# CHAPTER 1

# CREEPING BENTGRASS AND ANNUAL BLUEGRASS NON-STRUCTURAL CARBOHYDRATE RESPONSE TO IRRADIANCE QUANTITY AND QUALITY

## Introduction

Carbohydrate levels have been evaluated as a factor in various types of turfgrass winterkill (Dionne et al., 2000; and Rossi and Buelow, 1997). It has been suggested that carbohydrates may act as cryoprotectant agents that increase plant hardness (Levitt, 1980; Olien and Lester, 1985; and Tognetti et al., 1990).

Most of the research pertaining to carbohydrate levels and freezing tolerance has been done in small grain crops (McKersie et al., 1982; Olien and Lester, 1985; and Tognetti et al., 1990) and forages (Guinchard et al., 1997; Turner and Pollock, 1998), although a few studies have been done to turfgrasses. Dionne et al. (2001) observed a significant increase in crown fructan levels in cold acclimated annual bluegrass plants, but the increase did not correlate with increased freezing tolerance. In contrast, Fry et al. (1993) reported a strong correlation ($r = 0.78$) between stolon sucrose levels and cold tolerance of centipedegrass.

Irradiance quality and quantity can affect turfgrass carbohydrate levels (Weston and Rossi, 2000; Wu and Torries, 1988); and therefore, the effects of irradiance may also affect freezing and ice coverage tolerance. Decreased carbohydrate production, especially during the hardening period, may decrease tolerance to ice encasement damage. The objectives of this study were to determine if selected irradiance sources and temperature treatments differentially affected the total non-structural carbohydrate (TNC) levels of creeping bentgrass and annual bluegrass selection crowns and verdure.

## Materials and Methods

Plant material in this experiment was grown in 24 mm diameter by 124 mm long plastic root tubes in Hagerstown silt loam soil. The soil was steam sterilized to decrease weed infestations at 82 C for four hours prior to use. Tubes were planted with 4-5 tillers of creeping bentgrass or annual bluegrass.

The annual bluegrass was selected from a putting green at the Village Green Golf Course in Hickory, PA. Selections were made following a winter in which extensive ice damage occurred to the putting green. Annual bluegrass selections were cultivated from areas that exhibited either good (VG18A) or poor (VG18D) tolerance to the ice coverage. 'Penncross' and 'Penn A-4' creeping bentgrass selections were removed from 3-yr-old field plots maintained as a putting green at the Valentine Turfgrass Research Center, University Park, PA.

Turf was clipped with scissors as needed to maintain heights at approximately 4 to 5 mm. Soluble fertilizer (28-7-14) was sprayed at 97 kg N/ha at a delivery rate 374 l/ha. Fertilizing was applied to minimize growth and was approximately 291 kg N/ha/year. Fertilizer applications were lightly watered in after application to remove the spray solution from the foliage. Tubes were placed in shallow trays that were filled with water to provide ample moisture for growth.

Three Conviron (Model #CMP3244) growth chambers were used to provide three different irradiance levels with an 8-hr photoperiod and high or low temperature treatments (Table 1.1). Non-treated control tubes were maintained in a greenhouse under natural irradiance conditions. Irradiance measurements were made with a Licor 1000 data logger equipped with a Licor LI-190SA quantum sensor measuring the photosynthetically active radiation (PAR), and radiation spectral distribution of the light sources was measured with a Licor 1800 spectro-radiometer (Fig. 1.1).

Table 1.1. Irradiance and temperature treatments.

| Treatment | Light Source | Light Intensity | Air Temp C |
|---|---|---|---|
| | | ($\mu$mols $m^{-2}$ $s^{-1}$) | (day/night) |
| High light-high temp | Incandescent/florescent | 266 | 18/10 |
| High light-low temp | Incandescent/florescent | 266 | 10/3 |
| Low light-high temp | Metal halide | 114 | 18/10 |
| Greenhouse | Sunlight | 684 (full sun at noon) | 21/14 |

Two root tubes of each bentgrass or annual bluegrass selections were placed in their respective treatment environment for 35 days. Two tubes of each selection per rep were used to provide enough tissue for TNC analysis. Tubes were randomized within each treatment and placed in trays containing approximately 3 cm depth of water.

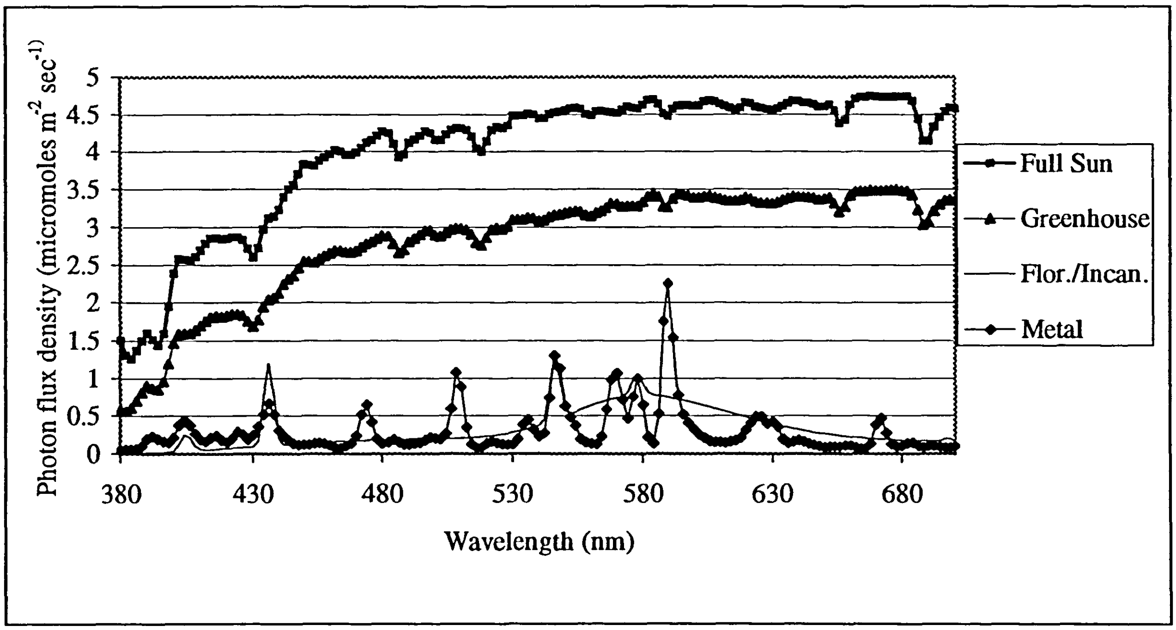



Fig. 1.1. Spectral distribution of photosynthetic photon flux densities of selected irradiance sources.

At the end of the treatment periods, tubes were removed from the growth chambers; and the turf plugs were removed from the tubes. The roots were washed with water to remove the root-zone material; and the turf was separated into small sections using a scalpel to remove the shoots and roots from the crowns. The remaining crowns included a short section of shoots and roots and were approximately 5 mm in length. The crown and shoot samples of the duplicate selection tubes were combined and placed in coin envelopes for drying.

The samples were placed in a 100 C-drying oven for 1 hour and then into a 65 C-drying oven for 24 hours. Samples were reduced with an Udy Corporation Cyclone mill using a 60-mesh screen and placed in 25 cc glass scintillation vials with screw-on caps. Samples were analyzed by the West Virginia University Rumen Profiling Laboratory for TNC contents using the method described by Smith et al. (1964).

Treatments were arranged in a completely random design with three replicates in a two species by two selections by four irradiance levels complete factorial. The trial was repeated (experiment 1 – Sept. 22, 2001-Jan. 19, 2001; experiment 2 – Jan. 21, 2001-Apr. 24, 2001), and variances were homogeneous across trials (Levene, 1960), so the data were pooled and analyzed using mixed models analysis (Littell et al., 1996) with the mixed procedure of SAS (1999). Trials and replicates were considered random effects.

## Results and Discussion

### Comparison of Crown TNC Concentrations by Species

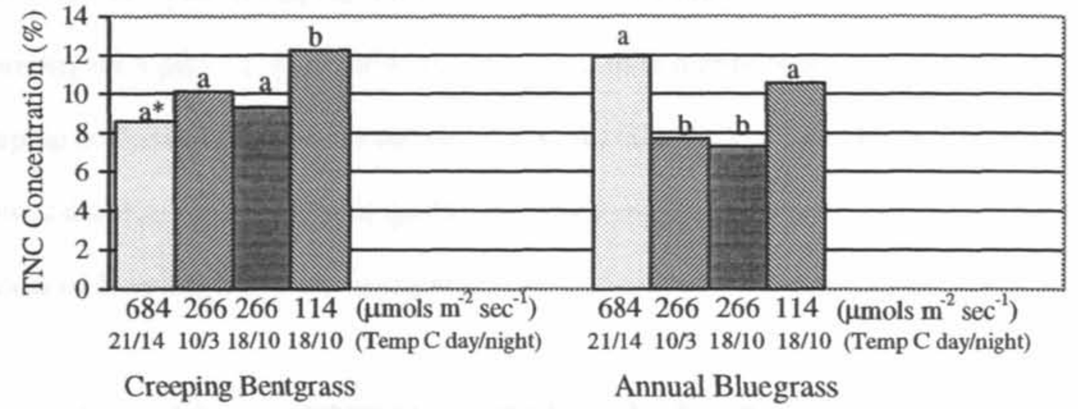
There were distinct visual differences between species after the treatments were completed. The annual bluegrass plants under the incandescent/florescent lights (266 μmols $m^{-2}$ $sec^{-1}$) at both the 18/10 C and 10/3 C (day/night temps) were very chlorotic, and stems and leaves were thin and upright. The plants were also difficult to separate due to a loss of tissue structural integrity. The creeping bentgrass plants became elongated under the artificial irradiance treatments, but maintained prostrate growth and a dark green color.

The treatment by species interaction for the crown TNC concentrations was significant (Table 1.2). Creeping bentgrass and annual bluegrass crown TNC concentrations had opposite responses to decreasing irradiance levels.

Table 1.2. Analysis of variance for crown TNC concentrations of creeping bentgrass and annual bluegrass exposed to selected levels of irradiance and temperature treatments.

| Source | df | F-Value | Pr > F |
|---|---|---|---|
| Treatment | 3 | 7.07 | 0.0003 |
| Species | 1 | 1.95 | 0.1668 |
| Treatment * species | 3 | 6.43 | 0.0006 |
| Treatment * selection (species) | 8 | 0.98 | 0.4590 |
| Error mean square = 6.5, df = 75 | | | |

The crown TNC concentrations for creeping bentgrass under the lowest irradiance level was significantly higher than the other three irradiance treatments (Fig. 1.2). The ability of creeping bentgrass to maintain TNC levels under varying irradiance levels was also reported by Bell and Danneberger (1999). They reported that there were no significant differences in creeping bentgrass TNC levels between full sun and perpetual shade treatments. They suggested that creeping bentgrass may have the ability to conserve carbohydrates by limiting growth in shaded conditions. Interestingly, they also reported that creeping bentgrass in perpetual shade had the highest TNC average, which is similar to the response observed in this study where the lowest irradiance treatment (114 $\mu$mols $m^{-2}$ $sec^{-1}$) provided the highest crown TNC average in creeping bentgrass.




*Bars within the same group with the same letter are not significant different according to the Least Squares Means Test with $p = 0.05$.

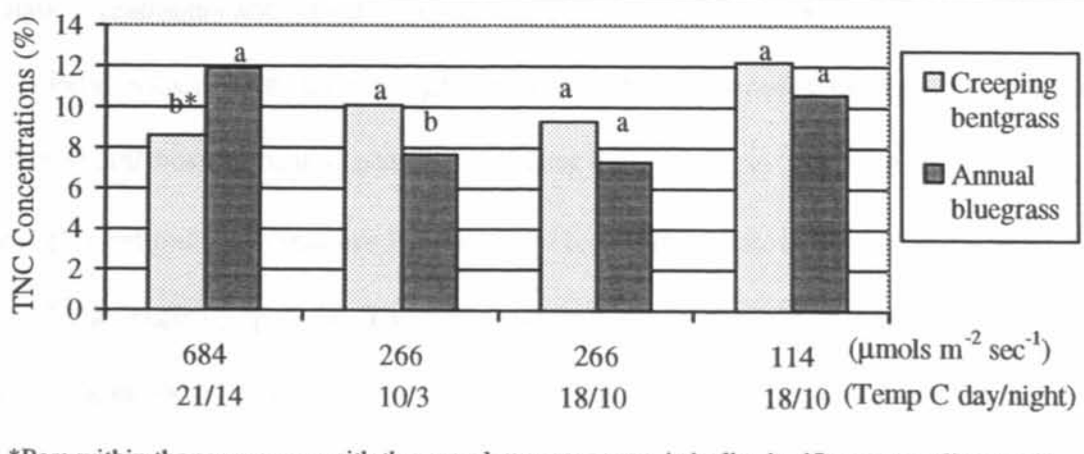
Fig 1.2. Comparison by species of crown TNC concentrations of creeping bentgrass and annual bluegrass exposed to selected irradiances and temperature treatments.

Annual bluegrass TNC levels were significantly lower in florescent/incandescent irradiance treatments as compared to the greenhouse treatment, regardless of temperature regime of the florescent/incandescent treatments (Fig. 1.2). The metal halide lamp treatment provided similar TNC levels as the greenhouse treatment. This may be the result of a response to irradiance quality rather than irradiance intensity. The irradiance sources used in this experiment had varying spectral properties (Fig. 1.1). The metal halide lamp was especially lacking in the red-light portion of the spectrum as compared to the growth chamber combination of florescent and incandescent sources and may have contributed to the differences between creeping bentgrass and annual bluegrass TNC concentrations.

Changing the ratios of different irradiance fractions has been shown to alter turfgrass growth. Weston and Rossi (2000) observed a strong relationship between the ratio of red to far-red irradiance and the carbohydrate status of cool-season grasses. Lowering the ratio of red/far-red to 0.2 decreased tiller numbers and TNC levels in creeping bentgrass, fine fescue, and perennial ryegrass. McVey and Mayer (1969) showed that Kentucky bluegrass quality increased and yield decreased by increasing the amount of blue irradiance transmittance.

## Comparison of Crown TNC Concentrations by Irradiance Treatment

Creeping bentgrass had significantly higher crown TNC levels compared to annual bluegrass under the artificial irradiance treatment at a low temperature (Fig. 1.3). Though, in the greenhouse treatment, annual bluegrass had significantly higher TNC levels. This data suggests that creeping bentgrass maintains higher TNC levels when grown under limited irradiance conditions. These findings are similar to those of Bell




*Bars within the same group with the same letter are not statistically significant according to the Least Squares Means Test with p = 0.05.

Fig. 1.3. Comparison by irradiance level and temperatures of crown TNC concentrations of creeping bentgrass and annual bluegrass exposed to selected irradiance and temperature treatments averaged across selections.

and Danneberger (1999) who reported higher TNC levels in bentgrass when exposed to perpetual shade as compared to full sun. They suggested that the increased carbohydrates were the result of decreased shoot growth.

The ability to conserve or increase crown carbohydrates would be a significant advantage to surviving long periods of reduced photosynthesis. My data indicated that creeping bentgrass had the ability to conserve TNC when exposed to reduced irradiance levels at low temperatures, whereas annual bluegrass did not. This could be an important attribute to surviving prolonged ice or snow cover.

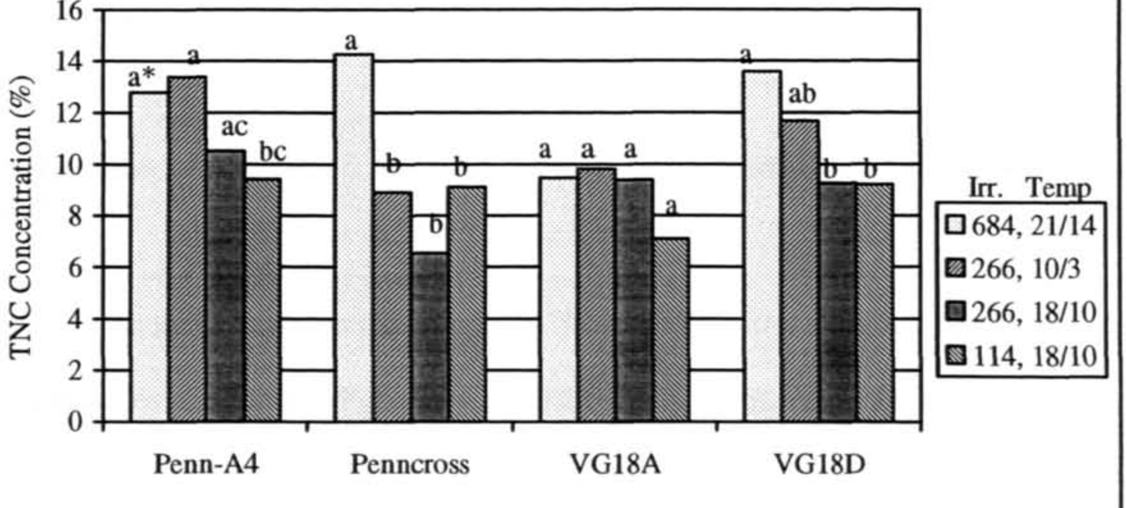
## Comparison of Verdure TNC Concentrations by Selection

The treatment by selection interaction was the only significant factor in the verdure TNC concentration analysis of variance (Table 1.3). Unlike the crown TNC concentrations, the verdure TNC concentrations were affected differently by the irradiance treatments and was dependent upon selection, not species.

Penn-A4 had significantly higher verdure TNC concentrations in the greenhouse, and florescent/incandescent irradiance treatment (266 µmols $m^{-2}$ $sec^{-1}$) at 10/3 C (day/night) temperature compared to the metal halide (114 µmols $m^{-2}$ $sec^{-1}$) treatment at 18/10 C (day/night) (Fig. 1.4). The difference may be attributed to a difference in irradiance quality; however, the TNC concentration of the greenhouse treatment and the florescent/incandescent (266 µmols $m^{-2}$ $sec^{-1}$) treatments were equivalent to those of the metal halide (114 µmols $m^{-2}$ $sec^{-1}$) treatment.

Table 1.3. Analysis of variance for verdure TNC concentrations of creeping bentgrass and annual bluegrass exposed to selected levels of irradiance and temperature treatment.

| Source | df | F-Value | Pr > F |
|---|---|---|---|
| Treatment | 3 | 8.04 | 0.0001 |
| Species | 1 | 0.97 | 0.3276 |
| Treatment by species | 3 | 0.81 | 0.4934 |
| Treatment by selection (species) | 8 | 2.36 | 0.0256 |
| Error mean square = 10.00, df = 75 | | | |




*Bars within the same group with the same letter are not significantly different according to the Least Square Means Test with p = 0.05.

Fig. 1.4. Comparison by species of verdure TNC concentrations of creeping bentgrass and annual bluegrass exposed to selected irradiance and temperature treatments.

Penncross had significantly higher TNC concentrations in the greenhouse (684 µmols $m^{-2}$ $sec^{-1}$) treatment compared to the florescent/incandescent irradiance source at both high and low temperatures and the lowest irradiance metal halide (114 µmols $m^{-2}$ $sec^{-1}$). This may indicate that Penncross photosynthate production is significantly reduced under decreased irradiance and that temperature is not a factor.

There were no TNC concentration differences in verdure tissue among treatments associated with light quality for the VG18A annual bluegrass selection. The VG18D annual bluegrass had significantly higher TNC concentrations in the natural sunlight treatment compared to the florescent/incandescent and metal halide treatments at the 18/10 C temperatures. This data suggests that the photosynthetic capacity of VG18D may be more dependent on temperature than irradiance level. Similar reduction in annual bluegrass photosynthesis due to reduced temperatures has been reported by Borland and Farrar (1987).

## Comparison of Verdure TNC Concentrations by Irradiance and Temperature Treatment

In the greenhouse treatment, only VG18A had significantly lower TNC concentrations compared to Penncross and VG18D (Fig. 1.5). In the florescent/ incandescent treatment at 10/3 C, Penn-A4 had significantly higher TNC concentrations than the other three selections. At the same irradiance at higher temperatures (18/10 C), Penn-A4 had significantly higher TNC concentrations than the Penncross. There were no differences among selections with the metal halide treatment of 114 µmols $m^{-2}$ $sec^{-1}$.

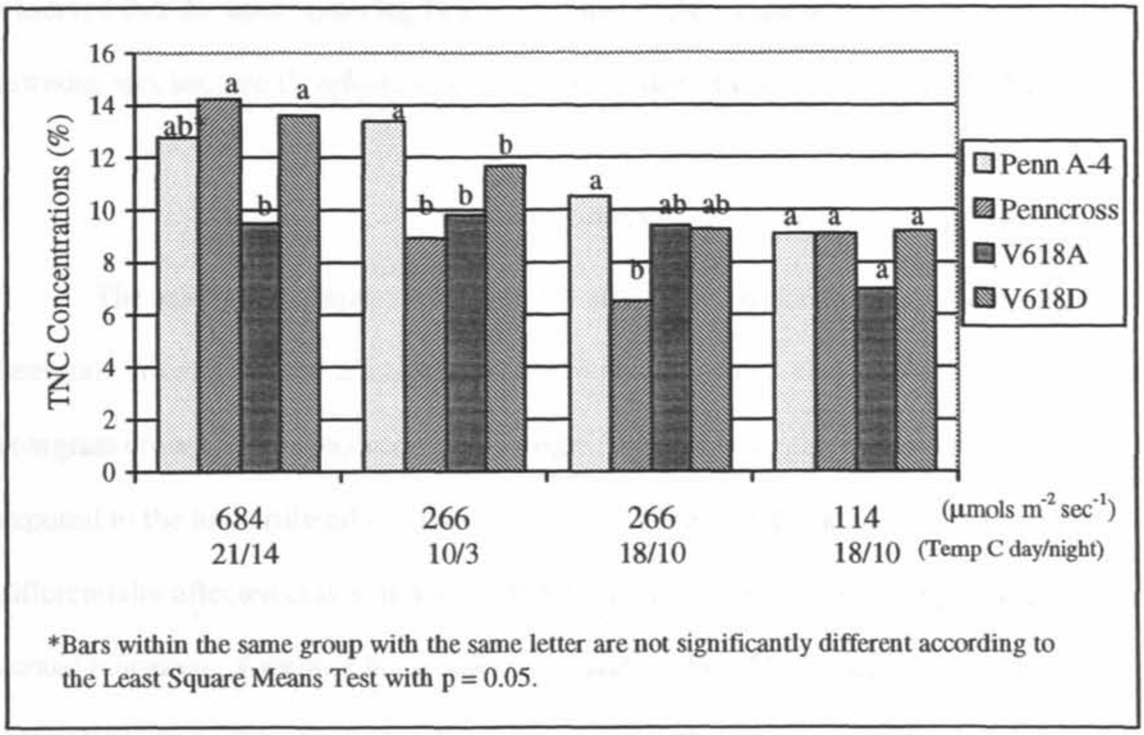



Fig. 1.5. Comparison by irradiance levels and temperatures of verdure TNC concentrations of creeping bentgrass and annual bluegrass exposed to selected irradiance and temperature treatments.

The ability to have higher verdure TNC concentrations under reduced irradiance may be the result of genetic differences between the selections. The ability to conserve carbohydrates would enable an increase in sequestering of carbohydrates during times of low-light (e.g., cloudy days, early spring, late fall). The increased TNC concentrations may provide an advantage to withstand stresses related to low carbohydrate reserves (e.g., prolonged snow or ice cover).

The verdure TNC concentration differences may also be the result of differential leaf respiration. Atkin et al. (1997) reported differential leaf respiration rates in two Poa species (*Poa annua* L. and *Poa costiniana* Vick.) with different growth rates. They observed that the faster growing Poa species had higher respiration rates than the slower growing species, and therefore, a higher consumption of carbohydrates in the leaf tissue.

## Conclusions

The selected irradiance levels did not affect TNC concentrations in creeping bentgrass crowns, but did affect the concentrations of annual bluegrass crowns. Creeping bentgrass crown TNC concentration was significantly higher than annual bluegrass when exposed to the low artificial irradiance treatment. The selected irradiance levels differentially affected crown and verdure TNC concentrations of creeping bentgrass and annual bluegrass. Creeping bentgrass crown and verdure TNC concentrations were equivalent or significantly higher than those of annual bluegrass at all lower irradiance treatments. This indicates that for the selections and irradiance levels used in this study, creeping bentgrass had the ability to conserve carbohydrates better than annual bluegrass under decreased irradiance conditions.

More research is needed to better understand the carbohydrate budgets of annual bluegrass and creeping bentgrass. Carbohydrate concentrations can affect a plant's ability to tolerate various stresses (Levitt, 1980). Further understanding of dark respiration and photorespiration rates of unhardened and hardened cool-season grasses, especially creeping bentgrass and annual bluegrass, would also be important in describing carbohydrate balance.