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The Relative Digestibility of the Constituents of the Carbohydrate Complex of Grasses at Successive Stages of Growth with reference to their Partition into Crude Fibre and Nitrogen-Free Extract according to the Standard Method for Feeding Stuff Analysis.*

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Introduction.

Ever since its introduction the so-called " standard feeding stuffs analysis " has been the subject of criticism. According to Browne (1940) the shortcomings of this method were realised by Henneberg himself when he announced this system for the evaluation of feeding stuffs in 1863. Even at that time it was known that the chemical make-up of the " crude fibre " isolated by the successive treatment of the material with dilute acid and dilute alkali from various plants may be quite different. At the same time the " nitrogen-free extract " was designated " a mixture of substances ", the nature of which was subsequently investigated (Tollens, 1897).

The partition of the carbohydrate fraction into " crude fibre " and " nitrogen-free extract " was no doubt an attempt to divide this fraction into an indigestible part (crude fibre) and a digestible part (nitrogen-free extract). That this differentiation did not come up to expectations was soon realised when it became known that cellulose, the chief constituent of the crude fibre, is susceptible to attack by the micro-organisms in the digestive tract of ruminants and that the nitrogen-free extract contained poorly digestible substances such as lignin. It was, however, shown (see Browne loc. cit.) that the sum of the digestible crude fibre and of the digestible nitrogen-free extract agreed approximately with the figure obtained for the nitrogen-free extract of the feed. Furthermore, since the digestible part of the crude fibre was taken to have the same nutritive value as the digestible part of the nitrogen-free extract, it was concluded that the practical value of the Henneberg, or Weende, method for the evaluation of feedstuffs was not seriously impaired; digestible crude fibre " compensates " for indigestible nitrogen-free extract so that the nitrogen-free extract of the feeding stuff may still serve as an approximate index for its digestible carbohydrate content.

* The data here presented formed the subject of a paper read before the Pretoria Section of the South African Chemical Institute on July 1, 1941.

If such is the case, then the substitution of the time-honoured Henneberg system of analysis for one which will entail the determination of the actual plant constituents (cellulose, hemicelluloses, lignin, sugars, etc.) comprising the crude fibre-nitrogen-free extract complex, an undertaking which is of a time-consuming character and often quite complicated, is, admittedly, not justified for the ordinary routine of feeding stuff analysis. On the other hand, it must be emphasized that the validity of the assumption that digestible crude fibre " compensates " for indigestible nitrogen-free extract, a compensation which according to Maynard (1940) does not work out satisfactorily in many cases, is dependent upon the correctness of Kellner's finding, viz., that digested cellulose has as great a fattening value as starch. Although as pointed out by Maynard (loc. cit.), the work of Kellner has not been disproved the explanation offered for its validity, viz., that starch, like cellulose and pentosans may be subject to rumen fermentation, does not seem adequate. Simple sugars, starch, cellulose and hemicelluloses are all more or less attacked and broken down by micro-organisms in the forestomach of the ruminant. Products of this breakdown have been identified in *in vitro* studies but the nature and nutritive value of the ultimate fission products elaborated by the organisms *in vivo* are by no means so wellknown: we do not know the relative nutritive value of, for instance, a pound of digestible sucrose, a pound of digestible cellulose and a pound of digestible polyuronide material. It is with problems of this nature, the elucidation of which may result in a more rational and more accurate system for the evaluation of foodstuffs, and in work on crop composition as influenced by stage of majority or other conditions that the old Henneberg system of analysis is wholly unsuited.

Crampton and Maynard (1938) recently proposed a modified procedure for feeding stuff analysis involving the determination of cellulose and lignin. It is no doubt a step in the right direction; the least digestible constituent, lignin, is determined separately but the undetermined fraction (" other carbohydrates ") may still be composed of a mixture of substances of greatly varying digestibility. Thus, to quote extreme cases, in oats it will consist largely of highly digestible starch while in a mature grass hay it will contain a high percentage of pentosans and polyuronides, substances which according to the results to be reported on in this paper have a digestibility lower than that of cellulose. In this connection the marked differences in the digestibility of individual constituents (excluding lignin) of the nitrogen-free extract of the same feed and in the digestibility of the same constituent of the nitrogen-free extract in different foodstuffs as reported by Fraps (1930) are of special interest.

In this paper data are presented which demonstrate the relative digestibilities of the individual cell-wall constituents of grasses as affected by stage of maturity. Results on the composition of the crude fibre of these materials (grass and faeces) are also presented.

Experimental Materials.

The samples of grass hays and faeces analysed were chosen from materials left over from digestion trials conducted on the customary lines and previously reported on by Louw (1938). The coefficients of digestibility for the dry matter obtained in these trials were utilized to calculate the coefficients of digestibility of the actual plant constituents here determined. The samples chosen for analysis were 12 in number. Of these 4 were grass samples and the rest consisted of 8 composite samples of the faeces voided by two sheep in four successive digestion trials with the four samples of grass. These grass samples were obtained by cutting a mixture of veld grasses growing under the prevailing weather conditions

of the summer rainfall area of South Africa at four different stages of growth, viz., after periods of 1, 2, 3 and 4 months of undisturbed growth. For convenience, when discussing the results, these samples will be designated 1M-, 2M-, 3M- and 4M-grass respectively, and the corresponding samples of faeces 1M-, 2M-, 3M- and 4M-faeces.

Analytical Methods.

The following analyses were carried out on the air-dry material:—

(1) *Cellulose:* Norman and Jenkins (1933).

(2) *Lignin* after hydrolysis: Norman and Jenkins (1934, 1), the material being pretreated with alcohol-benzene.

(3) *Total furfural yield:* By distillation with 12 per cent. HCl and precipitation as the phloroglucide.

(4) *Xylan from cellulose:* Furfural from cellulose and expressed as xylan according to Kröber's table. [A.O.A.C. Methods of analysis (1930).]

(5) *Uronic acid anhydrides:* Dickson, Otterson and Link (1930) as modified slightly by Phillips, Goss and Browne (1933).

(6) *Pentosans:* Pentosans here represent the difference between the total furfural and the furfural derived from the uronic acids *plus* the furfural from the cellulose expressed as pentosan (Kröber's table).

Note.—$\%\ \text{Furfural from uronic acids} = \frac{\%\ \text{uronic acid anhydride}}{4{\cdot}60}$

[Phillips *et al* (1939)].

(7) *Hemicelluloses* = Pentosans + Uronic acid anhydrides.

(8) *Crude protein, Ether extract* and *total ash* were also determined by the usual procedures.

(9) *Cold water-soluble fraction:* 24 hours at room temperature, nitrogen and ash being determined in the residue so as to arrive at a figure for a cold water soluble fraction which excludes water-soluble crude-protein and ash.

Results.

(1) *The Carbohydrate Constituents of Grass Hays and Their Digestibility.*

The analyses of the grass samples and of the samples of faeces (average for two sheep) are given in Tables 1 and 2, respectively. Figures for ash, crude protein and ether extract have been included in these tables in order to show to what extent the undetermined fraction, or so-called nitrogen-free extract, of the old system of analysis can be reduced by the determination of the actual structural constituents of the plant material. In Table 3 the coefficients of digestibility of the constituent parts of the carbohydrate complex are presented.

(a) General.

The analysis of the cell-wall constituents of plants involves the determination of natural cellulose, the hemicelluloses, and of lignin. Before proceeding to a discussion of the values obtained for these constituents a few remarks concerning the analytical methods employed are necessary.

Natural cellulose is represented as consisting of " true " cellulose or α-cellulose and other polysaccharides intimately associated with the α-cellulose. According to Norman (1937) the polysaccharide found in most structural celluloses is a xylan. Furthermore, this " cellulosan ", as it is called, is the most susceptible part of the cellulose aggregate, being hydrolised by relatively dilute acids and extractable to a considerable extent by dilute alkalis. It should, according to Norman (loc. cit.), not be regarded as an extraneous substance but as an integral part of the natural cellulosic fabric of the tissue. Any method for the isolation of natural cellulose must therefore take cognisance of the properties of its cellulosan fraction: only approximately neutral reagents should be used as in the method of Norman and Jenkins (loc. cit.) employed in the present investigation. The method of filtering off on fine smooth poplin stretched over a Buchner funnel and closely fastened with tight rubber bands with the application of slight suction has been found especially useful as this prevents too long contact between reagent and material as would happen with a slow filtering process.

As already indicated the lignin has also been determined by a method of Norman and Jenkins. This method embodies all the improvements of the original Ost and Wilkening (1910) procedure made necessary by recent researches dealing with the interference of substances, such as protein and pentosans, with the determination. The figures obtained can, however, not be taken as accurate since the lignin, as isolated, still contains small quantities of nitrogenous material (see Tables 1 and 2).

TABLE 1.

Composition of Grass Samples.

(All results expressed as percentages of the original oven-dried material.)

Constituent.	Period of growth (months).			
	1.	2.	3.	4.
Ash	11·00	11·40	10·90	10·60
Crude protein	10·42	8·81	6·92	5·53
Ether extract	2·81	2·39	2·20	2·00
Natural cellulose	44·20	45·00	47·90	49·50
Hemicelluloses (see below)	11·45	12·02	12·03	12·68
Lignin	9·90	10·00	10·50	11·40
Substances not accounted for (by diff.)	10·22	10·38	9·55	8·29
TOTAL	100·00	100·00	100·00	100·00
Additional Data.				
(1) Uronic acids as anhydrides	4·30	4·26	4·04	3·89
(2) Pentosans	7·15	7·76	7·99	8·79
Total hemicelluloses (1) + (2)	11·45	12·02	12·03	12·68
(3) Xylan from natural cellulose	11·51	12·19	13·55	13·83
(4) True cellulose	32·69	32·81	34·35	35·67
(5) Nitrogen in lignin	0·35	0·26	0·20	0·18
(6) Cold water-soluble substances	11·80	10·50	9·20	8·30
(7) Digestion coefficient of the dry matter (average for 2 sheep)	60·40	54·90	51·35	45·50

TABLE 2.

Composition of Faeces (Average for 2 Sheep).

(All results expressed as percentages of the original oven-dried material.)

Constituent.	Period of growth (months).			
	1.	2.	3.	4.
Ash	20·40	19·50	17·71	17·05
Crude protein	9·65	7·70	6·81	6·00
Ether extract	4·10	3·60	3·10	2·72
Natural cellulose	27·90	31·70	35·50	36·81
Hemicelluloses (see below)	13·31	13·32	13·49	13·06
Lignin	18·90	18·60	18·80	18·50
Substances not accounted for (by diff.)	5·74	5·58	4·59	5·86
TOTAL	100·00	100·00	100·00	100·00
Additional Data.				
(1) Uronic acids as anhydrides	3·96	3·90	3·87	3·75
(2) Pentosans	9·35	9·42	9·62	9·31
Total hemicelluloses (1) + (2)	13·31	13·32	13·49	13·06
(3) Xylan from natural cellulose	8·04	9·28	10·65	11·20
(4) True cellulose	19·86	22·42	24·85	25·61
(5) Nitrogen in lignin	0·50	0·44	0·34	0·32
(6) Cold water-soluble substances	5·90	6·20	6·00	5·30

TABLE 3.

Coefficients of Digestibility of the Constituents of the Carbohydrate Complex of Grass Samples.

Constituent.	Period of growth (months).			
	1.	2.	3.	4.
Natural cellulose	75·00	68·20	63·92	58·14
(*a*) True cellulose	75·90	69·10	64·80	60·90
(*b*) Xylan in cellulose	72·40	65·60	61·70	55·90
Hemicelluloses	54·00	50·08	45·50	43·85
(*a*) Uronic acids	63·48	58·65	53·45	47·55
(*b*) Pentosans	48·25	45·20	41·45	42·20
Lignin	24·47	16·10	12·95	11·58
Cold water-soluble substances	80·10	73·30	68·24	65·20

A wholly satisfactory method for the determination of the hemicelluloses of plants has not so far been developed. The shortcomings of the available procedures have recently been commented on by Phillips (1940) and need not be repeated here. Suffice it to say that the figures reported in this paper must be looked upon as only approximate, the figure for hemicelluloses being a summation of the values obtained for uronic acid anhydrides and pentosans.

It should be mentioned that no allowance has been made in the figure for uronic acid anhydride for the presence of small quantities of gums, mucilages or pectic substances in the materials analysed, substances which will also yield CO_2 on distillation with 12 per cent. HCl. The total CO_2 content, excluding, of course, CO_2 derived from carbonates, has been determined on the unextracted material and reported as uronic acid anhydride by multiplying the figure for CO_2 with the factor 4. Lastly, the value for " pentosan " excludes the furfural derived from both the uronic acids and the xylan associated with the natural cellulose.

(*b*) *Natural Cellulose.*

The natural cellulose content of the four samples of grass increases from 44·2 per cent. in the sample cut after one month's growth to 49·5 per cent. in that harvested after four months of undisturbed growth. The relatively small difference in the cellulose content of samples IM and 4M, between the youngest and the most advanced stages of maturity, is due to the fact that sample IM, after a growth period of only 30 days has already reached a high degree of maturity in comparison with grasses growing for approximately the same period in temperate regions. Thus Norman (1936) in work done at Rothamsted (England) found for Rye Grass cut at the youngest stage of growth on April 26, a cellulose content of only 20·89 per cent. whereas the samples of hay cut on June 22, almost 2 months later, contained no less than 40·85 per cent. cellulose, a figure comparable with that for sample IM of the present investigation. The content of xylan associated with the cellulose also increases with age in the grass samples from 11·51 per cent. in IM to 13·83 per cent. in 4M. If the xylan is expressed as a percentage of the natural cellulose containing it the values 26·0, 27·1, 28·3 and 28·0 are obtained for samples IM, 2M, 3M and 4M, respectively. A change in the ratio of xylan to true cellulose thus occurs with increasing age in the plant [c.f. also Norman (loc. cit.) and Phillips *et al* (loc. cit.)]. The results under discussion cover only four stages of growth but from the limited data it would appear that the increase in the ratio of xylan to true cellulose is arrested at some stage (in this case 3M) in the development of the plant, thereafter remaining constant or even decreasing.

An inspection of the coefficients of digestibility given in Table 3 reveals that cellulose is the most digestible constituent of the cell wall structure. Its digestibility is, however, markedly influenced by stage of maturity in the plants; in sample 1M it is digested to the extent of 75·0 per cent. while only 58·1 per cent. of the natural cellulose is digestible in sample 4M. To what extent this lowering of the digestibility is dependent upon possible changes in chemical complexity or physical condition of the natural cellulose itself with age is not known. On the other hand it is generally assumed and has often been demonstrated [Kellner (1905), Prjanischnikow and Tomme (1936), Quin and Louw (1940)] that lignin, a constituent of the cell-wall, has a profound influence on the digestibility of cellulose. This influence is so great that for all practical purposes the decrease in the coefficients of digestibility for cellulose from samples 1M to 4M may be wholly ascribed to lignin.

A noteworthy feature in the digestibility of natural cellulose is that on splitting up this constituent into true cellulose and xylan the latter is found to be slightly less digestible than the former. If the xylan in the natural cellulose isolated from the samples of faeces (see Table 2) is expressed as a percentage of the natural cellulose containing it the figures 28·8, 29·3, 30·0, and 30·4 are obtained for faeces samples 1M, 2M, 3M and 4M, respectively. This higher xylan content

of the cellulose of the faeces is, of course, responsible for the slightly lower digestibility obtained for it in comparison with that for true cellulose. This result, suggesting a preference for true cellulose over xylan by the digestive agencies of the animal, may, however, be only apparent, since the explanation for the higher xylan content of the faeces cellulose may be somewhat as follows: Natural cellulose *in situ* and as isolated from the feed is a mixture of celluloses of varying xylan content and those portions with the highest xylan content remain undigested and are thus recovered in the faeces. Whatever the explanation it should be pointed out that such results afford further proof for the necessity of splitting up the carbohydrate complex of feeds and faeces into its ultimate chemical components when dealing with digestibility studies of a fundamental nature. The determination of natural cellulose as such is inadequate since the ratio of its components, xylan and true cellulose, is, as indicated, altered in passing through the alimentary tract.

(*c*) *Lignin.*

The increases in the lignin content of the grass samples (see Table 1) with advancing maturity are quite small. A growing period of three months, representing the difference in degree of maturity between samples 1M and 4M, resulted in an increase in the percentage of lignin from 9·9 (1M) to only 11·4 (4M). This increase is in reality, no doubt slightly greater since the nitrogen content of the lignin isolated from sample 1M is almost twice as great as that in the lignin from sample 4M. An appropriate correction should, therefore, reduce the figure for 1M more than it will the figure for 4M. Nevertheless from published data [Phillips *et al.* (loc. cit.), Phillips and Goss (1935), Norman (loc. cit.), etc.] it would appear that while appreciable and fairly rapid increases in the lignin content of plants occur during the younger stages of growth the rate of increase slows down markedly when the plant approaches maturity. Thus Phillips *et al.* (loc cit.) reported the lignin content of oat plants as 2·03 per cent. when they were 28 days old. This figure for lignin increased to 6·66 per cent. at the age of 63 days, which meant that in the course of 35 days the lignin content was more than trebled. After a further 28 days it rose to 9·35 per cent.—the rate of increase was slowing down—and at the age of 105 days, 14 days afterwards, the lignin content was only slightly higher, viz., 9·71 per cent. The small increases in the lignin content of samples 1M to 4M in spite of appreciable differences in the duration of the growth periods must, therefore, be attributed to the circumstance, previously referred to, that sample 1M has already reached a high degree of maturity in comparison with plants growing for a similar period in temperate regions.

The generally accepted fact that lignin is the least digestible portion of the structural constituents of plants is again revealed by an inspection of the relevant data in Table 3. At the same time, as pointed out by Phillips (1940) there is considerable difference of opinion as to whether lignin is or is not decomposed by bacteria and fungi in general. In an earlier publication [Phillips (1934)], dealing with a review of the chemistry of lignin, he concluded from a consideration of the more recent lignin metabolism experiments that this substance is, at least in part, broken down by the digestive processes of the animal body. Maynard (1940) also cited figures to indicate the extent to which lignin may be digested by different species fed alfalfa hay. According to his data a guinea pig digested 5 per cent. and a lamb no less than 28 per cent. of the lignin in alfalfa hay. No reliable data on the magnitude of the digestibility of lignin, is, of course, possible until the method for its determinaton has been perfected. In the mean time it should be borne in mind that the errors in the figures for the lignin

in the feed and in the faeces will cancel each other out to a certain extent so that coefficients calculated from these approximate figures may still be quite reliable. If, for instance, the nitrogen contents of the lignin isolated from grass sample 1M (0·35 per cent. N) and faeces sample 1M (0·5 per cent. N) are taken as bases for correcting the lignin values, then the grass lignin value (9·9 per cent.) will have to be reduced by $0{\cdot}35 \times 7{\cdot}14$ and the faeces lignin value (18·90 per cent.) by $0{\cdot}50 \times 0{\cdot}40$ in order to obtain a coefficient of digestibility of 0 for the lignin. The conversion factor (0·40) for the nitrogen in the faeces lignin is obviously absurd. Furthermore, it is extremely unlikely that the nitrogen content of the complex contaminating the lignin isolated from the feed will differ from that of the complex associated with the lignin from the faeces to quite the extent assumed by the factors 7·14 and 0·40, respectively. Rather may it be assumed that the nitrogen content of the extraneous substance is in both cases the same. The conclusion that at least some of the lignin is digestible seems, therefore, inescapable.

Unlike the actual amount, the digestibility of the lignin is profoundly influenced by the stage of maturity of the plants containing it. The coefficient (see Table 3) for grass sample 1M is 24·47 per cent. and for sample 4M, the most advanced stage of maturity, only 11·58 per cent. In this connection it will be interesting to refer to work done by Beckmann, Liesche, and Lehmann (1923) on the qualitative and quantitative differences in the lignin of woods and straws. By employing a 1·5 per cent. caustic soda solution and varying the conditions from extraction at room temperature for 48 hours through a series of intermediate steps to extraction in an autoclave at 9 atmospheres these authors were able to demonstrate the changes in chemical make-up and physical condition which accompany the ageing of lignin of plant materials. The conclusion drawn was that the total amount of lignin in any plant material at any stage of its development is a heterogeneous mixture of varying solubility in alkali, accompanied by variations in the methoxyl content, in such a manner that the younger the plant the easier does its lignin dissolve in dilute alkali and the smaller is its methoxyl content, and *vice versa*. It may, therefore, be inferred that while the difference in absolute amount of lignin in two samples may be negligibly small, it may nevertheless be appreciable as regards quality. These changes in solubility and methoxyl content are probably also responsible for the differences in susceptibility to attack by the digestive agencies of the animal body, reflected in decreasing coefficients of digestibility for the lignin of grass samples 1M to 4M, corresponding with advancing stage of maturity.

(*d*) *Hemicelluloses.*

As determined in this investigation the figure for hemicelluloses in the grass samples is only about 25 per cent. of that obtained for natural cellulose. It increases slightly from 11·45 per cent. for sample 1M to 12·68 per cent. for sample 4M. The uronic acid anhydrides which form part of the hemicelluloses decrease, however, in percentage amount from 4·30 for 1M to 3·89 for grass sample 4M. This is in agreement with results obtained by, for instance, Phillips *et al* (1939), who pointed out that uronic acids are constituents of pectins as well as of hemicelluloses, and since the pectins, which contain a much greater percentage of these acids than do the hemicelluloses, decrease in percentage content as the plants grow older, the total percentage of uronic acids in the plant would necessarily decrease with age.

The figures here reported for hemicelluloses would have been about twice as great if the xylan associated with the cellulose had been included with the figures for the pentosan portion of the hemicelluloses. By virtue of its behaviour

towards dilute acids and alkalis xylan in cellulose should, as is generally done, have been classified with the hemicelluloses. The reason for not doing so will emerge from the discussion to follow.

From an inspection of the data given in Table 3 it is seen that the digestibility of the hemicelluloses lies somewhere between that of lignin and cellulose, decreasing from 54·0 per cent. for sample 1M to 43·85 per cent. for sample 4M. In fact, the digestibility of the hemicelluloses in the foodstuffs in question is considerably lower than that of cellulose. The higher digestibility of the uronic acids in comparison with that of the pentosans, the other constituent of the hemicelluloses, may not have any special significance in view of the fact, previously referred to, that small amounts of pectic substances, possibly of high digestibility, are included with the figure for uronic acids. According to Maynard (1940) " hemicellulose appears to have a somewhat higher digestibility than cellulose ". He, however, pointed out that generalisations are dangerous and proceeded to state that " the digestibility of pentosans by sheep, as determined by the furfuraldehyde method, has been reported to vary from 55 to 95 per cent. in different feeds ". From this it seems warranted to infer that the digestibility of the hemicelluloses is wholly or largely determined by a substance or substances with which they are associated in the plants. In this connection the indirect evidence for the existence of a lignin-hemicellulose complex in which the two substances exist *in situ* in some form of chemical combination, the rupture of the linkage by chlorination resulting in the solution of the hemicelluloses, discussed by Norman (1937), is considered to be of more than ordinary significance. In fact, the relatively low coefficients of digestibility obtained for the hemicelluloses of the more or less mature materials tested in this investigation coupled with the accepted poor digestibility of lignin are deemed to be addtional indirect evidence for the existence of a lignin-hemicellulose complex in plant material. Furthermore, the relatively high digestibility obtained for xylan in cellulose seems to suggest that this substance is not in combination with lignin, or at least not in the same manner or to the same extent as are the other hemicelluloses. It is for this reason that the xylan associated with the cellulose has not been grouped with the hemicelluloses in the present investigation. Earlier in this paper the higher xylan content of cellulose from the faeces as compared with cellulose from the feed has been explained by assuming that the feed cellulosic fabric is a mixture of celluloses of varying xylan content and that the portion remaining undigested contains the higher percentage of xylan. In view of the existing evidence and that discussed above for the existence of a lignin-hemicellulose complex, it is possible that some sort of combination also exists between lignin and some of the xylan in the cellulose rendering it thereby relatively indigestible.

It was originally maintained by Payen (1840) that the substance or substances which could be separated from cellulose by treating the plant material with caustic soda, potassium hydroxide or nitric acid and designated as " incrusting materials ", surrounded or impregnated the cellulose in lignified material. This view was supported by some and opposed by other investigators who claimed to have produced evidence for their veiw that lignin is chemically combined with cellulose. This controversy has not been settled and is being continued [cf. Phillips (1940)]. Nevertheless, from what has been said in the above discussion the assumption that lignin is partly or wholly chemically combined with hemicellulose appears to rest on firm ground, and this combination may be held responsible for the low digestibility of the hemicelluloses of lignified materials. Whether the decreasing digestibility of cellulose with age in the plants (see

Table 3) is also due to a similar combination between lignin and cellulose, possibly through its associated xylan, or to the protective influence of a poorly digestible lignin-hemicellulose incrustation remains to be decided by future research. It should, however, be pointed out that judging from what is stated by Phillips (1940) about Payen's work, the possibility for the existence of a lignin-hemicellulose combination is not excluded from his " incrustation hypothesis " since his " incrusting materials ", isolated by solution in caustic soda, were no doubt a mixture containing both lignin and hemicelluloses. Evidence for the existence of a lignin-hemicellulose complex is, therefore, not necessarily opposed to Payen's hypothesis, as is apparently considered to be the case (c.f. Phillips loc. cit.).

(e) The Undetermined Fraction.

In Table 1 the percentages of substances not accounted for in the scheme of analysis followed are given as 10·22, 10·38, 9·55 and 8·29 for grass samples 1M, 2M, 3M and 4M, respectively. On the other hand, if the nitrogen-free extract is calculated in the usual way from the relevant data in Tables 1 and 4 the undetermined fractions will be found to be 42·57, 43·70, 43·68 and 42·97 as percentages of the dry matter for the four samples of grass in ascending order of maturity. On an average the undetermined fraction has thus been reduced from 42·7 per cent. to only 9·6 per cent. an achievement which for the purposes of fundamental research in crop composition and nutritive value should fully justify the extra time and labour involved in the additional analyses. The relatively small fraction not accounted for is no doubt still a mixture of substances probably also of varying nutritive value, but judging from the relevant data presented in Table 1 it must be composed mainly or wholly of water-soluble substances (c.f. figures for substances not accounted for and substances soluble in cold water in Table 1).

In the analysis of the faeces (see Table 2) the figures for substances not determined are considerably smaller than those for the grass samples. It is to be noted that also here the undetermined fraction is composed mainly of substances of a water-soluble nature. Coefficients of digestibility have been calculated for these water-soluble substances and found to be higher than those for any other constituent determined (see Table 3). In the strictly scientific sense, however, no undue importance is attached to these coefficients as it is realised that the individual constituents of the water-soluble fraction of the feed may, and mostly are, quite different from those present in the water-soluble fraction of the faeces. It is not, for instance, expected that reducing sugars, like glucose and fructose or possibly water-soluble polymerisation products of them, that are no doubt present in the feed will also be found in the faeces. These sugars are most likely completely digested.

(2) The Composition of the Crude Fibre.

In discussing the implications of his work on the composition of the crude fibre of various materials Norman (1935) referred to the usual assumption that the residual fibre in the faeces is directly comparable with that from the original material fed to the animal. He doubted the validity of the assumption, and mentioned the necessity for a detailed series of analyses on the food material and the faeces, and the crude fibre from each, in order to settle this important point. Since detailed analyses of food materials and faeces were undertaken for the main object of the present investigation it was thought worth while to extend the scope of the work slightly to include detailed analyses also of the crude fibre isolated by the usual Weende method from the same food materials and faeces.

The results of these analyses are given in Tables 4, 5 and 6. Before discussing them it should be pointed out that natural cellulose was determined on crude fibre preparations in the wet state, in view of an observation by Norman (1936) that cellulose preparations containing cellulosan undergo an irreversible change on oven-drying, as a result of which a fraction becomes soluble in hot water—crude fibre will be seen to be nothing but crude cellulose. Also, figures for hemicelluloses have in this case been arrived at by expressing the difference between total furfural and furfural in natural cellulose as pentosan.

TABLE 4.

Amount and Composition of the Crude Fibre.

Material.	Crude Fibre. Percentage of Dry Matter.	Composition of Crude Fibre (percentages).					
		Natural Cellulose.	True Cellulose.	Xylan in Cellulose.	Pentosan from Hemicellulose.	Lignin.	Crude Protein.
1M Grass.....	33·20	96·3	84·8	11·6	0·21	3·7	0·18
2M Grass.....	33·70	96·1	84·1	11·9	1·12	4·5	0·38
3M Grass.....	36·30	97·8	83·8	14·0	1·24	4·6	0·25
4M Grass.....	38·90	95·9	81·6	14·3	0·82	4·5	0·33
1M Faeces.....	23·65	91·5	78·2	13·3	1·86	12·2	2·20
2M Faeces.....	25·55	93·7	80·8	12·8	1·64	11·0	1·50
3M Faeces.....	28·30	92·7	78·7	14·0	1·59	9·7	1·16
4M Faeces.....	29·30	93·5	79·3	14·2	1·23	9·6	1·09

TABLE 5.

Comparison of the Composition of Crude Fibre with that of the Original Material.
(All Results expressed as Percentages of the Original Oven-dried Material.)

Material.	Crude Fibre.	True Cellulose.	Xylan in Cellulose.	Furfuraldehyde from Hemicellulose as Pentosan.	Lignin.	Protein.
1M Grass.....	33·20	32·69	11·51	8·76	9·90	10·42
Crude Fibre.....	—	28·15	3·85	·07	1·23	·06
2M Grass.....	33·70	32·81	12·19	9·35	10·00	8·81
Crude Fibre.....	—	28·37	4·03	·38	1·51	·13
3M Grass.....	36·30	34·35	13·55	9·48	10·50	6·92
Crude Fibre.....	—	30·41	5·09	·45	1·66	·09
4M Grass.....	38·90	35·67	13·83	10·23	11·40	5·53
Crude Fibre.....	—	31·75	5·55	·32	1·74	·13
1M Faeces.....	23·65	19·86	8·04	10·83	18·90	9·65
Crude Fibre.....	—	18·51	3·14	·44	2·90	·52
2M Faeces.....	25·55	22·42	9·28	10·84	18·60	7·70
Crude Fibre.....	—	20·67	3·28	·42	2·81	·39
3M Faeces.....	28·30	24·85	10·65	11·02	18·80	6·81
Crude Fibre.....	—	22·30	3·96	·45	2·75	·33
4M Faeces.....	29·30	25·61	11·20	10·69	18·50	6·00
Crude Fibre.....	—	23·23	4·17	·36	2·81	·32

TABLE 6.

Recoveries of Cell-wall Constituents in Crude Fibre. Each Constituent expressed as a Percentage of that present in Original Material.

Material.	True Cellulose.	Xylan in Cellulose.	Pentosan in Hemicellulose.	Lignin.
1M Grass	86	33	0·8	12·4
2M Grass	86	33	4·1	15·1
3M Grass	88	38	4·7	15·8
4M Grass	89	40	3·1	15·3
1M Faeces	93	39	4·1	15·3
2M Faeces	92	35	3·9	15·1
3M Faeces	90	37	4·1	14·7
4M Faeces	91	37	3·4	15·2

An inspection of the data in Table 4 reveals that on an average 96·5 per cent. of the crude fibre from the grass samples is composed of natural cellulose, the rest, again taking averages, being made up of 4·4 per cent. lignin, 0·8 per cent. pentosan, and 0·3 per cent. protein. That the sum of all these percentages mount up to more than 100 must be ascribed mainly to the inaccuracy of the lignin determination. On the other hand, the average composition of the crude fibre of the faeces samples is found to be 92·8 per cent. natural cellulose, 10·6 per cent. lignin, 1·6 per cent. pentosan, and 1·5 per cent. protein. The average figures quoted above apply with minor deviations, also to the crude fibre from individual samples of feed and faeces. It is noteworthy that xylan associated with cellulose is responsible for approximately 90 per cent. of the furfural content of the crude fibre. This finding is deemed to be further proof for the necessity for discriminating between pentosan associated with cellulose and pentosan associated with the lignin-hemicellulose complex in studies of a more exact nature.

To sum up, the crude fibre of the food materials and faeces analysed is composed almost exclusively of natural cellulose. On the other hand, strictly speaking, the crude fibre of the food material is not directly comparable with the crude fibre of the faeces, that of the latter containing less cellulose and more of the lignin-hemicellulose complex than the crude fibre of the food material. For purposes of research this state of affairs is inadmissible.

Table 6 has been drawn up from data compiled in Table 5 and represents the recovery of structural constituents in the crude fibre. An inspection of the data reveals that " true " cellulose, i.e. natural cellulose *minus* its xylan, is the most resistant constituent of the cell-wall to alternate boiling with dilute alkali, 86 to 89 per cent. of that present in the grass samples and 90 to 93 per cent. of that present in the faeces samples being recovered in the crude fibre. The losses of cellulose which did occur may no doubt be ascribed mainly to the removal by solution in the alkali or by hydrolysis in the acid of a glucosan which according to Norman (1937), is associated with the cellulose of plant materials.

Unlike the true cellulose, its associate xylan, is extensively removed by the Weende procedure. This susceptibility to attack by the reagents used in the Weende method of xylan is, however, greatly surpassed by that of the other

furfural producing substances. In fact the hemicellulose pentosans are almost completely removed. The recovery of lignin in the crude fibre is also small but none the less greater, on a percentage basis, than that of hemicellulose.

(3) *Concluding Remarks.*

Perhaps the outstanding feature in the results obtained in this investigation is that, except for the small amount of water-soluble substances, natural cellulose is the most digestible portion of the carbohydrate complex of the grass samples analysed, irrespective of stage of maturity. On the other hand, from the results on the composition of the Weende crude fibre it is evident that this method isolates a fraction which is almost entirely composed of cellulose. In the case of the type of roughage investigated, therefore, the Weende procedure does not answer the purpose it was intended to serve, viz., to isolate the least digestible portion of feeding stuffs; the contrary is, in fact, nearer the truth. The method, furthermore, underestimates the cellulose in an irregular manner, depending on the stage of maturity and nature of the material analysed. This circumstance further adds to the heterogeneous nature of the mixture which is determined by difference and designated as nitrogen-free extract under the old scheme of analysis. The widely differing extent to which individual constituents of this mixture may be digested has been demonstrated by the results of the present investigation.

If the object of a modified system of analysis is to divide the carbohydrate fraction of feeding stuffs according to digestibility then the scheme followed in this investigation must be considered a success which cannot be claimed for the existing method of analysis.

The need for an improved system of analysis is no doubt imperative in certain fields of research. Thus in the case of the plant physiologist who, as pointed out by Denny (1940), has to prepare " a balance sheet by which an accounting as complete as possible may be made for income, outgo, and transformation of materials " it is, of course, essential to determine every single chemical entity in the plant. For the biochemist engaged in the evaluation of food materials this may, however, not be necessary in view of the possibility that the digestible portions of chemically different carbohydrate constituents may be biologically the same. Such, indeed, is actually the case, if the finding of Kellner (1909), viz., that digestible cellulose and digestible starch have equal fattening values, is accepted as final. If, furthermore, equal production values can be assigned to the digestible portions of all the carbohydrate constituents then it seems logical to consider the digestible part of the whole carbohydrate complex as a single biological entity indicative of the energy value of the feed just as its phosphorus content is taken as a measure of its value for fulfilling the requirements of the animal for this mineral constituent. It should, on the other hand, be remembered that Kellner found his conclusion to be valid only when the nutrients fed were in the pure isolated form or present in concentrates such as cotton seed cake meal. Digestible cellulose, for instance, present in coarse fodders has, however, a fattening value very much smaller than that of digestible cellulose isolated from the fodder before being fed. The feeding value of a nutrient may, therefore, be greatly influenced by its physical condition and the substances with which it is associated in the plant. It seems, therefore, definite that if the relative production values of feeding stuffs are to be determined by chemical analyses and digestion trials then some system of partitioning the carbohydrates will have to be devised. The shortcomings of the Henneberg system, involving the determination of the crude fibre, have been dealt with. It remains to suggest an improved system. In this connection it is, however, felt that, irrespective

of the validity, or otherwise, of Kellner's results, fundamental research on the relative nutritive values of the several constituents comprising the carbohydrate complex with special reference to their occurrence in different food materials, is a necessary preliminary to the adoption of an efficient system of analysis for the evaluation of these food materials. With regard to grassland products it will probably be found useful to consider the so-called carbohydrate complex as consisting of:

(1) a smaller amount of water-soluble substances, e.g., the sugars, which vary in amount with stage of growth in the plant and are readily available to the animal;

(2) natural cellulose and the hemicelluloses, which form the major portion of the complex. The percentage amount is also influenced by stage of maturity in the plant and the production value is greatly reduced by the work of mastication and of digestion which are necessary preliminary steps to its utilisation by the animal;

(3) lignin, whose quality and quantity depend on stage of maturity in the plant, is poorly digested and as a result has a marked detrimental influence on the digestibility of the other structural constituents of the plant, especially on that of the hemicelluloses with which it is intimately associated, a circumstance which may necessitate the removal of the hemicelluloses from Group 2 above and considering them as a separate group.

SUMMARY.

The structural constituents, natural cellulose, hemicelluloses and lignin, in graminaceous food materials, faeces, and the crude fibre isolated from these have been determined. The results showed that:

(1) crude fibre is almost wholly composed of natural cellulose but that the method for its isolation underestimates the natural cellulose content of the feed and of the faeces;

(2) natural cellulose is the most digestible portion and lignin the least digestible portion of the cell-wall structure. From this finding it is inferred that a closer association exists between the lignin and the hemicelluloses than between the former and the natural cellulose of the cell-wall complex;

(3) with regard to roughages the standard feeding stuffs analysis does not divide the carbohydrate complex into substances of relatively low and substances of relatively high digestibility.

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