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QUALITY AND HYGIENIC CONDITIONS OF WHITE LUPIN SILAGE, AFFECTED BY FORAGE STAGE OF GROWTH AND USE OF SILAGE ADDITIVES

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ABSTRACT

A two-factor field experiment with white lupin cv. Butan was carried out. The first factor was the green forage harvest date (the flat pod stage – Cut 1 and the stage of green ripe seeds – Cut 2), while the second one – application of silage additives: biological (strains of lactic acid bacteria) and chemical (a mixture of organic acids), and the control treatment (without additives). In Cut 2 higher fresh matter (FM) and dry matter (DM) yields were obtained. Silage inoculated with the biological additive contained a significantly greater count of lactic acid bacteria. Both additives reduced counts the *Clostridium* bacteria, yeasts and mould fungi. The silage with the chemical additive had a three-fold higher content of water-soluble carbohydrates (WSC), while the biological additive increased lactic acid (LA) levels. White lupin can be used as a silage raw material, but plants before ensiling should be partially wilted and silage additives should be applied.

Key words: harvest date, lupin silage, silage additives

INTRODUCTION

White lupin (*Lupinus albus* L.) is an annual legume belonging to the *Fabaceae* family, it is used for human consumption, as green manure and forage crop (Huyghe, 1997). Forages are major constituents of dairy and beef cattle diets (Mustafa et al., 2002). The purchase of compound feed represents a substantial part of variable costs in on-farm ruminant production, thus the use of protein-rich alternative forage crops, grown on-farm, needs to be considered (Frasel et al., 2001). Annual legumes and cereals such as common vetch, hairy vetch, grasspea, oat (Dumont at al., 2005), barley and triticale (Rojas at al., 2004), are potentially the most viable fodder sources (Karadag and Buyukburc, 2003), while the use of lupin in animal nutrition may increase profitability of production (McNaughton, 2011). According to (Idziak et al., 2013), based on FAOSTAT data corn is one of the most commonly cultivated plants worldwide. Maize silage is a high-quality forage that is used on many dairy farms and on some beef cattle farms (Budakli Carpici et al., 2010; Iptas and Yavuz, 2008; Kusaksiz, 2010), whereas Doležal et al. (2008) reported that some researchers, e.g. Carruthers et al. (2000) and Egorov et al. (2001), studied the potential for lupin application as a silage raw material also in mixtures with cereals and grasses. Voytekhovich (2000) argued that narrow-leaved lupin silage is of better quality in terms of its nutritive value than white lupin silage. In contrast, Fraser et al. (2005a,b) reported that both lupins can be successfully ensiled as the whole-crop. An appropriate harvest date of a forage crop has a significant effect on silage quality. Delaying of harvest adversely affects the ensiling ability due to an increase in buffer capacity and a decrease of sugar contents. However, the green fodder ensiling capacity may be improved by adding different substances and preparations. They are designed to improve the conditions of lactic fermentation and aerobic stability, to reduce the amount of silage juices and the content of undesirable spores, such as *Clostridium*, and also to improve the collection, palatability and digestibility of fodder. The study conducted by Borreani et al. (2009) showed that field pea, faba bean and lupin may be successfully ensiled after a wilting period under good weather conditions and with the addition of a lactic acid bacteria inoculant.

The experiment was to test the suitability of ensiling white lupin harvested at two different growth stages and to analyze the quality of silage produced using silage additives.

MATERIALS AND METHODS

Experimental site: A field experiment with white lupin cv. Butan was conducted at the Teaching and Experimental Station in Gorzyń (52˚33'53 N, 15˚53'42 E), belonging to the Poznań University of Life Sciences, Poland. The trial was carried out from 2005 to 2007 on grey-brown podzolic soil under ordinary growing conditions. Four replicate plots of 20 m^2 were prepared by ploughing and power-harrowing. A fertiliser (P_2O_5 60 kg ha^{-1} and K_2O 80 kg ha^{-1}) was applied to the seedbed. No irrigation or fertiliser was applied after sowing. In early April, white lupin seeds were inoculated with *Bradyrhizobium* bacteria (cv. Butan) and drilled at a rate of 225 kg ha^{-1}. Weeds were controlled by post-emergence treatment with 2.0 l ha^{-1} of linuron (Agan Chemical Manufacturers Ltd.). Two effects were studied: 1) harvesting dates (stages) and 2) application of silage additives, corresponding to three treatments: wilted crop ensiled either with no additives (0), with a microbial inoculant (B) Polmasil, which contained strains of lactic acid bacteria: *Enterococcus faecium* M74, *Lactobacillus casei*, *Lactobacillus plantarum* and *Pediococcus* spp. at a concentration of 10^9 CFU (Polmass S.A., Poland), and with a chemical additive (CH) KemiSile 2000, which contained in %: formic acid 55, propionic acid 9, benzoic acid 5, ammonium formate 24, and benzoic acid ester 7 (Kemira OY, Finland). The two harvesting dates and stages were: flat pod (Cut 1) and green ripe seed (Cut 2). At each harvest date the crop at a stubble height of 4-6 cm was cut from the plot area using a plot harvester, and sub-samples of the crop were collected to determine their chemical composition. At each stage of growth the harvested crop was wilted in the field for 24 h.

Ensiling

The wilted crop was chopped with an experimental mechanical chopper to a length of 20–30 mm. Then the inoculant/additive treatment was applied by means of a hand sprayer. About 2.2 kg of the crop was ensiled in sterile 5 dm^3 jars (150 mm diameter × 280 mm height). The crop mass was thoroughly mixed before being placed in the mini-silos and then it was compacted in each silo. Four jars - replications of each treatment - were prepared in this way.

The material was stored in the dark at a temperature of 22-25 °C for ten weeks. After that time the jars were opened and representative samples of the ensiled material were collected for analyses of their nutritive value and basic fermentation characteristics. Each year green matter was ensiled in 24 jars (2 harvest dates x 3 (2 silage additives + the control) x 4 replications). The chemical composition of silage was determined by analysing an average sample in each combination (a total of 6 average samples per year); the years were replications. One microbial sample was collected from each jar and its chemical composition was determined: 3 series x 24 (a total of 72 samples per year).

Chemical analysis

The basic composition of forage was determined according to AOAC (1990). The content of water-soluble carbohydrates (WSC) was determined according to the methodology given by McDonald and Henderson (1964), ammonia nitrogen ($N\text{-}NH_3$) (Conway 1962). The pH values were determined, using the pH Meter by Hann Instruments, in a suspension prepared from 10 g of silage and 90 cm^3 of deionised water, homogenized for 20 minutes. The concentration of fatty acids was determined using a gas chromatograph equipped with the Supelco FID detector, a 80/100 Chromosorb® WAW glass column of 2 m, I.D. 2 mm with GP filling of 10% SP-1200/1% H_3PO_4 and a Varian 8200 CX autosampler. The carrier gas was hydrogen (flow rate = 30 cm^3 min^{-1}), oven temperature was 120˚C, injection temperature was 250˚C and detector temperature was 300˚C. Fluka acid patterns were the reference standards.

Microbiological analysis

The count of *Clostridium* bacteria was determined on MERCK TSC® Agar, the count of lactic acid bacteria – on MERCK ATP Agar, *Enterobacteriaceae* – at a base Fluorocult® LMX Broth, modified according to Manofi,and OSSMER from MERCK solidified with DIFCO agar. The count of mould fungi was determined on a bengal rose agar base, and the counts of yeasts on a wort agar (BTL spółka z o. o., Zakład Enzymów i Peptonów in Łódź). Culture plate was made by successive dilutions.

Statistical analysis

All data were processed using analysis of variance (ANOVA) with the SAS package (SAS Institute, 1999). The means of treatments were compared by means of Tukey's least significant difference test (LSD) at $P<0.01$ and $P<0.05$. Field experiments were arranged as a split-plot randomized complete block design with four replications. All data were subjected to analysis of variance based on the general linear model for repeated measurements.

RESULTS AND DISCUSSION

The analysis of variance indicated that there were statistically significant differences between forage crop harvest date and individual years of the study for FM and DM yields and DM content in unwilted and wilted crop (Table 1). The highest yield of green crop (30.1 t ha^{-1}) was recorded in the year 2005, which had the most beneficial weather conditions when lupin vegetation proceeded without major disruptions in water supply. Harvest of lupin in Cut 2 contributed to an increased FM yield and DM yield, and wilting of the crop increased the DM content. The NDF and WSC contents were similar for both harvest dates in the three years, but CP content was on average lower in Cut 2. In studies conducted by Mihailović et al. (2008), FM yield and DM yield of white lupin were also differentiated over the years and amounted respectively to 21.3-50.3 t ha^{-1} and 3.6-8.6 t ha^{-1}, depending on the cultivar. In the case of pea, FM yield can

reach 24.4-30.1 t ha^{-1}, DM yield 4.4-5.5 t ha^{-1} (Turk et al., 2011) and DM content – 318-360 g kg^{-1} (Borreani et al., 2006). The DM yield of soybean intercropped with corn is also higher (Reta Sanchez et al., 2010). The FM yield of narrow-leaved lupin may be 36.6-37.0 t ha^{-1}, and its DM content after wilting is 182-231 g kg^{-1} (Fraser et al., 2005b). In the experiment carried out by Borreani et al. (2009), following a wilting period, the DM content of field pea, faba bean and white lupin increased from 482 to 618 g kg^{-1}, from 237 to 295 g kg^{-1} and from 142 to 173 g kg^{-1}, respectively. The significance of the appropriate choice of harvest date is presented in an experiment conducted by Fraser et al. (2001), who examined, among other things, the effect of harvest date on the suitability of pea and faba bean for ensiling. It turned out that the best term for pea was 12 weeks after sowing, and for faba bean – 14 weeks, when the FM yield, DM content and DM yield were the largest. In the opinion of Turk and Albayrak (2012), harvesting at the late stages caused a reduction in forage quality. Contents of CP decreased with the progress in plant growth, while DM yield, CP yield, and NDF contents increased.

Table 1. The effect of harvest date on fresh matter yield (FM), content of dry matter (DM), dry matter yield and chemical composition of lupin forage in successive years

Parameter	Harvest date (H)	Years (Y)			Significance		S.E.D.	
		2005	2006	2007	H	H × Y	H × Y within H	H × Y between H
FM yield (t ha^{-1})	Cut 1	20.9	15.8	3.8	**	**	2.90	17.25
	Cut 2	39.2	21.0	11.6				
	Mean	30.1	18.6	7.7				
DM yield (t ha^{-1})	Cut 1	3.7	5.3	1.2	**	**	0.95	8.71
	Cut 2	11.3	6.1	3.9				
	Mean	7.5	5.7	2.6				
DM (g kg^{-1}) unwilted forage	Cut 1	158	173	176	**	**	3.15	120.1
	Cut 2	253	182	189				
	Mean	205	177	183				
DM (g kg^{-1}) wilted forage	Cut 1	177	292	321	**	**	8.54	192.0
	Cut 2	288	334	334				
	Mean	233	313	327				
CP (g kg^{-1})	Cut 1	154.0	172.9	108.0	-	-	-	-
	Cut 2	141.0	162.8	115.7				
NDF (g kg^{-1})	Cut 1	261.8	221.3	232.3	-	-	-	-
	Cut 2	266.1	218.3	288.2				
WSC (g kg^{-1})	Cut 1	399.9	394.4	438.7	-	-	-	-
	Cut 2	422.1	386.4	416.5				

Cut 1 flat pod stage; Cut 2 - stage of green ripe seeds; CP - crude protein; NDF - neutral-detergent fibre; WSC - water-soluble carbohydrates; * significant at P<0.05.; ** significant at P<0.01.

According to Gallo et al. (2006), silage quality depends on weather conditions during harvest. In our experiment significant interactions were found between harvest date and the applied silage additive (Table 2). Under the influence of the microbial inoculant the count of lactic acid bacteria significantly increased by about 22.5% in the first dates of harvest. The two additives decreased the number of mould fungi in both the first and second date. On average, in silage from the first harvest date the count of lactic acid bacteria was by 5.8% higher, that of mould fungi was higher by 1.9%, while the count of yeasts was lower by 2%. It was found that the number of lactic acid bacteria under the influence of the microbial inoculant significantly increased by 12.3%, whereas the content of undesirable *Clostridium* bacteria (16.1-59.2%), yeast (2-6%) and fungi (4.5-14%) significantly decreased as a result of application of both additives. At the same time, it should be mentioned that the chemical additive was more effective, as it significantly decreased also the count of *Enterobacteriaceae* bacteria. According to Faligowska and Selwet (2012), in yellow lupin silage the microbial inoculant caused a marked increase in the level of lactic acid bacteria. Both additives caused a noticeable decrease in the content of undesirable bacteria from the *Enterobacteriaceae* family, *Clostridium*, as well as yeast and mould fungi, still the chemical additive was also more effective than the microbial inoculant.

The chemical composition of white lupin silage was not affected by diverse harvest dates (Table 3). In the case of additives, their addition differentiated only the content of LA and WSC, and decreased the content of N-NH_3. When compared to the control, silage with the chemical additive contained three times more WSC. However, silage with the microbial inoculant contained about 50% more LA. CP content was not significantly differentiated in white lupin silage, ranging from 141.7-157.2 g kg^{-1} DM. The crop harvested in Cut 2 contained more WSC, but probably required a higher consumption of WSC in the process of respiration in the early stages of fermentation, because the Cut 2 silage contained about half as much WSC. The results of silage composition,

specifically the low level of acetic acid and the very low level of butyric acid, above all suggest a lactic acid homofermentative process both in the control and in silages containing silage additives (McDonald et al., 1991).

Table 2. The effect of harvest date and application of additives on the microbiological composition of silage (log 10 JTK g^{-1})

Parameter	Harvest date (H)	Inoculation treatment (I)			Significance			S.E.D	
		0	B	CH	H	I	H × I	H × I within H	H × I between H
Lactic acid bacteria	Cut 1	6.88	8.43	6.96	**	**	**	0.171	0.175
	Cut 2	6.94	7.09	6.99					
	Mean	6.91	7.76	6.98					
Enterobacteriacea	Cut 1	3.76	3.60	2.65	NS	**	NS	0.423	0.393
	Cut 2	3.55	3.41	2.50					
	Mean	3.66	3.51	2.58					
Clostridium	Cut 1	3.34	3.02	1.15	NS	**	NS	0.621	0.655
	Cut 2	3.47	2.70	1.62					
	Mean	3.41	2.86	1.39					
Yeasts	Cut 1	4.96	4.83	4.67	**	**	NS	0.094	0.096
	Cut 2	5.04	4.98	4.73					
	Mean	5.00	4.90	4.70					
Mould fungi	Cut 1	4.01	3.85	3.51	**	**	**	0.055	0.049
	Cut 2	4.00	3.79	3.37					
	Mean	4.00	3.82	3.44					

Cut 1 – flat pod stage; Cut 2 - stage of green ripe seeds; 0 - control; B - microbial inoculant; CH - chemical additive;
NS – non-significant; * significant at P<0.05.; ** significant at P<0.01.

Table 3. The effect of harvest date and application of additives on the chemical composition of silage

Parameter *g kg^{-1} DM*	Harvest date (H)	Inoculation treatment (I)			Significance			S.E.D.	
		0	B	CH	H	I	H × I	H × I within H	H ×I between H
DM	Cut 1	220.9	250.6	245.7	NS	NS	NS	57.00	81.10
	Cut 2	218.6	217.9	226.3					
$N\text{-}NH_3$	Cut 1	87.5	32.5	75.0	NS	**	NS	18.0	21.2
	Cut 2	85.0	35.0	78.2					
CP	Cut 1	152.5	141.7	157.2	NS	NS	NS	22.16	19.77
	Cut 2	151.4	143.1	148.2					
NDF	Cut 1	319.4	303.1	290.8	NS	NS	NS	45.51	79.20
	Cut 2	351.4	326.2	321.7					
Ash	Cut 1	104.6	102.3	97.0	NS	*	NS	11.20	59.74
	Cut 2	84.1	76.4	67.0					
Fat	Cut 1	24.4	35.6	23.9	NS	NS	*	7.48	17.47
	Cut 2	33.9	32.8	33.9					
WSC	Cut 1	13.7	17.6	42.5	NS	**	NS	14.57	33.13
	Cut 2	7.3	9.4	20.2					
LA	Cut 1	8.5	12.5	5.0	NS	**	NS	4.39	6.02
	Cut 2	8.5	13.1	7.2					
AA	Cut 1	2.3	2.0	5.1	NS	NS	NS	3.98	3.92
	Cut 2	2.7	2.2	2.5					
BA	Cut 1	1.1	0.2	0.5	NS	NS	NS	1.09	1.01
	Cut 2	0.9	0.2	0.1					
pH	Cut 1	4.6	4.1	4.4	NS	**	NS	0.29	0.66
	Cut 2	4.5	3.9	4.2					

Cut 1 – flat pod stage; Cut 2 - stage of green ripe seeds; 0 - control; B - microbial inoculant; CH - chemical additive; DM - dry matter;
$N\text{-}NH_3$ - ammonia-N; CP - crude protein; NDF - neutral-detergent fibre; WSC - water-soluble carbohydrates; LA - lactic acid; AA - acetic acid; BC - butyric acid; NS – non-significant; * significant at P<0.05.; ** significant at P<0.01.

In the experiment conducted by Fraser et al. (2005a), harvest date had a significant effect on DM, N-NH_3, LA, AA, and WSC concentration in white lupin silage. Fraser et al. (2001) also studied the suitability of pea and field bean as silage materials. It turned out that changes in plant ripeness had little effect on the chemical composition of green forage, but harvest dates differentiated, among other things, the DM content, N-NH_3, CP, WSC, LA and pH of silage. Borreani et al. (2006) reported that the stage of growth affected the LA and AA, WSC concentrations in pea silage. In the experiment conducted by Borreani et al. (2009), BA was detected in silages, except for wilted silages made from field pea and white lupin, inoculated with *Lactobacillus plantarum*. As a result, BA was over 25 g kg^{-1} DM in the control silages with the DM content lower than 300 g kg^{-1} DM. The pH and fermentation products were also greatly influenced by the crops and the application of silage additives. Doležal et al. (2008) found that the chemical additive decreased LA, AA, ethanol, N-NH_3 and pH, while it raised the CP content of yellow lupin silage. Fraser et al. (2005a) reported that inoculation with *Lactobacillus plantarum* significantly reduced pH and N-NH_3, AA, and CP concentrations, while it increased the DM and WSC concentrations in white lupin silages. Similarly, when investigating suitability of pea and faba bean silage Fraser et al. (2001) found that inoculation increased the LA concentration and reduced the pH and N-NH_3 and AA concentrations in the silages. Microbial inoculation lowered the pH and N-NH_3 values and increased the LA concentrations in all tested pea silages, except for the silages from the earliest harvest date (Borreani et al., 2006).

CONCLUSIONS

White lupin can be used as a silage material, but plants before ensiling should be partially wilted and silage additives should be applied.

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