ERRATA, VOL. XXXI

No. 4, page 297, lines 4 and 7. *Nitrate* should read *nitrite.*
No. 8, page 717, line 32. *High* should read *low.*
No. 9, page 768, line 18. *763–768* should read *769–777.*

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IN MEMORY OF ROBERT BEAR STOLTZ

On October 2, 1948, the bottom seemed to drop out of many, many lives, for on that day Robert Bear Stoltz was called to a greatly needed rest by his Maker. Great was the loss felt by his family and the host of friends he left behind. On the day of his burial, even the heavens wept unashamedly.

The greatness of Robert Stoltz can best be measured by the record of his achievements. His motto could easily have been, "What do we live for, if it is not to make life less difficult for others".

He was first of all a wonderful husband and father of four children, and took great pride and joy in his five grandchildren.

He was a true friend and wise councilor, none ever were better, for he had the wisdom, tact and diplomacy that rarely is found today.

He truly was a great educator, administrator and organizer.

Professor Stoltz was 58 years old, a relatively young man, but one who had crammed more genuine greatness and achievement in that short span than many who live 20 to 30 years longer. He was an untiring and thorough worker.

Professor Stoltz had been associated with the Ohio State University, College of Agriculture since his graduation in 1912. In 1923 he was promoted to full professor, and in 1929 he was made the chairman of the then newly organized department of Dairy Technology. This department soon became outstanding under Professor Stoltz' guidance for the backing it received from the dairy industry in the State. He was instrumental in the organization of the Ohio Swiss Cheese Association in 1918, and was its secretary and treasurer until 3 years ago. He also promoted the organization of the Columbus Milk Distributors Association in 1934 and was its secretary until 3 years ago. This organization has done much to promote friendly relations among its members. He was also instrumental in their adoption of the universal milk bottle shortly after the organization was founded.

Other organizations recognized Professor Stoltz' ability. He was at one time secretary of the National Cheese Association, and since 1936 he was secretary-treasurer of the American Dairy Science Association, after being president of this organization in 1934. He did much to increase the membership of this organization and put it on a sound financial basis. In 1947 he was presented with the Association Award giving him honorary life membership in the ADSA. He was also a member of the Advisory Council of Sealtest, Inc.

In 1937 he made a study of dairying in New Zealand and Australia.

Ever mindful of both education and the dairy industry, he believed in turning out students well versed in the fundamentals of dairying and in the psychology of "how to win friends and influence people."

Because sufficient funds were unavailable at Ohio State University for research in dairy technology, Professor Stoltz, as an honorary member of the Board of Trustees of the Ohio Dairy Products Association in an advisory capacity, suggested and worked diligently toward the promotion of a research fund by the dairy industry in the State. This fund, known as the Ohio Dairy Products Research Fund, became a reality and today amounts to considerably more than $100,000, the interest on which at 6 per cent is used for research in dairy technology at The Ohio State University. It has since been suggested that the name of this fund be changed to the Robert B. Stoltz Memorial Research Fund.

Professor Stoltz was listed in *Who's Who in America, Who's Who in American Education,* and in *American Men of Science.* He was a member of the Ohio Post-War Program Commission, and of the Columbus Rotary Club.

Professor Stoltz was very active in Masonry, and if ever a man was a true Mason and lived up to the Masonic Creed, it was Bob Stoltz. He was a 33rd degree Mason, and was elected this year as Deputy General Grand Master of the General Grand Council, R. & S. M., of the United States; had served as Grand Master of the Grand Council, R. & S. M. of Ohio; was Past Master of University Lodge and for 14 years its secretary; a member of York Chapter, R. A. M.; York Council, R. & S. M.; Columbus Commandery, Scottish Rite; Aladdin Temple; and Red Cross Constantine.

He was a member of Acacia Fraternity, and Delta Theta Sigma and Gamma Sigma Delta honorary fraternities.

Professor Stoltz was a native of Bradford, Ohio, where he was born March 6, 1890. Surviving are his widow, Mrs. Marie Cassel Stoltz, a son, Philip, three daughters, Mrs. Bonnie Marie Downes, Mrs. Susan Ann George, and Mrs. Roberta Mary Miles.

"Patience, kindness, generosity, humility, courtesy, unselfishness, good temper, guilessness, sincerity—these make up the supreme gift, the stature of the perfect man." If that is so, then Bob Stoltz was a perfect man because he possessed all of them to a very marked degree.

L. H. BURGWALD

THE INFLUENCE OF WATER LEVEL AND TEMPERATURE OF STORAGE ON CAROTENE PRESERVATION IN DEHYDRATED ALFALFA, CEREAL GRASSES AND MIXED FEEDS[1]

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In earlier papers (2, 3), it was pointed out that the carotene of dehydrated alfalfa or dehydrated cereal grasses could be preserved completely if the water content was adjusted to 12 to 20 per cent level and the material stored at 22 to 25° C. in airtight containers. It was postulated that this preservation was related to the speed of reaction of respiratory enzymes which in turn was related to the moisture content of the material.

EXPERIMENTAL

Further studies now have been made with lower water levels in these dehydrated materials stored for 3 months at 22 to 25° C. and 33 to 36° C., respectively. The water levels ranged from 0.9 to 15 per cent with graded increments generally of 2.5 per cent. The materials used were: (a) A dehydrated commercial alfalfa prepared in October, 1947, with an initial carotene content of approximately 350 γ per g. and a water content of 3.6 per cent. (b) An alfalfa cut from a University field in September, 1946, and dried in the laboratory at 50° C. This product was dried further in a vacuum oven for 24 hours at 50° C. prior to starting the experiment. The material, when put up for experimental observation, had a carotene content of approximately 150 γ per g. It consisted of both stem and leaf. (c) A dehydrated alfalfa[2] which was dried for 2.5 hours at 95° C. before being used in these water level experiments. It contained 154 γ of carotene per g. (d) A dehydrated cereal grass[2] which contained approximately 160 γ of carotene per g. and was dried for 2.5 hours at 95° C. before being used in the experiments.

All of these materials were adjusted to different water levels and placed in ice cream cartons holding about 250 g. The control was unwaxed, and the remaining cartons were dipped several times in Flexowax to insure complete exclusion of oxygen. They then were stored at 22 to 25° C., and duplicate sets stored at 33 to 36° C. for 3 months. At the end of that time, the cartons were opened, sampled for carotene determination and observations on color and aroma made. The data giving the results are found in tables 1 through 4.

Since it was possible to preserve the carotene in these dehydrated materials

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[2] Supplied by the Cerophyl Laboratories, Inc., Kansas City, Mo.

TABLE 1

Effect of water level and temperature on carotene, color and pressure of dehydrated alfalfa a. (Carotene data on water free basis)

Treatment	Color	Gas pressure	% water	Carotene content		
				Initial (γ/g.)	3 months (γ/g.)	% loss
		Stored at 22 to 25° C.				
No seal	Green	None	5.2	338	118	67.0
Sealed	Green	None	0.9	338	223	34.0
Sealed	Green	None	2.5	338	248	26.0
Sealed	Green	None	5.0	356	338	5.3
Sealed	Green	None	7.5	356	344	3.6
Sealed	Green	None	10.0	356	362	0.0
Sealed	Slight olive green	None	12.5	356	371	0.0
Sealed	Olive green	None	15.0	356	376	0.0
		Stored at 33 to 36° C.				
No seal	Green	None	3.6	338	66	81.5
Sealed	Green	None	0.9	338	230	32.0
Sealed	Green	None	2.5	338	259	23.0
Sealed	Green	None	5.0	356	329	8.0
Sealed	Green	None	7.5	356	348	2.5
Sealed	Olive green	Positive	10.0	356	350	2.0
Sealed	Brown	Positive	12.5	356	363	0.0
Sealed	Brown	Positive	15.0	356	356	0.0

by the procedure outlined, it seemed important to study the losses of carotene in a mixed feed such as is often used in dairy, poultry and hog rations. The ration fed consisted of: soybean meal, 20 per cent; wheat middlings, 20 per cent; wheat bran, 10 per cent; white corn, 21 per cent; oats, 10 per cent; alfalfa

TABLE 2

Effect of water level and temperature on carotene, color and pressure of air dried alfalfa b. (Carotene data on water free basis)

Treatment	Color	Gas pressure	% water	Carotene content		
				Initial (γ/g.)	3 months (γ/g.)	% loss
		Stored at 22 to 25° C.				
No seal	Green	None	6.9	164	122	25.6
Sealed	Green	None	3.4	157.6	129	18.2
Sealed	Green	None	5.0	157.6	136	13.7
Sealed	Green	None	7.5	157.6	144	8.4
Sealed	Green	None	10.0	157.6	146	7.0
Sealed	Slight olive green	None	12.5	157.6	159	0.0
Sealed	Olive green	None	15.0	164	162	1.2
		Stored at 33 to 36° C.				
No seal	Green	None	5.2	164	70	57.0
Sealed	Green	None	3.4	157.6	115	27.0
Sealed	Green	None	5.0	157.6	120	24.0
Sealed	Green	None	7.5	157.6	131	17.0
Sealed	Slight olive green	None	10.0	157.6	140	11.0
Sealed	Slight olive green	Positive	12.5	157.6	136	14.0
Sealed	Olive green	Positive	15.0	164	147	11.0

TABLE 3

Effect of water level and temperature on carotene, color and pressure of dehydrated alfalfa c. (Carotene data on moisture free basis)

Treatment	Color	Gas pressure	% water	Carotene content		
				Initial (γ/g.)	3 months (γ/g.)	% loss
		Stored at 22 to 25° C.				
No seal	Green	None	4.4	154	95	38.0
Sealed	Green	None	3.7	154	111	28.0
Sealed	Green	None	5.0	154	117	24.0
Sealed	Green	None	7.5	154	138	10.4
Sealed	Green	None	10.0	154	139	9.7
Sealed	Slight olive green	None	12.5	154	149	3.2
Sealed	Slight olive green	None	15.0	154	159	0.0
		Stored at 33 to 36° C.				
No seal	Green	None	3.1	154	68	55.0
Sealed	Green	None	3.7	154	104	32.0
Sealed	Green	None	5.0	154	116	24.0
Sealed	Green	None	7.5	154	132	14.0
Sealed	Slight olive green	None	10.0	154	144	6.5
Sealed	Olive green	None	12.5	154	154	0.0
Sealed	Olive green	Positive	15.0	154	159	0.0

meal, 15 per cent; $CaCO_3$, 2 per cent; $Ca_3(PO_4)_2$, 1 per cent; and iodized salt, 1 per cent. The only major source of carotene in this feed was the 15 per cent alfalfa meal. The feed was stored at 33 to 36° C. for 3 months with water levels

TABLE 4

Effect of water level and temperature on carotene, color and pressure of Dehydrated cereal grass d. (Carotene data on water free basis)

Treatment	Color	Gas pressure	% water	Carotene content		
				Initial (γ/g.)	3 months (γ/g.)	% loss
		Stored at 22 to 25° C.				
No seal	Green	None	4.4	166	110	34.0
Sealed	Green	None	1.6	166	115	31.0
Sealed	Green	None	2.5	166	123	26.0
Sealed	Green	None	5.0	166	133	20.0
Sealed	Green	None	7.5	166	146	12.0
Sealed	Green	None	10.0	166	155	6.3
Sealed	Faint olive green	None	12.5	166	161	3.0
Sealed	Olive green	None	15.0	166	174	0.0
		Stored at 33 to 36° C.				
No seal	Green	None	3.0	166	74	55.0
Sealed	Green	None	1.6	166	104	37.0
Sealed	Green	None	2.5	166	112	33.0
Sealed	Green	None	5.0	166	136	18.0
Sealed	Faint olive green	None	7.5	166	150	9.7
Sealed	Olive green	None	10.0	166	164	1.2
Sealed	Olive green	None	12.5	166	164	1.2
Sealed	Brown	None	15.0	166	172	0.0

ranging from 2.3 to 15 per cent under sealed and unsealed conditions. The initial moisture was reduced by drying for 2.5 hours at 95° C. A companion series also was set up but with the addition of certain trace elements now commonly used in such mixed feeds. This series which contained the trace elements was dried initially for 32 hours at 50° C. in a vacuum oven. The trace elements used were 0.02 per cent $FeCl_2 \cdot 4H_2O$, 0.02 per cent $CuSO_4 \cdot 5H_2O$, 0.25 per cent $MnSO_4 \cdot H_2O$ and 0.02 per cent $CoCl_2 \cdot 6H_2O$.

At the end of 3 months, these cartons were opened, carotene determinations made, as well as observations on color, flavor, aroma, rancidity and other characteristics. The initial carotene content of this feed was 39.6 γ per g. It is assumed that practically all of the carotene was in the 15 per cent of alfalfa meal.

The determination of carotene in the mixed feed by the Wilkes method (4) gave high results. This method seemed entirely satisfactory where only alfalfa or a cereal grass was involved, but when applied to the mixed feed with 15 per cent of alfalfa, the results often were 50 per cent too high. This assumes that there was complete carotene conservation with 12.5 to 15 per cent of water when sealed. Apparently, certain interfering pigments which developed during storage were registering as carotene by the Wilkes method.

The Wilkes method was abandoned for the mixed feed analysis, and the general procedures for chromatographic analysis of carotene which are given in *Methods of Vitamin Assay* (1) were incorporated in the determination. The extraction consisted of allowing 1 to 2 g. of sample to stand 16 to 18 hours (in the dark) in 60 ml. of a 2:1 mixture of Skelly B and acetone. After filtering to remove the sample, the extract was evaporated on a steam bath to reduce the total volume to about 50 ml. Saponification was accomplished next by adding 80 ml. of 5 per cent alcoholic KOH to the extract and allowing the solution to stand in the dark for at least 15 minutes.

The Skelly B phase was obtained by the addition of 40 ml. of water. Following re-extraction of the alcoholic phase twice with 25 ml. portions of Skelly B, the combined Skelly extracts were washed five times with distilled water. The extract was evaporated down, and the final traces of moisture were removed under vacuum. Ten to 15 ml. of Skelly B were added immediately to the dried pigments which now were ready to be chromatogramed. The adsorbent employed in the column was a 1:1 mixture of MgO (Micron Brand, no. 2641, Westvaco Corp., Newark, Cal.) with Hyflo Super-Cel (Johns Manville). Following adsorption, the pigments were eluted with a 2 per cent solution of dry acetone in Skelly B. The pigment passing through the column was considered as carotene, and the carotene content of the sample was calculated by standard procedures using pure β-carotene as the reference standard.

The data secured in this experiment are shown in table 5. The striking observation made on these samples was the rancid odor and bleached color in the non-waxed carton containing the trace elements and the total absence of these characteristics when the oxygen was excluded by waxing. When the oxygen was excluded, the greenish color and clean pleasant aroma persisted in all the samples, although with 15 per cent of water the color was slightly olive green.

TABLE 5

Effect of water level, temperature, trace elements, on carotene, color, aroma and pressure of mixed feed with 15 per cent alfalfa stored at 33 to 36° C. (Carotene data on water free basis)

Treatment	Color	Gas pressure	% water	Carotene content		
				Initial (γ/g.)	3 months (γ/g.)	% loss
		No trace elements, Fe, Cu, Mn, Co				
No seal	Bleached	None	3.6	39.6	2.7	93.2
Sealed	Green	None	2.3	39.6	13.7	65.4
Sealed	Green	None	5.0	39.6	16.6	58.1
Sealed	Green	None	7.5	39.6	34.4	13.1
Sealed	Green	None	10.0	39.6	40.8	0.0
Sealed	Green	None	12.5	39.6	41.0	0.0
Sealed	Slight olive green	Positive	15.0	39.6	42.6	0.0
		Plus trace elements, Fe, Cu, Mn, Co				
No seal	Bleached[a]	None	4.3	39.6	1.1	97.3
Sealed	Green	None	3.2	39.6	3.8	90.5
Sealed	Green	None	5.0	39.6	6.9	82.5
Sealed	Green	None	7.5	39.6	27.0	31.8
Sealed	Green	None	10.0	39.6	41.8	0.0
Sealed	Green	None	12.5	39.6	46.0	0.0
Sealed	Slight olive green	None	15.0	39.6	46.3	0.0

[a]Rancid aroma; all other samples had a pleasant aroma.

In the series without the trace elements, the unwaxed material was bleached but possessed a clean non-rancid aroma. In the waxed samples, the greenish color and pleasant aroma persisted in all the samples, although with 15 per cent of water the product was slightly olive green with a slight fermentation aroma.

Involved in the development of the process of carotene preservation in the materials investigated is the question of an economical, practical airtight re-

TABLE 6

Record of preservation of the carotene of dehydrated alfalfa in single thicknesses of Saran tubes (200 gauge). Stored at 33 to 36° C. for 2 months. (Carotene data on water free basis)

Treatment	Color	Gas pressure	% water	Carotene content		
				Initial (γ/g.)	3 months (γ/g.)	% loss
No seal	Green	None	4.2	377	127	67.0
Sealed[a]	Green	None	7.5	377	368	2.3
Sealed	Green	None	7.5	377	364	3.4
Completely waxed	Green	None	7.5	377	366	2.8
Sealed	Green	None	10.0	377	372	1.3
Sealed	Green	None	10.0	377	346	8.0
Completely waxed	Green	None	10.0	377	372	1.3
Sealed	Olive green	None	12.5	377	363	3.7
Completely waxed	Olive green	None	12.5	377	361	3.9

[a] Sealed refers to Saran tubes, waxed only at the end tube joint.

ceptacle. Materials such as tin and iron would be available, but prices probably would prohibit their general use. Sheet aluminum has been tried but is liable to have pin holes and was not effcetive. Fiber cartons allowed air transmission and gave negative results even when lined with asphalt or paraffin paper liners. Waxed cartons were very effective but the process of waxing did not seem practical. Bags made with Kraft paper treated with Melamine resin were not effective. Among the plastics, polyethylene pouches in Kraft paper bags were tried but found ineffective. The oxygen and carbon dioxide transmission rates of cellophane, nylon, parafilm, pliofilm and polyvinyl alcohol were considered too high to warrant a trial.

Saran, a vinyl-vinylidene chloride copolymer manufactured in various thicknesses was investigated. The 200 gauge material was made into tubes about 1 foot long and sealed at the ends and seams with Flexowax. These tubes were filled with dehydrated alfalfa with varying water content (7.5, 10 and 12.5 per cent) and stored at 33 to 36° C. for 2 months. The records with Saran are shown in table 6. The carotene losses were practically zero. While no claim is made that this material is absolutely negative to oxygen and carbon dioxide transmission, yet the rates of transmission must be very low, even where the alfalfa contained 15 per cent of water. Methods of using this material for the production of a bag or a carton suitable for use in the dehydrated alfalfa and dehydrated cereal grass industry are in progress. It also would appear that such an oxygen-impervious bag or receptacle would find large application in the feed and food industry, where exclusion of oxygen from materials surrounded by an inert gas such as carbon dioxide or nitrogen is desirable.

DISCUSSION

The data on the four dehydrated materials show that a definite level of water with exclusion of oxygen can preserve the carotene of these materials. In a previous study (3), the authors demonstrated the effect of moisture upon the rate of respiration in dehydrated materials. A water content of 5 to 7 per cent or lower apparently does not allow a sufficiently rapid rate of oxygen utilization to prevent significant carotene destruction. Dehydrated materials containing 7.5 per cent or more of moisture when sealed showed good carotene preservation; hence, it is concluded that the oxygen tension in such samples is reduced to a low level in a period of a few days. When the water level is above 10 per cent, the partial destruction of chlorophyll generally supervenes. This is especially true when the storage temperature is as high as 33 to 36° C. for 3 months. Shorter periods of storage at such high temperatures may not affect seriously the green color. Since a green color of the product is much prized by the trade, a water level of about 10 per cent under sealed conditions is recommended to achieve a high preservation of the carotene and maintain the green color.

One must expect variation in the behavior of these dehydrated plant materials to the process outlined. Early harvested materials may have a different rate of respiration than those harvested late in the season. A leaf meal would be

expected to behave differently than a meal composed of both leaf and stem. The season's rain fall, the latitude, the type of soil and the method of dehydration all may have their influence on the behavior of these plants under storage. The length of the period between havesting and storage also may be an important factor. These problems might well be studied.

The results on carotene preservation in a mixed feed under sealed conditions, with or without trace elements, are especially interesting. That the carotene from only 15 per cent of alfalfa in a mixed feed can be preserved when the oxygen is excluded is important information. It is believed that not only does the alfalfa respire and use up the oxygen when there is a proper moisture content, but that other plant materials also will respire and supplement the activity of the alfalfa. However, this point has not been proven definitely, but since the carotene was preserved in a mixed ration containing only 15 per cent alfalfa (principal carotene source), it is logical to conclude that other plant tissues are contributing to the respiration.

The green color was well preserved when the mixed feed was sealed while the unsealed product became distinctly bleached. Further, in the presence of the trace elements, the unsealed material developed a definite rancid odor, a condition that did not develop in the absence of the trace elements under sealed and unsealed conditions. Consequently, it would seem unwise to add these trace elements to a mixed feed that is to be stored for months and where free access to oxygen is allowed. Some other vehicle, probably common salt, should be used for providing additional trace elements when needed by our livestock.

Many trials were made of materials presumed to be airtight. It is imperative that receptacles for carrying out the outlined process for carotene preservation be airtight, that is, made of materials that will retain the carbon dioxide generated within and prevent the transmission of oxygen into the receptacle. Flexowax was effective but probably impractical. There may be other suitable waxes, but this was the only one tried. Among the plastic films, Saran (Dow Chemical Company) possessed a high preservation quality. It has a high tensile strength and should lend itself to the solution of the problem involved in these studies. Other suitable plastic films may be found.

In practice it is correctly assumed that storage of feed materials with a high water content may lead to the growth of molds and even spontaneous combustion. Both conditions are governed by access to oxygen. With a process that excludes oxygen or greatly lowers its tension, common molds cannot grow and combustion cannot start.

SUMMARY

1. The effect of moisture level and temperature on carotene losses in dehydrated alfalfa and cereal grasses was studied under sealed conditions. The moisture levels studied were 2.5 to 15 per cent and the temperatures employed were 22 to 25° C. and 33 to 36° C.

2. In most instances, almost complete carotene preservation resulted with 10 to 15 per cent of water. Preserving both the carotene and the green color

was best accomplished at 7.5 to 10 per cent of water with 10 per cent as the preferred level because of the more optimum carotene preservation with no detrimental color change. At 7.5 per cent of water, the amount of loss was unpredictable and varied from 2.5 to 17 per cent. The losses increased with decreasing water levels below 7.5 per cent and at 2.5 to 5 per cent varied from 5 to 32 per cent.

3. Storage at 22 to 25° C. (room temperature) was more favorable for the preservation of the green color at 10 to 15 per cent of water level than storage at 33 to 36° C. Little difference in color preservation was observed at either temperature with the moisture below 10 per cent. Postitive pressures seldom were observed with 10 per cent moisture or less and storage at 22 to 25° C.

4. Storage under sealed conditions at 33 to 36° C. of a mixed feed containing 15 per cent alfalfa as the main source of carotene resulted in complete carotene retention with 10 per cent of moisture. Below 7.5 per cent the losses were large. The feed became bleached in the unwaxed carton but retained a pleasant aroma. In waxed cartons feed at any moisture level remained green and had pleasant aromas.

5. Where the mixed feed contained the added trace elements Fe, Cu, Mn and Co, the contents of the unwaxed carton were bleached and also possessed a rancid or tallowy odor. Under sealed conditions the green color and fine aroma were retained, and at 10 per cent and above of water, the carotene was preserved completely.

6. Investigation of many materials as barriers to oxygen and carbon dioxide transmission finally led to the use of Saran, a plastic film. It was found effective for the preservation of carotene in dehydrated alfalfa, with a proper water level.

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STUDIES ON RUMINAL GAS FORMATION AND ON CONSUMPTION OF ALFALFA PASTURE BY CATTLE

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This paper is concerned with the effect of diet on ruminal gas formation and with studies on the consumption of legume pasture. It was shown earlier (2) that no more gas was formed on a bloat-provoking diet such as green alfalfa tops than on a non-bloat-provoking diet such as alfalfa hay and grain. This observation led to the conclusion that increased gas production in itself could not explain acute bloat. In the same paper, it was shown that the rate of gas production depended upon the amount of feed consumed. Furthermore, both the present authors (2) and Quin (6) have shown by introducing gas into the rumen that much more can be expelled by belching than ever is produced in the rumen. Consequently, the hypothesis that acute bloat is due to a lack of sufficient coarse roughage in the rumen to induce eructation has been suggested (2). With this hypothesis, the rate of gas production is still an important consideration because belching rarely, if ever, is completely inhibited on diets low in coarse roughage. On the basis of this theory, it has been possible to induce and prevent bloat at will (3). Nevertheless, fatal bloat is not always produced on all succulent fields, and this failure has appeared to be due to a low consumption of alfalfa.

Quin (6) has suggested that bloat depends on both a high sugar content of the legumes at certain times which accelerates gas production, and a high saponin content which results in foaming with a consequent trapping of the gas and which thus prevents eructation. In support of the importance of the first factor, he submits evidence that glucose added to ruminal contents speeds up gas production more than does the addition of starch. The second postulate was based on his observation that ruminal ingesta from animals fed on alfalfa had a greater tendency to foam than ingesta from animals on other feeds, and on the report of Jacobson (4) that alfalfa contains a saponin with strong foam-producing properties. The results reported in the present paper confirm Quin's finding that glucose, under certain conditions, results in a more immediate increase in gas production than does starch, but further work is necessary to establish the view that changes in sugar content play a major role in determining the incidence or severity of bloat. The present authors have stuck a number of bloated cattle and find that foaming is not the cause of many cases of bloat. In one animal near death, the excess gas easily escaped when the animal was stuck with a trocar cannula, and in another severely bloated animal the excess gas with withdrawn by means of a stomach tube without obstruction by foaming. However, foaming may prevent eructation under certain conditions.

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EXPERIMENTAL

Dairy cows, in most instances lactating Jerseys, were used in the present studies. The method used for determining the rate of gas production has been described (2) as also has the method for determining food consumption on pasture (1). The rumen was tapped for gas production by means of a trocar cannula intended for bleeding horses.

Ruminal Gas Production Studies

Comparison of gas production on green alfalfa tops and on green Sudan grass. Although the rate of gas production on non-bloat-provoking diets such as alfalfa hay and grain has been compared to that on green alfalfa tops,

TABLE 1

Comparison of ruminal gas formation following feeding of Sudan and alfalfa tops. The cows were fed ad libitum throughout the 4-hour experimental period

Cow no.	Pounds of Sudan or alfalfa tops consumed[a]	Cubic feet of gas formed:		
		Half-hour before feeding	Half-hour after feeding	First 4 hours after feeding
		Sudan tops		
760	69.7	0.08	0.28	4.02
760	50.4	0.26	0.47	5.43
757	7.5	0.32	0.47	2.77
760	63.0	0.34	0.59	6.70
Av.	47.6	0.25	0.45	4.73
		Alfalfa tops		
760	26.9	0.06	0.48	4.17
757	2.4	0.34	0.28	3.29
760	19.7	0.30	0.78	6.82
832	12.9	0.34	0.43	3.00
Av.	15.5	0.26	0.49	4.32

[a] In addition to the tops, cow 760 received 4 pounds of a concentrate mix the night before the trial. Cows 757 and 832 received 4 pounds of concentrates the night before and the morning of the trial.

grasses have not been compared with legumes. Because bloat rarely occurs on grasses, this comparison seemed desirable.

The alfalfa and Sudan tops were cut and fed in the barn. The cows were pastured on the field from which the tops were to be taken for two days preceding the trial. No hay was fed the night previous or on the morning of the trial, but the regular concentrate allowance was given (4 to 8 lb. per day, depending on milk production). Gas production was determined over a 30-minute control period before feeding the alfalfa or Sudan. The object of the experiment was to determine the amount of gas formed on the two feeds when cows were given free access to the feed over a 4-hour experimental period. The results are shown in table 1. The gas formation during the first 4 hours after the beginning of feeding was approximately the same for the two feeds, but the average consumption of Sudan was three times that of alfalfa. Conse-

quently, it appears that the rate of gas production would be greater with alfalfa than with Sudan if equal quantities were fed.

Cow 757 ate very little during the gas determination period, as she was distressed by the presence of the cannula. Cow 760, on the other hand, evidenced no discomfort upon insertion of the trocar cannula; she continued to eat and ruminate in a normal manner. These individual differences are mentioned to point out that the use of the trocar cannula in gas production studies necessitates some discrimination in the selection of suitable experimental subjects.

It may be noted that there is an increase in gas production during the first 30 minutes after feeding (table 1). The promptness of acceleration of ruminal gas formation following ingestion of feed is an interesting phenomenon. On a given feed, there are some discrepancies between the amount consumed and the volume of gas formed which are difficult to explain. The amount of feed consumed was not measured on the 2 days preceding the test, and it may be that variations in the volume of ingesta present in the rumen at the beginning of the trial may provide an explanation.

TABLE 2

Comparative effects of glucose and starch on ruminal gas formation in cows given free access to green alfalfa tops for 4 hours preceding the experimental period. Two kg. of starch or glucose in 6 liters of H_2O were administered through a cannula directly into the rumen

Cow no.	Drench	Cubic feet of gas formed:		
		Hour before drench	Hour after drench	Second hour after drench
760	glucose	1.50	1.56	1.69
832	glucose	0.73	0.85	0.76
	Av.	1.12	1.21	1.23
760	starch	1.14	1.14	1.53
760	starch	1.45	1.39	1.34
	Av.	1.30	1.27	1.45

Comparative effects of glucose and starch on gas formation. In the light of Quin's hypothesis and data cited above, it seemed desirable to obtain more information on the effects of glucose and starch on gas formation. The tests were run under two conditions: in the first, the cows were given free access to green alfalfa tops fed in the barn for 4 hours preceding the test period; in the second, the cows were fed 9 lb. of alfalfa hay and 6 lb. of rolled barley 20 hours before the experimental period. Gas production was determined for 1 hour before the experimental period in the first experiment and for 30 minutes in the second. To introduce the test substance, glucose or starch, a rubber tube with a funnel attached to one end was connected to the side arm of the cannula. During the introduction of the test substance, the tube leading from the cannula to the gas meter was clamped off. The solution of starch or glucose was poured into a funnel elevated 3 or 4 feet above the level of the entrance of the cannula into the rumen, the fluid flowing into the rumen by gravity. Five to ten minutes were needed in introducing the solution.

In table 2 is shown the effect of administering starch or glucose to cows fed alfalfa tops for 4 hours preceding the test period. No significant change in the rate of gas formation resulted with either glucose or starch.

The results obtained when the cows were fed 20 hours before the experimental period are given in table 3. The amount of glucose or starch administered was reduced from 2 kg., as in the previous experiment, to 1 kg., because the higher dose of glucose on a partially empty rumen had an adverse effect on the cow, resulting in diarrhea and loss of appetite. Under this regime, 1 kg. of glucose in 3 liters of water increased gas production regularly within the first half hour after its introduction. The response to an equal amount of

TABLE 3

Comparative effects of glucose and starch on ruminal gas formation in cows fed 20 hours before the experimental period. One kg. of starch or glucose in 3 l. of H_2O was administered through a cannula directly into the rumen

Date of trial	Cubic feet of ruminal gas formed:					
	Hour before drench[a]	1st hour after drench	2nd hour after drench	3rd hour after drench	4th hour after drench	Total after drench
	1 kg. glucose administered					
Mar. 11	0.33	0.67	0.68	0.51	0.53	2.39
Mar. 23	0.31	1.11	1.11	0.78	0.76	3.77
Mar. 30	0.42	1.09	0.85	0.85	0.52	3.31
Apr. 13	0.44	1.00	0.96	0.83	0.45	3.24
Av.	0.38	0.97	0.90	0.74	0.57	3.18
	1 kg. starch administered					
Mar. 18	0.56	0.80	0.94	0.84	0.56	3.13
Mar. 25	0.48	0.45	0.62	0.67	0.56	2.29
Apr. 6	0.48	0.43	0.76	0.98	0.81	2.98
Av.	0.51	0.56	0.77	0.83	0.64	2.80

[a] Gas was determined for only 0.5 hr. before drenching. The figure obtained was multiplied by 2 to facilitate comparison of gas production before and after drenching.

starch was not marked until the second hour after drenching, but during the third and fourth hours more gas was liberated than with glucose. The tests on starch and glucose were run for 30 minutes longer than is shown in table 3. During this last half hour, there was an average production of 0.22 cubic feet of gas with glucose and 0.32 cubic feet with starch. Thus the effect of starch is more prolonged and the total gas produced is apparently the same as with glucose.

Studies on Consumption of Alfalfa Pasture

For these studies, the cows were weighed in and out of pasture and during the intervening period all excreta were collected and weighed. The pasturing period extended from 8 a.m. to 2:30 p.m. The studies were made between June 14 and October 10. Insensible losses were determined on 2 days and amounted to approximately 3 lb. per hour, but the insensible losses were not taken into account in calculating feed consumption. On a few occasions, the

insensible losses exceeded feed consumption, thus explaining the apparent negative consumption values shown in figures 1 and 2.

One of the objectives of the experiment was to determine if palatability varied in different fields. Further, it was desired to ascertain the influence of maturity on palatability. Decisive answers were not obtained to either question for reasons which will be explained. The results of the study are summarized in figures 1 and 2.

Figure 1 gives the feed consumption on two different fields, 1-C South and Dairy Field 4. Two lactating cows were used in this part of the study; cow

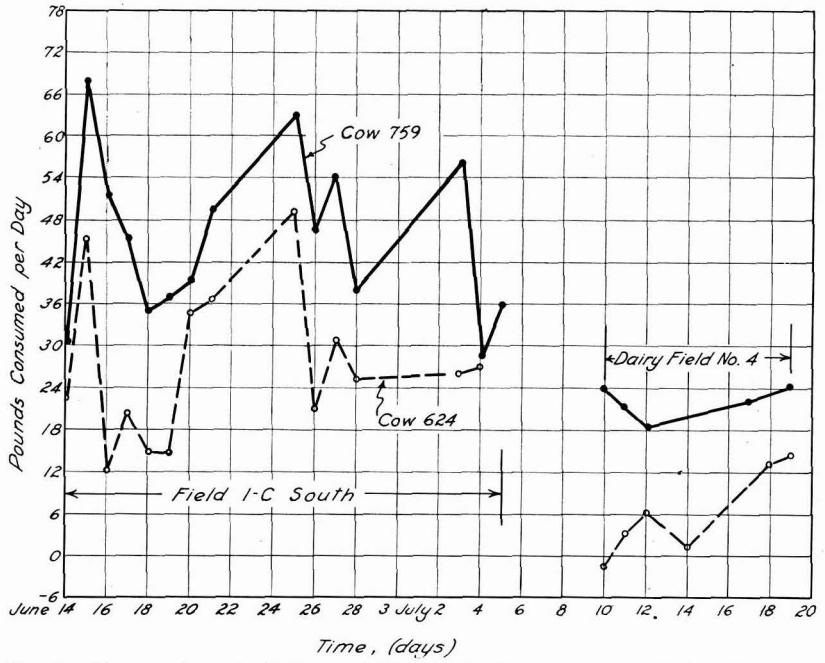

FIG. 1. Consumption of alfalfa on fields 1-C South and Dairy Field 4. The cows, 759 and 624, were on pasture for 6.5 hours daily. In determining feed consumption, insensible losses were not considered.

624 received 5 lb. of a concentrate mix at 3 a.m. and 3 p.m. and cow 759 received 7 lb. night and morning. Cow 759 had been shown to be susceptible to bloat in previous studies, whereas cow 624 never had bloated severely, even under conditions in which the majority of the herd had bloated. No hay was fed. The cows were pastured intermittently on 1-C South from June 14 to July 5. There were 12 acres in this field, and thus the amount consumed by the 2 cows had no appreciable influence on the amount of feed available during this period. Although the stand of alfalfa on 1-C South appeared to be fairly clean on cursory examination, there were some weeds and annual grasses on the irrigation checks. When the cows were first put on the field, the alfalfa was about 1 foot tall and very succulent. Furthermore, the alfalfa was relatively

unpalatable and during the first week the cows ate approximately as much weeds and grasses as alfalfa. One cow did not bloat on this field, and the other, cow 759, bloated slightly on 3 different days. We attribute this relative lack of bloat to the consumption of sufficient weeds and grasses to induce belching. In support of this view is the fact that both cows ruminated more than one would expect when pasturing on succulent alfalfa without access to hay. Cows ruminate very little on fields causing severe bloat. Experience in the next field, Dairy Field 4, with the same cows adds weight to this interpretation. This field had been pastured earlier in the season and was devoid of contaminating

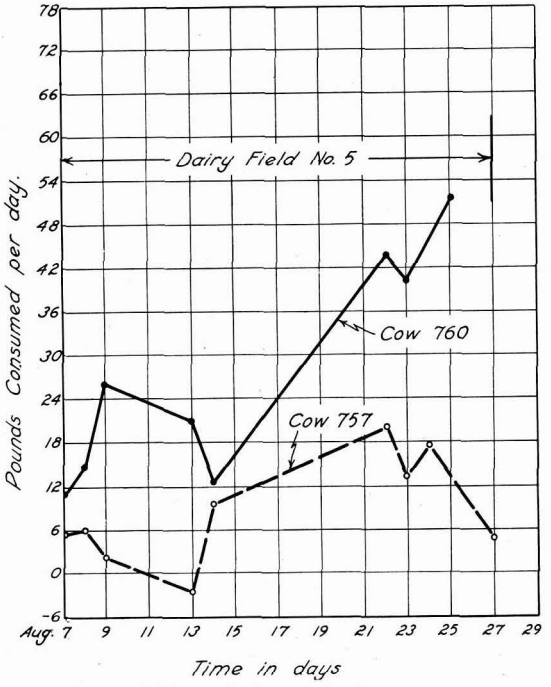

FIG. 2. Consumption of alfalfa on Dairy Field 5. The cows, 760 and 757, were on pasture for 6.5 hours daily. In determining feed consumption, insensible losses were not considered.

weeds and grasses. Cow 624 bloated on 4 of 6 pasturing days on this field, and cow 759 bloated every day—on two occasions the bloat was sufficiently severe to require treatment with turpentine. "Severe bloat" refers to a condition in which there is marked distress, frequent urination and defecation and a ruminal pressure of 45 to 70 mm. Hg (1).

Palatability of the alfalfa on the two fields was considered. Field 1-C South was pastured over a 3-week period during which the alfalfa became progressively more mature. It was in the late bud stage at the termination of the trial. This field was irrigated on June 11. The fact that the cows ate a considerable proportion of weeds, particularly during the first week, presents

some difficulties in interpretation. However, the cows were under constant observation during the pasturing period by the attendants collecting the excreta, and there is little doubt but that the cows ate a greater amount of alfalfa as it became more mature. Furthermore, there is little doubt that the alfalfa in field 1-C South was more palatable than in Dairy Field 4. The results are difficult to evaluate, however, for two reasons: on field 1-C South, the exact consumption of alfalfa is unknown because the cows ate grasses and weeds in addition to alfalfa; secondly, the consumption of alfalfa on Dairy Field 4 was depressed as the result of bloat. In other words, when the cows bloated, they stopped eating.

The results on Dairy Field 5 with cows 760 and 757 are shown in figure 2. Here the evidence seems a little more clear-cut that the alfalfa becomes more palatable as it matures. On August 13 and 14, cow 760 bloated, on the latter date sufficiently severely to require treatment with turpentine. This explains her relatively low consumption on these days. By August 22nd, the alfalfa was in the early bloom stage and was sufficiently coarse to induce frequent rumination. No adequate explanation is available for the relatively low and sporadic feed consumption of cow 757.

DISCUSSION

The results on ruminal gas formation following feeding of green Sudan and alfalfa tops indicate that one might expect a greater amount of gas formed from alfalfa if equal amounts of the two feeds were given. With *ad libitum* feeding, the total gas formed from the two feeds, however, was approximately the same because of the greater consumption of Sudan. Increased gas production on legumes does not in itself provide an adequate explanation of bloat; cows will bloat on amounts of alfalfa comparable to those consumed in these experiments but bloat did not occur when normal animals were given an amount of Sudan producing an equivalent volume of gas. Previous studies (2) have shown that dry legume hay results in as much gas production as green alfalfa. These data give further confirmation, therefore, that it is the inability of animals to eructate the gas on legumes which makes alfalfa and clover dangerous from a standpoint of bloat. Nevertheless, the rapid gas formation on legumes undoubtedly is a contributing factor in bloat.

Quin (5) has compared the rate of gas formation on alfalfa and grass hay. He reports a rapid production of gas on alfalfa hay, a result in accord with our studies (2). On the contrary, he found no gas formed over a 90-minute period in two of three trials with sheep on a basal diet of grass hay. In the light of the data reported herein on a green grass (Sudan), this result needs further confirmation.

When cows were fed 20 hours before the experimental period, glucose caused an earlier increase in gas formation than did starch, but the total gas produced from the two substances over a period of 4.5 hours was about the same. Quin reported that when starch, in the form of maize, was given to sheep maintained on a basal diet of green alfalfa, there was no gas formed over a 90-minute

period. When cows were given a full feed of alfalfa during a 4-hour interval preceding the test period, no difference in gas formation between glucose and starch was observed. Conceivably the sugar content of alfalfa could be a contributing factor in bloat as postulated by Quin, but further studies are necessary to establish the point.

The present studies on palatability of legumes at different stages of maturity were not conclusive but indicated that alfalfa increases in palatability as it matures. Two main difficulties in these studies were encountered: first, cows ate weeds and grasses along with the alfalfa when the fields were contaminated; second, cows on pure alfalfa stands bloated and this in turn depressed feed consumption and made it impossible to obtain a true estimate of palatability. Therefore, it appears that a more desirable procedure would be to cut the alfalfa tops and feed them in the barn. In this way, the weeds and grasses could be avoided. Further, it would appear necessary to supplement the diet with sufficient Sudan grass hay or with green Sudan to obviate bloat.

SUMMARY AND CONCLUSIONS

In an average of four trials, 4.7 cubic feet of gas were produced when cows consumed 47.6 lb. of green Sudan tops fed *ad libitum* over a 4-hour period as compared to 4.3 cubic feet when cows consumed an average of 15.5 lb. of green alfalfa tops over a similar period.

The amount of gas formed following drenching with glucose or starch was determined both by feeding cows 20 hours before the experimental period or feeding them with alfalfa tops *ad libitum* 4 hours preceding drenching. In the former instance, glucose caused a more prompt increase in gas formation, whereas the effect of starch was more prolonged. When cows were fed immediately preceding drenching, on the other hand, no difference between glucose and starch as regards gas formation was discernible.

Studies on the consumption of alfalfa pasture indicate that alfalfa becomes more palatable as it matures up to the early bloom stage, but the results were inconclusive.

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A STUDY OF THE USE OF THE ANTIOXIDANT NORDIHYDROGUAIARETIC ACID IN DAIRY PRODUCTS. II. ITS ANTIOXYGENIC PROPERTIES IN UNSWEETENED FROZEN CREAM[1]

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The use of antioxidants in retarding the development of oxidized flavor during the storage of frozen cream has been studied by several investigators (2, 3, 4, 5, 6). The work reported herein consists of a study in which nordihydroguaiaretic acid (NDGA) was used to retard the development of oxidized flavor during the storage of unsweetened frozen cream containing 40 per cent milk fat.[2]

EXPERIMENTAL PROCEDURE

Two grades of cream, one of high and the other of low quality, were used in this study. Standard plate count (1), acidity and pH were the criteria upon which quality was based (table 1).

TABLE 1

The standard plate count, titratable acidity and pH of the raw and pasteurized cream

	High quality		*Low quality*	
	(a)	(b)	(c)	(d)
Raw cream				
Standard plate count	340,000	32,000	345,000,000	6,000,000
Titratable acidity, as % lactic acid	0.120	0.140	0.155	0.125
pH (25° C.)	6.74	6.63	6.50	6.66
Pasteurized cream	*150° F.*	*170° F.*	*150° F.*	*170° F.*
Standard plate count	900	55	2,000	2,860
Titratable acidity, as % lactic acid	0.125	0.135	0.145	0.140
pH (25° C.)	6.62	6.60	6.50	6.39

The different batches of cream were standardized to contain 40 per cent milk fat. NDGA was added after pasteurization as a 10 per cent solution in glycerol or as a 5 per cent water suspension. The concentrations of NDGA were computed on the basis of the fat content of the cream. When used, copper was added at a concentration of 0.5 p.p.m. in the form of a 0.5 per cent aqueous solution of copper sulfate.

The cream was pasteurized in well-tinned equipment, cooled to 40–45° F., sealed in tinned cans holding 300 ml. and stored at –12 to –20° F. For monthly flavor criticisms, the frozen cream was thawed by holding it 24 hours at 40° F.

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[1] The data contained in this paper are from a thesis submitted by the senior author to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of Master of Science, 1947.

[2] The legality of adding antioxidant material to dairy products would need to be established before its use could be recommended. The authors' interest in the product studied was mainly scientific, although the practicable possibilities of a study of this nature always must be recognized.

TABLE 2

The antioxidant effect of NDGA added to unsweetened cream stored at sub-zero temperatures

Treatment	Flavor criticisms											
	1 mo.		3 mo.		5 mo.		7 mo.		9 mo.		11 mo.	
	(1)[a]	(2)[b]	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Pasteurized at 150° F. for 30 min.												
Series A. Low quality cream.	No copper added.											
Control	...[c]	...	...	...	...	...	1[d]	1	1	2	2	4
Control + 0.00125% NDGA	...	...	...	...	...	...	...	...	...	...	...	...
Control + 0.005% NDGA	...	...	...	...	...	...	...	...	...	...	...	...
Series B. Low quality cream.	0.5 p.p.m. copper added.											
Control	2	3	3	4	4	5	5[fe]	5[f]	5[f]	5[f]	f	f
Control + 0.00125% NDGA	...	...	1	1	1	1	2	2	4[f]	4[f]	5[f]	5[f]
Control + 0.005% NDGA	...	...	1	1	1	1	2	2	4[f]	4[f]	5[f]	5[f]
Series C. High quality cream.	No copper added.											
Control	...	...	...	...	...	1	...	1	...	1	.....	.....
Control + 0.00125% NDGA	...	...	...	...	...	...	...	...	...	...	.....	.....
Control + 0.005% NDGA	...	...	...	...	...	...	...	...	...	...	.....	.....
Series D. High quality cream.	0.5 p.p.m. copper added.											
Control	...	2	3	3	4	5	5	5	5	5[f]	.....	.....
Control + 0.00125% NDGA	...	±[f]	...	±	1	1	2	2	2	2	.....	.....
Control + 0.005% NDGA	...	±	...	...	1	1	1	1	...	...	.....	.....
Pasteurized at 170° F. for 15 min.												
Series E. Low quality cream.	No copper added.											
Control	...	±	...	±	...	1	...	1	±	1	±	1
Control + 0.00125% NDGA	...	...	...	...	...	...	...	...	...	...	...	...
Control + 0.005% NDGA	...	...	...	...	...	...	...	...	...	...	...	...
Series F. Low quality cream.	0.5 p.p.m. copper added.											
Control	...	±	...	±	...	1	...	...	±	±	±	±
Control + 0.00125% NDGA	...	...	...	...	±	±	...	...	...	...	...	...
Control + 0.005% NDGA	...	...	...	...	±	±	...	...	...	...	...	...
Series G. High quality cream.	No copper added.											
Control	...	...	...	...	...	...	...	1	...	1	...	1
Control + 0.00125% NDGA	...	...	...	...	...	...	...	...	...	...	...	...
Control + 0.005% NDGA	...	...	...	...	...	...	...	...	...	...	...	...
Series H. High quality cream.	0.5 p.p.m. copper added.											
Control	...	...	...	...	1	1	2	3	3	4	3	5
Control + 0.00125% NDGA	...	...	...	...	...	...	...	...	...	...	...	...
Control + 0.005% NDGA	...	...	...	...	...	...	...	...	...	...	...	...

[a] Flavor judged immediately after taking cream out of storage and thawing it.
[b] Flavor judged after thawing cream and then holding it at 40° F. for 1 week.
[c] No oxidized flavor present.
[d] The numbers 1 to 5 indicate increasing levels of oxidized flavor defect.
[e] Fishy flavor.
[f] Flavor slightly 'off.' Not typically oxidized.

It then was held at 40° F. for 1 week after which it was judged again for flavor. The judging panel was composed of three or more persons.

RESULTS

The data in table 2 are typical of results obtained with cream which was placed in storage during the months of August and September. There were no significant differences in the antioxygenic effectiveness of the NDGA when added in glycerol solution and when added in a water suspension. Therefore, the data presented in table 2 includes only results from cream treated with NDGA in glycerol solution.

The effect of concentration of NDGA. A concentration of 0.005 per cent NDGA was more effective than one of 0.00125 per cent. This is demonstrated in the results obtained with the high quality cream which contained added copper and which was pasteurized at 150° F. for 30 minutes (table 2, series D). Oxidized flavor had developed at the end of 3 months storage in the control sample. While this off-flavor had developed at the end of 5 months in the cream treated with both 0.00125 per cent and 0.005 per cent NDGA, the intensity of the off-flavor at the end of 7 months was less in the cream containing 0.005 per cent than in the cream containing 0.00125 per cent NDGA.

The effect of pasteurization temperature. Development of oxidized flavor was retarded by pasteurizing the cream at 170° F. for 15 minutes. Oxidized flavor had developed at the end of 7 months storage in the control sample of series A which was pasteurized at 150° F. for 30 minutes, while it did not develop during storage at sub-zero temperatures in the similar cream pasteurized at 170° F. for 15 minutes. Oxidized flavor was present at the end of 1 month in the control sample of series B which was pasteurized at 150° F. for 30 minutes, whereas it did not develop in the similar cream pasteurized at 170° F. for 15 minutes.

There was no oxidized flavor development during storage for 11 months at sub-zero temperatures in the high quality cream which contained no added copper (table 1, series C and G). The off-flavor was present at 3 months in the control sample of series D which was pasteurized at 150° F. for 30 minutes but was not detected until the end of 5 months in the similar cream pasteurized at 170° F. for 15 minutes. While the keeping quality of the cream pasteurized at 170° F. for 15 minutes was superior to that pasteurized at 150° F. for 30 minutes, it had a cooked flavor which persisted throughout the storage period. The keeping quality of the cream which was pasteurized at 150° F. for 30 minutes and which contained NDGA but no added copper was comparable to that of cream pasteurized at 170° F. for 15 minutes.

The effect of quality of the cream. The cream which was pasteurized at 150° F. for 30 minutes developed the oxidized flavor in the control sample containing added copper (series B) at the end of 1 month, but the off-flavor was not detected in the similar high quality cream until the end of 3 months (series D).

There was no oxidized flavor development in any of the low quality cream pasteurized at 170° F. for 15 minutes during storage for 11 months at sub-zero

temperatures. However, the off-flavor was present at the end of 5 months in the control sample of the similar high quality cream containing added copper (series H).

The effect of quality as indicated in this study was variable. The high quality cream which was pasteurized at 150° F. for 30 minutes had a better keeping quality than the similar low quality cream with respect to the oxidized flavor development. The converse of this was true in the cream which was pasteurized at 170° F. for 15 minutes.

The effect of holding the thawed cream at 40° F. for 1 week. Oxidized flavor developed frequently in the control samples which were held at 40° F. for 1 week, although they did not have the off-flavor when they were taken out of storage. This relationship was illustrated in the control sample of series D pasteurized at 150° F. for 30 minutes which had been stored for 1 month at sub-zero temperatures and did not have the oxidized flavor when first removed from the low temperature storage, but developed it after the sample had been held at 40° F. for 1 week. The same observation was made after 5 months in the control samples of series E and after 7 months in the control samples of series H, both of which were pasteurized at 170° F. for 15 minutes.

After storage for 1 week at 40° F., the oxidized flavor usually increased in intensity in the control samples which had that off-flavor when they were first taken out of storage. This is evident in the cream pasteurized at 150° F. for 30 minutes in the control samples of series A after 9 and 11 months, in series B after 1, 3 and 5 months and in series D after 5 months. This trend also was evident after 7, 9 and 11 months in the control samples of cream in series H which had been pasteurized at 170° F. for 15 minutes. However, the intensity of the oxidized flavor did not increase during storage at 40° F. in the samples which contained NDGA.

CONCLUSIONS

1. Concentrations of 0.00125 to 0.005 per cent nordihydroguaiaretic acid were found to retard the development of oxidized flavor in unsweetened frozen cream during storage for 11 months.

2. In the absence of added copper, the keeping quality of the cream which contained nordihydroguaiaretic acid and was pasteurized at 150° F. for 30 minutes was comparable to that pasteurized at 170° F. for 15 minutes but to which the antioxidant had not been added.

3. In this study, the high quality cream pasteurized at 150° F. for 30 minutes had a better keeping quality than the low quality cream similarly pasteurized. The converse of this was true in the cream which was pasteurized at 170° F. for 15 minutes.

4. During storage for 1 week at 40° F., an oxidized flavor developed frequently in the control samples, although these samples did not have the off-flavor when they were taken out of storage at sub-zero temperatures. This did not occur in the cream which contained nordihydroguaiaretic acid.

5. During storage for 1 week at 40° F., the intensity of the oxidized flavor usually increased in the control samples which had the off-flavor when they were

first taken out of storage at sub-zero temperatures. This did not occur in the oxidized samples which contained nordihydroguaiaretic acid.

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THE RELATION BETWEEN THE MONTH OF CALVING AND YEARLY BUTTERFAT PRODUCTION[1]

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Approximately 71 per cent of the dairy cows of Oregon are located west of the Cascade Mountains; and about two-thirds of these, or 46 per cent of the total, are found on farms in the ten Willamette Valley counties, a region with an average temperature of 52° F. and a monthly mean range from a high of 65° F. in July to 38° F. in December. The average rainfall of about 42 inches comes mostly during the winter months. Since the climate under these conditions is mild and does not show large seasonal variations in comparison with some other parts of the United States, it became of interest to study the effect of the month of calving on butterfat production.

Differences in yearly milk production between cows freshening in the different months of the year, in various parts of the United States, have been found to exist (1, 2, 5, 7, 8). The season of the year in which the cow freshens also was reported to exert an effect on her butterfat production (1, 4, 8). In Connecticut, Frick *et al.* (2) found that the differences in milk production of cows calving in the different months of the year were highly significant statistically.

PROCEDURE

Data for the present study were obtained from the record books of the dairy herd owned by Oregon State College and from official test records of cows tested in Oregon covering the years 1910 through 1946. Only first-calf, 2-year old records were used. The official records of butterfat production were tabulated separately for cows milked twice a day during 305-day and 365-day lactations. Production of cows milked three times daily, part or all of the milking period, was reduced to a 2-times a day milking basis by using the factor 0.0655 of 1 per cent for each day the cow was milked 3 times. The distribution of the 2690 records between breeds was 1881 Jerseys, 358 Guernseys, 301 Holstein-Friesians and 150 Ayrshires. The number of records available from the College herd was 359, while 2331 were from private herds.

An analysis of variance (6) was applied to the data to find out the significance of the difference in butterfat production of cows calving each month of the year.

RESULTS AND DISCUSSION

Information on the butterfat records of first calf heifers used in the study is given in table 1.

Table 2 gives a summary of the results of the statistical analysis of the data. The variations in butterfat production among cows freshening within each month

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TABLE 1

Average yearly butterfat production of five groups of first calf heifers

Month of freshening	Group 1[a]		Group 2		Group 3		Group 4		Group 5	
	No. of heifers	Av. prod.	No. of heifers	Av. prod.	No. of heifers	Av. prod.	No. of heifers	Av. prod.	No. of heifers	Av. prod.
		(*lb.*)		(*lb.*)		(*lb.*)		(*lb.*)		(*lb.*)
January	80	455	34	372	66	488	19	483	42	320
February	78	464	38	390	68	489	23	479	28	295
March	91	469	32	375	64	474	20	477	24	330
April	102	421	26	379	76	473	11	467	25	304
May	72	425	27	380	81	464	13	479	27	372
June	53	438	18	359	62	465	10	458	19	326
July	48	434	26	338	47	458	18	471	22	326
August	69	459	19	384	78	482	23	467	33	322
September	104	446	19	397	105	466	35	472	49	312
October	70	444	25	398	84	450	24	470	29	341
November	64	461	25	389	69	484	9	459	31	329
December	78	445	29	387	72	498	27	474	30	305
Total & mean	909	447	318	379	872	474	232	473	359	323

[a] Group 1. Jersey, 305 day, Register of Merit
Group 2. Guernsey, Holstein, Ayrshire, 305 day, Advanced Registry
Group 3. Jersey, 365 day, Register of Merit
Group 4. Guernsey and Holstein, 365 day, Advanced Registry
Group 5. Ayrshire, Guernsey, Holstein and Jersey herd test (college)

were large, and a definite trend was not followed when the monthly averages were studied, but rather an up-and-down line. Jersey cows milked for 305 days were

TABLE 2

Analysis of variance of the butterfat records used in the study

Group	Source of variation	Degrees of freedom	Sum of squares	Variance	Variance ratio	Significance level	
						5%	1%
1 Jersey R. of M. 305 days	Month	11	209,932.14	19,084.74	2.25	1.80	2.26
	Error	897	7,594,314.99	8,466.35			
	Total	908	7,804,247.13				
2 Guernsey Holstein Ayrshire A.R. 305 days	Month	11	77,933.46	7,084.86	1.45	1.82	2.31
	Error	306	1,496,925.13	4,891.91			
	Total	317	1,574,858.59				
3 Jersey R. of M. 365 days	Month	11	160,275.05	14,570.46	1.02	1.80	2.26
	Error	860	12,275,470.21	14,273.80			
	Total	871	12,435,745.26				
4 Guernsey Holstein A.R. 365 days	Month	11	8,700.71	790.97	0.09	1.83	2.34
	Error	220	2,009,556.38	9,134.35			
	Total	231	2,018,257.09				
5 All breeds College Herd Test	Month	11	124,238.84	11,294.44	1.71	1.81	2.29
	Error	347	2,297,328.15	6,620.54			
	Total	358	2,421,566.99				

the only group that showed significance at the 5 per cent level, although not significant at the 1 per cent level. Since the other four groups showed insignificant differences between the production of cows freshening in different months of the year, the significance of the first group is of doubtful value.

SUMMARY

The butterfat records of 2690 first-calf heifers in herds located in western Oregon, a region with rather uniformly mild temperature, were studied to determine the effect of the month of calving on yearly butterfat production.

It seems that under western Oregon conditions the season of the year in which a cow freshens has no appreciable effect on her yearly butterfat production.

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SOME FACTORS INFLUENCING THE MALE HORMONE CONTENT OF COW MANURE[1]

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Riley and Hammond (8) discovered that the feeding of dried cow manure to day-old chicks caused marked stimulation of the comb growth. Evidence was presented indicating that the factor present was an androgenic rather than a gonadotrophic substance. They reported that "feces from bulls were entirely without effect, whereas feces from pregnant cows, as well as from unbred heifers, had marked androgenic effects."

Turner (10, 11, 12) confirmed the report of the presence of orally-active androgens in the feces of lactating cows when dried at 45° C. The androgen content of the manure of other ruminants, including goats and sheep of both sexes and conditions, was either low or absent. The feces of dairy bulls showed indications of only small androgen excretion by that route.

Gassner and Longwell (1, 4) reported that the concentration of androgens in feces reached a peak during the last week of pregnancy and then dropped sharply to zero at calving. Steer and bull manures were relatively inactive biologically.

The present studies were initiated to throw further light upon the functional relationship between the male hormone eliminated in the feces of dairy cows of the several breeds and reproduction and lactation. Further, in connection with studies concerned with the characterization of the androgens excreted and with methods for their extraction, it was considered helpful to know when the greatest concentration of hormone might be expected.

EXPERIMENTAL PROCEDURE

The fresh manure was collected from individual cows of the Guernsey, Holstein and Jersey breeds in the University of Missouri dairy herd. Complete samples were not collected, rather the feces dropped during the milking period in the morning or afternoon were combined until a sufficient quality was collected for an assay. This usually required 2 to 3 days. Cows in various stages of lactation and pregnancy were included. When a series of samples from the same cow was collected, at least a month intervened between samples. Each collection of fresh manure was placed quickly in a Freas electric drying oven maintained at a temperature of 45° C. Samples were stirred daily. At least 48 hours were required to dry the collection. The dried manure was placed in a large lard can and, when collection was complete, the entire sample was ground in a small hammer mill and thoroughly mixed before assay.

The androgen content of each sample was assayed biologically by the method

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previously described (12). Groups of about 20 sexed White Plymouth Rock chicks were used throughout and the dried cow manure was fed uniformly at the 10 per cent level by substituting the dried manure for an equal weight of alfalfa meal in the basal chick starter ration. These assays were conducted monthly throughout the year.

Each month a group of control chicks and a group fed methyl testosterone at the rate of 20 mg./kg. of feed were included to determine the possible seasonal variation in the responsiveness of the chick comb to androgen. Since no seasonal trend was observed in the average comb weight of either sex in the control group or those fed methyl testosterone, it was decided that no correction factor for season was required (13).

As a measure of the presence of biologically active androgens in the samples, the average comb weight per 100 g. body weight of each sex was determined. The average comb weights per 100 g. body weight for the two sexes were added together and divided by two. This value should represent the average comb weight per 100 g. body weight of a population of chicks containing equal numbers of the two sexes.

As a measure of the biological activity of the male hormone present in the various samples of dried cow manure, comparison may be made with the average comb weight of groups of control chicks of the same age and with groups of chicks fed 10 and 20 mg. of methyl testosterone per kilogram of starter feed. Since the biologically active androgenic hormones present in cow manure are not known, it seems preferable to indicate differences in the various samples in terms of average comb weight rather than in terms of any single androgen. For a comparison of the oral effectiveness of several androgens in fowls the reader is referred to a paper by the writer (14). The average comb weight of all the chicks fed samples of dried cow manure assayed in this study is presented for comparison (table 1).

RESULTS

Effect of pregnancy upon androgen excretion. In the dairy cows available for our study, no clear-cut separation of pregnancy and lactation could be made, since the heifers pregnant for the first time were not stabled. The group of cows included in table 2 were all lactating cows, but they were classified on the basis of the month of pregnancy. Since lactating cows are not bred until 90 days or more after parturition, the group of cows whose manure was assayed during the first month of pregnancy included cows lactating an average of 124 days. By the eighth month of pregnancy, most of the cows had been dried up; only two of eight cows still were lactating slightly. The cows in the ninth month of pregnancy all were dry.

It will be seen that, aside from the second month, the assay results did not vary greatly from month to month until the eighth and ninth months of pregnancy. Apparently there is a tendency for the male hormone excretion rate to increase at the approach of parturition. This rise occurred at a time when the cows were either dry or almost dry.

TABLE 1

Comparison of comb weight of chicks fed control feed, methyl testosterone and cow manure

Treatment	Male chicks					Female chicks					Male + female / 2	
	No. of chicks	Av. body weight	Av. testes weight	Av. comb weight	Comb weight / 100 g. body wt.	No. of chicks	Av. body weight	Av. ovary weight	Av. comb weight	Comb weight / 100 g. body wt.	comb weight / 100 g. body wt.	Ratio of control group
		(*g.*)	(*mg.*)	(*mg.*)	(*mg.*)		(*g.*)	(*mg.*)	(*mg.*)	(*mg.*)	(*mg.*)	
Control	185	242.4	48.88	102.69	42.37	199	224.8	48.61	51.17	22.76	32.59	1:
10 mg. MT[a]/kg.	52	248.4	42.86	240.80	96.94	46	236.3	45.45	246.20	104.18	100.56	3.09
20 mg. MT[a]/kg.	157	239.2	43.61	360.50	150.74	158	226.2	49.78	252.67	111.70	131.22	4.03
10% cow manure	872	251.8	31.47	202.25	80.31	855	236.1	38.10	137.66	58.31	69.31	2.13

[a] MT = Methyl Testosterone

TABLE 2

Effect of the stage of pregnancy upon the androgen excretion of dairy cattle (assay with white rock chicks—28 days of age)

Month of pregnancy	Lactation days. av.	No. of cows (breed)[a]	Male Chicks					Female Chicks					Male + female / 2
			No. of chicks	Av. body weight	Av. testes weight	Av. comb weight	Comb weight / 100 g. body wt.	No. of chicks	Av. body weight	Av. ovary weight	Av. comb weight	Comb weight / 100 g. body weight	comb weight / 100 g. body weight
				(*g.*)	(*mg.*)	(*mg.*)	(*mg.*)		(*g.*)	(*mg.*)	(*mg.*)	(*mg.*)	(*mg.*)
1	124	3(2H, 1J)	32	235.1	28.85	147.24	62.63	43	232.6	33.74	106.19	45.65	54.14
2	169	7(5H, 1G, 1J)	64	248.9	29.93	233.48	93.80	67	236.3	38.87	195.93	82.92	88.36
3	180	6(5H, 1J)	69	243.4	32.95	175.53	72.12	48	224.2	51.14	137.18	61.19	66.66
4	216	4(4H)	34	257.4	37.72	153.73	59.72	46	243.9	45.25	111.33	45.65	52.69
5	284	5(2H, 3G)	56	266.1	40.10	161.61	60.73	47	242.2	46.67	102.10	42.16	51.44
6	286	5(2H, 3J)	49	260.5	33.30	210.05	80.63	51	236.6	66.09	124.19	52.49	66.56
7	294	3(2H, 1J)	29	248.0	28.47	148.70	59.96	29	243.6	39.90	142.10	58.33	59.15
8	Almost dry	8(5H, 1G, 2J)	73	234.1	28.45	281.66	120.32	83	224.2	40.41	190.05	84.78	102.55
9	Dry	7(4H, 3J)	73	272.4	32.12	256.81	94.27	69	252.2	37.88	169.31	67.13	80.70
Non-pregnant, dry cows		4(1H, 3J)	33	252.6	28.15	205.81	81.48	43	253.3	38.93	168.53	66.53	74.01
Breed													
Holstein		22	226	249.0	32.04	164.63	66.12	219	235.4	43.52	126.40	53.70	59.91
Guernsey		4	49	267.6	37.68	202.67	75.74	38	250.5	45.61	123.66	49.36	62.55
Jersey		7	58	249.4	27.03	233.96	93.81	74	233.6	54.36	165.85	70.99	82.40

[a] H = Holstein, G = Guernsey and J = Jersey.

Whether the apparent high level of androgen excretion during the second month is significant is not clear. One of the Holstein cows appeared to excrete an unduly large amount of androgens at this time in comparison with her other assays. Furthermore, since the tabulation according to the stage of lactation shows no similar increase, the writer prefers to believe that this does not represent a general increased level of androgen excretion.

In order to interpret the fluctuation in the androgen excretion rate from month to month during pregnancy due to possible breed variation, the data were classified on the basis of the breed for the first 7 months of pregnancy. The eighth and ninth months were excluded due to possible effect of the preparturient rise in the androgens. This tabulation indicates little difference in the excretion of androgens by Holstein and Guernsey cows, but the Jersey cows appear to excrete greater quantities of androgens under similar conditions.

A small group of non-pregnant dry cows also is included. The relatively higher androgen excretion rate by this group, as compared to the pregnant group, is believed to be due to the presence of a predominant number of Jersey heifers. It would appear that neither pregnancy nor lactation is necessary for the excretion of relatively large amounts of androgens. This confirms the report of Riley and Hammond (8).

Effect of lactation upon androgen excretion. The data on the individual cows were tabulated according to the stage of lactation (table 3). It will be seen that no trend in the average comb weight with the advance of lactation is present during the first 8 months. Comb weight values above normal in the ninth and eleventh months of lactation are interpreted as indications of the prepartum rise in androgen excretion rather than relationship to the advance in the period of lactation.

The tabulation of the data by breeds, up to the time of the preparturient rise, again indicated little difference in the androgen excretion by Holstein and Guernsey cows. The Jersey cows, however, again were higher but not quite as high as in the tabulation of pregnant animals.

DISCUSSION

While the data are limited, they indicate that a relatively high average level of androgen excretion occurs in unbred heifers and non-pregnant, non-lactating cows. Since this observation is in agreement with that of Riley and Hammond (8), it would appear that this hormone is excreted at relatively high levels in sexually mature heifers without reference to pregnancy. Whether there is a cyclic variation in the androgens in relation to the period of heat in heifers has not been investigated. Since there is much experimental work indicating that androgens can stimulate the growth of the mammary duct system, the cyclic growth of the pubertal duct system of heifers may be due, in part, to the presence of androgens as well as estrogens in the blood at this time.

Following conception, the rate of androgen excretion is not believed to increase markedly. It is true that these data show a high level during the second month of pregnancy with a reduction until the seventh month. Further data

TABLE 3

Effect of the stage of lactation upon the androgen excretion of dairy cattle (assay with white rock chicks—28 days of age)

Month of lactation	Stage of pregnancy av.	No. of cows (breed[a])	Female Chicks					Male Chicks					Male + female / 2
			No. of chicks	Av. body weight	Av. testes weight	Av. comb weight	Comb weight / 100 g. body wt.	No. of chicks	Av. body weight	Av. ovary weight	Av. comb weight	weight Comb / 100 g. body weight	comb weight / 100 g. body wt.
				(*g.*)	(*mg.*)	(*mg.*)	(*mg.*)		(*g.*)	(*mg.*)	(*mg.*)	(*mg.*)	(*mg.*)
1	open	8(6H, 1G, 1J)	86	263.2	33.48	205.85	78.21	72	234.6	44.21	127.46	54.33	66.27
2	open	8(3H, 1G, 4J)	73	238.2	30.36	151.05	63.41	70	230.8	46.40	93.05	40.32	51.87
3	3 open 2 bred	5(4H, 1J)	52	261.7	32.56	206.27	78.82	51	251.1	47.25	128.58	51.21	65.02
4	3 open 2–29 days	6(3H, 1G, 2J)	54	262.5	30.55	205.68	78.35	62	234.2	37.05	149.69	63.92	71.14
5	4 open 2–31 days	6(5H, 1G)	74	256.1	36.87	204.55	79.87	50	238.8	47.73	133.99	56.11	67.99
6	1 open 5–67 days	6(4H, 1G, 1J)	61	249.6	31.86	206.65	82.79	58	220.5	49.40	134.94	61.20	72.00
7	2 open 2–76 days	4(4H)	33	269.3	40.75	183.79	68.25	45	239.3	41.58	117.17	48.96	58.61
8	116	6(4H, 1G, 1J)	59	257.1	35.76	179.51	69.82	58	234.8	50.93	93.19	39.69	54.76
9	137	7(4H, 1G, 2J)	70	233.3	28.92	215.99	92.58	67	235.1	39.98	173.83	73.94	83.26
10	164	3(1H, 1G, 1J)	31	267.3	36.03	178.73	66.86	30	244.4	43.26	127.50	52.17	59.52
11	217	4(3H, 1G)	44	256.3	28.10	324.40	126.41	30	232.0	34.68	286.09	123.30	124.85
Breed													
Jersey	all	33	374	251.6	34.62	184.49	73.33	364	236.1	50.68	126.34	53.51	63.42
Holstein	all	6	93	269.3	34.67	200.47	74.44	76	247.7	44.17	110.01	44.41	59.43
Guernsey	all	10	167	255.3	28.93	212.65	83.29	166	234.9	41.44	139.72	59.46	71.39

[a] H = Holstein, G = Guernsey and J = Jersey

will be required to indicate whether the rise during the second month is significant. Until that time, it seems preferable to believe that early pregnancy is not a period of increased androgen excretion.

It is well known that the first half to two-thirds of pregnancy is a period of rapid duct and lobule-alveolar growth of the udder. This growth is stimulated by the hormone of the corpus luteum, called progesterone, acting upon the anterior pituitary thus stimulating the secretion of the mammogenic hormone. It has been proved that the estrogenic hormones augment the action of progesterone and mammogen. It also has been shown that certain androgenic hormone derivatives can stimulate slight lobule-alveolar mammary growth (7). The fact that the androgenic hormones are not excreted in increased amounts during the first two-thirds of pregnancy suggests that they do not play a predominant role in the great growth of the udder at this time. They may supplement the progesterone and balance physiologically the increasing secretion of estrogen.

The most striking change in the androgen excretion rate occurs during the period preceding parturition. It is well known that the excretion of estrogen both in the urine (15) and feces (2) increases rapidly at the approach of calving in dairy cattle. It is possible that the rise in androgen excretion at this time indicates a mechanism designed to counter-balance or offset, in part, the physiological effect of the rapidly rising prepartum estrogen secretion. It is believed that the secretion of progesterone may decline at this time, thus permitting estrogen to become predominant and to initiate parturition and, by stimulation of the pituitary, to increase the secretion of the lactogenic hormone (5, 6).

Since estrogen has been shown to stimulate the secretion of adrenocorticotrophic hormone by the pituitary, there would be expected increased gluconeogenesis of protein and resultant loss of nitrogen in the urine due to the hormones of the adrenals (3, 9). The androgens are known to have the opposite effect, increasing the retention of nitrogen and body growth by reduction of the secretion of the adrenal cortical hormones (16).

The concurrent rise in both estrogen and androgen during late pregnancy may indicate the presence of an adaptive mechanism of the body by which certain effects of one hormone can be balanced by the opposite effects of the other yet permitting necessary stimulation to prevail, *i.e.*, the estrogen stimulation of the lactogenic hormone.

The rise in androgen secretion at the approach of parturition suggests the need of further study of the relation of estrogen to androgen in the stimulation of the lactogenic hormone. It has been shown that androgenic hormones are capable of stimulating an increase in the lactogenic hormone of the pituitary (5). Does the androgen secreted prepartum supplement estrogen in the stimulation of the lactogenic hormone?

Since the level of excretion of androgenic hormones during most of lactation is rather uniform, there is no reason to believe that the androgens play a dynamic role in the maintenance of milk secretion.

SUMMARY AND CONCLUSIONS

1. Manure from cows of the Guernsey, Holstein and Jersey breeds during various physiological states has been dried at 45° C. and assayed biologically for its content of male (androgenic) hormone.

2. It was observed that sexually mature non-pregnant heifers excrete male hormone at a level comparable to those of mature cows.

3. During the first two-thirds of pregnancy, no tendency for a rise in androgen excretion was observed. There was evidence of a preparturient rise in androgens.

4. With the advance of lactation, no change in androgen excretion was noted except when associated with the approach of the subsequent parturition.

5. Dried cow manure from the Guernsey and Holstein breeds appeared comparable in biological activity; the Jersey cows appeared to excrete slightly more male hormone.

6. It is suggested that the preparturient rise in androgen may be related to the marked rise in estrogen at the same time.

7. It is possible that androgens as well as estrogens play roles in the stimulation of the secretion of the lactogenic hormone by the pituitary at the time of parturition.

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THE INFLUENCE OF THE RATION AND RUMEN INOCULATION ON THE ESTABLISHMENT OF CERTAIN MICROORGANISMS IN THE RUMENS OF YOUNG CALVES[1]

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INTRODUCTION

Previous investigations concerning the etiology of digestive disturbances in young calves included a study of the microorganisms which appeared in their rumino-reticular cavities (7). It was observed at that time that rumen fauna and certain characteristic flora similar to those seen in samples from mature animals were not established in the majority of the calves examined until they were several weeks old. Upon direct inoculation of organisms from cows into the rumens of a few calves, the organisms became established in some of them. These calves, which were on various rations, were among those in the herd at that time which progressed most satisfactorily, but proper controls were not included. The investigations were continued for the purpose of attempting to determine if there was any material advantage in stimulating the development in calves of early rumen activity comparable to mature animals.

Limited studies with a few young calves indicated that certain microorganisms characteristic of the rumen flora and fauna failed to become established regardless of how often inoculations were made when most of the dry feed ingested was grain. It generally was possible, on the other hand, to establish these particular rumen microorganisms in calves even before they were a week old, provided they were ingesting good quality hay and no grain. Variations in ruman flora which were related to the feed ingested have been reported for sheep by Elsden (4), who also cited van der Wath's findings on the same subject. Phillipson (6) also makes reference to this variability of the flora associated with ration differences. It would be expected that a similar situation would exist as regards young calves.

As a result of the preliminary investigations, it was decided to place 4-day-old calves on various systems of feeding, both with and without rumen inoculations, in order to study further the significance of the previous observations. Clinical studies and repeated examinations of the rumen flora and fauna were carried out on these calves during their first 6 weeks of age. Blood plasma vitamin A, carotene and ascorbic acid determinations were made at frequent intervals on many of the calves used in this experiment. The results are reported elsewhere (5).

METHODS

Young calves of both the Jersey and Holstein breeds which had received colostrum usually for 3 days were placed on twice-a-day pail feeding of pasteur-

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ized milk between their second and fourth days of age. They were fed at the daily rate of approximately 0.9 lb. for each 10 lb. of body weight at birth, but within the limits of a minimum of 5 lb. and a maximum of 10 lb. per day. It was hoped to encourage the consumption of dry feeds at an early age by limiting milk consumption to this comparatively low amount. They were treated variously as regards the dry feeds given them. Calves which received hay had access to it while with their dams or were provided on their first day of age with 1 lb. of green alfalfa hay which occasionally had some grasses mixed in it. This was replenished or replaced frequently so that the calves had fresh hay before them at all times. Grain, when included in the ration, was fed once per day as long as the quantities did not exceed 0.5 lb. per day. It consisted of a 14 per cent protein herd mixture of corn, oats, wheat bran and soybean oil meal.

Rumen inoculations were given to some of the calves by passing pieces of freshly obtained cuds from cows into the posterior of the calves' mouths. The cows were being fed alfalfa hay, grain and silage. The inoculations generally were carried out on the fifth, tenth, fifteenth and twenty-first days of age. Samples were obtained from the fore-stomachs of the calves with stomach tubes, using the syphoning method developed for use with the Colorado rumen lavage tube, and, in some instances, samples were taken at the time of slaughter. No difference as regards flora and fauna was observed in samples obtained from the same calves by the two methods.

Calves which did not receive rumen inoculations were cared for by the same personnel as the others and were housed in the same buildings. However, they were separated from direct contact with the other calves and mature animals by partitions and passageways. A limited effort was made to avoid transferring organisms from one calf to another on milk buckets and other equipment.

The rumen samples were examined under the microscope in the fresh condition, using slides and cover glasses, for the purpose of observing the protozoa. For the most part, Gram stained smears were relied upon for bacteriological purposes. In preparing the slides, some of the thick soupy materials containing particles of feed were included, since Baker (2) has shown that some varieties of bacteria tend to remain rather closely attached to feed particles and may not always be readily visible in the free liquid. Descriptions of microorganisms based on morphological and staining characteristics leave much to be desired. However, this method was considered the most advantageous to use for evaluating rumen bacteriological activity under the conditions of this investigation.

RESULTS

The Microorganisms

The protozoa encountered in the rumen samples from the calves appeared to be similar to those mentioned by others, including several investigators whose observations were cited by Baker and Harriss (3) in their recent review article. All those commonly seen in samples from cows were established readily upon

inoculation into calves which were ingesting suitable feeds including hay and hay-plus-grain rations.

Many bacteria differing in morphology and staining characteristics were visible in the rumen samples. Many of them have been described previously by others including Baker (1, 2) and Baker and Harriss (3). Some varieties of organisms were observed to be noticeably present only when appreciable quantities of hay were being consumed and certain other varieties when grain was the principal dry feed ingested. This does not mean that these particular organisms alone were present under such conditions, but merely that they at least were readily visible in the smears. However, when the proportion of grain consumed was high, a few varieties of organisms including those described herein sometimes would appear to make up the majority of the bacterial population. Proof neither was sought nor obtained that the organisms mentioned were the most important ones in the digestion of the feeds present. They were used in this study

TABLE 1

Classification and description of some calf rumen flora observed to vary with the type of feed eaten

Hay Flora[a]	
Group I	Quite large G+ coccoids in closely knit pairs
Group II	Large G+, thick, fairly square-ended rods
	Very large G– cigar-shaped rods
	Smaller G– short rods in fours and multiples of 4
Grain Flora[a]	
Group I	Medium-sized, comparatively thin, G+ rods (sometimes granular stain and variable length)
Group II	G– rods resembling coliform

[a] Flora which appeared to be characteristically associated with the ingestion of these feeds.

as indicators of the presence or absence of characteristic bacterial populations. As reported elsewhere, further studies showed that the same organisms possibly might be used as indicators, within certain limits, of the relative ratios of grain and hay being ingested by young calves (8).

It was possible to subdivide the varieties of the organisms which were noticed to be associated with hay consumption into two sub-groups (table 1). The first consisted of large Gram-positive coccus organisms in closely knit pairs and sometimes in groups of four or more, but not in chain formations. The approximate size of the pairs was 2.8×2.3 microns, and some groups composed of more than a single pair were as much as 4.4×4.0 microns. They possibly were similar to those called large sarcina packets by Baker (1). Among the organisms included in the second hay sub-group were large Gram-positive, thick, rather square-ended rods whose length varied between 3.2 and 5.5 microns and which were approximately 2.5 microns wide. They were observed quite frequently in pairs. Gram-negative, extremely large, cigar-shaped organisms, which often were as much as 21.5 microns long and approximately 4.0 microns wide, also were included in this sub-group. It is probable that these latter organisms are similar to those referred to by others as giant elipsoidal forms (3) or Ocillospira (1, 2). Besides

these, in this group were Gram-negative rods of approximately 1.0×0.8 microns in size that tended to group in fours and multiples of four in shapes suggestive of window panes.

Organisms associated with grain consumption also could be sub-divided into two groups (table 1). The first consisted of Gram-positive rods which ranged between 1.7 and 3.4 microns in length and were approximately 0.8 microns in width. They appeared to resemble lactobacilli (10). Present in some smears were masses of either short varieties of this organism or a different organism of similar Gram staining property. Sometimes, especially when considerable numbers were present, a tendency existed for these organisms to stain in a granular manner. The second sub-group were Gram-negative and morphologically resembled coliform organisms or did not differ much from them.

Photomicrographs of the various organism types are reproduced in figure 1.

Variations in the establishment of microorganisms

Calf group 1 (hay plus rumen inoculation). Protozoa and bacteria of the two groups noted to be associated with hay ingestion were established in the rumens of eight calves, which had good quality alfalfa hay available to them from birth, before they reached the age of 3 weeks. The organisms still were present several weeks later in the seven calves which were continued on the experiment. "Grain-type" organisms were extremely scarce in samples from these calves (table 2).

TABLE 2

The influence of the ration and rumen inoculation on the establishment of certain microorganisms in calf rumens

Calf group	Ration	Age	No. calves examined	No. calves protozoa present	No. calves hay flora present		No. calves grain flora present	
					Group I	Group II	Group I	Group II
		(*wks.*)						
I	Hay plus inoculation	3	8	8	8	8	0	0
		6	7	7	7	7	0	0
II	Hay, uninoculated	3	8	0	8	3	0	0
		6	6	0	6	5	0	0
III	Hay and grain plus inoculation	3	7	6	2	1	2	0
		6	7	7	6	2	4	0
IV	Hay and grain, uninoculated	3	5	1	2	2	4	1
		6	5	1	2	2	5	3
V	Calf starter, uninoculated	3	4[a]	0	0	0	4	4
		6	2[a]	0	0	0	2	1

[a] 2 Calves received hay.

Calf group 2 (hay without rumen inoculation). Protozoa failed to make their appearance in samples from eight similarly-fed but uninoculated calves up to the time they reached the age of 3 weeks and up to 6 weeks in the case of the six of them that were continued on experiment. "Hay flora" of the paired coc-

cus type developed in all eight calves by the time they were 3 weeks old and continued to be present in samples from these calves throughout the experimental period. Samples from all but three of the eight calves contained organisms of the second hay sub-group when the calves were 3 weeks old. By 6 weeks of age, samples from five of the six calves contained some of at least one of the organisms of this sub-group. Organisms of the two grain types also were extremely scarce in samples from these calves (table 2). A fairly large Gram-positive rod, thicker and more tapered at the ends than the one seen in association with grain feeding, was observed in great numbers in samples from two of these calves by the time they were 3 weeks old and in five of the six at 6 weeks of age. These organisms never were observed in appreciable numbers in samples from any of the other calves.

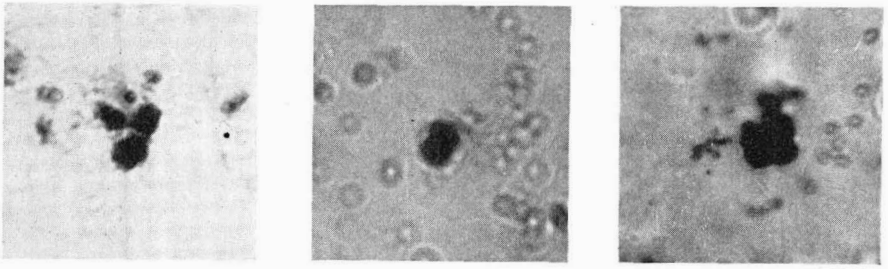
Calf group 3 (*hay, grain, plus rumen inoculation*). Seven inoculated calves were fed good quality alfalfa hay from birth and a simple 14 per cent protein grain mixture starting on the 14th day of age, both free choice. Protozoa became established in six, hay flora of the paired coccus organism sub-group in two, and hay flora of the second hay sub-group in one, by the time the calves were 3 weeks of age. By 6 weeks of age, samples from all seven had protozoa present, six contained hay flora of the first sub-group and two of the second. Gram-positive grain-type flora developed in two calves by 3 weeks and in four calves by 6 weeks of age (table 2).

Calf group 4 (*hay plus grain without rumen inoculation*). Only one of five uninoculated calves on a schedule of hay and grain similar to group III developed rumen protozoa by 4 weeks of age or even by 6 weeks of age. Flora of both hay groups were present in samples from two of the five calves by the time they were 3 weeks old but had not yet appeared in the other calves at 6 weeks of age. Bacteria of the first grain sub-group were visible in samples from four calves at 3 weeks of age and in all five calves at 6 weeks of age. Gram-negative bacteria of the second grain sub-group were observed in samples from one calf at 3 weeks and from three calves at 6 weeks of age (table 2).

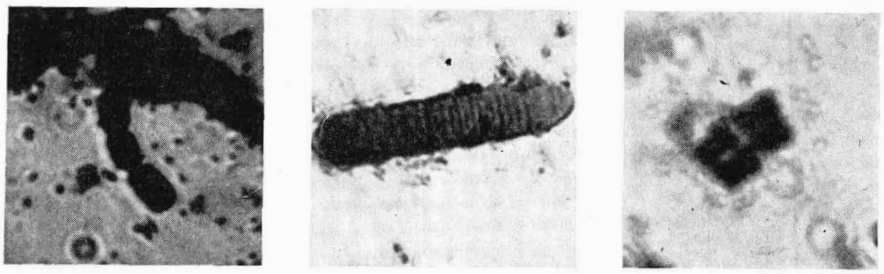
Calf group 5 (*calf starter grain ration without inoculation*). Four uninoculated calves were fed a commercial calf starter grain ration in pellet form. Two of them received good quality alfalfa hay in addition. Both feeds were given free choice starting on the fourth day of age. The amount of milk fed one of the two calves receiving hay gradually was reduced as the calf increased its consumption of the grain. At 3 weeks of age, no protozoa or hay-type bacteria could be seen in samples from any of these four calves. However, great numbers of the grain-type organisms were present almost to the exclusion of all other bacteria. Only the two calves receiving hay were continued on experiment beyond 3 weeks of age. A similar condition was noted in them when they were 6 weeks of age, although the Gram-negative bacteria resembling coliform organisms appeared to be less prevalent (table 2).

Samples from approximately 20 calves of similar age and on rations fairly similar to those used for the last two groups had been examined repeatedly the previous year. The results were very much the same.

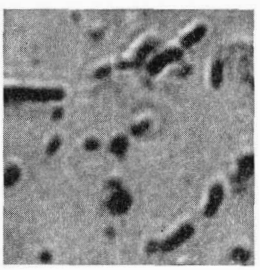
Calf group 6 (shavings and straw bedding without inoculation). Neither protozoa nor hay-type flora were established in any one of seven uninoculated calves which were fed milk alone and bedded with shavings or straw up to the time they were 3 weeks of age. It also was questionable whether any of the



Hay Group I Organisms
(pairs and multiples of pairs)



Hay Group II Organisms
(thick rod, cigar-shaped rod, and group of small rods)



Grain Group I Organisms
(medium-sized rods)

FIG. 1. Photomicrographs of calf rumen microorganisms
(approximately $\times 1800$)

grain-type flora were present in the samples. In fact, relatively few organisms of any kind were present even though all calves ate some of the bedding. Medium-sized, spindle-shaped bacteria which often contained spore-like bodies were present in appreciable numbers in samples from five out of six of these calves

which were bedded with shavings. These were the only ones in which this organism was ever observed.

Growth of the calves. An effort was made to have the two breeds about equally represented in each group with the exception of group VI, which was composed entirely of Jerseys. The average gains in weight made by the calves in all six groups were between 7 and 9 lb. per calf for the first 3 weeks. The calves in groups I, II, III and IV made an average gain of 15 to 16 lb. per calf during the second 3 weeks, while those in group V gained an average of 22 lb. Group VI was discontinued at the end of the first 3 weeks. These gains are lower than the standards given for calves of comparable breeds by Ragsdale (9). Although gain in weight was desired, it was not an objective in these experiments.

Health of the calves. The hair coats of calves on hay alone without rumen inoculation (Group II) appeared to be rougher than those on similar feed which received the inoculation (Group I). Any difference resulting from inoculation was less noticeable or non-existent between the groups of calves receiving rations containing grain. As reported elsewhere (5), calves in group I receiving hay and inoculation maintained on the average uniformly higher blood plasma levels of ascorbic acid during their first 6 weeks of age than any other group. The clinical manifestations of sickness were limited to digestive tract upsets. No trouble was experienced in this respect among the calves in groups I and II, although two calves in each group had rather soft feces on a single day each. The incidence of diarrhea among the calves in group III was 57 per cent, group IV 66 per cent, group V 75 per cent and for group VI 70 per cent. The duration of individual attacks ranged between 2 and 8 days.

Sources of the organisms other than from rumen materials. The organisms designated as Hay Group I and those associated with grain established themselves more readily by natural means in the young calves than did the protozoa and the organisms in Hay Group II. This indicates greater availability of sources of the former. Feces would be a logical source of organisms, augmenting the organisms spread through slobbers from cud-chewing mature animals. Baker (2) examined the feces from a bovine fed on hay and various concentrate rations for some of the characteristic organisms. He concluded that the majority of these organisms were destroyed on passage through the intestines. This seemed to be true based on our Gram stain examinations of fecal samples. However, limited numbers of organisms resembling those designated as Hay Group I and also the Gram-positive varieties of those associated with grain ingestion were present in seven fecal samples from rumen inoculated calves on hay and milk alone. They apparently also were present to a lesser extent in two of four samples from similar calves eating both hay and grain, and in six out of seven samples from cows on mixed rations. Although the varieties associated with grain ingestion were visible in three samples from calves on calf starter ration, no hay-type bacteria could be observed. A young calf was given, by stomach tube, repeated rumen inoculations with feces from a 5-month-old inoculated calf on a hay and skim-milk diet. None of the second-hay-group bacteria or rumen protozoa became established in its rumen even though the first-hay-group organisms did so. Thus,

it would appear that feces from older stock may provide a source from which some of the rumen microflora may be obtained by young calves.

DISCUSSION

It would appear from the present observations that rumen protozoa encounter difficulty in being transferred to young calves under conditions which frequently exist on dairy farms. However, their importance as regards the well-being of the host animals has not been fully established. It is not possible from our data to deduct that they were responsible for the higher blood plasma ascorbic acid levels reported elsewhere (5), or the better appearance of the inoculated calves which received hay alone. In fact, there is more indication that flora were involved because the calves in group I maintained higher blood plasma levels of ascorbic acid than group III, yet both had protozoa in their rumens. Furthermore, a much less satisfactory condition as regards hay-type flora existed in group III as compared to group I.

The type of feed ingested appeared to be the principal factor which influenced the establishment of the organisms designated as group I of those noted to associate with hay and both groups of organisms observed in association with grain ingestion. Evidently the same was partly true of organisms designated as group II of those observed to associate with hay, although the figures indicate a less satisfactory source of organisms for inoculation of the calves than existed for the former. The failure of inoculation to establish hay varieties of flora in calves provided with both hay and grain was unexpected and difficult to explain until later experiments were conducted. These showed that once the ratio of grain ingested exceeded the hay, the proportion of hay varieties of flora appeared to markedly decrease in the Gram stain preparations of rumen samples (8). Thus, it appears that the logical explanation is that some of the young calves tended to eat proportionately more grain than hay when both were offered free choice.

The practical observation that the early development of mature rumen function in young calves may be influenced for the better by inoculation, under some conditions, is probably of some significance. Whether the microscopic observations as outlined here are sufficiently sound and adequately described must await further experimental work under varied conditions. The varieties described as being associated with hay ingestion are sufficiently characteristic in morphology that their recognition probably is quite reliable. However, because of the lack of definite morphological individuality, recognition of organisms designated as associated with grain ingestion possibly is less accurate.

Quite probably the rumen flora may vary somewhat between herds. A slight indication of this has been obtained from examinations conducted on calves in a few other herds and from the reports of others. However, fairly similar conditions probably exist in the majority of herds as regards rumen microorganisms, and observations made on calves in various locations may be comparable.

The comparatively low milk consumption of the calves used in these experiments probably was responsible, especially during the first 3 weeks of age, for

the fact that weight gains were lower than accepted standards (9). The total consumption of milk during the 6-week-period was as low as 210 lb. each for most of the Jersey calves and only two Holsteins received more than 336 lb.

Apparently, rumen inoculation did not influence the ability of the calves to withstand the factors which existed in the herd that stimulated attacks of diarrhea. On the other hand, the type of ration fed, especially good alfalfa hay and milk, appeared to be of more value in preventing the occurrence of this malady. This naturally raises the question as to whether or not, under some conditions, the health of the digestive tracts of young calves may be jeopardized as the result of attempts to make rapid gains in weight at very early ages.

SUMMARY

The rumens of young calves being fed milk and various dry feed rations were inoculated with microorganisms from the rumens of mature stock by placing pieces of cuds from the latter in the posterior of the mouths of the calves. The inoculations were omitted from similarly fed calves used as controls.

The inoculations assisted in the establishment of protozoa in the rumens of calves eating either hay alone or both hay and grain. They assisted in the establishment of some, but not all, of the characteristic varieties of rumen microflora which were associated with hay ingestion in calves fed on alfalfa hay alone. The establishment of varieties of organisms which were associated with the ingestion of grain was not assisted by the inoculations. The establishment of the varieties of flora which were associated with hay ingestion was inhibited in some calves when grain was fed.

The inoculated calves on a diet of alfalfa hay and milk alone were considered to have a better appearance than the controls, but this difference was not apparent between the inoculated and uninoculated groups fed on both hay and grain. Data reported elsewhere (5) show that uniformly higher levels of ascorbic acid in the blood plasma were maintained during the first 6 weeks following birth in the inoculated calves fed alfalfa hay and milk alone than in the calves of any other group. Gains in weight by the calves were very similar in all groups during the first 3 weeks of age. During the second 3-week period, all groups made similar gains except group V, which received a commercial calf starter grain ration. The two groups of calves fed on alfalfa hay and milk alone were free of diarrhea, but the incidence in all other groups was in excess of 50 per cent.

Feces were examined in a search for sources of organisms which resembled natural inhabitants of rumens and some appeared to be present.

The authors wish to acknowledge the assistance of Mr. John Tate, Mr. R. L. Johnson, and Mr. C. E. Knoop in conducting this investigation.

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THE INFLUENCE OF THE RATIO OF GRAIN TO HAY IN THE RATION OF DAIRY CALVES ON CERTAIN RUMEN MICROORGANISMS[1]

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Variations were observed to occur in certain flora and fauna present in rumen samples from young calves which apparently depended upon the ratio of grain to hay that the calves ingested (1). This indication was pursued further to determine if the observed variations were consistent. Should this be the case, it was hoped that examinations of rumen contents and rumen microorganisms might prove to be more valuable in making differential diagnoses of some calf problems. For instance, information of what feeds have been ingested often is essential in order to determine with any degree of accuracy if a relationship exists between the feed and the unhealthy condition of the calves. Yet, it often is difficult to estimate what the calves actually have been eating when more than one feed has been offered free choice, including the bedding. This problem is complicated further when several calves are fed together in groups because of the individual variations that exist between calves in their choice of feeds.

It perhaps is of interest to add here that extreme variations in rumen microfauna and microflora were observed to be more frequent among young calves fed dry feeds free choice during their first few weeks of age than among similarly-fed older calves. This situation probably resulted from the fact that younger calves ate limited quantities of feed and often limited themselves to only one feed at a time. Because of the relatively small capacity of their rumens, the influence of eating a single feed on the microorganisms was much greater than in older calves in which the buffering effect of larger amounts of previously-eaten feeds existed.

METHODS

The 19 calves used were between 1 and 4.5 months of age. Rumen flora and fauna, which had been classified as quite characteristic, had been present in all the calves prior to the time this particular study was undertaken. Most of them had been inoculated by use of cuds from mature animals in the manner outlined previously (1). It was difficult to determine accurately the relative quantities of hay and grain ingested by calves younger than these and, consequently, data from such calves were omitted.

The quantities of hay and grain consumed during the 4 days prior to the examinations were used in arriving at the relative ratios of the two feeds eaten by the calves. However, they actually were consuming approximately the same proportions for some days longer than this. This period was chosen on the basis of experience with the establishment of characteristic flora and fauna in the rumens of young calves.

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Some calves received different ratios of hay to grain at different times either by design or their own choice and, consequently, data from them were included in more than one category. The hay was fairly good quality alfalfa hay and the grain was a simple 14 per cent protein mixture containing 4 parts of corn, 3 of oats, 1 of bran and 1 of soybean oil meal.

Rumen samples were obtained by use of stomach tubes and examined microscopically in the fresh state and by use of Gram stains as outlined previously (1). The preparations were examined for the same organisms as those discussed in the previous paper (1). Two main groups of bacteria were noted, one associated with hay ingestion and the other with grain, and two sub-groups were recognized in each group.

RESULTS

The results of 35 examinations are summarized in table 1. Column 3 in the

TABLE 1

The influence of the ratio of grain to hay on some calf rumen microorganisms

Ratio	No. rumen samples	No. samples protozoa present	No. samples Hay flora present		No. samples Grain flora present	
Grain : Hay			Group I	Group II	Group I	Group II
0 : 1	19	19	19	19		
1 : 3	6	6[b]	6	6	1	1
					4[a]	
2 : 3	4	3[b]	4	2	4	2
		1		2[a]		
1 : 1	6	6[b]	6	3	3	5
				1[a]		
3 : 2	3	2[b]	3	2	3	2
		1[a]				
3 : 1	2	2[a]			1[b]	1[b]
					1	1
1 : 0	5	1[a]			4[b]	3[b]
						1

[a] = few; [b] = masses; unmarked = moderate or appreciable numbers.

table shows that protozoa were present in all samples except four of the five from animals on grain without hay. Moderate numbers were observed to be present when the dry feed ration consisted of hay alone. With the addition of some grain to the ration, the numbers of protozoa in the samples increased greatly. This is in agreement with the findings of others as summarized by Phillipson (2). A marked reduction in numbers followed, once the ratio exceeded 3 parts of grain to 2 parts of hay. Limited numbers only were present in samples from calves eating three or more times as much grain as hay. The one calf receiving grain without hay, but still having protozoa present in the rumen, was one of the oldest calves used. It had been fed grain with straw for bedding for 10 days at the time of the examination.

Organisms which were classified as belonging to the hay flora groups were visible in Gram stain preparations of all 19 samples from calves on rations of hay alone. The prevalence of these organisms in the smears appeared to in-

crease with the addition of some grain to this ration. However, as the proportion of grain ingested approached quantities equal to the hay, a reduction was rather apparent. Organisms of the second hay group were reduced more noticeably than those of the first hay group in samples from animals eating as much or more grain than hay. As shown in columns 4 and 5, both groups of the organisms associated with hay were missing from the samples once the ratio reached 3 parts of grain to 1 of hay. Thus, the organisms associated with hay consumption disappeared from the rumen samples at lower ratios of grain to hay than did the protozoa. The apparent increases in the flora associated with hay ingestion on the addition of some grain to rations of hay alone may have resulted from the eating of more balanced rations by the calves. Such is suggested by the observations of Van der Wath, as cited by Phillipson (2), that bacterial numbers were influenced by the diet, with balanced rations being the most satisfactory.

Only limited numbers of the bacteria which were observed to associate with grain rations were visible in the samples until a proportion of 3 parts of grain to 2 of hay was being consumed (columns 6 and 7). These organisms increased in relative prevalence in comparison with other flora as the proportion of grain increased. On rations of grain alone, some samples appeared to contain practically no other organisms.

Very small Gram-negative organisms were noticeably prevalent in samples from calves on rations containing hay alone or high proportions of hay. Small Gram-positive short rods or cocci were observed in increasing proportions on the addition of grain to rations of hay.

Although data collected on this group of calves are very limited, they indicate that by observing certain flora and fauna present in rumen samples from calves, it may be possible to estimate the relative ratio of grain to hay that they are ingesting.

SUMMARY

A total of 35 rumen samples from 19 calves between the ages of 1 and 4.5 months were examined microscopically. The calves received rations of alfalfa hay or grain alone, or various proportions of these. Most of them had received rumen inoculations and the remainder had been exposed to usual rumen microorganisms in a natural manner.

Moderate numbers of protozoa and flora of varieties observed to associate with hay ingestion accompanied the ingestion of hay without grain.

Masses of protozoa along with fairly numerous flora of the 2 hay groups were associated with the consumption of hay and moderate quantities of grain.

Similar concentrations of protozoa, accompanied by rather limited numbers of organisms of the hay groups and fairly numerous bacteria of the varieties observed to associate with grain consumption, accompanied the ingestion of approximately equal quantities of hay and grain.

Limited numbers of protozoa accompanied by great numbers of bacteria of the grain groups, but no organisms of the hay groups, were present when the ration consisted of almost all grain.

Protozoa and organisms of the varieties associated with hay ingestion generally were absent entirely in samples from calves on strictly grain rations.

The authors wish to acknowledge the assistance of Mr. John Tate, Mr. R. L. Johnson and Mr. C. E. Knoop in conducting this investigation.

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THE INFLUENCE OF THE RATION AND EARLY RUMEN DEVELOPMENT ON THE CHANGES IN THE PLASMA CAROTENOIDS, VITAMIN A AND ASCORBIC ACID OF YOUNG DAIRY CALVES[1]

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The period between birth and the time a calf has developed into a normally-functioning ruminant is recognized as critical from the standpoint of nutritional well-being and health. As pointed out by Pounden and Hibbs (8), this period may extend for many weeks in some cases, when judged by the presence or absence of characteristic rumen microorganisms.

Several reports have appeared in the literature showing the usual changes in the blood plasma carotenoids and vitamin A of calves from birth to several weeks of age (1, 2, 3, 6, 11, 12, 14, 15). The changes in plasma ascorbic acid have been reported by Phillips *et al.* (7), Hibbs and Krauss (2), Sutton and Kaeser (12), and Teeri *et al.* (14).

Wise *et al.* (15) have reported results showing that after the blood carotenoids reach a peak on about the third day, as the result of colostrum feeding, there is a rapid decline for from 4 to 5 weeks and then a gradual rise to the post-colostrum feeding level or slightly above at 8 to 10 weeks of age. Vitamin A follows a somewhat similar trend. Teeri *et al.* (14) report that, on the average, blood carotenoid values level off between 15 and 23 weeks of age at about 44 γ per 100 ml. in Holstein calves and at about 65 γ per 100 ml. in Jersey and Guernsey calves. Considerable individual variation is indicated by the high and low values obtained in calves apparently fed and managed alike.

It is striking that until most calves are several months old their blood carotenoid levels do not even approach the levels found in mature animals fed on dry feeds. This may be the result of the ability of the mature animal to consume relatively large quantities of roughage. It is not illogical, however, in the light of our previous observations regarding the variations in the rate of establishment of characteristic rumen microorganisms in calves (8), to assume that the differences in the blood carotenoids and vitamin A levels between calves and adult animals might be due, at least in part, to their relative ability to digest roughage in the rumen. Furthermore, many of the individual variations found among calves may be due, in part, to the differences in the age at which normal rumen function begins.

Investigations were undertaken, therefore, to study the influence of the ration and rumen inoculations on the establishment of rumen function in young calves, the results of which are reported elsewhere (9, 10). Concurrently, a study was made on the influence of the ration and early rumen development on the changes

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occurring in the plasma carotenoids, vitamin A and ascorbic acid of dairy calves during their early postnatal development.

EXPERIMENTAL

Plasma vitamin A and carotenoids were determined by the method described by Kimble (4). The method of Mindlin and Butler (5) was used to determine plasma ascorbic acid.

After a colostrum feeding period, usually 3 days, all calves were fed whole milk for the entire experimental period of 6 weeks at the rate of 0.9 lb. per 10 lb. of body weight at birth. This relatively low level of milk feeding was adopted in order to encourage the calves to consume more of the dry feeds offered.

Beginning on the fourth day of age, calves born in the herd from January until April, 1948 were assigned to one of six groups and fed whole milk and various dry feeds with and without rumen inoculations as follows: Group I, alfalfa hay plus rumen inoculations; Group II, alfalfa hay alone; Group III, alfalfa hay plus grain (14 per cent protein herd ration) plus rumen inoculations; Group IV, alfalfa hay plus grain (14 per cent protein herd ration); Group V, alfalfa hay plus standard calf starter pellets; Group VI, whole milk only (this group was continued on experiment for only 3 weeks).

The rumen inoculations in groups I and III were accomplished by direct transfer of small pieces of cud material to the mouths of the calves from cows in the herd. This was done in order to make certain that these calves had access to the microorganisms normally present in the rumens of the adult animals.

All calves were bled as nearly as possible on the fourth and seventh days of age and weekly thereafter until the forty-second day of age and the plasma carotenoids, vitamin A and ascorbic acid were determined.

The results of the blood analyses are shown in table 1. No beneficial effect of rumen inoculations on the blood plasma carotenoids was observed. Average figures for the calves in groups III, IV and V, which were fed grain, show that when grain was included in the ration, plasma carotenoids did not increase during the first 6 weeks to the extent observed in groups I and II, which were fed alfalfa hay as the only dry feed. The calves in groups I and II made an average steady increase in plasma carotenoids from 4 days until 42 days of age, reaching extremely high levels as compared to any of the other groups.

Plasma vitamin A was found to decrease markedly from the fourth until the forty-second day of age in all groups except group V, which received a calf starter containing 5,000 U.S.P. units of supplemental vitamin A per lb. No marked beneficial effect from rumen inoculations was noted on the plasma vitamin A level although the values for vitamin A appear to be somewhat higher in group III as compared to group IV.

The level of plasma ascorbic acid was found to decline between the seventh and fourteenth days of age in all groups except group I, which was fed alfalfa hay plus rumen inoculations. This group maintained the most uniformly high level of plasma ascorbic acid during the first 4 weeks of any of the groups.

TABLE 1

The influence of the ration on the changes in the plasma carotenoids, vitamin A and ascorbic acid of the blood of young calves

Group[a]	No. of calves	Plasma carotenoids Ages of animals						
		4 days	7 days	14 days	21 days	28 days	35 days	42 days
				Plasma carotenoids (γ/100 ml.)				
I	6	32.3 ± 6.8[b]	34.9 ± 5.5	42.5 ± 4.4	58.2 ± 16.3	76.9 ± 14.2	93.2 ± 20.0	99.2 ± 23.3
II	6	35.9 ± 5.7	38.2 ± 5.8	49.9 ± 14.0	53.6 ± 19.4	67.9 ± 23.9	92.6 ± 20.0	96.6 ± 22.0
III	7	20.6 ± 2.9	20.7 ± 3.1	34.0 ± 7.7	37.0 ± 12.7	32.7 ± 9.1	36.2 ± 10.7	49.7 ± 3.3
IV	5	21.5 ± 3.9	32.7 ± 9.8	36.2 ± 7.5	35.4 ± 13.7	35.2 ± 10.3	44.4 ± 12.3	56.8 ± 15.4
V	4	38.4 ± 20.0	32.7 ± 8.4	35.1 ± 8.9	31.4 ± 10.3	33.0 ± 0.0	35.5 ± 10.7	28.3 ± 2.6
VI	6	19.4 ± 3.5	22.4 ± 4.5	27.5 ± 4.6	20.0 ± 2.6			
				Plasma Vitamin A (γ/100 ml.)				
I	6	20.3 ± 3.3	16.4 ± 3.1	14.8 ± 1.5	12.1 ± 0.9	10.8 ± 1.2	9.2 ± 1.8	8.1 ± 1.0
II	6	16.1 ± 2.8	13.7 ± 2.3	11.7 ± 1.5	9.6 ± 2.2	6.8 ± 1.6	7.5 ± 1.1	7.7 ± 1.1
III	7	13.1 ± 1.8	12.2 ± 2.5	11.6 ± 1.3	10.5 ± 1.6	7.9 ± 1.1	8.9 ± 1.5	9.0 ± 1.2
IV	5	13.5 ± 1.4	9.9 ± 1.2	8.6 ± 1.4	8.8 ± 1.0	6.5 ± 0.9	7.0 ± 0.7	6.3 ± 0.3
V	4	15.1 ± 5.3	13.4 ± 4.6	12.8 ± 3.1	11.7 ± 1.8	15.0 ± 0.0	16.0 ± 2.5	14.7 ± 1.8
VI	6	9.2 ± 1.7	7.9 ± 1.3	9.4 ± 0.9	6.3 ± 1.4			
				Plasma ascorbic acid (mg./100 ml.)				
I	5		0.47 ± 0.06	0.46 ± 0.03	0.42 ± 0.04	0.47 ± 0.03	0.43 ± 0.09	0.36 ± 0.05
II	5		0.47 ± 0.06	0.27 ± 0.05	0.25 ± 0.01	0.29 ± 0.05	0.46 ± 0.04	0.42 ± 0.03
III	7		0.49 ± 0.02	0.26 ± 0.03	0.38 ± 0.07	0.33 ± 0.04	0.38 ± 0.06	0.41 ± 0.05
IV	5	0.44 ± 0.03	0.36 ± 0.04	0.33 ± 0.09	0.32 ± 0.02	0.30 ± 0.05	0.40 ± 0.08	0.50 ± 0.07
V	3		0.41 ± 0.05	0.30 ± 0.04	0.40 ± 0.00	0.39 ± 0.00	0.30 ± 0.00	0.44 ± 0.00
VI	6	0.53 ± 0.01	0.59 ± 0.03	0.33 ± 0.08	0.35 ± 0.04			

[a] Group I. Whole milk plus alfalfa hay plus rumen inoculations.
Group II. Whole milk plus alfalfa hay.
Group III. Whole milk plus alfalfa hay plus grain plus rumen inoculations.
Group IV. Whole milk plus alfalfa hay plus grain.
Group V. Whole milk plus commercial calf starter.
Group VI. Whole milk only.

[b] Standard error.

By the fifth week there was very little difference among all the groups. Group II, which received alfalfa hay alone without the rumen inoculations, declined in plasma ascorbic acid to the lowest level of any of the groups during the first 4 weeks. Groups III, IV and V, in which grain was included in the ration, declined sharply but recovered to a level intermediate between groups I and II by the twenty-first day of age.

In view of these results, it was decided to investigate the possible effects of the addition of grain to the ration of older calves which had been fed hay as the only dry feed. Four calves from groups I and II were continued on whole milk plus alfalfa hay to an average of 64 days of age.

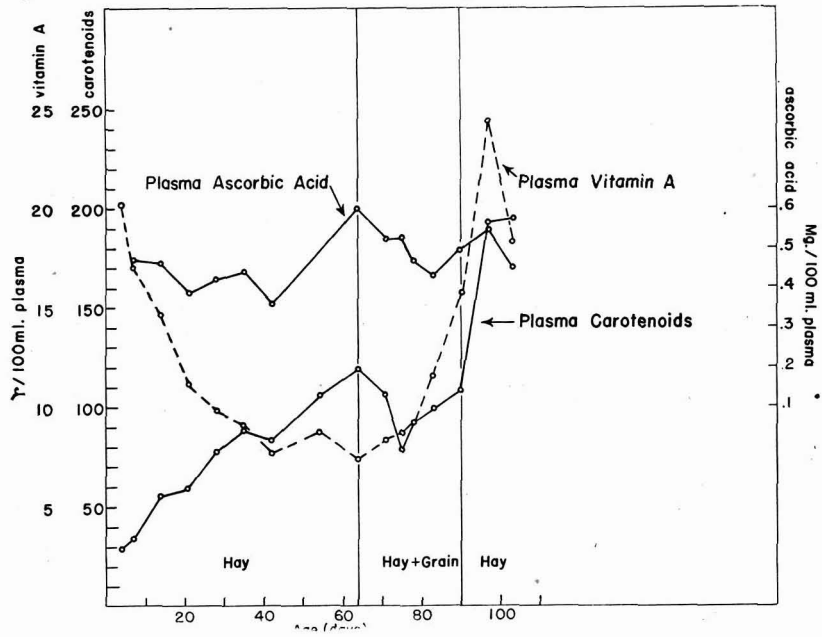

FIG. 1. The influence of adding grain to the ration of 64-day-old calves fed only whole milk and alfalfa hay until that time.

As shown in figure 1, the average plasma carotenoids had increased consistently from 29 γ per 100 ml. at 4 days of age to 118 γ per 100 ml. at 64 days of age. Plasma vitamin A had declined from 20.2 to 7.4 γ per 100 ml. during the same period, and plasma ascorbic acid values had increased from 0.47 to 0.60 mg. per 100 ml.

At this point, a 14 per cent protein grain mixture was included *ad libitum* in the ration. The week prior to the change in ration the calves were eating an average of 2.4 lb. of hay per day. Hay consumption decreased during the grain feeding period. After 4 weeks of grain feeding, the calves were eating 1.7 lb. of hay and 2.6 lb. of grain per day. Grain then was removed from the ration, and during the following week the calves consumed an average of 2.6 lb. of hay per day.

Figure 1 indicates the changes that occurred in the levels of blood plasma carotenoids, vitamin A and ascorbic acid as the result of adding grain to the ration. The plasma carotenoid level was shown to decrease, the plasma vitamin A increased and the plasma ascorbic acid decreased during this period. When grain was removed from the ration, the carotenoids rapidly increased from 108 to 193 γ per 100 ml. within a week, and the plasma vitamin A continued to rise to a peak of 24.3 γ per 100 ml. No marked changes occurred in the plasma ascorbic acid level after the grain was removed from the ration.

DISCUSSION

So far as the blood picture is concerned, the only marked beneficial effect of rumen inoculations appeared to be the higher plasma ascorbic acid level maintained in group I as compared to group II, where alfalfa hay was the only dry feed fed. The mode of action through which this effect was elicited is not readily explainable.

The suppressing action of grain feeding on the blood plasma carotenoids is strikingly demonstrated by the differences in the plasma carotenoid levels between groups I and II, which were fed hay alone, and groups III, IV and V, which received grain in addition to the hay. Accurate records of hay consumption were difficult to obtain during the first few weeks. Therefore, data are not available to demonstrate conclusively whether increased hay consumption or increased digestibility of the hay played the leading role in causing the relatively higher plasma carotenoid level of groups I and II as compared to groups III, IV and V. Indications from the data obtained were that the calves fed grain consumed less hay than those fed hay alone. It would seem that decreased digestibility of the hay possibly was a factor contributing to the low carotenoid levels observed in the grain-fed groups based on the conditions observed with respect to the microorganisms in the rumen when high proportions of grain to hay were fed (10). This would be likely especially when grain consumption reached a level equal to or higher than the hay consumption, as was the case in many instances.

It was noted that the plasma vitamin A level decreased when the plasma carotenoids were increased in groups I and II. The opposite effect on plasma vitamin A was observed when plasma carotenoids declined following the addition of grain to the ration of 64-day-old calves (fig. 1). This suggests that the plasma vitamin A level of the blood is not always a reliable indicator of the state of vitamin A metabolism in the young calf.

Sutton and Soldner (13) have presented data showing that in adult cattle the seasonal changes in blood plasma vitamin A do not closely follow blood plasma carotene changes but tend to lag behind. Plasma vitamin A often was observed to increase when plasma carotene was on the decline.

There are several factors which may be responsible for these apparent discrepancies in the behavior of plasma vitamin A in relation to the plasma carotenoids. Possibly, one of these complicating factors is the liver storage of vitamin A. The degree of saturation of the liver and whether the vitamin A

stores are being increased or depleted may influence the plasma vitamin A level, independent of the effect of the intake of carotene from the roughage. It also is possible that factors affecting the conversion of carotene to vitamin A complicate the blood picture. The answers to these questions must await further work involving the relationship between blood plasma vitamin A and liver storage, the sources of vitamin A and carotene and the physiology of the conversion of carotene to vitamin A in the calf.

SUMMARY AND CONCLUSIONS

Preliminary investigations indicated that the development of the rumen in young calves is influenced by the type of ration fed. Experiments were conducted, therefore, to determine the effect of different rations and early rumen development on the levels of vitamin A, carotenoids and ascorbic acid in the blood of young dairy calves.

Rumen inoculations, accomplished by direct transfer of cud material from cows in the herd to the calves, were supplied to about one-half the calves in order to make certain that they had access to the microorganisms present in the rumens of adult animals.

Rumen inoculations were effective in preventing the usual drop in blood plasma ascorbic acid between the seventh and fourteenth days of age when only alfalfa hay and milk were fed but were ineffective when grain was included in the ration. A ration of whole milk and alfalfa hay alone resulted in carotenoid levels considerably higher after 14 days of age than was observed when grain was included in the ration. Rumen inoculations had no marked effect on the blood carotenoid levels. Neither the inoculations nor the type of ration fed markedly influenced the blood plasma vitamin A.

When grain was introduced into the ration of 64-day-old calves, which had been fed only alfalfa hay and milk until that time, a marked reduction in hay consumption and blood carotenoids resulted. Plasma vitamin A increased and ascorbic acid declined during the same period.

These results, when correlated with the effect of different rations on the development of various rumen microorganisms in these calves, indicate that palatable, high quality hay stimulates the early development of rumen function in the young calf and appears to have a favorable physiological effect in meeting the vitamin needs of these animals.

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THE EFFECT OF SUPPLEMENTAL VITAMIN A UPON GROWTH, BLOOD PLASMA CAROTENE, VITAMIN A, INORGANIC CALCIUM, AND PHOSPHORUS OF HOLSTEIN HEIFERS[1, 2]

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The nutritional value of vitamin A for dairy cattle has been generally accepted and numerous investigators have studied the minimum vitamin A and carotene requirements of calves and heifers for growth. Boyer *et al.* reported that 75 γ of carotene per kg. body weight was adequate for yearling Holstein heifers. Jones and Haag (5) found that heifers did not require supplemental vitamin A for growth and reproduction if they were pastured during the summer. Keyes *et al.* (6) obtained more gain in body weight by supplementing a standard calf starter with vitamin A. With this in mind, a study was undertaken to determine the value of supplementing one of the commonly used heifer rations with vitamin A. The effect of supplemental vitamin A upon blood plasma carotene, vitamin A, inorganic calcium and phosphorus concentrations and growth was studied.

EXPERIMENTAL PROCEDURE

A preliminary experiment was conducted with 22 Holstein heifers from February 1 to May 24, 1946. These animals were divided into two similar groups based upon age and body weight. Both groups were fed and managed identically except that the vitamin A group received supplemental vitamin A. The vitamin A supplement used in these trials was prepared from a fish liver oil source in linseed oil meal and soybean oil meal in the amount of 250,000 USP units per lb. based on manufacturer's analysis. The basal and experimental rations were prepared with similar composition except for the supplemental vitamin A. The animals were fed mixed hay *ad libitum* and 10 lb. of grass silage and 8 lb. of a grain mixture containing 14 per cent crude protein per day. After April 1 the amount of grain was increased to 10 lb. per day.

Growth was determined by measuring body weight and height at withers of the animals. They were weighed and measured at the beginning and end of the experiment with one intermediate weight taken in April.

Blood plasma carotene and vitamin A were determined at monthly intervals using the methods of Moore (8) and Kimble (7), respectively. Blood plasma inorganic calcium and phosphorus were determined at monthly intervals using

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the Clark-Collip modifications (3) of the Kramer-Tisdall method for calcium and the method of Gomori (4) for phosphorus, respectively.

The main experiment was conducted from January 1 to May 6, 1947, using 28 Holstein heifers. These heifers were divided into two similar groups based upon age, body weight, blood plasma carotene and vitamin A, and blood plasma inorganic calcium and phosphorus concentrations. Both groups were fed and managed identically except that the vitamin A group received supplemental vitamin A in the form used in the preliminary trials. The heifers were fed a weighed amount of mixed hay and grass silage at each feeding. They also received 10 lb. of grain per day.

Growth of the heifers was measured by determining the body weight and height at withers at the beginning and end of the experiment with one intermediate weight in March. Chemical analyses were the same as in the preliminary experiment, except that in the determination of blood plasma carotene and vitamin A the carotene was removed (1) previous to determining vitamin A when over 200 γ of carotene per 100 ml. were present. All data obtained in these trials were treated statistically where applicable (10).

RESULTS

In the preliminary experiment, the 11 heifers in the vitamin A group received an average daily intake of 40,382 USP units of supplemental vitamin A. This resulted in no significant differences in gain of body weight or height at withers between the two groups of 11 heifers each. The control group gained 130 lb. and 4.9 cm. and the vitamin A group gained 135 lb. and 4.9 cm.

In the main experiment the basal ration contained an average of 114,000 USP units of vitamin A per daily allowance per heifer. This was supplemented with 129,400 USP units of vitamin A per day for the 14 heifers in the vitamin A group. An analysis of variance of the gains in weight and height at withers as presented in table 1 showed a significant increase in gain in weight but no significant difference in gain in height at withers resulting from feeding supplemental vitamin A. The vitamin A group of 14 heifers gained an average of 235.9 lb. and 9.4 cm. while the control group gained 187.6 lb. and 8.4 cm., or a difference of 48.3 lb. and 1.0 cm. The heifers in the vitamin A group exhibited better condition, being smoother throughout and showing more flesh. The hair was glossier and smoother, and the hides seemed to be more pliable than those of the control group.

Feeding supplemental vitamin A at either level increased the blood plasma vitamin A concentrations and decreased the blood plasma carotene concentrations of the heifers. The mean blood plasma vitamin A concentrations of the heifers used in the preliminary trial were 18.14 γ per 100 ml. for the control group and 21 γ per 100 ml. for the vitamin A group during this trial. Similarly, during the main trial the mean blood plasma vitamin A concentrations were 15.82 and 21.71 γ per 100 ml. for the control and vitamin A-fed groups, respectively. These differences were highly significant statistically. The data from the main trial are presented in table 2. During the course of this trial, the blood plasma vitamin

A concentrations of the control group increased an average of 1 γ of vitamin A per 100 ml., whereas that of the vitamin A group increased 9 γ per 100 ml. The mean for each group was 14 γ of vitamin A per 100 ml. at the beginning of the trial.

The feeding of supplemental vitamin A resulted in a depression of the blood plasma carotene concentrations. In the preliminary trial, the blood plasma carotene concentration of the vitamin A-fed group decreased from 183

TABLE 1

Growth of heifers during main experiment

Heifer no.	Body weight			Height at withers		
	Initial	Final	Gain	Initial	Final	Gain
	(*lb.*)	(*lb.*)	(*lb.*)	(*cm.*)	(*cm.*)	(*cm.*)
			Control group			
729	700	875	175	120	124	4
732	668	800	132	113	120	7
734	749	967	218	120	128	8
737	565	716	151	113	120	7
738	600	800	200	115	121	6
740	513	732	219	112	120	8
742	637	871	234	110	120	10
744	526	675	149	106	117	11
747	513	700	187	106	115	9
749	456	579	123	106	115	9
752	374	622	248	100	110	10
755	404	610	206	101	106	5
756	334	513	179	96	107	11
757	344	550	206	94	107	13
$\overline{X}$	527	715	188	108	116	8
			Group fed vitamin A			
731	766	908	142	121	126	5
733	716	1027	311	117	123	6
735	732	1069	337	116	124	8
736	668	930	262	110	118	8
739	668	970	302	114	120	6
741	555	750	195	108	116	8
743	637	890	253	109	119	10
745	501	700	199	109	121	12
746	489	725	236	105	117	12
748	539	755	216	104	118	14
751	404	615	211	101	111	10
753	384	560	176	99	110	11
754	344	593	249	100	109	9
759	275	489	214	93	106	13
$\overline{X}$	548	784	236	108	117	9

to 96 γ per 100 ml., whereas the control group decreased from 220 to 153 γ per 100 ml. During the main trial, the blood plasma carotene concentrations decreased from 278 to 156 and from 271 to 85 γ per 100 ml. of blood plasma for the control and vitamin A-fed groups, respectively. Analyses of variance proved this difference to be highly significant statistically.

Blood plasma carotene and vitamin A determinations were continued during June, July and August, while the cattle were on pasture after the preliminary experiment, to determine if there was a carry-over effect from feeding supple-

TABLE 2

The effect of supplemental vitamin A upon blood carotene, vitamin A, calcium and phosphorus

Heifer no.	Carotene			Vitamin A			Calcium			Phosphorus		
	Nov. 26	March 11	May 6	Nov. 26	March 11	May 6	Nov. 26	March 11	May 6	Nov. 26	March 11	May 6
	(γ/*100 ml.*)			(γ/*100 ml.*)			(*mg./100 ml.*)			(*mg./100 ml.*)		
						Control group						
729	393	175	153	17	19	15	8.80	8.68	8.28	8.10	8.63	7.15
732	650	184	134	13	16	13	9.80	9.18	9.31	9.18	7.94	7.56
734	260	142	140	21	24	21	8.93	8.18	8.68	10.11	8.69	9.18
737	501	232	178	11	20	13	10.17	8.25	8.68	10.39	8.75	8.32
738	375	119	108	18	16	17	9.11	9.05	9.08	9.06	9.12	8.21
740	404	190	170	12	18	27	9.36	9.67	9.03	10.11	9.25	7.35
742	210	246	199	13	18	13	10.11	9.05	9.72	8.81	8.81	6.52
744	175	184	178	12	16	17	9.73	9.36	9.40	9.44	9.30	8.57
747	307	236	167	17	15	18	9.67	8.74	8.91	8.93	10.54	7.93
749	156	187	151	4	13	9	9.67	9.30	9.72	8.57	10.54	7.72
752	94	131	126	16	11	13	9.49	9.55	9.72	8.69	9.63	8.45
755	94	134	151	19	15	14	9.55	9.11	9.66	9.97	10.25	8.93
756	148	151	164	15	14	15	9.24	9.55	8.80	10.04	11.21	9.44
757	123	156	159	14	11	12	9.80	9.67	9.14	9.13	9.57	8.45
$\bar{X}$	278	176	156	14	16	15	9.53	9.10	9.15	9.32	9.45	8.13
						Group fed vitamin A						
731	634	96	108	17	18	19	8.93	9.05	9.14	9.70	6.76	6.72
733	522	116	112	23	19	18	8.56	8.87	8.51	9.18	7.88	6.81
735	398	100	105	20	22	24	9.73	9.18	9.14	7.46	8.75	6.47
736	466	156	134	17	28	34	9.61	9.61	9.26	7.05	7.88	8.10
739	561	114	85	20	26	40	9.42	9.55	9.08	8.81	7.67	6.95
741	149	112	67	1	15	12	9.61	9.11	9.89	7.25	9.30	7.20
743	183	137	105	13	26	22	9.49	9.30	9.89	8.10	6.86	6.76
745	123	103	76	7	18	19	10.11	9.92	9.77	10.39	9.44	7.72
746	224	98	74	11	18	20	10.11	8.87	8.97	6.81	9.44	6.66
748	123	69	52	7	21	24	9.73	9.49	9.08	9.70	9.57	7.15
751	96	78	63	17	17	23	9.80	8.93	10.23	9.97	9.37	7.56
753	96	33	48	13	13	20	9.42	9.42	9.43	8.57	7.88	7.35
754	105	80	63	16	20	23	9.80	10.04	9.31	10.39	9.18	7.15
759	110	91	94	15	19	30	8.99	8.68	9.49	8.95	9.97	8.93
$\bar{X}$	271	99	85	14	20	23	9.52	9.29	9.37	8.74	8.57	7.25

mental vitamin A. It was found that the heifers receiving supplemental vitamin A had lower blood plasma carotene and vitamin A concentrations while on pasture than the heifers that did not receive supplemental vitamin A. The mean blood plasma vitamin A concentrations were 22.15 γ per 100 ml. for the control group and 17.45 γ per 100 ml. for the vitamin A group. This difference was significant. The mean blood plasma carotene concentrations were 874 γ per 100 ml. for the control group and 696 γ per 100 ml. for the vitamin A group. This difference was highly significant.

Feeding supplemental vitamin A had no effect upon the blood plasma inorganic calcium and phosphorus concentrations of the heifers. In the preliminary experiment there were no significant differences between the two groups; however, both groups had higher blood plasma inorganic calcium concentrations and lower blood plasma inorganic phosphorus concentrations during the summer pasture period than during the feeding trial. In the main experiment there was no significant difference in the blood plasma inorganic calcium concentrations of the two groups of heifers, but the control group had a higher (highly significant) mean blood plasma inorganic phosphorus concentration than the vitamin A group. Too much emphasis must not be placed upon this, however, since the control group had a higher mean blood plasma inorganic phosphorus concentration than the vitamin A group at the start of the trial.

CONCLUSIONS

Feeding an average of 40,400 USP units of supplemental vitamin A per day to Holstein heifers receiving a normal ration resulted in no increase in the rate of growth. Increasing the vitamin A supplement to an average of 129,400 USP units per day in addition to the 114,000 USP units supplied daily in the basal ration resulted in a significant increase in body weight gains of Holstein heifers.

Feeding supplemental vitamin A significantly increased the blood plasma vitamin A concentrations and decreased the blood plasma carotene concentrations of Holstein heifers.

Blood plasma inorganic calcium and phosphorus concentrations were not altered by feeding supplemental vitamin A.

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ASSOCIATION ANNOUNCEMENT

COLLEGIATE STUDENTS' INTERNATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

Atlantic City, N. J.—October 25, 1948

Teams from twenty-six State Agricultural Colleges, participated in this, the fourteenth annual contest sponsored by the Dairy Industries Supply Association, Inc., and the American Dairy Science Association.

Following is a list of those who won high standings in the Contest:

ALL PRODUCTS

Individuals

1. Donald R. Moore, Michigan State College
2. Donald H. Pflueger, Iowa State College
3. W. E. Shiffermiller, Ohio State University
4. William H. Hoagland, University of Connecticut
5. Richard E. Lewis, Ohio State University
6. John N. Lewis, Ohio State University
7. Charles H. Fitch, Iowa State College
8. Lester Hankin, University of Connecticut
8. N. V. Kennedy, Mississippi State College
10. Arnold D. Nelson, Iowa State College

Teams

1. Iowa State College
2. Ohio State University
3. Michigan State College
4. Mississippi State College
5. University of Tennessee
6. University of Connecticut
7. University of Maryland
8. University of Massachusetts
9. Pennsylvania State College
10. Cornell University

BUTTER

Individuals

1.	Donald H. Pflueger, Iowa State College	4.33
2.	Donald R. Moore, Michigan State College	8.17
3.	William H. Hoagland, University of Connecticut	9.00
4.	William C. Flynt, Jr. Mississippi State College	10.42
5.	Delmer A. Boyce, University of Tennessee	11.50
6.	Charles H. Fitch, Iowa State College	11.75
7.	Harold McCracken, University of Tennessee	11.84
8.	William R. Vial, Purdue University	12.25
9.	Richard E. Lewis, Ohio State University	12.67
10.	John N. Lewis, Ohio State University	13.50

Teams

1.	Iowa State College	34.58
2.	Ohio State University	39.84
3.	Mississippi State College	41.92
4.	Michigan State College	42.34
5.	University of Tennessee	43.51
6.	University of Connecticut	44.00
7.	Purdue University	50.59
8.	University of Nebraska	53.34
9.	North Carolina State College	56.25
10.	Cornell University	57.25

CHEESE

Individuals

1.	William J. Deisley, Pennsylvania State College	28.17
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3.	Arnold D. Nelson, Iowa State College	29.50
4.	Donald R. Moore, Michigan State College	29.75
5.	Ralph Whitehead, Michigan State College	31.25
6.	Harold A. Newlander, Cornell University	31.34
7.	Henry H. Sprowls, Texas Tech.	33.00
8.	Donald H. Pflueger, Iowa State College	33.09
9.	Robert J. Schutrumpf, University of Maryland	33.43
10.	M. V. Kennedy, Mississippi State College	33.50

Teams

1.	Michigan State College	94.84
2.	Iowa State College	98.09
3.	Cornell University	100.50
4.	Ohio State University	102.35
5.	Texas Tech.	106.85
6.	Mississippi State College	110.34
7.	University of Tennessee	111.18
8.	University of Illinois	112.58
9.	University of Connecticut	117.50
10.	Purdue University	120.52

ICE CREAM

Individuals

1.	Donald H. Pflueger, Iowa State College	29.00
2.	John A. McLeod, Jr. North Carolina State College	31.51
3.	Ralph Whitehead, Michigan State College	32.00
4.	W. E. Shiffermiller, Ohio State University	33.00
5.	Donald R. Moore, Michigan State College	33.50
6.	George H. Brink, University of Tennessee	34.50
6.	Roland I. Zeller, University of Minnesota	34.50
8.	Lester Hankin, University of Connecticut	35.00
8.	William H. Hoagland, University of Connecticut	35.00
10.	Delmer A. Boyce, University of Tennessee	35.50

Teams

1.	University of Tennessee	108.50
2.	Iowa State College	110.00

3.	Ohio State University	111.50
4.	University of Minnesota	114.00
5.	Pennsylvania State College	116.00
6.	Michigan State College	117.00
7.	University of Connecticut	118.50
8.	University of Nebraska	119.50
9.	University of Massachusetts	127.17
10.	University of Maryland	129.00

MILK

Individuals

1.	William J. Deisley, Pennsylvania State College	14.92
2.	Arnold D. Nelson, Iowa State College	20.45
3.	Dan Guyer, Oklahoma A. & M.	22.00
4.	Luther O. Meadows, Mississippi State College	23.25
5.	William H. Hoagland, University of Connecticut	23.95
5.	Donald R. Moore, Michigan State College	23.95
7.	W. E. Shiffermiller, Ohio State University	24.35
8.	Richard E. Lewis, Ohio State University	25.20
9.	Charles D. Spencer, University of Maryland	25.35
9.	Alan D. Young, University of Massachusetts	25.35

Teams

1.	Iowa State College	76.43
2.	Ohio State University	78.45
3.	Pennsylvania State College	81.42
4.	University of Maryland	85.70
5.	University of Massachusetts	86.37
6.	Mississippi State College	86.90
7.	University of Connecticut	88.65
8.	Michigan State College	90.83
9.	Oklahoma A. & M.	93.87
10.	University of Illinois	94.92

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November 1, 1948

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