Tydskrif Weidingsveren. S. Afr. (1990), 7(3)

The relationship between the nitrogen and nitrate content and nitrate toxicity potential of *Lolium multiflorum*

R.J. Eckard

Department of Agriculture and Water Supply (Natal Region), Private Bag X9059, Pietermaritzburg 3200,
Republic of South Africa

Accepted 13 October 1989

The interrelationships between the nitrate-N and nitrogen content and dry matter yield of Midmar ryegrass (*Lolium multiflorum* cv. Midmar) were investigated. Data were collected from N fertility trials, from two seasons (1985 and 1987) on four soil sites (Metz, Griffin, Clovelly and Katspruit) with N fertilizer rates ranging from 0 to 1 080 kg N/ha/yr, but varying on different sites. The data showed little accumulation of nitrate-N with increasing nitrogen levels up to 3,2–3,5% nitrogen, followed by a sharp increase in nitrate-N with little further increase in nitrogen content. Dry matter yields approached a maximum between 3,2 and 3,5% nitrogen and between 0,15 and 0,25% nitrate-N, thereafter an accumulation of nitrogen or nitrate-N occurred with little increase in dry matter yield. Above these limits, applied N fertilizer appeared to be in excess of the growth requirements of the plant and potentially toxic to ruminants. This indicated a wasteful use of N fertilizer. The data are reviewed in terms of potential toxicity to ruminants.

Die verwantskap tussen nitraatstikstof, stikstofinhoud en droëmateriaalopbrengs van Midmar raaigrass (*L. multiflorum* cv. Midmar) is ondersoek. Die data is ingesamel in N-kalibrasie proewe van twee seisoene (1985 en 1987) op vier grondtipes (Metz, Griffin, Clovelly en Katspruit). Die N-bemestingspeile het gevarieer tussen 0 en 1 080 kg N/ha/jaar en tussen die verskillende grondtipes. Die data toon dat nitraat-N weinig akkumuleer met verhoogde stikstof vlakke, tot op 3,2–3,5% stikstof in die plant, gevolg deur 'n skerp styging in nitraat-N, met geringe verdere verhoging in stikstofinhoud. Droëmateriaalopbrengs was naby maksimum tussen 3,2 en 3,5% stikstof en tussen 0,15 en 0,25% nitraat-N. Indien toediening verder verhoog is, was daar 'n akkumulasie van stikstof of nitraat-N met 'n geringe verhoging in droëmateriaalopbrengs. Bokant hierdie perke blyk toegediende N-bemesting hoër as die plant se groeibehoeftes te wees, en potensieël toksies vir herkouers. Die data dui op 'n verkwisting van N bemesting. Die data is in verband gebring met potensieël toksiese vlakke vir herkouers.

Additional index words: Midmar, protein toxicity, ryegrass

Introduction

Irrigated Midmar ryegrass pastures are widely utilized under intensive grazing in the Natal midlands. With the increased utilization of nitrogen (N) fertilizer in intensive pasture production, an increase in the number of animal health problems, attributed to the high N fertility status of the pasture, has occurred. The high nitrate-N (Deinum & Sibma, 1980) and nitrogen (Wilman, 1970; Bredon, unpub. data, 1979. Animal Science Section, Department of Agriculture and Water Supply, P.Bag X9059, Pietermaritzburg 3201) content of the pastures, resulting from the liberal use of N fertilizers, appear to be the forms of N alleged to be deleterious to animal health.

An investigation of the interrelationships between the nitrogen, nitrate-N and dry matter yield of the plant was undertaken to formulate preventative management recommendations.

Methods

Data were extracted from Midmar ryegrass (*Lolium multiflorum* Lam. cv. Midmar) N fertility trials, the experimental design of which was detailed by Eckard (1989). Three of the trials were located on the Cedara Research Station (altitude 1 067 m, mean annual rainfall 885 mm and pan evaporation 1 478 mm) in the Natal Mistbelt, and a fourth on the Tabamhlope Research Station (altitude 1 450 m, mean annual rainfall 1 166 mm and pan evaporation 1 436 mm) in the Highland Sourveld. The three trials at Cedara were established on a Metz, Clovelly and Katspruit soil form, being warm north-facing, level and relatively cold bottomland sites, respectively. The Griffin soil form at Tabamhlope was located on a west-facing slope. Data are reported for the 1985 season on the Metz and Griffin soil sites and the 1987 season on the Katspruit and Clovelly soil sites.

In the 1985 season N rates of 360, 720 and 1 080 kg N/ha/yr were applied on the Metz and Griffin sites. In the 1987 season of experimentation on the Clovelly and Katspruit sites, N rates were 200, 300 and 400 kg N/ha/yr and 200, 400 and 600 kg N/ha/yr, respectively. Data are reported for N applied at seedling emergence and every four weeks thereafter, from April to December. Data are meaned for three sources of N fertilizer (LAN, urea and ammonium sulphate), as there were no significant differences between the N sources with respect to the data presented (Eckard, 1986; Eckard, 1990). A sufficiency of nutrients other than N were supplied to all plots, based on initial soil analyses of the sites (Eckard, 1989). Irrigation was supplemented to ensure a minimum application of 25 mm of water per week.

Samples were taken at intervals of four weeks by cutting them to a height of 5 cm between 10:00 am and 11:30 am. Net sample mass was recorded and subsamples were taken for dry matter determination (oven-dried at 90°C for 24 h) and subsequent chemical analysis. All samples were analysed for their nitrogen (%) content by Near Infra-Red spectroscopy (NIRS), according to the method of Eckard *et al.* (1988). The NIRS calibration was based on and regularly checked against the standard Kjeldahl method (Crude protein = nitrogen × 6,25) (AOAC, 1980). Nitrate-N analyses were performed using the standard nitrate electrode technique (Barker, 1974; Orion, 1979; Carlson & Schneider, 1986;

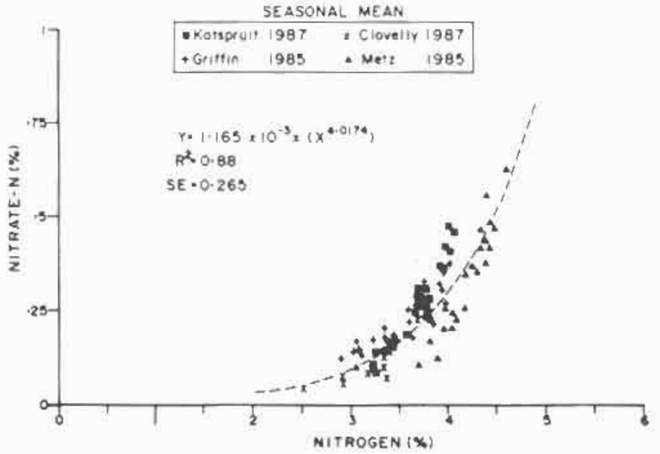

Figure 1 The relationship between the seasonal mean nitrate-N and nitrogen contents of Midmar ryegrass. Data are presented for four soil sites in different seasons (Griffin 1985, Metz 1985, Clovelly 1987 and Katspruit 1987).

Eckard, 1986) and reported as nitrate-N (%). All laboratory analyses are reported on a dry matter basis.

Results and Discussion

The relationship between nitrogen and nitrate-N content is illustrated in Figures 1 & 2, being the mean for the season (mean of 9 defoliations) and for selected defoliation dates in the season (April–December), respectively. The relationship between nitrogen and nitrate-N content, for all the sites, is shown in Figures 1 & 2. The relationship in Figure 1 is best described by the model $Y = a \times X^b$.

A similar relationship between nitrogen and nitrate-N to that presented in Figures 1 & 2 was found by ap Griffith (1960) for a number of temperate grasses. His findings show a consistent pattern of little or no accumulation of nitrate-N with increasing nitrogen levels, up to 3,2–3,5% nitrogen (20–22% crude protein), followed by a sharp (largely linear) increase in nitrate-N with little further increase in nitrogen content. Darwinkel (1975) reported a similar relationship in Italian ryegrass, with the point of accumulation of nitrate-N varying between 2,0 and 4,0% nitrogen, depending on the age of the plant, N application rate and the season of the year.

Darwinkel (1975) attributed the rapid accumulation of nitrate-N, relative to nitrogen, to the uptake of nitrate-N exceeding the demand by the plant for protein production. The reasons for this uptake/demand imbalance could be water stress, an excessive supply of N in the soil or the physiological limit of the protein production of the plant being exceeded (Darwinkel, 1975; Deinum & Sibma, 1980). In the present investigation, water stress was largely avoided by the irrigation of all plots.

In Figure 2 the relationship for all sites and seasons is shown at four defoliation dates for the growing season,

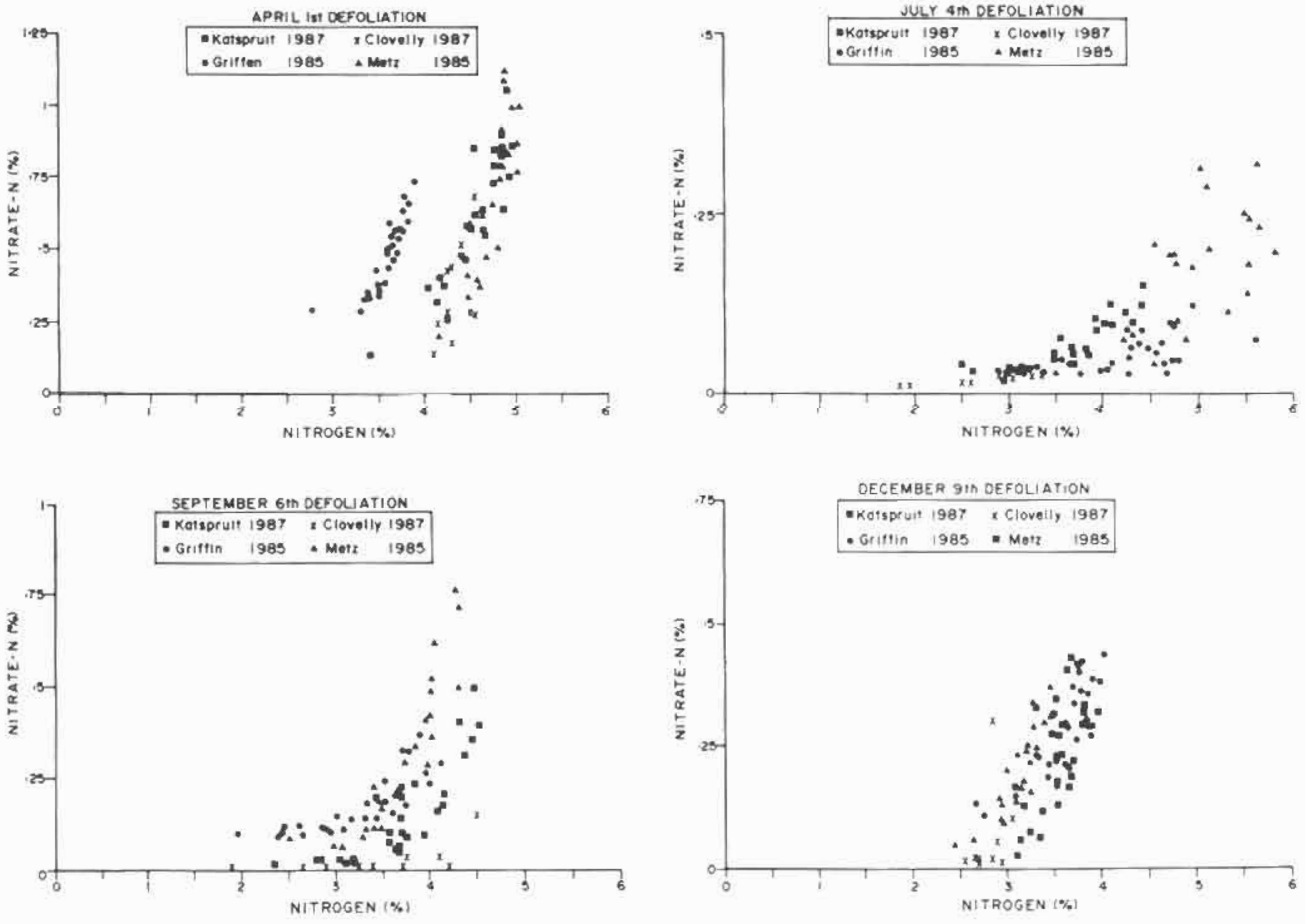

Figure 2 The relationship between the nitrate-N and nitrogen contents of Midmar ryegrass at four defoliation dates (April, July, September and December) for the growing season. Data are presented for four soil sites in different seasons (Griffin 1985, Metz 1985, Clovelly 1987 and Katspruit 1987).

representing four seasonal growth phases. The data from the Griffin site (April 1985, Figure 2a) show nitrate-N accumulation at lower nitrogen levels than the rest of the sites and seasons. This apparent accumulation of nitrate-N could have resulted from moisture stress at the Griffin site in April 1985. A number of problems were experienced in the initial stages of irrigation management on this site, which resulted in a reduced and erratic water supply. Such a low moisture supply would cause a nitrate-N accumulation at a lower than normal nitrogen level (Wright & Davison, 1964; Deinum & Sibma, 1980). As the extent of the moisture deficiency during this period was not known, reliable conclusions could not be drawn from the data, except to point out the possible effect of moisture stress on nitrate-N accumulation.

The data from the July defoliation (Figure 2b) show the plant accumulating higher nitrogen levels, relative to nitrate-N, than during the rest of the season. Jones *et al.* (1982) demonstrated the preferential uptake of ammonia (70–90% of total N) from the soil by ryegrass plants at root temperatures between 3 and 10°C. Low temperatures restrict nitrification in the soil and would therefore restrict the uptake of nitrate-N by the plant (Schmidt, 1982).

The data from Figures 1 & 2 appear to indicate a lower accumulation of nitrate-N in the herbage on the Clovelly site than on the other sites at all defoliation dates. The Clovelly site was topdressed with lower N levels than all the other sites. Nitrogen fertilizer was, therefore, never applied much in excess of the requirements of the plant (350 kg N/ha/yr; Eckard, 1989).

The general trend in the data presented in Figure 2 shows a decline in nitrate-N accumulation in the colder months (Figure 2b). With the warming of the season, from September to December, a greatly increased biomass of the plant was observed (data not shown), resulting in a relative dilution of the nitrogen content of the plant (Figure 2c & 2d).

The April and September defoliations (Figure 2a & 2c) appear to be periods when nitrate-N in the plant could reach levels potentially toxic to ruminants. The April defoliation, being the first defoliation of the season, represents a supply of soil N released in response to the working of the soil at planting. The September defoliation coincides with increased soil temperatures in early spring, which lead to increased mineralization of soil N as well as to the possible supply of unutilized fertilizer N built up during the winter fertilizer applications.

Although it is generally recognized that nitrate-N accumulation in forages may be potentially toxic to livestock, considerable disagreement exists regarding the concentrations of nitrate-N which result in lethal or sub-clinical toxicity in ruminants. Most literature agrees on a 'safe' limit of 0,21–0,35% nitrate-N in the dry matter, beyond which toxicity could occur either clinically or sub-clinically (McCreery *et al.*, 1966; Lawrence *et al.*, 1968; Lovelace *et al.*, 1968; White & Halvarson, 1980; Olsen & Kurtz, 1982). Some reports set these 'safe' limits as low as 0,07–0,15% nitrate-N (George *et al.*, 1973), while more recent investigations indicate that they may be as high as 0,57–0,60% nitrate-N (Coombe & Hood, 1980; Deinum & Sibma, 1980). Sheep grazing Midmar ryegrass pastures on the Cedara research station, with a nitrate-N content in excess of 1,2% nitrate-N, have shown positive mass gains with no apparent ill effects (Eckard, unpub. data, 1987. Pasture Science Section, Department of Agriculture and Water Supply, P.Bag X9059, Pietermaritzburg 3201). The validity of setting a 'safe' limit is questionable as toxicity levels vary widely with the type of animal, quantity of nitrate-N ingested, condition of the animal, previous diet of the animal, carbohydrate content of the diet and other factors (Wright & Davison, 1964; George *et al.*, 1973).

The most reliable means of expressing toxic, or sub-clinically toxic, nitrate-N levels would be in terms of dietary nitrate-N (g/kg live mass) levels required to produce death in a set percentage of a given animal population. The lethal dose (LD) required to produce death in 50% of the population is called the LD_{50} (Wright & Davison, 1964). The LD_{50} for nitrate-N toxicity in ruminants lies between 160 and 224 mg nitrate-N/kg live mass, when nitrate-N is a constituent of the herbage on offer (Wright & Davison, 1964). Calculating from these figures (a broad generalization using daily requirements for maintenance), the LD_{50} of nitrate-N for ruminants on a pure Midmar ryegrass diet (no supplements) lies between 0,65 and 0,90% nitrate-N. These levels of nitrate-N are well above the critical limit for plant growth of 0,15–0,25% nitrate-N, below which dry matter yield is restricted by a lack of N (Van Burg, 1966; Eckard, 1986). Nitrate-N levels in excess of this critical limit therefore indicate a wasteful and uneconomical use of N fertilizer.

The level of nitrogen in the diet appears to be of greater importance than nitrate-N. Wilman (1970), in a review of literature, indicates that nitrogen levels above 3% may cause adverse effects in ruminants. This agrees closely with the data of Bredon, Dugmore & Lesch (unpub. data, 1979. Animal Science Section, Department of Agriculture and Water Supply, P.Bag X9059, Pietermaritzburg 3201) who set this level at 3,2–3,5% nitrogen. The adverse effects of high nitrogen in the diet may be in the form of bloat (Hegarty, 1981), ammonia toxicity with consequent reduced feed intake, or a number of other minor disorders that lead to unthrifty animals as concluded by Hibbit (1984). Referring to the relationships presented in Figures 1 & 2, it may also be concluded that at nitrogen levels of 3,2–3,5%, potentially toxic levels of nitrate-N did not appear in the herbage. If the pasture was subjected to conditions predisposing the accumulation of high nitrate-N, without increasing the nitrogen content, i.e. moisture stress (Griffin site, Figure 2a), potentially toxic levels of nitrate-N could accumulate in the herbage at relatively low nitrogen levels. Nitrate-N toxicity in ruminants, grazing Midmar ryegrass pastures, would therefore occur only under conditions of stress to the pasture and the animal. Stress conditions can occur when animals are in poor condition, starved, unadapted to the pasture, or if N fertilizer is applied to a stressed pasture (i.e. moisture stress) at high rates in April or September (Eckard, 1986).

The soluble carbohydrate content of the forage is an important factor affecting the susceptibility of ruminants to both high nitrate-N and nitrogen levels in the diet. Ruminants are known to tolerate potentially toxic levels of nitrate-N or nitrogen in forages if the energy supply, required to assimilate and metabolize these nitrogenous fractions, is not limiting (Hibbit, 1984). Bryant & Ulyatt (1964) produced evidence to show that the soluble carbohydrate content of forages decreases with increasing N fertilization rate, nitrogen and total N in the herbage. If the energy supply is lacking in the diet, rumen function is impaired and the rumen rate of passage is slowed down, resulting in a reduced voluntary intake (Hibbit, 1984). It may be concluded that excessive nitrogen (above 3,5%) in the forage is likely to be uneconomical, not only because dry matter production is not stimulated when levels rise above this level (Eckard, 1986), but also because of reduced intake and toxicity in the animal which leads to a production loss.

In Figures 3 & 4 the relationship between nitrogen and dry matter yield, and nitrate-N and dry matter yield are presented, respectively. In both Figures 3 & 4, the dry matter yield approaches its maximum before an accumulation of nitrogen or nitrate-N occurs. The observed points in the lower ranges were therefore vital in describing the relationships presented.

From the data presented in Figure 3, as mentioned earlier, it would appear that nitrogen levels in excess of 3,2–3,5% may be in excess of the plant's requirement for growth, may be detrimental to the ruminant, and may indicate a wasteful use of N fertilizer. These limits are indicated by the vertical dashed lines in Figure 3. It is of interest to note that the level of nitrogen at which animal health may be adversely affected corresponds to a possible wasteful use of N fertilizer.

As mentioned earlier, Van Burg (1966) and Eckard (1986) reported that, as long as nitrate-N levels of 0,15–0,25% or above are maintained in the plant, dry matter yield is not restricted. These limits are indicated by the vertical dashed lines in Figure 4. It would appear that above these limits applied N fertilizer is in excess of the growth requirements of the plant and potentially toxic to ruminants, and therefore indicates a wasteful use of N fertilizer.

Conclusions

From the data presented, it would appear that N-related animal health problems on Midmar ryegrass pastures can be largely attributed to a wasteful use of N fertilizer. A number of poor management practices can, however, predispose ruminants to N-related problems. One of the most common mistakes is to allow unadapted and hungry animals free access to highly fertilized ryegrass pastures.

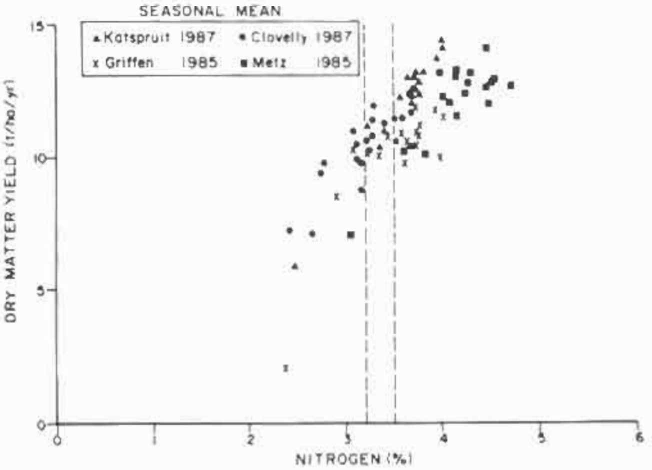

Figure 3 The relationship between the seasonal total dry matter yield and mean nitrogen content of Midmar ryegrass. Data are presented for four soil sites in different seasons (Griffin 1985, Metz 1985, Clovelly, 1987 and Katspruit 1987). Vertical lines indicate the 3,2–3,5% nitrogen limits.

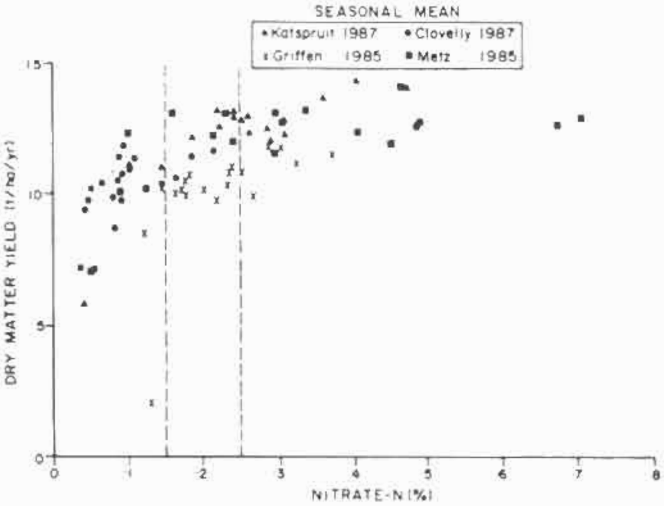

Figure 4 The relationship between the seasonal total dry matter yield and mean nitrate-N content of Midmar ryegrass. Data are presented for four soil sites in different seasons (Griffin 1985, Metz 1985, Clovelly 1987 and Katspruit 1987). Vertical lines indicate the 0,15–0,25% nitrate-N limits.

The merit of reducing N fertilizer applications during periods of potentially high nitrate-N and nitrogen accumulation requires investigation. Dry matter yields do not appear to be restricted by a reduction in N applications during these periods (April and September). The reduction of livestock and production losses caused by the build-up of toxic nitrogenous compounds during these periods could be avoided by a more strategic application of N fertilizer.

The reduction of N fertilizer application in the mid-winter period might represent a cost effective saving in colder areas where no mid-winter growth of Midmar ryegrass takes place. The merits of the above strategies are currently being investigated.

Acknowledgements

Dr N. Miles (Soil Science, Cedara) and Mr D. Naicker (Pasture Science, Cedara) are thanked for their cooperation and the analyses.

References

AOAC, 1980. *Official methods of analysis of the association of official analytical chemists*. W. Horwitz (ed.). 13th edn, Washington D.C. p.15.

Barker, A.V., 1974. Nitrate determinations in soil, water and plants. Research Bulletin no. 611. Massachusetts Agricultural experimental station. pp.6 & 22.

Bryant, A.M. & Ulyatt M.J., 1964. Effects of nitrogenous fertilizer on the chemical composition of short-rotation ryegrass and its subsequent digestion by sheep. *N.Z. J. Agric. Res.* **8:** 109–117.

Carlson, M.P. & Schneider, N.R., 1986. Determination of nitrate in forages by using selective ion electrode: Collaborative study. *J. AOAC.* **69:** 196–199.

Coombe, N.B. & Hood, A.E.M., 1980. Fertilizer-nitrogen: effects on dairy cow health and performance. *Fert. Res.* **1:** 157–176.

Darwinkel, A., 1975. Aspects of assimilation and accumulation of nitrate in some cultivated plants. Agric. research reports no. 843. Pudoc, Wageningen: Centre for agric. publishing and documentation.

Deinum, B. & Sibma, L., 1980. Nitrate content of herbage in relation to nitrogen fertilization and management. In: *The role of nitrogen in intensive grassland production*. W.H. Prins & G.H. Arnold (eds). Proc. int. Symp. Eur. Grassl. Fed. Pudoc, Wageningen, the Netherlands. pp.95–100.

Eckard, R.J., 1986. The nitrogen nutrition of Italian ryegrass (*Lolium multiflorum*). M.Sc. thesis, University of Natal.

Eckard, R.J., Miles, N. & Tainton, N.M., 1988. The use of Near Infra-Red spectroscopy for the determination of plant nitrogen. *J. Grassl. Soc. South. Afr.* **5**(3): 175–177.

Eckard, R.J., 1989. The response of Italian ryegrass to applied nitrogen in the Natal midlands. *J. Grassl. Soc. South. Afr.* **6**(1): 19–22.

Eckard, R.J., 1990. The effect of source of nitrogen on the dry matter yield, nitrogen and nitrate-N content of *Lolium multiflorum*. *J. Grassl. Soc. South. Afr.* **7**: In press.

George, J.R., Rhykerd, C.L., Noller, C.H., Dillon, J.E. & Burns, J.C., 1973. Effect of N fertilization on D.M. yield, total-N, N recovery and nitrate-N concentration of three cool-season forage grass species. *Agron. J.* **65**: 211–216.

Griffith, G., 1960. Nitrate content of herbage at different manurial levels. *Nature* **185**: 627–628.

Hegarty, M.P., 1981. Deleterious factors in forages affecting animal production. In: *Nutritional limits to animal production from pastures*. J.B. Hacker (ed.). Proc. int. Symp. St. Lucia, Queensland, Australia: CSIRO, Commonwealth Agric. Bureau. pp.133–146.

Hibbit, K.G., 1984. Effect of protein on the health of dairy cows. In: *Recent advances in animal nutrition - 1984*. W. Haresign & D.J.A. Cole (eds). London: Butterworths. pp.189–200.

Jones, L.H.P., Hopper, M.J., Hatch, D.J. & Clarkson, D.T., 1982. Uptake of ions from solutions of controlled composition. Grassland Research Institute Annual Report, 1981. pp.19–20.

Lawrence, T., Warder, F.G. & Ashford, R., 1968. Nitrate accumulations in intermediate wheatgrass. *Can. J. Plant Sci.* **48**: 85–88.

Lovelace, D.A., Holt, E.C. & Anderson, W.B., 1968. Nitrate and nutrient accumulation in two varieties of bermuda-grass (*Cynodon dactylon*) as influenced by soil-applied fertilizer nutrients. *Agron. J.* **60**: 551–554.

McCreery, R.A., Hojjati, S.M. & Beatty, E.R., 1966. Nitrates in annual forages as influenced by frequency and height of clipping. *Agron. J.* **58**: 381–382.

Olsen, R.A. & Kurtz, L.T., 1982. Crop nitrogen requirements, utilization and fertilization. In: *Nitrogen in agricultural soils*. F.J. Stevenson (ed.). Agronomy no. 22. Madison, Wisconsin. pp.567–604.

Orion Research Inc., 1979. Instruction manual; nitrate electrode. Massachusetts, U.S.A.

Schmidt, E.L., 1982. Nitrification in soil. In: *Nitrogen in agricultural soils*. F.J. Stevenson (ed.). Agronomy no. 22. Madison, Wisconsin. pp.253–288.

Van Burg, P.F.J., 1966. Nitrate as an indicator of the nitrogen-nutrition status of grass. Proc. X Int. Grassl. Cong. Helsinki, Finland. pp.267–272.

White, L.M. & Halvarson, A.D., 1980. Nitrate levels in vegetative and floral tillers of western wheatgrass and green needlegrass as affected by nitrogen fertilization. *Agron. J.* **72**: 143–148.

Wilman, D., 1970. The effect of nitrogenous fertilizer on the rate of growth of Italian ryegrass. 3. Growth up to ten weeks. *J. Br. Grassl. Soc.* **25**: 242–245.

Wright, M.J. & Davison, K.L., 1964. Nitrate accumulation in crops and nitrate poisoning in animals. *Adv. Agron.* **16**: 197–247.