CXCIII. THE COMPOSITION OF FORAGE CROPS.

I. RYE GRASS. (WESTERN WOLTHS.)

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MANY papers dealing with the composition and digestibility of forage crops are to be found in the literature, but the analyses are usually restricted to those few groups included in the conventional methods of agricultural analysis. These rarely extend beyond the determination of ash, or mineral constituents, crude protein, crude fibre and ether-soluble material. The sum of these analyses is far below 100 % in the case of grasses, and the difference from 100, often 40–60 %, is expressed vaguely as “N-free extractives” or “soluble carbohydrates”. In the belief that more information should be obtained about the carbohydrate constituents of forage crops in general, and this ill-defined fraction in particular, detailed analyses have been undertaken using methods which have been developed in the past few years. Not only is it desirable to obtain further knowledge as to the final composition of agricultural materials used as feeding stuffs, but also to be able to follow the changes which take place during development, maturation, drying and storage. This paper records a preliminary study of the development of rye grass and its conversion into hay.

Sampling.

The samples were taken from Pastures Field, Rothamsted, in April, May and June 1935, the seed having been sown under oats the previous season. The selected area, 21 yd. sq., was divided into small plots each $1\frac{1}{2}$ yd. sq. Sampling was effected by cutting with shears an area of 1 sq. yd. within the small plot, this being conveniently determined by the use of a wooden frame of that size. The rejection of margins permitted passage between the areas to be sampled and obviated the necessity of avoiding plots adjacent to one previously sampled on account of possible edge effects. At each time of sampling eight plots, selected at random in the usual way, were cut. The grass from these plots was bulked, again at random, into two samples (A and B), each containing the yield from four plots. In this way an estimate of error due to variation in composition is possible and, since all determinations were subsequently made in duplicate on these A and B samples, an estimate of analytical error has also been obtained. The number of plots cut at each time of sampling is adequate since the purpose of these investigations is a study of composition and not of yield or rate of growth. Many more plots would have to be sampled to obtain reliable figures for the latter.

Treatment of samples.

The grass when cut was bulked into A and B samples and removed to the laboratory. By hand-picking, all weeds and stubble were separated, and the sample placed in an oven at 100° for a short period to inactivate enzymes. Drying was then effected in a drying room at a moderate temperature. After chaffing the samples were ground in a high speed mill to pass 60-mesh.

Other information.

The weather conditions of early summer of 1935 were exceptional, the rainfall in the latter part of May and in June being much above average and the sunshine deficient. Between 26 April, when the first samples were taken, and 21 June, when the last were cut, the rainfall totalled 4.9 in. The sunshine in June, up to the 21st, was 92.8 hours, the average for the period being 143.4 hours. As a result the grass when mature was greener and more stemmy than is usually the case.

The whole area was mown on 22 June and the grass left for hay in the usual way, being turned once. A severe thunderstorm (0.28 in.) soaked it when partly dry. When dry, a large random sample was taken (28 June) so that information might be obtained as to the changes normally occurring in the process of hay making.

Analytical methods.

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

1. *Ash*: in an electric muffle furnace.
2. *Crude protein*: N by Kjeldahl $\times 6.25$.
3. *Cold water-soluble fraction*: 24 hours at room temperature, nitrogen being determined in the residue.
4. *Crude fibre*: official method.
5. *Total pectin* (expressed as calcium pectate yield).
6. *Cellulose*: Norman & Jenkins [1933].
7. *Total furfuraldehyde yield*: by distillation with 12% HCl and precipitation as the phloroglucide.
8. *Furfuraldehyde* from cellulose.
9. *Lignin* after hydrolysis: Norman & Jenkins [1934, 1], the material being pretreated with alcohol-benzene.
10. *Ether-soluble fraction*: by extraction in a Soxhlet apparatus.

RESULTS.

Some information about the rate of growth of the grass may be obtained from the dry weights of the samples (Table I), each representing the yield from 4 sq. yd.

Table I. *Yield of rye grass from 4 plots each of 1 sq. yd.*

Sample		
1	April 26	A 97.6
		B 102.8
2	May 10	A 201.2
		B 206.4
3	May 24	A 323.5
		B 290.9
4	June 7	A 494.4
		B 381.8
5	June 21	A 786.5
		B 605.3

Non-structural constituents.

Figures relating to the non-structural constituents are given in Table II and call for no special comment except in the case of the water-soluble fraction, which was very high in the younger samples. More than half of the material of the first three samples was extracted by cold water and of this the protein accounted for only a small part. Hot water was no more effective and indeed removed rather less protein, no doubt owing to coagulation. The nature of the

Table II. *Some non-structural constituents of rye grass.*

All analyses expressed as % on oven-dried samples.

Each figure is the mean of 4 analyses—duplicate determinations on 2 independent composite samples.

Sample period	Ash	Crude protein	Cold water-soluble	Protein in cold water extract	Hot water-soluble	Protein in hot water extract	Ether extract
1	9.52	10.11	53.92	3.17	52.43	3.01	3.46
2	9.11	8.56	52.28	2.90	53.02	2.39	3.42
3	7.84	7.27	54.16	2.23	55.46	2.29	6.34
4	7.66	7.15	46.21	2.45	47.76	2.64	4.12
5	7.50	4.72	37.21	0.89	38.27	0.89	4.84
Hay	7.58	5.46	32.07	2.65	31.29	2.12	2.62
*S.E. $\pm$ (sampling error)	0.30	0.09	0.55	0.12	0.63	0.22	0.15
S.E. $\pm$ (analytical error)	0.19	0.09	0.36	0.10	0.30	0.08	0.44
Analytical error % $\pm$	2.3	1.2	0.8	4.2	0.6	3.8	10.6

* S.E. = Standard error. Sampling error includes analytical error.

Since each figure in the table is the mean of 4 analyses, the sampling and analytical errors per sample are obtained by doubling the errors given.

non-nitrogenous water-soluble fraction will be dealt with later. The increase in crude protein content on drying for hay was of course due to carbohydrate losses, and the apparent increase in crude protein passing into solution in cold and hot water in the same sample to some protein degradation resulting in the production of soluble products. This will be discussed later.

Structural constituents.

Analyses of some structural constituents are given in Table III. With increasing age the cellulose content rose rapidly, particularly in the samples 4 and 5. For comparison, crude fibre determinations were made by the official method. The deficiencies of the crude fibre figure have been dealt with in detail

Table III. *Some structural constituents of rye grass.*

All analyses expressed as % on oven-dried samples.

Each figure (except N in lignin) is the mean of 4 analyses—duplicate determinations on 2 independent composite samples.

Sample period	Cellulose	Furfuraldehyde from cellulose	Xylan in cellulose	Total furfuraldehyde yield	Furfuraldehyde from polyuronides	Lignin	N in lignin	Crude fibre	Calcium pectate
1	20.89	2.63	4.11	7.29	4.66	4.63	0.29	18.76	0.91
2	20.45	2.75	4.30	7.73	4.98	4.37	0.28	18.17	0.41
3	23.15	3.99	6.19	7.77	3.78	5.08	0.27	17.08	0.52
4	29.69	5.44	8.45	9.85	4.41	6.30	0.27	21.54	0.60
5	36.30	7.09	11.02	11.31	4.22	8.48	0.28	26.34	0.42
Hay	40.85	7.61	11.80	13.15	5.54	9.32	0.28	31.60	0.19
S.E. $\pm$ (sampling error)	0.31	0.14	0.21	0.16	0.24	0.17	*	0.42	0.05
S.E. $\pm$ (analytical error)	0.21	0.09	0.14	0.22	*	0.07	*	0.33	0.04
Analytical error % $\pm$	0.76	1.92	1.79	2.4	5.3	0.90	*	1.54	7.85

* Not determined.

elsewhere [Norman, 1935, 1] but it is sufficiently apparent from the rye grass samples that the cellulosic structure of the plant is underestimated in an irregular manner in the fibre determination. Whereas in sample 1 the crude fibre figure accounts for about 90 % of the cellulose, in samples 4 and 5 only 72 % is estimated. A contributory factor in this is the increase of xylan in the cellulose with increasing age. This change is more obvious if the xylan be expressed as a percentage of the cellulose containing it (Table IV).

Table IV. *Xylan content of rye grass at different ages. Expressed as % of the cellulose.*

Sample	Xylan
1	19.7
2	21.0
3	26.7
4	28.5
5	30.4

Similar, but not entirely regular, increases in the content of xylan associated with the cellulose have been found in the barley plant by Norman [1933] and by Phillips & Goss [1935]. It is not easy to see why there should be this change in the nature of the cellulosic framework of the plant with increasing age. The glucose and xylose units forming the cellulose and xylan are presumably laid down together by the same mechanism, the balance altering in favour of the pentose as the plant becomes more mature. The pentose metabolism of plants is little understood as yet and provides a number of such problems. It has previously been pointed out that a calculation of total pentosans from the furfuraldehyde yield of the whole material is misleading because of the changing proportion of xylan in association in the cellulose [Norman, 1933] and this fact is again evident from the rye grass results. The total furfuraldehyde yield increased with age, but whilst in the youngest material 26 % of the total was derived from the xylan in the cellulose, the amount from this source increased to 62 % in the mature material. The nature of these changes may be seen from Fig. 1. Since the direct determination of hemicelluloses is unsatisfactory, the furfuraldehyde not from cellulose is taken as a measure of this group of encrusting polyuronides. No correction has been made for the furfuraldehyde derived from pectin, since the pectin content was quite low throughout. The proportion of encrusting polyuronides apparently remained much the same over the whole period of growth. Information as to the actual amount is not given by these analyses. However, the furfuraldehyde yields of hemicellulose preparations that have been obtained indicated that the factor for conversion of furfuraldehyde from this source to polyuronide is a little over 2.

Since the ripening of grass is always said to be accompanied by lignification, and since the extent of lignification considerably affects digestibility, the lignin contents of these samples are of especial interest. The determination of lignin has been a matter of much research lately, and whilst some of the disturbing factors have been recognized, it cannot be said that a method of general applicability has been evolved. In the presence of protein, errors are introduced by the linkage of protein fission products with the lignin [Norman & Jenkins, 1934, 2]. Some workers have corrected for this disturbance by determining the N present in the lignin product, calculating this as protein and subtracting. Such a correction is not justified, and until such time as a method which shall be free from protein interference is devised, it is preferable simply to record the N found in the lignin. The pretreatment advocated by Norman & Jenkins [1934, 1]

for reducing the errors due to the presence of pentose groups does at the same time considerably reduce the protein disturbance. The lignin figures given in Table III were obtained on materials which had been given this pretreatment following extraction with alcohol-benzene, as usually advocated for woods. The apparent lignin residue contained over 6% N on an ash-free basis in the case of the youngest sample. It was curious however that the actual amounts of N retained by the lignin were almost identical in each case although the actual yield of apparent lignin increased progressively with age, the final sample containing

Fig. 1. Distribution of furfuraldehyde yield from rye grass samples.

nearly double that of the youngest. The error due to protein may therefore be taken to be more or less constant in this series and the increase in lignin content to be from about 3% in the youngest sample to about 7% in the most mature. The importance of the alcohol-benzene extraction for the removal of fatty and waxy compounds in the case of such green materials was made evident when lignin determinations were carried out on the ether-extracted residues, an acid pretreatment being given in both cases. For comparison, the determination was also made without a solvent extraction (Table V).

Table V. *Effect of solvent extraction on lignin determinations.*

All results expressed on 100 g. oven-dry material.

Rye grass sample	Alcohol-benzene extraction		Ether extraction		No solvent extraction	
	Apparent lignin	N in lignin	Apparent lignin	N in lignin	Apparent lignin	N in lignin
1	4.63	0.29	*	*	6.58	0.25
2	4.37	0.28	5.27	0.33	6.37	0.21
3	5.08	0.26	5.99	0.31	5.72	0.21
4	6.30	0.27	6.79	0.32	7.14	0.20
5	8.48	0.28	9.21	0.28	9.73	0.19
Hay	9.31	0.28	11.15	0.32	10.64	0.20

* Not determined.

It is evident that a further examination of the effect of solvent extraction on the determination of lignin in green plant materials is called for. Although treatment with alcohol-benzene resulted in the lowest yield of lignin residue, the nitrogen in the lignin was actually increased by extraction with either ether or alcohol-benzene. The differences between ether-extracted and alcohol-benzene-extracted samples are greater than could be accounted for by the higher N content of the lignin from the former.

Water-soluble constituents.

The astonishingly high content of material soluble in cold water in these samples led to an investigation of the nature of the substances removed. The major constituent of the extract appears to be a fructose anhydride or laevan. The presence of such fructose polysaccharides in members of the Gramineae has been reported at various times, and recently Challinor *et al.* [1934] have studied the laevan from rough-stalked meadow grass. The yield of pure laevan obtained by them was less than 1 % of the dry weight of the grass taken. Buston [1934], in the course of an investigation on the polyuronides of grasses, determined the amount of reducing sugar in the aqueous extract of young grass samples before and after hydrolysis with 1 % H_2SO_4 for 40 min. The increases on hydrolysis, by no means all necessarily due to fructose, suggest that the fructosan content of the samples investigated by him was low, except perhaps in Crested dog's tail (*Cynosurus cristatus*). Preparations of fructosan have also been obtained from rye [Kizel & Kretovitsch, 1934] and from barley [Archbold & Barter, 1935].

The accurate determinations of the fructosan in the rye grass samples presented difficulties which have not been entirely overcome. It was first determined that an aqueous extract could be hydrolysed readily by low concentrations of acid and that 0.5 % oxalic acid gave no increase in fructose after heating for 30–45 min. Even at such a dilution, hydrolysis was extremely rapid in the first 15 min. Determinations were carried out first on cold aqueous extracts of the samples, the extraction being made for 24 hours. This was found to give an incomplete extraction of fructosan and accordingly a second treatment for the same time was given. After clearing with a small amount of basic lead acetate and de-leading with sodium phosphate, the total reducing value was determined by the Shaffer-Somogyi [1933] micro-method, and the aldoses by hypiodite oxidation for 2 hours in a refrigerator. Similar determinations were made after hydrolysing the extract by boiling with 0.5 % oxalic acid in a water-bath for 1 hour, conditions which were found to result in the complete hydrolysis of the fructosan. The results are given in Table VI, the figures obtained by the copper method being calculated as fructose and those by the iodine method as glucose.

Table VI. *Carbohydrate in aqueous extracts of rye grass samples.*

Results calculated on the basis of 100 g. oven-dry grass.

Sample	First extraction				Second extraction			
	Total sugar before hydrol.	Total sugar after hydrol.	Apparent glucose before hydrol.	Apparent glucose after hydrol.	Total sugar before hydrol.	Total sugar after hydrol.	Apparent glucose before hydrol.	Apparent glucose after hydrol.
1	3.10	27.47	9.66	9.96	0.06	0.98	0.56	0.70
2	7.00	30.88	8.12	9.18	0.11	0.77	0.07	Trace
3	6.37	35.15	9.20	10.49	0.05	1.16	0.50	0.56
4	4.28	26.66	7.97	8.50	0.11	1.06	0.47	0.42
5	6.54	21.77	7.02	6.69	0.12	1.09	0.47	0.47
Hay	5.60	15.68	7.83	7.83	0.07	0.34	0.50	0.25

Two important facts are at once apparent. First, the increase in reducing sugar produced by hydrolysis is due almost solely to the liberation of fructose, since the apparent glucose figures show little difference after hydrolysis. Second, the extract must contain oxidizable material unaffected by the copper reagent, but easily oxidized by hypiodite, because the total reducing value by the copper reagent is far lower than the apparent glucose content by hypiodite oxidation. This is, of course, a common observation with plant extracts and makes it impossible to get true figures for fructose and fructosan contents by this method. Hot water extraction for 1 hour gave figures for total reducing sugar very similar to the sum of the cold extracts with the difference that the amounts of apparent glucose after hydrolysis determined by hypiodite oxidation were a little higher. Direct hydrolysis of the fructosan in the grass was effected by heating for 30 min. with 5% oxalic acid in a water-bath. As far as could be judged, the results obtained indicate that hot aqueous extraction or repeated cold aqueous extraction does result in complete removal of the fructosan. To avoid difficulty due to the presence of aldose sugars and non-sugar reducing substances, determinations were carried out by the copper reagent on extracts which were first oxidized by hypiodite, the excess iodine being removed with sulphite. The procedure adopted was similar to that recently described by Van der Plank [1936]. The grass samples, finely powdered, were extracted for 30 min. in a boiling water-bath, and a portion of the extract, after clarification, hydrolysed by boiling with 2% oxalic acid for 30 min.

5 ml. portions of the unhydrolysed and hydrolysed extracts were treated with 2 ml. $N/10$ iodine (containing 34 g. KI per litre) and 1 ml. $N/4$ NaOH and kept in a refrigerator just above freezing point for 2 hours. After acidification with 1 ml. $N/3$ H_2SO_4 the excess iodine was removed by titrating with dilute sulphite. The reducing value of this solution was then determined in the usual way with the Shaffer-Somogyi reagent. As a result of the pretreatment with hypiodite, considerable quantities of iodide are present which appreciably affect the apparent reducing value of fructose. The values for the fructose content must therefore be taken from a curve specially determined for the quantity of iodide that is present. Unless this be done the error introduced is over 10%. The results obtained are given in Table VII. The fructose or fructosan present passed

Table VII. *Fructosan in rye grass samples.*

Results calculated on the basis of 100 g. oven-dry grass.

Sample	Before hydrolysis as fructose	After hydrolysis as fructose	Fructosan as fructose
1	1.06	28.21	27.15
2	3.00	26.62	23.62
3	3.10	37.53	34.43
4	2.69	25.33	22.64
5	3.73	18.87	15.14
Hay	5.23	14.19	8.96

through a sharp peak and then rapidly declined as maturity approached. A further considerable fall took place in the process of hay making. The intervals at which the samples were taken were not sufficiently short to provide a full picture of the changes in the fructosan content. It is however quite evident that this polysaccharide must be the chief carbohydrate reserve of rye grass, and, because of the high availability of fructose, must be also of great nutritional importance. The method of drying adopted was such that some slight hydrolysis of fructosan to fructose might take place. A more detailed investigation of the

distribution of this polysaccharide is in progress. Young wheat plants have also been found to contain fructosan, the amount decreasing rapidly as the ears form (unpublished).

Hydrolysis of rye grass fructosan.

A crude preparation of fructosan was obtained from an aqueous extract of young dried rye grass (collected 17 May). Proteins were removed with basic lead acetate and lead with phosphate. After concentration to a small volume *in vacuo*, inorganic salts were precipitated by the addition of alcohol to give a concentration of 47%. After further concentration, the solution was poured into a large excess of absolute alcohol giving a creamy white precipitate, which was immediately filtered off. Purification of the precipitate was attempted by several means, including the baryta fractionation described by Archbold & Barter [1935], but no method was very successful. The crude precipitate had a specific rotation $[\alpha]_D -40^\circ$ approximately and very slight reducing properties. After hydrolysis for 15 min. with 0.5% oxalic acid the sugar produced amounted to 87% on an ash-free basis, 84.4% being fructose. This polysaccharide was very susceptible to hydrolysis and yields of sugar of a similar magnitude were obtained on heating for 30 min. with 0.05% oxalic acid; using 0.025% oxalic acid the sugar produced did not exceed 58%. Slight hydrolysis was effected on prolonged boiling with water, 19.0% sugar being obtained after 1 hour and 20.8% after 3½ hours.

The changes occurring during hay making.

No direct determinations of the total losses occurring during hay making were made, as the whole area was cut. Some indication of the magnitude of these losses may, however, be obtained by comparing in the mature sample and the hay the percentage contents of constituents such as cellulose and lignin which would be unlikely to be reduced by the treatment. Such a comparison suggests that the losses were in the neighbourhood of 10%. Of this more than half was fructosan, presumably as a result of respiration, or enzymic conversion of the polysaccharide into fructose which would be liable to be washed out by rain. As evidence of the breakdown it is significant that the hay contained the highest percentage of free fructose. Some losses of ash and ether-soluble material also occurred. There does not appear to have been any loss in the nitrogenous constituents as judged by the total N figures, but extensive changes must have taken place because the cold water-soluble N increased from approximately 20 to 50% of the total. The xylan in the cellulose was slightly reduced, with a concurrent increase in furfuraldehyde from the polyuronides. The pectin content, though low in the mature grass, fell sharply.

DISCUSSION.

The system of analysis described in this paper though not complete accounts far more satisfactorily for the various constituents of grass than conventional methods. It is imperfect in that a reliable method for the determination of the polyuronide hemicelluloses has not been found. Buston [1934] attempted this determination by direct preparation and found the apparent hemicellulose content of various grasses by this method to be 16–20%. His procedure is, however, very laborious and is open to certain criticisms [Norman, 1935, 2], the chief perhaps being that any alkaline extraction also removes cellulosan from the cellulose, with the result that the product does not only consist of encrusting polyuronides. An attempt to avoid this difficulty will be described elsewhere.

In the case of sample 5, only 43.4% of the grass is accounted for by the conventional methods, leaving 56.6% to be described as soluble "carbohydrates". Excluding any estimate of the hemicelluloses, the analyses given in this paper on the same sample total to over 80%. The presence of a considerable amount of soluble carbohydrates in the form of fructosan is now established and the role of this in nutrition will have to be examined. Presumably it is readily utilized by the animal, though the action of digestive enzymes on this polysaccharide has not been studied. Archbold & Barter [1935] found that their fructosan preparations were but slowly attacked by invertase. The position of fructosan in the metabolism of the plant can as yet be only a matter of speculation. In the young plant fructosan is produced more rapidly than cellulose, but later cellulose production predominates. This is shown in Table VIII in

Table VIII. *Amounts of various constituents as percentages of that present in final sample (5).*

Sample	Dry matter	Fructosan	Cellulose	Lignin	Crude protein
1	14.4	22	8	8	31
2	29.4	41	17	15	54
3	44.1	87	28	26	69
4	63.0	84	52	48	96

which the chief constituents in each sample are expressed approximately as percentages of that present in the final sample, the increases in yield being taken from the means of the figures in Table I.

Some of the advantages that have been ascribed to the higher protein content and greater digestibility of the cellulose of young grass may in fact be due to its much higher content of fructosan, if other species of grass are at all similar to rye grass.

SUMMARY.

1. Samples of rye grass cut at fortnightly intervals have been analysed, particular attention being given to the structural constituents.

2. The contents of cellulose and lignin increased rapidly as maturity approached. The percentage of xylan in the cellulose also increased with age. The polyuronide hemicelluloses, as judged by furfuraldehyde yield did not exhibit any regular increase and were lower in the mature grass than in the young grass.

3. A water-soluble fructosan, or laevan, was found in considerable amounts in the younger samples, reaching at one stage over 37% expressed as fructose. The percentage content of this polysaccharide fell rapidly on maturity.

4. Losses in hay making were of the order of 10%, mostly accounted for by loss of fructosan.

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