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# The Variation of Crude Protein and Total Fat of the Main Grassland Plants, in Various Stages of Growth, in "Kostilata" Subalpine Grassland in Theodoriana, Arta, Greece

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**ABSTRACT**

The chemical composition of different plant parts varies, both within the plant itself, as well as among different plants, which is due to their structure, stage of growth and phenological characteristics. In this study, the results of a research conducted in 2013, in a subalpine ecosystem in Epirus (Greece) are presented. The objectives of this research were the determination of crude protein and total fat (ether extract) of the ecosystem's main grassland plants, per group (grasses, legumes, other forbs), at different stages of growth. These plant species were: a) grasses (*Alopecurus gerardil* Vill, *Stipa pennata* L., *Phleum alpinum* L.), b) legumes (*Trifolium repens* L, *Lathyrus aphaca* L, *Lotus corniculatus* L.) and c) other forbs (*Ranunculus repens* L., *Achillea millefolium* L. and *Geranium lucidum* L.). The results showed that: a) the largest amount in crude protein was found in *Trifolium repens* L. with a percentage of 17.05%, with a statistically significant difference only with grasses, b) in all plant species, the largest amounts of crude protein and total fat was observed during the initial stage of plant growth, showing a gradual decrease until the final stage of growth, c) statistically significant differences appeared in the amount of crude protein and total fat, between the same plant species, at different sampling dates, and between different plant species in the same sampling dates and d) the amount of total fat in all plant species was on average 3%.

***Keywords:*** Subalpine grassland, crude protein, total fat.

## Introduction

The subalpine grasslands have a rich flora and are used primarily by pastoralism. Animal nutrition is the main factor that determines the quality of animal products (Boyazoglu and Morand-Fehr, 2001; Coulon *et al.*, 2004). It is well known that ruminant animals cover a large part of their dietary needs, by grazing, which ranges from 25% to 75% (Zervas, 1998), while, animal nutrition represents 50% to 90% of the production costs of each animal product (Ruiz *et al*., 2009). Finally, rearing systems which are based on grazing, give animal products of high nutritional value (Buchin *et al.*, 1999; Viallon *et al.*, 2000; Noziere *et al.*, 2006).

The amount of crude protein in grassland plants is one of the most important factors that define the quality of the produced forage (Buxton, 1996; Bell, 2003; Mlay *et al.,* 2006). At a specific time, the stage of plant growth varies between different plant species (Tallowin and Jefferson, 1999; Bruinenberg *et al.*, 2002) and the main factors that affect the growth of plants in natural conditions are precipitation and air temperature (Frank and Ries, 1990; Papanastasis *et al*., 1997;

Tallowin and Jefferson, 1999; Lemaire *et al.*, 2000). The amount of crude protein in grassland plants, at an early stage of growth is higher than that of plants at a mature stage of growth (Buxton, 1996; Minson, 1990; Tzialla *et al.* 2000; Ammar *et al*., 2004; Duru and Ducrocq, 1997; Hejcman *et al.*, 2010; Mountousis, 2008; Roukos *et al.*, 2006; Perez Corona *et al.*, 1998). More specifically, plant leaves contain higher percentage of crude protein than the stems and shoots, even at higher stage of growth (Cook, 1972; Ganskopp and Bohnert, 2001). As the plants grow, the ratio of leaf and stem usually decreases (Albrecht *et al.*, 1987; Buxton, 1996). The amount of crude protein in legumes is higher than that of grasses (Minson, 1990), while the amount of crude protein in forbs lies between that of legumes and grasses (Cook, 1972; Krysl *et al.*, 1984; Meyer and Brown, 1985; Ruyle, 1993).

The total fat or in other words, ether extract comprises the group of nutrient fat, which plays very important role in the animal body (Liamadis, 2000) and it is a very important energy component of the ruminant feed (Bauman *et al*., 2003). The amount of fat in forage is, generally, low (less than 3% of the dry matter) (Coleman and Henry, 2002; Bruinenberg, 2003). The amount of total fat in plants decreases with growth (SCA, 1990), while the leaves of the plants contain higher amount of fat than the stems (Cook, 1972). The milk fat and the rate and type of fatty acids are affected by the rate of feed coming from grazing (Avondo *et al.*, 2003; Nudda *et al.*, 2003), as well as the plant species and their phenological stage (Addis *et al.*, 2005; Cabiddu *et al.*, 2005).

The use of the grasslands by extensive livestock farming, contributes to the production of quality animal products, the preservation of biodiversity and the protection of ecosystems themselves from natural hazards (Hadjigeorgiou *et al*., 2005; Chatzitheodoridis *et al.*, 2007). Also, the rational use of grasslands requires both the knowledge of the nutritional needs of animals, as well as the quantity and quality of rangeland production in specific soil and climatic environments (Holechek *et al.*, 1995).

The "Kostilata" subalpine grassland is used only by pastoralism, it is of low production, dominated by grasses and it needs to be rationally managed (Roukos *et al.*, 2014). In this study, the variation in chemical composition (crude protein, total fat) of the main plants, per group (grasses, legumes, other forbs), in different stages of growth is described.

## Materials and Methods

The research was conducted in 2013, in "Kostilata" subalpine grassland, and it extends at an altitude of 1400 to 2393 m., it is located, approximately, 80 km northeast of Arta, in Theodoriana, in the mountain range of Tzoumerka. Sixty (60) fixed experimental cages, one meter high, made of mesh, with dimensions 4 m x 4 m, were installed, to protect plants from grazing. The cages were placed, randomly, in such a way so as to be representative of the grassland's vegetation. The aboveground biomass was collected, with the aid of a metallic frame, with dimensions of 50 x 50 cm, from five (5) different positions, within each of the cages, in order to have homogeneity, according to the method of harvesting (Odum; 1971, Cook and Stubbendieck, 1986; Sarlis, 1998). The samplings were carried out from April 30th to July 15th and specifically at 30/4, 16/5 8/6, 17/6 28/6 and 15/7, during which the animals were grazing. Forage was separated, from each sample, in three main groups: grasses, legumes and other forbs. From each group, three plant species, which had the highest proportion of biomass were selected: a) from grasses (*Alopecurus gerardil* Vill, S*tipa pennata* L., *Phleum alpinum* L.), b) from legumes (*Trifolium repens* L, *Lathyrus aphaca* L, *Lotus corniculatus* L. and c) from other forbs *(Ranunculus repens* L., *Achillea millefolium* L. and *Geranium lucidum* L. For the determination of the plant species the encyclopedia "Mountain Flora of Greece I and II" (Strid, 1986, Strid and Tan, 1991), the book "The main grasses of natural grasslands" (Papanastasis *et al*., 1993) and the book "Vascular Plants of Greece" (Dimopoulos *et al*., 2013) were used, whereas, for receiving the climate parameters (air temperature and precipitation), the weather station which is installed in Theodoriana, was used (Table 1). Likewise characteristics of soil of study area are given in Table 2.Then, the samples were placed in an oven for drying, at 65°C for 48 hours (Deinum and Maassen, 1994). The determination of crude protein was made according to the Kjeldahl method (A.O.A.C., 1999), while for the determination of total fat, an extraction of the samples was made, in petroleum ether, by using the Soxherm apparatus, according to the Soxhlet method (A.O.A.C., 1990).

The results were compared for significant differences by one-way ANOVA test while mean differences were checked using Tuckey's test ($p<0.05$). Statistical analyses were performed also with OriginPro 9.0 software.

## Results and Discussion

### Crude Protein

The largest amount in crude protein, on average, was found in *Trifolium repens* L. at a rate of 17.05%, in *Lotus corniculatus* L. at a rate of 14.86% and in *Lathyrus aphaca* L. at a rate of 14.76%. The other forbs followed and especially, *Ranunculus repens* L. with 13.88%, *Achillea millefolium* L., with 12.52% and *Geranium lucidum* L. with 11.80%, while, grasses showed the lowest percentage rates, 9.68%, 9.34%

and 10.79%, in *Alopecurus gerardii* Vill, in *Stipa pennata* L. and in *Phleum alpinum* L. respectively, with a statistically significant difference to be observed only between *Trifolium repens* L. and grasses (Table 3). Legumes contain larger quantity of crude protein compared to other plants (Minson, 1990; Ruyle, 1993). The results of our research agree with those of Minson (1990), who found that legumes contain crude proteins at a rate of around 16% to 17%, of the dry matter, while grasses contain crude proteins, at a rate of around 10% to 13%. Also, the rates of crude protein recorded by Meyer and Brown, (1985) and Ruyle, (1993) in other forbs, were lying between those of grasses and legumes.

The higher rates in all plant species occurred, with a statistically significant difference, in their early growth stages and decreased as the plants matured. The decrease in the amount of crude protein in plants is due to the fact that leaves have a higher content of protein, compared to the stems (Ganskopp and Bohnert, 2001), whereas, as the plants mature, the ratio of leaf / stem, usually decreases (Albrecht *et al*., 1987; Buxton, 1996). Also, the decrease of crude protein in plants, during plant growth, has been reported by Duru and Ducrocq, (1997) and Hejcman *et al.*, (2010).

Also, a statistically significant difference was observed between plant species, on the same sampling dates, mainly, between both legumes and other forbs and grasses. These differences are due to the fact that, at a specific time, the stages of growth vary between different plant species (Tallowin and Jefferson, 1999; Bruinenberg *et al.* 2002).

**Total fat**

The largest amount of total fat, on average, was observed in *Lathyrus aphaca* L. with 2.90%, while the other forbs and grass showed lower rates. Statistically, significant difference was observed both between the different growth stages of the same plant, as well as between different plant species at the same sampling dates, mainly between both legumes and other forbs and grasses. Also, the higher rates in all plant species occurred, with a statistically significant difference, in their early growth stages and decreased as the plants matured (Table 4). All the above are due to the fact that leaves have a higher content of total fat, compared to the stems (Cook, 1972) and that the content of the plants in total fat decreases as the plants mature (SCA, 1990), while, at a specific time, the stages of growth vary between different plant species (Tallowin and Jefferson, 1999; Bruinenberg *et al.*, 2002).

Also, according to Albrecht *et al*., (1987) and Buxton, (1996), as the plants mature, the ratio of leaf / stem, usually decreases. The results of our research agree with those of Coleman and Henry, (2002) and Bruinenberg, (2003), who report that the content of forage dry matter, in total fat is less than 3%.

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Table 1. Climatic data of the region, the years 2010, 2011, 2012 and 2013 (National Meteorological Station, 2014)

| Year | Average, annual air temperature ($^0$C) | Average, annual precipitation (mm) |
|---|---|---|
| 2010 | 11.27 | 2.887 |
| 2011 | 10.66 | 1.549,1 |
| 2012 | 11.53 | 3.240,8 |
| 2013 | 11.55 | 3.143,4 |
| Mean | 11.25 | 2.705,2 |

Table 2. Characteristics of the soil of the study area (Roukos *et al*., 2014)

| Parameter | Clay | Slit | Sand | pH | Organic matter | $CaCO_3$ | P |
|---|---|---|---|---|---|---|---|
| | (%) | (%) | (%) | | (%) | g / kg | g / kg |
| Mean | 14.5 | 36.0 | 49.5 | 5.6 | 6.8 | 0.465 | 14.5 |
| Typ. error | 0.72 | 0.77 | 1.05 | 0.06 | 0.24 | 0.39 | 2.60 |

Table 3. Content of crude protein in plants, at various stages of growth, on average.

| Group | | Sampling dates of the year 2013 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Species** | **30/4** | **16/5** | **27/5** | **8/6** | **17/6** | **28/6** | **15/7** | **Average** |
| Grasses | *Alopecurus gerardii* L. | …… | 13.27 ± 3.15a$^{14}$ | 10.47 ± 0.53ab$^{1}$ | 11.06 ± 0.46ab$^{13}$ | 7.93 ± 0.74 bc$^{1}$ | 8.36 ± 0.12 bc$^{1}$ | 6.96 ± 0.63bc$^{1}$ | 9.68 ± 0.62$^{1}$ |
| | *Stipa pennata* L. | …… | 11.97 ± 0.86a$^{1}$ | 10.78 ± 0.04ab$^{1}$ | 9.88 ± 0.05ab$^{1}$ | 8.88 ±1.46abc$^{1}$ | 8.38 ± 0.39bc$^{1}$ | 6.16 ± 0.33c$^{1}$ | 9.34 ± 0.46$^{1}$ |
| | *Phleum alpinum* L. | …… | ……. | ……. | 11.52 ± 1.27a$^{1}$ | 12.36 ± 2.21a $^{1}$ | 10.71 ± 1.09a$^{13}$ | 8.57 ± 0.56a$^{1}$ | 10.79 ± 1.29$^{1}$ |
| Legumes | *Trifolium repens* L | …… | 18.91 ± 1.60a $^{2}$ | 21.43 ± 0.68a $^{2}$ | 18.53 ± 0.48a$^{2}$ | 19.62 ± 0.80 a$^{2}$ | 12.45 ± 0.37b$^{2}$ | 11.33 ± 0.60b$^{2}$ | 17.05 ± 0.8$^{12}$ |
| | *Lathyrus aphaca* L. | …… | 13.50 ± 0.48ab$^{1}$ | 17.50 ± 3.03a$^{23}$ | 19.29 ± 1.59 a$^{2}$ | 17.34 ± 0.32a$^{2}$ | 14.11 ±1.66ab$^{2}$ | 6.8 ± 0.16b$^{2}$ | 14.76 ±1.21$^{12}$ |
| | *Lotus corniculatus* L. | …… | 16.68 ± 0.58a$^{24}$ | 16.02 ± 0.26a$^{3}$ | 18.87 ± 0.48a$^{2}$ | 17.12 ± 1.31a$^{2}$ | 11.74 ± 0.32 b$^{23}$ | 8.74 ± 0.11b$^{1}$ | 14.86 ± 0.68$^{12}$ |
| Other forbs | *Ranunculus repens* L. | 19.90 ± 0.84a | 15.63 ±1.36b$^{234}$ | 14.13 ± 0.21b$^{34}$ | 13.11 ± 0.46b$^{13}$ | 11.85 ± 0.25bc$^{13}$ | 8.65 ± 0.49c$^{1}$ | …… | 13.88 ± 0.60$^{12}$ |
| | *Achillea millefolium* L. | …… | 13.68 ± 1.95a$^{1}$ | 14.81 ±1.78a$^{34}$ | 13.82 ± 0.37a$^{13}$ | 12.39±1.09a$^{3}$ | 12.17 ± 1.33a$^{2}$ | 8.23 ± 0.17b$^{1}$ | 12.52 ± 1.11$^{12}$ |
| | *Geranium lucidum* L. | …… | 18.35 ± 0.21a$^{23}$ | 15.81 ± 0.38b$^{34}$ | 9.49 ± 0.19c$^{1}$ | 10.51±0.38c$^{13}$ | 9.65 ± 0.37c$^{1}$ | 6.98 ± 0.12d$^{1}$ | 11.80 ± 0.28$^{12}$ |
| | **Mean** | 19.9 ± 0.84a | 15.20 ± 0.80a | 15.21 ± 0.96a | 15.98 ± 0.70a | 14.58 ± 0.85a | 10.76 ± 0.53b | 8.97 ± 0.54b | |

* Means with different superscripts (a ,b, c, d) in each row, differ significantly (P < 0,05 )

** Means with different exponent (1 2 3 4) in each column differ significantly (P < 0,05 )

Table 4. Content of total fat in plants, at various stages of growth, on average.

| Group | Species | Sampling dates of the year 2013 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 30/4 | 16/5 | 27/5 | 8/6 | 17/6 | 28/6 | 15/7 | Average |
| Grasses | *Alopecurus gerardii* L. | …… | 2.95 ± 0.29a[1] | 1.78 ± 0.23 ab[1] | 1.50 ± 0.11b[1] | 0.86 ± 0.09b[1] | 1.09 ± 0.49b 1 | 1.73 ± 0.13b[1] | 1.65 ± 0.73[1] |
| | *Stipa pennata* L. | …… | 3.04 ± 0.02a[1] | 1.92 ± 0.03b[1] | 1.88 ± 0.09bc[2] | 2.76 ± 0.05ad[2] | 1.94 ± 0.26bcd[12] | 2.09 ±0.23bcd[1] | 2.27 ± 0.50[1] |
| | *Phleum alpinum* L. | …… | …….. | ……. | 2.13 ± 0.37a[2] | 2.65 ± 0.36[2] | 1.55 ± 0.22b[13] | 1.29 ± 0.18bc[1] | 1.90 ± 0.60[1] |
| Legumes | *Trifolium repens* L | …… | 3.24 ± 0.47 a[1] | 2.57 ± 0.22ab[2] | 1.74 ± 0.19b[21] | 1.86 ± 0.12b[34] | 1.83 ± 0.23b[13] | 1.44 ± 0.42b[1] | 2.11 ± 0.66[1] |
| | *Lathyrus aphaca* L. | …… | 4.0 ± 0.20a[1] | 2.97 ± 0.14ac[2] | 2.18 ± 0.22cb[2] | 2.51± 0.31abc[2] | 2.15 ± 0.14bc[23] | 3.62 ± 0.60a[2] | 2.90 ± 0.77[1] |
| | *Lotus corniculatus* L. | …… | 3.80 ± 0.41a[1] | 3.88 ± 0.56a[2] | 3.40 ± 0.86a[3] | 3.40 ± 0.86b[2] | 1.97 ± 0.16 b[12] | 1.43 ± 0.32c[1] | 2.7 ± 1.04[1] |
| Other forbs | *Ranunculus repens* L. | 3.30 ± 0.15a | 2.58 ±0.29ab[1] | 2.81 ± 0.31a[2] | 2.41 ± 0.10ab[2] | 1.63 ± 0.21b[3] | 1.51 ± 0.04b[1] | …… | 2.37 ± 0.69[1] |
| | *Achillea millefolium* L. | …… | 2.74 ± 0.76a[1] | 3.07 ± 0.44a[2] | 1.85 ± 0.01a[2] | 1.81 ± 0.03a[3] | 2.8 ± 0.52a[234] | 2.32 ± 0.12a[1] | 2.43 ± 0.52[1] |
| | *Geranium lucidum* L. | …… | 3.67±0.14a[1] | | 2.08 ± 0.31bc[2] | 2.02 ± 0.33bc[34] | 3.03 ± 0.19a[4] | 1.64 ± 0.14dc[1] | 2.58 ± 0.79[1] |
| | **Mean** | 3.30 ±0.15a | 3.09 ± 0.35 a | 2.75 ± 0.29a | 2.13 ± 0.25a | 2.03 ± 0.17a | 2.03 ± 0.26b | 1.94 ± 0.22b | |

* Means with different superscripts (a, b, c, d) in each row, differ significantly (P < 0,05 )
** Means with different exponent (1 2 3 4) in each column differ significantly (P < 0,05 )

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