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# THE CHEMICAL COMPOSITION OF RAPIDLY GROWING RYEGRASS AND ITS RELATION TO ANIMAL PRODUCTION

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IN NEW ZEALAND, there is generally a marked acceleration of grass growth in the autumn, coinciding with warm, moist conditions. This is particularly the case for the ryegrasses in high-producing pastures. Clarke and Filmer (1956, 1958a, b) and Butler and Rae (1957) have observed that "autumn flush" grass growth coincides with losses of weight in grazing sheep. Hoggets are particularly prone, leading to the condition known as "hogget ill-thrift". The outstanding feature of this metabolic disorder appears to be a loss of appetite, with the animals starving in the midst of plenty. This paper deals with chemical studies made on rapidly growing autumn ryegrass at Plant Chemistry Division; the results are discussed in relation to work being done elsewhere in New Zealand on hogget ill-thrift and to overseas work on other animal nutritional problems.

The most obvious characteristic of the chemical composition of rapidly growing autumn ryegrass is the comparatively high content of non-protein nitrogen compounds, and in particular of inorganic nitrate. It has long been recognized that levels of nitrate in the feed of ruminants in excess of 1.5 per cent.  $\text{KNO}_3$  equivalent on a D.M. basis can result in the characteristic symptoms of nitrate poisoning. The toxicity is due to the reduction of nitrate to nitrite by rumen bacteria, nitrite being two to three times as toxic as nitrate when fed orally. The question therefore arises whether continued ingestion of sub-clinical amounts of nitrate by ruminants is likely to be deleterious.

The paper is divided into three parts:

- (a) Factors affecting herbage nitrate content.
- (b) The metabolism of nitrate by ruminants.
- (c) Other non-protein nitrogen constituents of herbage.

## Factors Affecting Herbage Nitrate Content

It should first be stated that the nitrate content of grass herbage is capable of wide variation from zero to 6.6 per cent.  $\text{KNO}_3$  equivalent on a D.M. basis, the extreme figure being re-

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corded in 1956 near Ashburton for rapidly growing short-rotation ryegrass (I. J. Cunningham, pers. comm.). At Plant Chemistry Division, similar high nitrate contents have been observed in pure ryegrass stands after fertilizing with sulphate of ammonia or urea. The factors known to control this variation are as follows.

#### AVAILABILITY FROM SOIL

The nitrogen cycle in soils of grazed grass-clover associations is summarized in Fig. 1, slightly modified from Walker (1956). The extent of conversion of ammonium ions to nitrate ions in the soil is dependent on the activity of the soil nitrifying bacteria, which is favoured by warm, moist aerobic conditions. The ammonium content of soils of European grass-clover pastures normally exceeds that of nitrate (Richardson, 1938), but in New Zealand on high fertility pastures the nitrate concentration increases to high levels when light, warm rains follow lengthy dry periods. This is shown by the high nitrate levels of drainage waters from high-producing pasture, collected after onset of autumn rains (G. W. Butler and H. G. Hopewell, pers. comm.). Concentrations of 21 p.p.m. were observed after the first autumn rains in 1954 and 1955, compared with no detectable amounts in

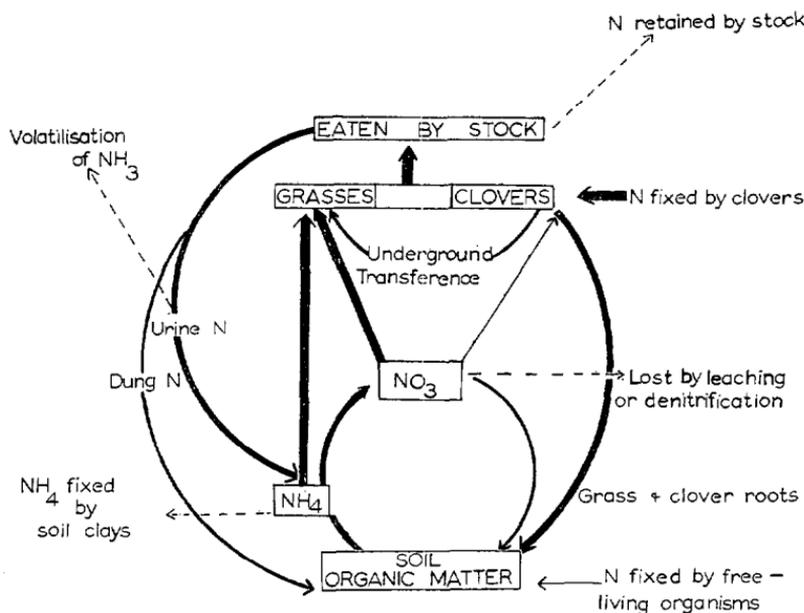


Fig. 1: The nitrogen cycle in grazed clover-grass associations (modified from Walker, 1956).

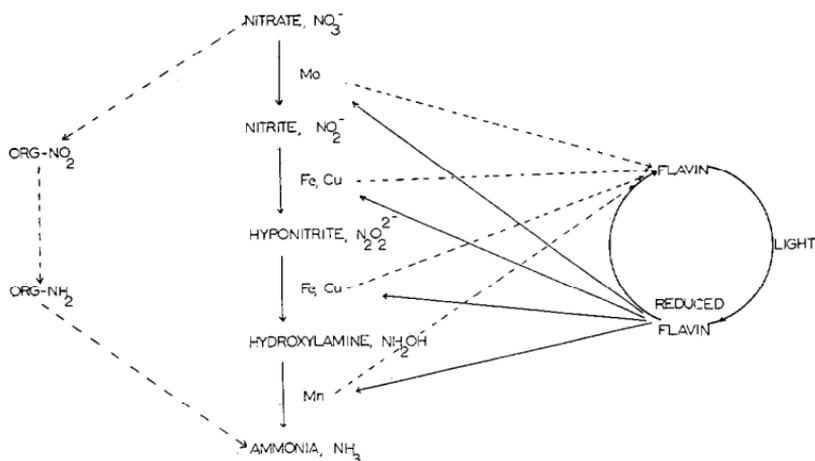


Fig. 2: Scheme for assimilation of nitrate by plants, proposed by Nicholas (1957, 1958).

the spring. In urine patches, ammonia-N may rise rapidly to several hundred p.p.m. (Thompson and Coup, 1943) and nitrate-N then rises to high levels as ammonia-N declines. Heavy rains lower the soil nitrate content because of the ease with which nitrate is leached. Another point is that fallowing of land promotes nitrification, so that newly-sown pasture is more likely to have high nitrate contents than established pasture.

#### FACTORS AFFECTING ASSIMILATION BY PLANTS

The enzymic steps proposed by Nicholas (1957, 1958) for assimilation of nitrate by plants are summarized in Fig. 2. It will be seen that the metals molybdenum, iron, copper and manganese are required for successive steps. Several workers (Anderson and Spenser, 1950; Ergle, 1953) have demonstrated that sulphur deficiency also leads to nitrate accumulation; this is thought to be an indirect effect. For each of the steps in Fig. 2, reduced flavin, which has been formed by photosynthetic activity, is required. Hence low light intensities slow down the reduction steps and favour accumulation of nitrate. The light requirement also means that there is a diurnal fluctuation, with maximum nitrate contents at dawn. In practice, it is observed that in pasture species nitrate always accumulates, rather than one of the other intermediates. Thus in the case of deficiencies of molybdenum, iron, copper, manganese, sulphur and light, nitrate always accumulates in pasture species.

## SPECIES AND TISSUE DIFFERENCES

There are considerable species differences in nitrate accumulation. For example some weeds (*Amaranthus* spp., *Cirsium* spp.) accumulate large quantities and the term "oat-hay poisoning" has been used because of the tendency for oats to be high in nitrate. Of the grasses and clovers, the *Lolium* species generally have the greatest tendency to accumulate nitrate (Bathurst and Mitchell, 1958). In New Zealand, short-rotation ryegrass has given the highest nitrate contents; in Florida, Italian ryegrass was second only to oats of a number of forages tested (Kretschmer, 1958); and in Aberystwyth, Griffith (1958) has observed threefold differences in nitrate content between ryegrass varieties.

There is also variation in nitrate content in different parts of the plants and with stage of growth. With the grasses, the stems and leaf bases have the greatest content and the leaf tips the least, as shown in Table 1. In clovers, stems and petioles have greater nitrate contents than leaves. Rapidly growing immature tissues are most likely to contain much nitrate.

In grass-clover swards nitrate is taken up preferentially by the grasses, but in pure stands of clover or clover-dominant pastures, the legume may contain high nitrate levels under particularly suitable conditions (Bathurst and Mitchell, 1958).

Summarizing the above, the following factors are all conducive to high herbage nitrate contents:

- (1) High rate of nitrification by soil bacteria.
- (2) Urine patches and high fertility.
- (3) Little or no leaching.
- (4) Newly-sown pasture after fallowing.
- (5) Deficiencies of molybdenum, iron, copper, manganese and sulphur.
- (6) Low light intensities.
- (7) Particular pasture species.
- (8) Immature growth.

TABLE 1: THE VARIATION IN NITRATE CONTENT ALONG THE LENGTH OF RYEGRASS LEAVES IN A 4 IN. PASTURE

Leaf Segment (in)	Perennial		Short Rotation	
	Nitrate (% KNO <sub>3</sub> )	D.M. (%)	Nitrate (% KNO <sub>3</sub> )	D.M. (%)
0 to 1	2.48	17.1	1.48	15.4
1 to 2	1.97	18.2	1.20	16.8
2 to 3	1.05	21.2	0.71	19.4
3 to 4	0.50	25.6	0.34	23.7

It will be realized, therefore, that in the field there is ample scope for variation in nitrate content of herbage, with respect to both situation and time. In any paddock there will be variations in nitrate content dependent on botanical composition, height and maturity of herbage, urine patches, stock camps, etc. Another point is that open pasture and grass adjacent to bare pasture tend to be higher in nitrate, because of the greater availability of soil nitrate. The type of variation which is possible even with a single

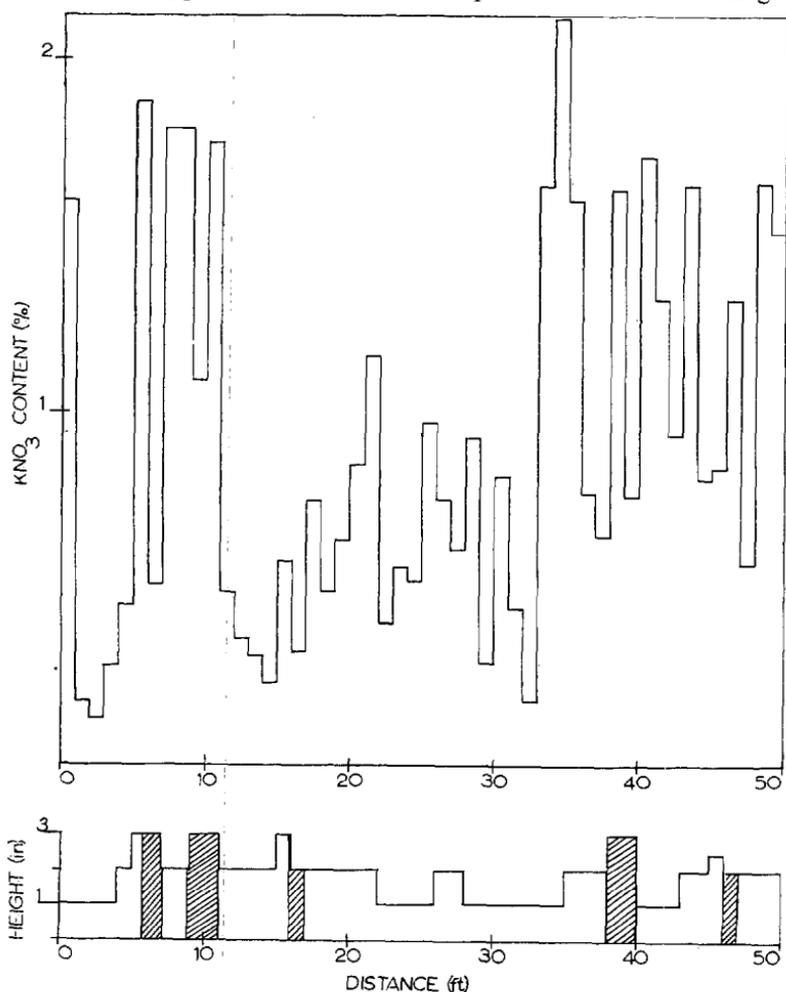


Fig. 3: Variation in herbage nitrate content in a pure short-rotation ryegrass pasture over a distance of 50 ft. The variations in height of pasture are also shown and "urine patches" are shaded.

species is shown in Fig. 3; here herbage from a pure stand of short-rotation ryegrass was collected over each foot of a 50 ft. length and analysed. The area was under grazing by sheep and was uniformly fertilized by drill with sulphate of ammonia from time to time. Obvious urine patches and the height of pasture are also shown.

In Fig. 4, the variation in mean nitrate content of herbage from pure stands of short-rotation and perennial ryegrass is shown over the period 21 January to 14 May, 1957. Rainfall data are also given; in general high nitrate levels followed freshening of the pastures as a result of rain.

From the viewpoint of ruminant nutrition, it is important to note the violent fluctuation in nitrate content which can occur in rapidly-growing immature herbage under conditions of high fertility. With pasture species used in New Zealand, this applies particularly to the ryegrasses. It is also true of some crops, such as the Brassicae (kale, rape and choumoellier) and oats.

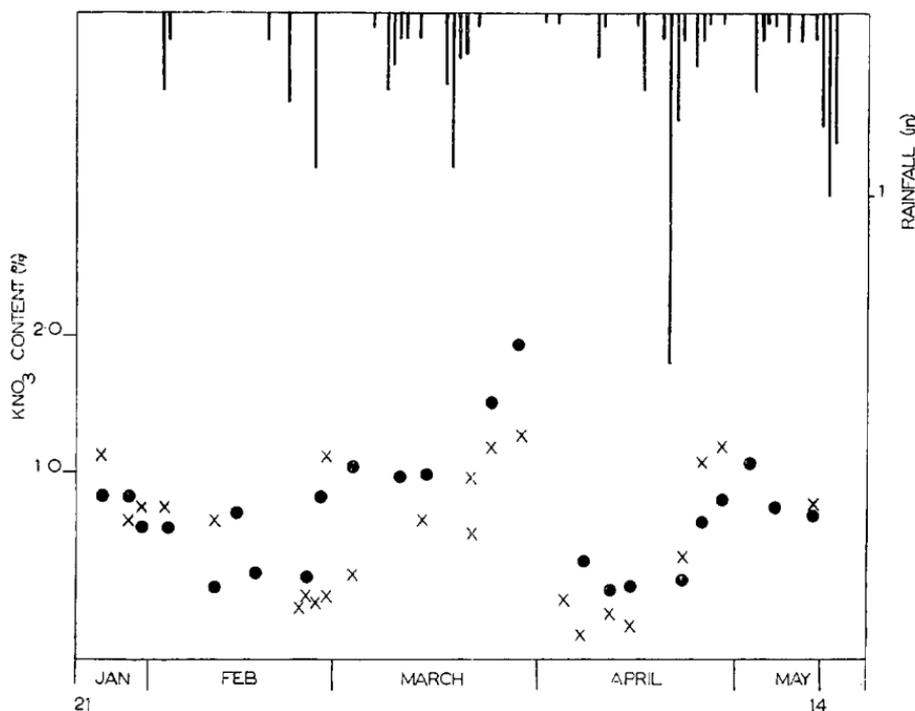


Fig. 4: Variation in mean nitrate content of herbage in pure stands of short-rotation and perennial ryegrass in the period 21 January to 14 May, 1957. Crosses designate short-rotation, and circles perennial ryegrass. Rainfall data are also given.

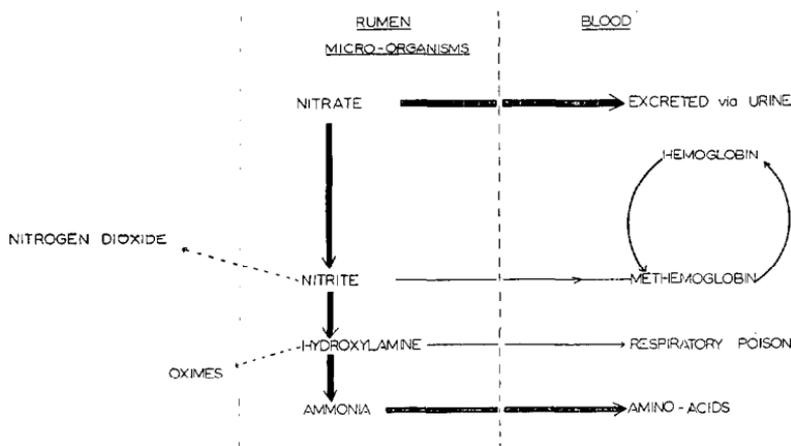


Fig. 5: Pathways of metabolism of nitrate by ruminants.

### The Metabolism of Nitrate by Ruminants

This has been studied by Sapiro *et al.* (1949), Lewis (1951a, b), Muhrer *et al.* (1955), Holtenius (1957), Pfander *et al.* (1957), Jamieson (1958), Prewitt and Merilan (1958) and Stewart and Merilan (1958). The pathways are summarized in Fig. 5. Certain rumen microorganisms reduce nitrate through nitrite and probably hydroxylamine to ammonia; the rate of reduction is dependent on the presence of suitable hydrogen donors, particularly readily available carbohydrates. If the ratio of available carbohydrate to nitrate in the feed is low, the supply of hydrogen donors is likely to be insufficient, leading to the accumulation of the toxic intermediates nitrite and possibly hydroxylamine. Several investigators have reported that the feeding of various carbohydrate supplements accelerates the reduction of nitrate completely to ammonia.

If nitrite accumulates, blood haemoglobin may be converted to methaemoglobin, leading in severe cases to the characteristic symptoms of nitrate poisoning and often culminating in death. Regeneration of haemoglobin from methaemoglobin occurs slowly over a period of several hours (Prewitt and Merilan, 1958).

N. D. Jamieson (pers. comm.) has pointed out that if free nitrous acid is formed in the rumen (because of acidic pH of the rumen liquor), there is a possibility for the formation of nitrogen dioxide; this could result in pneumonic symptoms. The nitrogen

dioxide would arise in an analogous manner to its formation by bacteria in ensiled forages which are high in nitrate (Petersen *et al.*, 1952).

Jamieson (1958) presented evidence that toxicity from hydroxylamine is also a factor and by feeding hydroxylamine to sheep he has observed lysis of the red blood corpuscles leading to a haemolytic anaemia, which he has also observed in the field in hoggets which have ill-thrift.

The question then arises: to what extent could continued ingestion of sub-lethal amounts of nitrate account for hogget ill-thrift? No clear-cut affirmative or negative answer can be given at present but the points "for" and "against" are these:

#### FOR

- (1) Analyses carried out at Plant Chemistry Division show that mean herbage nitrate is usually greater than 1 per cent.  $\text{KNO}_3$  equivalent on a D.M. basis on pastures causing weight losses in hoggets.
- (2) Muhrer *et al.* (1956) reported that animals fed sub-lethal amounts of nitrate (0.5 to 1.5 per cent.  $\text{KNO}_3$  equivalent) have reproduction and lactation difficulties.
- (3) As mentioned above, Jamieson (1958) has observed that the clinical blood picture of sheep given hydroxylamine orally and intravenously is similar to that of hoggets with ill-thrift.

#### AGAINST

- (1) Methaemoglobin has not, to the writer's knowledge, been observed in the blood of hoggets diagnosed as having ill-thrift. This may be due to a reversion of low proportions of methaemoglobin to haemoglobin in drawn blood (Heubner and Stuhlmann, 1942), or to the blood sampling being done at the wrong time. The fact that it has not been observed is, however, a strong argument against sub-clinical nitrate poisoning.
- (2) The toxic symptoms observed in stall feeding of sub-lethal levels of potassium nitrate to sheep (Jamieson, 1958; Clarke, 1959) and dairy and beef cattle (Prewitt and Merilan, 1958) are invariably milder than observed in the field at comparable or even lower levels of nitrate intake.

In considering these points, two additional aspects need to be taken into account. First, as mentioned earlier a high ratio of available carbohydrate to nitrate is more important in avoiding

toxicity than the absolute nitrate content. In lush autumn ryegrass this ratio tends to be low, because total soluble sugars are low and nitrate relatively high.

Secondly, there is the question of variability of nitrate dose. Stall feeding experiments with both sheep (Jamieson, 1958) and cattle (W. K. Kennedy, pers. comm.) show that irregular feeding with nitrate at a given level causes greater distress than steady feeding at that level. An adjustment or adaptation of the rumen microflora to a steady level of nitrate would appear likely. As described earlier, there is great scope for variation of nitrate in the feed, and it would seem that this could contribute to the effects seen in the field.

Taking all these points into consideration, it appears unlikely that nitrate in herbage is *per se* responsible for ill-thrift in sheep. Because of this, other non-protein nitrogen constituents of autumn ryegrass which may be of importance are being studied at Plant Chemistry Division.

#### **Other Non-Protein Nitrogen Constituents**

Early in this work, it was observed that the non-protein nitrogen of rapidly growing autumn ryegrass contained a fraction which was either absent or present only in very small amounts in mature herbage (Butler and Rae, 1957). This fraction can be determined as total nitrogen by the Dumas micro-combustion procedure, but not by the Kjeldahl procedure. It is also determined as inorganic nitrate by the phenoldisulphonic acid method after digestion with alkaline peroxide.

With the collaboration of C. C. Watson, Dominion Laboratory, work has continued on the characterization of this fraction. At this stage a small amount of a mixture of compounds which contain amino- and nitro-groups has been separated in amounts of 0.1 per cent. of the original dried herbage, using chromatographic techniques. On pharmacological grounds it would be expected that this type of compound might exert harmful effects on animals and therefore preparations are being scaled up in order to do more chemical characterization and to do biological testing of the fraction. A major stumbling block in the prosecution of this work is that there is no clear cut pathological condition associated with hogget ill-thrift. Hence suitable tests with laboratory animals are difficult to devise. It is not clear, therefore, whether those concerned are straying far from the nutritional

problem of ill-thrift or not in following up this fraction, but it is of great interest in the elucidation of pathways of nitrogen metabolism in the plant.

It might be asked how such a fraction might be expected to arise in rapidly growing ryegrass herbage? Two hypotheses can be advanced. First, referring to Fig. 2, it will be seen that another route for assimilation of nitrate is postulated in higher plants. Here nitrate is first combined with an organic molecule to give an organic nitro-compound which can be reduced to the corresponding amine. Although the inorganic route for nitrate reduction is thought to be generally dominant, there may be occasions when the alternative organic route becomes important, leading to a build-up of organo-nitro- and amino-compounds, *e.g.*, when the nitrate content of the herbage becomes high. There are an increasing number of reports of organic nitro-compounds occurring naturally in higher plants and in fungi. (Carter, 1951; Bush *et al.*, 1951; Cooke, 1955; Raistrick and Stossel, 1958; M. P. Hegarty, pers. comm.).

The second hypothesis is one that has been considered elsewhere in these *Proceedings* in another connection. The warm moist periods which are associated with outbreaks of hogget ill-thrift are just those conditions when soil microbiological activity, and particularly fungal activity, can be expected to be maximal. It is possible that this is of significance for ill-thrift and also for the fraction under study. For example, it is known that two soil fungi, *Aspergillus niger* and *Penicillium atrovenetum*, can secrete a nitro-compound,  $\beta$ -nitropropionic acid, in large quantities into the culture medium (Bush *et al.*, 1951); Raistrick and Stossel, 1958). Such a compound might be taken up from the soil by the grass and ingested by grazing animals. Hutton *et al.* (1958) have shown that  $\beta$ -nitropropionic acid fed to rabbits causes losses in weight.

### Conclusion

The connection between the data on chemical composition of autumn ryegrass presented here and the hogget ill-thrift condition is still to be regarded as a hypothesis and nothing more. Much detailed work remains to be done. It should be mentioned that there are two aspects which have not yet been considered in this approach to the problem, which may nevertheless be important.

- (1) The thinking has been in terms of deleterious chemical constituents present in the herbage. The absence of an essential constituent remains a possibility.

- (2) Many of the animal observations suggest that the herbage is unpalatable to stock. Because of the complexity of the palatability effect, there has been no attempt as yet to base the chemical attack on this possibility. Unpalatability could, however, be due to the presence of one or more unusual chemical constituents such as those present in the organic fraction under investigation. Some of these have a most pungent and irritating odour.

Finally, although "high nitrate" grass is observed most frequently during the autumn "flush", it has been observed once at Palmerston North during the spring "flush" of September, 1957. The weather sequence on this occasion was an unusually dry winter with warm temperatures in September. Sheep grazing this pasture showed checks in liveweight gain during the period that the "flush" growth had a high nitrate content.

\* \* \*

The data reported here are drawn from a trial carried out jointly by officers of the Sheep Husbandry Department, Massey Agricultural College, Plant Chemistry Division, Grasslands Division and Dominion Laboratory. The following persons have been associated in the work: Dr A. T. Johns, Prof. A. L. Rae, Dr D. S. Flux, R. A. Barton, Dr G. W. Butler, N. O. Bathurst, C. C. Watson, Mrs. G. W. Butler, Dr L. Corkill, R. W. Brougham, A. C. Glenday, R. W. Schwass.

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## DISCUSSION

Q.: *What happens to the nitrate content of pasture by May-June when the hogget ill-thrift problem nearly always ceases to exist?*

A.: With the onset of cold weather and heavy rains which leach nitrate from the soil, it is found that the nitrate content of pasture declines to zero or very low values.

Q.: *Concerning the role of trace elements in the reduction of nitrate through hydroxylamine to ammonia. Presumably, if there is a deficiency of manganese, for example, this could result in an accumulation of nitrate in pasture and perhaps a shunting of the metabolism through an organic path rather than through the inorganic path. Would this be so and should we be investigating the importance in practice of some of these trace elements on nitrate production?*

A.: Nitrate is known to accumulate in pasture in the cases of molybdenum, manganese, and sulphur deficiencies in the field. It is quite possible that such deficiencies might lead to shunting of the metabolism through an organic path, but we have no evidence for this as yet.