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Diurnal variations in the contents of water-soluble carbohydrates in *Vicia sativa* L.

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Abstract

Diurnal variation in water-soluble carbohydrate (WSC) may influence the grazing behavior and dietary preferences of ruminants. Feeding forage with high WSC content could increase growth rates of livestock. Quantifying diurnal variation in WSC will be helpful in designing the management strategies to obtain the herbage in higher WSC content. The objective of the present study was to investigate the diurnal variation in WSC content of *Vicia sativa on* Qinghai-Tibetan plateau. Two sets 24h continuous sampling were conducted on 20% and 80% blooming stage, respectively. And sampling was conducted every 3 hours from 06:00 to 06:00 (the following day). It found that, the content of WSC and sucrose were lowest at sunrise and then increased till the following midday, but fructan reached its highest level at nightfall. In addition, the content of sucrose was higher in 80% blooming stage than that in 20% blooming stage during daytime, but lower in the night. Conversely, the concentration of fructan was higher in 20% blooming stage than that in 80% blooming stage. The result may suggest that carbon fixed during photosynthesis is directed firstly towards sucrose, andfructan synthesis occurred mostly during the end of the light period as the synthesis of sucrose decreased. In conclusion, *Vicia sativa* should be harvest between noon and sundown for greater WSC concentration on Qinghai-Tibetan plateau.

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Introduction

Water-soluble carbohydrate (WSC) is the source of readily available energy for plant growth and survival (Burns *et al*., 2005). The content accumulates during daylight because photosynthesis produces more WSC than the amount consumed in the metabolization for plant growth and maintenance. Water-soluble carbohydrate of grasses, mainly consisting of sucrose and fructan, is made in the cytoplasm during periods of photosynthesis. Previous studies found that leaves accumulated sucrose and fructan, and then exported sucrose to the rest of the plant during the day. At night, sucrose is remobilized to maintain the export of sucrose to sink tissues and to support respiration in the leaves (Lunn and Hatch, 1995; Aggarwal and Michael, 2014). The net balance of leaf photosynthesis, respiration and carbohydrate export produces a diurnal variation in WSC whose minimum concentrations occur in early morning and maximum in late afternoon (Bowden *et al*., 1968; Lechtenburg *et al*., 1972). Some studies (Holt and Hilst, 1969; Lechtenburg *et al*., 1972) showed that the WSC contents in grasses increased linearly during daylight hours. And some revealed that WSC contents increased only during the morning and kept stable during the afternoon, but declined during the night (Waite and Boyd, 1953; Marais *et al*., 1993).

The diurnal cycles in WSC may influence the grazing behavior and dietary preferences of ruminants because it could provide readily available energy for rumen microbial activity, and thus improve the forage utilization (Mayland *et al*., 2000; Wilkins and Humphreys, 2003). Feeding forage with high WSC content could increase growth rates of livestock (Lee *et al*., 2001; Kanwar and Kumar, 2009; Szalay *et al*., 2013) and the absorption of amino acids from the small intestine in beef steers (Ujan *et al*., 2017). The content of WSC in pasture is variable and some evidence proves that the magnitude of the diurnal change in WSC could affect the temporal herbage intake. For instance, Orr *et al*. (2001) found that sheep increased their rate of intake of both grass and legume pastures as WSC content increased over the day. Hay harvested in the afternoon with higher WSC content was reported to be preferred instead of the hay cut in the morning (Burns *et al*., 2005). These findings suggested how to alter the WSC composition of forage by taking advantage of diurnal WSC cycle. Plant breeders selected plant materials based on WSC contents (Humphreys, 1989) with an expectation to increase the energy content and thereby improve nutrient-use efficiency and livestock production ultimately. Quantifying the changing pattern of WSC contents or rates of accumulation will be helpful in designing the management strategies to obtain the herbage in higher WSC content. The objectives of this study were to determine the diurnal variation of WSC contents of common vetch (*Vicia sativa* L.), and to investigate the daily rates of WSC accumulation in two different growing stages on Qinghai Tibet plateau, China.

Materials and methods

Geographic locations

The field experiment was performed in 2010 at Gannan Tibetan Autonomous Prefecture, which is located on Qinghai-Tibetan plateau, China. The average annual precipitation is 426 mm, mean daily temperature is 2.9℃, and over 60% of area in the region was grassland. The soil was chernozem, slightly acidity (pH is 6.7), deficit in P (7.5 mg/kg) and rich in K (17.5g/kg), the content of organic matter was 42 g/kg.

Experimental design

A local common vetch (*V. sativa* L.), the commercial cultivar "333/A" was used in the trail. Seeds were sown under field on May 1st. The field plots were 4×5m^2 each and the seeding rate was 75 kg/ha. No irrigation or fertilizer was applied to the field during the study period. A completely randomized design with three replications was adopted in the study.

Sampling

Sampling was carried out in blooming stage by manual clipping. Two sets 24h continuous sampling were conducted on Jul. 10-11 (20% blooming stage) and 13-14 (80% blooming stage), respectively. The sampling was conducted every 3 hours from 06:00 to

06:00 (the following day) and that required to complete within 5 minutes. Fresh samples (500g) were immediately frozen after being collected and subsequently frozen dried, and ground to pass a 1-mm screen in a Wiley shear-mill, then stored at -20℃ in refrigerator before chemical analysis.

Chemical analysis

Water-soluble carbohydrate was measured by near-infrared reflectance spectroscopy (NIRS) (NIRSytems Model 6500, MD, USA). The WSC content was estimated by use of calibration equations described by Sanada *et al.* (2007). Total WSC was extracted from a 0.25 g ground sample by boiling de-ionized water containing 1 mg/ml of propylene glycol as the internal standard for 1 h. The extract was passed through a 0.45-um pore filter and analyzed using high-performance liquid chromatography (HPLC). Sucrose and fructan in the extract were separated using gel permeation HPLC columns (Shodex KS-802 and KS-803 combined; Showa Denko, Tokyo, Japan) with a flow rate of 0.8 ml/minof HPLC-grade water at 50°C, and were detected using a refractive index detector (L-2490; Hitachi, Tokyo, Japan). Carbohydrates were identified using sucrose and fructan as the external standards and quantified using propylene glycol as the internal standard.

Statistical analysis

Data was processed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Analysis of variance in WSC content s among different sampling time was performed using one way ANOVA, results in graphs showing changes in WSC content of *V. sativa* over the day of the experiment with standard error of the mean.

Results and discussion

Weather conditions on sampling days

Sward weather conditions were monitored to ensure no climate change out of sampling time between 20% and 80% blooming stage that might affect pasture growth and carbon partitioning (Table 1).

Table 1. Weather data recorded in field experiment station, 10-14 July, 2010.

Date	Air temperature (°C)		Radiation (MJ m^{-2})	Rainfall (mm)
	Minimum	Maximum		
10 July	2.5	15.8	20.1	0
11 July	0.5	16.3	17.5	0.5
12 July	2.1	16.7	17.7	0
13 July	3.8	18.0	18.6	0
14 July	3.7	17.7	19.3	0

During sampling periods, sunrise and sunset were approximately at 06:26 and 19:50 respectively.

The air temperature between the two sampling dates were more similar for the majority of the day, the only small difference was that the mean daily minimum temperature approximately 2.2℃ lower and mean daily maximum temperature approximately 1.8℃ higher in 20% blooming stage than that in 80% blooming stage.

In addition, during the two sampling periods, days were generally sunny, with only intermittent periods of cloud cover in the end of 20% blooming stage sampling period. The mean intensity of solar radiation during the sampling periods was between 17.5 and 20.1 MJ/m^2.

Diurnal variation in dry matter

The dry matter (DM) contents collected in 15:00 were highest and that in 24:00 hours were lowest, the levels were higher in 80% blooming stage than that in 20% blooming stage (Fig. 1).

The diurnal cycles in DM contents of the grass were similar to those measured in other grasses by

Ciavarella *et al.* (2000), and reflected the daily fluctuation in water content (both internal and external to the plants) that occurred as a result of dew formation, guttation from the tips of the leaves overnight, and the subsequent loss of moisture by evaporation and transpiration after sunrise.

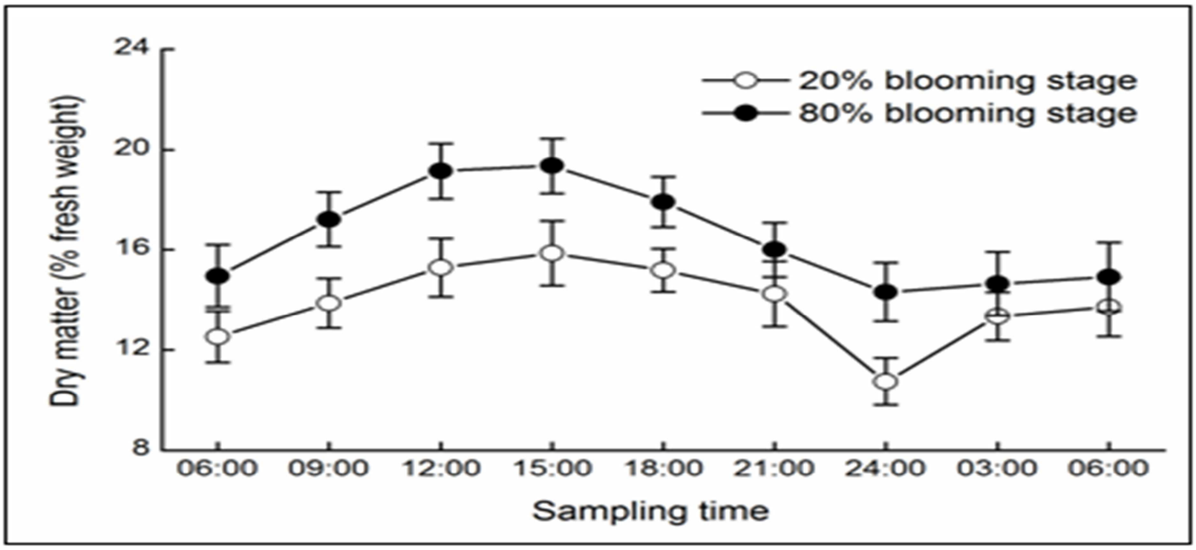

Fig. 1. The diurnal variations in contents of dry matter (DM) at 20% blooming stage (Jul. 10-11) and 80% blooming stage (Jul. 13-14) in *V. sativa* in 2010. Vertical bars indicate the standard errors of the mean (n = 3).

The DM level was increased along with the advancing maturity of grass. This may have been partly associated with much higher fiber levels and lower leaf-to-stem ratio (Milchunas *et al.*, 2005).

Diurnal variation in water-soluble carbohydrate

The diurnal changes in WSC of the present study were 8.07-12.55 mg/g in 20% blooming stage and 6.63-15.08 mg/g in 80% blooming stage (Fig. 2). And the same change was found in WSC concentrations for both sampling periods.

However, the content of WSC was higher in 80% blooming stage than that in 20% blooming stage during the day, but that was much lower in the night.

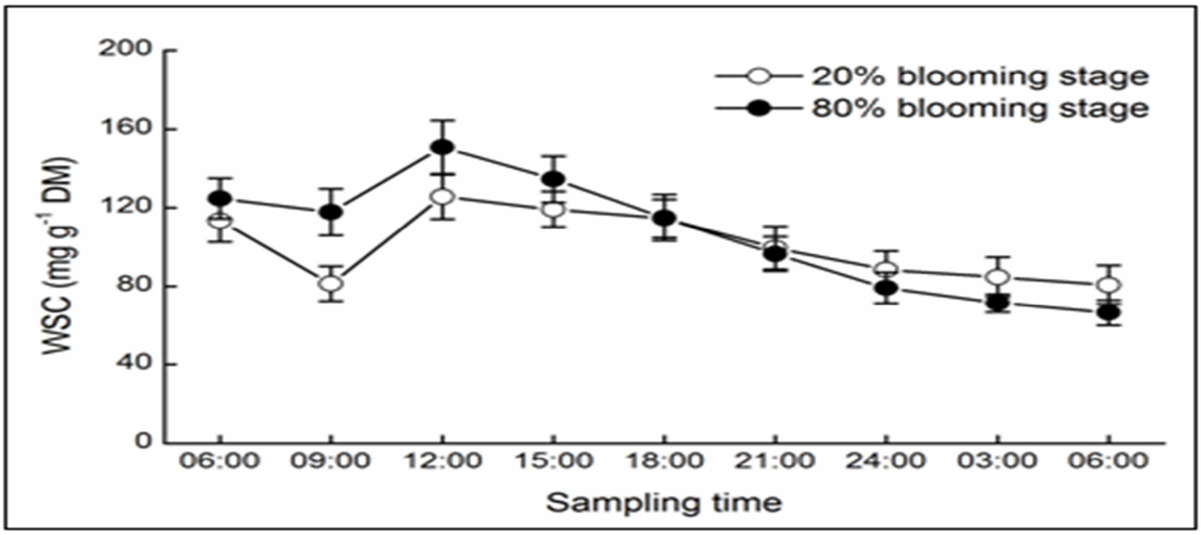

Fig. 2. The diurnal variations in contents of water soluble carbohydrate (WSC) at 20% blooming stage (Jul. 10-11) and 80% blooming stage (Jul. 13-14) in *V. sativa* in 2010. Vertical bars indicate the standard errors of the mean (n = 3).

The different changing tendencies were found among the diurnal variations of WSC contents in the present study, there was a marked increase in the level of WSC collected between 09:00 and 12:00 hours, and most of WSC in *V. sativa* accumulated in this period of time during the day.

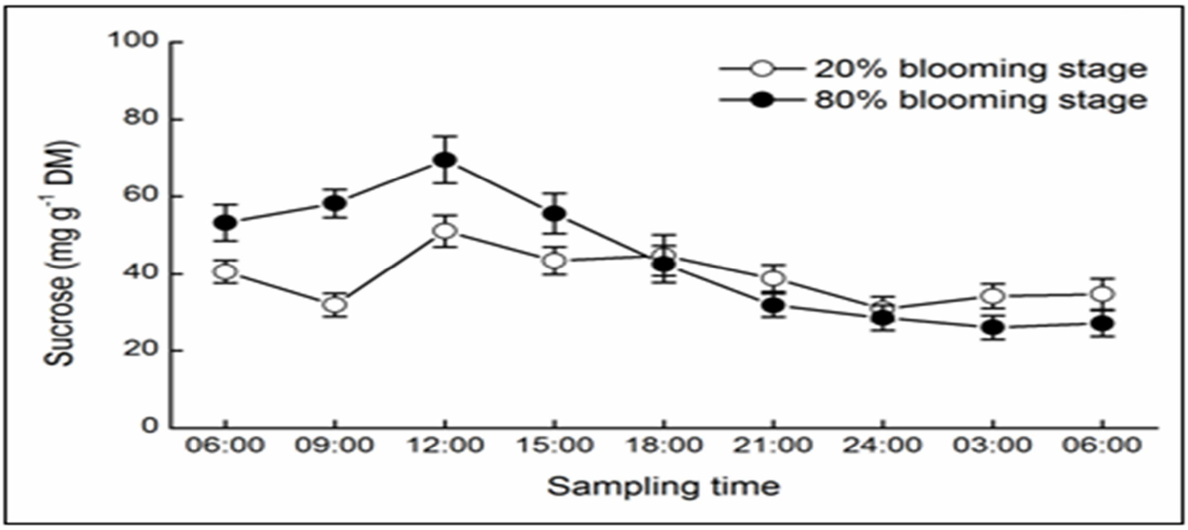

Fig. 3. The diurnal variations in contents of sucrose at 20% blooming stage (Jul. 10-11) and 80% blooming stage (Jul. 13-14) in *V. sativa* in 2010. Vertical bars indicate the standard errors of the mean (n = 3).

The highest content of WSC was found in that collected at 12:00 hours, and then the content was reduced gradually.

The result in the present study was of some difference with previous researches (Orr *et al.*, 1997; Fisher *et al.*, 1999). Orr *et al.* (1997) found that the WSC contents in both grass and legume pastures increased during the daylight hours and declined overnight, the only difference was magnitude of the diurnal change, the similar result was found by Fisher *et al.* (1999). In some studies (Holt and Hilst, 1969; Lechtenburg *et al.*, 1972), the WSC contents in grasses increased linearly during daylight hours.

In others, WSC contents increased only during the morning, became stable during the afternoon, but declined during the night (Waite and Boyd, 1953; Marais *et al.*, 1993).

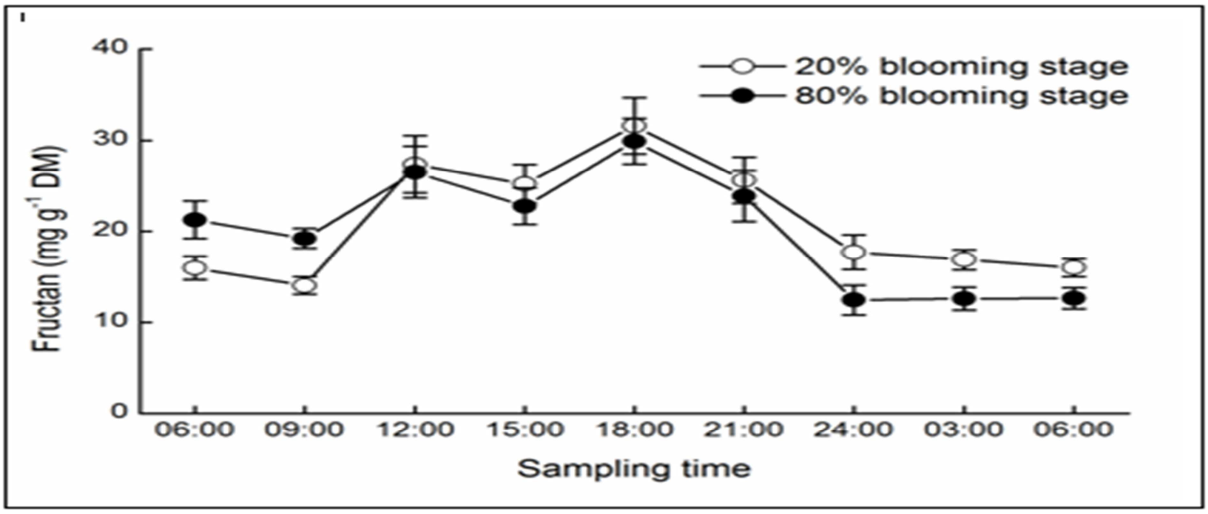

Fig. 4. The diurnal variations in contents of fructan at 20% blooming stage (Jul. 10-11) and 80% blooming stage (Jul. 13-14) in *V. sativa* in 2010. Vertical bars indicate the standard errors of the mean (n = 3).

Diurnal variation in sucrose

The diurnal changes in sucrose of *V. sativa* were 30.85-51.06 mg/g in 20% blooming stage and 26.02-69.51 mg/g in 80% blooming stage (Fig. 3). The diurnal variation was the same for both sampling periods. The content increased from 09:00 to 12:00, and declined gradually from 12:00 to 24:00 and then remained constant till 06:00 the next day. The similar change has been found in other grass species (Marais *et al*., 1993; Ciavarella *et al*., 2000). In addition, this diurnal variations were similar to the change of WSC in the present study, it could be concluded that as a major component of WSC, sucrose plays a key role in the variation of WSC. Sicher and Kremer (1984a) found that the rapid increase of WSC is accounted by sucrose, which is the major photosynthetic product synthesized during the initial hours of the light period. And sucrose in barley (*Hordeum vulgare* L. subsp. *vulgare*) leaves increased three-fold during the first 12 h of light (Sicher *et al*., 1984b). It is well known that leaves accumulate sucrose, and export sucrose to the rest of the plant during the day. At night sucrose is remobilized to maintain the export of sucrose to sink tissues and to support respiration in the leaves (Lunn and Hatch, 1995), this could partly explain the diurnal variation of sucrose during the day and night time.

Diurnal variation in fructan

The diurnal variation in fructan was difference with sucrose. The contents were 14.00-31.58 mg/g in 20% blooming stage and 12.42-29.88 mg/g in 80% blooming stage (Fig. 4). The contents of fructan were increased from 09:00 to 12:00, and decreased from 12:00 to 15:00in a day, then gradually increased to the highest level at 18:00. The result detected in the present study were of some difference with previous studies, In this study, fructan contents did not show the linear increase late in the day that had been stated by Sicher and Kremer (1984a). In addition, the variations in fructan contents that were measured were more similar to the small and irregular variations described by Waite and Boyd (1953). Furthermore, compared with sucrose, the highest level of fructan was found in the later of the day, and in that time the content of sucrose was declined. This was in accordance with Sicher *et al*. (1984b), they found that fructan synthesis occurred primarily towards the end of the light period as rate of sucrose synthesis decreased, which was probably related with activity of fructan biosynthesis enzyme (Vijn and Smeekens, 1999).

Conclusion

On the basis of above findings, we concluded that the changing balance between photosynthesis and respiration caused diurnal variations in WSC and its component contents of *V. sativa* throughout the day. And *V. sativa* should be harvest between noon and sundown for a higher WSC content than the daily mean, which could improve protein utilization and increase DM intake for the consumed ruminants.

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Declaration of Interests

Authors declare that there is no conflict of interest. The authors alone are responsible for the content and writing of this article.

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