the above findings, although neither amino acid alone resulted in improved growth. The addition of both, however, improved growth, feed efficiency, and protein efficiency. This suggests that both are limiting to about an equal degree. Even with the addition of these two amino acids, however, or with the addition of seven amino acids, the growth was only 88% of that obtained with skim milk. There are, apparently, other limiting amino acids besides methionine and threonine, a situation which could be due either to a lack in availability or to the fact that the amounts of threonine and methionine added were not adequate for maximum improvement in growth. To obtain a maximum response to an amino acid addition, it is necessary to add the optimum amount of the most limiting amino acids (*22*).

In general, the proteins of the pepitoria are of good nutritional quality. Pepitoria should, therefore, receive more attention since it provides an oil which could have practical uses and could provide a good protein to be used in combination with other food products. In Guatemala, the whole pepitoria seed combined with corn masa in mixed preparations is used to a limited extent as a human food.

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FORAGE DIGESTIBILITY

Benzene–Ethanol Extracts of Forage and Feces as Indicators of Digestibility

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To circumvent some of the difficulties encountered in chromogen determination, dried forage and feces samples were extracted with benzene–ethanol (2 to 1), and the resulting crude pigment was determined gravimetrically. Correlations between these results and dry-matter digestibilities with mixed forages indicate that this method is at least as good as chromogen or methoxyl for prediction of digestibility. The method can be applied either in place of or following a regular crude fat analysis without affecting the crude fiber determination.

Plant pigments or chromogens have been used extensively as a measure of the indigestibility of forage crops. Reviews by Reid and Kennedy (*8*), Valentine (*12*), and more recently Harris, Cook, and Butcher (*4*) of the published work in this field all emphasize the usefulness of the technique, while at the same time indicating certain limitations in its use.

One of the limitations of the chromogen method was pointed out by Cook and Harris (*1*) who concluded that "the chromogen method was not suited for determining digestibility of winter range forage since, in some cases, there was considerably less chromogen material recovered in the feces than was actually consumed." Davidson has reported (*2*) that the various components of the chromogen fraction had apparent digestibilities as high as 87%. Even where these special circumstances are not a factor, there is lack of agreement on the wave length which should be used. Kane and Jacobson (*6*) analyzed the pigments and concluded that the wave length to be used should be that at which the pigment pheophytin absorbs since, during passage through the animal, the chlorophylls are converted to pheophytin.

More recently, Deijs and Bosman (*3*) have advocated addition of oxalic acid to the 85% acetone which is used to extract the chromogens in order to

Table I. Regression Analysis of the Relationship between Dry-Matter Digestibility and Methoxyl, Chromogen, and Pigment Values on Forage and Feces

Experiment	No. of Samples	Methoxyl Values			Chromogen Values			Pigment Values		
		b[a]	S_E	r	b	S_E	r	b	S_E	r[b]
				FORAGE, SHEEP						
408	17	−13.205	3.295	−0.616[b]	...	...	...	1.969	3.098	0.672
460	10	−13.405	6.664	−0.414	...	...	...	2.874	2.477	0.941
482	10	...	...	...	...	...	...	2.047	4.050	0.873
Total	37	...	...	...	...	...	...	2.004	3.404	0.829
				FECES, SHEEP						
408	17	−14.635	2.629	−0.776[b]	...	...	...	2.195	2.868	0.728
460	15	−12.396	1.854	−0.965[b]	10.814	1.974	0.960[b]	2.325	1.332	0.982
482	29	−10.264	3.615	−0.888[b]	5.063	5.007	0.771[b]	2.020	3.849	0.872
Total	61	−8.506	4.409	−0.752[b]	...	...	...	2.081	3.296	0.870
				FECES, COWS						
459	9	...	...	...	7.140	3.621	0.517	3.224	2.433	0.818
477	26	...	...	...	9.633	3.781	0.848[b]	2.693	4.743	0.747
Total	59	...	...	...	8.758	3.941	0.787[b]	2.702	4.247	0.747

[a] b = regression coefficient, S_E = standard error of estimate, r = correlation coefficient.
[b] Significant at $P = 0.01$.

Table II. Regression Analysis of the Relationship between Organic-Matter Digestibility Using Sheep and Per Cent Benzene–Ethanol Extract on an Organic-Matter Basis

Experiment	No. of Samples	Regression Coefficient	Standard Error of Estimate	Correlation Coefficient[a]
		FORAGE		
408	17	1.899	3.188	0.690
460	10	2.684	2.674	0 934
		FECES		
408	17	2.240	2.613	0.805
460	15	1.807	1.163	0.989

[a] Significant at $P = 0.01$.

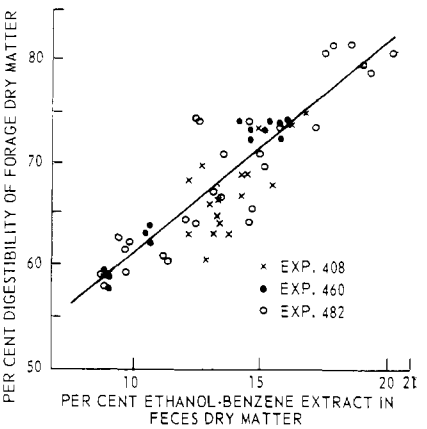

Figure 1. Relationship between dry-matter digestibility of forages fed to sheep and ethanol–benzene extracts of feces

convert the chlorophylls to pheophytin.

Smart, Matrone, and Smart (*11*) have added copper salts to stabilize the chlorphylls and pheophytins against change by acid, alkali, and light. As pointed out by Valentine (*12*), this modification of the chromogen method may be a real improvement.

However, the spectrometric method still has some inherent disadvantages. Generally, best results are obtained only when fresh forage and feces are extracted, and the extracts must be protected from exposure to light (*8*). There is also uncertainty associated with the choice of a spectrophotometric standard, although most workers have apparently used only the absorbance value without conversion to concentration units.

In an effort to circumvent these difficulties, a new approach was taken to the problem. Rather than measure the extracted pigments spectrophotometrically, they have been measured gravimetrically.

Materials and Methods

The samples used in this investigation were from a number of digestibility trials using both sheep and cows. The forages were mixtures of grasses and alfalfa at various stages of maturity. Chromogen determinations on the fresh feces were carried out by the method of Reid *et al.* (*9*); the other determinations were carried out on oven-dried samples. The methoxyl determination was carried out by the method of Mathers and Pro (*7*), as modified by Shearer (*10*).

Pigment was determined by extraction of a 1-gram sample in a Goldfisch apparatus for 16 hours with a 2 to 1 (v./v.) mixture of benzene and ethanol (95%). The extract was finally concentrated on a steam bath, care being taken not to overheat, and the residue dried in vacuo for 5 hours at 70° C. and weighed. All results are reported on a dry-matter basis.

Results and Discussion

Figure 1 shows the relationship between the dry-matter digestibility of forages fed to sheep and the pigment extracted with benzene-ethanol from feces dry-matter (experiments 408, 460, and 482). Also shown is the line plotted from the regression equation $y = 40.25 + 2.08x$ where y = dry-matter digestibility and x = per cent pigment in feces dry-matter.

Similar scatterings were found for pigment in the forage of experiments 408, 460, and 482, and for the feces of experiments 459 and 477. Regression equations for these two sets of data are $y = 26.85 + 2.00x$ and $y = 32.02 + 2.70x$, respectively. Regression analyses were carried out on the data, and the regression coefficients (b), the standard errors of estimate (S_E), and the correlation coefficients (r) are given in Table I for the relationship between the dry-matter digestibilities and methoxyl, chromogen, and pigment values. The correlations are highly significant in all cases except two, namely, methoxyl in the forage of experiment 460 and chromogen in the feces of experiment 459.

A test proposed by Hotelling (*5*) was applied to the data, where available, to ascertain which variable might be best for prediction of dry-matter digestibility. In no case were methoxyl values found to be significantly better

than pigment values for prediction of digestibility. On the other hand, the pigment results are significantly better than the methoxyl ones in two cases, one at the 1% level (experiment 460, forage) and one at the 5% level (total feces from sheep). The chromogen determination proved significantly better (1% level) than pigment in only one experiment (experiment 477).

Thus, the data in Table I show that the pigment determination is at least as good as the methoxyl or chromogen determinations for prediction of dry-matter digestibility.

In the case of experiments 408 and 460, the pigment results were calculated on an organic-matter basis and compared with the organic-matter digestibilities. A regression analysis was carried out, and the results are shown in Table II. In general, the results are similar to those obtained on the dry-matter basis.

Since many workers routinely carry out proximate constituent analyses on their samples, it would be desirable to include this crude pigment determination in the analysis of the proximates either instead of, or in addition to, the crude fat determination. In a further investigation using the samples of experiment 482, the pigment extraction with benzene–ethanol was carried out following a crude fat determination using petroleum ether (b.p. 30° to 60° C.). By this method, the crude fat-free pigment averaged 19.49 and 9.50% in the forage and feces, respectively, and the correlation coefficients with dry-matter digestibility were 0.901 and 0.872, the regression equations were $y = 27.09 + 2.18x$ and $y = 42.67 + 2.84x$, and the standard errors of estimate (8 and 27 degrees of freedom) were 3.603 and 3.849, respectively. Without this pre-extraction of the crude fat, the average pigment values were 21.49 and 13.87% for the forage and feces, respectively.

This result indicated that the pigment determination could be carried out along with the proximate constituent analysis, provided the crude fiber results were not altered. To test this, 12 samples of forage and 12 of feces were analyzed for crude fiber both in the normal way after extraction with petroleum ether and also following a further extraction with benzene–ethanol. An analysis of variance carried out on the results showed that there was no significant difference between the two methods. Thus, the crude fiber results were not affected by the prior extraction of pigment.

This means that if crude fat values are not required then the crude pigment determination can be substituted in the regular proximate constituent analysis. If, however, the crude fat values are wanted, then the pigment determination can be carried out following the extraction of the crude fat and preceding the crude fiber determination. The appropriate regressions would be used in each case.

Conclusion

The results obtained indicate that the gravimetric determination of pigment described here can be used to predict digestibility in much the same manner as chromogen and methoxyl values are used. The determination is readily carried out, requiring only an extraction apparatus, a vacuum oven, and a balance. Satisfactory results are obtained on dried samples, precautions such as must be taken with the chromogen method are not needed, and satisfactory correlations with digestibility are obtained using either forages or feces. The determination can, if desired, be carried out along with the regular proximate constituent analysis.

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CHEMICAL CONTROL OF FLOWERING

Concentration of a Floral-Inducing Entity from Plant Extracts

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DURING the past 25 years, experimental evidence has been accumulating to support the hypothesis that a flowering hormone or class of hormones exists. It has been proposed that such a hormone is in effect the stimulus which signals differentiation of the cells of a growing plant site from the vegetative to the flowering state. A comprehensive and critical summary of current knowledge concerning the physiology and chemistry of the flowering processs is given by Hillman (*3*).

Chemical control of the flowering process, obviously, would be of great assistance in the elucidation of the mechanism of plant reproduction and would have applications in various specialized areas of agriculture. Attempts to isolate from the living plant and to identify chemically the hypothetical hormone, named florigen by Chailakhyan (*2*), have been vigorous but singularly unsuccessful. Lang and Reinhard (*4*) have shown that the various gibberellins have a function, perhaps indirect, in the process of flower formation in plants which are classified as long-day (*3*) as far as day-length or photoperiod requirements for flowering are concerned.

To date, no single chemical entity or small number of chemical compounds

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