INTERNATIONAL JOURNAL OF BIOASSAYS
ISSN: 2278-778X
CODEN: IJBNHY
OPEN ACCESS

ORIGINAL RESEARCH ARTICLE

Biochemical composition and phosphorus use efficiency in some mixtures

Anna Ilieva and Viliana Vasileva*
Institute of Forage Crops, 89 "General Vladimir Vazov" Str., Pleven 5800, Bulgaria.

Received: April 06, 2016; **Revised**: April 23, 2016; **Accepted**: May 25, 2016
Available online: 1st July 2016

Abstract: In pot trial the biochemical composition and phosphorus use efficiency of birdsfoot trefoil, sainfoin and subterranean clover grown pure and in mixtures with perennial ryegrass in the next ratios were studied in the Institute of Forage Crops, Pleven, Bulgaria: birdsfoot trefoil + perennial ryegrass (50:50%); sainfoin + perennial ryegrass (50:50%); subterranean clover + perennial ryegrass (50:50%); birdsfoot trefoil + subterranean clover + perennial ryegrass (33:33:33%); sainfoin + subterranean clover + perennial ryegrass (33:33:33%). The highest crude protein content was found in the aboveground mass of birdsfoot trefoil (19.17%) and sainfoin (19.30%). The water soluble sugars contents in mixtures was found higher compared to the pure grown legumes. Birds foot trefoil showed the highest phosphorus use efficiency for plant biomass accumulation and nodules formation. In mixtures the phosphorus use efficiency was found be higher as compared to the same in pure grown legumes.

Key words: Biochemical Composition; Mixtures; Phosphorus Use Efficiency

Introduction

Mixtures between legumes and perennial grasses make possible to ensure higher productivity and quality of the forage as compared to pure grown crops (Komarek *et al.,* 2007). Legumes in mixtures contributed to higher crude protein content and the grasses contributed to higher water-soluble sugars content, both favorably affect the digestion and use of forage (Jolaosho *et al.,* 2009; Smahel *et al.,* 2013).

Biochemical composition is related to nutritive value and its determination is necessary for the optimal forage rations for livestock and better forage absorption. It is changing under the influence of many factors, including the species and the variety characteristics of crops, soil and climatic conditions, application of mineral and organic fertilizers (Lukasiewicz *et al.,* 2008). Phosphorus content influences on the nutritive value of forage. Phosphorus performs an extremely important role in the processes of exchange of matter and energy and its deficiency affects practically all the processes occurring in plants (photosynthesis, respiration, reproductive function) (*http://fizrast.ru/kornevoe pitanie fiz-rol/makro/mikro-URmakroelementy.html*).

Phosphorus is essential for stimulation the process of nodulation in legumes (Richardson and Beria, 2009; Tairo and Ndakidemi, 2013). Both, the development of the roots and nodulation (number, size, activity, duration of the life cycle) depend on the phosphorus use efficiency (Serraj *et al.,* 1999; Armstrong, 1999). It is known that in mixtures the efficiency of resource use is greater (Albayrak *et al.,* 2011; Kusvuran *et al.,* 2014). It would be interesting to follow the efficiency of the use of phosphorus for the formation of plant biomass when crops were pure cultivated and in legume grass mixtures. In the present study traditional pasture forage crops (birdsfoot trefoil, sainfoin and perennial ryegrass) were included, the use of which in the pastures of temperate countries is known practice (*Vučković*, 2004; Chourkova, 2014; Ilieva *et al.,* 2015).

Subterranean clover (*Trifolium subterraneum* L.) is widely distributed in pastures of temperate climatic regions of Middle and Northern Europe and America (Nichols *et al.,* 2012), but the studies in recent years showed that it has the practical applicability of the climatic conditions of Bulgaria as well (Vasilev, 2006; Vasilev and Vasileva, 2012; Ilieva *et al.,* 2015).

The purpose of this work was to determine the biochemical composition and phosphorus use efficiency (PUE) of birdsfoot trefoil, sainfoin and subterranean clover grown pure and in mixtures with perennial ryegrass.

Materials and Methods

The trial was carried out at the Institute of Forage Crops, Pleven, Bulgaria (2013-2014). The objects of study were birdsfoot trefoil (*Lotus corniculatus* L.) cv. "Targovishte 1"; sainfoin (*Onobrychis Adans.*) local population, subterranean clover (*Trifolim subterraneum ssp. brachycalicinum*) cv. "Antas" and perennial ryegrass (*Lolium perenne* L.) cv. "IFK - Harmoniya". Crops were studied pure (100%) and in two- and three components mixtures in the next ratios: birdsfoot trefoil + perennial ryegrass (50:50%); sainfoin + perennial ryegrass (50:50%); subterranean clover + perennial ryegrass (50:50%); birdsfoot trefoil + subterranean clover + perennial

***Corresponding Author:**
Prof. Viliana Vasileva,
Institute of Forage Crops,
89 "General Vladimir Vazov" Str.,
Pleven 5800, Bulgaria.
E-mail: viliana.vasileva@gmail.com

http://dx.doi.org/10.21746/ijbio.2016.07.006

ryegrass (33:33:33%); sainfoin + subterranean clover + perennial ryegrass (33:33:33%).

Plastic pots with capacity of 6 l were used filled with soil (leached chernozem subtype). Water soluble phosphorus fertilizer was applied (2.62 g/pot). The sowing was made on the depth of 1-1.5 cm for birdsfoot trefoil, subterranean clover and perennial ryegrass, and 3 cm for sainfoin. Treatments were four replicated.

Two cuts for forage were harvested. In dry plant samples from dry aboveground mass (dried at 60 ^{o}C) total nitrogen content by Kjeldahl (as percentage of absolute dry matter), crude fiber (CF) (Weende method), calcium (Ca), phosphorus (P) and magnesium (Mg) (Sandev, 1979), water soluble carbohydrates (Ermakov *et al.,* 1987).

Phosphorus use efficiency for dry aboveground mass formation (PUE, P, g/kg DM) (g/g)=dry mass (g)/phosphorus applied (g) was calculated; phosphorus use efficiency for dry root mass formation (PUE, P, g/kg DRM) (g/g)=dry root mass (g)/phosphorus applied (g); phosphorus use efficiency for nodule formation (g/g)=nodule weight (g)/phosphorus applied (g), according to formulae of Bowen and Zapata (1991). Phosphorus in yield of dry mass was calculated as productivity of dry mass multiplied by the percentage of phosphorus, (P, g/kg DM). Data were averaged for two years and statistically processed using SPSS (2012).

Results and Discussion

The data from the biochemical analysis of aboveground mass showed that in pure grown crops, the highest crude protein and the lowest crude fiber content were found in birdsfoot trefoil (19.17% crude protein, crude fiber 17.02%) and sainfoin (19.30% crude protein, crude fiber 18.29%) (Table 1). In the subterranean clover crude protein content was 15.26% and that of crude fiber was 21.92%. The data obtained for ryegrass (crude protein 17.59% and crude fibre 22.01%) were close to those of the cultivar's characteristics (Katova, 2011). The water soluble sugars contents in legumes ranged from 2.60% to 4.00% and in ryegrass was 4.90%. The highest calcium (2.08%) and phosphorus (0.425%) content also was found in subterranean clover.

In two components mixtures of legumes with ryegrass, crude protein content was similar to that of one of legume components. In the mixture of birdsfoot trefoil with ryegrass crude protein contents was 19.22%, in the mixture of sainfoin with ryegrass - 19.14% and in the mixture of subterranean clover with ryegrass - 16.16%. Crude fiber content in these mixtures was on the level of content in ryegrass grown pure (22.01%). A similar trend was found in three components mixtures of birdsfoot trefoil and sainfoin with subterranean clover and ryegrass. Contents of crude protein (18.56% and 19.71%, respectively) was similar to that in birdsfoot trefoil and sainfoin and the crude fiber content (22.00% and 21.86%, respectively) to ryegrass (22.01%) grown pure.

The contents of calcium in the mixtures of birdsfoot trefoil and sainfoin with ryegrass was on the level of the content of calcium in ryegrass (1.02%). In the mixture of subterranean clover with ryegrass and in three components mixtures calcium content was lower than the content in the legume component, and higher than that of the ryegrass.

The contents of phosphorus in the mixtures studied was lower than the phosphorus contents in the legume components as well in ryegrass. The exception were mixtures of subterranean clover with ryegrass and mixtures of birdsfoot trefoil with ryegrass, where the phosphorus contents was similar to that in ryegrass grown pure (0.373%). The content of magnesium in mixtures was close to subterranean clover (0.303%).

The water soluble sugars contents in mixtures of legumes with ryegrass was higher compared to the pure grown legumes and lower compared to ryegrass. Only in the mixture of subterranean clover with ryegrass the water soluble sugars contents (4.90%) was as in ryegrass grown pure.

Changes in biochemical composition of plants are probably related to changes in the height and development of the plants, as well as competition and compensatory mechanisms in their mixed growing. Phosphorus use efficiency (PUE) (P, g/kg DM) is agronomic parameter representing the ratio between the productivity and the amount of applied phosphorus. In our study we calculated the efficiency of the use of phosphorus for the formation of plant biomass (dry aboveground and root mass) and nodules. Data showed that for pure cultivated crops the highest phosphorus use efficiency for dry aboveground mass formation was recorded in birdsfoot trefoil (PUE 1.128), followed by sainfoin and subterranean clover (PUE 1.038) (Figure 1).

Phosphorus use efficiency in mixtures for the formation of dry aboveground mass was higher than the same in pure cultivated crops. Thus, in birdsfoot trefoil, the excess for two components mixtures (with perennial ryegrass) was 22.7% and for three components mixtures (with subterranean clover and perennial ryegrass) by 27.2%, respectively. For the mixtures of sainfoin the differences were smaller for both, two components mixtures (by 10.7%), and for three components ones (by 15.8%). Phosphorus use efficiency for dry aboveground mass formation in subterranean clover was by 22.1% higher than in a mixture with perennial ryegrass.

It is seen that phosphorus use efficiency for the accumulation of dry aboveground mass in mixtures of birdsfoot trefoil and subterranean clover was similar in values and was over 20% higher as compared to pure cultivated crops.

In terms of phosphorus use efficiency for formation of dry root mass the highest phosphorus use efficiency was found in sainfoin, followed by birdsfoot trefoil and subterranean clover. In mixtures the phosphorus use efficiency for dry root mass formation was also higher for two- as well for three components mixtures of birdsfoot trefoil and the differences were close (29.6-32.8%). Perhaps the root system of birdsfoot trefoil has this ability to absorb phosphorus and accumulate dry mass.

The phosphorus use efficiency for dry root mass formation in two components mixtures of sainfoin was only by 4.9% higher than that of pure cultivated sainfoin. This is related to its overall weaker development, both pure and in mixtures. Hardarson and Atkins (2003) considered that the main factor for the formation of plant mass in sainfoin is the nitrogen from the soil and from the nitrogen fixation. In this culture, however, has a special feature for fixing of relatively small amounts of nitrogen from the atmosphere. It was found in the conduct of research with a variety of methods, i.e. 15N isotopic method (Provorov and Tikhonovich, 2003; Campillo *et al.,* 2005; Prosser *et al.,* 2006), as well as by the method of reference culture (Carlsson and Huss-Danell, 2003; Hardason and Atkins, 2003). Authors measured low amounts of fixed nitrogen in comparison to other legumes (white and red clover, and alfalfa). As a possible reason they cited the fact that sainfoin needs about 20 mol CO2 for 1 mol N2, unlike others legumes, included in the study needed around 10 mol. Shakirov *et al.,* (2010) considered that sainfoin has less ability to absorb carbon, as well as smaller leaf surface.

In three components mixtures the difference in the phosphorus use efficiency for dry root mass formation as compared to pure sainfoin was 17.8%. In a mixture with perennial ryegrass phosphorus use efficiency for dry root mass formation in subterranean clover was by 20.3% higher. Competition for soil nitrogen may have a beneficial effect on the stimulation of some processes in mixtures, where more components were included in. Thus, in birdsfoot trefoil phosphorus use efficiency for formation of plant biomass was higher in its three components mixtures with subterranean clover and perennial ryegrass (by 27% for dry aboveground and by 33% for dry root mass). In sainfoin phosphorus use efficiency was also higher in three components mixtures (by 15.8% for dry aboveground and by 17.8% for dry root mass).

In terms of phosphorus use efficiency for formation of nodules the variation in values obtained for this characteristic was found higher and is associated with different nodulating ability of legumes (Figure 2). Competitiveness with grass component is a major factor influencing nodulation in mixtures (Ledgard and Steel, 1992). The ingestion of mineral nitrogen in mixtures is greater than when crops were pure cultivated and this reflect on the nodule number. In the mixtures of birdsfoot trefoil the phosphorus use efficiency for nodule formation was by 16-18% higher than that of the pure cultivated birdsfoot trefoil. In sainfoin no evidence and the differences were very close, which is associated with the specifics of this culture, discussed above. At a lower phosphorus use efficiency and nitrogen assimilation is less (van der Velde *et al.,* 2014).

The highest phosphorus use efficiency for nodules formation was found in the mixture of subterranean clover with perennial ryegrass (by 26.2%). Perennial ryegrass stimulates the biological fixation of nitrogen (Davidson *et al.,* 1986; Rogers *et al.,* 1998). Legumes and perennial ryegrass in mixtures have different type of root system and the ability to absorb nutrients as well efficiency of their use are different (Matsunaka and Takahasi, 2001. From the phosphorus use efficiency depends the content of phosphorus in dry mass yield. The calculation of this characteristic showed that in pure cultivated crops the highest phosphorus content in dry mass yield was found in subterranean clover (1.085 g/kg DM) and was close on values to birdsfoot trefoil (0.949 g/kg DM) and sainfoin (0.919 g/kg DM) (Figure 3). Due to the higher productivity of dry aboveground mass, the phosphorus content in the dry mass yield of three components mixtures of birdsfoot trefoil and sainfoin was significant higher (by 17.6% and 47.4%, respectively). A small but proved the difference was found for two components mixture of subterranean clover with perennial ryegrass (by 7.7%).

The tendencies found in our study were associated with different type of nitrogen metabolism of the components included in mixtures, which affects the efficiency of use of the resources. Thus, in perennial ryegrass the assimilation of nitrogen was carried out through the roots from nitrate reductase, while in legumes the nitrogen fixation process was included.

Conclusions

The highest crude protein contents were found in aboveground mass of birdsfoot trefoil (19.17%) and sainfoin (19.30%). In two components mixtures of legumes studied with ryegrass, and in three components mixtures of birdsfoot trefoil with subterranean clover and ryegrass, as well of sainfoin with subterranean clover and ryegrass, crude protein contents was close to that of one of the legumes components. Calcium and phosphorus contents was found be higher in pure grown subterranean clover (2.08% and 0.425%, respectively), and magnesium content in ryegrass (0.341%). The water soluble sugars contents in mixtures was higher compared to the pure grown legumes and lower compared to ryegrass. Birdsfoot trefoil showed the highest phosphorus use efficiency for plant biomass accumulation and nodules formation as compared to sainfoin and subterranean clover. In mixtures of legumes studied with perennial ryegrass the phosphorus use efficiency was found be higher as compared to the same in pure grown legumes. The highest phosphorus use efficiency for dry aboveground and root formation was found in three components mixtures of birdsfoot trefoil and for nodules formation in two components mixtures with perennial ryegrass. The highest content of phosphorus in the dry aboveground mass yield was obtained from three components mixtures of birdsfoot trefoil (1.399 g P/kg DM) and from two components mixtures of subterranean clover with perennial ryegrass (1.169 g P/kg DM).

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Cite this article as:
Anna Ilieva and Viliana Vasileva. Biochemical Composition and Phosphorus Use Efficiency in Some Mixtures. *International Journal of Bioassays* 5.7 (2016): 4694-4698.
http://dx.doi.org/10.21746/ijbio.2016.07.006

Source of support: Nil
Conflict of interest: None Declared