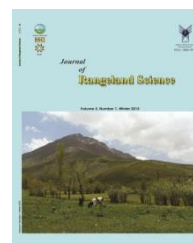


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Research and Full Length Article:

Variations of Water Soluble Carbohydrate in Plant Organs of *Bromus tomentellus* and *Festuca ovina* in Three Phenological Stages

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Abstract. Information on quality characteristics of forage species help the range managers to select a suitable grazing program that minimizes the damages to vegetation. Water Soluble Carbohydrates (WSC) -the main photosynthetic products- are stored in various plant parts and are more likely to be consumed when plants require them. In order to investigate the changes of WSC in distinguishable organs in three phenological stages, samples were taken from *Bromus tomentellus* and *Festuca ovina* in three phenological stages (vegetation, flowering and maturity). Samples of roots and aerial parts were taken for both species using a completely randomized factorial arrangement-based design with the phenological stages regarded as the first factor and herbage parts as the second factor with 5 replications. Higher WSC values were observed in the leaves of *B. tomentellus* (4.6%) and *F. ovina* (4.5%) in maturity and flowering stages, respectively. Maximum WSC values of roots were obtained in *B. tomentellus* (3.2%) and *F. ovina* (3.5%) at vegetation and maturity stages, respectively. It was suggested that range utilization in a phenological stage might be managed in which forage species can withstand the defoliation without severe reserve depletion. Results indicate that except flowering stage in *B. tomentellus*, WSC was increased with the increase of panicle weight in the maturity stage and there was an inverse relationship between plant components and WSC content percent in the other phenological stages.

Key words: Soluble carbohydrates, Phenological stages, Plant parts, Grass species

Introduction

Water Soluble Carbohydrates are regarded as the critical nutrient storages in the range plants. Therefore, it is principal to know the amounts and locations of WSC stored in the plants while determining an appropriate grazing period (Arzani, 2009). Determining the composition and concentration of plant WSC is frequently necessary to estimate their available resources for the plant growth to evaluate the forage energy value for grazing animals (Watts, 2004; Longland & Byrd, 2006). Determining the relationship between WSC reserve and phenological stage is important in understanding the relationship between phenology and physiology, forecasting the possible reactions of plant species to harvest and understanding the rangeland ecological changes (Holecheck *et al.*, 2005). Hall (2003) based on various studies had divided the carbohydrates into two groups; the first group forms the contents

inside the cell and the second one forms the cell wall. These compounds form over 75% dry matters and constitute a vast part of animal diet (Hall, 2003). WSCs are completely dissolved in the cytoplasm of plant cells including sugars (Sucrose, Fructose and Dextrin) and Starch that make a large part of nutrient storages in the plants. These materials move in the plant and are consumed in the growth process, respiration and so on. There is no agreement about the location of material storage in the plant organs. Perhaps, it can be indicated that at any development forms, material storage location is distinguishable from the others. Jafari (2012) evaluated the environmental and genetic variations for Water Soluble Carbohydrate (WSC) content in the forage grasses of cool season. He stated that WSC content depends on the environmental factors, gene action and genotype and environment interactions. The amount of these substances is distinct

in the plant organs in various phenological stages. The most significant storage organs involved the roots, tubers, rhizomes, stems, branches and canopy cover (Arzani, 2009). Adequate rest periods after grazing will provide the sward time to accumulate the additional carbohydrate reserves. If re-grazing was conducted before the adequate rest period, the root would eventually deplete its stored reserves leading to increase the possibility of plant mortality (Brueland *et al.*, 2003). Hoffman *et al.* (2003) indicated that the ruminants for absorbing proteins need WSC to provide the energy necessary for protein yeast microbes. Through the process of photosynthesis, green plants absorb light energy and reduce carbon dioxide (CO₂) to produce carbohydrates that serve as raw materials for further chemical synthesis (Hoffman *et al.*, 2003). In most cases, carbohydrate production rates in plants are greater than physiological consumption.

Excess amounts will be stored in various parts of the plant and will be available for future requirements such as respiration during dormancy, growth following winter or after drought dormancy and reproduction. Plants need time during their growth cycles to produce and store the excess carbohydrates for using in critical stress periods (Arzani, 2009; Arzani, 2004).

Souza *et al.* (2010) reported that the patterns of WSC and starch from the concentrations of shoots and underground organs are related to species, time and space. Similarly, Stream *et al.* (2011) stated that the WSC concentrations were independent of developmental stages and plants in distinct developmental stages were characterized by various concentrations and patterns of WSC accumulation before winter.

In the same way, results of Alcoverro *et al.* (2001) demonstrate that seasonal changes in total WSC (Sucrose and Starch) were consistent across the plant organs and age classes of leaf with the highest values in the rhizomes and lowest

values in the older leaves (Alcoverro *et al.*, 2001). In *Stipa barbata* species in semi-arid rangelands of Yazd in central part of Iran, the lower and higher values of WSC reserves were observed in panicles and seed falling stages, respectively (Miraskarshahi, 2004). Results obtained by Souza *et al.* (2010) indicated that WSC content from the shoots of *Melinis minutiflora* and *Echinolaena inflexa* varied due to the time of the year while in the underground organs, variations were primarily observed in relation to the transects. Marked differences were also observed in the pattern of above-ground biomass production between these two grasses related to their location with two peaks of July and January at the reserve border. The differences in carbohydrate accumulation, partitioning and composition of individual sugars concerning time of the year and location in the reserve were more related to the annual growth cycle of both grasses and possibly to specific physiological responses of *Melinis minutiflora* to the disturbed environments in the reserve border (Souza *et al.*, 2010). Richburg (2005) illustrated that WSC concentration had no significant fluctuations in two storage organs from vegetative growth stage (low) to the flowering one and it could be stated that stabilizing these low values in vegetation stage to panicles' appearance stage in the pod was the result of inverse relationship between the plant growth rate and carbohydrate storage amount. This occurred simultaneously with temperature and rainfall conditions during April and May. Fluctuation patterns of root and rhizome WSC reserves were distinguishable in *Agropyron intermedium* species. At the first harvest, the reserve level of WSC in the roots was the lowest one which represented the amount of energy needed to produce photosynthetic organs. Considering the continued plant growth, carbohydrate reserves of root and rhizome have an increasing pattern that can be resulted from high rates of photosynthetic

productions being more than the plant needs (Richburg, 2005). Results obtained by Brueland *et al.* (2003) illustrated that the WSC over a growth period might be distinguishable. This result will demonstrate distinct reactions to the timing, frequency and severity of harvest. According to the fixed time plans and regardless the phenological and physiological relations, the use of distinct pasture species can be expressed as one of the rangeland degradation causes.

To determine the appropriate time for grazing and investigate the plant sensitivity to the grazing in a systematic model of livestock and pasture management, investigating the carbohydrate reserve process of dominant plants was necessary. Consequently, the present study aims to investigate the variations in the ratio of plant parts and WSC in distinguishable parts (stems, leaves and panicles) of two grasses in three phenological stages (vegetative, flowering and maturity) while analyzing species were *Astragalus verus* and *F. ovina*. Due to the exceeding elevation, plant delay growth was experienced in site 2. Soil was slightly lighter with a depth less than soil in the first site.

Two common grass herbage for both sites (*B. tomentellus* and *F. ovina*) were selected for the analysis. These species are observed in the most mountainous rangelands of Iran and are quite palatable. They are normally able to re-growth rapidly after grazing or harvesting. They belong to the sub-family of Pooideae (Mobayen, 1980).

Samples were taken in three phenological stages: early vegetative growth, flowering and ripening. In each site, 5 points were randomly selected for sampling of each species. At each time, samples were collected from each point for both species. Samples included the roots and aerial parts of plants. They were taken between 9-11 am and were placed in paper bags and then put in black plastic bags. Then, they were immediately transferred to the laboratory using a

the relationship between plant components and WSC content percent in the phenological stages.

Materials and Methods

The study was carried out in Zagros Mountain of Iran with the mean annual precipitation of 1183 to 742 mm. Exclosures on two sites were selected for sampling with the distance of 5 km. The first site was located in southwest to northeast aspect and had been excluded from grazing since 1969. Average elevation in this site was 1960 m above sea level. Soil type in both sites was classified as Entisols (orchrepts suborder) based on USDA method (Hemmati, 1997). Dominant species were *Festuca ovina* and *Bromus tomentellus*.

The second site was located in northwest and had been excluded from grazing since 1995. Its average elevation was 2200 m above sea level. Dominant

portable refrigerator. Plant parts (leaves, stems, panicles and roots) were carefully separated and dried in an oven at 75°C for 24 hours and weighed in the laboratory. Then, the plant part ratios were determined.

Dried samples were ground to pass through a 40-mesh screen (0.42 mm opening) and stored at room temperature in the sealed bottles during a chemical analysis. Extraction and determination of WSC were accomplished using the Colorimetric method (Dubois *et al.*, 1956) by Chaplin and Kennedy 1992 modification.

Effects of phenological stage and plant parts were analyzed using completely randomized factorial experiment design with 5 replications of each species. Also, SAS9 software was used. Samples were taken in three phenological stages (vegetation, flowering and maturity) from four organs (leaf, stem, panicle and roots). Locations were combined into one analysis conducted by Cochran and Cox (1957) and Chaplin &

Kennedy (1992). The differences between treatments were compared using LSD test. Then, the relationships between plant components and WSC content percent in phenological stages were investigated.

Results

Growth stage comparisons

Leaf WSC in *B. tomentellus* was increased from vegetative stage through flowering to maturity ones; in contrast, root WSC was increased during vegetative and flowering stages as compared to the maturity stage. Stem WSC was high during the flowering stage and low during vegetative and maturity ones. WSC of panicles at maturity was higher than panicle appearance stage (Table 1 and Fig. 1).

For *F. ovina*, leaf and stem WSC were higher during flowering stage and lower during vegetative and maturity ones. In contrast, root WSC was in the lowest level

during flowering stage; however, it increased during vegetative stage and it was in the highest level at maturity stage. Panicle WSC was higher at maturity than flowering (Table 1 and Fig. 1).

In both species during vegetative growth, WSC was lower in the roots than leaves and stems. During flowering stage, WSC was higher in leaves. At maturity, panicles had higher WSC than the other parts; In general, stem and leaf carbohydrates were less than both panicles and roots (Table 1).

Table 1. WSC content (%) of *Bromus tomentellus* and *Festuca ovina* leaves, stems, panicles, and roots in vegetative, flowering, and maturity stages

Species	Plant Part	Vegetative	Flowering	Maturity
<i>Bromus tomentellus</i>				
	Leaf	2.34 b (C)	2.88 b (B)	4.64 a (A)
	Stem	3.09 a (B)	4.08 a (A)	3.37 b (B)
	panicles	-	1.87 c (B)	2.31 c (A)
	Root	3.21 a (A)	3.07 b (A)	2.41 c (B)
<i>Festuca ovina</i>				
	Plant part	Vegetative	Flowering	Maturity
	Leaf	3.12 a (B)	4.49 a (A)	1.92 c (C)
	Stem	2.31 b (B)	3.29 b (A)	1.09 b (C)
	panicles	-	2.03 c (B)	3.65 a (A)
	Root	2.16 b (B)	1.25 d (C)	3.49 a (A)

Plant part means of species in each growth stage (column) followed by the same lower case letter (a, b, c, d) are not significantly different ($P>0.05$)

Phenological growth stage means of each plant organ (rows) followed by the same upper case letter (A, B, C) are not significantly different ($P>0.05$)

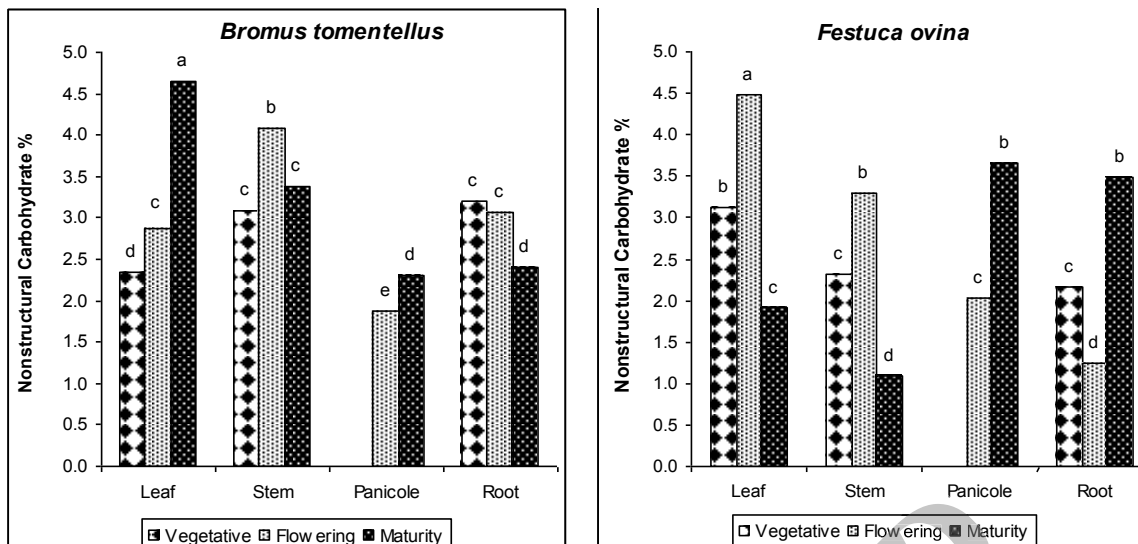


Fig. 1. WSC% of *Bromus tomentellus* and *Festuca ovina* leaves, stems, panicles, and roots in vegetative, flowering, and maturity stages

Plant part components (stems, leaves and panicles)

In comparisons between plant organs of two species, results showed that *F. ovina* had higher leaves but lower stem and root WSC% during both vegetative growth and flowering stages as compared to *B. tomentellus*. In contrast, at maturity stage, *B. tomentellus* had higher values for leaf and stem WSC and lower values for root WSC in comparison with *F. ovina*. Panicle WSC was lower in *B. tomentellus*'s than that for *F. ovina*'s during flowering and maturity stages.

(Fig. 2), illustrates that leaves are the crucial parts of forage in the vegetative stage. The highest leaf percent was recorded for *B. tomentellus* with the average value of 94.3% out of its total forage weight in the vegetative stage. In the flowering stage, panicles became the main part of total forage. In this stage,

ratios of stem to total weight were exceeded than the vegetative stage. However, in the flowering stage, the ratio of leaf to total forage weight was still exceeded the stem and panicle ratios for both species. In the maturity stage, ratios of leaves were exceeded the stems for *F. ovina*. However, for *B. tomentellus*, higher ratios were obtained for the stems. Distinct conditions were discovered for panicles related to the anatomic characteristics of each species. Higher ratios of panicles were recorded for *B. tomentellus* in flowering stage. However, the ratio of its panicles was lower than the ratio of its leaves and stems in flowering and maturity stages. For *F. ovina*, ratio of panicles in the maturity was lower as compared with flowering stage. In this species, panicles did not make up a considerable part of its forage in the flowering or maturity stages.

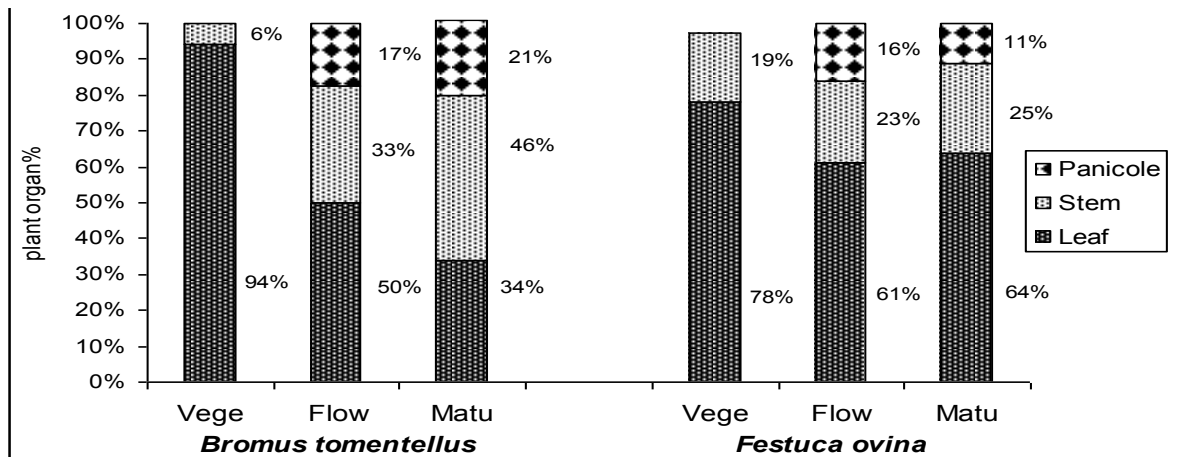


Fig. 2. Changes of plant organ percentage (stems, leaves, and panicles) of *Bromus tomentellus* and *Festuca ovina* in three phenological stages

Discussion

Measurements of WSC in plant tissues were significant to estimate the plant organ resources available for plant growth and stress tolerance or feed value for grazing the animals (Zhao *et al.*, 2010). WSC is the major source of energy in the diet of dairy cattle (Zhao *et al.*, 2008).

Location and level of WSC in the distinguishable parts of species in each phenological stage were not similar. The results of our study illustrated the highest value of WSC (4.6%) obtained for the leaves of *B. tomentellus* in the maturity stage and the lowest value (1.9%) was obtained in its panicles for the flowering stage. Maximum WSC was recorded for the stems of this species in the flowering stage and minimum rates of stems were recorded in the vegetative growth stage.

Higher WSC levels in leaves were obtained in the maturity stage and lower values were observed in the vegetative growth stage. For panicles, WSC levels in the maturity stage were higher than the flowering stage. While in the roots, higher values of WSC were observed in the vegetative growth stage and lower values were recorded during the maturity stage. Reviewing the changes in the stored WSC of other species in the arid region of Yazd in central part of Iran, Baghestani Meybodi *et al.* (2005) reported the differences among various plant species, distinguishable parts and

various phenological stages. Stored WSC reduced to minimum level at early spring growth and also re-growth at cutting (Baghestani Meybodi *et al.*, 2005).

After forage harvesting, the concentrations of WSC were decreased in the remaining sections. This decrease reached the minimum values within a week after the harvest and plant photosynthetic activity increased at the same time. If the harvest was carried out successively before the initial storage, the forage percent and its WSC are reduced finally leading to the plant death. Accordingly, Hoekstra and Schulte (2007) concluded that for the germination and regrowth, the stored carbohydrates are the considerable nutrient sources in plants. WSC reserves play a significant role in the recovery of plants after grazing or harvesting (Hoekstra & Schulte, 2007).

Caballero *et al.* (2001) in a survey studied the protein variations and WSC particles in *Vicia sativa* in three phenological stages. Their results revealed that the fibrous components vary with maturity in the plant in the vegetative growth stage and fibrous compounds frequently increase except ADF (Fiber washed with acid). They indicated that in the flowering stage from flowering to seed trashing, it increases and then, decreases in secondary seed losing (Caballero *et al.*, 2001).

Results of Charehsaz *et al.* (2010) indicated that there was a significant difference of phenological growth. In their study, WSC percent was increased according to the growth rate. Quality of grass species in terms of WSC illustrated a significant difference. WSC% in *Agropyron intermedium* and *Dactylis glomerata* increased by the growth. The highest WSC value was measured in *A. intermedium* in maturity stage. This demonstrates that this species is valuable for the delayed grazing (Charehsaz *et al.*, 2010).

In present study, the highest WSC level in *F. ovina* was obtained in the leaves in the flowering stage (4.5%) and the lowest level was in the stems (1.1%) in the maturity stage. Concentration of WSC in the leaves and stems of this species in the flowering stage was more than two other growing stages. In panicles and roots, WSC percent in the maturity stage was exceeded the flowering stage. Minimum WSC levels for the roots were obtained in the flowering stage. This might be due to the transportation of WSC from the roots to the above-ground parts of the plant during rapid growth of this species. As Miraskarshahi (2004) reported, in *Stipa barbata* species in semi-arid rangelands of Yazd, the lowest and highest amounts of WSC reserves were observed in flowering and maturity stages, respectively (Miraskarshahi, 2004).

WSC content of forage can widely vary due to the interaction of plants and their environment. These variables involve species and forage variety, growth stage and environmental conditions during the plant growth. Very young grasses that are rapidly growing have frequently lower WSC in the ideal conditions than later stages of vegetative growth. Conditions in spring and fall are frequently favored for WSC accumulation regardless the plant growth stage (Watts, 2005).

The reason for location differences of WSC in the species might be due to their morphological characteristics. WSC may

be temporarily stored in all of the plant tissues. At millet forage or perhaps other broad-leaved plants, WSC reserves tend to be focused in the basic parts of upper sections. The most WSC reserves are stored in the lower parts of stems, bulb, rhizome and roots; however, the appropriate storage organs in most plants are useless parts that are living during the plant dormancy (Hoecheck *et al.*, 2005).

There were significant differences between WSC of *B. tomentellus* in three phenological stages and plant parts in the most cases ($P < 0.05$). There were no significant differences for WSC in stems within vegetative and maturity stages, roots in vegetative and flowering stages, leaves and stems in the vegetative growth stage and panicles and roots in the maturity stage. However, for *F. ovina*, there were significant differences among WSC contents of all parts within all phenological stages ($P < 0.05$). There were no differences between WSC contents of its panicles and roots in the maturity stage. The results of Dusek (2002), Holcheck *et al.* (2005), Zohdi (2002) and Gharadaghi *et al.* (2004) also indicated the effects of seasonal changes in the storage of WSC for the studied plants (Dusek, 2002, Zohdi, 2002 & Gharadaghi *et al.*, 2004). Results demonstrated that weight ratios of plant parts were distinguishable ($P < 0.05$) in distinct growth stages. Leaves and stems indicated more variations than the panicles in both species. In the vegetative growth stage, plants had no panicles and leaves made up 78-94% of the forage.

Variations in the ratios of forage were more extreme for *B. tomentellus* than *F. ovina*. In this species, leaves made up 94.3% of forage in the vegetative growth stage while stems made up 5.7% of forage in this stage. In *F. ovina*, leaves were also the crucial parts of forage. In the flowering stage, panicles appeared and stems extended. In this stage, the ratio of stems and leaves was changed and stems made up a considerable part of forage. Panicles also comprised 17.3 and 16.2% of total

weight. However, in this stage, leaves also constituted the crucial part of forage in both species. In the maturity stage, stem growth was fully developed while leaf growth was completed in flowering stage. Thus, the ratio of stems and leaves was distinguishable as compared with the flowering stage. *F. ovina* had elegant and thin stems in the maturity stage while it still had numerous leaves. Thus, the ratio of leaves for this species exceeded the stems and panicles even when its growth was completed. In contrast, the stems of *B. tomentellus* were extensive and stout in the maturity stage. Stems made up 45.7% of its forage while leaves made up 34% of forage in this stage. Leaves in *B. tomentellus* were closely gathered around the base of plant. They did not grow much during the third stage of growth. Because of that, the ratio of stems exceeded the ratio of its leaves.

In terms of panicles, the ratio of weights varied in the flowering and maturity stages. In *F. ovina*, panicle seeds were shed after maturity and the ratio of panicles in forage of this species was lower in the maturity stage than the flowering stage. Results of surveying the relationship between plant components and WSC content percent in distinct species in various phenological stages illustrate that considering panicles in *Bromus tomentellus*, WSC is also increased as the panicles weight increases in the maturity stage and there was an inverse relationship between the plant components and WSC content percent in the other phenological stages; in other words, WSC content percent decreases Arzani, H., 2009. Forage quality and daily requirement of grazing animal, Tehran University Press, 329 p. (In Persian).

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when the weight of organs increases and vice versa.

Conclusions

WSC is the primary source of energy stored in the vegetation organs of perennial plants. A comprehensive knowledge of carbohydrate synthesis, translocation, utilization and storage as influenced by various ecological parameters is valuable in determining when and to what extent plants may be utilized for optimum productivity with minimum damages to the plant resource. Depletion of WSC storage is believed to be a primary factor for the loss in plant vigor and subsequent range deterioration. Consequently, in designating the time of grazing, attention to plant parts and the quality of the grass which is influenced by WSC is critical. In the time of livestock exclusion according to the fact that the analyzed plants are various kinds of grass, it is necessary that an adequate amount of WSC remains in the roots of plant for its regrowth.

Acknowledgment

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تغییرات کربوهیدرات‌های محلول در آب در اندام‌های دو گونه *Bromus tomentellus* و *Festuca ovina* در سه مرحله فنولوژیکی

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چکیده. اطلاعاتی از ویژگی‌های فیزیولوژیکی گیاهان می‌تواند به مدیران مرتع در انتخاب برنامه چرای برای به حداقل رساندن خسارات وارده به پوشش گیاهی کمک کند. کربوهیدرات‌های محلول حاصل از تولیدات فتوسنتزی در بخش‌های مختلف گیاه ذخیره می‌شوند، تا در زمان نیاز به مصرف برسند. در تحقیق حاضر به منظور بررسی تغییرات کربوهیدرات‌ها در اندام‌ها و مراحل رشد متفاوت، از ریشه و اندام هوایی *B. tomentellus* و *F. ovina* در سه مرحله فنولوژیکی (رویشی، گلدهی و رشد کامل) نمونه‌برداری شد. تجزیه و تحلیل داده‌ها با استفاده از آزمایش فاکتوریل مرحله فنولوژیکی و اندام گیاهی در قالب طرح کاملاً تصادفی با ۵ تکرار صورت گرفت. نتایج نشان داد که حداکثر میزان کربوهیدرات محلول در برگ *B. tomentellus* و *F. ovina* به ترتیب در مرحله رشد کامل (۴/۶٪) و گلدهی (۴/۵٪) مشاهده شد. بالاترین میزان کربوهیدرات‌ها در ریشه *B. tomentellus* (۳/۲٪) و *F. ovina* (۳/۵٪) به ترتیب در مرحله رویشی و رشد کامل مشاهده شد. استفاده از مرتع در مرحله فنولوژیکی که گونه علفی بتواند در برابر ریزش برگ‌ها بدون افت شدید ذخیره مقاومت کند توصیه می‌شود. همچنین نتایج نشان داد که به استثنای اندام خوشه در *B. tomentellus* کربوهیدرات‌ها با افزایش وزن گل در مرحله رشد کامل افزایش یافته و نیز رابطه معکوسی بین درصد اجزای گیاهی و میزان کربوهیدرات‌های محلول در سایر مراحل فنولوژیکی مشاهده شد.

کلمات کلیدی: کربوهیدرات‌های محلول، مراحل فنولوژیکی، اندام‌های گیاه، گندمیان