jan., 1951, 23.1 and 31.4% of the milk bottled and sold in Milwaukee was in 1- and 0.5-gal. containers, respectively. In July, 1951, 22.2% was in 1-gal. containers and 35.3 in 0.5-gal. containers. In the Chicago market the main shift has been toward less milk sold in quarts

and greater amounts sold in 1- and 0.5-gal. packages. The 1-qt. container presently represents only a little more than 1/3 of the fluid milk sold. In Oklahoma City, the shift to 0.5-gal. containers is clearly indicated. In the Quad Cities of Ill. and Iowa, gallon sales are confined primarily to restaurants, bakeries, soup companies, etc. During Aug., 1951, gallon sales showed a decrease of 9.5 and 12.8% from Aug., 1950, and Aug., 1949, respectively. On the other hand, sales in 0.5-gal. containers showed an increase this last Aug. of 48% over 1950 and 3,767% over sales during Aug., 1949. In the Dubuque, Ia. market, gallon sales this last Aug. showed a decrease while 0.5-gal. sales showed an increase of 127% over Aug., 1950, and 2,334% over Aug., 1949. In New York City, the use of 0.5-gal. bottles has fallen off sharply since 1949. In 1949, 13% of the milk was marketed in this size container, while in 1951 less than 2% of the milk was distributed in 2-qt. containers. No gallon containers were used in either Knoxville or Kansas City but the use of 0.5-gal. containers has shown a steady increase in these areas. C. J. Babcock

356. Dating—an essential and necessary public health control measure. G. F. KIRCHOFF, Jefferson County Board of Health, Birmingham, Ala. Milk Dealer, **41**, 6: 49-50, 120-130. Mar., 1952.

Dating of milk by expressing the day of the week on the cap, hood or container has public health significance. It is of considerable value as a control measure by furnishing the consuming public information on the age of milk distributed. Data show that the age factor has a bearing on wholesome, palatable quality of milk. Possible evils of not dating are: (a) The consumer is placed in a position of questioning the milk supply because of the age factor. (b) Removal of the day of week will develop the practice of milk remaining on sale until sold regardless of quality. (c) Stores, in many instances, will become overstocked by competing dairies. (d) Milk returned to the dairy at completion of the day's delivery after having remained on delivery trucks a full day, instead of being utilized for by-products, will be stored in the plant and reloaded for the following day's delivery and subjected to a 2nd day of varying temperatures and quality deterioration. (e) Making use of coding as an alternative in place of the day of week gives knowledge of age only to health department authorities and the distributor. (f) Without dating and depending on consumer complaints to determine if stocks have remained in the store too long may well be a costly practice when judged on the loss of consumer confidence in the ability of the distributor to keep fresh milk, free from objectionable flavor or quality deterioration on the market. (g) Attacks on the dating requirement in markets where it is in effect are very largely a matter of commercial expediency to extend the age of milk when placed on sale to the extent the consumer is penalized when quality becomes questionable. Data show dating as required in 44 cities. C. J. Babcock

357. Le problème zootechnique de la production laitière (The animal husbandry problem of milk production). G. THIEULIN. *Lait*, **32**: 19-30. Jan.-Feb., 1952.

An extensive report on milk production and consumption throughout the world is given.

S. Patton

358. Method for producing cocoa-milk beverage material. G. GRINDROD. U. S. Patent 2,589,801. 14 claims. Mar. 18, 1952. Official Gaz. U. S. Pat. Office, **656**, 3: 811. 1952.

A cocoa, milk and sugar concentrate testing about 38% total solids is agitated at coagulating temps. to cause adsorption of milk solids on the cocoa particles. The product then is diluted with water, canned and sterilized. R. Whitaker

359. Chocolate milk. 3. Its place in the school

lunchroom. Anon. *Milk Dealer*, **41**, 4: 46, 92-94. Jan., 1952.

Evidence indicates that total milk consumption increases when chocolate and plain milk are simultaneously available. Children and young people choose chocolate milk when it is available in preference to soft drinks and/or candy where they would not choose plain milk. All milk sold under the National School Lunch Program must be whole milk, whether plain or chocolate flavored. Many schools not participating in the National School Lunch Program require that all milk sold in the school lunchroom be whole milk, whether plain or chocolate flavored. Increasing milk consumption among school children is nutritionally desirable and, where making chocolate milk available to them along with unflavored milk is a means to that end, the practice has merit. C. J. Babcock

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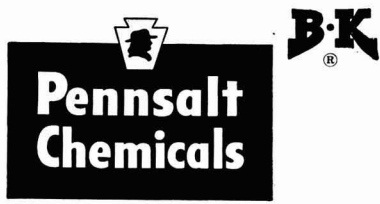
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