

Seasonal variation in water buffaloes' diet grazing in wet grasslands in Northern Greece

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Key words: vegetation composition, dietary preferences, grazing livestock, plant functional types, wetlands.

Ključne besede: vrstna sestava vegetacije, prehranske preference, pašna živina, rastlinski funkcionalni znaki, mokrišča.

Abstract

Seasonal variability in grasslands' vegetation affects animals' diet selection. We studied the seasonal changes in water buffaloes' diet during grazing in wet grasslands in Northern Greece. We recorded each month the plant species of the vegetation and the species consumed by buffaloes. We categorized the former into three functional groups (graminoids, legumes, and forbs) and the latter into five groups (graminoids, legumes, forbs, shrubs, and trees). There were significant differences in the proportions (%) of functional groups in the vegetation and in buffaloes' diet between seasons (χ^2 test, $P < 0.05$). Graminoids, legumes, and forbs participated in buffaloes' diet in all seasons, while the less preferred functional groups were trees and shrubs. Buffaloes consumed ten species in all seasons but we observed the most diverse diet, in terms of plant species, in spring. The most frequently consumed species in each functional group were *Cynodon dactylon* (graminoids), *Trifolium repens* (legumes), *Cichorium intybus* (forbs), *Rubus* sp. (shrubs), and *Populus* sp. (trees). However, the majority of plant species in buffaloes' diet was in very low proportions (<1%), while buffaloes did not sample at all 38 herbaceous species. Researchers need to conduct further research to understand water buffaloes' foraging strategy regarding plants' anti-quality characteristics.

Izveček

Sezonska spremenljivost traviščne vegetacije vpliva na selektivnost prehranjevanja pašnih živali. Proučevali smo sezonske spremembe v prehrani vodnih bivolov med pašo na mokrotnih travnikih v severni Grčiji. Vsak mesec smo beležili vrstno sestavo vegetacije in vrste s katerimi so se bivoli prehranjevali. Združili smo jih v tri funkcionalne skupine (trave, metuljnice in zeli) in nato v pet skupin (trave, metuljnice, zeli, grmovnice in drevesa). Ugotovili smo značilne razlike v deležih (%) funkcionalnih skupin v vegetaciji in v prehrani bivolov med sezonami (χ^2 test, $P < 0.05$). Trave, metuljnice in zeli so bile prisotne v prehrani bivolov celotno obdobje, manj so izbirali drevesa in grmovnice. Bivoli so se celotno obdobje prehranjevali z desetimi vrstami, najbolj raznoliko prehrano glede vrstne pestrosti smo opazili spomladi. Najbolj pogoste vrste v vsaki od funkcionalnih skupin so bile *Cynodon dactylon* (trave), *Trifolium repens* (metuljnice), *Cichorium intybus* (zeli), *Rubus* sp. (grmovnice) in *Populus* sp. (drevesa). Večina rastlinskih vrst v prehrani bivolov je bila zastopana z majhnimi deleži (<1%) in bivoli niso izbirali vseh 38 zeliščnih vrst. Nadaljne raziskave so potrebne za razumevanje pašne strategije vodnih bivolov v povezavi z značilnostmi rastlinskih vrst, ki kažejo na njihov slabšo kakovost.

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1. Introduction

Domestic livestock face multiple challenges during their foraging routine as they have to cope with heterogeneous landscapes and diverse environmental conditions (Launchbaugh et al. 2006, Ginane et al. 2015). The choice of their diet could be considered a strategy to obtain maximum quantity and sufficient quality of forage (Mládek et al. 2013). Therefore, the plant species that the animals included in their diet are the result of a careful decision in order to meet their physiological demands (Owen-Smith & Novellie 1982, Soder et al. 2009).

The Greek water buffalo is an endangered local breed of the Eurasian water buffalo (*Bubalus bubalis*) (FAO 2007, Tsiobani et al. 2016), which has been extensively raised in the wetlands of the country since the end of the 19th century (Georgoudis 1993). Water buffaloes, like cattle, are characterized as bulk grazers, meaning that they are less selective in order to fill their stomachs (Whitmore 2000). However, due to the large volume, the slow movement, and the high bacterial activity of their rumen, they have a different diet than cattle and sheep (Napolitano et al. 2013, Jerrentrup et al. 2015, Tóth et al. 2018). Although there are numerous studies in the literature about the diet selection of cattle and other domestic animals (Cibils et al. 2004, Fraser et al. 2009, Soder et al. 2009, Ginane & Dumont 2010), data concerning the water buffaloes' dietary behaviour is very limited (Napolitano et al. 2007, Antkowiak et al. 2012, Tsiobani et al. 2014). This knowledge is significant for the protection of this local breed as well as for the achievement of proper management of wet grasslands' biodiversity because the selective grazing affects the structure and the dynamics of plant communities (Duncan et al. 2006).

Wet grasslands constitute a particular category of grasslands (Papanastasis & Ispikoudis 2012) and a type of wetlands. They are either found on the shorelines of lakes or along floodplains of rivers, wherein they are temporarily flooded or saturated with water. This periodic flooding allows the growth of plants typical on moist soils (Keddy 2010). Wet grasslands, in general, have unique ecological characteristics, that resemble both terrestrial and aquatic ecosystems, providing a natural habitat for several plant and animal species (van der Valk 2006, Verhoeven et al. 2006). According to Kazoglou (2007), the vegetation of the wet grasslands in Northern Greece is mainly herbaceous, including plant species from the genera *Agrostis*, *Alopecurus*, *Carex*, *Cyperus*, *Festuca*, *Juncus*, *Poa*, and *Scirpus*, that belong to graminoids, *Trifolium* and *Vicia* from legumes, and *Althaea*, *Cirsium*, *Eleocharis*, *Galium*, *Lysimachia*, *Mentha*, *Myosotis*, *Potentilla*, *Ranunculus*, *Roripapa*, *Rumex*, and *Veronica* from forbs.

Throughout their life history, plant species are either dormant or under growth (Moen et al. 2006). This means that there is seasonality in their abundance in the grassland as well as variety in their phenological stage and nutritive value (Villalba & Provenza 2009). This seasonal cycle of forage availability and quality directly affects grazing animals' diet composition (Hejman et al. 2008) and imposes constraints on animals (Zweifel-Schielly et al. 2012). Plant species in grasslands according to their response to environmental factors are classified into functional groups such as graminoids (including grasses, sedges, and rushes), legumes, forbs, shrubs, and trees (Blondel 2003). Since environmental conditions change between seasons, the ratio of different plant functional groups in wet grasslands also differs between seasons (Keddy 2010), affecting thus the water buffaloes' diet. Therefore, the proportion of different plant functional groups in the buffaloes' diet is not expected to coincide with their proportion in the wet grasslands' vegetation as stated by other researchers (Galt et al. 1982, Hejman et al. 2008) for different kind of grazing animals and grasslands vegetation. In this context, the aim of this study was to investigate the seasonal changes of different plant functional groups in the diet of Greek water buffaloes during grazing in wet grasslands in Northern Greece.

2. Material and methods

2.1 Study area and animal facilities

The study was conducted in the wet grasslands of Chrysochorafa (longitude 23°10'08.48" E, latitude 41°10'46.67" N), a rural village in Northern Greece, from May 2015 to April 2016. The grasslands cover an area of 91.19 ha and are located on the shores of Lake Kerkini, which is an artificial freshwater lake created at the site of a former natural swamp. The lake is characterized by large fluctuations of water level, where the minimum levels are observed between September and February and the maximum levels are observed between early May and early June (Papastergiadou & Balabonas 1992). The wider area of the Lake Kerkini is under protection according to the Ramsar Convention.

The climate of the region is intermediate between Mediterranean and Mid-European, characterized by hot, dry summers and cold, wet winters. The seasonal yearly mean air temperature during the study period ranged from 4.6°C in the winter and 25.4°C in the summer. Also, the seasonal yearly mean precipitation ranged from 24.4 mm during summer to 72.3 mm during autumn (Weather Station of Chrysochorafa 2018). Autumn was the wettest season with normal expected temperature levels, while summer was the driest season of all.

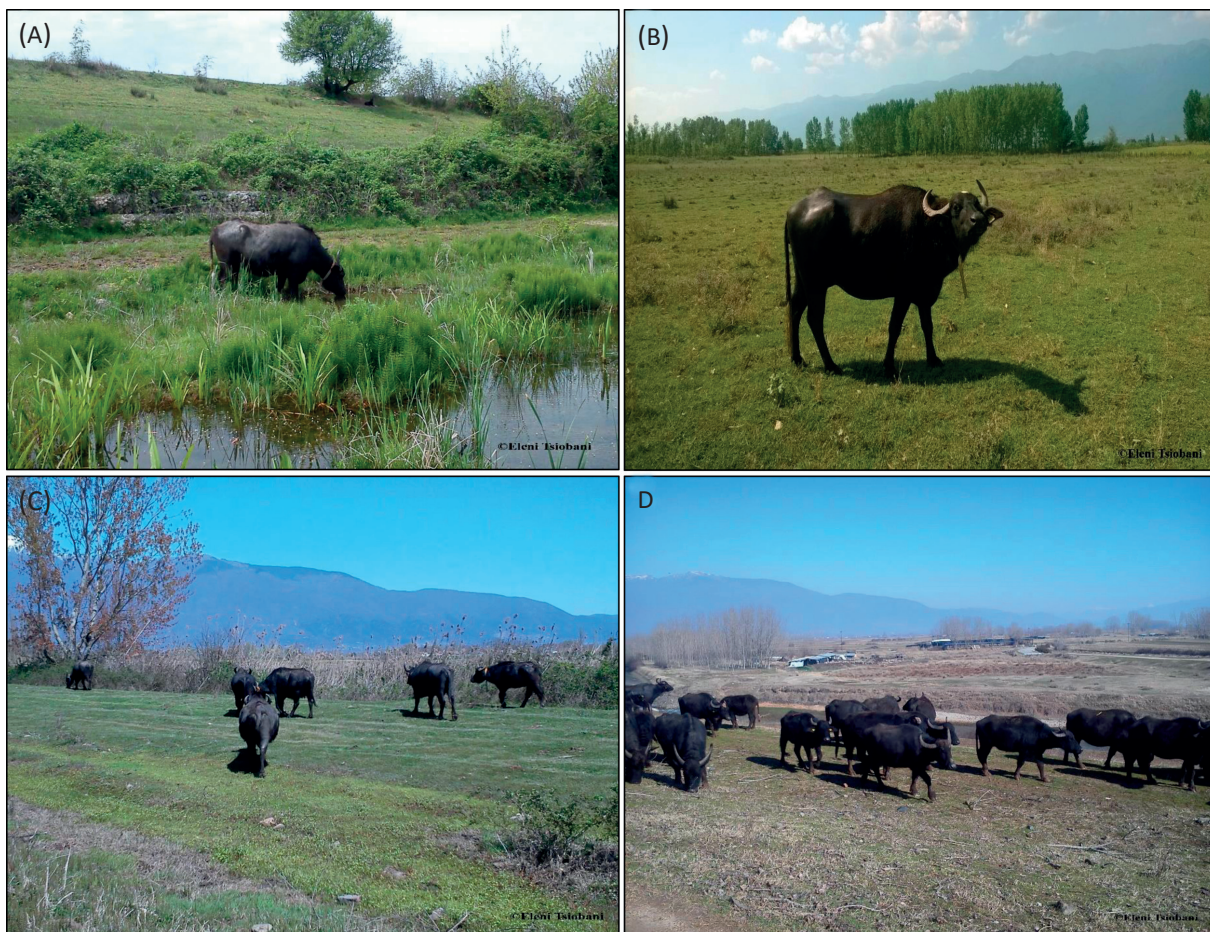


Figure 1: Greek water buffaloes grazing on the wet grasslands of the Lake Kerkini in Northern Greece during (A) spring, (B) summer, (C) autumn, (D) winter. Photos provided by Eleni Tsiobani.

Slika 1: Grški vodni bivoli na paši na mokrotnih travnikih ob jezeru Kerkini v severni Grčiji: (A) spomladi, (B) poleti, (C) jeseni, (D) pozimi. Fotografije Eleni Tsiobani.

The soils are alluvial, supporting vegetation of great variety (Psilovikos & Margoni 2010), mainly herbaceous species. According to our observations, woody species (shrubs and trees) were present but scattered at the edges of the study area. *Clematis vitalba*, *Cornus sanguineus*, *Cytisus monspessulanus*, *Genista tinctoria* (an endemic species of the Balkan Peninsula), *Paliurus spina-christi*, *Rubus* sp., and *Sambucus nigra* were the present shrub species, while *Acacia* sp., *Fagus* sp., *Morus* sp., *Olea europaea*, *Platanus* sp., *Populus* sp., and *Salix alba* were the trees. *Phragmites australis*, *Typha latifolia*, and *Scirpus sylvaticus* were present in the marshes of the irrigation ditches in the study area. The areas adjacent to the wet grasslands were cultivated by extended crop cultures, principally maize and wheat.

A herd of 91 Greek water buffaloes was selected for the purposes of the study. The animals grazed on the wet grasslands every day (Figure 1) and returned to the stable in the late afternoon. The buffaloes also grazed while wal-

lowing into the canals as well as in the several mudholes present in the area. The herd was constantly supervised by two herders in order to guide the animals to areas of better forage quantity and quality according to the season, and also to prevent them from entering cultivated fields. Supplementary feedstuff was not given to the animals for three days prior and during the observation days.

2.2 Data collection

The composition (%) of herbaceous species of the wet grasslands was determined on transects with the line intercept method (Bonham 2013). Every month, prior to the observations of the animals, we placed three non-permanent 25 m long line transects in relatively homogeneous areas that were representative of the wet grasslands' vegetation type. Along each line, we dropped vertically a single pin every 0.25 m and we recorded all species the

pin hit (each species was counted only once per pin-drop, even in the cases where the pin hit several parts or more individuals of the species). The total number of hits was 100 per transect, and 900 per season (see Table 1, the columns regarding vegetation). The recorded herbaceous species were identified and categorized into three functional groups (graminoids, legumes, and forbs). The proportion of each plant species was calculated as the number of hits a plant received per transect divided by 100. The monthly proportion (%) of each species was the average of the three transects. Thus, the seasonal proportion (%) of each species was the average of their three monthly proportions. The same procedure was followed for the calculation of the seasonal proportions (%) of plant functional groups. It should be mentioned here that it was not feasible to measure *Phragmites australis*, *Scirpus sylvaticus*, and *Typha latifolia* in the canals, as they were covered by water throughout the year. Thus, although these species were consumed by the buffaloes and contributed to their diet, they were not included in the plant species composition of the wet grasslands' vegetation.

The focal sampling technique (Altmann 1974) was applied to the whole group in order to determine water buffaloes' diet selection. All the animals had numbered collars around their neck so that they could be easily identified from a distance. The observations were carried out over a period of twelve consecutive months, for two consecutive days per month and for five hours per observation day. Each hour was divided into two half-hour observation periods. Two well-trained observers conducted the observations. The observers had already spent several days within the herd prior to the observations so that the animals would get acquainted to them and not be disturbed by their presence. The first observer, with the help of a synchronized chronometer, constantly followed the herd, observing each animal for 18 seconds. If the observed animal was grazing, the observer recorded in protocol sheets the consumed plant species. The second observer collected in separate paper bags representative samples of the consumed plant species for identification. We had 240 observation periods for the whole study (2 observation periods per hour × 5 hours per observation day × 2 days per month × 12 months). These 240 observation periods corresponded to 11416 recorded animal observations regarding their diet (N=3930 in spring, N=3866 in summer, N=2083 in autumn, and N=1537 in winter: see Table 1 at the columns relevant to water buffaloes' diet). The different number of animal observations per season is due to the fact that in each observation period not all animals grazed. They might perform other activities, such as moving, standing, ruminating, wallowing, lying, or drinking water. The collected plants species were identified and cat-

egorized into five functional groups, e.g. graminoids, legumes, forbs, shrubs, and trees. We calculated the monthly proportion (%) of each plant species in the buffaloes' diet and we determined the seasonal proportion (%) as the average of the three monthly values. We followed the same procedure for the calculation of the seasonal proportions (%) of plant functional groups in the diet. All plant species were identified with the use of the Treatises Mountain Flora of Greece (Strid 1986, Strid & Tan 1991) and Flora Europaea (Tutin et al. 1964–1980).

2.3 Statistical analysis

The chi-square (χ^2) test was applied to compare the distribution of proportions (%) of the plant functional groups in the wet grassland's vegetation across seasons. Specific comparisons for their proportions (%) were performed with the z-test at significance level $P < 0.05$. Chi-square (χ^2) was also applied for the proportions (%) of the plant functional groups in water buffaloes' diet. Additionally, the Spearman rank correlation was used to examine whether the proportions (%) of different plant functional groups in buffaloes' diet are related with their proportions (%) in the wet grasslands' vegetation. A species-wise Spearman rank correlation was also performed for each season. The significance level was set at $P < 0.05$. All statistical analyses were performed with SPSS v.25 software (SPSS Inc., Ill: Chicago, USA).

3. Results

3.1 Plant species composition of the wet grasslands' vegetation

The chi-squared test showed that there was a statistically significant relationship between plant functional groups of the wet grasslands' vegetation and seasons ($\chi^2 = 251.297$, $df = 6$, $P < 0.001$) (Table 1). The seasonal proportion (%) of graminoids in the grasslands' vegetation was at significantly higher levels during summer and autumn compared to the two other seasons. On the contrary, forbs had their lowest values ($P < 0.001$) during that time. The proportion (%) of legumes during summer was significantly higher than the other seasons ($P < 0.001$). *Alpecurus pratensis*, *Carex* sp., *Cynodon dactylon*, *Hordeum murinum*, *Trifolium repens*, *Anagallis arvensis*, *Cichorium intybus*, *Fumaria officinalis*, and *Plantago lanceolata* were the present plant species in the grassland throughout the year. *Cynodon dactylon* was the most dominant species from the graminoids, *Trifolium repens* from the legumes and *Cichorium intybus* from the forbs (Table 2).

Table 1: Seasonal proportions (%) of plant functional groups in the vegetation (graminoids, legumes, and forbs) and in the diet (graminoids, legumes, forbs, shrubs, and trees) of water buffaloes grazing in the wet grasslands of the Lake Kerkini, Northern Greece. Comparisons of distributions of their proportions (%) were performed with the chi-square test at $P < 0.05$. Specific comparisons for their proportions (%) were performed with the z-test.

Tabela 1: Sezonski deleži (%) rastlinskih funkcionalnih skupin v vegetaciji (trave, metuljnice in zeli) in v prehrani vodnih bivolov (trave, metuljnice, zeli, grmovnice in drevesa), ki se pasejo na mokrotnih travnikih ob jezeru Kerkini (Severna Grčija). Primerjave porazdelitev deležev (%) smo testirali z hi-kvadrat testom ($P < 0,05$). Posebne primerjave njihovih deležev (%) smo naredili z z-testom.

Plant functional group	Season							
	Spring		Summer		Autumn		Winter	
	Vegetation (n=900) ¹	Water buffaloes diet (N=3930) ²	Vegetation (n=900)	Water buffaloes diet (N=3866)	Vegetation (n=900)	Water buffaloes diet (N=2083)	Vegetation (n=900)	Water buffaloes diet (N=1537)
Graminoids	39.3 ^a	44.5 ^B	57.1 ^b	68.2 ^C	60.8 ^b	76.8 ^A	45.2 ^a	64.5 ^C
Legumes	25.6 ^a	22.1 ^B	32.6 ^b	23.8 ^B	25.2 ^a	9.3 ^A	23.4 ^a	16.3 ^C
Forbs	35.1 ^a	25.7 ^B	10.3 ^b	4.6 ^C	14.0 ^b	10.5 ^A	31.3 ^a	16.0 ^D
Shrubs	-	6.6 ^B	-	2.1 ^A	-	2.8 ^A	-	2.6 ^A
Trees	-	1.0 ^A	-	1.2 ^A	-	0.7 ^A	-	0.6 ^A
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

¹ The letter n in the parentheses indicates the total number of hits per season regarding the wet grasslands' vegetation.

² The letter N in the parentheses is the total number of observations per season regarding the water buffaloes' diet. N is different per season as not all animals grazed during the observation periods.

^{a,b} Superscript lowercase letters denote the significant differences in the proportions (%) of plant functional groups in wet grasslands' vegetation between seasons at the level 0.05 ($\chi^2 = 251.297$, $df = 6$, $P < 0.001$).

^{A,B,C,D} Superscript capital letters denote the significant differences in the proportions (%) of plant functional groups in water buffaloes' diet between seasons at the level 0.05 ($\chi^2 = 1219.255$, $df = 12$, $P < 0.001$).

Table 2: Seasonal proportion (%) of plant species in the wet grasslands vegetation of the Lake Kerkini, Northern Greece and their proportions (%) in the diet of water buffaloes. The bold format, both in vegetation and buffaloes diet, indicates values beyond 1%.

Tabela 2: Sezonski deleži (%) rastlinskih vrst na mokrotnih travnikih ob jezeru Kerkini (severna Grčija) in njihovi deleži (%) v prehrani vodnih bivolov. Krepko so označene vrednosti nad 1 % v vegetaciji in v prehrani.

Plant functional group	Season							
	Spring		Summer		Autumn		Winter	
	Vegetation	Water buffaloes' diet	Vegetation	Water buffaloes' diet	Vegetation	Water buffaloes' diet	Vegetation	Water buffaloes' diet
Graminoids								
<i>Alopecurus pratensis</i>	0.2	3.1	5.1	1.7	10.3	2.3	0.1	1.5
<i>Bromus erectus</i>	0.9	0.5						
<i>Bromus hordeaceus</i>	2.1	0.8						
<i>Bromus intermedius</i>	0.2	0.1						
<i>Carex</i> sp.	1.0	2.0	1.1	2.7	4.9	0.2	14.4	2.7
<i>Cynodon dactylon</i>	29.5	25.7	49.8	56.6	43.4	60.5	29.9	61.1
<i>Cyperus fuscus</i>						0.1		
<i>Festuca valesiaca</i>	0.2							
<i>Hordeum murinum</i>	3.1	2.5	0.2	2.6	0.3	0.1	0.8	0.1
<i>Juncus subulatus</i>	0.1	0.0*	0.1	0.1				
<i>Phragmites australis</i>		2.0		1.0		5.3		0.3
<i>Poa arvensis</i>			0.1					
<i>Poa pratensis</i>	0.6							
<i>Poa trivialis</i>	0.7	3.6	0.1	0.6				
<i>Scirpus sylvaticus</i>		0.1						

Plant functional group	Season							
	Spring		Summer		Autumn		Winter	
	Vegetation	Water buffaloes' diet	Vegetation	Water buffaloes' diet	Vegetation	Water buffaloes' diet	Vegetation	Water buffaloes' diet
<i>Setaria verticillata</i>					1.4	6.8		
<i>Sorghum halepense</i>					0.2	0.2		
<i>Sorghum scoparium</i>			0.6	2.7				
<i>Typha latifolia</i>		0.2						
<i>Vulpia myuros</i>	0.8	0.5						
Legumes								
<i>Melilotus neapolitanus</i>	0.9	0.7	0.6	0.0				
<i>Trifolium repens</i>	24.7	22.4	31.0	23.9	24.4	8.0	23.4	12.1
<i>Vicia</i> sp.			1.0	0.1	0.8	0.1		
Forbs								
<i>Ajuga reptans</i>	0.2							
<i>Anagallis arvensis</i>	0.8		0.3	0.2	0.4	0.4	0.6	0.1
<i>Anagallis tenella</i>	0.2	0.4			0.1			
<i>Anthemis arvensis</i>	0.6							
<i>Apium nodiflorum</i>					0.1			
<i>Bellis perennis</i>	0.3							
<i>Berteroa obliqua</i>	0.4							
<i>Calystegia silvatica</i>	0.2	0.0						
<i>Capsella bursa-pastoris</i>	0.3	0.9						
<i>Cardamine hirsuta</i>							0.3	
<i>Carduus nutans</i>	0.1	0.5						
<i>Carlina acanthifolia</i>	1.1							
<i>Carlina vulgaris</i>	0.3	1.3	0.1	0.0	0.1	0.0		
<i>Cerastium arvense</i>	1.2	2.7						
<i>Cerastium brachypetalum</i>	0.2							
<i>Chrysanthemum morifolium</i>	0.1							
<i>Cichorium intybus</i>	7.8	8.2	6.0	2.3	4.8	7.4	4.1	14.0
<i>Convolvulus arvensis</i>			0.1	0.4				
<i>Crepis setosa</i>	0.7		0.1					
<i>Daucus muricatus</i>	0.9	0.3						
<i>Diplotaxis muralis</i>							0.1	
<i>Erodium cicutarium</i>	0.1	2.7			0.8		0.1	
<i>Erodium moschatum</i>	0.2	0.5						
<i>Erysimum graecum</i>							1.7	
<i>Euphorbia helioscopia</i>	0.2							
<i>Fumaria officinalis</i>	2.1	0.7	0.1	0.0	1.2	0.1	2.6	
<i>Geranium rotundifolium</i>	2.7						11.6	0.6
<i>Inula hirta</i>					0.9			
<i>Knautia orientalis</i>							0.8	
<i>Leontodon crispus</i>							0.2	
<i>Leontodon tuberosus</i>	1.2							
<i>Lepidium latifolium</i>							1.6	
<i>Malva sylvestris</i>	0.2	0.1						
<i>Mentha pulegium</i>	0.1	0.4	0.2	0.0	0.2	0.0		
<i>Myosotis arvensis</i>	0.1	0.4						
<i>Nigella damascena</i>			0.1	0.0				
<i>Origanum vulgare</i>					0.1	0.1		
<i>Oxalis corniculata</i>					0.7			

Plant functional group	Season							
	Spring		Summer		Autumn		Winter	
	Vegetation	Water buffaloes' diet	Vegetation	Water buffaloes' diet	Vegetation	Water buffaloes' diet	Vegetation	Water buffaloes' diet
<i>Picris sprengeriana</i>	0.1	0.0						
<i>Plantago amplexicaulis</i>	1.9	1.0					1.9	0.3
<i>Plantago coronopus</i>	0.2							
<i>Plantago lanceolata</i>	4.2	4.0	0.7	0.4	1.7	0.0	1.1	0.4
<i>Plantago major</i>					0.1	1.2		
<i>Polygonum aviculare</i>					0.9			
<i>Portulaca oleracea</i>	0.1	0.1			0.4	1.6		
<i>Potentilla reptans</i>	0.4		1.4				2.0	
<i>Prenanthes purpurea</i>	0.3	0.2			1.0		0.1	0.7
<i>Pulicaria vulgaris</i>							0.6	
<i>Pulmonaria officinalis</i>	0.3							
<i>Ranunculus repens</i>	0.1	2.3	0.1					
<i>Ranunculus sardous</i>	0.3		0.3	0.1				
<i>Reichardia picroides</i>	1.0	0.3	0.3					
<i>Reichardia vulgare</i>							0.4	
<i>Rorippa sylvestris</i>	0.8	0.0					0.1	
<i>Rumex crispus</i>	0.3		0.1	0.0				
<i>Rumex pulcher</i>					0.1		0.6	2.5
<i>Ruta graveolens</i>			0.1	0.7				
<i>Scleranthus perennis</i>	0.2							
<i>Silybum marianum</i>			0.1	0.0				
<i>Sisymbrium altissimum</i>	0.9							
<i>Stellaria palustris</i>	0.7						0.1	
<i>Taraxacum</i> sp.							0.2	
<i>Tordilium maximum</i>					0.1			
<i>Verbascum sinuatum</i>	0.1				0.2			
<i>Veronica arvensis</i>	0.2							
<i>Veronica hederifolia</i>							0.7	
<i>Veronica serpyllifolia</i>	0.1							
Shrubs								
<i>Clematis vitalba</i>		0.9		0.1				
<i>Cornus sanguineus</i>		0.6						
<i>Cytisus monspessulanus</i>		0.4						
<i>Genista tinctoria</i>		0.3						
<i>Paliurus spina-christi</i>		0.1						
<i>Rubus</i> sp.		5.6		2.5		1.3		2.8
<i>Sambucus nigra</i>						3.8		
Trees								
<i>Acacia</i> sp.		0.1				0.1		
<i>Fagus</i> sp.		0.0						
<i>Morus</i> sp.		0.1						
<i>Olea europaea</i>				0.1				
<i>Platanus</i> sp.		0.0						
<i>Populus</i> sp.		0.4		1.1		0.4		0.7
<i>Salix alba</i>		0.2		0.0		0.1		
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

* The value 0.0 in water buffaloes' diet corresponds to plant species with proportions (%) less than 0.05.

3.2 Plant species composition of the water buffaloes' diet

According to the chi-square test, a statistically significant relationship was detected between plant functional groups of the water buffaloes' diet and seasons ($\chi^2 = 1219.255$, $df = 12$, $P < 0.001$) (Table 1). The herbaceous vegetation (graminoids, legumes, and forbs) was the main component of the water buffaloes' diet for all four seasons, as it constituted 92.3% (spring), 96.6% (summer and autumn), and 96.8% (winter) of the buffaloes' diet. On the other hand, the woody species (shrubs and trees) were less consumed (from 3.2% in winter to 7.6% in spring). Alterations in the composition of the water buffaloes' diet were observed in all seasons, with graminoids remaining predominant. However, they had a lower contribution during spring, due to the increased participation of legumes, forbs, and shrubs ($P < 0.001$). Legumes had their lowest value during autumn ($P < 0.001$), while trees were constantly a negligible component of animals' diet throughout the year.

A total of 66 plant species (16 graminoids, 3 legumes, 33 forbs, 7 shrubs, and 7 trees) were included in the water buffaloes' diet (Table 2). The animals consumed a broader variety of plant species in spring (49 species) compared to summer (28), autumn (23), and winter (15). The same trend had also appeared for the plant functional groups. Specifically, 13 graminoids were recorded in spring, eight in summer, seven in autumn, and five in winter. Five graminoids (*Alopecurus pratensis*, *Carex* sp., *Cynodon dactylon*, *Hordeum murinum*, and *Phragmites australis*) were present in water buffaloes' diet during all four seasons. From these species, *Cynodon dactylon* was the dominant graminoid in the diet, ranging from 25.7% in spring to 61.1% in winter, while the rest ones were observed in much lower proportions. Specifically, *Alopecurus pratensis* contributed to the diet from 1.5% in winter to 3.1% in spring, *Carex* sp. from 0.2% in autumn to 2.7% in winter and summer, *Hordeum murinum* from 0.1% in autumn and winter to 2.6% in summer, and *Phragmites australis* from 0.3% in winter to 5.3% in autumn (Table 2). Regarding the contribution of other graminoids to the buffaloes' diet within seasons, it was noted that *Bromus erectus*, *Bromus hordeaceus*, *Bromus intermedius*, *Juncus subulatus*, *Poa trivialis*, *Scirpus sylvaticus*, *Typha latifolia*, and *Vulpia myuros* were also present during spring apart from the constantly consumed species. Also, *Juncus subulatus*, *Poa trivialis*, and *Sorghum scoparium* participated in the buffaloes' diet during summer, while *Setaria verticillata* and *Sorghum halepense* were consumed during autumn. The buffaloes' graminoid diet

during winter consisted only of the five species that were constantly consumed as mentioned above.

From legumes, *Trifolium repens* was the dominant species in all seasons. Also, *Melilotus neapolitanus* and *Vicia* sp. were consumed by buffaloes but in minor proportions and not in all seasons.

A total of 22 forbs participated in the water buffaloes' diet during spring, 12 in summer, nine in autumn, and seven in winter. *Cichorium intybus* and *Plantago lanceolata* were consumed during all seasons, with the former being predominant. Apart from these plants, the relative contribution of *Carlina vulgaris*, *Cerastium arvense*, *Erodium cicutarium*, *Plantago amplexicaulis*, *Plantago lanceolata* and *Ranunculus repens* in the diet was greater than 1% in spring, whereas the remaining forbs were in lower percentages. Forbs were also included in the diet in small proportions (<1%) during summer, except *Cichorium intybus*. In autumn, only *Plantago major* and *Portulaca oleracea* were consumed by buffaloes at percentages higher than 1%, while during winter one species (*Rumex pulcher*) was above this level.

Similarly to the other plant functional groups, a greater variety of shrub species in the buffaloes' diet was observed during spring (six species) in contrast to summer (two), autumn (two), and winter (one). *Rubus* sp. was present in the buffaloes' diet in all seasons, ranging from 1.3% in autumn to 5.6% in spring. Apart from *Rubus* sp., *Sambucus nigra* was also included in the diet during autumn, although in greater amounts. In general, all the other shrubs were consumed in small proportions (0.1% to 0.9%).

Trees were a negligible component of the buffaloes' diet. Six trees were recorded in spring, three in summer, three in autumn and one in winter. *Populus* sp. was the tree consumed in all seasons, followed by *Salix alba*, which participated in buffaloes diet with very low percentages (<0.2%).

The Spearman's test revealed a strong correlation ($\rho = 0.947$, $P < 0.001$) between the seasonal proportion (%) of each plant functional group in the wet grasslands' vegetation and their proportion (%) to the water buffaloes' diet (Table 3). Also, Spearman's test detected a significant association between the proportion (%) of plant species in the vegetation and their proportion (%) in the water buffaloes' diet in each season ($\rho = 0.475$ for spring, $\rho = 0.840$ for summer, $\rho = 0.719$ for autumn, and $\rho = 0.616$ for winter; $P < 0.01$ in all cases).

Table 3: Correlation coefficient (Spearman's rho) and its significance level between the proportion (%) of plant functional groups in the wet grasslands vegetation and their proportion (%) to the buffaloes' diet across seasons.

Tabella 3: Korelacijski koeficienti (Spearman rho) in statistična značilnost med deleži rastlinskih vrst (%) v funkcionalnih skupinah na mokrotnih travnikih in in njihovim deležem (%) v prehrani bivolov v različnih sezonah.

Season	Plant Functional group	Proportion (%) in the vegetation	Proportion (%) in the water buffaloes' diet	Correlation coefficient	Significance level
Spring	Graminoids	9.85	10.87	rho = 0.947	P<0.001
	Legumes	6.39	6.11		
	Forbs	8.76	7.16		
Summer	Graminoids	14.28	17.95		
	Legumes	8.14	6.34		
	Forbs	2.58	1.14		
Autumn	Graminoids	15.19	19.92		
	Legumes	6.31	2.13		
	Forbs	3.50	2.87		
Winter	Graminoids	11.31	17.38		
	Legumes	5.86	3.20		
	Forbs	7.83	4.93		
Total		100.0	100.0		

Discussion

Water buffaloes in our study were mainly grazers than browsers as graminoids, legumes, and forbs made up the fundamental part of their diet in all seasons. These findings are in accordance with Tsiobani et al. (2014) who reported that water buffaloes spent more time feeding on grasses (74%) in comparison to woody species (21.5%) and forbs (4.5%). Napolitano et al. (2007) also highlighted the importance of herbaceous vegetation and reported the absence of woody species in the water buffaloes' diet. It seems that water buffaloes, like cattle, due to their anatomical characteristics (wide mouth and inflexible upper lips) are not well adapted to harvest woody vegetation (Phillips 1988).

Moreover, seasonal changes in the water buffaloes' dietary composition were revealed in our study. Specifically, during spring the contribution of graminoids to the water buffaloes' diet was decreased (44.5%) in comparison to the other seasons. This could be mainly attributed to the low consumption of *Cynodon dactylon*, despite its high nutritive value (Ramirez et al. 2003), in favor of other graminoids, such as *Alopecurus pratensis*, *Carex* sp., *Hordeum murinum*, *Phragmites australis*, and *Poa trivialis*. The observed low contribution of graminoids to the water buffaloes' diet during this period was compensated by the increased consumption of highly nutritious legumes (22.1%), forbs (25.7%), and shrubs (6.6%) (Ulyatt et al. 1988, Yiakoulaki et al. 2007). *Trifolium repens* was the dominant legume in their diet while *Melilotus neapolitanus*

was consumed in very low proportions (0.7%). Among the forbs, *Cichorium intybus* was the most favored species (8.2%) followed by *Plantago lanceolata* (4%). Both these species have high nutritive value and their abundance in the vegetation could enhance the nutritional profile of grasslands (Sanderson et al. 2003).

The consumption of *Ranunculus repens* (2.3%), although its poisonous effects on livestock, may be justified by the fact that grazing animals are able either to tolerate toxic plants – if they are familiar in the grazing area, or metabolize them efficiently into their rumen or outcome their poisonous impact by consuming other herbs (Valentine 1990, Provenza et al. 1992). *Rubus* sp. and *Populus* sp. were the main woody species in the water buffaloes' diet in spring. According to our observations, the animals used to consume the outer foliage and thin twigs (2–3 cm diameter) of *Rubus* sp., which are soft and without thorns. It was observed that despite the numerous plant species that contributed to buffaloes diet during this period, a large proportion of them (69%) were just tested by the animals, meaning that they were selected in very low percentages (<1%). In this regard, Hejcman et al. (2008) stated that grazing sheep also tested a wide scale of plant species but in low selection frequency.

In the summer, the graminoids and legumes accounted for the vast majority (92.0%) in the water buffaloes' diet. This can be attributed to the high participation of *Cynodon dactylon* (56.6%) and *Trifolium repens* (23.9%). These species with their numerous rhizomes and stolons (Mislevy 1979) are able to spread and persist in various envi-

ronmental conditions (Harlan et al. 1970, Grime 1979, Evans 1973). They can also form associations between them competing thus with species of a smaller presence in the grassland (Bogdan 1977, Orr et al. 1990). *Sorghum scoparium* was also included in the diet in percentage of 2.7%, despite its possible toxic effects on cattle (Vough 1978). Also, during this period 64% of the plant species that participated in buffaloes' diet were grazed in percentages lower than 1%. Legumes, such as *Melilotus neapolitanus* and forbs, like *Carlina vulgaris*, *Convolvulus arvensis*, *Fumaria officinalis*, *Mentha pulegium*, *Nigella damascena*, *Ranunculus sardous*, *Silybum marianum* etc. seems to be undesirable to water buffaloes. This can be attributed either to their morphological structure (thorns) or the presence of toxic substances, such as alkaloids, saponins, coumarin, glycosides etc (Mardirossian et al. 1983, Todd et al. 1995, Al-Sultan et al. 2003). It appears that the water buffaloes tended to test the numerous available plant species on the wet grassland awaiting for a post-ingestive response (Provenza et al. 2003).

In autumn and winter, fewer species were found in the buffaloes' diet compared to the other seasons. During these periods, the same pattern was also followed as in spring and summer regarding the participation of plant species with percentages lower than 1%.

In our study, a strong correlation was found between the abundance of species in the wet grassland's vegetation and those that were consumed by animals. These findings are consistent with Soder et al. (2009) who stated that grazing animals alter their diet not only due to their preference for certain species but also from the availability of these species in the grassland's vegetation. However, the proportion of plant species in animals' diet does not always coincide with their proportion in grasslands' composition (Galt et al. 1982, Hejzman et al. 2008). According to our results, 38 herbaceous species in total (four graminoids and 34 forbs) that were present in the wet grassland's vegetation did not appear in buffaloes' diet. This was more evident during winter when only 46% of the available herbaceous flora was grazed by buffaloes. This finding can be attributed either to the low availability of these plant species in the wet grasslands' vegetation or their low palatability. Particularly, forbs are markedly decreased and disintegrated in winter or remain as stemmy remnants, which make them undesirable for the animals (Valentine 1990).




Conclusion

Water buffaloes grazing on wet grasslands have to face several challenges in terms of consuming both herbaceous and woody species. Few species (10) from all plant

functional groups were constantly consumed by buffaloes in all seasons while the greater number of plants constituting their diet was simply tested by them. In this study, the seasonal changes in water buffaloes' diet composition were revealed. However, it is important to understand the water buffaloes' foraging strategy in relation to anti-quality characteristics of plant species.

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