ALCOHOL-SOLUBLE CARBOHYDRATES IN VARIOUS TROPICAL AND TEMPERATE PASTURE SPECIES

A. NOBLE* and K. F. LOWE†

ABSTRACT

Variation in total alcohol-soluble carbohydrate concentration of seven pasture species was studied over a 24-month period. The highest levels of carbohydrate (8.0%-9.0%) *were found in the temperate grasses* (Bromus unioloides *cv. Priebe and* Lolium perenne × L. multiflorum *cv. Grasslands Manawa) during the months December to January and May to August. One of the tropical grasses,* Setaria anceps (*CPI* 32930), *maintained a level of between* 2.5% *to* 4.0% *total alcohol-soluble carbohydrate through most of the period. However,* Panicum maximum *cv. Gatton had much lower percentages, especially in winter when the foliage was damaged by frost.*

The temperate legume Trifolium repens *cv. Ladino maintained high levels of total alcohol-soluble carbohydrate* (3.0%-9.0%). *The carbohydrate levels in the tropical legumes* (Desmodium intortum *cv. Greenleaf and* Glycine wightii *cv. Tinaroo) were lower than those of* T. repens *in summer, the differences increasing in autumn and winter.*

Application of nitrogen fertilizer did not increase carbohydrate levels in grasses above those attained in grass/legume pastures.

INTRODUCTION

There is considerable evidence that the digestibility of tropical grasses and legumes is lower than that of temperate species (Milford and Minson 1966). Hamilton, Lambourne, Roe and Minson (1970) and Stobbs (1972) reported that milk production from unsupplemented tropical pastures is lower than from temperate species at a similar stage of growth. Low intake of digestible energy is suggested as the main cause of this low production. The carbohydrate composition is one of the factors which influences digestibility (Wilson and Ford 1971, 1973). As utilization of feed depends on the readily fermentable carbohydrate content, considerable interest is being shown in the comparative levels of carbohydrate in herbage species.

In ryegrass this readily fermentable carbohydrate fraction was mainly alcohol-soluble carbohydrates (Bailey 1964). However in a comparative study Wilson and Ford (1973) found that there was also a considerable amount of the 100°C water-soluble fraction in ryegrass grown under controlled environment conditions. In the tropical species they found that alcohol-soluble carbohydrates constituted the largest fraction of soluble carbohydrates.

Wilson and Ford (1971) demonstrated that over a range of growth temperatures ryegrass generally had higher levels of soluble carbohydrate and lower levels of starch than the tropical species *Setaria anceps* and *Panicum maximum*. There was also a greater difference between the tropical and temperate grasses in levels of alcohol-soluble, than in levels of water-soluble carbohydrates. In a recent experiment, Wilson and Ford (1973) found that levels of 100°C water-soluble carbohydrate showed much larger variation in temperate compared with tropical grasses. Alcohol-soluble carbohydrate levels showed less variation than in their previous work.

*Dept. of Primary Industries, Indooroopilly. 4068.
†Dept. of Primary Industries, Ipswich. 4305.

In this investigation the pattern of alcohol-soluble carbohydrate levels of a range of tropical and temperate grasses and legumes, grown under irrigation, was studied. The alcohol-soluble carbohydrate fraction was chosen as it showed least variation under different climatic conditions and cultural practices.

MATERIALS AND METHODS

The experiment was located on a black earth soil at the Gatton Research Station in south-east Queensland. Since levels of all nutrients were extremely high at the commencement no establishment fertilizer was used. A maintenance dressing of 112 kg ha^{-1} of "superphosphate with sulphur"* (7.6% P, 25.8% S) and 112 kg ha^{-1} of "pasture mixture 1"* (1.75% N, 8.3% P, 8.3% K) with copper (0.25% Cu), zinc (0.24% Zn), molybdenum (0.03% Mo) was applied in November, 1969.

A randomized block design with five replications was used. Plot size was 10.7 m × 10.7 m. Tropical and temperate pasture treatments were able to be irrigated separately. Irrigation timing was determined by readings from gypsum conductance blocks situated under two selected treatments in each replicate.

Table 1 lists the ten simple pasture mixtures, four containing temperate species and six containing tropical species, which were sampled for alcohol-soluble carbohydrates.

TABLE 1

The 10 *Grass* × *Legume or Nitrogen Combinations*

Grass	*Nitrogen source*
Bromus unioloides cv. Priebe (prairie grass)	*Trifolium repens* cv. Ladino (white clover)
Bromus unioloides cv. Priebe	336 kg ha^{-1} N as urea
Lolium perenne × *L. multiflorum* cv. Grasslands Manawa (ryegrass)	*Trifolium repens* cv. Ladino
Lolium perenne × *L. multiflorum* cv. Grasslands Manawa	336 kg ha^{-1} N as urea
Setaria anceps CPI 32930 (setaria)	*Desmodium intortum* cv. Greenleaf (Greenleaf desmodium)
Setaria anceps CPI 32930	*Glycine wightii* cv. Tinaroo (Tinaroo glycine)
Setaria anceps CPI 32930	336 kg ha^{-1} N as urea
Panicum maximum cv. Gatton (Gatton panic)	*Desmodium intortum* cv. Greenleaf
Panicum maximum cv. Gatton	*Glycine wightii* cv. Tinaroo
Panicum maximum cv. Gatton	336 kg ha^{-1} N as urea

The pasture was sampled at approximately monthly intervals. Grass tillers were cut 2.5 cm above ground level from 30 random positions in each treatment. In the case of legumes whole runners (to the base of crowns) were sampled. Sampling took place in mid morning and cut material was treated in the field laboratory within thirty minutes. From September 1968 until September 1969 all replicates were analysed separately; subsequently samples were bulked to give two composite duplicates. Legumes from the ryegrass/white clover, setaria/Greenleaf desmodium and setaria/Tinaroo glycine were analysed during the period February to October, 1970. Following each sampling the experiment was mown and raked.

In the field laboratory samples were cut into 1 cm lengths and 20 g of this material were treated with 200 ml of boiling 95% ethanol to prevent post harvest respiration. Loss of simple carbohydrates by enzymatic breakdown during respiration has been demonstrated by Waite and Boyd (1953).

---

*Proprietary fertilizers.

In the chemical laboratory the samples were macerated in a blender. Three half-hour extractions with 95% ethanol were conducted on this material. Appropriate aliquots of the extract were treated with water to remove the alcohol. The water solution was then clarified using a method based on that of Hassid (1936) but modified by using charcoal as the clarifying agent.

Reducing carbohydrates were determined by oxidation with potassium ferricyanide based on the method of Hoffman (1937). In the determination of total alcohol-soluble carbohydrates, an initial hydrolysis step to convert all carbohydrates to the reducing form preceded the oxidation step with potassium ferricyanide. Acid hydrolysis was achieved using 0.05N sulphuric acid for thirty minutes on a steam bath; the solution was then neutralized with NaOH. Results were expressed as percentage glucose on a moisture-free basis. The same analytical technique was used for both grasses and legumes.

RESULTS

Climate

During the experimental period of 24 months extremes of rainfall and temperature occurred, thus enabling the effects of these on carbohydrate levels to be studied.

Figure 1 presents the rainfall and temperature data at the site during the experimental period. Even with irrigation there were periods of possible soil water deficit. Details of the irrigation schedules are published elsewhere (Lowe, in press). Summer and winter temperatures during the first year were above average; only August and September exhibited normal winter temperatures. Colder conditions

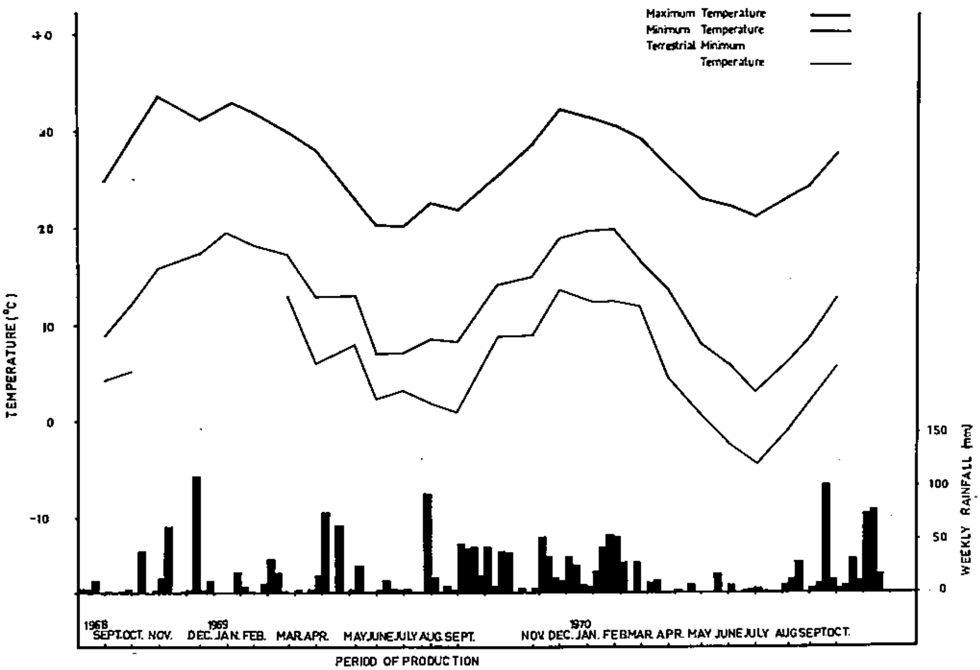

FIGURE 1

Maximum, minimum, and terrestrial minimum temperatures at Gatton for the experimental period September 1968 to October 1970.

were experienced in the second winter when considerably more frosts were recorded. For 5 weeks during midwinter, weekly terrestrial minimum temperatures were below —4°C with daily readings as low as —11°C.

Cloud cover data was collected for each sampling day and for the preceding 3 days. Cloud cover was rarely heavy and thus was of little value for interpreting differences in carbohydrate levels.

Effect of Nitrogen and Legumes on Carbohydrate Levels of Grasses

There was no consistent difference in total alcohol-soluble carbohydrate levels of grasses grown in association with a legume or fertilized with nitrogen (Table 2). In addition, the levels of total alcohol-soluble carbohydrate did not show a consistent response immediately after the application of nitrogen fertilizer to pure grass stands (data not presented). Similarly, there was no consistent trend in reducing carbohydrate levels (data not presented).

TABLE 2

Total alcohol-soluble carbohydrate percentage in grasses at sampling dates when significant differences were measured between grasses grown with a legume or fertilized with nitrogen

		% Total alcohol-soluble carbohydrate		
Grass	Cutting Date*	Grass with legume	Grass with nitrogen	L.S.D. (5%)
Prairie grass	1.x.68	6.08	4.37	1.04
	18.vi.69	6.49	4.35	1.08
	15.xii.69	4.08	2.93	1.12
	10.ii.70	4.29	2.95	1.07
Ryegrass	7.v.69	4.07	5.29	0.65
	18.vi.69	8.76	7.61	1.08
Setaria (with Tinaroo glycine)	6.viii.69	3.23	4.57	0.97
Setaria (with Greenleaf desmodium)	3.ix.70	1.88	3.16	1.22
	1.x.70	1.52	3.29	1.38
Gatton panic	—	—	—	N.S.†

*Differences not significant at other harvest dates
†Gatton panic mixtures showed no significant differences throughout the experimental period

Accordingly, the data from pasture mixtures containing similar grasses have been combined; from now on only these average values will be presented and discussed.

Comparison of Carbohydrate Levels in Tropical and Temperate Grasses

Total alcohol-soluble carbohydrate levels of temperate grasses (ryegrass and prairie grass) were higher than those of the tropical grasses (Gatton panic and setaria) over most of the experimental period (Figure 2). During winter* and spring the levels in temperate grasses were significantly higher ($P < 0.01$).

Total alcohol-soluble carbohydrate levels in ryegrass varied from 3.0% to 9.5% while those of prairie grass varied from 2.5% to 8.5%. Comparative levels in the tropical grasses varied from 1.5% to 4.5% for setaria and from 0.2% to 3.5% for Gatton panic. In general, the seasonal variation of carbohydrate levels was smaller in the tropical grasses than in the temperate grasses.

*Winter = June-August Summer = December-February
Spring = September-November Autumn = March-May

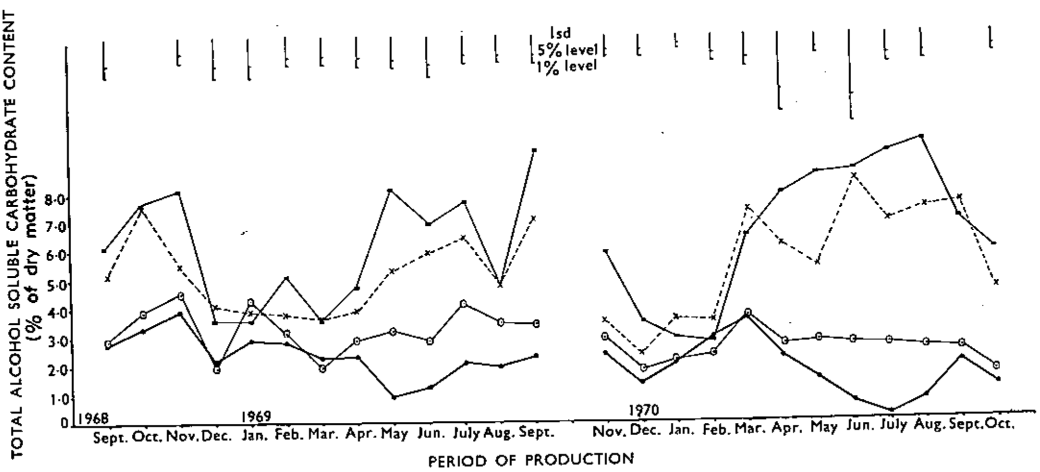

FIGURE 2

Levels of total alcohol soluble sugars extracted from various pasture grasses defoliated monthly. *Bromus unioloides* cv. Priebe x-----x; *Lolium perenne* × *L. multiflorum* cv. Gr. Manawa ■———■: *Setaria anceps* C.P.I. 32930 ⊙———⊙; *Panicum maximum* cv. Gatton •———•.

Similar trends were recorded in the reducing carbohydrate content of the various tropical and temperate grasses.

Pattern of Carbohydrate Levels in Grasses

Total alcohol-soluble carbohydrate levels of setaria were generally greater than those of Gatton panic and showed less seasonal variation. The general pattern exhibited by both species showed peak levels attained in late summer and spring. Both grasses demonstrated a drop in levels in early summer. The patterns exhibited in both years by Gatton panic agreed more closely than those of setaria.

Although there were few significant differences between levels in setaria and Gatton panic in summer, larger differences were recorded in autumn and winter. Whereas levels increased in setaria under the colder growing conditions, those in Gatton panic dropped sharply.

In the temperate grasses levels were high in late spring and mid-winter but low in summer and early autumn. Although a similar pattern was generated in both years it was about one month later in the second year.

Pattern of Carbohydrate Levels in Legumes

Both tropical legumes showed a similar pattern of total alcohol-soluble carbohydrate content (Figure 3), although Tinaroo glycine achieved higher levels in autumn. During winter, frosting reduced carbohydrate levels significantly in both legumes. No material was available for sampling during the period July to September as frosted material had been removed by post-sampling defoliation.

The total alcohol-soluble carbohydrate pattern for white clover resembled that of the tropical legumes until late autumn; however, levels were very much higher especially in the winter and spring period.

Except in the summer, there was very little difference between levels of total and reducing carbohydrates of all three legumes.

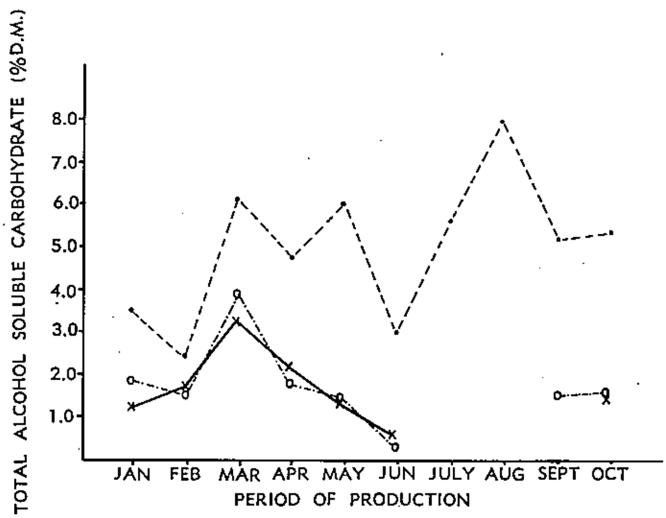

FIGURE 3
Total alcohol soluble carbohydrate content of selected tropical and temperate legumes grown in mixed pastures defoliated monthly.
Trifolium repens cv. Ladino •----•; *Desmodium intortum* cv. Greenleaf ×———×; *Glycine wightii* cv. Tinaroo ○—·—·—○.

DISCUSSION

The temperate grasses, ryegrass and prairie grass, had the highest total alcohol-soluble carbohydrate content throughout the experimental period. This result agrees with the data of Wilson and Ford (1971, 1973) which showed these differences to occur over a range of temperature regimes. However, the values for total alcohol-soluble carbohydrates in ryegrass obtained in our experiment (2.5%-11%) were lower than those obtained by Wilson and Ford (1971) of 8%-11% and Wilson and Ford (1973) of 6.9%-14.2%. Values obtained for setaria showed similar differences 0.5%-4.5% and 7%-11% respectively. Wilson and Ford (1973) obtained values of 7.5% to 12% for *Panicum maximum* var. *trichoglume*. However their research was conducted in controlled environment rooms where temperature extremes were smaller and more regular than those experienced in the field. Under field conditions in New Zealand, which are closer to optimum for temperate species than those of south-east Queensland, Bailey (1964) obtained similar values to those of Wilson and Ford.

Smith and Grotelueschen (1966) found mean seasonal levels of reducing alcohol-soluble carbohydrates of 6.6% for perennial ryegrass and 2.0% to 2.5% for *Bromus* sp. These are similar to our experimental values of 3.0%-4.8% for ryegrass and 2.5% to 3.8% for prairie grass.

The carbohydrate values obtained by other authors and quoted for comparative purposes were based on a 90% ethanol extraction of freeze-dried (Bailey 1964) and oven-dried (Smith and Grotelueschen 1966) material. The fresh material in this experiment was extracted with 95% ethanol, which would equate to extraction of dried material with 88% ethanol (Smith and Grotelueschen 1966).

Our experiment showed considerable differences between species in levels of total alcohol-soluble carbohydrates. Ryegrass attained significantly higher carbohydrate levels in autumn and winter. There were also differences between seasonal levels in the tropical grasses; levels of carbohydrate in setaria were generally higher than those of Gatton panic over the entire period, and these differences were emphasised during winter. It is possible that these are intrinsic differences dependent on the altitude and latitude of the region from which the plant material was originally collected.

The tropical grasses reacted differently to frosting. *Setaria anceps* CPI 32930 was an introduction chosen for its ability to withstand frost damage and this attribute was well demonstrated under the conditions experienced during the experiment. Gatton panic showed a rapid decline in carbohydrate levels at the onset of winter in both years; this coincided with severe leaf damage from frost and subsequent leaching by rainfall and irrigation. On the other hand, total alcohol-soluble carbohydrate levels in setaria increased during the mild winter in 1969. In the severe second winter some minor leaf damage occurred in setaria together with a slight drop in carbohydrate levels.

The lack of response of total alcohol-soluble carbohydrate levels in all grasses to nitrogen applications generally agrees with findings of Nowakowski (1962 and 1969) with Italian ryegrass (*Lolium multiflorum*). However, he did find that other carbohydrate fractions were affected (Nowakowski 1962). As other carbohydrate fractions were not investigated in the present experiment, it was not possible to determine if other fractions had been influenced by nitrogen application. Although nitrogen reduced the level of soluble carbohydrate in temperate grass foliage (McIlroy 1967) there was a corresponding increase in nitrogen content, resulting in little net change in the cell constituents.

There was no evidence in this current experiment to indicate that there was any inverse relationship between levels of alcohol-soluble carbohydrate and levels of nitrogen (Lowe, in press).

Mackenzie and Wylam (1957) have shown that low light intensity causes lower levels of various carbohydrate fractions in plants. Also, Wilson and Ford (1971) have demonstrated that growth temperatures can influence carbohydrate levels in both tropical and temperate grasses. However, from data presented on rainfall and temperature plus data on cloud cover (Lowe in press) it is not possible to readily distinguish specific climatic effects on carbohydrate levels, as these all interact in the field situation.

To our knowledge there has been no published work on the alcohol-soluble carbohydrate levels of tropical legumes. The values for white clover of 2.5% in late autumn and 8.0% in winter agree with results published by Bailey (1964) in New Zealand of 4.5% and 11.4% for the same periods. The pattern of carbohydrate level also agreed closely with that of Bailey.

The similarity of reducing and total alcohol-soluble carbohydrate levels in white clover during winter suggests that during this period white clover converts non-reducing carbohydrate to reducing carbohydrate. A similar conversion also seems likely in Tinaroo glycine and Greenleaf desmodium during late autumn and early spring.

Low levels of water-soluble carbohydrate have been measured in temperate species (Bailey 1964) and in Nandi setaria (Anonymous 1971). On the other hand, very high levels of water-soluble carbohydrates were reported in other work on temperate species (Bowden, Taylor and Davies 1968, Wilson and Ford 1971, 1973). Wilson and Ford (1971, 1973) also report considerable levels of water-soluble carbohydrates in tropical grasses, although their work was confined to controlled environment conditions and on very young material. It is indeed possible that there are considerably higher levels of carbohydrate in temperate plants than is indicated by the alcohol-soluble carbohydrate levels measured here. The evidence suggests however, that in tropical species, under field conditions alcohol-soluble carbohydrates constitute a large part of the readily utilisable energy.

This study was part of a wider investigation to determine the value of tropical species as components of irrigated pastures. The majority of irrigable land in south-east Queensland occurs on alluvial flats which are subject to severe frosts in winter. Traditionally, irrigated pastures have been based on temperate species; if tropical species are to be successfully integrated, they must show superiority in at least some characteristics. Setaria maintained reasonable levels of alcohol-soluble carbohydrate in summer and winter. Although of lower quality than the temperate grasses, its value in terms of increased dry matter and nitrogen yield together with higher efficiency of water use (Lowe, in press) would compensate and make it a valuable irrigated pasture component. In terms of alcohol-soluble carbohydrate levels however, Gatton panic and the tropical legumes make a poor contribution to the feeding value of irrigated pastures, especially in autumn and winter. Hence, they would be of doubtful value in this situation.

ACKNOWLEDGEMENTS

Thanks are due to the Biometry Branch of the Department of Primary Industries, Queensland for the statistical analyses. The project was supported by the Australian Dairy Produce Board.

REFERENCES

ANON (1971)—Annual Report 1970-71, Division of Tropical Pastures, C.S.I.R.O., Brisbane p. 57.

BAILEY, R. W. (1964)—Pasture quality and ruminant nutrition. I. Carbohydrate composition of ryegrass varieties grown as sheep pasture. *New Zealand Journal of Agricultural Research* **7**: 496-507.

HAMILTON, R. I., LAMBOURNE, L. J., ROE, R., and MINSON, D. J. (1970)—Quality of tropical grasses for milk production. Proceedings of the XIth International Grasslands Congress, Surfers Paradise pp. 860-864.

HASSID, W. Z. (1936)—Determination of reducing sugars and sucrose in plant materials. *Industrial and Engineering Chemistry*, Analytical Edition **8**: 138-140.

HOFFMAN, W. S. (1937)—A rapid method for the determination of glucose in blood and urine. *Journal of Biological Chemistry* **89**: 51-55.

LOWE, K. F. (in press)—The growth, nutritive value and water use of irrigated pasture mixtures at Gatton, south-east Queensland. Master of Agricultural Science Thesis, University of Queensland.

MCILROY, R. J. (1967)—Carbohydrates of grassland herbage. *Herbage Abstracts* **37**: 79-87.

MACKENZIE, D. J., and WYLAM, C. B. (1957)—Analytical studies on the carbohydrates of grasses and clover. VIII. Changes in carbohydrate composition during the growth of perennial ryegrass. *Journal of the Science of Food and Agriculture* **8**: 38-45.

MILFORD, R. and MINSON, D. J. (1966)—The feeding value of tropical pasture. "Tropical Pastures" (Ed. W. Davies and C. L. Skidmore) Faber and Faber, London pp. 106-114.

NOWAKOWSKI, T. Z. (1962)—Effects of nitrogen fertilizers on total nitrogen, soluble nitrogen and soluble carbohydrate content of grass. *Journal of Agricultural Science* **59**: 387-392.

NOWAKOWSKI, T. Z. (1969)—Effects of nitrogen and potassium fertilizers on contents of carbohydrate and free amino acids in Italian ryegrass. I. Effects on growth and soluble carbohydrate contents of leaves, stubble and roots. *Journal of the Science of Food and Agriculture* **20**: 666-670.

SMITH, D. and GROTELUESCHEN, R. D. (1966)—Carbohydrates in grass. I. Sugar and fructosan composition of the stem bases of several northern adapted grasses at seed maturity. *Crop Science* **6**: 263-266.

STOBBS, T. H. (1972)—Suitability of tropical pasture for milk production. *Tropical Grasslands* **6**: 67-69.

WAITE, R. and BOYD, J. (1953)—The water-soluble carbohydrates of grasses. I. Changes occurring during the normal life-cycle. *Journal of the Science of Food and Agriculture* **4**: 197-204.

WILSON, J. R. and FORD, C. W. (1971)—Temperature influences on the growth, digestibility, and carbohydrate composition of two tropical grasses, *Panicum maximum* var. *trichoglume* and *Setaria sphacelata*, and two cultivars of the temperate grass *Lolium perenne*. *Australian Journal of Agricultural Research* **22**: 563-571.

WILSON, J. R. and FORD, C. W. (1973)—Temperature influences on the in vitro digestibility and soluble carbohydrate accumulation of tropical and temperate grasses. *Australian Journal of Agricultural Research* **24**: 187-198.

(Accepted for publication October 14, 1974)