

## Fertilizer application (P, K, S, Ca and Mg) on pasture in calcareous dehesas: effects on herbage yield, botanical composition and nutritive value

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### ABSTRACT

The objective of the present study was to evaluate the effect of P, Ca, S, and/or K and/or Mg application at different rates on the botanical composition, herbage yield and its nutritive value in dehesas developed on calcareous soils. In two growing seasons, 2009/10 and 2010/11, five surface-broadcast fertiliser treatments were applied once in autumn. Two grazing periods were simulated by cutting the pasture in mid-spring and late-spring. Treatment K1, with the highest amount of Ca and without K in its composition, provided higher crude protein values in the herbage than controls in 2009/10. When the effect of each nutrient was analysed separately, a strong and highly positive response of Mg application on herbage production was recorded. A surface application of fertilisers containing 25 kg Mg/ha in autumn could increase herbage yield by 40% under the studied conditions. However, Mg was not applied alone, and thus this increase could be due to an effect of Mg by itself or to a synergistic effect between Mg and other nutrients applied. The application of Ca, P and Mg may also favour the production of high-quality forage by yielding a greater cover of legumes and digestible protein.

**Keywords:** permanent grassland; forage; magnesium; crude protein; legumes

Dehesas are grasslands with scattered trees and well-developed herbaceous understory used mainly for extensive rearing of sheep, cattle and pigs. They cover a total area of about 3.5–4 million ha in the Iberian Peninsula. Natural pastures in this area have low and very variable biomass productivity and low nutritive value, as a consequence of low and irregular rainfall of Mediterranean climate, low soil fertility, inadequate livestock management and poor plant species composition (Vázquez de Aldana et al. 2006). Herbage yield is about 1440 kg dry matter (DM) per ha and year, and the resulting forage contains 4–20% legume fraction, 9–12% crude protein (Olea and San Miguel-Ayán 2006), 44–59% neutral detergent fibre and 28–37% acid detergent fibre (Viguera et al. 2006).

Traditionally, to improve productivity, in this area management was focused in three aspects

(Rossiter 1966): low livestock grazing intensity, sowing of well-adapted and very productive pasture species, and fertilizer application. Because fertilizer application is an expensive practise, it will be only recommended if a significant increase in both productivity and quality of the herbage is obtained. Phosphorus was widely applied in dehesas on acidic soils, as P available for plants is low there. Application of P generally results in substantial increase in the productivity and quality of pastures and promotes legumes (Olea et al. 2005). Conversely, the application of N is considered inappropriate in dehesas because it reduces the cover of legumes and the most productive grasses in sward (Hejcman et al. 2012). The effectiveness of K to increase productivity is controversial, although it was shown also to promote legumes (Sarunaite and Kadziulienė 2010). In very acid

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soils, Mg and Ca are essential nutrients able to increase pH, thus increasing the bioavailability of other essential nutrients like P. Mg participates also in important plant physiological processes such as photosynthesis (Cakmak and Yazici 2010).

Research in fertiliser application in dehesas was focused on P, traditionally applied in autumn in the form of superphosphate. Because dehesas are located mainly on acidic soils (around 70% of their total area), most of the studies were carried out under those conditions (Olea and San Miguel-Ayaz 2006). However, the effectiveness of other forms of P, and the application of other nutrients on dehesas developed in alkaline conditions has not been as intensively studied. Therefore, the main objective of this study was to evaluate the effect of different nutrients (P, K, Ca, Mg and S) applied in different combinations, on the herbage yield, nutritive value (protein, fibre and lignin content) and botanical composition of pastures in the semi-arid conditions of the dehesa ecosystem on calcareous soils.

## MATERIAL AND METHODS

The study was conducted in two growing seasons, 2009/2010 and 2010/2011, in the dehesa Los Varales in south-western Spain (UTM 30N,  $x = 686\ 864$  m;  $y = 4\ 293\ 812$  m;  $z = 250$  m a.s.l.). The main soil properties for 0–30 cm depth are shown in Table 1. Climatic data for the two experimental years differed from each other (Figure 1) and both years differed from 30-year annual mean. Both study years had annual precipitations (723 mm in 2009/10, 579 mm in 2010/11) greater than the 30-year average (445 mm). In the study area, the tree layer was dominated by holm oak (*Quercus ilex* subsp. *ballota* (Desf.) Samp.). Natural pastures consisted of spontaneous annual species

(*Helianthemetalia*, *Thero-Brometalia*, *Sisymbrietalia*, etc.), although perennials (*Agrostietalia*, *Poetalia*, etc.) were also present.

The experiment was a randomized block design with four replications and five treatments: (B) a non-fertilized control; (S) 250 kg/ha superphosphate; (T) 370 kg/ha Thomaskali (K + S KALI GMBH, Kassel, Germany); (K1) S + 150 kg/ha Kieserita (K + S KALI GMBH); (K2) 125 kg/ha Thomaskali + 150 kg/ha Kieserita. The rate of each nutrient applied per treatment is given in Table 2. The area of each experimental plot was 500 m<sup>2</sup> (50 × 10 m). Fertilizers were uniformly hand-applied once per year in autumn after first showers. To obtain measurements, a 2 m<sup>2</sup> area per plot relocated each year in areas free of trees, was maintained free of grazing.

Each year, four representative soil samples per plot (0–30 cm depth) were taken before fertiliser application. They were air dried (70°C) and sieved to < 2 mm using a roller mill. Texture was determined gravimetrically. Soil pH was determined using a calibrated pH meter (ratio 10 g soil: 25 mL deionized H<sub>2</sub>O), organic carbon (C<sub>org</sub>) by oxidation with dichromate, extractable P by Olsen procedure, and cation exchange capacity (CEC) by the Drouineau procedure. Ca, Mg and K were extracted with ammonium acetate (1 mol/L) and quantified by atomic absorption. Electrical conductivity (EC) was determined using an EC-meter (Crison Instruments, S.A., Barcelona, Spain). Total N was determined using the Dumas combustion method (Leco FP-428 analyzer, Leco Corp., St. Joseph, USA).

In each free-grazing area, before the pasture biomass harvesting (1 or 2 times per year, at early and/or late spring), botanical composition was determined by visual cover estimation of legumes, grasses and other families. In the laboratory, samples were air dried (70°C) and the follow-

Table 1. Soil properties at the study site

Parameter	Mean ± SE	Parameter	Mean ± SE
Clay (%)	24.0 ± 4.5	P <sub>Olsen</sub> (mg/kg)	5.11 ± 0.30
Silt (%)	16.8 ± 2.2	K (mg/kg)	132.9 ± 0.4
Sand (%)	59.1 ± 6.5	Ca (g/kg)	6.8 ± 1.6
pH (1:2.5)	7.98 ± 0.29	Mg (mg/kg)	685.4 ± 184.7
C <sub>org</sub> (g/kg)	5.86 ± 0.52	CEC (mmol <sub>+</sub> /kg)	135.4 ± 46.4
EC (S/m) (1:5)	0.06 ± 0.01	Ca/Mg ratio	10.6 ± 2.8
N <sub>tot</sub> (g/kg)	0.6 ± 0.1	C <sub>org</sub> /N <sub>tot</sub> ratio	10.0 ± 1.6

EC – electrical conductivity; SE – standard error; CEC – cation exchange capacity

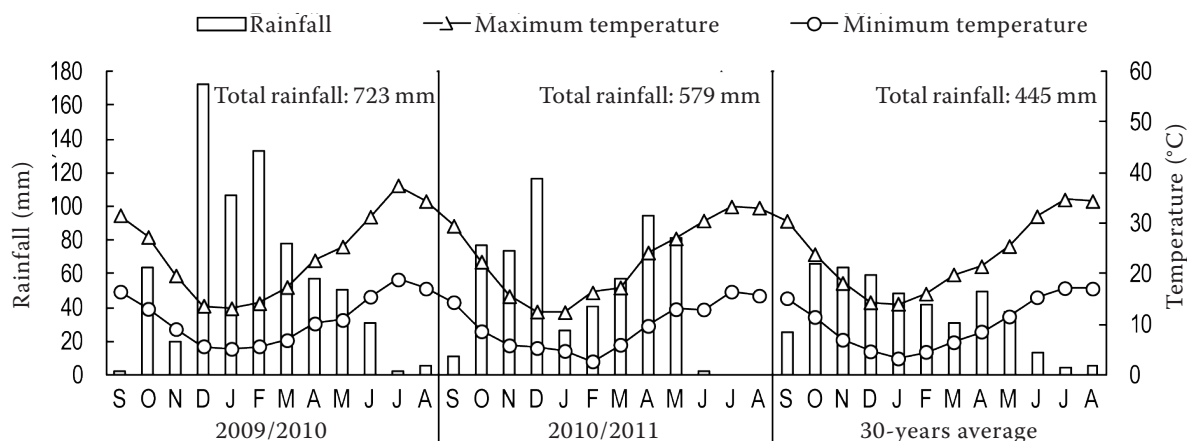


Figure 1. Monthly and annual rainfall and mean maximum and minimum temperatures in 2009/10, 2010/11 and in an average year from a 30-year period at the study site

ing parameters were determined: biomass yield, total N as described above, crude protein (CP) by multiplying N and 6.25 as conversion factor, digestible protein (DP) according to Andrieu et al. (1981), neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) by means of a fibre analyzer (ANKOM 8-98, ANKOM Technology, Macedon, USA) following the official procedures (AOAC 2012).

For each year separately, soil and plant parameters data were subjected to ANOVA and multiple comparison test (Fisher's *LSD* test), including treatment, and/or harvest time and/or their interaction in the model. In order to explore a potential single effect of any nutrient separately, linear regressions between each plant response variable and the rate of each nutrient applied were also performed. Regression models were carried out also for each 2-, 3- or 4-components combination in order to evaluate eventual combined or synergic effects between them. The analyses were performed with the statistical program SAS (Statistical Analysis Software v. 9.1.3, SAS Institute Inc., Cary, USA).

Table 2. Rates (kg/ha) of each nutrient in each treatment applied once per year in autumn

Treatment	P	Ca	S	K	Mg
B	0	0	0	0	0
S	19.64	51.82	27.03	0	0
T	19.38	31.73	11.10	55.29	8.93
K1	19.64	51.82	57.07	0	22.61
K2	6.55	10.72	33.78	18.68	25.63

B – control; S – 250 kg/ha superphosphate; T – 370 kg/ha Thomaskali; K1 – S + 150 kg/ha Kieserita; K2 – 125 kg/ha Thomaskali + 150 kg/ha Kieserita

## RESULTS AND DISCUSSION

As shown in Table 1, soils had a sandy-clay-loam texture, alkaline pH, low  $C_{org}$ , total N and K, very low  $P_{Olsen}$ , and high levels of total Ca and Mg. They did not exhibit salinity problems and CEC and C/N ratio were normal (Horneck et al. 2011). According to ANOVA (data not shown) most of the soil parameters remained unaltered a year later, regardless of fertilizer treatments. Only in the case of Mg, its concentration decreased significantly after one year when treatments were considered together (on average  $685.4 \pm 184.7$  mg/kg in the first year and  $269.8 \pm 26.7$  mg/kg in the second one). Thus, the Ca/Mg ratio in the second year increased to 17–23.

Due to climatic conditions, herbage was only harvested once in 2009/10, and twice in 2010/11. According to ANOVA the fertiliser treatment only affected significantly ( $P \leq 0.05$ ) the crude protein in 2009/10 and the cover of grasses and other herbs in 2010/11. Treatment K1 provided forage with CP values significantly higher than that from not fertilized plots. In plots fertilized with K2 and T, the grass cover was higher than that observed in controls. Such increments were obtained in detriment of the other herbs cover (Table 3). As expected, a significant effect of the harvest time on the nutritive value of the forage was observed. In the first harvest the values of CP, DP and ADL were significantly higher than in the second harvest; whilst NDF and ADF were lower (Table 3).

When nutrients were analysed separately (Figure 2), a strong significant positive relationship was found between herbage yield and Mg applied, especially

Table 3. Mean  $\pm$  standard error of herbage yield (kg/ha), legumes (Leg, %), grasses (Gras, %) and other herbs (Oth, %) cover, neutral detergent fibre (NDF, %), acid detergent fibre (ADF, %), acid detergent lignin (ADL, %), crude protein (CP, %) and digestible protein (DP, %) as affected by the treatment and harvest time

Treatment	Yield	Leg	Gras	Oth	NDF	ADF	ADL	CP	DP
<b>2009/2010</b>									
B	1684 $\pm$ 425	17 $\pm$ 7	19 $\pm$ 9	64 $\pm$ 9	53 $\pm$ 1	38 $\pm$ 1	7 $\pm$ 1	8 $\pm$ 0 <sup>b</sup>	6 $\pm$ 0
S	1750 $\pm$ 548	33 $\pm$ 15	18 $\pm$ 10	49 $\pm$ 15	53 $\pm$ 1	37 $\pm$ 1	6 $\pm$ 1	9 $\pm$ 0 <sup>ab</sup>	7 $\pm$ 0
T	2333 $\pm$ 1093	49 $\pm$ 15	34 $\pm$ 13	17 $\pm$ 5	53 $\pm$ 2	35 $\pm$ 1	6 $\pm$ 1	9 $\pm$ 1 <sup>ab</sup>	7 $\pm$ 1
K1	2635 $\pm$ 284	56 $\pm$ 17	12 $\pm$ 5	32 $\pm$ 18	51 $\pm$ 2	36 $\pm$ 1	6 $\pm$ 1	10 $\pm$ 1 <sup>a</sup>	8 $\pm$ 0
K2	2269 $\pm$ 449	59 $\pm$ 14	17 $\pm$ 6	24 $\pm$ 9	53 $\pm$ 2	37 $\pm$ 1	6 $\pm$ 0	9 $\pm$ 1 <sup>b</sup>	6 $\pm$ 0
Average	2134 $\pm$ 237	43 $\pm$ 6	20 $\pm$ 4	37 $\pm$ 6	52 $\pm$ 1	36 $\pm$ 0	6 $\pm$ 0	9 $\pm$ 0	7 $\pm$ 0
<b>2010/2011</b>									
B	1587 $\pm$ 164	3 $\pm$ 1	42 $\pm$ 7 <sup>c</sup>	55 $\pm$ 7 <sup>a</sup>	55 $\pm$ 4	33 $\pm$ 1	6 $\pm$ 1	10 $\pm$ 1	5 $\pm$ 0
S	1463 $\pm$ 211	6 $\pm$ 2	53 $\pm$ 10 <sup>abc</sup>	41 $\pm$ 9 <sup>abc</sup>	54 $\pm$ 4	32 $\pm$ 2	7 $\pm$ 1	11 $\pm$ 1	5 $\pm$ 0
T	1947 $\pm$ 450	2 $\pm$ 1	77 $\pm$ 9 <sup>a</sup>	22 $\pm$ 9 <sup>c</sup>	58 $\pm$ 5	31 $\pm$ 2	7 $\pm$ 1	11 $\pm$ 1	5 $\pm$ 1
K1	2208 $\pm$ 238	7 $\pm$ 3	47 $\pm$ 8 <sup>bc</sup>	46 $\pm$ 10 <sup>ab</sup>	56 $\pm$ 4	34 $\pm$ 2	7 $\pm$ 1	10 $\pm$ 1	5 $\pm$ 1
K2	2340 $\pm$ 256	3 $\pm$ 1	69 $\pm$ 7 <sup>ab</sup>	28 $\pm$ 7 <sup>bc</sup>	57 $\pm$ 4	32 $\pm$ 1	7 $\pm$ 1	11 $\pm$ 1	5 $\pm$ 1
<b>Harvest time</b>									
I	376 $\pm$ 35	3 $\pm$ 1	55 $\pm$ 6	43 $\pm$ 6	47 $\pm$ 2 <sup>B</sup>	31 $\pm$ 1 <sup>B</sup>	9 $\pm$ 1 <sup>A</sup>	13 $\pm$ 0 <sup>A</sup>	6 $\pm$ 0 <sup>A</sup>
II	1533 $\pm$ 119	6 $\pm$ 1	61 $\pm$ 5	34 $\pm$ 5	65 $\pm$ 1 <sup>A</sup>	34 $\pm$ 1 <sup>A</sup>	5 $\pm$ 0 <sup>B</sup>	8 $\pm$ 0 <sup>B</sup>	4 $\pm$ 0 <sup>B</sup>
Average	1909 $\pm$ 128	4 $\pm$ 1	58 $\pm$ 4	38 $\pm$ 4	56 $\pm$ 2	32 $\pm$ 1	7 $\pm$ 0	11 $\pm$ 0	5 $\pm$ 0

B – control; S – 250 kg/ha superphosphate; T – 370 kg/ha Thomaskali; K1 – S + 150 kg/ha Kieserita; K2 – 125 kg/ha Thomaskali + 150 kg/ha Kieserita. I – mid-spring harvest; II – late spring harvest. In 2010/11 for herbage yield, average is the sum of the values obtained in both harvests. For each year and parameter, different lowercase letters mean significant differences ( $P \leq 0.05$ ) between treatments according to *LSD* test. Different uppercase letters indicate significant differences between harvest times. When letters do not appear, averages were not significantly different according to ANOVA

during 2010/11 ( $R^2 = 0.96$ ). At Mg application doses of 8.9 kg/ha or 25.6 kg/ha, increments ranged from 250 to 780 kg DM/ha respectively, which means herbage productions 15–40% higher than controls. However, such results should be taken as preliminary due to the limited time length of the experiments (just two growing seasons). Further studies, including more study years and a broader range of conditions should be carried out to confirm them. Although the initial Mg in soil was normal or high for plant growth, it could be considered as deficient according to the Ca:Mg:K ratio of 8.2:1:0.8 proposed by Frank (2000) as the ideal for growth of crops. In our case the Ca:Mg ratio in the second year ranged from 7 to 23. It is common knowledge that Ca, Mg and K are exchangeable cations in soil solution and a high concentration of any of them can displace the others.

In dehesas P application was traditionally recommended because it produces an important increment in biomass and promotes legumes (Olea et

al. 2005). In our study, as the initial values of P were very low, a high response of biomass yield to P was expected, but it was not observed. This unexpected lack of response could be due to the alkaline conditions of our soils, quite different from those usually found in dehesas of southern Spain, which are usually acidic. In calcareous soils P was shown to react with Ca forming minerals such as dicalcium phosphate dihydrate or octocalcium phosphate (Freeman and Rowell 1981, Tunesi et al. 1999). Likewise, in these soils the low efficiency of P application is more important when the product is applied in a granular format (Lombi et al. 2004), which was also our case.

A positively significant relationship was also observed between DP and Ca and Mg in the first harvest (2010/11), but negative again with Mg in the second harvest of this year (Figure 2). An increase in legume cover was associated with an increase in protein content (Viguera et al. 2006). In the present study, legumes were not affected by any treatment,

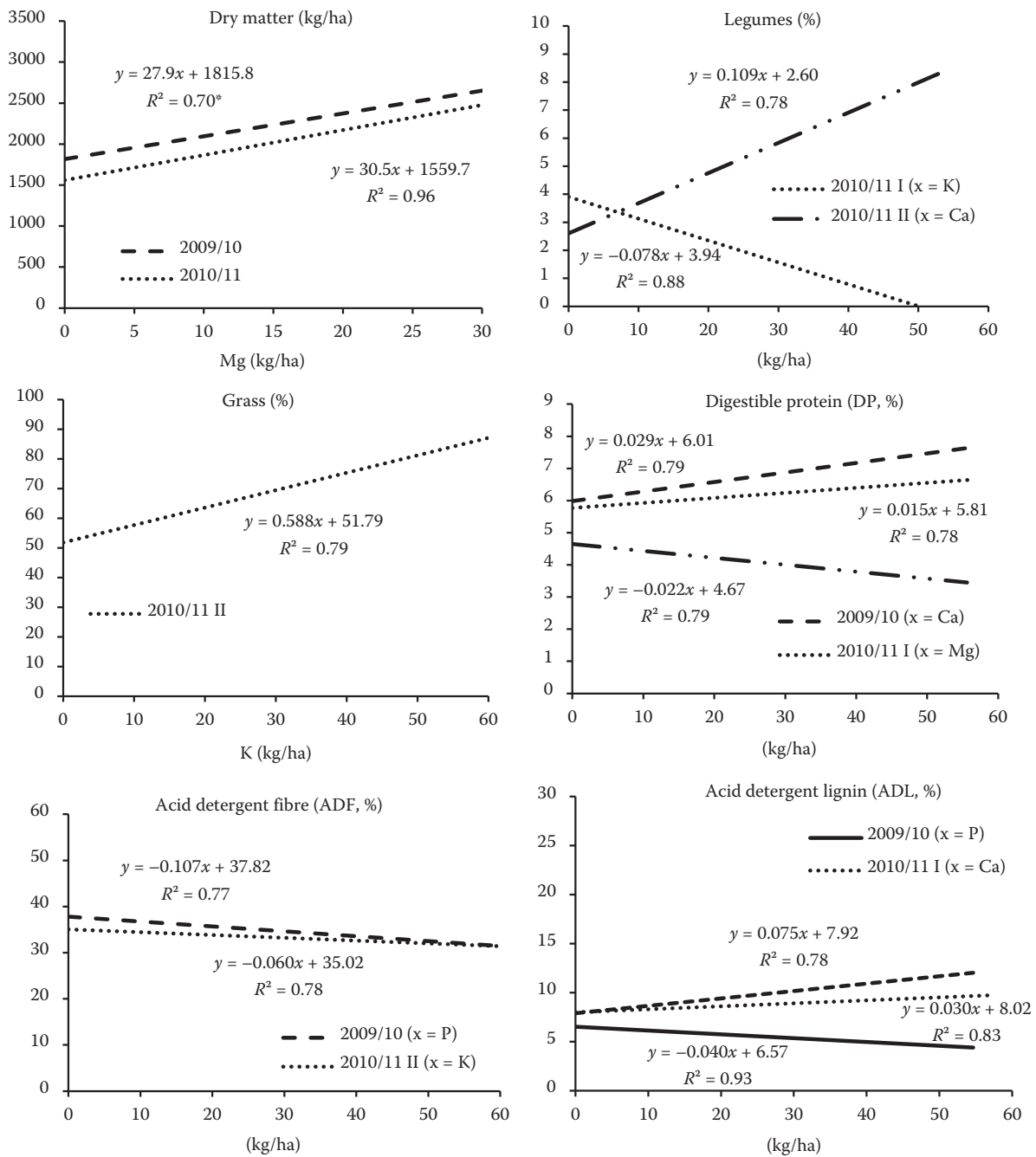


Figure 2. Significant ( $P < 0.05$ ) linear regressions between the amount of nutrient applied and herbage yield (dry matter), botanical composition (legumes and grasses % cover) and nutritive value variables (DP, ADF and ADL). 2010/11 I – mid-spring harvest of 2010/11; 2010/11 II – late spring harvest of 2010/11. In this regression, the  $P$ -value was 0.07

however, a slight relationship was found (Figure 2) between them and both, K (negative correlation) and Ca (positive correlation). This result agrees well with the fact that treatment K1, which produced forage with the highest CP content, included in its composition the highest amount of Ca and did not contain K (Table 2). ADF was negatively correlated with P and K, whereas ADL correlated

positively with Ca and negatively with P (Figure 2). Changes in the availability of several nutrients could enlarge or shorten the biological cycle of the plant species involved in the forage. This fact may affect the nutritive value of the biomass because fibre and protein contents change along plant life cycle (Olea et al. 2005). In order to evaluate the results more precisely, it is important to indi-

cate that the produced forage matter is used for grazing in the study area. Livestock consists of sheep at a stocking rate of 3–4 sheep/ha grazing in a continuous extensive system. Supplementary feeding is provided as needed, especially during summer and winter. Therefore, increases in forage yield and quality of forage could prompt a higher stocking rate or a reduction in the supplementary feeding cost. Considering that a good nutritive value is linked to a high percentage of legumes, high protein content and low fibre values (i.e. a high digestibility), under the current conditions, the application of Ca, P and/or Mg may favour the production of high-quality forage. Conversely, the application of K may favour the growth of grasses over legumes (Figure 2).

In conclusion, the present paper provides an alternative to the conventional P application in dehesas. In calcareous soils, Mg application increased pasture herbage yield by 40%. However, Mg was not applied alone, and thus this increase could be due to an effect of Mg itself or to a synergic effect between Mg and other nutrient applied. The application of P, Ca and Mg may favour the cover of legumes in the sward, increasing then the protein content of the forage.

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