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ORIGINAL PAPER

HEAVY METAL CONTENT IN GREEN FODDER MADE FROM NARROW-LEAVED LUPINE AND SPRING TRITICALE TO BE USED IN CATTLE FEEDING*

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Abstract

Legumes mixed with cereals grown to produce green fodder are a valuable source of feed for cattle providing they are not contaminated with heavy metals. The content of heavy metals in soil is related to the soil's chemical composition, which is affected by amounts of pollutants emitted to the atmosphere by industries and transport. This also concerns vast areas of agricultural land cropped with fodder plants consumed mainly by dairy cattle. The objective of the research was to determine the effect of a component share in a mixture and harvest date on the heavy metal content in green fodder of narrow-leaved lupine, spring triticale and their mixtures intended to be used for cattle feeding. Two factors were examined: factor I − component share in a mixture: narrow-leaved lupine – pure stand 100%, spring triticale – pure stand 100%, narrow-leaved lupine 75% + spring triticale 25%, narrow-leaved lupine 50% + spring triticale 50%, narrow-leaved lupine 25% + spring triticale 75%; factor II − harvest date: the stage of narrow-leaved lupine flowering, the stage of narrow-leaved lupine flat green pod. Plant material samples were taken to determine Cu, Zn, Cd, Pb, Cr and Ni contents. Of the tested mixtures, narrow-leaved lupine mixed with spring triticale at the 75 + 25% and 50 + 50% component shares had the lowest heavy metal content. Cadmium and lead content in the green fodder composed of narrow-leaved lupine/spring triticale mixtures was significantly affected by the experimental factors, and remained below the detectability level of an emission spectrophotometer Perkin Elmer Optima 8300. Even if the feed produced from the tested narrow-leaved lupine/spring triticale mixtures was safe for cattle, regular monitoring of the heavy metal content of such feed is recommended.

Keywords: narrow-leaved lupine, spring triticale, mixture, harvest date, heavy metals.

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INTRODUCTION

Heavy metals in soil are taken up by plants, which are then consumed by animals, after which they pass to foodstuffs for human consumption (SHARMA et al. 2004, OCIEPA-KUBICKA, OCIEPA 2012). Thus, it is necessary to assess the safety of animal feed, including green fodder, in terms of heavy metal content. Monitoring the concentration of toxic and potentially toxic elements is one of the most important aspects of feed quality control (ABDULMOJEED, ABDULRAHMAN 2011, MAHAMED et al. 2012). Research by SNAPP et al. (2005) and DABNEY et al. (2001) has demonstrated that legumes have a favourable effect on the soil environment, including organic carbon content and cation exchange, which in turn reduces heavy metal uptake from soil. The above finding indicates that leguminous plants are a valuable animal feed and, when mixed with cereals, they can positively affect the chemical composition of green fodder (SOBUKOLA et al. 2010, ALI, AL-QATHANI 2012). As there are no Polish studies assessing the heavy metal content in green fodder of legume/cereal mixtures, a need arises to fill in this gap. The objective of the study reported here was to determine the effect of mixture components and harvest date on the heavy metal content in the green fodder of narrow-leaved lupine, spring triticale and their mixtures dedicated to cattle feeding.

MATERIAL AND METHODS

Field research was conducted from 2016 to 2018 at the Zawady Experimental Farm (52°03′39″ N, 22°33′80″ E) owned by the Siedlce University of Natural Sciences and Humanities. The experimental soil was Albic Luvisol (Arenic). The chemical composition of the soil is shown in Table 1. The experiment was set up in a split-block arrangement with three replicates.

The following two factors were examined: factor I − component share in a mixture: narrow-leaved lupine – pure stand 100%, spring triticale – pure stand 100%, narrow-leaved lupine 75% + spring triticale 25%, narrow-leaved lupine 50% + spring triticale 50%, narrow-leaved lupine 25% + spring triticale 75%; factor II − harvest date: the stage of narrow-leaved lupine flowering, the stage of narrow-leaved lupine flat green pod. The following sowing doses were used: narrow-leaved lupine 240 kg ha^{-1}, spring triticale 220 kg ha^{-1}, narrow-leaved lupine 180 kg ha^{-1} + spring triticale 55 kg ha^{-1}, narrow-leaved lupine 120 kg ha^{-1} + spring triticale 110 kg ha^{-1}, narrow-leaved lupine 60 kg ha^{-1} + spring triticale 165 kg ha^{-1}. Oat preceded the mixtures in all the study years. In autumn, phosphorus and potassium fertilisers were applied at the following doses related to the soil chemical composition: 35.2 kg ha^{-1} P and 99.6 kg ha^{-1} K. In spring, nitrogen fertilisers

Table 1

Chemical composition of the soil before the experiment

Sample	Elemental content mg kg^{-1} soil								pH in KCl	Humus content (%)
	P	K	Cu	Zn	Pb	Cd	Cr	Ni		
Means of years 2016-2018	0.523	1.145	0.110	0.750	<1.530	0.011	<1.280	1.270	6.2	1.38

were applied at a dose of 30 kg ha^{-1} N to all the plots prior to seed sowing, excluding ones under narrow-leaved lupine grown in pure stand. Additional amounts, that is 50 kg ha^{-1} N preceding spring triticale, and 30 kg ha^{-1} N preceding narrow-leaved lupine/spring triticale mixtures, were applied at the stem elongation stage. Narrow-leaved lupine and spring triticale – the first experimental factor – were sown in early April. Harvest date – the second factor – was late June and early July.

During harvest, fresh matter samples were taken to determine microelements. Cu, Zn, Pb, Cd, Cr and Ni contents were determined using *inductively coupled plasma* optical *emission* spectrometry (ICP-OES) with a spectrometer Perkin Elmer Optima 8300. Each characteristic was subjected to analysis of variance following the linear model of the split-plot design. Separation of means for significant sources of variation was achieved using the Tukey's test. Statistical calculations were performed using the authors' own algorithms in MS Excel 12.0.

RESULTS AND DISCUSSION

Statistical analysis revealed a significant effect of the experimental factors and their interaction on the copper content in the green fodder composed of narrow-leaved lupine/spring triticale mixtures (Table 2). The highest copper content was recorded in narrow-leaved lupine and the lowest one was in spring triticale. Also, Mohamed et al. (2012), Jarecki and Bobrecka-Jamro (2015) as well as Woźniak and Soroka (2016) determined a lower copper content in the biomass of cereals than in leguminous biomass. A higher copper content in legumes was reported by Zarcinas et al. (2004), Chandra Sekhar et al. (2002) and Adefarati et al. (2017). In the current experiment, the copper content, although higher in narrow-leaved lupine green fodder, did not exceed the WHO/FAO standards, thus rendering the feed safe for animals (Zarcinas et al. 2004, Adefarati et al. 2017). Copper taken up by plants at low amounts, and then passed on to animals, is necessary for the body of an animal as it participates in oxidation and reduction processes, in which it is not only a coenzyme component, but it also regulates metabolism, iron

Table 2

Copper content in the green fodder (means across 2016-2018), mg kg^{-1} d.m.

Component share in mixture (%)	Harvest date		Means
	stage of narrow-leaved lupine flowering	stage of narrow-leaved lupine flat green pod	
Narrow-leaved lupine – pure stand 100%	4.974	4.150	4.562
Spring triticale – pure stand 100%	4.147	3.278	3.713
Narrow-leaved lupine 75% + spring triticale 25%	4.595	3.816	4.206
Narrow-leaved lupine 50% + spring triticale 50%	4.360	3.610	3.985
Narrow-leaved lupine 25% + spring triticale 75%	4.109	3.439	3.774
Means	4.437	3.659	4.048
LSD 0.05			-
Component share in mixture			0.198
Harvest date			0.127
Interaction			0.212

transportation and collagen metabolism (Cabrera et al. 2003). In this research, the addition of spring triticale to mixtures with narrow-leaved lupine significantly reduced the copper content in green forage. In the 75 + 25% mixture of narrow-leaved lupine with spring triticale, the copper content was by 0.356 mg kg^{-1} lower than its concentration found in narrow-leaved lupine. In the present work, spring triticale mixed with narrow-leaved lupine significantly reduced the content of copper in green fodder. Its lowest concentration was determined in the 50 + 50% and 25 + 75% mixtures of narrow-leaved lupine and spring triticale. A similar relationship was reported by Trąba and Wolański (2003), who examined the dry matter of grass/legume mixtures and observed that the copper content was nearly twice as low as in legumes grown in pure stand. In the experiment reported here, the harvest date had a significant influence on the copper content in the green feed of narrow-leaved lupine, spring triticale and mixtures of these plants. A higher concentration of copper was recorded in narrow-leaved lupine/spring triticale mixtures harvested at the stage of narrow-leaved lupine flowering compared with mixtures with lupine harvested at the flat green pod stage. Also, the research by Ladipo and Doherty (2011), Fytianos et al. (2001), Abdulmojeed and Abdulrahman (2011) revealed influence of plant maturity at harvest on copper uptake. Demirezen and Aksoy (2006) as well as Muchuweti et al. (2006) demonstrated that plants harvested at earlier development stages contained more copper. In the present work, an interaction between the experimental factors was confirmed, indicating that narrow-leaved lupine grown in pure stand and harvested at the flowering stage had the highest copper content, whereas the least copper was determined in spring triticale grown in pure stand and harvested at the stage of grain milky maturity. This research showed some interaction of the examined factors, which demonstrates that the green fodder composed of mixtures of narrow-leaved lupine with spring triticale harvested in the flowering stage of narrow-leaved lupine was characterized by a higher copper content, the highest being determined in the green fodder of narrow-leaved lupine, followed by the green fodder of mixtures collected in the later development stage. In the flat green pod stage of narrow-leaved lupine, the green fodder of mixtures of narrow-leaved lupine with spring triticale contained less copper, and the least amount was in spring triticale. Among the mixtures, the lowest concentration of copper was recorded in the 25 + 75% mixture of lupine and spring triticale. All the tested narrow-leaved/ /spring triticale mixtures harvested at the stage of narrow-leaved lupine flat green pod had a lower copper content than lupine grown in pure stand and harvested at the same development stage.

The zinc content in the green fodder from the tested plants was significantly affected by the experimental factors and their interaction (Table 3). Zinc plays a significant role in plant metabolism. Both excess and shortage of this element result in hindered plant growth and development (Ociepa-Kubicka, Ociepa 2012). Zinc is necessary in plant nutrition, but plants growing in a polluted environment may accumulate high concentrations of this element, which poses a serious threat to human and animal health

Table 3

Zinc content in the green fodder (means across 2016-2018), mg kg^{-1} d.m.

Component share in mixture (%)	Harvest date		Means
	stage of narrow-leaved lupine flowering	stage of narrow-leaved lupine flat green pod	
Narrow-leaved lupine – pure stand 100%	39.35	54.64	46.99
Spring triticale – pure stand 100%	27.35	36.50	31.95
Narrow-leaved lupine 75% + spring triticale 25%	37.50	48.59	43.05
Narrow-leaved lupine 50% + spring triticale 50%	35.24	47.05	41.15
Narrow-leaved lupine 25% + spring triticale 75%	31.39	38.81	35.10
Means	34.17	45.12	-
LSD 0.05			-
Component share in mixture			2.28
Harvest date			1.37
Interaction			3.02

(Srinivos et al. 2002, Sharma et al. 2004, Yu-Wei et al. 2013). In our experiment, the highest zinc content was recorded in narrow-leaved lupine grown in pure stand, while the lowest one occurred in spring triticale grown in pure stand. Research by Fytianos et al. (2001), Demirezen and Aksoy (2006), Muchuweti et al. (2006) and Mohamed et al. (2012) demonstrated that zinc content was higher in legumes than in cereals. However, the legume content of zinc is lower than the WHO/FAO standards and is not harmful to human or animal health (Adefarati et al. 2017). The lowest content of zinc was found in the mixture of narrow-leaved lupine with spring triticale at the 25 + 75% ratio of the components. In the experiment reported here, narrow-leaved lupine mixed with spring triticale increased the concentration of this element in green fodder, the values being lower than in narrow-leaved lupine but higher than in spring triticale. The finding corresponds to results reported by Goliński et al. (2007) and Szpunar-Krok et al. (2009). In the present work, harvest date had a significant effect on zinc content in the green fodder of narrow-leaved lupine/cereal mixtures. The zinc content was higher in mixtures containing narrow-leaved lupine harvested at the stage of flat green pod than at the flowering stage. As zinc is a microelement, its higher content in green fodder has a tremendous effect on human and animal health (Zarcinas et al. 2004, Chandra Sekhar et al. 2009, Adefarati et al. 2017, Płaza et al. 2018). In this experiment, the interaction of the studied factors was demonstrated, from which it follows that the green fodder of mixtures of narrow-leaved lupine collected in the stage of the flat green pod with spring triticale was characterized by a higher content of zinc than the mixtures containing cereal and narrow-leaved lupine collected at the flowering stage. In the experiment reported here, some interaction between the experimental factors was confirmed. The highest zinc content was determined in narrow-leaved lupine grown in pure stand and harvested at the stage of flat green pod, whereas the lowest one was in spring triticale grown in pure stand and harvested at the stage of grain milky maturity. Among the tested mixtures, the highest zinc content was recorded in narrow-leaved lupine/spring triticale mixtures whose component shares were 75 + 25% and 50 + 50% and which were harvested at the stage of narrow-leaved lupine flat green pod.

Lead and cadmium contents were insignificantly affected by the experimental factors and, determined in the green fodder of narrow-leaved lupine/spring triticale mixtures, were lower than the detectability level of an emission spectrophotometer Perkin Elmer Optima 8300. A possible explanation is that the experimental area is situated far away from roads. As a result, cadmium and lead contents of narrow-leaved lupine/spring triticale mixtures did not exceed standards set for greed fodder in the *Commission Regulation* (EU) 2013, thus rendering the green fodder a safe feed for cattle. Despite the low concentrations of Cd and Pb in the green fodder of narrow-leaved lupine/spring triticale mixtures, there is an urgent need to monitor and control the content of these elements on a regular basis.

Table 4

Chromium content in the green fodder (means across 2016-2018), mg kg^{-1} d.m.

Component share in mixture (%)	Harvest date		Means
	stage of narrow-leaved lupine flowering	stage of narrow-leaved lupine flat green pod	
Narrow-leaved lupine – pure stand 100%	1.476	1.819	1.648
Spring triticale – pure stand 100%	3.683	7.037	5.360
Narrow-leaved lupine 75% + spring triticale 25%	1.997	3.546	2.772
Narrow-leaved lupine 50% + spring triticale 50%	2.280	4.195	3.238
Narrow-leaved lupine 25% + spring triticale 75%	3.104	5.463	4.284
Means	2.508	4.412	-
LSD 0.05			-
Component share in mixture			0.610
Harvest date			0.227
Interaction			0.812

Chromium content in the green fodder of narrow-leaved lupine/ spring triticale mixtures was significantly affected by the experimental factors and their interaction (Table 4). The highest chromium content was recorded in spring triticale grown in pure stand, whereas the lowest one was in narrow-leaved lupine grown in pure stand. Legume mixed with cereal contributed to a significant decline in the green fodder content of chromium. The lowest concentration of this element was determined in mixtures with the following legume/cereal ratios: 75 + 25% and 50 + 50%. The chromium content in the mixture of narrow-leaved lupine with spring triticale in a 25 + 75% ratio was lower than in spring triticale. Research by Ali and Al-Qantan (2012), Brigide et al. (2014) revealed an excess of chromium in cereal and rice. In contrast, legumes had a lower concentration of this element (Brigide et al. 2014, Akinyele, Shokunbi 2015). In the experiment reported here, there was a significant effect of the harvest date on the chromium content in green fodder made from narrow-leaved lupine/spring triticale mixtures. The content of this element was higher in green fodder harvested at the stage of narrow-leaved lupine flat green pod than at the flowering stage. This may be explained by the fact that plants harvested at later development stages take up higher quantities of this element and consequently their content of chromium is higher (Adefarati et al. 2017). In our experiment, the chromium content in the green fodder composed of narrow-leaved lupine/spring triticale mixtures harvested at the stage of narrow-leaved lupine flat green pod was low and did not pose a threat to animal health. Similar results were reported by Sobukola et al. (2010), Ladipo and Doherty (2011) as well as Adefarati et al. (2017), who demonstrated that the chromium content in the plants they tested (cereals and legumes) did not exceed the WHO/FAO standards. The level of chromium in plants is affected by their maturity at harvest (Lapido, Doherty 2011, Adefarati et al. 2017). Low amounts of chromium are necessary for life as the element plays a significant role in the metabolic processes of glucose, some proteins and fats (Sobukola et al. 2010, Ociepa-Kubicka, Ociepa 2012). The study reported herein showed some interaction between the experimental factors, for example the content of chromium in the green fodder composed of mixtures collected in the earlier development stage, i.e. in the flowering stage of narrow-leaved lupine, was lower, and it was the lowest in the green fodder made of narrow-leaved lupine in comparison with the mixtures made from lupine collected in the stage flat green pod. Among all the mixtures of narrow-leaved lupine with spring triticale, the ones mixed in 75 + 25% and 50 + 50% ratios were characterized by the lowest content of chromium. In contrast, the highest content of chromium was recorded in spring triticale harvested at a time when narrow-leaved lupine was in the stage of flat green pod, which was when triticale reached the stage of grain milky maturity. The current research confirmed interaction between the experimental factors. The lowest chromium content was determined in the green fodder of narrow-leaved lupine grown in pure stand and harvested at the flat green pod or flowering stage, as well as narrow-leaved lupine/spring triticale mixtures which had the following component shares: 75 + 25% and 50 + 50%, and were harvested at the stage of narrow-leaved lupine flowering. The highest concentration of chromium was determined in spring triticale grown in pure stand and harvested at the stage of grain milky maturity.

Statistical analysis revealed a significant effect of the experimental factors and their interaction on the nickel content in the green fodder composed of narrow-leaved lupine/spring triticale mixtures (Table 5). The lowest nickel content was recorded in narrow-leaved lupine grown in pure stand, while the highest one was determined in spring triticale grown in pure stand. It should be noted that nickel present in plants at low concentrations is necessary for their growth and development (Akinyele, Shokunbi 2015). In the experiment reported here, the nickel content in spring triticale did not exceed the WHO/ /FAO standards (Adefarati et al. 2017). Nickel is toxic when its plant content is too high (Cabrera et al. 2003). According to WHO (1996), the acceptable Ni norm in feed is 10 mg kg^{-1}. In our research, the Ni content in mixtures was much lower. Moreover, narrow-leaved lupine mixed with spring triticale significantly reduced the nickel content in the green feed, with the lowest concentrations of this element in mixtures whose legume/cereal component shares were 75 + 25% and 50 + 50%. The mixture of narrow-leaved lupine with spring triticale at a 25 + 75% ratio of these crops contained less nickel than spring triticale. Harvest date had a significant effect on the nickel content in the green fodder made of narrow-leaved lupine, spring triticale and

Table 5

Nickel content in the green fodder (means across 2016-2018), mg kg^{-1} d.m.

Component share in mixture (%)	Harvest date		Means
	stage of narrow-leaved lupine flowering	stage of narrow-leaved lupine flat green pod	
Narrow-leaved lupine – pure stand 100%	1.671	2.086	1.879
Spring triticale – pure stand 100%	3.396	5.819	4.608
Narrow-leaved lupine 75% + spring triticale 25%	2.040	2.861	2.451
Narrow-leaved lupine 50% + spring triticale 50%	2.329	3.652	2.991
Narrow-leaved lupine 25% + spring triticale 75%	2.857	4.330	3.594
Means	2.459	3.750	-
LSD 0.05			-
Component share in mixture			0.584
Harvest date			0.156
Interaction			0.712

mixtures of these plants. It was significantly lower in mixtures harvested at the stage of narrow-leaved lupine flowering than at flat green pod stage. Also Sobukola et al. (2010) as well as Lapido and Doherty (2011) demonstrated that the amount of nickel in plants is affected by their maturity stage at harvest. In the discussed experiment, the interaction of the studied factors was demonstrated, which shows that the mixtures of narrow-leaved lupine with spring triticale collected in the earlier development stage, i.e. in the flowering stage of narrow-leaved lupine, contained less nickel than those collected later, that is in the flat green pod stage. The lowest nickel content was found in narrow-leaved lupine, and among mixtures it was the lowest when narrow-leaved lupine was mixed with spring triticale at 75 + 25% and 50 + 50% ratios. However, the highest nickel content was recorded in spring triticale harvested at a time when narrow-leaved reached in the flat stage of the green pod, that is when triticale was at the stage of grain milky maturity. In the experiment reported here, the interaction between the experimental factors was confirmed. The lowest nickel content was found in the green fodder of narrow-leaved lupine grown in pure stand and harvested at the flowering and flat green pod stage, as well as in the mixtures whose lupine/triticale component shares were 75 + 25% and 50 + 50% and which were harvested at the stage of lupine flat pod. The highest concentration of nickel was determined in spring triticale harvested at the stage of grain milky maturity.

CONCLUSIONS

The highest copper and zinc contents, and the lowest chromium and nickel contents were found in the green fodder of narrow-leaved lupine grown in pure stand. Among the tested mixtures, narrow-leaved lupine mixed with spring triticale at ratios of 75 + 25% and 50 + 50% had the lowest heavy metal content.

Narrow-leaved lupine/spring triticale mixtures harvested at the stage of lupine flowering had a higher copper content and a lower concentration of zinc, chromium and nickel in their green feed compared with mixtures in which lupine was harvested at the stage of flat green pod.

Cadmium and lead contents in the green fodder of narrow-leaved lupine/ /spring triticale mixtures were below the detectability level of an emission spectrophotometer Perkin Elmer Optima 8300.

The heavy metal content in the green fodder of narrow-leaved lupine, spring triticale and mixtures of these plants harvested at the stage of narrow-leaved lupine flowering and flat green pod was low and did not pose a threat to animal health. The permissible thresholds for heavy metal content in cattle feed according to WHO (1996) are as follows: copper 10 mg kg^{-1} feed, zinc 0.60 mg kg^{-1} feed, chromium 1.30 mg kg^{-1} feed, nickel 10 mg kg^{-1} feed.

Although the feed produced from the tested mixtures of narrow-leaved lupine and spring triticale is safe for cattle, it is recommended to regularly monitor their heavy metal content.

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