SEASONAL VARIATION OF CHEMICAL COMPOSITION AND DRY MATTER DIGESTIBILITY OF RANGELANDS IN NW GREECE

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ABSTRACT

This study was carried out to determine the chemical composition and in vitro dry matter digestibility of grazable material, during the growing season of plants, in three different altitudinal zones, in native rangelands, northwestern Greece. Samples were collected during the period from May to October of the years 2004 and 2005. Sample collection was accomplished by cutting aboveground biomass at a height similar to that grazed by small ruminants. The results showed that herbage production was significantly affected ($P<0.001$) by sampling year, growing season and altitudinal zone respectively, as well as ($P<0.01$) by the "month x year" and ($P<0.05$) "altitude x month" interactions. CP, ash, EE and CF content and IVDMD affected significantly ($P<0.01$) by both harvest month and altitudinal zone, while there was no significant affection by the sampling year and the interaction between altitude, month and year (except EE which affected ($P<0.01$) by the "month x year" interaction). Herbage production strongly related ($P<0.01$) to the altitude ($r= +0.247$), harvest month ($r= -0.479$) and CP content ($r= -0.274$). IVDMD related positively ($P<0.01$) to CP ($r= +0.729$), ash ($r= +0.369$) and EE ($r= +0.351$) content and negatively to harvest month ($r= -0.779$) and to CF content ($r= -0.663$). It was recommended that additional protein sources should be supplied in order to cover the needs of the grazing animals. It is necessary the transhumance of herds from lower to higher altitude for better utilization of rangelands.

Key words: Herbage, chemical composition, in vitro digestibility, rangelands, northwestern Greece

INTRODUCTION

Traditional stockfarmings of ruminants (sheep, goat and cattle) utilize in a high degree the Greek native grazing lands. Both, herbaceous and woody plants should occur in grazing lands as they are considered important contributors to grazing animals' nutrition [15, 16]. However, because of the thoughtless use of pasturelands, supplementary feed is often required to compensate animals for pasture deficiencies [10]. Grazing land productivity and nutritional quality are affected by abiotic and biotic environmental factors including topographic factors such as slope, aspect and altitude, together with soil characteristics, climatic regime, botanical composition and range improvement practices [18, 6, 12]. On open rangelands, the quality and quantity of forage varies appreciably with climate and often leads to nutritional inadequacy [19].

Protein content and digestibility of dry matter have been emphasized as the main determinants of forage quality [18]. The evaluation of quality of feeds is important for the prediction of animal performance [22]. Forage evaluation implies the description of feedstuffs with respect to their capacity to sustain diverse kinds and levels of production [5, 9]. Thus, to improve the quality of the forage consumed by range animals, it is necessary to obtain information of the chemical components of the feed in relation to the requirements.

The objective of this study was to determine the seasonal variations in chemical composition and dry matter digestibility of the grazable material, over a grazing period in the Greek mountainous areas, among three altitudinal zones of native rangelands in north-western Greece.

MATERIALS AND METHODS

Study area

This study was conducted in the mountainous and subalpine rangelands of Mt. Varnoudas, north-western Greece (40º 46΄ to 40º 53΄ N, 21 º 07΄το 21 º 24΄E, 900-2334m above sea level). The average altitude of Mt. Varnoudas is approximately 1700m while the highest peak reaches 2334m. The basic geological substrate of the whole area is consisted of metamorphic rock textures (i.e. phyllites, gneisses and micas shcists) of the Pelagonic geotectonic zone. The fertility of the soil varies depending on slope, exposure, and vegetation. The mountain and topographic lie is quite tense and, in combination to the climate conditions, which vary from zone to zone, create an impressive variation of flora from the lowest to the upper most zones. There are large areas covered by beech and oak forests as well as by other small trees and shrubs. The pasturelands in openings of forests as well as those in the sub-alpine region are covered by herbs of a high diversity. The area under study has special climate characteristics, which differ from those of the typical Greek mediterranean climate [17]. The climate approaches the middle – European type having as major characteristics a quite cold and damp winter and a rather dry summer. Sometimes in winter there can be observed very low temperatures, which can reach -23ºC, a fact, which is very unusual for the Greek climatic conditions. The monthly average air temperature as well as rain precipitation in the decade 1991 – 2000 was 12.2ºC and 52.2mm respectively. The mean monthly rain precipitations during experimental period (2004 - 2005), was approximately 54.0 mm [8], a fact that shows a uniformity of both, rain precipitation and air temperature (~ 12ºC).

The pasturelands of Mt. Varnoudas were graded as seasonal [17]. That is, from 600-1500m, they are grazed during spring and autumn, while over 1500m they are grazed in summer.

Sampling and Experimental Analyses

The research work was conducted during the years 2004 and 2005, from May to October. Twenty-four experimental cages, sized 4m x 5m, fenced with metallic net 1.5m high in order to obstruct free – range grazing, were placed. In each of the three altitudinal zone (lower altitudinal zone: 900-1300m, middle altitudinal zone: 1301 – 1700m, upper altitudinal zone: >1700m) were placed eight experimental cages. In the lower zone, the flora was constituted by grasses, legumes and forbs, in the middle zone mainly by grasses and some forbs, while in the upper zone mainly by grasses of subalpine regions.

Each experimental cage was divided into 36 equal parts. In the beginning of each month, from May to October aboveground biomass (as a mix by grasses, legumes and forbs) was collected from 6 different of the 36 equal parts. Sample collection was accomplished by cutting aboveground biomass imitating the way of small ruminant grazing [14]. The collected biomass was stored in paper bags and was weighted immediately afterwards.

Samples were analysed for dry matter (DM), ash, ether extracts (EE), crude fibre (CF) and crude protein (CP) contents. DM contents of herbage were determined by drying the samples at 68ºC until a steady weight. Ash was determined and ash by igniting the samples in a muffle furnace at 500 ˚C for 8 h. Nitrogen (N) content was measured by the Kjeldahl method [2]. CP content was calculated as N*6.25. Crude fibre (CF) and ether extracts (EE) were determined following the methods of A.O.A.C. [2]. Each sample was analysed in triplicate. In vitro dry matter digestibility was determined according to the two-stage method described by Tilley and Terry

[23].

Three mature sheep (body weight 48-49 kg) fitted with a permanent ruminal cannula were used for the extraction of rumen fluid to carry out the in vitro incubation (digestibility) of the browse material. Animals were not fed one day before fluid extraction but they had free access to water. Dry forage samples (0.5g) were subjected to a 48 h digestion period with McDougall's buffer/rumen fluid mixture in sealed glass bottles followed by 24 h digestion with pepsin in weak acid [23]. All incubations were carried out in triplicate. Three blank tubes (without sample) were used in each run. After 24 h digestion with pepsin in weak acid, the samples were filtered by gravity in pre weighed pyrex crucibles with spongy bottom and the residues dried at 103±2.0 °C for 24h. The dry residues were weighed and digestibility was calculated using the equation:

$$IVDMD(\%) = \frac{\text{initial DM input} - (\text{Residue} - \text{Blank})}{\text{initial DM input}} x100$$

The data were analyzed statistically using univariate ANOVA testing for the effects of sampling year, month and altitudinal zone separately as well as "altitude x month", "altitude x year", "month x year" and "altitude x month x year" interactions, using the SPSS 12.0 [21]. It was also carried out an analysis of correlation of the variables and stepwise linear regression. The significance level was assessed to P<0.05, except for the cases of the existence of a different indication.

RESULTS AND DISCUSSION

Herbage Production

Herbage production was affected significantly (P<0.001) by the sampling year, harvest month and altitudinal zone respectively. It was also affected (P<0.01) by the "month x year" and "altitude x month" interactions, while there was no affection by "altitude x year", "altitude x month x year" interactions (Table 1).

Herbage production showed a differentiation among subsequent or different months in all altitudinal zones, in both years. However, it presented similar fluctuation in production, which was increasing from May to October, showing its peak in August in the lower (205.06 ± 60.41 g DM . m^{-2}) and upper zone (248.03±80.64 g DM . m^{-2}), while in the middle zone (223.25± 101.98 g DM . m^{-2}) this occurred in July in 2004 (Fig 1). Production of herbage mass was much lower in the year 2005, showing its peak in July, in the middle (127.79± 15.30 g DM . m^{-2}) and upper zone (194.64± 62.95 g DM . m^{-2}) respectively, and in June in the lower zone (110.08± 4.80 g DM . m^{-2}).

Table 1 Influence of harvest time and altitudinal zone to the herbage production, chemical composition and digestibility of the grazable material in Mt Varnoudas pastures

PARAMETER	ALTITUDE	MONTH	YEAR	ALTITUDE x MONTH	ALTITUDE x YEAR	MONTH x YEAR	ALTITUDE x MONTH x YEAR
DM	***	***	***	*	NS	**	NS
ASH	*	***	NS	NS	NS	NS	NS
EE	***	*	NS	NS	NS	***	NS
CF	***	***	**	NS	NS	NS	NS
CP	***	***	NS	NS	*	NS	NS
IVDMD	**	***	NS	NS	NS	NS	NS

Level of significance: ***: P<0.001, **: P<0.01, *: P<0.05, NS: Not Significant

DM: Dry Matter, EE: Ether Extracts, CF: Crude Fibre, CP: Crude Protein, IVDMD: In Vitro Dry Matter Digestibility.

According to George et al. [6] rain precipitation determines the beginning and the end of growing period of plants, while the air temperature usually determines the amount of aboveground biomass production during the vegetative period. In the study area, at the beginning of the experimental period, herbage production was greater in lower zone (900-1300m) due to propitious blooming circumstances of the vegetation of the pastures (temperature, moisture and sunlight) in the first year. The same variation has been observed in the second sampling year. The typical shape of grassland growth is a sigmoid curve, increasing to a maximum and then decreasing [18]. Herbage production in all zones resembled this pattern (Fig.1).

Herbage production had a negative relation ($P<0.01$) to sampling year ($r= -0.205$) as well as to ash ($r= -0.272$) and CF ($r= -0.274$) content. On the contrary herbage production presented positive relation ($P<0.01$) to the altitudinal zone ($r= +0.247$) and ($P<0.05$) to the EE ($r= +0.235$) content (Table 2).

Crude protein, ash, ether extracts and crude fibre content

For all studied samples, CP content was maximum at the beginning of growing season, during the initial leaf growth that is accompanied by a high mitotic activity and a strong demand for nutrients, particularly nitrogen [20, 1]. Thereafter, the CP content declined through the growing season, as a response to tissue ageing, particularly during the autumn, when nutrients are transferred to perennial tissues before abscission (Fig. 2). The annual mean contents of CP, in the year 2004, were 8.70±2.02, 10.09±2.65 and 9.73±2.52 (DM %) in the lower, middle and upper altitudinal zone respectively. During 2005 mean annual CP contents were 7.90±1.96, 10.55±2.71 and 9.58±2.41 (DM %) in the three altitudinal zones respectively. Statistical analyses showed that, CP

Table 2 Correlation coefficients of measured parameters

	ALTITUDE	MONTH	YEAR	DM	ASH	EthExt	CF	CP	IVDMD
ALTITUDE	1								
MONTH	0.000	1							
YEAR	0.000	0.000	1						
DM	0.247**	0,091	-0.479**	1					
ASH	-0,127	-0.479**	0.012	-0.272**	1				
EE	0.462**	-0.095	-0.072	0.235*	-0.142	1			
CF	-0.285**	0.726**	0.176	0.095	-0.389**	-0.185	1		
CP	0.222*	-0.776**	-0.033	-0.274**	0.512**	0.235*	-0.730**	1	
IVDMD	0.149	-0.779**	-0.018	-0.080	0.369**	0.351**	-0.663**	0.729**	1

Level of significance: *: $P<0.05$, **: $P<0.01$

DM: Dry Matter, EE: Ether Extracts, CF: Crude Fibre, CP: Crude Protein, IVDMD: In Vitro Dry Matter Digestibility.

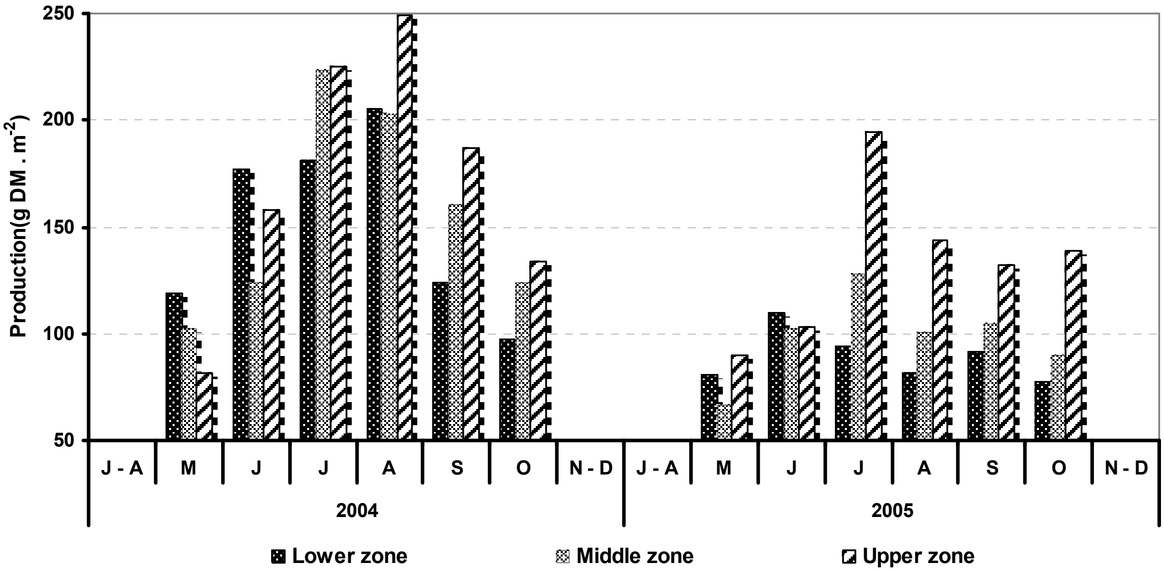

Fig. 1 Seasonal variations of herbage production (g/m2) of Mt Varnoudas pastures at lower, middle and upper altitudinal zone (Means of eight experimental cages per zone)

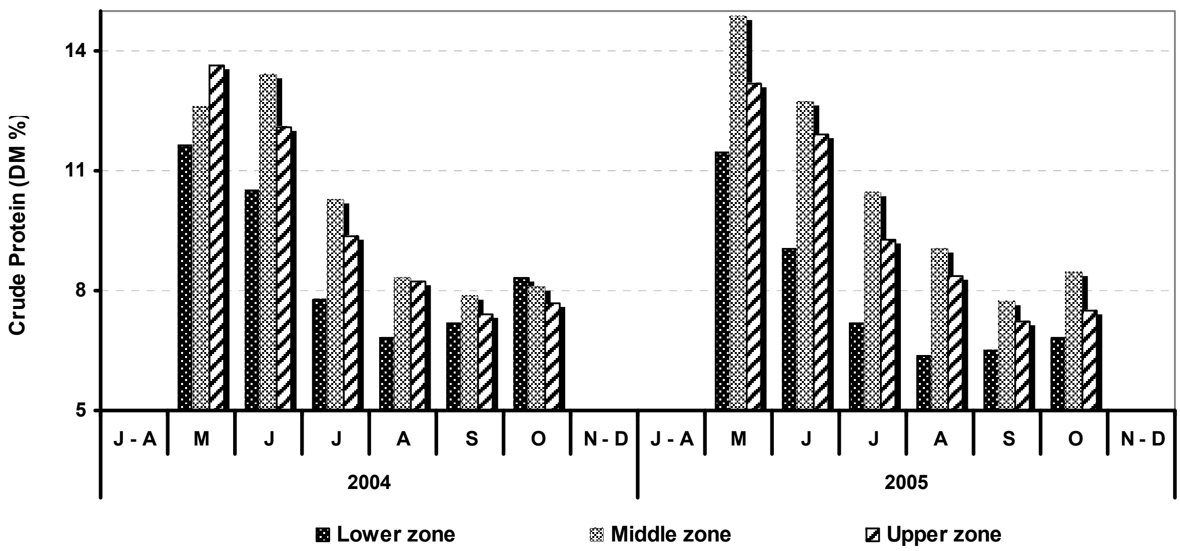

Fig. 2 Seasonal variations of crude protein (DM %) of Mt Varnoudas pastures at lower, middle and upper altitudinal zone (Means of eight experimental cages per zone)

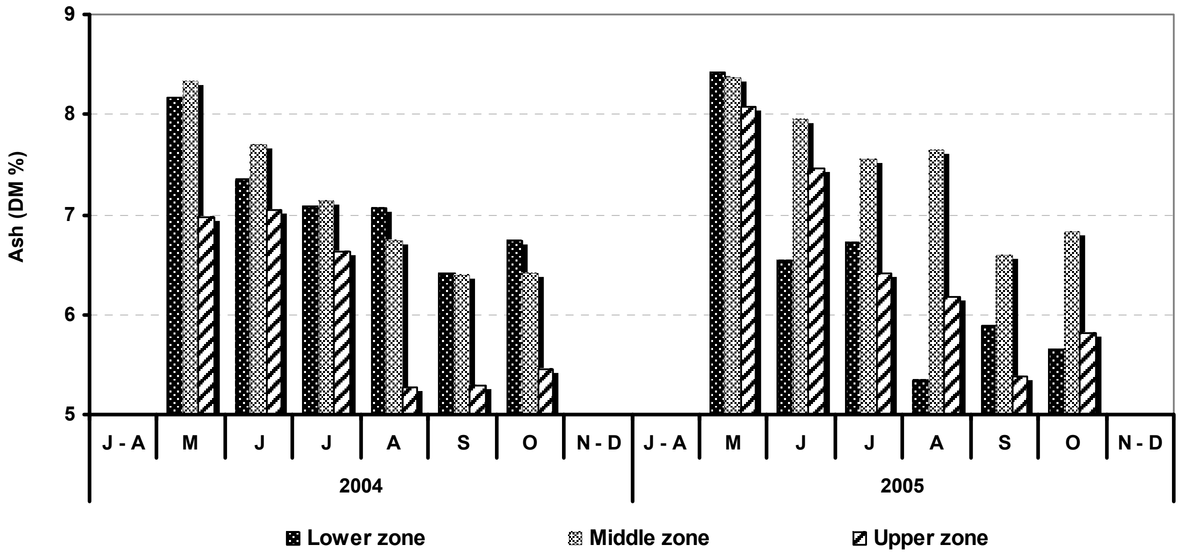

Fig. 3 Seasonal variations of ash (DM %) of Mt Varnoudas pastures at lower, middle and upper altitudinal zone (Means of eight experimental cages per zone)

content of herbage, affected significantly ($P<0.001$) by altitudinal zone and harvest month as well as ($P<0.05$) by the "altitude x year" interaction (Table 1). CP content decreases significantly as the autumn approaches, as consequence of the decrease in their leaf/stem ratio [4, 18, 25, 3, 1], considering the differences in composition between leaves and stems. Correlations between CP content and the other shown parameters are shown in Table 2.

To ewes that weigh approximately 50 kg the daily protein needs of preservation come up to 95g kg^{-1} (or 9.5%) of dry matter [13]. In both sampling years, in the studied rangeland, these needs were adequately covered from May to August in the middle and upper altitudinal zone, while in the lower zone that was from May to July (Fig 2). For the rest of the period additional protein sources should be supplied in order to cover the needs of preservation of the grazing animals.

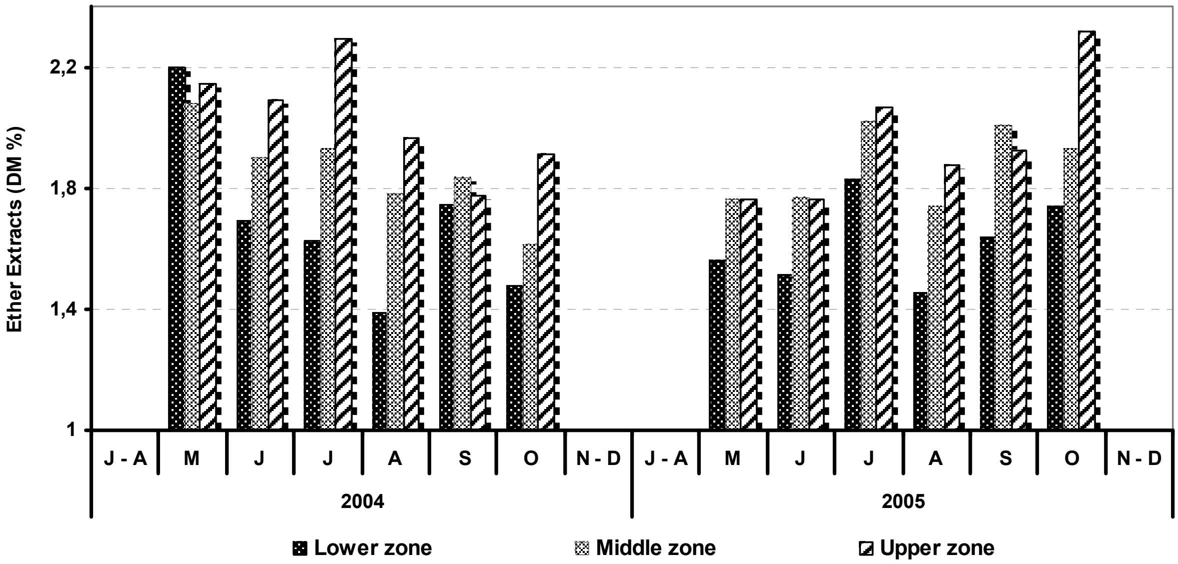

Fig 4 Seasonal variations of ether extracts (DM %) of Mt Varnoudas pastures at lower, middle and upper altitudinal zone (Means of eight experimental cages per zone)

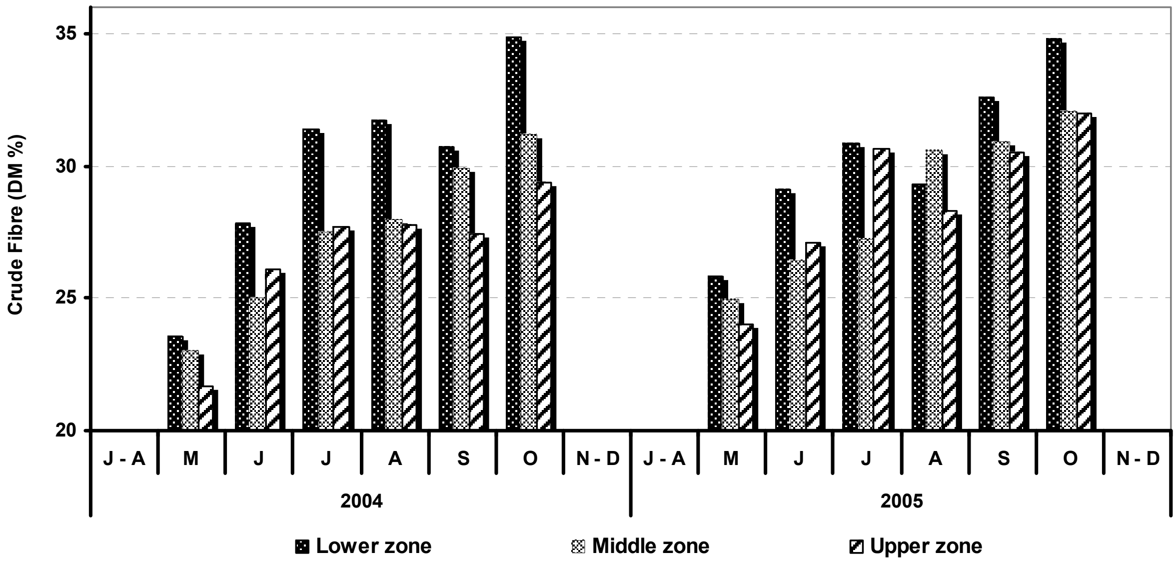

Fig 5 Seasonal variations of crude fibre (DM %) of Mt Varnoudas pastures at lower, middle and upper altitudinal zone (Means of eight experimental cages per zone)

Ash contents were different between years in all altitudinal zones as growing season processed. Annual mean ash content in 2004, was found to be 7.13±1.08, 7.12±0.99 and 6.11±1.15 (DM %) in the lower, middle and upper altitudinal zone respectively, while in 2005 these values were 6.42±1.48, 7.49±1.12 and 6.55±2.19 (DM %) in the three zones respectively (Fig. 3). Ash content affected significantly (P<0.01) by the harvest month and (P<0.05) by the altitudinal zone (Table 1). Ash correlated positively (P<0.01) with altitude (r= +0.247), CP (r= +0.512) content and IVDMD (r= +0.369) whereas it had been negatively correlated to sampling year (r= -0.479) and CF (r= -0.389) content (Table 2).

Year-to-year and month-to-month of ether extracts content was quite variable among different altitudinal zones. Annual mean EE content was found to be

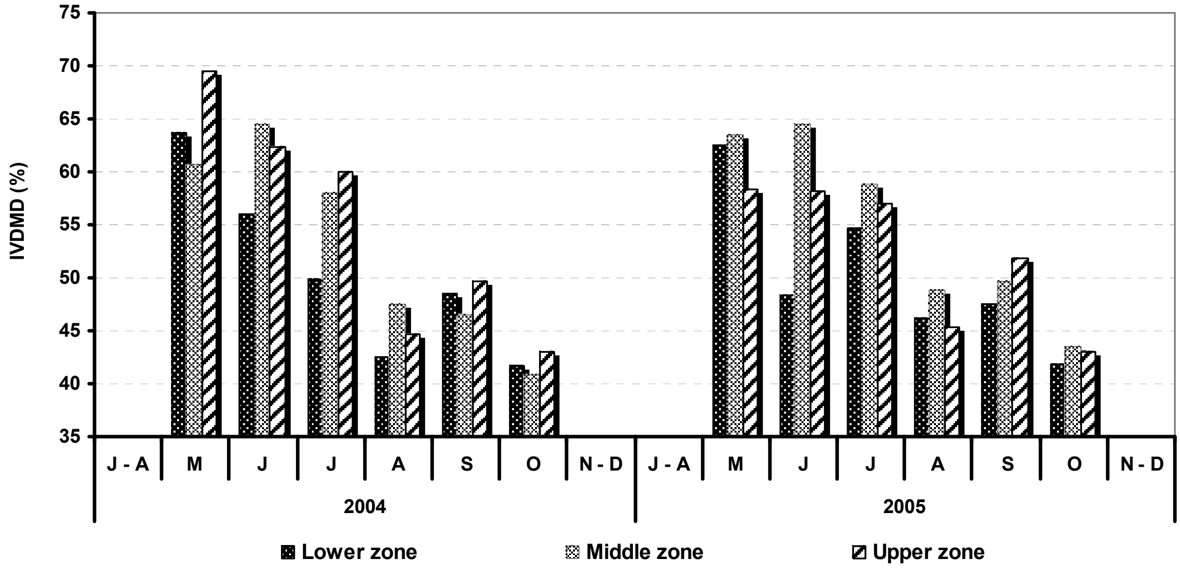

Fig 6 Seasonal variations of in vitro dry matter digestibility (DM %) of Mt Varnoudas pastures at lower, middle and upper altitudinal zone (Means of eight experimental cages per zone)

1.69±0.31, 1.86±0.23 and 2.03±0.28 (DM %) in the lower, middle and upper altitudinal zone respectively, while in 2005 these values were 1.62±0.23, 1.87±0.28 and 1.95±0.27 (DM %) in the three zones respectively (Fig. 4). Rangelands in the upper zone had greater level of EE, following by these in middle and lower zone, in both sampling years.

EE content affected significantly ($P<0.001$) by altitudinal zone and "month x year" interaction, as well as ($P<0.05$) by the harvest month (Table 1). Ether extracts content related significant ($P\leq0.05$) to the altitudinal zone (r= +0.462), herbage production (r= +0.235), IVDMD (r= +0.351) and CP content (r= +0.235), while it was not found strong relation with the other measured parameters (Table 2).

As the plants became older, the crude fibre tended to increase for all plants [7]. In studied rangelands, CF content increase uniformly with month in all altitudinal zones with no differences found between sampling years (Fig 5). Annual mean CF content was found to be 30.01±4.08, 27.44±3.61 and 26.67±2.68 in the lower, middle and upper zone respectively, during the first sampling year, while in the second year, these values were 30.41±3.47, 28.70±3.11 and 28.77±3.33 in the three zones respectively.

CF content affected significantly ($P<0.001$) by the harvest month and the altitudinal zone as well as ($P<0.01$) by the sampling year. However there was no affection by the interaction between the former parameters (Table 1). The correlation coefficients between CF content and the other measured parameters are shown in table 2.

In vitro dry matter digestibility

In vitro dry matter digestibility (IVDMD) of selected grazable material did not show extraordinary differences between sampling years. In general, IVDMD showed a trend to decrease progressively from spring to autumn in all altitudinal zones (Fig 6). In the year 2004, IVDMD ranged from 63.66 in May to 41.75 % in October, in the lower, from 60.74 to 40.84 % in the middle and from 69.46 to 42.99 % in the upper altitudinal zone respectively. In 2005, IVDMD ranged from 62.43 in May to 41.90 % in October, from 63.54 to 43.55 % and from 58.40 to 42.97 %, in the three zones respectively. In all cases, the highest in IVDMD value was reached when the CF content was lowest (at the beginning of growing season). These results are consistent with those of Van Soest [24] and Váskez-de Aldana et al. [25], who indicated that interannual variations in DMD are mainly related to those of crude protein and fibre. As referred above, the forage leaf, stem, and senescent fraction proportion changed during the evaluated months. According to Moreira et al. [11], the higher the leaves proportion in the forage, the higher the CP contents and the lower the cell wall contents. The consequence will be more highly digestible forage. During the summer, leaf proportion in the pasture linearly declined. So DMD linearly decreased during the summer. In the study area DMD followed the same pattern.

IVDMD affected significantly ($P<0.01$) by the altitudinal zone and ($P<0.001$) the harvest month, while it was not affected by the sampling year and the interaction between altitude, month and year (Table 1). IVDMD related negatively (P<0.01) with the harvest month (r= -0.779) and CF (r= -0.663) content, while it was found positive relation to ash (r= +0.369), EE (r= +0.351) and CP (r= +0.729) content (Table 2)

CONCLUSIONS

Native rangelands usually supply livestock with high food quality during spring but forage quality declines rapidly as grazable material matures. Pastures in the lower zone were most productive at the beginning of growing season, while those in the middle and upper zone at mid-summer. Crude protein content was enough to cover only the maintenance needs of ruminants at the beginning of the growing season and then declined. Fibre content was increasing continuously as the growing season processed. Ash and EE contents were quite variable among subsequent or different months in all altitudinal zones, in both years.

It was recommended that additional protein sources should be supplied in order to cover the needs of preservation of the grazing animals during the grazing period in the studied area. Furthermore rational range management techniques are required to elevate the quality and quantity of grazable material and maintain the traditional pastoralism of these landscapes.

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REFERENCES

[1] Ammar H., López S., González J.S., Ranilla M.J.,. Seasonal variations in the chemical composition and in vitro digestibility of some Spanish leguminous shrub species. J. Anim. Feed Sci. (2004) 115: 327–340.

[2] A.O.A.C. Official methods of analysis, 16th ed. (930.15) Association of Official Analytical Chemists. Washington, D.C., 1999.

[3] Brueland B.A., Harmoney K.R., Moore K. J., George J.R., Brummer E.C., Developmental Morphology of Smooth Bromegrass Growth Following Spring Grazing. Crop Sci. (2003) 43:1789-1796.

[4] Buxton D.R., Quality-related characteristics of forages as influenced by plant environment and agronomic factors. Anim. Feed Sci. Technol. (1996) 59: 37-49.

[5] France J., Theodorou M.K., Lowman R.S., Beever D.E., Feed evaluation for animal production. In: Theodorou, M.K., France, J. (Eds.), Feeding systems and feed evaluation models. CABI Publishing, 2000, pp. 1-9.

[6] George M, Bartolome J.W., McDougald N., Connor M., Vaughn C., Markegard G., Annual range forage production. Rangeland Management Series. University of California, Division of Agriculture and Natural Resources Publ. 8018, 2001, pp. 1-9.

[7] Ghadaki M.B., Van Soest P.J., McDowell R.E., Malekpour, B. Chemical composition and in vitro digestibility of some range forage species of Iran. In Proceed. Evaluation and mapping of tropical African rangelands, Bamaco – Mali. International Livestock Centre of Africa, Addis Abeba, Ethiopia, 1975.

http://www.fao.org/wairdocs/ilri/x5543b/x5543b00.HTM

[8] HNMS Hellenic National Meteorological Service. Athens, 2006.

[9] Juαrez A.S., Montoya R., Nevarez G., Cerrillo M.A., 2004. Seasonal variations in chemical composition and dry matter degradability of the forage consumed by goats in a highly deteriorated rangeland of North Mexico. SA J. Anim. Sci., (2004) 34 (5): 68-71.

[10] McDowell L.R., Free-choice mineral supplementation and methods of mineral evaluation. In: Nutrition of Grazing Ruminants in Warm Climates, Academic Press Inc. San Diego, 1985, pp 383-407.

[11] Moreira, F.B., Prado I.N., Cecato U., Wada F.Y., Mizubuti I.Y., Forage evaluation, chemical composition, and in vitro digestibility of continuously grazed star grass. Anim. Feed Sci. Technol. (2004) 113: 239–249.

[12] Mutanga O., Prins H.T., Skidmore A.K., Van Wieren S., Huizing H., Grant R., Peel M., Biggs H., Explaining grass-nutrient patterns in a savannah rangeland of southern Africa. Journal of Biogeography (2004) 31: 819-829.

[13] NRC, Nutrient requirements of sheep. National Academy Press, Washington, D.C., U.S.A., 1985.

[14] Odum E.P., Fundamentals of ecology. 3rd edition. W. B. Saunders Co., Philadelphia and London., 1971.

[15] Papachristou T.G, Nastis A.S., Diets of goats grazing oak shrublands of varying cover in Northern Greece. J. Range Manage. (1993a) 46: 220-226.

[16] Papachristou T.G, Nastis A.S., Nutritive value of diet selected by goats grazing on kermes oak shrublands with different shrub and herbage cover in Northern

Greece. Small Rum. Res. (1993b) 12: 35-44.

[17] Papanikolaou K., Nikolakakis I., Imamidou A., Pappa V., Ntotas V., 2002 Botanical and chemical composition of grazable material in Florina – Greece rangelands and its role in developing of organic stock breeding. Anim. Sci. Rev. (2002) 27: 48-49.

[18] Pérez-Corona M.E., Vázquez de Aldana B.R., García-Criado B., García-Ciudad A. Variations in nutritional quality and biomass production of semiarid grasslands. J. Range Manage. (1998) 51: 570-576.

[19] Ramirez R.G., Feed values of browse. In: VI International Conference on Goats Editorial. International Publishers, Beijing, China, 1996, pp. 510-517.

[20] Ryan, D.F., Bormann, F.H., Nutrient resorption in Northern hardwood forests. Bioscience (1982) 32: 29–32.

[21] SPSS Inc. SPSS 12.0 for Windows, 2003.

[22] Tatli Seven P., Çerçi İ. H., Relationship between nutrient composition and feed digestibility determined with enzyme and nylon bag (in situ) techniques in feed sources. Bulg. J. Vet. Med., (2006) 9 (2): 107−113.

[23] Tilley J.M.A., Terry R.A., A two stage technique for in vitro digestion of forage crops. J. British Grassland Society (1963) 18:104-111.

[24] Van Soest P.J., (1982) Composition, fibre quality and nutritive value of forages. In: Heath M.E., Barnes R.F., Hetcalfe D.S. (Eds) Forages: the Science of Grassland Agriculture, Ames, IA: Iowa State University Press, 1982, pp. 412-421.

[25] Vázquez-de-Aldana B.R., García-Ciudad A., Pérez-Corona M.E., García–Criado B., Nutritional quality of semi-arid grassland in western Spain over a 10-year period: changes in chemical composition of grasses, legumes and forbs. Blackwell Science Ltd. Grass and Forage Science (2000) 55: 209-220.