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Content of cyanogenic glycosides in forage biomass of birds'-foot trefoil (*Lotus corniculatus*) grown alone and in mixed population

VILIANA VASILEVA[1], ANNA ILIEVA[2] and EMIL VASILEV[3]

Institute of Forage Crops, 89 General Vladimir Vazov 5800 Pleven, Bulgaria

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Birds'-foot trefoil is a perennial legume forage crop which is resistant to adverse weather conditions, including long dry periods, withstands the strike and grows well after cutting (Vučković S 2004, Stevanovic *et al*. 2015). Due to its good compatibility with many legumes and grasses it participates in the composition of natural and artificial meadows and pastures being a desirable component of grass mixtures (Radovic *et al*. 2003, Chourkova B 2010; Golubinova I and Marinov-Serafimov P 2015).

To make optimal rationales for animal feeding it is necessary to know the chemical composition of the forage. It also determines the nutritive value of the forage (Lukashevich *et al*. 2008). In addition to characteristics determining the nutritive values (crude protein, crude fiber, carbohydrate, mineral composition and digestibility) the content of secondary metabolites affecting the animal health status is also important (Mazid *et al*. 2011).

Birds'-foot trefoil, besides having a high content of crude protein and good digestibility of the dry matter (Chourkova 2012) contains also cyanogenic glycosides. They are harmless under normal conditions, but during enzymatic digestion they release the strongest poison - hydrocyanic acid that causes disorder or poisoning. The specific enzyme is contained in plant cells, but acts as a rupture of the tissues or when digests the green mass in the rumen of the animals. The amount of free hydrocyanic acid directly depends on the amount of cyanogenic glycosides contained in the plants (Vough and Cassel 2002).

All factors that lead to distortion of normal growth and development of plants (ecological, soil-climatic, etc.) contribute to increasing the content of cyanogenic glycosides in plants. The growth stage of plants also has a strong influence on the content of cyanogenic glycosides (Vough L and Cassel E 2002, Golubinova *et al*. 2016a). Often the birds'-foot trefoil is included as a component of mixtures because of the advantages, but in the mixed cultivation of different species, the biochemical composition of the plants also changes (Vough and Cassel 2002, Lukashevich *et al*. 2008, Vasileva and Ilieva 2017). The purpose of the work was to study the changes in the content of cyanogenic glycosides in forage biomass of birds'-foot trefoil grown alone and in mixtures with subterranean clover, cocksfoot and tall fescue.

The experiment was carried out in the experimental field of the Institute of Forage Crops, Pleven, Bulgaria on lached chernozem soil subtype (haplic) without irrigation. The objects of study were birds'-foot trefoil (*Lotus corniculatus* L.) (cv. Targovishte 1), three subclover subspecies, i.e. *Trifolium subterraneum* sp. *brachycalicinum* (cv. Antas), *Trifolium subterraneum* sp. *yaninicum* (cv. Trikkala) and *Trifolium subterraneum* sp. *subterraneum* (cv. Denmark), and two grasses - cocksfoot (*Dactylis glomerata* L.) (cv. Dabrava) and tall fescue (*Festuca arundinacea* Schreb.) (cv. Albena). They were grown alone (100%) and in mixtures birds'-foot trefoil:subclover (50:50%) and birds'-foot trefoil:grass (50:50%) as follows: birds'-foot trefoil + *Trifolium subterraneum* ssp. *brachycalicinum* (50:50%); birds'-foot trefoil + *Trifoilium subterraneum* sp. *yaninicum* (50:50%); birds'-foot trefoil + *Trifolium subterraneum* sp. *subterraneum* (50:50%), birds'-foot trefoil + cocksfoot (50:50%), birds'-foot trefoil + tall fescue (50:50%). The trial was based on the long plots method with the plot size of 70 m^2 (1.40 × 50 m) with 4 times replications of the variants. The content of cyanogenic glycosides (NCH/100 g DM) was determined in fresh plant samples by a mercury method (Ermakov *et al*. 1987). For this purpose, in two consecutive years of the first cut, plant samples were taken from the plants grown alone, from the total sample of the mixtures and from each component of the mixtures. The standard deviation (STDEV) was calculated using the computer software Excel for Windows XP.

Grazing animals usually avoid the birds'-foot trefoil when it is in the flowering stage. The data on the content of cyanogenic glycosides in the different parts of birds'-foot trefoil plants in the flowering stage shows that the leaves, stems and flowers contain cyanogenic glycosides.

Present address: [1,2]Senior Scientist (viliana.vasileva@gmail.com, anna_ibg@yahoo.com), Institute of Forage Crops, Pleven 5800, Bulgaria; [3]Senior Scientist (vasilev_642@abv.bg), Maize Research Institute, 5835 Knezha, Bulgaria.

The highest content of cyanogenic glycosides was found in the flowers (173.60 mg HCN/100 g DM), followed by leaves (90.39 mg HCN/100 g DM) and stems (38.40 mg HCN/100 g DM). According to the scale of toxicity, The Merck Veterinary Manual (2018) the leaves in flowering stage are highly toxic to animals, the flowers are very toxic and can cause death.

The content of cyanogenic glycosides in the aboveground biomass of the individual components of the mixtures of birds'-foot trefoil is presented in Table 1. In the three subclover subspecies grown alone it is found very low. For *Trifolium subterraneum ssp. subterraneum* is 2.84 mg HCN/100 g DM, in *Trifolium subterraneum* ssp. *brachycalicinum* is 3.18 mg HCN/100 g DM and in *Trifolium subterraneum* ssp. *yaninicum* is 7.87 mg HCN/100 g DM vs. Birds'-foot trefoil - 67.55 HCN/100 g DM. The cocksfoot and tall fescue do not contain cyanogenic glycosides (0.00 HCN/100 g DM).

In mixtures of birds'-foot trefoil with subclover the content of cyanogenic glycosides changes in both components of mixtures compared to their pure grown crops increasing by 8.8% - 19.9% in birds'-foot trefoil, and by 10.7 - 54.4% in subclover, strong in *Trifolium subterraneum ssp. brachycalicinum*. Changes in the content of cyanogenic glycosides are also observed in the forage biomass of mixtures of birds'-foot trefoil with grasses. The content of cyanogenic glycosides in birds'-foot trefoil increased by 6.0% in mixtures with tall fescue and by 17.9% in mixtures with cocksfoot compared to its pure crops. Increasing the content of cyanogenic glycosides in the components of mixtures is probably related to changes in plant height and development, to the competition, allelopathy and compensatory mechanisms in mixed growing (Lukashevich *et al*. 2008, Golubinova *et al*. 2016b).

When determining the content of cyanogenic glycosides in the total sample of birds'-foot trefoil with subclover plants (Table 2), opposite results obtained in the individual components. In both experimental years, the content of cyanogenic glycosides in the total sample decreases compared to the content of the birds'-foot trefoil pure grown and is higher than that of the three subclover subspecies. It is possible one of the reasons is the different percentages of the components of the mixture in the sample for the determination of cyanogenic glycosides and probably the predominance of subclovers.

In the mixtures of birds'-foot trefoil with cocksfoot and tall fescue, the content of cyanogenic glycosides in the total samples of the mixtures is close to that of birds'-foot trefoil. In order to establish the effect of dilution of the sample from mixture according to the percentage of components, the content of cyanogenic glycosides in the aboveground biomass of birds'-foot trefoil, subclover and tall fescue as monocultures and their mixtures in a different percentage of the components was measured (Table 2).

The data obtained show that the percentages of the components of the mixtures in the sample affect the cyanogenic glycosides content. It is low (decreasing in

Table 1 Content of cyanogenic glycosides in the components of mixtures

Variants	Cyanogenic glycosides mg HCN/100 g DM	2013/2014
	Pure crops	
Birds'-foot trefoil (100%)	67.55	69.45/87.55
Tr. subter. ssp. *brachycalicinum* (100%)	3.18	
Tr. subter. ssp. *yaninicum* (100%)	7.87	
Tr. subter. ssp. *subterraneum* (100%)	2.84	
STDEV	2.81	
Cocksfoot (100%)	0.00	
Tall fescue (100%)	0.00	
	Mixtures	
Birds'-foot trefoil +	81.03	
Tr. subter. ssp. *brachycalicinum* (50:50%)	4.91	63.15/34.43
Birds'-foot trefoil +	76.98	
Tr. subter. ssp. *yaninicum* (50:50%)	8.71	65.43/23.39
Birds'-foot trefoil +	73.47	
Tr. subter. ssp. *subterraneum* (50:50%)	4.05	63.02/30.93
STDEV for Birds'-foot trefoil	3.78	3.00/29.35
STDEV for subclover	2.48	
Birds'-foot trefoil +	79.68	
cocksfoot (50:50%)	0.00	70.34/96.56
Birds'-foot trefoil +	71.58	72.64/98.89
tall fescue (50:50%)	0.00	5.99/1.65

times) at a ratio of 20:80% when the proportion of subclover or grass is higher and approximates to the content of birds'-foot trefoil pure grown when the ratio is in favor of birds'-foot trefoil (80:20%). Similar data on the influence of the percentage of crops in the mixture on the chemical composition of the forage biomass were also obtained in the mixed cultivation of birds'-foot trefoil, alfalfa and sainfoin with wheatgrass (Ilieva *et al*. 2005) also find changes in the chemical composition of mixtures depending on the percentage of components (for alfalfa mixtures).

Leaves, stems and flowers of birds'-foot trefoil plants contain cyanogenic glycosides. The content of cyanogenic glycosides in the flowers of birds'-foot trefoil is very dangerous for the animal health in grazing. The content of cyanogenic glycosides in plants of *Trifolium subterraneum* sp. *subterraneum* (2.84 mg HCN/100 g DM), *Trifolium subterraneum* sp. *brachycalicinum* (3.18 mg HCN/100 g DM) and *Trifolium subterraneum* sp. *yaninicum* (7.87 mg HCN/100 g DM) was found very low. Cocksfoot and tall

Table 2 Content of cyanogenic glycosides in aboveground biomass of birds'-foot trefoil, subclover and tall fescue

Variants	Ratio	Cyanogenic glycosides
	%	mg HCN/100 g DM
Pure crops		
Birds'-foot trefoil	100	105.32
Tr. subter. ssp. *yaninicum*	100	13.92
Tall fescue	100	0.00
Mixtures		
Birds'-foot trefoil + *Tr. subter*. ssp. *yaninicum*	20:80	19.76
Birds'-foot trefoil + *Tr. subter*. ssp. *yaninicum*	50:50	58.59
Birds'-foot trefoil + *Tr. subter*. ssp. *yaninicum*	80:20	94.16
STDEV		37.21
Birds'-foot trefoil + tall fescue	20:80	26.50
Birds'-foot trefoil + tall fescue	50:50	56.50
Birds'-foot trefoil + tall fescue	80:20	87.02
STDEV		30.26

fescue do not contain cyanogenic glycosides.

In the mixtures of birds'-foot trefoil with subclover the content of cyanogenic glycosides compared to their pure crops changes in both components of mixtures – in birds'-foot trefoil increases from 8.8% to 19.9% and in subclover from 10.7 to 54.4%. In mixtures of birds'-foot trefoil with grasses the content of cyanogenic glycosides in birds'-foot trefoil also increases – by 6.0% in mixtures with tall fescue and by 17.9% in mixtures with cocksfoot. The content of cyanogenic glycosides in the total sample changes depending on the percent contribution of the components in the composition of mixtures.

SUMMARY

The work was aimed to study the changes in the content of cyanogenic glycosides in forage biomass of birds'-foot trefoil grown alone and in mixtures with subterranean clover, cocksfoot and tall fescue. The content of cyanogenic glycosides was determined in leaves, stems and flowers in fresh plant. Cyanogenic glycosides content in stems of birds'-foot trefoil was found 38.40 mg HCN/100 g DM, in leaves 90.39 mg HCN/100 g DM and in flowers 173.60 mg HCN/100 g DM, respectively. The content of cyanogenic glycosides in plants of three subclover subspecies was found very low, i.e. *Trifolium subterraneum* ssp. *subterraneum* (2.84 mg HCN/100 g DM), *Trifolium subterraneum* ssp. *brachycalicinum* (3.18 mg HCN/100 g DM), *Trifolium subterraneum* ssp. *yaninicum* (7.87 mg HCN/100 g DM). In the mixtures of birds'-foot trefoil with subclover the content of cyanogenic glycosides increased in both components compared to their pure grown – in birds'-foot trefoil from 8.8% to 19.9%, and in subclover from 10.7 to 54.4%. The content of cyanogenic glycosides in birds'-foot trefoil increased by 6.0% in mixtures with tall fescue and with 17.9% in mixtures with cocksfoot. The higher proportion of subclover in the mixtures with birds'-foot trefoil decreased the cyanogenic glycosides content.

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