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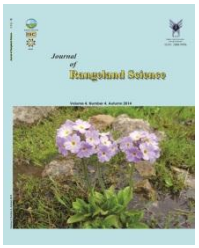

Research and Full Length Article:

Investigation of Forage Quality of *Vetiveria zizanioides* in Semi-Steppe Region of Maravehtappeh, Golestan Province, Iran

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Abstract. Information on forage quality in phenological stage could help range managers to choose suitable grazing method to achieve higher animal performance without detriment to vegetation. Vetiver grass (*Vetiveria zizanioides*) is a non-native perennial grass that was introduced to the Iran in 2008. This plant is a multi use species that could be considered from different viewpoints. Thus, its forage quality in three phenological stages (vegetative, flowering and maturity) was investigated. Samples of each stage were collected and analyzed for 7 quality traits such as Crude Protein (CP), Acid Detergent Fiber (ADF), Dry Matter Digestibility (DMD), Metabolizable Energy (ME), Digestible Energy (DE), Ash content (Ash) and Total Digestible Nutrient (TDN). Data were statically analyzed using One-way analysis of variance (ANOVA) and mean comparisons were done by Duncan method. The results indicate that there was a significant difference among phenological stages for all the traits ($p<0.01$). As the plant age increased, the quality values including CP (from 8.89% to 5.25%), DMD (34.79% to 31.63%), ME (3.91 to 3.38 MJ/Kg), Ash (6.7% to 5.5 %,), DE (1.71 to 1.64 Mcals/Kg) and TDN (23.07% to 20.79%) were decreased while ADF was increased (from 63.72% to 65.7%). This led to the reduction of Vetiver grass forage quality. Because of lower values of CP, ME and DMD (except vegetative stage) and higher values of ADF, it was suggested that this species had no prominent place in the ruminants' diet. However, it played important roles in soil conservation and it may be complementary to the native vegetation because it is available at different times of the year considering its phenology and growth season.

Key words: Forage quality, Phenological stages, *Vetiveria zizanioides*, Golestan, Iran

Introduction

The problems of animal food supply and its quality are aggravated in the arid and semi-arid regions with scarce and erratic rainfall that limits the growth of herbaceous species and biomass yield in rangelands. In addition, the uncontrolled and excessive use of rangelands has contributed to their degradation while reducing the availability of livestock feed resources further (Robles *et al.,* 2008). The chemical analysis of range forage plants serves as a comparative measure of differences between species and changes with respect to the season or phenology. Understanding of nutrient contents in the plants is useful for determining the range capacity, the most proper time of utilization of range plants, prediction of malnutrition and evaluation of nutrition requirements of plants (Asaadi and Khoshnood Yazdi, 2011). Information on forage quality of plant species, their chemical composition and factors affecting them such as climate and maturity stage are essential not only for determining the grazing capacity and reaching the best exploitation of range forage but also in recognizing probable deficiencies in the nutrition of livestock grazing there (Panahi *et al.,* 2012). Range forage quality has spatial and temporary variations. In order to obtain the optimum utilization of rangelands, it is necessary to consider temporal variations of forage quality (Asaadi and Khoshnood Yazdi, 2011). Stockmen and wildlife managers need to understand nutritional dynamics of forages in rangelands in order to sustain the adequate growth and reproduction of their animals (Murray *et al.*, 1978). Knowledge of nutritive value of forages is more important for planning the forage utilization during the grazing seasons and developing an optimal feeding regime for the ruminants (Ghanbari and Sahraei, 2012). Forage quality can be defined as the extent to which forage has the potential to produce a desired animal response (Ball *et al.,* 2001). Forage quality varies considerably due to several factors. Factors that affect forage quality include species, stage of growth, leaf-to-stem ratio, soil agents, climate, harvesting, diseases and pests (Arzani *et al.,* 2001). The most important factor influencing the forage quality is herbage maturity (Buxton *et al.,* 1996). Stiven and Fahey (1994) found that the effects of plant maturity are the most serious ones on forage quality related to plant environment including temperature. The quality of immature growth stages is generally higher than mature stages. Negative effects of age on the nutritive value of forages are mainly due to a low ratio of leaves to stem and an increase in stem cell walls and lignification (Marinas *et al.,* 2003). Buxton *et al.* (1996) stated that phenological stages affect the digestibility which is reduced by growth development because of stems growth and the reduction in ratio of leaf to stem. In the primary growth stage, grass stems are short and ratios of leaf to stem are high. When plant growth is completed, stems comprise a major part of the total forage (Asaadi and Khoshnood Yazdi, 2011). Information on nutritive value of plant species in each phenological stage could help range managers choose suitable grazing time and stocking rates to achieve higher animal performance without any detriments to vegetation (Arzani *et al.,* 2004). For determining forage quality, different variables are evaluated. Among various factors of forage quality, Crude Protein (CP), Dry Matter Digestibility (DMD) and Metabolizable Energy (ME) are mainly considered for the evaluation of forage quality (Arzani, 1994). Forage quality declines with the advancing of maturity which also influences forage consumption by the animals. As plants mature and become more fibrous, the cell wall concentration increases and the proportion of cell soluble content decreases. Acid Detergent Fiber percent (ADF) increases, cellulose and other

structural carbohydrates will be collected in cell wall (this process is called lignification). With increasing Crude Fiber (CF), digestibility and plant energy percent will decrease and forage intake drops dramatically (Panahi *et al.,* 2012). McDonald *et al. (*1995) reported that in early spring, DMD of plants might reach 80% or higher and it will decrease when plant growth is completed. Digestible energy, ME and DMD of forage decrease whereas fiber and lignin increase with the maturity of plants (Asaadi and Khoshnood Yazdi, 2011). Reduction of CP and DMD of forage and the increase in ADF and Neutral Detergent Fiber percent (NDF) have reported by many researchers when plants are matured (Behnamfar *et al.,* 2009; Goorchi, 1995 and Ghadaki *et al.,* 1974). There are reverse relationships between CP and fiber of a given species and significant differences between the quality of plant organs and phonologic stages (Azarnivand *et al.,* 2006). As fiber increases, forage quality declines (Ball *et al.,* 2001). Cabalero *et al.* (2001) reported the variations of CP of forage in three phenological stages for *Vicia sativa*. Their results indicated that fiber content of forage increased in the maturity stage. Rayburn (2002) found that nutrient values of forage are influenced by the stage of growth, severity of grazing and plant species. Among these, growth stage had been more important factor.

Considering all aspects related to livestock feeding, there is an increasing interest in the rational utilization of potential livestock food resources (Robles *et al.,* 2008). Range forage production increasing through the introduction of native and non-native plant species is a strategy to reduce pressure on rangelands. Vetiver System (VS) based on the use of Vetiver grass was developed in mid-eighties by the World Bank for soil and water conservation in India (Akhzari *et al.,* 2013). Vetiver grass (*V. zizanioides*) is a perennial sterile grass of Gramineae which is originated from Southeastern Asia, India and tropical Africa. At present, there are more than 100 countries cultivating and using vetiver (Chen, 1998; Xia *et al.,* 1996). The forage nutritional, industrial and medicinal values of this plant are of high importance. Although the use of Vetiver grass still has a crucial role in land management but forage obtained from this plant is appropriate to supply cattle, sheep and goats (Truong, 1997). Results obtained by Pingxiang *et al.* (2003) concerning the effects of time and space on variability of nutrients in Vetiver grass demonstrated that the nutrient content of Vetiver grass have strong correlations with the season, growth stage and various soil conditions. The grass was imported from Australia and introduced to the country by Iranian Forests, Range and Watershed Management Organization in 2008 (Maramaei, 2010).

Considering the importance of winter rangelands of Mravhteppeh for rangeland managers in the area and a successful experimental cultivation of Vetiver grass in Kechik watershed (Gholizadeh, 2012) and possibility of its use in range restoration plans in the east of Golestan province, Iran, the present study was done to evaluate its forage quality in phenological stages (vegetative, flowering and maturity) during the growing season.

Materials and Methods

Study area

This study was conducted in north east of Golestan province, Iran in Kechik watershed at an elevation of 620 to 1264 m above sea level. The climate in this region is cold semi-arid with mean annual precipitation of 482 mm and rainfall mainly in the autumn and winter. Its mean annual temperature and annual relative humidity are 16.7°C and 63.8%, respectively. The predominant vegetative covers are *Dactylis glomerata* and *Melilotus* sp. (Niknahad and Maramaei, 2011).

Sampling method

Samples were collected from Kechik station. They were clipped manually with special scissors at three grazing stages in 20th April (vegetative growth), 4th July (flowering) and 19th August (maturity) in 2013. All samples were ground through a 1 mm screen mesh for chemical analysis. Kjeldal method and electric furnace were used for measuring nitrogen and Ash content of the plant, respectively. Measurement of cell walls except hemi cellulose (ADF) was done based on the guidelines of AOAC (1980) using Fibertec device. The formula proposed by Oddy *et al.* (1983) was used for measuring the digestible dry matter (DMD). Metabolic Energy (ME) was measured using the equations proposed by the Australian Agricultural Standard Committee-SCA (1990). The regression equations presented by Fonnesbeck *et al.* (1984) were used for estimating Digestible Energy (DE) and total digestible nutrient percent (TDN) was calculated using the equation proposed by Linn and Martin (1999). Finally, the evaluation of DMD of the studied species in terms of values of forage quality indicators was done based on the guidelines of Arzani *et al.* (2013).

Statistical methods

Before subjecting data to a statistical analysis, the uniformity of data was checked (Verdoodt *et al.,* 2009). Then, One-way ANOVA was used to examine the differences in the measured quality factors of *V. zizanioides* in three growth stages conducted by the means of $SPSS_{21}$. In the case of significant differences, Duncan test was used for mean comparisons.

Results

Results of analysis of variance quality traits at three phenological stages are shown in Table 1. There were significant differences for all the quality traits in three phenological stages ($p<0.01$). Mean values of the measured quality indicators of the studied species at each sampling date are shown in Table 2 and Fig. 1. Results are summarized as follows:

Crude protein: The crude protein content of Vetiver grass was ranged from 5.25 to 8.89% and decreased with the progress of growth stages (Table 2). CP content at vegetative growth stage (8.89%) was significantly higher than two other stages of flowering stage (6.58%) and maturity stage (5.25%) (Table 2 and Fig. 1).

Acid Detergent Fiber (ADF): The highest ADF percent was recorded for maturity stage (65.7%), and the lowest value (63.72%) for vegetative growth stage (Table 2). There was no significant difference ($p>0.01$) between flowering and maturity stages (Table 2 and Fig. 1).

Digestible Dry Matter (DMD): The highest DMD% was observed at vegetative growth stages (34.79%) and the lowest one (31.63 %.) at maturity stage (Table 2). The DMD% at vegetative growth stage was significantly higher than two other stages and the values of flowering stage were significantly higher than maturity stage (Table 2 and Fig. 1).

Metabolic Energy (ME): Metabolic energy ranged from 3.91 MJ/kg in the vegetative stage to 3.38 MJ/kg of dry matter in maturity stage (Table 2). At vegetative growth stage, its values were significantly higher than two other stages and the ME content of flowering stage was significantly higher than maturity stage (Table 2 and Fig. 1).

Digestible Energy (DE): The highest DE content was obtained in vegetative growth stage 1.71 Mcals Kg^{-1} and the lowest value as 1.64 Mcals Kg^{-1} in maturity stage (Table 2). The DE content of Vetiver grass in vegetative growth stage was significantly higher than two other stages ($p<0.01$) but there was no significant difference between two late stages (Table 2 and Fig. 1).

Total Digestible Nutrient (TDN): TDN values ranged from 23.07% in vegetative stage to 20.79% in maturity stage and decreased with the progress of

growth stages (Table 2). The TDN content of Vetiver grass in vegetative growth stage was significantly higher than the other stages ($p<0.01$). But there was no significant difference between two late stages (Table 2 and Fig. 1).

Ash: Ash content of Vetiver grass was ranged from 6.7% in vegetative stage to 5.5 % in maturity stage (Table 2). The ash content in vegetative growth stage was significantly higher than two other stages ($p<0.01$), and its values in flowering stage were significantly higher than maturity stage (Table 2 and Fig. 1).

Table 1. Analysis of variance of seven quality traits at three phenological stages

S.O.V	df	CP (%)	ADF (%)	DMD (%)	ME (MJ/Kg)	DE (Mcals/Kg)	TDN(%)	Ash (%)
Phenological stage	2	16.66**	5.2**	12.82**	0.37**	0.006**	6.88**	1.89**
Error	12	0.34	0.4	0.27	0.008	0.001	0.52	0.04

**= Represents significant differences at $p<0.01$

Table 2. Mean± standard Deviation values of seven quality traits in three phenological stages of Vetiver grass

Phenological stage	CP (%)	ADF (%)	DMD (%)	ME (MJ/Kg)	Ash (%)	DE (Mcals/Kg)	TDN(%)
Vegetative	8.89±0.84[a]	63.72±0.67[a]	34.79±0.39[a]	3.91±0.07[a]	6.7±0.27[a]	1.71±0.02[a]	23.07±0.77[a]
Flowering	6.85±0.71[b]	65.13±0.36[b]	32.77±0.5[b]	3.57±0.08[b]	6.3±0.2[b]	1.66±0.01[b]	21.45±0.41[b]
Maturity	5.25±0.0[c]	65.7±0.78[b]	31.63±0.64[c]	3.38±0.11[c]	5.5±0.0[c]	1.64±0.03[b]	20.79±0.89[b]

Means of three phenological stages for each trait are significantly different ($P<0.01$)

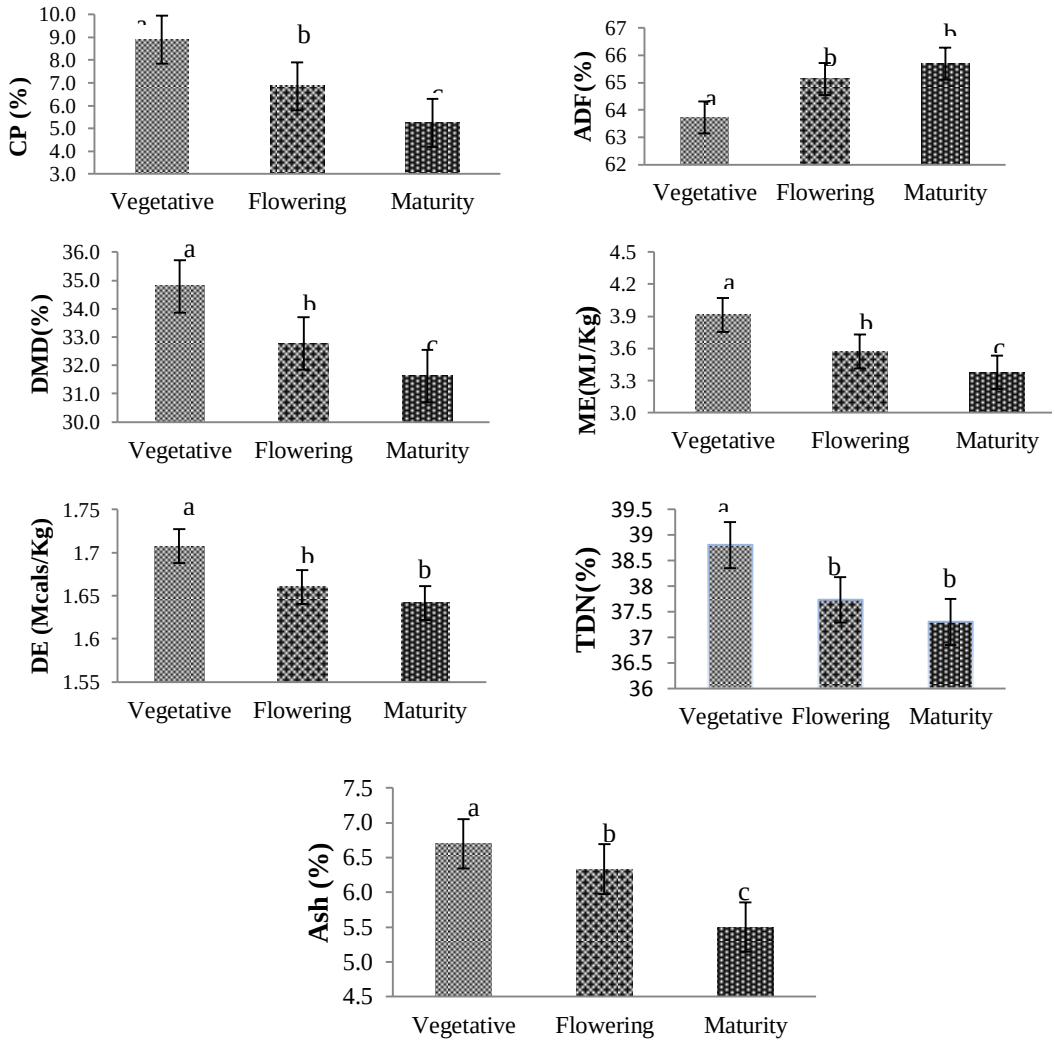

Fig. 1. Mean comparison of seven quality traits in three phenological stages using Duncan method ($p<0.01$)

Discussion

Results revealed that forage quality of vetiver grass declines as plants tend to mature that is in agreement with the studies carried out by Givens *et al.* (1990) indicating that forage quality varied significantly according to the growth stage. Daalkhaijav and Daltanzul (2000) have stated that nutritive value of plant generally decreases as the plant age is increased. Seasonal changes of CP during different phenological stages were reported by White (1983) and Akbarinia and Koocheki (1992). They found that when plants became older, CP decreased. In present study, CP of vetiver grass was different between phenological stages. For CP, as the plant age increased. Its values were decreased from 8.89% to 5.25%. The CP content in flowering and maturity stages is lower than the minimum level of 7-8% DM required for optimum rumen function and feed intake in ruminant livestock (Van Soest, 1994). Therefore, feedstuffs with CP content lower than 7% DM require a supplementation of nitrogen in order to improve their ingestion and digestion by the ruminants (Paterson *et al.*, 1996).

Due to low content of CP in tissues of vetiver grass, this plant is not a valuable source of CP for livestock feeding. The results of measured ADF indicated significant differences in phenological stages. Also, ADF showed an increasing trend (3.1%) during the development stages which is agreed with the results of Heshmati *et al.* (2006). Arzani *et al.* (2001) also reported that with the progress of plant growth, ratios of protector and firmness tissues which mostly consist of structural carbohydrates such as celluloses, hemicelluloses and lignin are increased. Therefore, maturity of plants and an increase in structural carbohydrates cause higher fiber amounts in forage in late the growing season. Torkan (1999) found that forage quality is decreased during plant growth due to the increase of ADF and decrease of CP. High level of fiber content in some of the forage species could be explained partly by the environmental conditions prevailing in the studied area (Boufennara *et al.*, 2012) as low precipitations (compared with the native place of vetiver grass) tend to increase the cell wall fraction and to decrease the soluble contents of the plants (Pascual *et al.*, 2000). It is well accepted that forage degradation in the rumen is mainly affected by the cell wall content and its lignification as lignin is an indigestible fraction and acts as a barrier that limits the access of microbial enzymes to the structural polysaccharides of the cell wall (Ghanbari and Sahraei, 2012).

The results indicated that the value of DMD of vetiver grass in all the measured stages was lower than critical level (Squires, 1981) of DMD (50%) for meeting daily need of one animal unit in the maintenance mod. The decrease of DMD% was observed with the progress of plant growth. This agreed with the results obtained by Akbarinia and Koocheki (1992), Asaadi and Khoshnood Yazdi (2011) and Arzani *et al.* (2004). They reported that a reduction of DMD with maturity of plants is due to the increase of structural tissues in the stems. It was also confirmed by Rayburn (2002) who pointed to the reduction of digestibility in the matured plants. These differences among phenoligical stages in digestibility may be partly attributed to the variations in cell wall content and composition (Boufennara *et al.*, 2012). Arzani *et al.* (2004) and Pinkerton (1996) reported that there is a close relationship between digestibility and cell wall characteristics. In contrast, the chemical structure of cell walls changes with the plant growth. As plant growth continues, fiber content increases and digestibility decreases. The results indicated that ADF had a negative correlation with digestibility. Ammar (2002) came to the same conclusion and reported that NDF,

ADF and lignin were negatively correlated with digestibility.

ME and DE of vetiver grass were decreased with the progress of growth that agreed with the results obtained by Arzani *et al.* (2006). Information on ME or DE content could guide range managers to estimate forage requirements of grazing animals based on the energy required for particular physiological status (Asaadi and Khoshnood Yazdi, 2011). The results of this study revealed that the value of ME of vetiver grass in all the measured stages is lower than critical level (Arzani *et al.*, 2013) of ME (8 MJ/Kg) for meeting daily needs of one animal unit in the maintenance mod.

The ash content of vetiver grass was decreased with the progress of growth that agreed with the results obtained by Ghanbari and Sahraei (2012) for *Festuca ovina* and *Alopecurus textilis.* As compared with the results of Ghanbari and Sahraei (2012), the ash content of vetiver grass was lower than *Festuca ovina* but higher than *Alopecurus textilis* in all phenological stages.

Decrease of TDN% was observed with the progress of plant growth. With the progress of plant growth, ratios of protector and firm tissues which mostly consist of structural carbohydrates such as celluloses, hemicelluloses and lignin are increased. Therefore, maturity of plants and an increase in structural carbohydrates cause higher fiber amounts in forage in late growing season (Arzani *et al.*, 2001). The above mentioned explications could be the reasons for such increasing trend.

Generally, all the measured quality factors of vetiver grass decreased during vegetative growth stage while ADF increased. Consequently, forage quality will be decreased (Azarnivand *et al.*, 2006). This result stresses the findings of other researchers indicating the phonologic effects on forage quality and variation of properties during the plant growth period (Kaboli, 2001, Ahmadi, 2004 and Holechek *et al.*, 2001). The results reported by the other researchers about the effects of phonologic stage on forage quality emphasizing that quality reduction is due to age, the increase of stem to leaf ratio, the increase of lignin and chollenchyma cells (Fahey, 1994; Zohdi, 2001).

Conclusion

Rangelands of Maraveh Tappeh usually supply livestock during autumn, winter and early spring. The results of current research reveal that vetiver grass should be considered with a low potential for range ruminants in Maraveh Tappeh rangelands. This species may be complementary to the native vegetation because it is available at different times of the year considering its phenology and growth season.

As final conclusions, it has to be mentioned that in addition to its important roles in soil conservation, the nutrient content of vetiver grass has strongly depended on soil conditions (Pingxiang *et al.*, 2003). The results of this study came to the same conclusions as Truong (1997) suggested that forage obtained from this plant is appropriate to supply cattle, sheep and goats. Therefore, the study of forage quality of vetiver grass in the other rangelands of country is recommended to identify its suitable cultivation area for livestock feeding.

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بررسی کیفیت علوفه *Vetiveria zizanioides* در منطقه نیمه استپی مراوه تپه، استان گلستان، ایران

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چکیده. اطلاعات مربوط به کیفیت علوفه هر گونه گیاهی در هر مرحله از فنولوژی آن گیاه به مرتعداران در خصوص انتخاب زمان مناسب چرا به منظور نیل به افزایش تولید فرآورده‌های دامی بدون آسیب رساندن به پوشش گیاهی کمک می‌نماید. وتیور گراس ***Vetiveria zizanioides*** گیاهی چندساله غیر بومی از خانواده گرامینه است که در سال ۱۳۸۷ وارد کشور شده است. این گیاه، گونه چند منظوره‌ای است که می‌تواند از جهات مختلفی مطالعه گردد. بنابراین، کیفیت علوفه این گیاه در ۳ مرحله فنولوژیک (ابتدای رشد، اواسط رشد و انتهای رشد) بررسی گردید و مقادیر پروتئین خام (CP)، انرژی متابولیسمی (ME)، قابلیت هضم ماده خشک (DMD)، دیواره سلولی منهای همی سلولز (ADF)، خاکستر، انرژی قابل هضم (DE) و کل مواد مغذی قابل هضم (TDN) در سه مرحله فنولوژیک تعیین شدند. به‌منظور مقایسه بین مراحل فنولوژیکی از روش تجزیه واریانس یک‌طرفه و آزمون دانکن استفاده گردید. نتایج نشانگر تفاوت معنی‌دار بین میانگین فاکتورهای بررسی شده در مراحل مختلف فنولوژیک بود ($p<۰/۰۱$). با افزایش سن گیاه، مقادیر CP، ME، DMD، خاکستر، DE و TDN به‌ترتیب از۸/۸۹٪ به ۵/۲۵٪، از ۳۴/۷۹٪ به ۳۱/۶۳٪، از ۳/۹۱ به ۳/۳۸ (MJ/Kg)، از ۶/۷ ٪ به ۵/۵٪، از ۱/۷۱ به ۱/۶۴ (Mcals/Kg) و از۲۳/۰۷ ٪ به ۲۰/۷۹ ٪ کاهش یافتند در حالیکه درصد ADF از ۶۳/۷۲٪ به ۶۵/۷٪ افزایش یافت. این امر منجر به کاهش کیفیت علوفه وتیور گراس گردید. این گیاه علاوه بر نقش مهمی که در حفاظت خاک دارد، به دلیل CP پایین (به استثنای ابتدای رشد)، ME پایین، DMD پایین و ADF زیاد نمی‌تواند در رژیم غذایی دام‌ها جایگاه برجسته‌ای داشته باشد اما به دلیل داشتن فصل رشد متفاوت، می توان از آن به عنوان مکمل گیاهان بومی منطقه مورد مطالعه استفاده نمود.

کلمات کلیدی: کیفیت علوفه، مراحل فنولوژیکی، ***Vetiveria zizanioides***، گلستان، ایران