Biological Forum – An International Journal **8**(2): 134-139(2016)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Estimating Qualitative Parameters of Three Halophytes using NIR Technology

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(Received 15June, 2016, Accepted 07 August, 2016)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Improvement of the traits related to forage quality including Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and CP in forage species has a significant impact in increasing livestock production. In breeding programs that the number of samples is occasionally high, the use of chemical methods is time-consuming and costly. For this reason, NIR technology has been introduced as a rapid and accurate method in estimating chemical com-position of agricultural products. This research was aimed to investigate the possibility of using this technology in estimation of NDF, ADF, CP, DMI, DMD, ME, RFV and WSC in *Suada fritucosa, Seidlitzia rozmarinos* and *Aeluropus littoralis*. A total of 45 samples of each species were selected during different phenological growth stages and from different vegetation types. According to the result, phonological stages of growth had a significant influence on forage quality. In all species, the amount of CP, DMD, ME and RFV decreased with progressing growth stages, while NDF, ADF and WSC contents increased. The index is measured on the basis of the highest quality forage species *Seidlitzia Rozmarinos* is with RVF=98.27 and *Suada fritucosa* species had the lowest forage quality (RVF=67.28). The results of this study showed the halophytes have potential for future applications to serve as sources of fodder, forage and other possible uses.

Keywords: halophytes, Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Crude Protein (CP), Near Infrared Reflectance Spectroscopy (NIRS).

INTRODUCTION

Knowledge on the nutritional value of plant species helps range managers and ranchers to balance between available food and livestock requirement to maximize livestock performance. Supplying livestock requirement is necessary in terms of energy, protein, minerals, and vitamins and it is possible when forage quality is studied in terms of chemical and physical compounds (Arzani, 2002). Several indicators are measured to determine forage quality including crude protein, crude fat, NDF, ADF, DMD, ME, WSC, nitrogen-free extract, crude fiber, lignin, minerals (phosphorus, potassium, calcium, etc.), relative nutritional value, and so forth. Among the mentioned factors, those must be considered that firstly less time and cost are spent measuring them and secondly provide a good estimation of forage quality. In recent years, NIR technology has developed and the measurement of agricultural and livestock products is possible with this system. Nowadays, quality traits of forage species are measured using this technology. This method is appropriate for selective programs in which plant breeders face a number of different plant populations and rapid and inexpensive methods are required to measure the traits. The percentage of crude protein and the content of ADF and NDF are important quality traits in improving the nutritional value of forage species including *Suada fritucosa, Seidlitzia rozmarinos* and *Aeluropus littoralis*. Several methods have been introduced for quality traits.

For example, currently, two chemical methods including the Kjeldahl and LECO nitrogen analyzer are used to measure the percentage of crude protein. In both methods, CP is calculated according to the formula (N × 6.25) with the difference that the speed and accuracy of LECO is higher compared to Kjeldahl. The method of Van Soest (1963) and the device of Fibertic 2010 are used to measure ADF. In addition, NDF is measured by the above method, with the difference that acid solution (ADS) and neutral solution (NDS) are used to measure ADF and NDF, respectively. NIR method is based on near-infrared absorption and reflection in the wavelengths of 700-2500 nm. In this method, radiation is emitted on samples and reflected energy (R) from samples is calculated according to log1/R. The device is calibrated based on multiple linear regressions (MLR) between the energy reflected from the object and chemical data. NIR measurement accuracy depends on calibration method. Therefore, the chemical methods must be accurate and standardized and forage samples should also have sufficient range for traits. Therefore, it would be better to collect the samples from different growth stages and different sites (Beerepoot and Angew, 1997). NIR spectrometry has been used since 1970 to analyze the factors, including the percentage of protein, NDF%, and the percentage of digestibility in cereals and forage species (Norris *et al.,* 1994, Deaville *et al.,* 2000). Norris *et al.,* (1976) estimated the standard error prediction to be 0.95, 3.1, 2.5, 2.1, and 3.5% for the percentage of crude protein, NDF, lignin, and digestibility, respectively. In a similar study, conducted on legumes and grasses by Garcia *et al.,* (2006), NIR calibration revealed relatively high correlation coefficients and low standard error for ADF, CP, DM, and DMD. Gatius *et al.,* (2004) applied the NIR in the estimation of crude protein (CP) and a calibration was performed for 27 samples with three growth stages (vegetative, flowering, and seeding). The raw content of the samples was measured by a comprehensive model, for all growth stages, and a unique model for the seeding stage. In the same results, Parnell and White (1983) showed NIR performance as an appropriate alternative to determining the crude protein of different forage species. Jafari (2001) evaluated the possibility of using NIR in estimation of digestibility and crude protein content in forage grasses and introduced the NIR method as a new, fast, accurate and efficient technology in measuring the forage quality of range species. The same results also have been reported by Ahmadi (2003), who compared laboratory and NIR methods for measuring the forage quality of a few rangeland species in different phenological stages. Charehsaz *et al.,* (2012) evaluated the performance of NIR method in estimation of crude protein, digestibility, ADF, and total ash. They reported SEC values of 0.15-1.09, 0.83-3.94, 0.52-4.96, and 0.21-0.86 and SEP values of 0.13-0.75, 84.62-3.34, 0.31-3, and 0.17-0.61 for the mentioned traits, respectively. Correlation coefficient between the experimental data and the results of NIR prediction for all traits, particularly CP, was very high (<95%). Arzani *et al.,* (2007) studied the forage quality of rangeland species in three provinces of Semnan, Markazi, and Lorestan to calculate daily animal unit requirement. According to the results, the changes of parameters were estimated to be 8.8, 9.5, and 9 for CP and 42% for ADF and 52% for DMD, and 6.86 MJ per kg dry matter.

MATERIALS AND METHODS

A. Study area

Aran o Bidgol City is located in the southwestern margin of the central desert of Iran and restricted from north by Salt Lake of Aran o Bidgol, Qom and Semnan provinces, from west by the city of

Kashan, from south by Natanz and from east by the city of Ardestan. Aran o Bidgol is one of the cities of Isfahan Province and is situated between 51°29'E and 34°14'N with an elevation of 912 m above the sea level. Aran o Bidgol with an area of 6051 km2is located in the central part of Iran; it lies 235 km by road southwest of the capital Tehran and 210 km from the province center. The climate of Aran o Bidgol is desert conditions; the annual rainfall is 100-150 mm, the minimum and maximum temperatures recorded were 5°C in winter and 48°C in summer.

B. Methodology

In the present study three halophyte species of were cut (harvested) at three phenological stages (ve-getative, flowering and seed ripening) in saline rangelands of Iran, Aran o Bidgol in 2015. In other words, a total of 45 samples of each species were selected at different growth stages from different vegetation types, dried at 70°C for 24 h, and ground with 1 mm screen mill and kept in closed containers. Eight quality traits (CP, ADF, NDF, DMD, DMI, WSC, ME and RFV) were estimated using near infrared spectroscopy (NIR). Details of the methodology and calibrations of NIR are given by Jafari *et al.*. Collected data were analyzed using a factorial experiment for simple effects of species and phenological stages and the first order interaction of species by phenological stages. All statistical analyses were conducted by SAS Inst.

RESULTS

The Near Infrared Reflectance Spectroscopy statistics values for the prediction of CP, ADF, NDF, DMD, ME, WSC and RFV of forage samples are shown in Table 1. According to the results, Significant differences were obtained between forage quality *of Suada fritucosa*, *Seidlitzia rozmarinos* and *Aeluropus littoralis* species (p<0.01) (Table 4).

Table 1: Average quality indices values in different plant species.

Species	CP(%)	ADF(%)	NDF(%)	DMD(%)	ME(MJ)	WSC(%)	RFV
Aeluropus littoralis	12/24 b	37/80 a	47/15b	46/04 a	5/83a	8/44c	91/28b
Seidlitzia rosmarinus	8/70 c	35/67 b	46/04 b	44/96 b	5/64b	19/02a	98/30a
Suada fruticosa	27/26 a	30/31 c	57/59 a	40/40 c	4/87c	11/00b	67/29c

Table 2: Comparison of means between phenological stages on all species.

Stage	CP(%)	ADF(%)	NDF(%)	DMD(%)	WSC(%)	ME(MJ)	RFV
Vegetative	19/68a	32/22c	43/28c	2/87a	10/86c	6/38a	111/13a
Flowering	15/50b	34/52b	50/67b	2/38b	12/43b	5/75b	84/26b
Seeding	13/01c	73/03a	56/82 a	2/14c	15/18a	4/21c	61/49c

Crude protein content of forage species ranged from 8.70 to 27.26% (Table 1). The minimum CP content was recorded for *Seidlitzia rozmarinos* and the maximum CP for *Suada fritucosa*. There was a significant difference in protein content growth stages of species (p<0.01). The highest protein content was 19.68% in vegetative growth stage and the lowest value (13.01%) was in seed ripening stage. The CP contents at different vegetative stages of species significant differences (p<0.01) (Table 3). Significant differences were observed among effects of the species and phenological stages for CP contents.

ADF (Acid Detergent Fiber) percentage of forage species ranged from 37.80 to 30.31% (Table 1). There were high ADF percentage for *Suada fritucosa* and low content ADF for *Aeluropus littoralis*. There was a significant difference in ADF percentage among growth stages of species (p<0.01). The lowest ADF percentage was 32.22% in vegetative growth stage and the highest value (73.03%) was in seed growth stage (Table 2).

The results indicated also that the ADF percentage of species were significantly different at three phenological stages. There were significant differences between effect of the species and phenological stages for ADF contents. There were significant differences (p<0.01) in digestible dry matter (DMD)contents of species at different vegetative stages (Table 3). The highest value of DMD at vegetative growth stage was 46.04% and the lowest one at seed ripening stage 40.40% (Table 2). The DMD contents at different vegetative stages of species significant differences (p<0.01) (Table 3). According to the results, significant differences were observed among effect of the species and phenological stages for DMD contents (p<0.01).

Metabolizable energy (ME) ranged from 5.83 to 4.87 MJ/kg dry matter. *Aeluropus littoralis* had the highest ME, while *Suada fritucosa* had the lowest value. The ME content at different vegetative stages of species showed significant differences (p<0.01).

Table 3: Average quality indices according to phenological stages in different species.

Stage	Species	CP%	ADF%	NDF%	DMD%	WSC%	ME(MJ)	RFV
1	*Aeluropus littoralis*	13/50a	36/93b	44/56c	48/54a	6/25a	7/44c	101/36a
2	*littoralis Aeluropus*	12/36b	37/97ab	46/71b	46/13b	5/84b	8/48b	91/88b
3	*Aeluropus littoralis*	10/85c	38/51a	50/17a	43/47c	5/39c	9/41a	80/61c
1	*Seidlitzia rosmarinus*	15/41a	32/35c	34/05c	53/45a	7/09a	15/35c	148/10a
2	*Aeluropus littoralis*	6/26b	35/48b	49/88b	46/74b	5/95b	17/89b	87/22b
3	*Aeluropus littoralis*	4/43c	39/17a	54/81a	34/68c	3/90c	23/84a	59/58c
1	*Suada fruticosa*	30/13a	27/39c	51/42b	45/96a	5/81a	9/78c	83/92a
2	*Suada fruticosa*	27/89b	30/12b	55/41b	43/81b	5/45b	10/93b	73/68b
3	*Suada fruticosa*	23/76c	33/41a	66/12a	31/43c	3/34c	12/28a	44/27c

Table 4: Analysis of variance quality indices values.

Source	df	CP(%)	ADF(%)	NDF(%)	DMD (%)	ME (MJ)	WSC(%)	RFV(%)
Species	2	1165/5**	178/85**	487/49**	107/67**	3/12**	365/84**	3173/5**
Stage	2	136/2**	69/28**	551/91**	518/47**	15/01**	57/32**	7409/2**
Species*Stage	4	25/5**	8/07**	82/81**	57/51**	1/67**	14/40**	1460/4**
Error	27	0/66	0/85	3/50	0/86	0/02	0/12	18/67
CV %		5/05	2/70	3/72	2/11	2/90	2/70	5/05

**,* denote significant differences in levels 0.01 and 001.

The highest value of ME at vegetative stage was 19.68 MJ/kg while the lowest value in seed ripening period was 13.01 MJ/kg. The ME content at different vegetative stages of species showed significant differences ($p<0.01$) (Table 3). According to the results, significant differences were observed among effects of species and phenological stages for ME contents ($p<0.01$) (Table 3). According to the results, significant differences were observed among effect of the species and phenological stages for WSC contents ($p<0.01$). The highest value of wsc at seed stage was 15.18 % while the lowest value in vegetative ripening period was 10.86%. *Aeluropus littoralis* had the lowest WSC, while *Suada fritucosa* had the highest value. WSC percentage ranged from 8.44 to 19.02 %.

DISCUSSION AND CONCLUSION

Arid and semi-arid regions of Iran have diverse and rich collection of plants. Vegetation cover of such regions has high resistance to difficult environmental conditions. Also, the forage, nutritional, industrial, medicinal and conservative values of these plants are of high importance. The nutrient value of range forage depends on plant composition and stage of growth. The close matching of nutrients requirements and feed quality is necessary for efficient animal production. This study suggests that adequate nutrients are available in vegetation communities including the evaluated species. Range forage quality has spatial and temporary variations. The chemical analysis of range forage plants serves as a comparative measure of differences between species and changes with season or phenology. Rangelands of Asadli and Sisab in poor condition usually supply livestock during spring and early summer. However, forage quality declines as plants mature. The results showed the evaluated forage species had different nutritive values. As Cook and Stubbendieck reported the chemical content of plant species may differ because of an inherent ability to withdraw certain nutrients from the soil and to concentrate them in tissues. Plants may also vary in susceptibility to leaching, or may produce different proportions of leaves, stems, and flower stalks at various stages of maturity or because of previous grazing treatments. Seasonal changes of CP during different phenological stages were reported by White, Akbarinia and Koocheki and Arzani *et al.* They found that when plants became older, CP decreased. In this study, CP of *Suada fritucosa, Seidlitzia Rozmarinos* and *Aeluropus littoralis* were different between phenological stages. Results also showed differences ($P<0.01$) between CP content in different species. Among species in the present study *Aeluropus littoralis* showed the lowest CP percentage. Stoddart *et al.* stated that declines in nutrient contents and leaching are especially serious in the case of herbaceous plants and forbs. In the same region, as the protein of bur clover (*Medicago hisida*) was leached, an increasing wide nutritive ratio resulted.

The results of ADF showed significant differences in phenological stages. Also, ADF showed an increasing trend during the development stages which is in accordance with Heshmati *et al.* Among species in the present study *Aeluropus littoralis* had the highest ADF percentage. Young plant cells have one external layer called a primary cell wall, but when they become mature, a secondary cell wall is also formed. Because of storage tissues in seeds, ADF and NDF contents varied with seed maturity between phenological stages and species. Arzani *et al.* also reported that with 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 late in the growing season. Dry matter digestibility of plant parts mainly decreased with growth progress, and DMD for all species in all phenological stages were different. This agreed with results obtained by Akbarinia and Koocheki. They reported that a reduction of DMD with maturity of plants is due to increasing structural tissues in stems. Pinkerton also reported a close relationship between digestibility and cell wall characteristics. In contrast, the chemical structure of cell walls changes with plant growth. As plant growth continues, fiber content increases and digestibility decreases.

Reduction of digestibility in matured plants also was reported by Kashki and Linn and Kuehn. In our experiment for different species DMD values of forages were above 50%. Generally, about 50% digestibility is sufficient for animal maintenance. As El-Shatnawi and Mohawesh and Ganskopp and Bohnert also reported, stems have relatively high fiber content. The results of measured ME showed significant differences in phenological stages. Information on ME content could guide range managers to estimate forage requirements of grazing animals based on energy required for particular physiological status. The results suggest that palatability, digestibility and nutritional values are decreased during the growth period due to accumulation of fiber in plant tissues. Arzani *et al.* proposed that metabolic energy is decreased during vegetative growth period. Current research showed that different species in this study are considered as a suitable source for livestock nutrition.

WSC percentage decreased with growth progression in species, as the highest and the lowest WSC percentage were measured in seed and vegetative ripening stage, respectively. Since in seed maturity stage WSCs are converted to structural carbohydrates and as a result structural and nonstructural carbohydrates will accumulate and increase. This was confirmed by the results obtained by Arzani *et al.*

Generally, comparison of forage quality of the plants shows that crude protein, metabolic energy and percentage of digestible dry matter decreased during vegetative growth stage while ADF increased. During the plant development, digestibility and nutritional content decreased and the content of non-digestible nutrients also decreased due to fiber increase of plant tissues. Consequently, forage quality with increasing growth stages will be decreased. Phenological stage of growth had a significant influence on forage quality. With increased plant development, CP, DMD and ME are reduced And also ADF, NDF are increased. Higher forage quality was recorded for the 1st stage of growth but quantity is Lowest. This study suggests that, since at desert areas least cover vegetated. Therefore livestock production is influenced by the nutrients in feed, so to achieve maximum production due to the impact of different growth stages on forage quality, the best time to be selected grazing.

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