CXLVII. THE COMPOSITION OF FORAGE CROPS

III. COCKSFOOT. CHANGES IN THE HERBAGE DURING GROWTH, WITH AND WITHOUT ADDITIONS OF NITROGENOUS FERTILIZER

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THE digestibility of forage crops varies widely with the age and composition of the tissues. A study of the developmental changes in the composition of rye grass [Norman, 1936; Norman & Richardson, 1937], using some newer analytical procedures, showed that apart from the diminution of protein content and the increase in lignin content, well recognized as accompanying the onset of maturity, important changes occurred in the carbohydrates. A water-soluble fructosan was found to be present in considerable amount in immature grass, apparently acting as a temporary reserve, later to be utilized for the production of insoluble structural constituents, predominantly cellulose. Such a change would substantially affect the nutritive value and digestibility of the herbage, but is not clearly evident from the results of the conventional analyses applied to feeding stuffs, as has been pointed out elsewhere [Norman, 1937, 1]. The purpose of the investigations reported here was to follow analytically the changes in composition during growth of another grass, cocksfoot (*Dactylis glomerata*; known in the U.S.A. as orchard grass) which is generally regarded as being coarser and less palatable than rye grass, and to determine if the latter is peculiar in its high content of fructosan or if this polysaccharide has an equally important role in another member of the Gramineae. Some evidence having been obtained in the case of both rye grass and barley that applications of N cause a reduction in the percentage content of fructosan, direct comparisons were made of grass taken from untreated and N-fertilized plots. Analyses were also carried out on the second growth of this grass by re-cutting all plots 4 weeks later.

Plan of experiment and source of samples

The experimental area was located on the north headland of Long Hoos field (section IV), Rothamsted, and consisted of a pure stand of cocksfoot in its second season. 160 small plots each $1\frac{1}{2}$ yd. square were laid out in two adjacent blocks each containing 80 plots. As before an area of 1 sq. yd. was cut from the plot at the time of sampling, and, because of the margin remaining, edge effects could be ignored. In view of the variation in yield obtained in the rye grass experiment in which only 6 plots were cut to form the composite sample, the number was increased to 8, which were chosen at random. The bulked samples were treated as in the previous season.

Sampling was commenced on 10 May 1937, when 8 plots were cut on each block. One block then received an application of sulphate of ammonia at

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the rate of 2 cwt. per acre. Each week until 28 June 8 plots were cut on each block. Supplementary applications of sulphate of ammonia at half the rate above were applied to the N block on 24 May and 7 June. The rainfall was well distributed and the grass made excellent growth. The month of May in this year was distinctly wetter than the average, rain falling on 15 days. All the plots were cut a second time 4 weeks after the first cut and bulked in the same combinations as before. In the following Tables sample numbers with the suffix N refer to grass taken from plots in the block receiving applications of sulphate of ammonia, and with the suffix O, from the untreated block. It is to be noted that all changes due to the application of N occurred after 10 May, since up to that time the treatment of the blocks had been identical.

Yield and composition of cocksfoot

The rate of growth of the grass is shown in Table I, the yield being expressed as dry weight per sq. yd. A substantial increase in dry weight was apparent in 3 weeks as a result of the application of N, and taken at the point of maximum yield the N plots outyielded the untreated plots by almost one-third. With additions of N the final yield of mature grass exceeded 2 tons per acre on the dry basis.

Table I. *Yield of cocksfoot per sq. yd. (first cut)*

Date of cutting	Sample	Oven-dry weight g.	Sample	Oven-dry weight g.
10 May	1-O	90	—*	—
18 "	2-O	130	2-N	124
24 "	3-O	166	3-N†	186
31 "	4-O	196	4-N	277
7 June	5-O	257	5-N†	377
14 "	6-O	284	6-N	413
21 "	7-O	335	7-N	456
28 "	8-O	314	8-N	460

* Ammonium sulphate applied at the rate of 2 cwt./acre.

† Ammonium sulphate applied at the rate of 1 cwt./acre.

Table II. *Some non-structural constituents of cocksfoot (first cut). All analyses expressed as % on oven-dried material*

Sample	Ash	Crude protein	Cold water-soluble	Protein in aqueous extract	Fructose before hydrolysis	Fructose after hydrolysis	Alcohol-benzene extract
1-O	10.8	12.3	33.9	4.4	2.7	11.3	18.7
2-O	9.7	11.0	32.1	4.1	3.6	11.1	16.9
2-N	10.1	15.4	31.8	6.8	3.5	9.4	18.3
3-O	9.3	9.9	28.3	3.3	2.8	8.7	13.0
3-N	9.7	12.1	25.0	3.8	2.6	5.8	12.6
4-O	9.8	7.5	24.2	2.3	3.1	8.3	13.0
4-N	10.2	12.3	23.5	3.0	3.4	4.8	16.0
5-O	8.5	7.0	23.6	2.3	3.6	7.5	14.2
5-N	8.1	10.3	22.8	3.4	3.4	5.7	13.4
6-O	8.0	5.3	20.7	2.1	2.6	6.9	12.3
6-N	7.9	10.6	21.3	3.1	2.7	5.0	14.4
7-O	7.0	5.4	22.3	2.3	2.1	7.8	12.1
7-N	8.8	9.1	24.0	3.1	2.9	7.3	13.0
8-O	7.8	5.4	23.6	2.2	1.9	9.7	11.0
8-N	8.0	7.9	24.0	2.5	2.9	8.2	13.8
S.E. mean $\pm$	0.09	0.15	0.21	0.05	0.04	0.06	—

Considering first some non-structural constituents (Table II), the N content of the herbage from the untreated plots diminished steadily, as is usually observed. The application of N was rapidly reflected in an increased protein content, even in as short a period as 1 week, and the protein content of the herbage receiving N was in all cases substantially higher. On an area basis the maximum yield of protein was obtained in sample 6-N, equivalent to 467 lb. per acre. In the untreated grass the peak was only 193 lb. in sample 7-O.

The water-soluble material in the grass was distinctly less in amount than in rye grass and was not much affected by the addition of N. It might have been expected that an increased N content would be accompanied by an increase in soluble constituents, but no indication of this was obtained. Indeed, in considering the fructosan, which in rye grass forms the major water-soluble constituent, the reverse appears to be the case, the addition of N to the herbage in every case having depressed the fructosan content. Unlike the rye grass too, the highest content was not found at the time of rapid emergence of the head, but about 3 weeks earlier in the first sample collected, when it amounted to only 11.3 % as against a peak of over 30 % in rye grass. The influence of N in reducing the accumulation of fructosan in barley has been described by Archbold [1938], who found that the fructosan content was highest when the N level was low. If fructosans are of secondary origin and arise only when the demand for soluble sugars in other parts of the plant is satisfied, as this author suggests, the addition of N might be expected to have the result observed by stimulating more extensive growth. This, however, is probably too simple a view. Although the peak content of fructosan in this grass was far lower than in rye grass, this constituent did not become so depleted on maturity. The fully mature samples still contained 8-9 %, and indeed there were indications of a slight rise in the final stages. No importance is attached to the amounts of free fructose found in these samples, since this is affected by the drying procedure and is considerably higher than that found by extraction of fresh material. Indications were obtained of the presence of yet another polysaccharide in the aqueous extract, as was the case also in rye grass. A careful study of the water-soluble carbohydrates of grasses would be desirable, since there is obviously some constituent unaccounted for.

Extraction with hot 1 : 1 alcohol-benzene was carried out primarily as a pre-treatment in the determination of lignin. Even in the mature samples the amount removed by the reagent was appreciable and consisted, of course, of a mixture of several constituents.

Analyses for the structural constituents are given in Table III. The cellulose content of this grass throughout its whole development was considerably higher than that of rye grass and this is expressed in the general belief that cocksfoot is a "coarse" grass of high fibre content. No large differences as a result of the N treatment were to be seen, though in most cases samples from plots so treated had a slightly lower cellulose content. Cellulose was at all times easily the largest single constituent. As the cellulose content increased the ratio of pentose to hexose groups in the newly formed cellulose also changed so that the amount of xylan in the cellulose rose from 25.7 % in the young grass to nearly 30 % in samples 5 and 6, thereafter declining a little. Similar changes in the nature of the cellulosic framework of the plant have been observed in both rye grass and barley, but at present no explanation of this change can be offered.

The direct determination of encrusting polyuronide hemicelluloses has not yet been achieved, and accordingly the furfuraldehyde yield from pentose and uronic groups not associated with the cellulose must still be used as a measure of

Table III. *Some structural constituents of cocksfoot (first cut). All analyses expressed as % on oven-dried material*

Sample	Cellulose	Furfur-aldehyde from cellulose	Xylan in cellulose	Total furfur-aldehyde	Furfur-aldehyde from polyuronides	Lignin	Nitrogen in lignin
1-O	38.5	6.3	9.9	10.2	4.0	7.9	0.5
2-O	42.4	7.2	11.2	11.3	4.1	7.1	0.4
2-N	41.3	7.2	11.1	10.8	3.7	7.0	0.5
3-O	44.4	7.8	12.1	12.4	4.6	8.6	0.5
3-N	46.0	8.3	12.9	12.7	4.4	8.7	0.6
4-O	46.8	8.7	13.4	13.8	5.2	10.2	0.4
4-N	44.7	8.0	12.4	13.1	5.1	11.1	0.5
5-O	45.8	8.7	13.5	13.5	5.8	11.1	0.4
5-N	45.8	8.7	13.5	14.0	5.3	12.0	0.5
6-O	48.9	9.1	14.1	14.0	4.9	11.1	0.3
6-N	46.2	8.9	13.8	13.4	4.4	12.4	0.6
7-O	48.6	9.0	13.9	14.1	5.1	11.9	0.3
7-N	45.6	8.5	12.3	13.1	4.6	12.4	0.5
8-O	46.4	8.6	13.3	13.6	5.0	12.1	0.2
8-N	44.3	7.9	12.3	12.6	4.8	11.8	0.4
S.E. of mean $\pm$	0.13	0.04	0.06	0.10	—	0.08	—

this constituent. The approach of maturity was not accompanied by any substantial increase in the hemicelluloses. The amount present in the herbage which had received applications of N was always slightly lower than in that from the untreated plots.

It has been shown in earlier studies that the deposition of lignin occurs slowly and steadily throughout the development of the plant. This was obviously the case in cocksfoot, though the amount, 7.9 %, found in the youngest samples was surprisingly high. Lignin is slightly over-estimated by the procedure used because of a disturbance caused by the presence of protein [Norman, 1937, 2], and although the herbage treated with N appeared to have a slightly higher lignin content, it is doubtful whether the differences are significant, since in every case more N was retained by the lignin residue.

Taking the structural constituents together, there is less difference between the immature and mature cocksfoot samples than was found between corresponding rye grass samples. That is to say, cellulose and lignin together increased only from 46 to 58 % in the former as compared with an increase of 29 to 47 % in the latter in an equal time interval. An interesting relationship between fructosan and structural constituents, particularly cellulose, appeared to hold in rye grass, the former being apparently utilized and transformed into the latter after the plant had ceased increasing in dry weight. There is no indication of a similar relationship in cocksfoot (Fig. 1), either with or without applications of N. When expressed on the basis of g. per sq. yd., both cellulose and fructosan continued to rise to maturity, the latter rather more rapidly in samples 7 and 8 when cellulose deposition was slackening. Although the percentage of fructosan in cocksfoot was distinctly lower than in rye grass, the maximum amount on an area basis was of the same order because of the heavier growth of the grass. Sample 8-N contained fructosan amounting to 402 lb. per acre, and sample 8-O, to 324 lb. per acre. If on nutritional grounds it was found that the fructosan was a particularly desirable constituent in herbage, then applications of N

might have to be considered carefully, inasmuch as in cocksfoot, though the percentage content is diminished, the yield on an area basis is increased after a certain stage (Fig. 2).

Fig. 1. Changes in cellulose and fructosan in cocksfoot expressed on an area basis.

Fig. 2. Yield of fructosan in cocksfoot with and without nitrogen applications.

Yield and composition of second growth cocksfoot

Samples from the re-cut areas must be compared on a different basis from first growth samples, inasmuch as they do not represent material of increasing maturity, but instead they are all of equal age, the 4-week period of growth moving progressively later. Any differences that may be found must then be due to the fact that the root systems from which the second growth developed were at different stages of maturity, and that soil and climatic conditions were variable. Since the plots cut were distributed at random within the blocks it was not feasible to apply late dressings of N at equivalent times. As described earlier, blanket applications were made to the whole block on 24 May and 7 June. The first would only directly affect the second growth on the plots pooled to form samples 1-3, and the latter that on the plots pooled to form samples 2-5. The later samples were therefore completely dependent on residual soil N.

The yields of second growth grass on the same groups of plots as originally sampled are given in Table IV. Both with and without added N the yields decreased as the season progressed. A substantial N effect was apparent, the highest yield, as might be expected, being obtained on the plots initially cut early and supplied with N. All the samples had a high ash content. The protein content of the herbage from the untreated plots (Table V) changed little as the

Table IV. *Yield of cocksfoot per sq. yd. (4 weeks' second growth)*

Date of cutting	Sample	Oven-dry weight g.	Sample	Oven-dry weight g.
7 June	1-O-1	55.1	1-N-1	137.4
14 "	2-O-1	48.6	2-N-1	119.0
21 "	3-O-1	41.0	3-N-1	122.0
28 "	4-O-1	34.8	4-N-1	106.0
5 July	5-O-1	21.3	5-N-1	67.8
13 "	6-O-1	20.2	6-N-1	61.1
20 "	7-O-1	22.8	7-N-1	44.7
27 "	8-O-1	11.9	8-N-1	24.4

Table V. *Some non-structural constituents of cocksfoot (4 weeks' second growth). All analyses expressed as % on oven-dried material*

Sample	Ash	Crude protein	Cold water-soluble	Protein in aqueous extract	Fructose before hydrolysis	Fructose after hydrolysis	Alcohol-benzene extract
1-O-1	12.9	12.0	27.4	2.8	1.9	6.7	14.1
1-N-1	12.0	19.3	27.5	4.8	1.9	3.8	16.3
2-O-1	13.1	12.4	26.5	3.1	1.4	3.5	15.8
2-N-1	12.4	17.9	25.0	5.0	1.9	2.8	12.9
3-O-1	13.0	12.2	28.8	3.4	1.6	6.1	14.2
3-N-1	12.9	14.3	26.9	3.9	2.3	5.0	15.7
4-O-1	13.4	11.7	28.6	2.3	1.6	6.7	11.9
4-N-1	12.6	14.1	27.9	3.6	1.8	6.0	11.8
5-O-1	13.7	12.0	26.6	2.8	1.1	5.1	13.1
5-N-1	13.4	14.0	27.2	3.7	1.7	5.4	12.5
6-O-1	13.7	12.2	24.6	2.9	1.1	5.6	13.1
6-N-1	12.1	13.6	27.4	3.5	1.8	5.7	11.4
7-O-1	13.0	10.9	23.5	3.0	0.8	3.5	14.4
7-N-1	12.1	12.3	23.7	3.1	1.2	4.4	11.4
8-O-1	12.4	10.1	21.6	1.8	0.9	2.6	13.2
8-N-1	13.0	13.5	24.2	2.9	1.1	3.7	12.9
s.e. of mean $\pm$	0.09	0.15	0.21	0.05	0.04	0.06	—

Table VI. *Some structural constituents of cocksfoot (4 weeks' second growth). All analyses expressed as % on oven-dried material*

Sample	Cellulose	Furfur-aldehyde from cellulose	Xylan in cellulose	Total furfur-aldehyde	Furfur-aldehyde from polyuronides	Lignin	Nitrogen in lignin
1-O-1	40.1	7.0	10.8	10.6	3.6	8.6	0.5
1-N-1	40.8	6.9	10.7	10.2	3.2	9.0	0.6
2-O-1	43.5	7.7	11.9	11.1	3.6	8.8	0.5
2-N-1	41.0	6.9	10.8	11.0	4.1	10.0	0.7
3-O-1	42.1	6.8	11.3	10.8	3.6	8.2	0.4
3-N-1	40.8	6.9	10.5	10.4	3.6	10.0	0.5
4-O-1	40.5	7.1	10.6	10.2	3.4	8.9	0.6
4-N-1	39.8	7.1	11.0	9.9	2.8	9.1	0.5
5-O-1	42.7	6.7	11.0	10.4	3.2	8.3	0.4
5-N-1	40.8	7.9	10.4	10.2	3.6	8.4	0.5
6-O-1	46.8	7.6	12.3	10.8	2.8	8.5	0.4
6-N-1	44.1	7.8	11.8	10.8	3.2	9.9	0.5
7-O-1	44.8	7.7	12.1	11.8	4.0	9.5	0.4
7-N-1	44.4	7.7	12.0	10.3	2.6	9.1	0.5
8-O-1	44.2	7.7	11.9	10.8	3.1	9.9	0.4
8-N-1	43.8	7.1	11.1	10.6	3.5	9.8	0.5
s.e. of mean $\pm$	0.13	0.04	0.06	0.10	—	0.08	—

season progressed, whereas that from the plots treated with N, at first over 19 %, decreased steadily. On an area basis the protein differences were very great, sample 1-O-1 containing 70 lb. per acre as compared with 282 lb. per acre in sample 1-N-1. No significant differences were found in the amount of total water-soluble material, though the fructosan content was lower than in young first growth herbage. This is in accord with similar observations on rye grass. In some, but not all, cases the effect of the application of N was again to reduce the fructosan content. The structural constituents were not higher in the second growth than in first growth herbage of approximately equal N content. Material from the plots treated with N had uniformly slightly lower cellulose contents than that from the untreated plots, but there were no consistent or significant differences between the two with respect to lignin or polyuronide hemicellulose contents. The amount of the latter present, as judged by furfuraldehyde yield, seemed unusually low, but this may be misleading in view of the observation that the hemicelluloses of immature tissues may have a lower pentose content than those of more lignified tissues.

In general, it cannot be said of cocksfoot that the composition of second growth herbage is as different from the first growth as seemed to be the case in rye grass, and a lower level of fructosan appears to be the most significant difference.

Recovery of nitrogen in the herbage

Apart from the questions of composition it is interesting to notice the recovery of N in the herbage. Because the applications were made on three occasions at intervals of 2 weeks, the uptake was variable. The results in Table VII were arrived at by determining the differences between the sums of the N contents of the first and second growths in the treated and untreated plots. A total of approximately 95 lb. of N per acre was added in the form of ammonium sulphate and the maximum recovery amounted to about 62 %, the highest figures occurring in samples 4 and 6, which were each cut initially 1 week after an application of N.

Table VII. *Recovery of N in the herbage (first and second growth combined)*

Samples	lb. of added N per acre absorbed
2	35
3	31
4	51
5	47
6	59
7	45
8	37

SUMMARY

1. The changes in composition of cocksfoot cut at weekly intervals up to maturity, with and without applications of ammonium sulphate, have been determined.

2. A water-soluble fructosan was found in this grass, the amount being depressed by applications of N. The maximum content was far lower than in rye grass and occurred in the youngest herbage collected. The most mature samples still contained an appreciable amount of this constituent.

3. Throughout development, the herbage was relatively high in structural constituents, the proportions being little affected by the treatments with N.

There was no apparent relationship between the disappearance of fructosan and the formation of structural constituents, as was the case with rye grass.

4. The composition of second growth herbage of equal age from the same plots was determined. The fructosan content of this material was lower than that of immature first growth but there was little difference in the structural constituents.

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