varieties with the lower sugar content. This average flavor rank increased progressively for the first group of squashes and decreased progressively for the second group.

A distinction between the two groups of squashes on the basis of texture did not become apparent until the third sampling period which coincides approximately with the time the sugar content had begun to exceed the starch content. At this time, and at the two remaining sampling periods, the average rank based on texture was higher for the Buttercup, Silver Bell, and Sweet Meat varieties than for the other three.

Since the squashes used in the taste tests were not the same fruits sampled for chemical analysis, the correlation coefficients between preference and constituent content were calculated using the mean of the chemical analyses of the four replicates with each of the three mean flavor and texture ranks obtained at each taste testing session from the five regular judges. These correlation coefficients ($n$ = 15) are presented in Table III.

Examination of this table indicates two distinct groups of squashes which can also be differentiated on the basis of the sugar-starch ratios in the latter part of the storage period. Buttercup, Silver Bell, and Baby Blue, the varieties with a relatively low sugar-starch ratio, had a positive association between sugar content and ranks for flavor and/or texture, and a negative association between starch content and ranks for flavor and/or texture. In contrast, Blue Hubbard and Butternut, the varieties with a relatively high sugar-starch ratio, had, with one exception, a negative association between sugar content and ranks for flavor and texture and a positive association between starch content and ranks for flavor and texture. The associations between total solids content and ranks for flavor and/or texture were in general similar to those between starch content and ranks for flavor and/or texture. The probability levels of significance are indicated in Table III.

Blue Hubbard and Butternut were relatively low in sugar. The tendency of a negative association between sugar content and degree of preference for these two varieties suggests that the "low sugar" varieties are less acceptable when the starch and total solids are at a minimum—that is, in the latter part of storage. Although Baby Blue also tended to be low in sugar, its total solids content as well as the sugar-starch ratio approximated that of the higher sugar content group. Preference appears to be influenced not only by the absolute sugar and starch content but also by the sugar-starch ratio and by total solids content.

Undoubtedly compounds other than sugar and starch, possibly protein and lipide degradation products, influence the quality of winter squashes. Investigations to identify such compounds would be warranted.


### Acknowledgment

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**QUALITY OF DAIRY PRODUCTS**

# Vitamin A, Carotenoid, Iodine, and Thiocyanogen Values, and the Refractive Index of Milk Fat as Influenced by Feed, and by Individual and Breed Differences


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THE VALUE of milk and its products in diet makes it very desirable that they should be produced in such a way as to make them palatable, nutritious, and fairly resistant to oxidation which accounts for objectionable flavors. Although fat in fresh milk is relatively stable, it is unstable in frozen cream and butter and may undergo deterioration if used in reconstituted milk (*9*, *12*).

The carotenoid–vitamin A–tocopherol relationship (*3*, *7*, *10*, *11*) and the part played by tocopherols and feed in the stabilization of fresh milk appear to be well established; other relationships such as between color intensity of milk fat, palatability of fresh milk, and inhibition of flavor defects (*4*, *6*) or between vitamin A activity and the keeping quality of butter (*13*) are not yet well known. This investigation presents a comparison of refractive index, vitamin A, carotenoid, iodine, and thiocyanogen values of milk fat from 28 cows of different breeds as influenced by the type of roughage fed consecutively to the same cows, breed differences, and physiological response of individual cows to feed consumed.

Studies on the effect of breed and feed on the palatability of fresh cream and butter and on the storage stability of fat in frozen cream and butter are now in progress.

### Experimental

Jersey, Brown Swiss, Holstein-Friesian, and Ayrshire cows were placed in groups

**The purpose of this work was to establish a basis for the study of milk fat constants and of storage life of fat in frozen cream and butter as influenced by feed and by breed. Feed, individual, and breed differences had statistically significant effects on milk fat constants. An inverse relationship was indicated between carotenoid and iodine values of fat. As an average, Ayrshire fat was lower in carotenoid and higher in iodine values than fat from any other breed; Jersey fat was highest in carotenoid and lowest in iodine values; Brown Swiss and Holstein fats were intermediate. Information on these relationships is of value in connection with studies of storage life of fat in milk products, and of fat metabolism. Data suggest that conversion of carotene to vitamin A in an animal body may vary with the degree of unsaturation of fat and is regulated by the same metabolic processes which control the degree of unsaturation of secreted fat.**

of seven by breeds. Only four breeds of cows were available at Cornell University. These cows were fed mixed legume-grass hay and well eared corn silage for 8 weeks. Then oats silage, with oats in full bloom, was substituted for corn, and the mixed hay was continued for another 4 weeks. The composition of hay was approximately 40% legumes (alfalfa, ladino clover, and red clover), and 60% early-cut timothy grass, with timothy headed, but prebloom. The average daily roughage intakes are presented in Table I.

Finally, all four groups of cows were transferred to orchard grass pasture. During the last 2 weeks of pasture feeding, the cows were given all of the freshly chopped legume-grass mixture and oats silage they were able to eat plus a commercial concentrate mixture. The early-cut legume-grass mixture contained ladino and red clovers, orchard grass, and timothy grass, with timothy headed, but prebloom.

A sample of milk was taken from each cow at the night milking at the end of 8-, 4-, and 2-week feeding periods, respectively. The milk was pasteurized at 61° C. for 30 minutes, and then held undisturbed for 2 days at 0° to 5° C. in glass bottles protected from light. In the following operation, gravity cream and skim milk were separated by siphoning, the cream was churned, and the fat was oiled, centrifuged, filtered, and analyzed for its vitamin A, carotenoid, iodine, and thiocyanogen values and refractive index.

Vitamin A and carotenoid contents of the milk fat were determined by the Koehn and Sherman method (*8*). Iodine (Hanus method) and thiocyanogen values and refractive index of milk fat were assayed using the A.O.A.C. method of analysis (*2*).

### *Results and Discussion*

**Iodine Values of Milk Fat.** Data in Figure 1 show iodine values of milk fat from individual cows, grouped by breeds, as determined at the end of 8-, 4-, and 2-week periods on the particular roughage studied. The cows were ranked on the graph in ascending order of iodine values of fat produced on legume-grass hay plus corn silage. Variations from cow to cow in the same group when other roughages were fed appear to be roughly consistent in their behavior. It can be seen that iodine values of fat were influenced not only by roughages fed to the cows, but also by the physiological response of the individual cow to the feed consumed and the breed differences. It is of interest to note that several cows from Ayrshire and Brown Swiss groups that produced fat with relatively high iodine values at the end of the first 8-week period on hay and corn silage ration have shown a tendency on subsequent feeding trials to produce fat of approximately the same high degree of unsaturation.

Data in Figure 1 and Table II reveal also that although the extent of rise in iodine values of fat, as affected by pasture feeding, varied roughly inversely with corresponding iodine values of fat produced on legume-grass hay and silage rations, the resulting rise in the total degree of unsaturation of fat from Jersey milk was found to be lower than that

**Table I. Average Daily Roughage Intake**

| Breed | Av. Daily Intake,[a] Lb. Legume-grass hay | Corn silage | Oats silage |
|---|---|---|---|
| Jersey | 18 | 40 | 30 |
| Brown Swiss and Holstein-Friesian | 30 | 60 | 45 |
| Ayrshire | 30 | 50 | 37 |

[a] Rations were supplemented with 8 (Jersey) and 12 pounds on the average of a commercial concentrate mixture daily, containing 18% of protein. The level of protein was adjusted with linseed, cottonseed, and soybean meal.

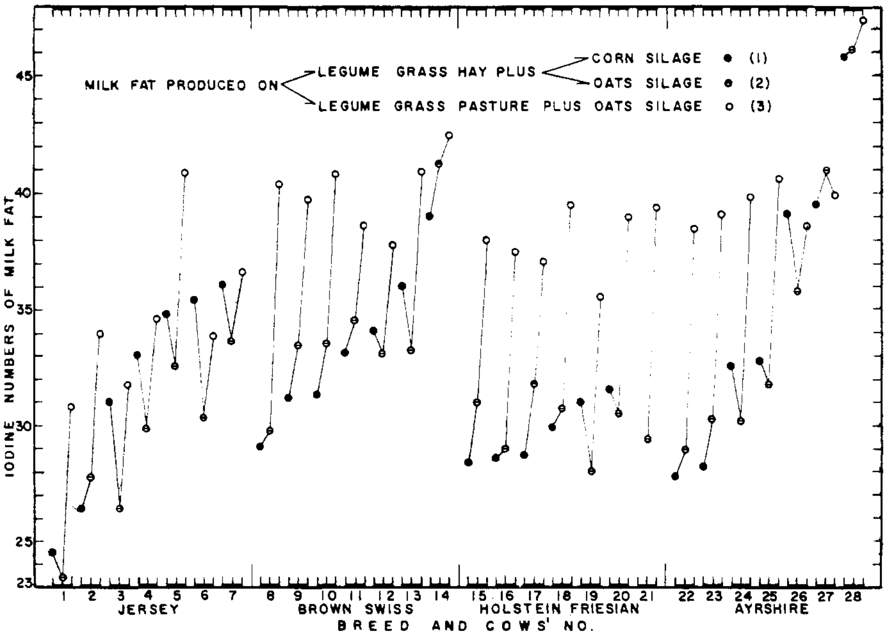


Figure 1. Iodine values of milk fat from each cow at the end of each of the consecutive feeding periods indicated on the graph

Periods were 8 weeks long (1), 4 weeks (2), and 2 weeks (3) shown by the fat from other breeds. As an average, samples of fat from Ayrshire milk were higher in their iodine values than those of fat from any other breed, while iodine values of fat from Brown Swiss, Holstein, and Jersey milk diminished progressively in this order.

It would appear therefore that among the breeds studied, Jersey cows were the slowest to readjust physiologically to pasture feeding and that the length of feeding period may play a part in this phenomenon.

The analysis of variance has also indicated that observed variations in iodine values of milk fat, as affected by individual cows in the breed, breed differences, and the roughages fed to the cows were statistically highly significant ($P<0.01$).

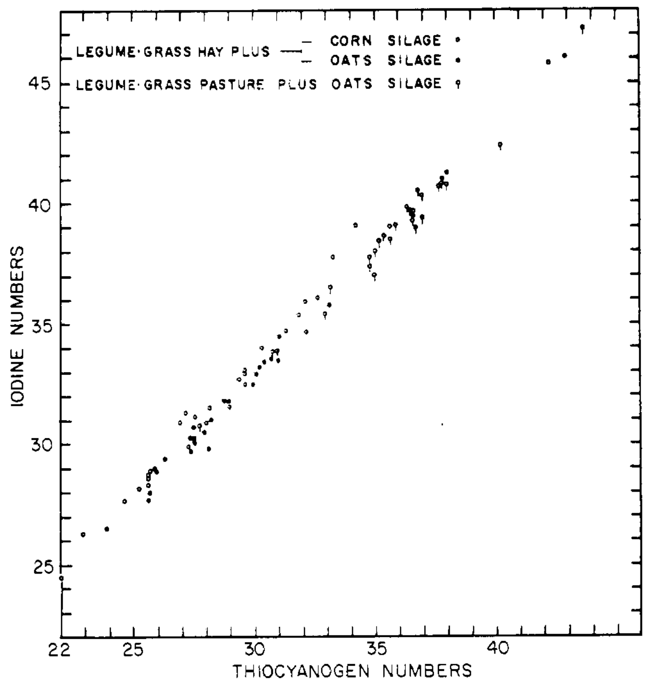


**Figure 2. Relationship between iodine and thiocyanogen values of milk fat as influenced by the roughages fed to cows, breed differences, and physiological response of the animal to feed consumed**

**Iodine and Thiocyanogen Values, and Refractive Index of Milk Fat.** The relationship between iodine and thiocyanogen values of milk fat described in the preceding paragraphs is presented in Figure 2, Tables II and III. Data in Figure 2 and Table III are conclusive in showing that, regardless of varietal factors involved, a highly significant correlation was found to exist between iodine and thiocyanogen values of fat (+0.987, +0.985, and +0.973 for the three sampling periods, respectively); the iodine values and refractive index of the fat (+0.941, +0.903, and +0.964); and the thiocyanogen values and refractive index (+0.917, +0.910, and +0.966). As before, variations in thiocyanogen values and refractive index of fat, as affected by individual cows in the breed, breed differences, and roughages were found to be highly significant ($P < 0.01$). These observations are in good agreement with data of Arup (*1*) and van der Burg, Brouwer, and Koppejan (*5*) on the correlation between iodine, thiocyanogen, and refractive index values of fat obtained at random from Dutch, Irish, and other foreign samples of creamery butter.

In Table IV are shown data on composition of hypothetically pure unsaturated triglycerides (*2*) in milk fat, as affected by hay-corn, hay-oats, and pasture-oats silage feeding, and by the breed of cows. Data are ranked in the table in ascending order of oleic acid glyceride content of fat. In general, they confirm the related data of Arup (*1*), obtained for fat from random samples of Irish summer and winter creamery butter and some foreign creamery butter. Both data indicate, as

**Table II. Vitamin A, Carotenoids, Iodine and Thiocyanogen Values, and Refractive Index of Milk Fat as Influenced by Breed and Feed**

| | | | Quantities/100 G. Fat | | | | | |
|---|---|---|---|---|---|---|---|---|
| Breed | Date | No. of Cows | Vitamin A, µg. | Carotenoids, µg. | Total vitamin A, µg. | Iodine No. | Thiocyanogen No. | Refractive Index |
| Jersey | 3/ 1/60 | 7 | 456 ± 102 | 628 ± 114 | 770 | 31.58 ± 4.21 | 28.32 ± 3.97 | 1.45327 ± 0.00053 |
| | 3/29/60 | 7 | 417 ± 61 | 666 ± 127 | 750 | 29.09 ± 3.27 | 26.57 ± 3.10 | 1.45307 ± 0.00038 |
| | 6/ 6/60 | 7 | 674 ± 91 | 932 ± 161 | 1140 | 34.64 ± 3.09 | 31.68 ± 3.08 | 1.45394 ± 0.00055 |
| | Av. | 21 | 515 | 742 | 886 | 31.77 | 28.85 | 1.45342 |
| Brown Swiss | 3/ 1/60 | 7 | 384 ± 47 | 502 ± 90 | 635 | 33.38 ± 3.11 | 29.72 ± 3.14 | 1.45384 ± 0.00033 |
| | 3/29/60 | 7 | 424 ± 53 | 362 ± 47 | 605 | 34.11 ± 3.25 | 31.27 ± 2.90 | 1.45420 ± 0.00040 |
| | 6/ 6/60 | 7 | 754 ± 99 | 666 ± 87 | 1087 | 40.09 ± 1.44 | 37.13 ± 1.59 | 1.45495 ± 0.00028 |
| | Av. | 21 | 520 | 510 | 775 | 35.86 | 32.70 | 1.45433 |
| Holstein-Friesian | 3/ 1/60 | 6 | 526 ± 82 | 420 ± 69 | 736 | 29.65 ± 1.23 | 26.55 ± 1.00 | 1.45321 ± 0.00042 |
| | 3/29/60 | 7 | 519 ± 53 | 448 ± 53 | 743 | 30.06 ± 1.23 | 27.17 ± 1.12 | 1.45335 ± 0.00029 |
| | 6/ 6/60 | 7 | 800 ± 49 | 707 ± 74 | 1153 | 38.03 ± 1.33 | 35.45 ± 1.36 | 1.45467 ± 0.00052 |
| | Av. | 20 | 619 | 530 | 884 | 32.72 | 29.88 | 1.45377 |
| Ayrshire | 3/ 1/60 | 7 | 593 ± 54 | 402 ± 135 | 794 | 35.08 ± 6.16 | 31.67 ± 5.90 | 1.45420 ± 0.00094 |
| | 3/29/60 | 7 | 520 ± 79 | 498 ± 103 | 769 | 34.87 ± 6.00 | 31.95 ± 5.85 | 1.45405 ± 0.00089 |
| | 6/ 6/60 | 7 | 850 ± 68 | 580 ± 112 | 1140 | 40.58 ± 2.93 | 37.08 ± 2.70 | 1.45497 ± 0.00043 |
| | Av. | 21 | 654 | 493 | 901 | 36.84 | 33.56 | 1.45440 |
| Av. of all breeds | 3/ 1/60[a] | 27 | 488 | 490 | 733 | 32.52 | 29.15 | 1.45364 |
| | 3/29/60[b] | 28 | 470 | 493 | 716 | 32.03 | 29.24 | 1.45366 |
| | 6/ 6/60[c] | 28 | 769 | 721 | 1130 | 38.33 | 35.33 | 1.45463 |
| Grand total av. | | 83 | 577 | 569 | 861 | 34.31 | 31.27 | 1.45398 |

Fat produced by dairy cows at the end of [a] 8-, [b] 4-, and [c] 2-week periods of feeding legume-grass hay plus corn silage, legume-grass hay plus oats silage, and legume-grass pasture plus oats silage, respectively.

## Table III. Correlation Coefficients for Milk Fat from the Same Group of Dairy Cows as Influenced by Feed

| | Correlation Coefficients for Milk Fat Produced on | | |
|---|---|---|---|
| | Legume-Grass Hay plus | | Legume-grass pasture plus oats silage |
| | Corn silage | Oats silage | |
| Vitamin A and carotenoids | −0.279 | +0.171 | −0.236 |
| Total vitamin A | +0.827 | +0.780 | +0.615 |
| Iodine value | −0.190 | +0.109 | +0.337 |
| Thiocyanogen value | −0.186 | +0.076 | +0.262 |
| Refractive index | −0.152 | +0.104 | +0.258 |
| Carotenoids and total vitamin A | +0.243 | +0.700 | +0.544 |
| Iodine value | +0.079 | −0.216 | −0.597 |
| Thiocyanogen value | +0.140 | −0.274 | −0.587 |
| Refractive index | −0.036 | −0.363 | −0.644 |
| Total vitamin A and iodine value | −0.144 | −0.039 | −0.158 |
| Thiocyanogen value | −0.100 | −0.097 | −0.208 |
| Refractive index | −0.159 | −0.125 | −0.273 |
| Iodine value and thiocyanogen value | +0.987 | +0.985 | +0.973 |
| Refractive index | +0.941 | +0.903 | +0.964 |
| Thiocyanogen value and refractive index (83 samples of milk) | +0.917 | +0.910 | +0.966 |

## Table IV. Average Composition of Hypothetically Pure Unsaturated Triglycerides[a] in Fat from Four Breeds of Dairy Cows on Hay + Corn Silage, Hay + Oats Silage, and Pasture + Oats Silage Rations

| | Composition of Milk Fat | |
|---|---|---|
| | Oleic acid glycerides, % | Linoleic acid glycerides, % |
| | 27.07 | 3.67 |
| | 27.87 | 2.95 |
| | 28.08 | 3.41 |
| | 28.93 | 3.86 |
| | 30.04 | 4.35 |
| | 32.67 | 4.02 |
| | 32.97 | 3.31 |
| | 33.29 | 3.46 |
| | 33.68 | 3.41 |
| | 38.24 | 2.96 |
| | 38.92 | 4.10 |
| | 39.71 | 3.42 |
| Av. all breeds | | |
| Hay + corn silage | 29.76 | 3.98 |
| Hay + oats silage | 30.65 | 3.27 |
| Pasture + oats silage | 37.54 | 3.48 |
| Single breed av.[b] | | |
| Jersey | 30.03 | 3.42 |
| Holstein-Friesian | 31.34 | 3.34 |
| Brown Swiss | 34.24 | 3.69 |
| Ayrshire | 35.09 | 3.84 |

[a] A.O.A.C. method (*2*).
[b] For the three sampling periods.

Arup has observed in his study, that despite wide differences in the origin of fat samples, their linoleic acid glyceride content varied but slightly, and thus showed a tendency to remain at a relatively constant level, while oleic acid glyceride content was definitely a variable factor.

It will be observed, however, that in addition to the seasonal variations in feed, hypothetically pure unsaturated triglyceride content of fat tends to vary as well with the breed of cows. Data in Table IV show that as an average, fat obtained from Ayrshire milk was higher in oleic acid glyceride content than milk fat of any other breed, while oleic acid glyceride content of fat from Brown Swiss, Holstein, and Jersey milk samples diminished progressively in this order.

**Vitamin A and Carotenoid Content of Milk Fat.** Average values for vitamin A and carotenoid content of milk fat as influenced by feed and breed are presented in Table II. Data show variations in vitamin A and carotenoid content of fat between individual cows of the same breed, between different breeds, and between roughages. Analysis of variance indicated that observed variations in vitamin A and carotenoid content of fat, as affected by roughages fed to cows and the breed of the cows, were highly significant statistically ($P < 0.01$). Likewise, variations in the vitamin A ($P < 0.01$) and carotenoid ($P < 0.05$) content of fat, as affected by individual cows within the breed, were found to be significant.

Comparison of data in Table II indicates that as an average fat from Ayrshire milk was lower in carotenoid content and higher in iodine value than fat from any other breed; and that the carotenoid content of fat from Ayrshire, Brown Swiss, Holstein, and Jersey milk samples increased progressively in this order. There is a definite tendency for iodine values of corresponding samples of fat to decrease as carotenoid content of fat increases. However, average total vitamin A content of fat from Ayrshire, Holstein, and Jersey milk samples has shown a tendency to remain at approximately constant level; and this constancy held to some extent for the three feeding periods, as is shown in Table II. Furthermore, a negative correlation (Table III) was also indicated between carotenoid content and iodine value of milk fat during hay + oats silage, and pasture + oats silage feeding (+0.079, −0.216, and −0.597 for the three sampling periods, respectively). Although these data are not sufficient to warrant a definite conclusion, they show a possibility that conversion of carotene to vitamin A in the animal body varies directly with degree of unsaturation of fat, and is regulated by the same metabolic processes which control the degree of unsaturation of secreted milk fat. This would explain the high carotene content of fat from Jersey milk.

### *Conclusions*

Highly significant positive correlation coefficients were obtained for fat between iodine and thiocyanogen values, iodine value and refractive index, and thiocyanogen value and refractive index; a highly significant negative correlation between carotenoid and iodine values was obtained for fat produced on pasture + oats silage ration.

As an average, fat obtained from Ayrshire milk was highest in iodine value, and lowest in carotenoid content; fat obtained from Jersey milk was lowest in iodine value, and highest in carotenoid content; Brown Swiss and Holstein samples were intermediate; the total vitamin A content of milk fat from Ayrshire, Holstein, and Jersey cows remained at a fairly constant level.

Data suggest that conversion of carotene to vitamin A in the animal body varies directly with the degree of unsaturation of fat and is regulated by the same metabolic processes which control the degree of unsaturation of secreted fat. This would explain variations in carotene content of milk fat produced by different breeds of cows.

Data show that under the experimental conditions described in this study the hypothetically pure linoleic acid glyceride content of fat varied but slightly, and that oleic acid glyceride content was a major variable factor. As an average, the Ayrshire milk fat was higher in oleic acid glyceride content than the milk fat of any other breed and oleic acid glyceride content of fat from Brown Swiss, Holstein, and Jersey milk samples diminished progressively in this order.


### *Acknowledgment*

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**FORAGE ESTROGENS**

# Detection of Daidzein, Formononetin, Genistein, and Biochanin A in Forages

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**Daidzein, not previously reported in forages, was found in alfalfa, and ladino, red, and subterranean clovers. Formononetin and genistein, isolated earlier from red and subterranean clovers, have now also been found in alfalfa and ladino clover. Biochanin A, previously reported in red and subterranean clovers, was found in alfalfa but not in ladino clover. A combination of three sequential purifications on paper chromatograms followed by one on silicic acid chromatostrips was used to isolate the isoflavones. The identity of the compounds was confirmed by absorption spectra as well as by comparison of paper and silicic acid chromatograms of the isolated compounds with those of authentic samples of the isoflavones.**

INTEREST in naturally occurring forage estrogens has increased in recent years, especially in view of the restrictions on the use of synthetic estrogens in animal feeds. Although coumestrol has been found in a number of more important forages (*2, 4*), the estrogenic isoflavones, formononetin, genistein, and biochanin A, known to be present in red and subterranean clovers (*7*), have not as yet been reported in alfalfa nor in any varieties of white clover. The closely related isoflavone, daidzein, has been reported only in *Pueraria sp.* root (*9*). Since genistein (4′,5,7-trihydroxyisoflavone) has been found together with its 4′-methyl ether, biochanin A, in some forages, it also seems logical to expect the presence of daidzein (4′,7-dihydroxyisoflavone) in those forages which contain its 4′-methyl ether, formononetin. The present experiments were undertaken to determine the presence or absence of daidzein, formononetin, genistein, and biochanin A in several important forages.

### *Experimental*

For this study four representative forage meals were employed: alfalfa (*Medicago sativa*), ladino clover (*Trifolium repens*, var. *ladino*), red clover (*Trifolium pratense*), and subterranean clover (*Trifolium subterraneum*). Each meal was shown to be estrogenically potent by a mouse assay (*7*).

Forage extracts were made in Soxhlet apparatus of 300 grams of each of the dried meals. Preliminary extraction of the meals with Skellysolve B for 24 hours removed the fats and lipochromes, which were discarded. A subsequent extraction of the meal with acetone for 24 hours removed the estrogens (*1*). The resulting acetone extracts were concentrated under reduced pressure to 300 ml. each and kept as stock solutions for all further work. The same chromatographic procedures were used to examine and to isolate the isoflavones, if present, from each of the four forages. To serve as guide spots, samples of the authentic isoflavones were applied in solution to each chromatogram in the purification procedures.

**Detection of Daidzein** (Table I). STEP 1. One hundred milliliters of the acetone extract was applied in 10-ml. portions to 10 sheets of Eaton and Dikeman No. 301 paper. The extract was applied as a narrow band 5 cm. from the long edge of an $18^1/_4 \times 22^1/_2$ inch sheet and a 5-cm. zone was left at one end, so that a 25-μg. reference spot of daidzein could be chromatographed at the same time. The sheets were developed simultaneously by ascending chromatography with the upper phase of a benzene–acetic acid–water (2:2:1) solvent mixture for 4 hours at room temperature. Daidzein and formononetin were located easily on the paper by visual inspection under an ultraviolet source with a peak at 3600 A. The exposure of the paper to ammonia vapors greatly increased the intensity of fluorescence of both daidzein and formononetin.

Under our laboratory conditions, the reference spots of daidzein had an average $R_f = 0.22$, measured to the leading edge of the spot. However, the daidzein from the extract appeared to have been carried somewhat further, probably because of the presence of other materials. After the developed chromatograms had been dried at room temperature, the paper in an area between the origin and $R_f = 0.40$ was removed, cut into small pieces, and treated with four 800-ml. portions of warm methanol.

STEP 2. The combined methanol solution was concentrated under reduced pressure to 100 ml. and applied to 33 sheets of Whatman 3-mm. paper (each $18^1/_4 \times 22^1/_2$ inches) in the manner described previously. These sheets were developed in acetone-water (3:7) for 16 hours. Daidzein reference spots advanced to $R_f = 0.85$. Corresponding fluorescent bands on the sheets were cut out, the absorbed material was extracted