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The effect of altitude on the botanical composition of forage and the variation of its chemical composition in a subalpine grassland

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Abstract: *This study presents the results of a research conducted in subalpine grasslands located in Epirus (Greece), for five consecutive years (2012-2016). The objective of the study was to determine the variation of the chemical composition {Crude Protein (CP), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Ether Extract (EE)} of grasses, legumes and other forbs of the area every 15 days, in three different altitudinal zones. Grasses were predominant in vegetation composition and were followed by other forbs. Legumes presented the highest mean CP content (P <0.05) and grasses presented the highest mean NDF and ADF content and the lowest mean EE content. The chemical composition of all botanical groups, in all altitudinal zones was statistically significantly (P<0.05) affected by the sampling time. The altitudinal zone affected (P<0.05) the mean CP, NDF and ADF content of all botanical groups, whereas it only affected the mean EE content of grasses. The sampling year affected (P<0.05) the average content of legumes and other forbs in the NDF and EE, whereas it affected the average ADF content of all botanical groups.*

Key words: *Subalpine grassland, grasses, legumes, forbs, chemical composition*

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I. Introduction

Subalpine grasslands are very important for animal production. They play an important role in the reaction of vegetation in relation to climate and therefore must be taken seriously into account as far as decisions on grazing management are concerned [1]. The quantity and quality of the forage produced is affected by climatic conditions, the physical and chemical properties of soil, botanical composition, the type of grazing animal and management [2, 3]. They play a key role in the viability of livestock farming in mountain areas, as their animal products are characterized by high quality, authenticity and originality [4, 5]. They also help to preserve biodiversity and protect the environment from natural hazards [4, 6, 7].

They are a very important source of food for animals, as they are characterized by a wide variety of plant species, with the result that the animals grazing on them produce quality products with significant organoleptic properties [8]. The rational grazing of farm animals in the grasslands can contribute positively to the conservation of protected species or habitats and therefore it is used as an ecological tool for the management of vegetation, internationally [9]. The organization of grazing by space and time must be rationalized in order to contribute to the preservation and protection of the ecosystem [10].

In Greece, the subalpine grasslands occupy an area of about 400,000hectares [11]. They are exploited during the summer months by nomadic livestock breeding and their production can meet animal welfare needs [12, 13]. The plant content in crude protein and crude fiber is one of the most important factors determining the quality of the produced forage [14 - 16]. Neutral Detergent Fiber (NDF) is negatively related to animal feed intake [17], while Acid Detergent Fiber (ADF) has a negative correlation with food digestibility [16, 18, 19]. The ether extract (EE) is an important energy component of ruminant food [20].

The aim of the present study was to record the botanical composition of the subalpine grassland of "Kostilata" and to determine its chemical composition at various altitudes, every fifteen days. The knowledge of the nutritional value of forage is a key factor for a more rational management of grasslands**.**

II. Material and methods

II.1. Study area

The study was conducted in the subalpine grassland of "Kostilata" in eastern Tzoumerka in northwestern Greece, for five consecutive years (2012-2016), from May to September. They occupy an area of approximately 950 hectares and range from 1100 m to 2393 m above sea level (a.s.l.). In winter they are covered with snow and during the summer months they are exploited by nomadic farming. The grazing animals (sheep) are of low weight (about 50 kg) and from their milk, the local dairy product "tsalafouti" is produced which is the most important factor in the local economy. The soil properties and slope degree of" Kostilata" grassland vary according to altitudinal zone (Table 1).

Table 1. Soil properties and slope degree of "Kostilata" grassland [21].

	Sand (%)	Silt (%)	Clay (%)	pH	Organic matter (%)	available P (mg Kg^{-1})	Slope degree (%)		
							0-30	30-60	> 60
Zone A	43.9 a	38.3a	17.8a	5.9a	6.9a	4.3a	37	38	25
Zone B	48.7b	35.1b	16.2ab	5.5b	6.7ab	5.6a	23	43	34
Zone C	52.9c	33.9b	13.2b	5.4b	5.5b	6.2b	18	42	40

*Mean values followed by different letter (a, b, c,), in the same column, differ significantly ($P< 0.05$).

II.2. Altitudinal zones-Forage samplings

The grasslands were divided into three altitudinal zones {1100 m – 1400 m (A zone), 1401 m -1800 m (B zone), 1801 m – 2393 m (C zone)}. Twenty (20) fixed experimental cages, with dimensions of 4 m × 4 m, were installed in each zone, consisting of a 1 m high fence to protect forage from grazing. Forage was collected every 15 days. In zones A and B, forage was collected from the beginning of May to the end of September, while in zone C from the middle of June to the end of September. After each sampling, the plants were separated into three main botanical groups (grasses, legumes and other forbs). The samples were placed in an oven for drying, at 65°C for 48 hours [22], they were weighed and they were milled to particle size 1 mm using a Kinematica Polymyx PX-MFC 90 D cutting mill.

II.3. Climatic data

For the collection of the required climatic parameters (air temperature and precipitation), three weather stations were used, one at 1100m (zone A),used throughout the research period and two other weather stations at 1600 m (zone B) and 2050 m (zone C), used from May 2013 to December 2015.

II.4. Chemical Analyses

The CP content was determined by the Kjeldahl method [23] using Gerhardt kjeldathermand Gerhardt Vapodest Type:40. The NDF content was determined according to the method of Van Soest et al [24] as modified by Vogel et al [25], using an ANKOM 2000 fiber analyzer. The Samples were analyzed using heat stable amylase without sodium sulfite in the neutral detergent reagent. The ADF content was determined according to AOAC [26]. NDF and ADF were expressed without residual ash. For the determination of EE, an extraction of the samples was made in petroleum ether, using the Soxherm apparatus [27].

II.5. Statistical analysis

One-way ANOVA test was used to compare the results for significant differences. Mean differences were checked using Tuckey's test ($P < 0.05$). Statistical analyses were performed with OriginPro 9.0 software.

III. Results

III.1. Precipitation and air Temperature

The highest mean annual air temperature and the highest mean annual precipitation were recorded in zone A. During the experimental period (May - September), in all altitudinal zones, the lowest mean monthly air temperature was recorded in May and the maximum one in August. The highest mean precipitation in zone A occurred in September and in zone B and C in May. The lowest mean precipitation in zone A occurred in July and in zone B and C in August (Tables 2, 3 & 4).

III.2. Forage composition

Grasses were dominant in terms of biomass in vegetation composition, with significant statistical difference ($P< 0.05$) over other botanical groups, in all altitudinal zones, followed by other forbs with significant statistical difference ($P <0.05$) over legumes. The altitudinal zone affected ($P < 0.05$) only legumes in the forage composition (Table 5). The sampling year did not affect ($P <0.05$) the participation of the botanical groups in forage composition (Table 6).

Table 2. Precipitation and air temperature at 1100 m (a.s.l.) during 2012 – 2016.

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Total
Year						Precipitation (mm)							
2012	224	383	233	522	205	34	24	125	121	481	342	547	3.241
2013	733	464	569	61	176	59	36	4	128	178	559	176	3.144
2014	349	137	246	236	127	107	43	49	136	258	187	473	2.350
2015	626	375	311	145	58	76	35	100	180	233	354	0.6	2.493
2016	506	494	259	144	197	66	28	55	373	176	435	2.4	2.734
Mean	488	371	324	222	153	68	33	67	188	265	375	240	2.792
Year						Air Temperature (ºC)							Mean
2013	2.9	3.4	6.6	11.4	15.6	17.4	19.3	20.9	16.3	12.8	8.3	3.7	11.6
2014	5	5.4	6.4	9.1	12.3	17.1	18.8	20.7	15.9	11.9	8.6	5.3	11.4
2015	3.2	2.4	5	9.2	14.8	16.4	21.7	20.3	18.3	12.6	9.9	5.5	11.7
2016	3.8	8.1	6.1	12.9	12.7	18.6	21.1	20.6	15.4	12.2	7.8	2.9	11.9
Mean	2.9	4.0	6.1	10.5	13.8	17.9	20.8	20.8	16.7	12.7	8.8	4.1	11.6

Table 3. Precipitation and air temperature at 1600 m (a.s.l.) during 2013 – 2015.

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Total
Year						Precipitation (mm)							
2013					93	72	66	7	140	201	581	172	
2014	259	178	164	309	192	121	75	76	143	262	203	377	2.358
2015	347	68	13	7	64	93	72	66	44	245	362	11	1.394
Mean	303	123	88	158	116	95	71	50	109	236	382	187	1.876
Year						Air Temperature (ºC)							Mean
2013					12.5	14.8	17.1	18.5	14.1	11.7	5.8	2.4	
2014	2.4	2.6	3.2	5.6	9.3	14.6	16.4	18.4	12.7	9.3	6.1	3.3	8.6
2015	0.5	-0.5	1.3	6.5	10.3	12.3	14.2	18.2	18.6	10.5	6.4	2.9	8.4
Mean	1.5	1.1	2.3	6.1	10.7	13.9	15.9	18.4	15.1	10.5	6.1	2.9	8.5

Table 4. Precipitation and air temperature at 2050 m (a.s.l.) during 2013 – 2015.

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Total
Year						Precipitation (mm)							
2013					110	65	63	4	102	204	376	117	
2014	72	98	98	267	226	140	102	57	152	203	191	256	1.863
2015	172	113	274	152	60	180	112	114	85	295	380	18	1.955
Mean	122	105	186	209	132	128	92	58	113	234	319	130	1.909
Year						Air Temperature (ºC)							Mean
2013					9.2	10.1	12.9	14.4	10.6	8.8	2.8	-0,6	
2014	0	0.1	-0.1	2	5.2	10.5	12.9	14.7	9.6	6.3	3.7	0,8	5.5
2015	-6.2	- 4	0.2	1.2	5.3	10.3	14.1	14.9	10.2	7.1	3.1	0.5	4.7
Mean	-3.1	-2.0	0.1	1.6	6.6	10.3	13.3	14.7	10.1	7.4	3.2	0.2	5.1

Table 5. Proportion of grasses, legumes and other forbs (g Kg^{-1} DM) in the forage of the subalpine grassland of "Kostilata", per altitudinal zone, from 2012 to 2016.

	Bot. Group*	Time of sampling										
		May		June		July		August		September		Mean
		1st half	2nd half	1st half	2nd half	1st half	2nd half	1st half	2nd half	1st half	2nd half	
Zone A	G.	593a^{1}	589a^{1}	703a^{1}	758a^{1}	798a^{1}	833a^{1}	841a^{1}	664a^{1}	725a^{1}	744a^{1}	724a^{1}
	L.	63b^{1}	145b^{1}	84b^{1}	84b^{12}	73b^{1}	44b^{1}	42b^{1}	22b^{1}	46b^{1}	34b^{1}	64b^{1}
	F.	344c^{1}	266c^{1}	213c^{1}	158c^{1}	129b^{1}	123c^{1}	117c^{1}	314c^{1}	229c^{1}	222c^{1}	212c^{1}
Zone B	G.	594a^{1}	614a^{1}	675a^{1}	701a^{12}	729a^{12}	783a^{2}	790a^{12}	82 a^{2}	779a^{12}	766a^{1}	725a^{1}
	L.	71b^{1}	160 b^{1}	155b^{2}	128b^{1}	101b^{1}	81b^{2}	76b^{2}	72b^{2}	82b^{1}	69b^{1}	100b^{2}
	F.	335c^{1}	226c^{1}	170 b^{1}	171c^{1}	170c^{12}	136b^{12}	134c^{1}	108b^{2}	139b^{2}	165c^{2}	175c^{1}
Zone C	G.				628a^{2}	678a^{2}	725a^{3}	761a^{2}	788a^{2}	792a^{2}	775a^{1}	735a^{1}
	L.				52b^{2}	81b^{1}	106b^{2}	79b^{2}	91b^{2}	75b^{1}	86b^{1}	82b^{12}
	F.				320c^{2}	241c^{2}	169c^{2}	160c^{1}	121b^{2}	133c^{2}	139c^{2}	183c^{1}

*Botanical Group**:** G., Grasses; L., Legumes; F., Other Forbs. **Mean values followed by different letter (a, b, c) in the same column and the same altitudinal zone differ significantly (*P*< 0.05).*** Mean values followed by different exponent ($^{1, 2, 3,}$) in the same column, in the same botanical group and in different altitudinal zone differ significantly (*P*< 0.05).

Table 6. Proportion of botanical groups (g Kg^{-1} DM) in the forage of the subalpine grassland of "Kostilata", per altitudinal zone and year.

	Botanical Group	Year				
		2012	2013	2014	2015	2016
Zone A	Grasses	750 ± 98a	725 ± 94a	721 ±121a	715 ± 103a	713 ± 80a
	Legumes	52 ±36b	5.7 ± 42b	6.4 ± 50 b	69 ± 38b	76 ± 34b
	Other Forbs	198 ± 106c	218 ± 86c	215 ± 93c	216 ± 9.0c	211 ± 6.c
Zone B	Grasses	725 ± 112a	712 ± 96a	712 ± 86a	728 ± 66a	716 ± 57a
	Legumes	57 ± 49b	102 ± 53b	99 ± 48b	104 ± 25b	105 ± 22b
	Other Forbs	218 ±103c	186±72c	18.9 ± 61c	169 ± 63c	179 ± 52c
Zone C	Grasses	721 ± 12a	738 ± 32a	727 ± 73a	721 ± 61a	721 ± 59a
	Legumes	64 ± 29b	93 ± 25b	76 ± 22b	82 ± 27b	81 ± 30b
	Other Forbs	215 ± 103c	169 ± 39c	197 ± 91c	197 ± 72c	198 ± 73c

*Mean values followed by different letter (a, b, c) in the same column differ significantly (*P*< 0.05).

III.3. Crude Protein (CP)

All botanical groups presented their highest CP content in the first measurements, following gradual decrease. Legume samples presented the highest mean values of CP content, in all altitudinal zones, with statistically significant difference (*P* <0.05), whereas the other forbs samples presented intermediate values. The CP content of botanical groups was influenced (*P*<0.05) by the altitude and the sampling time (Table 7), whereas their mean content was not affected at all (*P*<0.05) by the sampling year (Table 8).

III.4. Neutral Detergent Fiber (NDF)

All botanical groups presented the lowest NDF content in the first measurements following a progressive increase. Between the botanical groups, grasses samples showed the highest mean NDF values, in all three altitudinal zones, with statistically significant differences (*P* <0.05) from the other botanical groups. The NDF content of all botanical groups was affected *(P*<0.05) by the altitude and the sampling time (Table 9). The sampling year affected (*P*<0.05) the mean NDF content of legumes, in all altitudinal zones, the mean content of the other forbs only in zone C, while the mean content of grasses was not affected at all (*P*< 0.05) (Table 10).

Table 7. CP content of the botanical groups (g Kg^{-1} DM) of the subalpine grassland of "Kostilata", per altitudinal zone, from 2012 to 2016.

	Bot. Group*	Time of sampling										
		May		June		July		August		September		
		1st half	2nd half	1st half	2nd half	1st half	2nd half	1st half	2nd half	1st half	2nd half	Mean
Zone A	G.	139a^{1*}	119b^{1*}	112b^{1*}	79c^{1*}	67df^{1*}	60d^{1}	51e^{1*}	60d^{1*}	69dg^{1*}	73cdg^{1*}	83^{1*}
	L.	187a$^{1\bullet}$	177b$^{1\bullet}$	159c$^{1\bullet}$	127d$^{1\bullet}$	113e$^{1\bullet}$	100f$^{1\bullet}$	74g$^{1\bullet}$	87g$^{1\bullet}$	94f$^{1\bullet}$	100f$^{1\bullet}$	122$^{1\bullet}$
	F.	158a$^{1\square}$	141b$^{\square}$	131c$^{1\square}$	97d$^{1\square}$	86e$^{1\square}$	79eg$^{1\square}$	60f$^{1\square}$	72g$^{1\square}$	80eg$^{1\square}$	82e$^{1\square}$	99$^{1\square}$
Zone B	G.	130a^{2*}	131a^{2*}	117b^{1*}	101c^{2*}	93c^{2*}	81d^{2*}	64e^{2*}	73de^{2*}	76d^{2*}	78d^{1*}	94^{2*}
	L.	188a$^{1\bullet}$	193a$^{2\bullet}$	176b$^{\bullet}$	149c$^{2\bullet}$	142cd$^{2\bullet}$	132d$^{2\bullet}$	89e$^{2\bullet}$	99ef$^{2\bullet}$	99ef$^{1\bullet}$	102f$^{1\bullet}$	137$^{2\bullet}$
	F.	150a$^{1\square}$	152a$^{2\square}$	145a$^{2\square}$	121b$^{2\square}$	117bc$^{2\square}$	107c$^{2\square}$	82d$^{2\square}$	85d$^{2\square}$	86d$^{1\square}$	90d$^{2\square}$	114$^{2\square}$
Zone C	G.				131a^{3*}	126a^{3*}	113b^{3*}	81c^{3*}	97d^{3*}	98d^{3*}	97d^{2*}	106 ^{3*}
	L.				195a$^{3\bullet}$	178b$^{3\bullet}$	162c$^{3\bullet}$	135d$^{3\bullet}$	133d$^{3\bullet}$	129d$^{2\bullet}$	125d$^{2\bullet}$	151$^{3\bullet}$
	F.				165a$^{3\square}$	147b$^{3\square}$	133c$^{3\square}$	112d $^{3\square}$	116d$^{3\square}$	114d$^{2\square}$	109d$^{3\square}$	128$^{3\square}$

*Botanical Group**:** G., Grasses; L., Legumes; F., Others Forbs.**Mean values followed by different letter (a, b, c, d, e, f, g) in the same row differ significantly (*P* < 0.05).***Mean values followed by different exponent ($^{1, 2, 3,}$) in the same column, in the same botanical group and in different altitudinal zone differ significantly (*P*< 0.05).****Mean values followed by different symbol ($^{*, \bullet, \square}$) in the same column and in the same altitudinal zone differ significantly (*P*< 0.05).

Table 8. Mean CP content of the botanical groups (g Kg^{-1} DM) of the subalpine grassland of "Kostilata", per altitudinal zone and year.

	Botanical Group	Year				
		2012	2013	2014	2015	2016
Zone A	Grasses	86 ± 28a^{1*}	85 ±35a^{1*}	82 ± 32a^{1*}	80 ± 26a^{1*}	81 ± 34a^{1*}
	Legumes	120 ± 35b$^{1\bullet}$	123 ± 41b$^{1\bullet}$	124 ± 43b$^{1\bullet}$	119 ± 41b$^{1\bullet}$	122 ± 39b$^{1\bullet}$
	Other Forbs	101 ± 30c$^{1\square}$	99 ± 36c$^{1\square}$	98 ± 34c$^{1\square}$	98 ± 36c$^{1\square}$	96 ± 35c$^{1\square}$
Zone B	Grasses	98 ± 25a^{2*}	95 ± 30a^{2*}	96 ± 29a^{2*}	92 ± 25a^{12*}	93 ± 30a^{2*}
	Legumes	136 ± 38b$^{2\bullet}$	139 ± 42b$^{2\bullet}$	138 ± 42b$^{2\bullet}$	136 ± 37b$^{2\bullet}$	135 ± 42b$^{2\bullet}$
	Other Forbs	113 ± 31c$^{2\square}$	115 ± 32c$^{2\square}$	116 ± 33c$^{2\square}$	115 ± 31c$^{2\square}$	111 ± 22c$^{2\square}$
Zone C	Grasses	110 ± 21a^{3*}	109 ± 24a^{3*}	105 ± 25a^{2*}	106 ± 24α^{2*}	102 ± 23a^{2*}
	Legumes	148 ± 31b$^{3\bullet}$	153 ± 33b$^{3\bullet}$	151 ± 31b$^{3\bullet}$	150 ± 36b$^{2\bullet}$	151 ± 33b$^{3\bullet}$
	Other Forbs	128 ± 25c$^{3\square}$	130 ± 25c$^{3\square}$	128 ± 29c$^{3\square}$	128 ±27c$^{2\square}$	126 ± 27c$^{3\square}$

*Mean values followed by different letter (a, b, c) in the same row differ significantly (*P*< 0.05).**Mean values followed by different exponent ($^{1, 2, 3,}$) in the same column, in the same botanical group and in different altitudinal zone differ significantly (*P* < 0.05).***Mean values followed by different symbol ($^{*, \bullet, \square}$) in the same column and in the same altitudinal zone differ significantly (*P*< 0.05).

III.5. Acid Detergent Fiber (ADF)

All botanical groups showed the lowest ADF content in the first measurements followed by a progressive increase. The highest mean ADF content, in all three altitudinal zones, was presented in grasses samples, with a statistically significant difference observed in the A and B zones only in relation to legumes, while in zone C in relation to legumes and other forbs. The ADF content of all botanical groups was affected (*P* <0.05) by the altitudinal zone and the sampling time (Table 11). The year of sampling affected (*P* <0.05) the mean ADF content of grasses and legumes in all altitudinal zones, whereas the other forbs content was affected (*P*<0.05) only in zone C (Table 12).

Table 9. NDF content of the botanical groups (g Kg^{-1} DM) of the subalpine grassland of "Kostilata", per altitudinal zone, from 2012 to 2016.

	Bot. Group*	Time of sampling										
		May		June		July		August		September		
		1st half	2nd half	1st half	2nd half	1st half	2nd half	1st half	2nd half	1st half	2nd half	Mean
Zone A	G.	440a^{1*}	493b^{1*}	528c^{1*}	564d^{1*}	589e^{1*}	610eg^{1*}	659f^{1*}	646f^{1*}	626g^{1*}	614g^{1*}	577^{1}*
	L.	311a$^{1\bullet}$	329a$^{1\bullet}$	376b$^{1\bullet}$	405c$^{1\bullet}$	426cd$^{1\bullet}$	448de$^{1\bullet}$	469e$^{1\bullet}$	462ef$^{1\bullet}$	440df$^{1\bullet}$	4291cd^{1}	409$^{1\bullet}$
	F.	321a$^{1\bullet}$	334a$^{1\bullet}$	384b$^{1\bullet}$	434c$^{1\bullet}$	447cd$^{1\bullet}$	458d$^{1\bullet}$	477e$^{1\bullet}$	466de$^{1\bullet}$	459d$^{1\bullet}$	454d^{1}	423$^{1\bullet}$
Zone B	G.	422a^{2*}	470b^{2*}	488b^{2*}	526c^{2*}	554d^{2*}	578d^{2*}	607e^{2*}	599e^{2*}	592ed^{2*}	583ed^{2*}	542^{2}*
	L.	280a$^{2\bullet}$	305b$^{2\bullet}$	321b$^{2\bullet}$	347c$^{2\bullet}$	378d$^{2\bullet}$	403e$^{2\bullet}$	427fe$^{2\bullet}$	417ef$^{2\bullet}$	409ef$^{2\bullet}$	400e$^{2\bullet}$	369$^{2\bullet}$
	F.	318a$^{1\bullet}$	330a$^{1\bullet}$	347b$^{2\bullet}$	373c$^{2\bullet}$	393d$^{2\bullet}$	421e$^{2\bullet}$	441f$^{2\bullet}$	435fg$^{2\bullet}$	425eg$^{2\bullet}$	413e$^{2\bullet}$	390$^{2\bullet}$
Zone C	G.				420a^{3*}	479b^{3*}	535c^{3*}	575d^{3}*	572d^{3*}	564d^{3*}	558d^{3*}	529^{2}*
	L.				284a$^{3\bullet}$	305b$^{3\bullet}$	340c$^{3\bullet}$	358c$^{3\bullet}$	352c$^{3\bullet}$	342βc$^{3\bullet}$	341c$^{3\bullet}$	332$^{3\bullet}$
	F.				334a$^{3\square}$	339a$^{3\square}$	354b$^{3\bullet}$	375c$^{3\bullet}$	369cd$^{3\bullet}$	357bd$^{3\bullet}$	352b$^{3\bullet}$	354$^{3\bullet}$

*Botanical Group**:** G., Grasses; L., Legumes; F., Other Forbs.**Mean values followed by different letter (a, b, c, d, e, f, g) in the same row differ significantly (P< 0.05).***Mean values followed by different exponent ($^{1, 2, 3,}$) in the same column, in the same botanical group and in different altitudinal zone differ significantly (P< 0.05). Mean values followed by different symbol ($^{*, \bullet, \square}$) in the same column and in the same altitudinal zone differ significantly (P< 0.05).

Table 10. Mean NDF content of the botanical groups (g Kg^{-1} DM) of the subalpine grassland of "Kostilata", per altitudinal zone and year.

	Botanical Group	Year				
		2012	2013	2014	2015	2016
Zone A	Grasses	580 ± 69a^{1*}	590 ± 84a^{1*}	580 ± 59a^{1*}	557 ± 62a^{1*}	578 ±79a^{1*}
	Legumes	430 ± 61a$^{1\bullet}$	432 ± 66a$^{1\bullet}$	402 ± 51ab$^{1\bullet}$	394 ± 48b$^{1\bullet}$	389 ± 55b$^{1\bullet}$
	Other Forbs	420 ± 59a$^{1\bullet}$	427 ± 67a$^{1\bullet}$	438 ± 55a$^{1\bullet}$	413 ±48a$^{1\bullet}$	429 ± 61a$^{1\square}$
Zone B	Grasses	553 ± 68a^{1*}	541± 57a^{2*}	542 ± 58a^{2*}	532 ± 59a^{*12}	540 ± 64a^{2*}
	Legumes	400 ± 53a$^{2\bullet}$	386 ± 51a$^{2\bullet}$	352 ± 50b$^{2\bullet}$	352 ± 50b$^{1\bullet}$	354 ± 53b$^{1\bullet}$
	Other Forbs	389 ± 52a$^{2\bullet}$	387 ± 41a$^{2\bullet}$	391 ± 40a$^{2\square}$	388 ± 43a$^{1\square}$	393 ± 48a$^{2\square}$
Zone C	Grasses	554 ± 67a^{1*}	531 ± 53a^{2*}	531 ± 57a^{2*}	517 ± 48a^{2*}	511 ± 59a^{2*}
	Legumes	366 ± 25a$^{3\bullet}$	349 ± 21a$^{3\bullet}$	310 ± 35b$^{3\bullet}$	322 ± 33b$^{2\bullet}$	313 ± 38b$^{2\bullet}$
	Other Forbs	359 ± 12a$^{3\bullet}$	344 ± 18b$^{3\bullet}$	352 ±17ab$^{3\square}$	345 ± 15b$^{2\square}$	371 ± 26c$^{2\square}$

*Mean values followed by different letter (a, b, c) in the same row differ significantly (P< 0.05).**Mean values followed by different exponent ($^{1, 2, 3,}$) in the same column, in the same botanical group and in different altitudinal zone differ significantly (P< 0.05).***Mean values followed by different symbol ($^{*, \bullet, \square}$) in the same column and in the same altitudinal zone differ significantly (P< 0.05).

III.6. Ether Extract (EE)

In zone A the highest mean values in EE were presented in the other forbs samples, whereas in B and C zones were presented in the legume samples, with statistically significant differences (P<0.05), to be observed only in relation to grasses samples which presented the lowest mean content in all altitudinal zones. The altitudinal zone affected (P <0.05) the mean EE content of the grasses, while at the same sampling time (P<0.05) it affected the content of all botanical groups (Table 13). The sampling year affected (P<0.05) the mean EE content of grasses and legumes in zones A and C, whereas it affected the other forbs mean content only in zone C (Table 14).

Table 11. ADF content of the botanical groups (g Kg^{-1} DM) of the subalpine grassland of "Kostilata", per altitudinal zone, from 2012 to 2016.

	Bot. Group*	May 1st half	May 2nd half	June 1st half	June 2nd half	July 1st half	July 2nd half	August 1st half	August 2nd half	September 1st half	September 2nd half	Mean
Zone A	G.	265a^{1*}	302b^{1*}	331c^{1*}	357c^{1*}	376d^{1*}	390d^{1*}	411d^{1*}	400d^{1*}	383d^{1*}	373d^{1*}	359*
	L.	232a$^{1\bullet}$	264b$^{1\bullet}$	279b$^{1\bullet}$	326c$^{1\bullet}$	346cd$^{1\bullet}$	361d$^{1\bullet}$	386e^{1*}	365de$^{1\bullet}$	352d$^{1\bullet}$	342cd$^{1\bullet}$	325$^{\bullet}$
	F.	243a$^{1*\bullet}$	260a$^{1\bullet}$	308b$^{1\square}$	340c$^{1*\bullet}$	360c$^{1\bullet}$	378cd$^{1\bullet*}$	403d^{1*}	395d^{1*}	383cd^{1*}	375cd^{1*}	345*$^{\bullet}$
Zone B	G.	244a^{2*}	286b^{1*}	301b^{2*}	316bc^{2*}	333c^{2*}	353cd^{2*}	376d^{2*}	365d^{2*}	366d^{2*}	357d^{2*}	330*
	L.	216a$^{1\bullet}$	219a$^{2\bullet}$	242b$^{2\bullet}$	261b$^{2\bullet}$	278c$^{2\bullet}$	299cd$^{2\bullet}$	329d$^{2\bullet}$	326d$^{2\bullet}$	313cd$^{2\bullet}$	310cd$^{2\bullet}$	279$^{\bullet}$
	F.	238a$^{1*\bullet}$	253ab$^{1\square}$	278b^{2*}	300c^{2*}	322cd^{2*}	347d^{2*}	376d^{2*}	367d^{2*}	356d^{2*}	352d^{2*}	319*
Zone C	G.				250a^{3*}	277b^{3*}	316c^{3*}	337d^{3*}	333d^{3*}	332cd^{3*}	324cd^{3*}	310*
	L.				210a$^{3\bullet}$	229a$^{3\bullet}$	263b$^{3\bullet}$	284b$^{3\bullet}$	298b$^{3\bullet}$	267b$^{3\bullet}$	273b$^{3\bullet}$	261$^{\bullet}$
	F.				239a^{3*}	261b$^{3*\bullet}$	286c$^{3*\bullet}$	306c$^{3\bullet}$	308c$^{3\bullet}$	290c$^{3\bullet}$	288c$^{3\bullet}$	283$^{\bullet}$

*Botanical Group**:** G., Grasses; L., Legumes; F., Other Forbs.** Mean values followed by different letter (a, b, c, d) in the same row differ significantly (P< 0.05).*** Mean values followed by different exponent ($^{1,\ 2,\ 3}$) in the same column, in the same botanical group and in different altitudinal zone differ significantly (P< 0.05).****Mean values followed by different symbol ($^{*,\ \bullet,\ \square}$) in the same column and in the same altitudinal zone differ significantly (P< 0.05).

Table 12. Mean ADF content of the botanical groups (g Kg^{-1} DM) of the subalpine grassland of "Kostilata", per altitudinal zone and year.

	Botanical Group	Year 2012	Year 2013	Year 2014	Year 2015	Year 2016
Zone A	Grasses	373 ±55a^{1*}	367 ± 51ab^{1*}	344 ± 36b^{1*}	351 ± 54ab^{1*}	353 ± 51ab^{1*}
	Legumes	340 ± 60a$^{1\bullet}$	354 ± 55ac^{1}*	301±47b$^{1\bullet}$	31,6±57bc$^{1\bullet}$	299 ± 49b$^{1\bullet}$
	Other Forbs	341 ± 57a$^{1\bullet}$	357 ± 55a^{1*}	343 ± 53a^{1*}	338 ± 57a$^{*1\bullet}$	340 ± 62a^{1*}
Zone B	Grasses	357 ± 48a^{1*}	326 ± 41b^{2*}	317 ± 42b^{2*}	328 ± 48b^{12*}	320 ± 44b^{2*}
	Legumes	311 ± 58a$^{2\bullet}$	278 ±37bc$^{2\bullet}$	264 ± 46b$^{2\bullet}$	292 ± 54ac$^{1\bullet}$	263 ± 47b$^{2\bullet}$
	Other Forbs	327 ± 53a$^{1\bullet}$	315 ± 45a^{2*}	321 ± 46a^{1*}	328 ± 58a^{1*}	314 ± 47a^{12*}
Zone C	Grasses	328 ± 38a^{2*}	313 ± 35ab^{2*}	298 ± 41b^{3*}	312 ± 40ab^{2*}	302 ± 38b^{2*}
	Legumes	279 ± 36ac$^{3\bullet}$	238 ± 22b$^{3\bullet}$	243 ± 33b$^{2\bullet}$	298 ± 42c^{1*}	252 ± 38b$^{2\bullet}$
	Other Forbs	295 ± 30ac$^{2\bullet}$	267 ± 23b$^{3\square}$	274 ± 28b^{2*}	304 ± 36a^{1*}	82 ± 26bc$^{2*\bullet}$

*Mean values followed by different letter (a, b, c) in the same row differ significantly (P< 0.05).**Mean values followed by different exponent ($^{1,\ 2,\ 3,}$) in the same column, in the same botanical group and in different altitudinal zone differ significantly (P< 0.05).***Mean values followed by different symbol ($^{*,\ \bullet,\ \square}$) in the same column and in the same altitudinal zone differ significantly (P < 0.05).

Table 13. EE content of the botanical groups (g Kg^{-1} DM) of the subalpine grassland of "Kostilata", per altitudinal zone, from 2012 to 2016.

	Bot. Group*	May 1st half	May 2nd half	June 1st half	June 2nd half	July 1st half	July 2nd half	August 1st half	August 2nd half	September 1st half	September 2nd half	Mean
Zone A	G.	26.8a^{1*}	28.2b^{1*}	24.1c^{1*}	18.7d^{1*}	16.6e^{1*}	16.2ef^{1*}	14.8f^{1*}	15.3f^{1*}	14.9f^{1*}	14.7ef^{1*}	19.0^{12*}
	L.	29.4a$^{1\bullet}$	30.1a$^{1\bullet}$	25.6b$^{1\bullet}$	20.8c$^{1\bullet}$	18.3d$^{1\bullet}$	17.3de$^{1\bullet}$	17.0ef$^{1\bullet}$	16.7ef$^{1\bullet}$	16.5f$^{1\bullet}$	15.9f$^{1\bullet}$	20.8$^{1\bullet}$
	F.	28.5a$^{1*\bullet}$	39.8a$^{1\bullet}$	25.9b$^{1\bullet}$	20.4c$^{1\bullet}$	18.2d$^{1\bullet}$	18.0d$^{1\bullet}$	16.4e$^{1\bullet}$	16.8ed$^{1\bullet}$	15.7e$^{1\bullet}$	16.0e$^{1\bullet}$	21.6$^{1\bullet}$
Zone B	G.	27.0a^{1*}	29.0b^{1*}	24.9c^{1*}	19.8d^{1*}	17.8e^{2*}	17.2ef^{2*}	16.2fg^{2*}	16.0fg^{2*}	15.4g^{1*}	15.3g^{1*}	19.9^{1*}
	L.	28.9a$^{1\bullet}$	30.6b$^{2\bullet}$	26.4c$^{1\bullet}$	21.2d$^{1\bullet}$	18.9e$^{1\bullet}$	18.4e$^{2\bullet}$	17.1f$^{1\bullet}$	17.2f$^{2\bullet}$	15.6g^{1*}	16.0g^{1*}	21.0$^{1\bullet}$
	F.	28.6a$^{1\bullet}$	30.3b$^{1\bullet}$	26.5c$^{1\bullet}$	21.1d$^{1\bullet}$	19.2e$^{1\bullet}$	18.2e$^{1\bullet}$	16.8f$^{1\bullet}$	16.9f$^{1\bullet}$	15.9f^{1*}	15.7f^{1*}	20.9$^{1\bullet}$
Zone C	G.				22.3a^{2*}	21.8a^{3*}	19.5b^{3*}	17.1c^{3*}	16.0cd^{2*}	15.5d^{1*}	15.2d^{1*}	18.2^{2*}
	L.				27.6a$^{2\bullet}$	26.7a$^{2\bullet}$	23.4b$^{3\bullet}$	19.2c$^{2\bullet}$	17.6d$^{2\bullet}$	16.6de$^{1\bullet}$	15.9e$^{1\bullet}$	21.0$^{1\bullet}$
	F.				27.9a$^{2\bullet}$	27.1a$^{2\bullet}$	22.9b$^{2\bullet}$	18.7c$^{2\bullet}$	17.7cd$^{2\bullet}$	16.3de$^{1\bullet}$	15.8e$^{1\bullet}$	20.9$^{1\bullet}$

*Botanical Group: G., Grasses; L., Legumes; F., Other Forbs.** Mean values followed by different letter (a, b, c, d, e, f, g) in the same row differ significantly (*P*< 0.05).***Mean values followed by different exponent ($^{1,\ 2,\ 3}$) in the same column, in the same botanical group and in different altitudinal zone differ significantly (*P*< 0.05).****Mean values followed by different symbol ($^{*,\ \bullet}$) in the same column and in the same altitudinal zone differ significantly (*P*< 0.05).

Table 14. Mean EE content of the botanical groups (g Kg^{-1} DM) of the subalpine grassland of "Kostilata", per altitudinal zone and year.

	Botanical Group	Year				
		2012	2013	2014	2015	2016
Zone A	Grasses	$19.4 \pm 0.5a^{1*}$	$18.8 \pm 0.6a^{12*}$	$19.6 \pm 0.5a^{1*}$	$19.0 \pm 0.5a^{1*}$	$18.8 \pm 0.5a^{1*}$
	Legumes	$20.5 \pm 0.6a^{1*}$	$21.9 \pm 0.6b^{1*\bullet}$	$20.5 \pm 0.5a^{1*}$	$20.7 \pm 0.5a^{1*}$	$20.4 \pm 0.5a^{1*}$
	Other Forbs	$20.1 \pm 0.5a^{1*}$	$21.1 \pm 0.61b^{1\bullet}$	$20.6 \pm 0.53a^{1*}$	$20.4 \pm 0.52a^{1*}$	$20.6 \pm 0.5a^{1*}$
Zone B	Grasses	$19.9 \pm 0.6a^{1*}$	$20.1 \pm 0.5a^{1*}$	$19.9 \pm 0.5a^{1*}$	$19.7 \pm 0.5a^{1*}$	$19.6 \pm 0.5a^{1*}$
	Legumes	$20.7 \pm 0.5a^{1*}$	$21.1 \pm 0.6a^{12*}$	$20.8 \pm 0.5a^{1*}$	$21.0 \pm 0.5a^{1*}$	$21.0 \pm 0.4a^{1*}$
	Other Forbs	$20.8 \pm 0.4a^{1*}$	$21.5 \pm 0.6a^{1*}$	$20.9 \pm 0.5a^{1*}$	$20.8\alpha \pm 0.6a^{1*}$	$20.7 \pm 0.5a^{1*}$
Zone C	Grasses	$19.1 \pm 0.4a^{1*}$	$17.7 \pm 0.2a^{2*}$	$18.3 \pm 0.3a^{1*}$	$17.9 \pm 0.3a^{1*}$	$18.0 \pm 0.4a^{1*}$
	Legumes	$20.4 \pm 0.5a^{1*}$	$19.8 \pm 0.3a^{2\bullet}$	$21.1 \pm 0.5b^{1\bullet}$	$21.9 \pm 0.7b^{1\bullet}$	$21.7 \pm 0.6b^{1\bullet}$
	Other Forbs	$20.2 \pm 0.4a^{1*}$	$20.4 \pm 04b^{1\bullet}$	$21.3 \pm 0.6b^{1\bullet}$	$21.3 \pm 0.7b^{1\bullet}$	$21.4 \pm 0.6b^{1\bullet}$

*Mean values followed by different letter (a, b) in the same row differ significantly (*P*< 0.05).**Mean values followed by different exponent ($^{1,\ 2}$) in the same column, in the same botanical group and in different altitudinal zone differ significantly (*P*< 0.05).***Mean values followed by different symbol ($^{*,\ \bullet}$) in the same column and in the same altitudinal zone differ significantly (*P* < 0.05).

IV. Discussion

The dominance of grasses in vegetation composition of the area, over the other botanical groups is due to the low temperatures in the study area (Tables 2,3 & 4),to the low soil pH (Table 1) and possibly to grazing management that takes place in the "Kostilata" grasslands. The predominance of other forbs over legumes is due to the fact that legumes are more susceptible to lower temperatures, to the low pH of the soil, which is an inhibitor and possibly to grazing management.

Grasses are best adapted to adverse climatic conditions against other forbs and legumes, which are most affected by low winter and spring temperatures [28,29]. Grasses grow at low pH soils, while legumes at slightly acidic, neutral or even alkaline soils [15, 30]. In Mediterranean grasslands, low temperatures in winter and early spring reduce the participation of legumes in vegetation composition [15]. There is a possibility of reduction or disappearance of legumes after overgrazing [31].

Other researchers' studies [28, 29, 32,33] report a dominance of grasses in vegetation composition and greater participation of other forbs species, in comparison with legumes, results that are consistent with our study. Koutsoukis et al, 2010 [34] and Roukos et al, 2011 [35], report a dominance of grasses in grasslands in western Greece, but a greater participation of legumes versus other forbs, results that are inconsistent with ours. This differentiation is due to the different climatic and soil conditions prevailing between the grasslands and possibly to the different grazing management. The impact of management on vegetation composition has also been reported by other researchers [36, 37].

The highest mean participation of legumes in the vegetation composition in zone B, with statistically significant differences (P <0.05) compared to the zone A(Table5)is probably due to the different management (grazing) applied to the grasslands of the above altitudinal zones. Grazing management [36, 38] and climatic conditions in grasslands affect their botanical composition [39, 40]. Zhaiet al, 2018 [41] reported that the quantity and quality of plants in the grasslands are affected by grazing.

The statistically significant differences (P <0.05) observed between the mean values of botanical groups in CP, NDF, ADF and EE, in the same altitudinal zone (Tables 7, 9, 11 & 13) are due to the different leaf / stem ratio of the botanical groups. The leaves of the plants have higher CP and EE content than the stems and shoots, which have higher crude fiber content [42 - 44]. Grasses have a higher shoot / leaf ratio than other botanical groups [18, 45]. Grasses also have higher hemicellulose content than legumes [17]. Higher CP content of legumes in relation to other forbs and grasses and higher NDF and ADF content of grasses in relation to legumes and other forbs has been reported in other researchers' studies [46-50]. Also, lower mean EE content of grasses compared to legumes and other forbs was reported by Koutsoukis et al, 2016 [51]. The results of our study agree with those of the above researchers.

The highest CP and EE content of the botanical groups at the first measurements, with a gradual decrease over time ($P<0.05$) and the reverse trend of the NDF and ADF content of the botanical groups (Tables 7 ,9, 11 & 13) are due to the fact that: a) as the plant grows, its nutrient needs particularly in nitrogen are increasing [52, 53] and b) as the plants grow the leaf / stem ratio is reduced [14, 42, 54], so that stems and shoots occupy most of the biomass [43, 55]. Similar results were reported by other researchers' studies [16, 53, 56, 57, 58].

The statistically significant differences ($P<0.05$) observed in the CP, NDF, ADF and EE content of the same botanical group, at the same sampling dates, but in a different altitudinal zone (Tables 7, 9, 11 & 13) and in the mean content of the same botanical group, in the same zone, but in a different year (Tables 8, 10, 12 & 14), are due to the different climatic conditions prevailing in each altitudinal zone in the specific time period (Tables 2, 3, & 4). This resulted in the plants being at different stages of growth and therefore having a different leaf / stem ratio and different chemical composition.

At a specific time, the stage of plant growth varies between different plant species [59, 60] and the main factors that affect the growth of plants in natural conditions are precipitation and air temperature [59, 61, 62]. The chemical composition of the same plant species, at the same stage of growth differ significantly, when they grow in different environments [14, 63, 64]. Differences in the chemical composition of grassland plants every year, as well as between plants growing in different environments at the same time have been reported by researchers' studies [12, 59, 65, 66, 67], results consistent with those of our study.

The highest mean CP content of the botanical groups and the lowest mean NDF content observed in the plant samples of the highest altitudinal zones, compared to those of the lower zones, with statistically significant differences ($P<0.05$) (Tables 7, 9, 11 & 13) are attributed to the lowest temperatures observed in the highest zones during the research period (Table 2, 3 & 4). Temperature determines the rate of maturation of plants and affects leaf / stem ratio [68]. It contributes to the plant growth and increases the concentration of crude fiber [69].

The decrease in temperature as the altitude increases results to an increase in the protein content of the plants and a decrease in cellulose, hemicelluloses and lignin content [70 - 72]. Higher CP content of grassland plants and lower NDF and ADF content at higher altitudes has also been reported by Mezhunts, 2006 [49]; Roukos et al, 2011 [35]; Koidou et al, 2019 [58].

The results of the present study are in consistence with those of the above researchers regarding the CP, NDF and ADF content of plants, but our results on EE content are in contrast to those of Mountousis et al, 2006; 2008 [12, 73], which report a positive correlation between the EE content of the forage and the altitude. Also, our results on ADF content are in contrast with those of Koidou et al, 2019 [58], as they reported higher ADF content in plants of the higher altitudinal zones compared to those of the lower zones. This differentiation is probably due to the different plant species present in each grassland, as well as to the fact that plants growing in different environments have different chemical composition [14, 63].

According to National Research Council (NRC) [74], the daily maintenance requirements in CP of sheep weighing 50 kg amount to 95 g $Kg^{-1}$of feed [Dry Matter; (DM)]. The forage covers the CP maintenance needs of sheep grazing in zone A grasslands up to the first half of June, of sheep grazing in zone B grasslands, up to the first half of July and those grazing in zone C during the whole research period, except for the first half of August (Table 15). Mountousis et al, 2008 [12] in a study carried out in northern Greece and at the same altitudes as in the present study, report that forage covers the maintenance needs of 50 kg sheep from May to August, results which are consistent with ours, while Koidou et al, 2019 [58] in a study which was also conducted in northern Greece's grasslands and at same altitudes, report that forage covers the maintenance needs of 50 kg sheep from May to June. This differentiation is probably due to the lower temperatures observed in the "Kostilata" grassland during the months of the research.

When the CP content of forage is less than 70 gKg^{-1} DM, animal productivity is severely restricted and protein supplements are required [75, 76]. Protein supplementation in the feeding of sheep grazing in Zone A is required from mid-July to late August, in sheep grazing in Zone B it is necessary only in the first half of August, marginally (68g Kg^{-1} DM), and in sheep grazing in Zone C no protein supplements are necessary (Table 15).

According to Mertens, 1997 [77], no optimal level of NDF concentration has been established for sheep and goats. The minimum NDF content of forage is from 250 to 280g Kg^{-1} DM [74], whereas when the NDF content is greater than 600g Kg^{-1} DM, it results in reduced feed consumption by animals [78]. Also, according to Zervas, 2013 [79], a high NDF content of sheep nutrition causes reduced food consumption and reduced digestibility, resulting in a decrease in milk production.

The NDF content of forage in "Kostilata" grassland is at a satisfactory level as it exceeds, marginally, 600g Kg^{-1} DM only in zone A, in the first half of August (Table16).

The ADF content of forage in "Kostilata" grassland is generally at a satisfactory level, as it does not exceed 407 g Kg^{-1} DM in zone A, 374 g Kg^{-1} DM in zone B and 328 g Kg^{-1} DM in zone C (Table17). Similar results have been reported by Koidou et al, 2019 [58].

The EE content of the forage is at a low level (Table18). Low content of grassland plants in EE, less than 30 g Kg^{-1} DM has been reported by Coleman and Henry, 2002[80] and Mountousis et al, 2008 [12].

Table 15. CP content of forage (g Kg^{-1} DM) of the subalpine grassland of "Kostilata", per altitudinal zone, from 2012 to 2016.

Zone	May		June		July		August		September		Mean
	1st half	2nd half	1st half	2nd half	1st half	2nd half	1st half	2nd half	1st half	2nd half	
A	148a^{1}	134b^{1}	119c^{1}	86d^{1}	74e^{1}	64gh^{1}	53g^{1}	64eh^{1}	69ed^{1}	76d^{1}	89^{1}
B	141a^{1}	139a^{2}	131b^{2}	111b^{2}	102c^{2}	89d^{2}	68e^{2}	76ed^{2}	79d^{2}	81d^{2}	102^{2}
C				145a^{3}	131ab^{3}	123b^{3}	93c^{3}	103d^{3}	99d^{3}	100d^{3}	113^{3}

Time of sampling

*Mean values followed by different letter (a, b) in the same row differ significantly (P< 0.05).**Mean values followed by different exponent ($^{1, 2, 3}$) in the same column differ significantly (P< 0.05).

Table 16. NDF content of forage (g Kg^{-1} DM) of the subalpine grassland of "Kostilata", per altitudinal zone, from 2012 to 2016.

Zone	May		June		July		August		September		Mean
	1st half	2nd half	1st half	2nd half	1st half	2nd half	1st half	2nd half	1st half	2nd half	
A	391a^{1}	426b^{1}	469c^{1}	529d^{1}	559e^{1}	584f^{1}	632g^{1}	587f^{1}	578f^{1}	572ef^{1}	533^{1}
B	372a^{2}	412b^{2}	440c^{2}	477d^{2}	507e^{2}	545f^{2}	572g^{2}	568g^{2}	552f^{2}	542f^{2}	499^{2}
C				371a^{3}	433b^{3}	475c^{3}	517d^{3}	527d^{3}	520d^{3}	512d^{3}	479^{3}

Time of sampling

*Mean values followed by different letter (a, b, c, d, e, f, g) in the same row differ significantly (P< 0.05).**Mean values followed by different exponent ($^{1, 2, 3}$) in the same column differ significantly (P< 0.05).

Table 17. ADF content of forage (g Kg^{-1} DM) of the subalpine grassland of "Kostilata", per altitudinal zone, from 2012 to 2016.

Zone	May		June		July		August		September		Mean
	1st half	2nd half	1st half	2nd half	1st half	2nd half	1st half	2nd half	1st half	2nd half	
A	246a^{1}	285b^{1}	325c^{1}	351cd^{1}	369d^{1}	385de^{1}	407e^{1}	396e^{1}	379de^{1}	372d^{1}	351^{1}
B	238a^{1}	266b^{1}	285bc^{2}	307c^{2}	326cd^{2}	350de^{2}	374e^{2}	364e^{2}	359e^{2}	353de^{2}	322^{2}
C				258a^{3}	275b^{3}	311c^{3}	328c^{3}	327c^{2}	318c^{2}	317c^{3}	305^{3}

Time of sampling

*Mean values followed by different letter (a, b, c, d, e) in the same row differ significantly (P< 0.05).**Mean values followed by different exponent ($^{1, 2, 3}$) in the same column differ significantly (P< 0.05).

Table 18. EE content of forage (g Kg^{-1} DM) of the subalpine grassland of "Kostilata", per altitudinal zone, from 2012 to 2016.

Zone	May		June		July		August		September		Mean
	1st half	2nd half	1st half	2nd half	1st half	2nd half	1st half	2nd half	1st half	2nd half	
A	27.3a^{1}	28.6b^{1}	24.5c^{1}	19.1d^{1}	17.1e^{1}	16.4e^{1}	15.1f^{1}	15.2f^{1}	15.1f^{1}	15.0f^{1}	19.3^{12}
B	27.7a^{1}	29.5b^{2}	25.1c^{1}	20.4d^{1}	17.9e^{1}	17.7e^{2}	16.3f^{2}	16.0fg^{2}	15.3g^{1}	15.3g^{12}	20.1^{1}
C				24.1a^{2}	23.1a^{2}	20.4b^{3}	17.6c^{3}	16.3cd^{2}	16.0d^{2}	15.6d^{2}	19.0^{2}

Time of sampling

*Mean values followed by different letter (a, b, c, d, e, f, g) in the same row differ significantly (P< 0.05). **Mean values followed by different exponent ($^{1, 2, 3}$) in the same column differ significantly (P< 0.05).

V. Conclusion

The altitude influences the microclimate conditions that affect the time of plant growth and maturation, the botanical composition of the forage and the chemical composition of the plants. The plants that grow in higher altitudes are of better nutritional value, while between the botanical groups, legumes are of the best nutritional value.

The subalpine grasslands of "Kostilata" cover the maintenance needs of animals grazing on them, during the summer months.

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