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SUSTAINABLE YIELD INDEX IN SOME MIXTURES

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Sustainable yield index (SYI), nitrogen in dry aboveground mass yield and amount of fixed nitrogen of birdsfoot trefoil, sainfoin and subterranean clover pure grown and in mixtures with tall fescue in the next ratios: birdsfoot trefoil + tall fescue (50:50), sainfoin + tall fescue (50:50), subterranean clover + tall fescue (50:50), birdsfoot trefoil + subterranean clover + tall fescue (33:33:33), sainfoin + subterranean clover + tall fescue (33:33:33) were studied. Pot trial was carried out in the Institute of Forage Crops, Pleven, Bulgaria during 2011-2012. SYI in pure grown birdsfoot trefoil (0.767), sainfoin (0.720) and subterranean clover (0.666) was calculated. Sustainable yield index (SYI) in mixtures was found be higher - from 9.6% (sainfoin+tall fescue) to 18.2% (subterranean clover + tall fescue). Birdsfoot trefoil accumulated the highest amount of fixed nitrogen in dry aboveground biomass (8102 mg N/kg DM), followed by subterranean clover (6730 mg N/kg DM) and sainfoin (5598 mg N/kg DM). On averaged from mixtures studied nitrogen in dry aboveground biomass (7540 mg N/kg DM) was found be higher by 10.7% as compared to pure grown crops (6810 mg N/kg DM). Amount of fixed nitrogen obtained from mixtures (95.64 mg/kg DM) was by 15.2% higher as compared to pure grown forage legumes (81.79 mg/kg DM). Parameters as sustainable yield index, nitrogen in dry aboveground biomass, amount of fixed nitrogen were found be higher in mixtures.

Keywords: Sustainable yield index (SYI), nitrogen in dry aboveground mass yield, fixed nitrogen.

INTRODUCTION

Mixed crops between legumes and grasses have an essential role in building a system of sustainable and ecologically friendly farming (Mihovski and Sabeva, 2011; Luscher *et al.*, 2014; Kusvuran *et al.*, 2014). They are more effective than pure grown in using environmental resources, more productive of dry mass and long-term (Porqueddu *et al.*, 2003; Mahapatra, 2011). Legume component because of the ability to use the process symbiotic nitrogen fixation contributes to increase the nitrogen content in the mixture and nitrogen from biological nitrogen fixation is used directly by plants (Peeters *et al.*, 2006; Graham, 2008). The use of the nutrients is very important for enhancing yield of crops (Khan *et al.*, 2015; Asad *et al.*, 2014). Sustainability of the yield, the content and amount of fixed nitrogen and other morphological or physiological parameters has been changed under coexistence of species in the mixtures. The nutrients

Birdsfoot trefoil, sainfoin, tall fescue are traditional pasture forage crops, the use of which in the pastures of temperate countries is known practice (Vučković, 2004). Their mixed growing creates the potential for obtaining of forage with high energy and protein nutritive value (Demdoum *et al.*, 2010; Chourkova, 2010, 2014; Naydenova *et al.*, 2013).

Climate changes imposed new requirements to forage crops and mixtures namely adoption to the changed conditions, compatibility between components and efficient use of the recourses (Lelièvre and Volaire, 2009). Towards drought resistant components or drought tolerant ones should be involved in the mixtures. For the practice crops with self-seeding ability, which could present long time in the swards are of big importance (Carneiro, 1999).

Subterranean clover (*Trifolium subterraneum* L.) is an annual dry resistant ephemeral species with winter-spring type of development (Piano *et al.*, 1996). As nitrogen fixing crop it is widespread component in pastures of temperate areas of middle and northern Europe, and America (Nichols *et al.*, 2012). Studies with subterranean clover during last years as a component of pasture mixtures showed that this crop is suitable for the climatic conditions of Bulgaria and got a number of advantages over white clover (Vasilev, 2006; Mihovski and Goranova, 2007; Vasileva *et al.*, 2011; Ilieva and Vasileva, 2011; Ilieva *et al.*, 2015; Naydenova and Vasileva, 2015). Good winter resistance, an effective use of autumn-winter soil moisture, successful seed formation and self-seeding in late spring allowed to subterranean clover avoid the summer drought (Porqueddu *et al.*, 2003).

The aim of this work was to study the sustainable yield index (SYI), nitrogen in dry aboveground mass yield and amount of fixed nitrogen in mixtures of birdsfoot trefoil, sainfoin and subterranean clover with tall fescue in different ratios.

MATERIALS AND METHODS

The trial was carried out in the greenhouse in the Institute of Forage Crops, Pleven, Bulgaria (2011 - 2012) under semi controlled conditions. Birdsfoot trefoil (*Lotus corniculatus* L.) cv. "Targovishte 1"; sainfoin (*Onobrychis Adans*.) local population; subterranean clover (*Trifolium subterraneum ssp. brachycalicinum*) cv. "Antas" and tall fescue (*Festuca* *arundinacea* Schreb.) cv. “Albena” were used. Crops were grown pure (100%) and in mixtures in the next ratios: birdsfoot trefoil + tall fescue (50:50), sainfoin + tall fescue (50:50), subterranean clover+tall fescue (50: 50), birdsfoot trefoil + subterranean clover + tall fescue (33:33:33), sainfoin + subterranean clover + tall fescue (33:33:33). Plastic pots with capacity of 6 l were used, filled with soil (leached chernozem subtype) at four replications of the treatments. The sowing was made on the depth of 1-1.5 cm for birdsfoot trefoil and subterranean clover, 3 cm for sainfoin, and 0.5-1 cm for tall fescue.

Two cuts for forage were harvested and productivity of dry aboveground biomass (mg/pot), (dried at 60˚C) was recorded. Root biomass of the plants was washed with tap water and dry root biomass was recorded (mg/pot), (dried at 60^0C). Sustainable yield index for plant biomass was calculated using the formulae of Singh *et al*. (1990):

SYI (Sustainable yield index) = (Ym-Sd)/Ymax,

where: Ym - mean yield; Sd - standard deviation; Ymax - the maximum yield.

The SYI was calculated for dry aboveground biomass, dry root biomass and total productivity (dry aboveground biomass + dry root biomass). Nitrogen in yield of dry aboveground biomass (productivity of dry aboveground biomass multiplied by the percentage of nitrogen), expressed in N, mg/kg dry aboveground biomass was calculated. Previously total nitrogen content by Kjeldahl (as percentage of absolute dry matter) in aboveground biomass (AOAC, 1990) was quantified using dry plant material (aboveground biomass). Plant available nitrogen (PAN) was estimated using the equations of Sullivan and Andrews (2012):

PAN (Plant Available Nitrogen) (mg N/kg DM) = Total N (mg N/kg DM) x 0.4.

A formula of Carlsson and Huss-Danell (2003) was used for roughly estimation of fixed amount of nitrogen (mg/kg DM). Data were averaged and statistically processed using SPSS (2012).

RESULTS AND DISCUSSION

Data from our study showed that sustainable yield index for dry aboveground and dry root biomass in pure grown crops was the highest in birdsfoot trefoil (0.895 and 0.673), followed by sainfoin (0.632 and 0.444) and the lowest was in subterranean clover (0.070 and 0.045) (Table 1). Nyfeler *et al*. (2006) found higher the productivity of dry biomass in grass- legume mixtures as compared to pure cultivation due to more efficient use of natural resources, stimulating legumes to fix more nitrogen and uptake from grasses after its transfer. Each species in grass-legumes mixtures contribute in varying degrees to increase the productivity of dry mass (Vasilev *et al*, 2005; Vasileva and Vasilev, 2012). Sustainable yield index for dry aboveground biomass and dry root biomass in all tested mixtures was higher than that of pure grown crops. For birdsfoot’s mixtures the exceeding was about 20%. For the sainfoin’s mixtures sustainable yield index for dry aboveground biomass was by 28.9% for the mixtures with tall fescue (two component mixtures) and by 33.8% for the mixture with subterranean clover and tall fescue (three component mixtures) higher as compared to pure sainfoin.

Table 1: Sustainable yield index (SYI) of birdsfoot trefoil, sainfoin and subterranean clover, grown pure and in mixtures

Treatments	Sustainable yield index for					
	dry aboveground biomass	to pure	dry root biomass	to pure	total plant biomass	to pure
BT (100%)	0.895	-	0.673	-	0.767	-
BT+TF (50:50)	1.070	+19.6	0.827	+22.8	0.858	+11.9
BT+Subcl+TF(33:33:33)	1.089	+21.7	0.852	+26.6	0.888	+15.8
Sainfoin (100%)	0.632	-	0.444	-	0.720	-
S+TF (50:50)	0.814	+28.9	0.523	+17.7	0.789	+9.6
S+Subcl+TF (33:33:33)	0.845	+33.8	0.562	+26.5	0.834	+15.8
Subclover (100%)	0.070	-	0.045	-	0.666	-
Subcl+TF (50:50)	0.081	+15.9	0.053	+18.0	0.787	+18.2
±SD	0.404		0.311		0.073	
Max	1.089		0.852		0.888	
Min	0.070		0.045		0.666	
SE (P=0.05)	0.142		0.109		0.002	

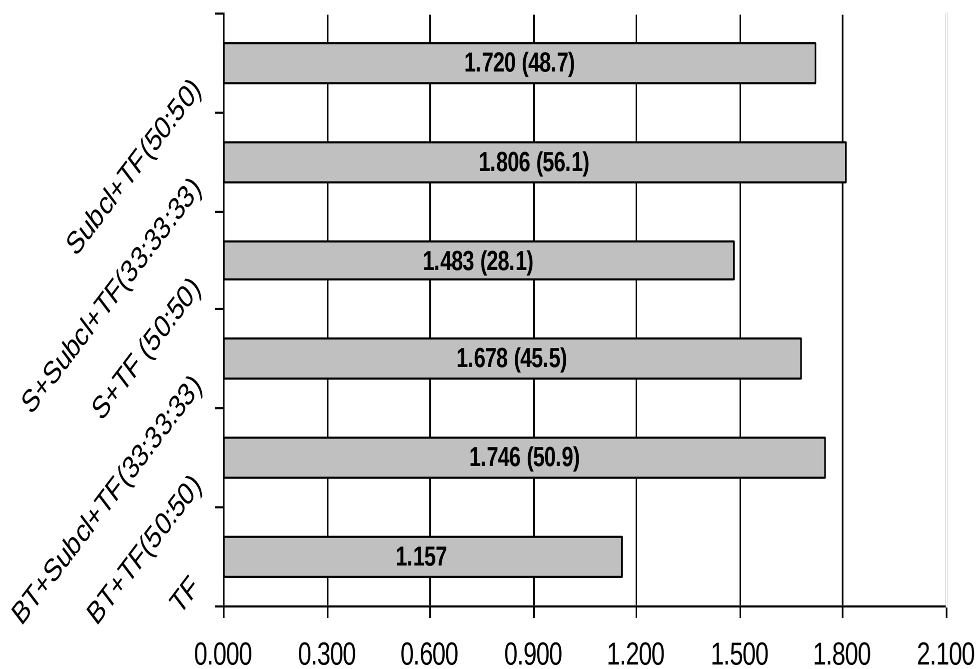
BT - Birdsfoot trefoil, TF - tall fescue, Subcl - subclover, S - sainfoin

With regard to the sustainable yield index for dry root biomass the differences were significantly larger for the two types of mixtures. Thus, in two component mixtures sustainable yield index was by 17.7 % and that of three component mixtures - by 26.5% higher than the sustainable yield index for pure grown sainfoin. A large amount of root biomass in mixtures increased the possibility for taking of immobile nutrients which contributed the better plant development as Datta *et al.* (2011) considered.

In terms of the sustainable yield index for total plant biomass in pure grown crops it was found be highest in birdsfoot trefoil (0.767), followed by sainfoin (0.720) and subterranean clover (0666). In mixtures this index was higher - from 9.6% (sainfoin + tall fescue) to 18.2%

(subterranean clover + tall fescue). The higher sustainable yield index in mixtures could be explained by the higher nitrogen use efficiency. The components included in the mixtures tested have different type of nitrogen metabolism. Nitrogen accumulated in tall fescue is the result solely of nitrate nitrogen assimilation through the roots due to the activity of nitrate reductase and nitrogen use efficiency was lower. In birdsfoot trefoil, sainfoin and subterranean clover nitrogen fixation process was included. Upon successful competition among the components for available nitrogen in mixtures the efficiency of use of nitrogen is higher which affects productivity and sustainability of mixture. Sustainable yield index depends on the portion and compatibility of the components in the mixture. The lower values of the parameters for subterranean clover are related to the morphology of this species.

Fig. 1 presents nitrogen content in the dry aboveground biomass. Legume components with nitrogen fixing ability increased nitrogen content in mixtures. Exceeding in all the mixtures were approximately 50% as compared to pure crops. The exception was the mixture of sainfoin with tall fescue (by 28.2%).



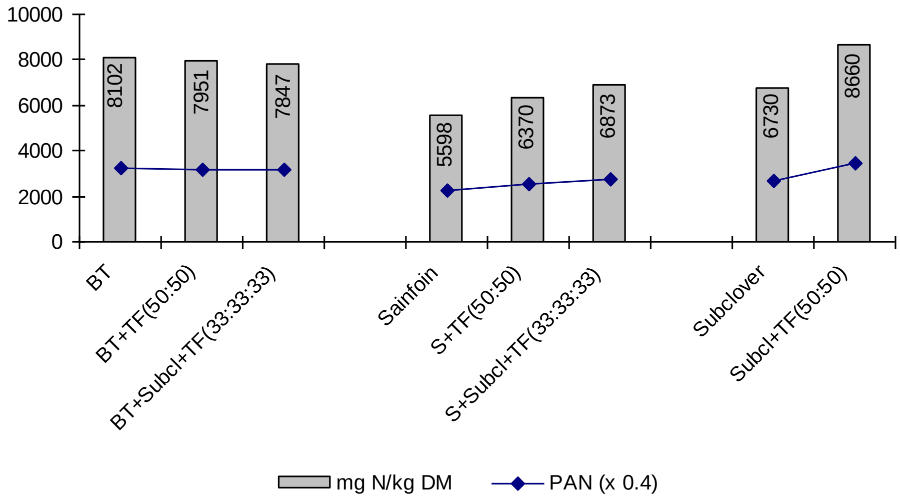
BT - Birdsfoot trefoil, TF - tall fescue, Subcl - subclover, S - sainfoin

Figure 1: Nitrogen content (in% abs. dry matter)

SE (P=0.05) 0.099, ±SD 0.243, in brackets - %, exceeding to tall fescue pure grown

When calculating the amount of nitrogen in dry aboveground biomass yield it is evident that in pure crops it was the highest in birdsfoot trefoil (8102 mg N/kg DM), followed by subterranean clover (6730 mg N/kg DM) and sainfoin (5598 mg N/kg DM) (Figure 2). In mixtures of sainfoin with tall fescue nitrogen content in the yield was by 13.8% higher than in pure crops, and significant more, by 22.8%, in the three components mixtures of subterranean clover and tall fescue. For the mixtures of subterranean clover with tall fescue the increase was by 28.7%, respectively. There are no proven differences for the birdsfoot trefoil's mixtures.

Averaged amount of nitrogen in dry aboveground biomass yield (7540 mg N/kg DM) obtained from all mixtures tested was by 10.7% more than in the pure crops (6810 mg N/kg DM



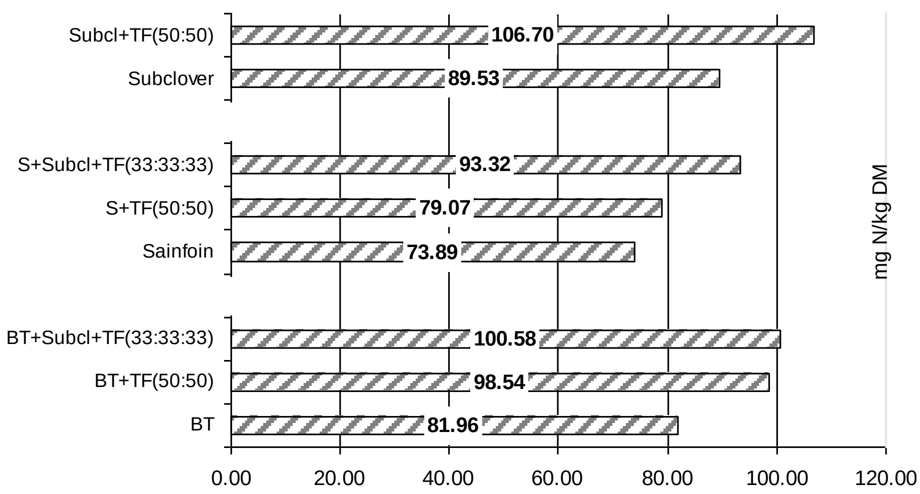
BT - Birdsfoot trefoil, TF - tall fescue, Subcl - subclover, S - sainfoin

Figure 2: Nitrogen in dry shoot mass yield and Plant Available Nitrogen (PAN)

(SE (P=0.05) 365, ±SD 1033)

A similar tendency was found for the plant available nitrogen. Based on the conservative estimate (0.4 = 40% nitrogen release) the contribution of legumes is about 40% of the nitrogen in the biomass (Sullivan and Andrews, 2012).

The data for the amount of fixed nitrogen calculated on a formula of Carlsson and Huss-Danell (2003) for rough estimation showed that in pure crops that amount ranged from 73.89 mg/kg DM (sainfoin) to 89.53 mg/kg DM (subterranean clover) (Figure 3).



BT - Birdsfoot trefoil, TF - tall fescue, Subcl - subclover, S - sainfoin

Figure 3: Fixed amount of nitrogen (by Carlsson and Huss-Danell, 2003)

SE (P=0.05) 4.04, ±SD 11.44

The potential for nitrogen fixation in grass-legume mixtures is bigger as compared to pure grown legume; in addition, legumes are less competitive with grasses for soil nitrogen (Høgh-Jensen and Schjørring, 1994; Carlsson and Huss-Danell, 2003; Nyfeler *et al*., 2005). Between legume and grass components in the mixture has a dynamic relationship in which ingestion of soil nitrogen from grasses reduces its suppressing effect on nitrogen fixation. As a result of the accumulation of soil nitrogen by biological nitrogen fixation, grass component dominates and nitrogen fixation decreases (Ledgard and Steel, 1992). Competition for soil nitrogen in mixtures can have a beneficial effect on the nitrogen-fixing process and accumulating more nitrogen in the grass component. Upon successful competition between species in a mixture, nitrogen is used efficiently and favors nitrogen fixation, which confirms the importance of the choice the appropriate components.

Significantly more fixed nitrogen amount was obtained from mixtures. Thus, for the mixtures of birdsfoot trefoil the exceeding was by over 20%, and for the mixtures of sainfoin with tall fescue with only 7%. This is related to the overall weaker development of sainfoin. Hume (1985) and Hardarson and Atkins (2003) considered that the main factor for the formation of plant biomass in sainfoin is nitrogen from the soil and from nitrogen fixation, feature, however, is the fixing of relatively small quantities of nitrogen from the atmosphere. It is found in conducting studies with different methods - isotopic method 15N (Provorov and Tikhonovich, 2003; Campillo *et al*., 2005; Prosser *et al.,* 2006), as well as the method of reference crop (Carlsson and Huss-Danell, 2003; Hardason and Atkins, 2003). Compared to other legumes (white and red clover, and alfalfa) authors measured lower amounts of fixed nitrogen. As a possible reason they point to the fact that sainfoin needs twice larger amount of CO_2 for 1 mol N_2 compared to other legumes included in the study. In addition, sainfoin has less ability to absorb carbon, as well has smaller leaf surface (Hume *et al*., 1985; Shakirov *et al*., 2010).

The results for the amount of fixed nitrogen in mixture of sainfoin with tall fescue are in line with those of Ilieva *et al.* (2015) for the physiological status of the plants expressed with a total content of plastid pigments in this mixture. In a third component, however (subterranean clover), when the legume: grass ratio was bigger; the excess in the amount of fixed nitrogen to pure sainfoin was significantly (26.3%).

The amount of fixed nitrogen from the mixture of subterranean clover with tall fescue was by 19.2% more than the pure subterranean clover. The amount of fixed nitrogen obtained from mixtures (95.64 mg/kg DM) was by 15.2% higher as compared to pure grown forage legumes (81.79 mg/kg DM).

Conclusions**:** Sustainable yield index (SYI) in pure grown birdsfoot trefoil (0.767), sainfoin (0.720) and subterranean clover (0.666) was calculated. In mixtures with tall fescue sustainable yield index (SYI) was found be higher - from 9.6% (sainfoin + tall fescue) to 18.2% (subterranean clover + tall fescue). Birdsfoot trefoil accumulated the highest amount of fixed nitrogen in dry aboveground biomass (8102 mg N/kg DM), followed by subterranean clover (6730 mg N/kg DM) and sainfoin (5598 mg N/kg DM). On averaged from mixtures studied nitrogen in dry aboveground biomass (7540 mg N/kg DM) was found be higher by 10.7% as compared to pure grown crops (6810 mg N/kg DM) and amount of fixed nitrogen obtained from mixtures (95.64 mg/kg DM) was by 15.2% higher as compared to pure grown forage legumes (81.79 mg/kg DM). Subterranean clover fixed the highest amount of nitrogen (89.53 mg/kg DM).

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