LXV. A STUDY OF THE RELATIVE AMOUNTS OF THE PROTEIN AND NON-PROTEIN NITROGENOUS CONSTITUENTS OCCURRING IN PASTURE HERBAGE, AND THEIR SIGNIFICANCE IN THE GRAZING OF THE HERBAGE BY STOCK.

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INTRODUCTION.

IN a comprehensive study [Greenhill, 1930, 1] of the chemical composition of pasture herbage as grazed under the intensive system of grass-land management, extending over the seasons 1926 to 1929, it was found that, of the total nitrogen of the herbage, the proportion present in protein form (as determined by the Stützer method) was more or less constant under all conditions. The value ranged usually between 85 and 90 % and seldom fell below 80 %.¹

Instances of abnormally high proportions of non-protein-nitrogen in pasture herbage have, however, been observed, accompanied by digestive disturbances in stock grazing the herbage.² Thus, a sample of intensively manured grass at Castle Douglas [Watson, 1929] in May 1929 was found to contain 49 % of its total nitrogen in non-protein form, whilst in another at Retford [Greenhill, 1930, 2] in April 1930 the value was 38 %. Samples of intensive pasture herbage from a farm at East Clandon [Greenhill, 1930, 3] in March and April 1930 were also found to contain between 30 and 40 % of their nitrogen in non-protein form; although in this instance the herbage was not being grazed at the time, the possibility of its causing digestive disturbances in stock was suspected.

Although instances of serious digestive disturbances in stock grazing grass, due apparently to an abnormally high proportion of non-protein-nitrogen or of a particular form of non-protein-nitrogen in the herbage, are of rare occurrence, instances of minor disturbances attributed, rightly or wrongly, to such an excess are less uncommon. It was decided, therefore, in 1930 to investigate further the amounts and relative proportions of the nitrogen compounds occurring in grass.

EXPERIMENTAL.

As practically no data were available in the literature, concerning the amounts of the various nitrogenous constituents occurring in grass, it was decided to determine the amounts of some of these constituents present normally in intensive pasture herbage at different times of the season. Accordingly, a number of samples were examined in 1930.

¹ The occasional lower values obtained were traceable usually to some hydrolysis of protein having occurred between the times of taking and drying the sample.

² The so-called "grass tetany" of stock is often ascribed to an excess of non-protein-nitrogen or of a particular non-protein nitrogenous constituent in the herbage.

Particulars of samples examined.

Twelve samples at the 4–6 in. growth stage, obtained from the intensively manured rotational grazing paddocks in Leak's Meadow and Feeding Ground at Jealott's Hill, were examined in the months of May, August, September and October. The herbage examined was not necessarily representative of the whole paddock; in some cases this was so, whilst in others the sample was taken from those areas where growth was most luscious. Also, although the samples were all of herbage at the grazing stage, the pasture was not necessarily grazed at the time the sample was taken. In those cases where it was grazed, however, no digestive disturbances in the stock were observed.

Methods of examination employed.

Determinations of the total nitrogen and of the protein-, amide-, ammonia- and nitrate-nitrogen were made on the dried, ground material.

The total nitrogen was determined by the Kjeldahl and the protein-nitrogen by the Stützer method.

The amide-nitrogen was determined by boiling with 5 % HCl for $1\frac{1}{2}$ hours and subsequent distillation with magnesia, allowance being made for the nitrogen originally present in the ammoniacal form.

The ammonia-nitrogen was determined by direct distillation with magnesia.

The nitrate-nitrogen (including any nitrite-nitrogen if present) was determined by the iron reduction method described by Vickery and Pucher [1929].

It should be noted [Greenhill, 1933] that grass kept for any appreciable length of time in the fresh condition is very liable, unless special precautions are taken, to undergo significant change in composition; besides some loss of dry matter, hydrolysis of protein, with a marked diminution in the value obtained for the protein-nitrogen and a corresponding rise in the non-protein-nitrogen value, is particularly likely to occur. For this reason it is important that as little time as possible should elapse between the taking and drying of the sample, and that the drying should be carried out as rapidly as possible, the grass being spread out in a thin layer and dried in an oven at a temperature of between 70° and 100°.

RESULTS.

The analytical data are summarised in the top section of Table I. The non-protein-N values, expressed as percentages of the total N, ranged from 12 to 23, the amide-N from 2.7 to 8.1, the ammonia-N from 1.0 to 2.0 and the nitrate-N from 0.1 to 4.0.

Comparing the individual samples, the proportions of the nitrogen present in amide and ammoniacal forms showed no relationship to the total nitrogen content of the herbage or to one another; the higher proportions of non-protein- and of nitrate-nitrogen, however, with exceptions, were associated with the higher total nitrogen contents.

DISCUSSION OF RESULTS AND CONCLUSIONS.

The samples of herbage examined included a wide range of total nitrogen contents (from the moderate figure of 2.8 to the high figure of 5.2 % of the dry matter, equivalent to 17.8 and 32.3 % of crude protein respectively) and also included samples taken under conditions of growth which might have been expected to be conducive to high proportions of non-protein-nitrogen. Also, the

Table I. *Summary of data for intensive pasture herbage (Jealott's Hill, Castle Douglas, Retford and East Clandon). 1929 and 1930.*

Particulars of samples	As % of dry matter		As % of total nitrogen				
	Total nitrogen	Equiv. to crude protein	Protein-N	Non-protein-N	Amide-N	Ammonia-N	Nitrate-N
12 samples intensive pasture herbage at grazing stage from Leak's Meadow or Feeding Ground, Jealott's Hill. May, August, September and October, 1930:							
Highest value	5.17	32.3	88	23	8.1	2.0	4.0
Lowest value	2.84	17.8	77	12	2.7	1.0	0.1
Average value	3.63	22.7	83	17	5.3	1.3	1.7
Intensive grass (first year permanent) from Castle Douglas [Watson, 1929]. May 1929. (Serious disturbance experienced in grazing stock.)	3.22	20.1	51	49	10.2	2.6	*
Intensive grass (seeds) from Retford [Greenhill, 1930, 2]. April 1930. (Serious disturbance experienced in grazing stock.)	5.21	32.6	62	38	11.3	4.1	2.5
4 samples intensive pasture herbage from East Clandon [Greenhill, 1930, 3]. March and April, 1930. (Pastures not being grazed at time as possibility of herbage causing some disturbance suspected.)	3.93 3.80 4.23 4.42	24.6 23.8 26.4 27.6	59 67 71 68	41 33 29 32	8.9 5.2 6.6 6.3	7.5 2.9 2.2 2.2	0.9 0.6 0.5 0.3

* Present in appreciable amount.

paddock from which the Leak's Meadow samples were taken¹ had received heavier dressings of artificial fertilisers, including nitrogen, than are usually applied to pastures. In no instance however did the proportion of the total nitrogen present in non-protein form rise above 23 %, whilst in the majority of samples it did not rise above 16 %.

In the three lower sections of Table I are given the corresponding nitrogen values for the intensive herbage samples from Castle Douglas [Watson, 1929], Retford [Greenhill, 1930, 2] and East Clandon [Greenhill, 1930, 3] referred to earlier. Comparing the values for the Jealott's Hill samples with the values for these samples, it will be observed that whilst the total nitrogen contents of the herbage were comparable, the proportions of the nitrogen present in non-protein form were much higher in the latter than in the Jealott's Hill samples. Amide- and ammonia-nitrogen were present generally in higher proportions; the comparison shows, however, no definite difference in respect of the proportions of nitrate-nitrogen, which were very variable.

From a consideration of the data presented and referred to, it is concluded that the proportion of the total nitrogen present in protein form, in intensive herbage as grazed by stock, is normally more or less constant at a value between 80 and 90 %, and that only in exceptional instances does the non-protein-nitrogen exceed 20 % of the total nitrogen. Such instances may very occasionally occur, however, and may be liable to cause digestive disturbances in stock grazing the herbage (the seriousness of which will depend on the actual amount of non-protein-nitrogen or on the excess of some particular form of non-protein-nitrogen present, and on the general grazing and other conditions) and should be guarded against.

In general, it seems probable that an unusually high proportion of non-protein-nitrogen in grazing herbage is likely to arise only under a peculiar combination of circumstances, namely on heavily manured grass under conditions of very rapid growth or when, after heavy manuring followed by a check to growth,

¹ The Leak's Meadow samples were all taken from Paddock No. 1.

growth suddenly recommences. A high proportion of non-protein-nitrogen is associated nearly always, for these reasons, with a particularly leafy growth and, in consequence, with a high total nitrogen content, though, of course, leafy growth is not of itself to be considered in any way indicative of an unusually high proportion of non-protein-nitrogen. Further, from the nature of the causes, such instances are most likely to occur in the early and late weeks of the grazing season and to be of very transient occurrence.

Reference may be made to the data otherwise noted for the fractionation of the nitrogenous constituents of grass, and to further work on this question at this Station. Sjollem [1931], at the Veterinary Laboratory of the University of Utrecht, in an investigation of the cause of "grass tetany", examined, also in 1930, a large number of samples of intensive pasture herbage taken in the early and late months of the grazing season, with results generally confirming those obtained by the writer in this country. Chibnall and Miller [1931], examining a sample of perennial rye-grass from a sewage farm, found it to contain 12.7 % of its nitrogen in nitrate form, or calculated as KNO_3 , equal to 4.4 % of the dry matter; this grass, however, was of rank growth and not such as would be grazed, and further, the sample examined consisted of the upper leafy part of the herbage only. In America, Leukel and Coleman [1930] and Leukel *et al.* [1934], at the University of Florida Agricultural Experiment Station, have given data for the influence of different fertiliser and cutting treatments on the main nitrogenous constituents of several pasture grasses, whilst Archibald and Bennett [1932], at the Massachusetts Agricultural Experiment Station, studying the fractionation of the nitrogenous constituents in samples of herbage from manured and unmanured pastures and pure stands of white clover, found the ammonia-nitrogen to average about 1.8 % of the total nitrogen for the manured and about 1.0 % for the unmanured herbage; none of the samples contained more than slight traces of nitrate-nitrogen. At Jealott's Hill, Eggleton [1935] has studied the uptake of nitrogen by pasture herbage in the spring, following the application of various nitrogenous fertilisers, including sodium nitrite, and of sulphate of potash, while other investigations upon which work has been in progress since 1930 by Blackman and others, include the fractionation of the nitrogenous constituents in "early bite" pasture herbage and in lawn herbage, the results of which will be published later.

It is possible, at the present, to do little more than state the values for, and the general observations which have been made concerning, the fractionation of the nitrogenous constituents of grass, the factors probably determining the relative proportions of the different constituents having so far been but little studied. The influence of fertilisers, of climatic and seasonal conditions, and of soil conditions both chemical and biological, require study, whilst the fractionation needs to be applied throughout the period of growth and to different parts of the plant.

SUMMARY.

Following the very occasional occurrence of digestive disturbances in stock grazing intensively manured pastures, due apparently to an abnormally high proportion of non-protein-nitrogen or of a particular non-protein-nitrogen compound in the herbage, samples of intensively manured pasture at the grazing stage from the rotational grazing paddocks at Jealott's Hill were examined in the months of May, August, September and October 1930, for their contents of total, protein-, amide-, ammonia- and nitrate-nitrogen.

The total nitrogen contents of the herbage ranged from 2.8 to 5.2 % of the dry matter; the non-protein-nitrogen values, expressed as percentages of the

total nitrogen, ranged from 12 to 23, the amide-nitrogen from 2·7 to 8·1, the ammonia-nitrogen from 1·0 to 2·0, and the nitrate-nitrogen from 0·1 to 4·0. Comparing the individual samples, the proportions of the nitrogen present in amide and ammoniacal forms showed no definite relationship to the total nitrogen content of the herbage or to one another; the higher proportions of non-protein- and of nitrate-nitrogen, however, usually but not always were associated with the higher total nitrogen contents.

These values are compared with those of pasture samples associated with digestive disturbances in grazing stock at other centres, and the possible bearing of the relative proportions of the different forms of nitrogen compounds in grass in relation to their effect on the grazing of stock is discussed.

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