Table IV. Melting Points and Relative Volatilities of 5-Propenyl Guaethol, Bourbonal, Vanillin, and Coumarin

	5-Propenyl Guaethol	Bourbonal	Vanillin	Coumarin
Melting point, °C.				
Closed capillary	85.5	78.0	82.0	68.0
Literature range	85–86	76.5 min.	81–82	67–70
Relative volatility, mg./sq. cm./hour				
24° C.[a]	0.0005	0.0003	0.0004	0.0016
50° C.	0.06	0.04	0.05	0.08
70° C.	0.18	0.10	0.12	0.21
90° C.	0.75	0.43	0.47	0.80
105° C.	1.80	1.03	1.14	1.90

[a] As determined in 55-mm. aluminum dishes; all other volatilities determined in 8-mm. glass petticups.

closed capillary melting points were run on the samples used.

Determinations of relative volatility above room temperature were actually run at 50°, 70°, 90°, and 105° C. in a forced-draft electric oven. Since this range extended well above the melting points of all of the compounds, duplicate samples of 0.1 to 0.2 gram of each compound were weighed into glass "petticups" (flat-bottomed glass cylinders, 8 mm. in inside diameter, 15 mm. high) and placed in an oven at 105° C. At intervals of 24 hours the petticups were removed, cooled, and weighed, until a substantially constant rate of loss had been established for each compound. The temperature was lowered to 90° C., and weighings were repeated at intervals of 1 to 2 days.

The temperature of the oven was then lowered to 70° C., at which temperature all the compounds except the coumarin were solid, and the procedure was repeated, with the period between weighings increased to 5 to 7 days. Then the temperature was lowered to 50° C., where all of the compounds were solid, and the procedure was repeated, with the period between weighings extended to 10 to 20 days.

Finally, as a check on relative volatility at lower temperatures, duplicate samples of 0.1 to 0.2 gram of each compound, ground to 50- to 80-mesh, were weighed into light-weight aluminum dishes, 55 mm. in inside diameter, 18 mm. deep, evenly distributed over the flat bottom, and placed in gently circulating air in a conditioned room at 24° C., 50% relative humidity, with an "umbrella" of paper over them to guard against possible falling dust. These were weighed at intervals of 10 to 20 days.

Results and Discussion

The solubilities of 5-propenyl guaethol at 24° C., read from the curves established as described, are given in Tables I and II. The values from these tables for 95% by volume ethanol and for 100% propylene glycol are in substantial agreement with those given by the manufacturer (*5*).

Solubilities of the four flavoring compounds in water, and of 5-propenyl guaethol in aqueous propylene glycol and in aqueous ethanol, from 0° to 50° C., read from the experimental curves, are given in Table III. The values for bourbonal, vanillin, and coumarin are in general agreement at most points with those given by the manufacturer (*4*), and the solubilities found for vanillin are within the rather wide range reported by Mange and Ehler.

Melting points and relative volatilities of the four compounds are given in Table IV. The samples used melted within the ranges given in the literature. Although the experimental work done on relative volatilities was rather limited, it appears that coumarin is somewhat more volatile than 5-propenyl guaethol over the temperature range investigated. Vanillin has substantially lower volatility than these, while bourbonal is slightly less volatile than vanillin. However, from the point of view of use as flavoring materials, the differences in volatility are probably not significant. This was confirmed experimentally in the course of the flavor studies cited (*1*).

Literature Cited

(1) Cartwright, L. C., and Kelley, P. H., *Food Technol.*, **6**, No. 9, 372–6 (1952).
(2) Hodgman, C. D., "Handbook of Chemistry and Physics," 33rd ed., pp. 1763–70, Cleveland, Ohio, Chemical Rubber Publishing Co., 1951.
(3) Mange, C. E., and Ehler, O., *Ind. Eng. Chem.*, **16**, 1258–60 (1924).
(4) Monsanto Chemical Co., "Something about the Senses," trade bulletin.
(5) Shulton, Inc., "Vanitrope Brand of Propenyl Guaethol," *Tech. Bull.* **5.**

Received for review October 2, 1953. Accepted April 16, 1953. Based on a paper presented before the Division of Agricultural and Food Chemistry at the 122nd Meeting of the AMERICAN CHEMICAL SOCIETY, *Atlantic City, N. J.*

Nutritive Values and Digestibility Are Studied Instrumentally

COTTONSEED MEAL EXTRACTS
Electrophoretic Patterns of Buffer Extracts Of Different Nutritive Value

MELVIN L. KARON, MABELLE E. ADAMS, and AARON M. ALTSCHUL
Southern Regional Research Laboratory, New Orleans, La.

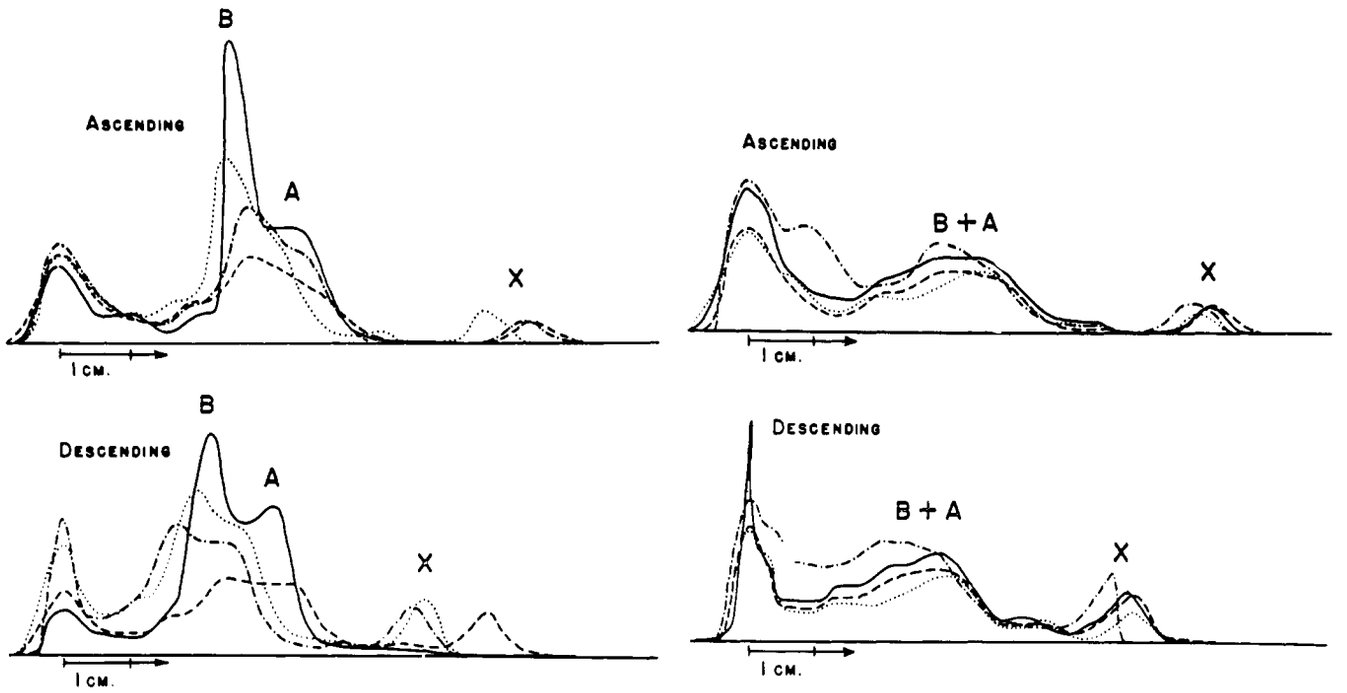

Figure 1. Electrophoresis patterns of total protein extracted from 10 grams of meal by 50 ml. of ethylamine-barbital buffer

Laboratory solvent-extracted meal (diluted 1 to 4).

Left.
... Series 1, No. 6 –.–. Series 1, No. 8 – – – Series 1, No. 7

Right.
–.–.–. Series 1, No. 1 ——— Series 1, No. 3 – – – – Series 1, No. 4
..... Series 1, No. 5

THE VALUE OF COTTONSEED MEAL as a protein supplement in feeds for swine and growing poultry is influenced by the extent of damage to the protein during processing and the presence of substances which may interfere with growth (gossypol and related compounds) (*14*). In meals having a free gossypol content below the level where it interferes with growth, overheating during processing reduces protein efficiency as measured by growth tests (*8, 9, 12*).

Conditions of processing also affect thiamine content and the amount of nitrogen that can be peptized from oil-free meal (*3, 12*). In general, meals of high nitrogen solubility and thiamine content also have high nutritive value, as measured by protein efficiency, and those of very low nitrogen solubility and thiamine content have poor nutritive value. The nutritive value of the protein in a meal, however, cannot be predicted accurately from these characteristics because in their intermediate range, which encompasses most commercial meals, these chemical determinations are not well correlated with animal feeding tests. Hence, more precise methods are needed for determining the effect of processing on the nutritive quality of cottonseed protein.

The present investigation was undertaken to determine whether differences in the nutritive value of cottonseed meals as a source of protein are reflected in the properties of the soluble proteins as determined by electrophoretic measurements of meal extracts. Previous investigations have shown that electrophoretic patterns of the major components of cottonseed meal extracts and of precipitated fractions of the proteins are essentially the same (*5*). Electrophoretic investigations have also been made of soybeans (*7*), and studies of soybean protein have shown that the electrophoretic patterns change with heating of the protein solutions (*7*).

Preparation of Cottonseed Meals

Two series of cottonseed meals produced in commercial plants by the screw-press method were investigated. In this process heat is applied to the cottonseed

In order to determine the nature of the changes taking place in cottonseed meal proteins as a result of differences in conditions of preparation, a series of investigations was conducted on the electrophoretic patterns of cottonseed meal extracts. This is part of a research program on the improvement of the nutritional value of cottonseed meal and the laboratory determination of differences in nutritive value of the meal as a protein supplement. As heat stress during processing increases, the electrophoresis patterns of the soluble protein fraction of the meal change. A new fast-moving component develops and the original two major components become more difficult to resolve. These changes seem to correlate well with changes in nutritive value within a single series of related meals.

Table I. Conditions Used in Processing Screw-Pressed Cottonseed Meals, Chemical Analyses, and Protein Efficiency of Meals

Meal	Max. Cooking Temp., °F.	Time of Cooking, Min.	Soluble Nitrogen, %	Thiamine, P.P.M.	Free Gossypol, %	Protein[a] Efficiency
Solvent-extracted			78.8	39.6	0.012	2.50
Series 1						
No. 8	180	15–20	14.3	6.2	0.01	2.06
No. 6	200	15–20	15.9	6.6	0.01	2.26
No. 7	200	70	12.8	4.5	0.01	2.06
No. 1	234	15–20	11.7	3.8	0.01	1.74
No. 3	260	20	10.0	2.1	0.02	1.74
No. 4	260	40	10.3	2.8	0.02	1.74
No. 5	280	100	8.2	1.1	0.03	0.54
Series 8						
No. 3	183	15	41.1	17.5	0.03	
No. 2	210	15	33.9	15.5	0.03	
No. 1	258	30	15.9	6.4	0.03	

[a] Grams gained per gram of protein (Nx 5.3) consumed (*4*).

kernels (meats) by cooking prior to pressing and also is developed during pressing to remove the oil. Accurate data are not available on the amount of heat developed in the press. Measurements of temperatures of cooked meats entering and of the oil leaving the press indicate that an increase in temperature of 50° to 70° F. takes place during pressing. Whether the observed difference in temperature represents the maximum rise in temperature in the press is not indicated by available data, nor do the data furnish information about the length of time that the meats are subjected to the highest temperature.

All meals in a series were produced on an experimental basis from the same seed and with the same equipment. The principal variables in series I were the conditions of cooking. As far as it was possible to maintain control, the work done in removing oil was the same for each meal. The maximum cooking temperature and length of cooking, therefore, were used as the basis for arranging the meals of each series in a probable order of minimum to maximum heat damage during processing, as is done in Table I.

In series 8 both the maximum temperature of cooking and the work done by the press were varied. Meal 1 was heated to the highest temperature during cooking and required the greatest load on the motors during the screw feed (95% of full load). Meal 3 was heated to the lowest temperature and operated under 60% load on the motors. Meal 2 was intermediate in both respects. Although the meals in series 8 differed one from the other by more than one operational variable, the changes in processing conditions were all in the same direction—that is, the meal cooked to the highest maximum temperature was subjected to the greatest work during processing and the one cooked to lowest maximum temperature was subjected to the least work. These considerations were used in arranging the meals of series 8, in Table I, in an order of heat damage during processing. This permits comparison of the effect of heat damage in the graded series upon physicochemical properties of the proteins in the meal.

Aside from the differences between the individual meals in each series, there were general differences between the processing conditions in the two series described in Table I. The screw presses were of different manufacture, the meats were in the press for different lengths of time, and the residual oil in the press cake differed. From the fact that the meals in series 1 had a lower residual oil content (3% in series 1 as compared to 5% in series 8), it may be presumed that more work was required in the pressing operation and more heat developed in the presses. For meats cooked to the same temperature, those from series 1 would therefore be subjected to greater total heat. This assumption was borne out by the results of the protein evaluations.

As a basis for comparison with the meals produced in commercial plants, a meal was prepared by laboratory solvent extraction under such conditions that the temperature did not exceed 120° F. The gossypol content of the meal was removed at the same time. Previous experience has shown that under these extraction conditions little heat damage to the protein has taken place.

Analytical Methods

Soluble nitrogen was determined by suspending 2.5 grams of the meal ground to pass a 1-mm. screen in Wiley mill No. 1 in 100 ml. of 0.5 *N* sodium chloride solution and shaking this suspension intermittently for 3 hours. The percentage of the total nitrogen extracted by this procedure is reported as soluble nitrogen.

Table II. Relative Concentrations and Mobilities of Components of Cottonseed Meals

Meal	X		A′		A		B		C		D	
	Mobility[a]	%	Mobility	%	Mobility	%	Mobility	%	Mobility	%	Mobility	%
Solvent-extracted			12.1	3	7.7	42	5.5	48	3.3	3	1.9	3
Series 1												
No. 8	14.7	11	12.0	4	7.7	35	5.6	37	3.5	7	2.3	6
No. 6	15.2	10	11.9	4	8.2	31	6.2	41	4.3	6	3.1	6
No. 7	16.3	12	13.1	5	8.7	33	6.2	36	4.0	8	2.3	7
No. 1	15.3	9	12.6	4	7.3[b]	56[b]	...	..	3.5	17	2.1	13
No. 3	15.3	16	11.5	9	7.3[b]	49[b]	...	..	4.4	15	2.5	11
No. 4	15.6	15	12.2	7	7.6[b]	58[b]	...	..	3.8	11	2.2	9
No. 5	15.9	10	12.4	7	8.3[b]	49[b]	...	..	4.3	19	1.6	15
Series 8												
No. 3			9.3	7	7.6	30	5.5	49	3.8	8	2.0	8
No. 2			10.9	6	7.0	39	4.8	47	3.1	5	1.7	4
No. 1	14.9	10	11.2	5	7.9	41	5.6	32	4.0	6	2.4	7

[a] Sq. cm./volt/sec. $\times 10^{-5}$ (all components migrate toward − pole).
[b] Components A and B are not clearly resolved, so values represent best measurement of combination.

Thiamine was assayed by the method of Sarett, in which the meal sample is digested with papain and takadiastase and then assayed microbiologically with *L. fermenti* 36 (A.T.C.C. No. 9338) in media complete except for thiamine, for 16 hours at 37° C. The extent of growth is determined by measuring the turbidity produced by means of an Evelyn colorimeter at 620 mμ. The thiamine contents obtained agreed well with those obtained by the thiochrome method (*13*).

Gossypol and gossypollike materials were determined according to the method of Pons (*10*), in which the gossypol extracted with a measured volume of 70% aqueous acetone reacts with *p*-anisidine and is analyzed colorimetrically.

Extracts from the meals were prepared for electrophoretic analysis as follows: The meal samples were extracted with butanone by cold percolation to remove residual oil and pigments, and were air-dried. Then 10 grams of the extracted meal were mixed with 50 ml. of ethylamine-barbital buffer (pH 10.4 at 25° C., ionic strength 0.10) in a dialysis tube and the solution was dialyzed in a mechanical dialyzer against 1 liter of the same buffer (*11*). The system was maintained at 5° and the buffer was changed every 24 hours. After 72-hour dialysis the meal suspension was centrifuged at 7000 g and the clarified protein solution was then dialyzed against 1 liter of fresh buffer for 48 hours. The nitrogen solubility in ethylamine-barbital buffer is approximately the same as in 0.5 *N* sodium chloride.

Protein solutions prepared in this manner were analyzed in the Tiselius moving boundary electrophoresis apparatus at 5° C. Electrophoretic patterns were recorded on photographic plates by the Longsworth scanning method. Analyses of the patterns were made on projected tracings, with the division into components, according to an acceptable method (*6*).

The meals of series 1 and the solvent-extracted meal were furnished to other investigators who tested them as sources of protein for chicks and rats (*2, 4, 8, 12, 14*).

Results and Discussion

Data on the nitrogen solubility, thiamine content, gossypol content, and protein efficiency of the meals are included in Table I. All commercial meals showed greatly reduced nitrogen solubility and greatly reduced values for thiamine, in comparison with the meal that had been solvent-extracted in the laboratory with mild heating. Gossypol content in all cases was below the level where it interfered with the growth and protein efficiency. In any given series of meals the greater the heat damage during processing, the greater was the reduction in nitrogen solubility and thiamine content.

As was suggested previously, the maximum temperature of cooking prior to pressing does not provide a complete picture of heat damage during processing, and therefore does not allow comparison between the meals in the two different series. For example, the maximum temperatures attained by meals 6 and 8, series 1, during cooking are comparable to those of meals 2 and 3, series 8; yet there is a vast difference in their nitrogen solubilities and thiamine contents.

The electrophoresis data for extracts of meals (Table II) and the trace patterns for the extracts of meals of series 1 (Figure 1) reveal that two major components, A and B, changed with processing conditions. The changes in the trace patterns appear to be due to the gradual replacement of these two components by a single component. In the solvent-extracted meal, components A and B accounted for 90% of all the soluble materials, with B slightly in excess of A. In meal 3 in series 8 (the one subjected to the least amount of heat damage) components A and B accounted for 79% of the total soluble protein, again with B in excess of A. In meal 2, series 8 (the intermediate meal with respect of heat damage), these two components accounted for 86% of the total soluble meal and again there was more of B than A. But meal 1 of series 8 (which has been subjected to the most heat) showed a greater amount of A than B and a 73% total for the two components.

As a result of the greater amount of heat stress applied to meals of series 1, more drastic changes are observed (Figure 1). In several of these meals it becomes difficult to distinguish between components A and B as the amount of heat damage increases. The formation of a single diffuse peak from components A and B in the meals subjected to greater heat may be the result of protein interaction between the components or the progressive decrease in extractability of these protein components as a result of the heating process, with this effect being greater on B than on A. It is difficult to decide what has happened to these components, but there seems to be evidence that the combined material has more of the physical properties of A than of B, inasmuch as its mobility is close to that of the original component A. A somewhat similar phenomenon was reported by Mann and Briggs (*7*) in their studies of soybean protein. When a solution of soybean proteins was heated, a single peak in the electrophoresis pattern was formed at the expense of several smaller peaks. The patterns of extracts of meals subjected to greater heat stress show increased proportion of components C and D and high delta and epsilon boundaries.

A new fast-moving minor component, X, was found in most of the commercial meals but not in the laboratory-prepared meal. The amount of component X did not seem to depend on the amount of heat to which the meal was subjected as long as the minimum amount of heating had taken place. For example, the amount of component X was practically the same in all the meals of series 1, even though there was a gradation in the amount of heat applied to the samples. The two members of series 8 that had been subjected to the milder heat conditions lacked this component. The data do not indicate whether component X is a protein breakdown product or a combination of one of the components with other constituents of the meal.

On the basis of their nutritional value for chicks, the meals of series 1 fall into three groups, the highest values being given by meals 6, 7, and 8, intermediate values by meals 1, 3, and 4, and a poor value by meal 5 (*8*). Rat feeding tests in which the laboratory-prepared, solvent-extracted meal was included indicated that there were four significant groups in the following decreasing order of protein efficiency: (1) solvent-extracted meal; (2) series 1, meals 6, 7, and 8; (3) meals 1, 3, and 4; and (4) meal 5 (*4*).

Comparison of the results of nutritional tests with the electrophoretic patterns in Figure 1 and with the data in Table II shows that there may be some correlation between the amounts and the relative separation of components A and B and nutritive value of the protein. For example, A and B can be distinguished in meals 6, 7, 8, whereas in the remaining meals in the series it is not possible to resolve these two components, the greatest tendency for a single diffuse peak being found in meal 5.

If clarity of resolution of components A and B is associated with highest nutritive value, the solvent-extracted meal would be expected to have the highest nutritive value, followed by the others in the order shown in Figure 1.

Although some correlations can be made between characteristics of the electrophoretic patterns and nutritive value, electrophoretic patterns are obtained on soluble proteins whereas nutritive value is dependent upon the entire protein ingested by the animal. As the heat damage during processing is increased, the percentage of total protein contributing to the electrophoretic pattern decreases, as shown by decrease in area of electrophoresis pattern, which parallels decrease in nitrogen solubility. Electrophoretic analysis has the advantage over mere solubility measurement, however, in that it provides information about the condition of the soluble pro-

tein components, degree of resolution, and presence of new components. This approach could be of greater usefulness if means were developed for greatly increasing the solubility of protein components in meals subjected to heat damage during processing.

Acknowledgment

The authors are pleased to acknowledge the helpful suggestions of Marjorie Z. Condon in preparation of this paper.

Literature Cited

(1) Briggs, D. R., and Mann, R. L., *Cereal Chem.*, **27,** 243–57 (1950).
(2) Haddon, R., *et al.*, *Cotton Gin and Oil Mill Press*, **52,** No. 9, 18 (1950).
(3) Hayward, J. W., *Feedstuffs*, **22,** No. 30, 30–5, 38, 40–2 (1950).
(4) Horn, M. J., Blum, A. E., Womack, Madelyn, and Gersdorff, C. E. F., *J. Nutrition*, **48,** 231–41 (1952).
(5) Karon, M. L., Adams, M. E., and Altschul, A. M., *J. Phys. Colloid Chem.*, **54,** 56–66 (1950).
(6) Longsworth, L. G., *Chem. Revs.*, **30,** 323–40 (1942).
(7) Mann, R. L., and Briggs, D. R., *Cereal Chem.*, **27,** 258–69 (1950).
(8) Milligan, J. L., and Bird, H. R., *Poultry Sci.*, **30,** 651–7 (1951).
(9) Olcott, H. S., and Fontaine, T. D., *Ind. Eng. Chem.*, **34,** 714–16 (1942).
(10) Pons, W. A., and Guthrie, J. D., *J. Am. Oil Chemists' Soc.*, **26,** 671–6 (1949).
(11) Reiner, M., and Fenichel, R. L., *Science*, **108,** 164–6 (1948).
(12) Research Conference on Processing as Related to Cottonseed Meal Nutrition, Southern Regional Research Laboratory, New Orleans, La., Proceedings, Nov. 13–14, 1950.
(13) Sarett, H. P., and Cheldelin, V. H., *J. Biol. Chem.*, **155,** 153–60 (1944).
(14) Second Conference on Cottonseed Processing as Related to Nutritive Value of the Meal, Southern Regional Research Laboratory, New Orleans, La., Proceedings, Nov. 5–7, 1951.

Received for review February 6, 1953. Accepted April 24, 1953. Presented at the Southwide Chemical Conference, Auburn, Ala., October 23 and 24, 1952. The use of trade names in this article is for identification and implies no endorsement by the Department of Agriculture of the product over similar products not mentioned.

DIGESTIBILITY OF FORAGES

Pigments Involved in the Chromogen(s) Ratio Method

WILLIAM W. G. SMART, JR., FRANK W. SHERWOOD, GENNARD MATRONE, and GEORGE H. WISE

Animal Nutrition Section, Department of Animal Industry, North Carolina Agricultural Experiment Station, Raleigh, N. C.

Although information as to the identity of the pigment or pigments is not essential to employment of the "chromogen(s)" ratio method, knowledge of its properties could lead to improvements in the ratio technique. The purpose of this study was to determine the identities of the pigments involved and to obtain further evidence of validity of the chromogenic method. The pigments contained in the chromogen mixture used as an indicator of digestibility were isolated and identified. Spectral absorption curves of the 85% aqueous acetone extracts from forage and feces displayed absorption maxima indicative of chlorophylls or their degradation products. Cold saponification of these acetone extracts demonstrated the presence of both chlorophyll products and carotenoids. Chromatographic separation of the pigments demonstrated the presence of at least seven pigments in the chromogen extract from hay and feces of animals fed this hay: chlorophyll a, chlorophyll b, pheophytin a, pheophytin b, luteol, violaxanthol, and β-carotene. The chromogen(s) involved in the chromogen ratio method for determining digestibility of forages is essentially a total chloroplast pigment extract. Changes in the chemical structure of these compounds on passage through the digestive tract have little effect on the light absorption of the mixture of pigments at the isosbestic point at 406 mμ.

A COMPARATIVELY SIMPLE CHEMICAL METHOD for estimating consumption and digestibility of forages by animals (7) is based on the principle that nonabsorbable material in a forage may be used as a marker and that its concentration in the feces is proportional to the digestibility of the forage. Based on experiments in which data from conventional digestion trials were used as the standard, Reid *et al.* proposed that an 85% acetone-water extract of forage contains a natural marker. This marker was labeled "chromogen(s)" (7), because its identity was unknown. Although information as to the chemical identity of the pigment or pigments is not essential to the employment of this procedure, a knowledge of their properties may lead to improvements in the ratio technique.

In view of the recognized abundance