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EFFECT OF SUPPLEMENTATION OF CHEMICAL PRESERVATIVE ON FERMENTATION PROCESS OF LUPINE SILAGE

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ABSTRACT

The aim of this study was to evaluate the effect of supplementation of a chemical preservative (mixture of organic acids) on the quality and nutritive value of lupine silage as compared with untreated ones. Relatively low water-soluble carbohydrate (WSC) content and the low buffering capacity of lupine crops necessitate the application of chemical preservatives.

The best quality of fermentation process and nutritive value was found in silages supplemented with 6 L/t of acid mixtures and they showed not only a better content of net energy (NEL) and CP but also a significantly higher ethanol content, a more favourable RDP content and a higher starch content than the control. The supplement of preservatives resulted in increased DM content in stored silage, increased escape of silage effluents and in the inhibition of acetic acid formation. In chemically treated silages (3L/t), the level of lactic acid and total acids per kilogram of dry matter was found to increase while the level of pH, formol titration acidity (TA), titration acidity of water extract (AWE) and content of NH_3 and acetic acid decreased. The results indicate that the lupine silage, when made at a lower dry matter content, can be expected to exhibit an improved stability against aerobic deterioration.

Key words: chemical preservative, fermentation process, lupine silage, nutritive value, quality of silage.

INTRODUCTION

The quality of silage is affected by poor climatic conditions in crop stage and also by additive supplementation (Gallo et al., 2006). Chemical preservatives are used as preserving agents either separately or in combination with other acids (mixture of acids). Bíro et al. (2006) presented the effect of chemical inhibitors on fermentation process of high moisture corn. Chemical additives are used for conservation of proteinaceous forages too (Gálik et all., 2007). Lupine (Lupinus, L.) is presently an important non-traditional leguminous crop whose economic importance is gaining worldwide. The genus Lupinus L. currently includes about 300 species, with the specially improved annual forms of Lupinus albus and Lupinus luteus with their low alkaloid content being of the greatest significance. The lupine seeds, especially those of the foreign alkaloid-free species, are frequently used for their high protein concentration in the production of feed mixtures for monogastric animals all over the world. The chemical composition and nutritive value of the seeds were paid great attention by researchers. The use of lupine was summarised in 1981 by Duke and later by Petterson (2000). A previous study by Milford (1994) also indicated the white lupine (Lupinus albus) as one of the potential feeds with high protein content. Many other studies confirmed the high nutritive value of lupine seeds, with the highest nutrient and fat content in legumes along with Soya (Gilbert, Acamovic, 2000; Campos–Andrada, Santana, Felgueiras et al., 2000; Ruis-Lopez, Garcia–Lopez Castaneda–Vazquez et al., 2000) and others. In addition to seeds of high protein content (Costa and Rezio, 2000), increased attention is currently paid also to the conservation and use of the whole plant.

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Lupine production for roughage is, according to Jones et al. (1999), a rule characterized by high yields of dry matter (up to 9 t/ha). Lupine as roughage has usually low DM content and insufficient amount of water-soluble carbohydrates (WSC), and therefore, it must be wilted before ensiling (Jones et al., 1999). Despite a higher content of nitrogenous substances, the buffering capacity of the whole ensiled lupine plant is similar to that of some grass varieties, but significantly lower in comparison with other legumes. The issue of lupine conservation has been dealt with by a number of authors. Lupine silage and its mixtures with cereals and grasses were studied by several investigators, for example, Egorov et al. (2001), Carrutheres et al. (2000), Jannasch, Martin (1999), Serrano (1989) and others. Silages prepared by them had a high content of digestible nitrogenous substances and a very high fermentation quality. However, the dry matter content was low (ca. 21 %). Voytekhovich (2000) informed that it is also possible to successfully ensile Lupinus angustifolius and the silages had better nutritive values than silages prepared from white lupine. Many authors recommended the use of ensilaging agents for the lupine conservation. The effect of partial wilting and the addition of bacterial inoculants on fermentation characteristics were studied by Jones et al. (1999). They noticed that inoculation of the ensiled lupine mass brings about significant acidification along with protein decomposition, formation of ammonia and preservation of a higher WSC amount than in the control silage.

The aim of this study was to evaluate the effect of chemical additive on the fermentation process and quality of lupine silage when ensiling lupine under exactly defined model conditions.

MATERIALS AND METHODS

In the model experiment, yellow lupine (Lupinus luteus) variety Juno was ensiled. It was harvested at a stage of wax ripeness of the seed using the Kemper field chopper. The lupine stand was not fertilised during the vegetation period. Green matter yield was 3.68 t/ha at a dry matter content of 187.15 g/kg. The whole lupine plants were cut, shortly wilted and subsequently chopped during collection to a particle length of greather than 50 mm. This model experiment used fresh crop lupine with 240.4 g/kg DM. The ensiled material was transported from the company Zichlinek to the laboratory of the Department of Animal Nutrition and Forage Production, Faculty of Agronomy (MZLU Brno) and homogenised using experimental preservatives. One control (A) without preservative and two experimental variants (B and C) with preservative (acid mixtures) were used. The chemical preservative comprised of propionic acid, formic acid, benzoic acid and ammonium formiate. The preservative was applied at a dose of 3 L/t (B) and 6 L/t (C) respectively. This material was ensiled in three replications in special experimental containers with content (volume) of 4 L, was hermetically sealed, and stored at a temperature of 22-25 °C for three months. After three months, the containers were opened and six representative samples of ensilage matter were taken for the analysis of nutritive value and basic fermentation characteristics. All experimental samples were compared with the untreated control without the addition of preservative. Dry matter content was estimated by drying the samples to a constant weight at a temperature of 103±2 °C. The analytical procedures were as described earlier (AOAC, 1980), according to existing norms (Public Notice No. 124/2001 Sb.) and Doležal (2002). Content of WSC was determined by the Luff-Schoorl titration method; pH was measured electrometrically. Lactic acid, acetic acid concentrations were determined by the gas chromatography method, and total NH_3 by the microdiffusion Conway methods, respectively AOAC (1980). Energy concentrations in the silages were calculated after Sommer et al. (2004). Rumen degradability of protein of silages was determined by in sacco method (Kacerovský et al., 1990). Dry matter losses was determined using method balance. Results were statistically analysed using One-way analysis of variance and compared by the Student t-test (Statgraphic).

RESULTS AND DISCUSSION

In the model experiment, yellow lupine (Lupinus luteus), variety Juno was ensiled.. The whole lupine plant was harvested at a stage of wax ripeness of the seed. The chemical composition of the whole ensiled lupine plant differed from other leguminous crops. One kilogram of the original biomass (dry matter) contained 208.2 g of crude protein, 221.7 g of crude fibre, 290.4 g ADF, 410.9 g NDF, and 140.5 g of starch. Rumen degradability of crude protein was 60.18 %. The content of water-soluble carbohydrates was 31.5 g of 1 kg DM. Dry matter of the whole lupine plant was low and after one-day wilting its value increased only to 187.2 g/kg, which is within the range recommended by Jones et al. (1999).

The regular course and quality of the fermentation process were mainly affected by the low content and composition of lupine dry matter and also by the addition of chemical agents. It is obvious that the content of water-soluble carbohydrates in the whole lupine plant during the specified vegetation stage before the harvest and conservation was insufficient (<5.80 g/kg of biomass) and in comparison with the grass it was too low. Therefore, it is necessary to ensure efficient wilting of the biomass to higher dry matter content or to use adequate ensiling agents.

The chemical composition of the control and treated model lupine silages from wilted forage is shown in table 1. The dry matter of model silages ranged from 187.99±0.68 g/kg in control silage up to 188.40±2.78g/kg in experimental silage with a higher dose of the chemical additive. With the increasing dose of chemical agents, changes were observed in some indicators which are important in terms of nutritive value. In the treated lupine silages, the preserved crude protein content was much higher as compared to the control, which indicates a more considerable conservation of proteins in the silage. The chemical conservation resulted in a reduced level of rumen degradability of protein in the silage in comparison with the control. This indicates that more considerate conservation of protein which is in accordance with the results published by other investigators (Jones et al., 1999), Jones and co-workers found a similar increase in the DM content of experimental silages by short-term wilting. The reduction in rumen degradability of CP by 4.42 %, resp. 9.14% is significant with respect to rumen fermentation. The better conserving effect is also documented in the model silages by the occurrence of a higher content of PDI compared to the control silages. The treated silages of lupine have a higher content of crude fibre and ADF and NDF fractions than the control silage. Jones et al. (1999) detected insignificantly lower content of ADF and NDF compared to the control silages in the inoculated unwilted or wilted forages of lupine. A great advantage of treated lupine silages with respect to the nutritive value is the preservation of a higher starch content, soluble sugar content and the higher concentration of energy. Bíro et al. (2004) reported that alfalfa silages with biological additive contain lowest amount of NFE, and significantly lower content of reduced sugars, while supplementation of lactic acid bacteria increased the dry matter content in silages.

The differential course of fermentation relates also to the extent of dry weight loss. The calculated values of DM losses due to the fermentation process correspond to the range and course of the fermentation process. The values indicate that the loss of dry matter depended on dry matter content and on the addition of the chemical additive. The addition of chemical preservative led to a reduced biomass loss (2.12 % vs. 1.62 %) as compared with the untreated control (7.89 %), a reduction in dry matter content to 5.38 % vs. 1.62 % with the application of 6 L of the agent per 1 t. Dry matter loss in the untreated control silage amounted to about 9.83 %.

Table 1: Chemical composition of lupine silage (per kg of DM)

Group / Characteristics		A	B	C
DM	(g/kg)	187.99±0.68	182.15±2.64	188.40±2.78
CP	(g/kg)	202.7	217.3	205.3
RDP	(g/kg)	699.1	668.2	635.2
Fat	(g/kg)	20.3	22.0	21.5
CF	(g/kg)	245.5	269.6	268.0
ADF	(g/kg)	312.8	337.9	339.3
NDF	(g/kg)	471.7	524.5	523.2
Ash	(g/kg)	104.1	101.0	92.2
Starch	(g/kg)	95.7	111.2	127.9
ME	MJ/kg DM	9.44	9.40	9.59
NEL	MJ/kg DM	5.56	5.51	5.59
PDIN	g/kg DM	107.98	109.56	108.25
PDIE	g/kg DM	76.32	76.71	76.44
WSC	%	0.21	0.95	1.19

DM – dry matter; CP – crude protein; RDP – rumen degradability of protein; CF – crude fibre; ADF – acido-detergent of fibre; NDF – neutral-detergent of fibre; ME – metabolisable energy; NEL – net energy of lactation; PDIN, PDIE – really digestible protein in the small intestine; WSC – water-soluble suger content

Table 2: Results of fermentation process of lupine silage

Silage Variant (n=6)	A		B		C	
Specification	Average± s.e.	vc	Average± s.e.	vc	Average± s.e	vc
DM (g/kg)	187.99±0.68 ^{A}	0.89	182.15±2.89 ^{B}	8.34	188.40±3.04 ^{A}	9.26
pH	4.07±0.01 ^{C}	0.80	3.81±0.02^{A}	0.00	3.90±0.02 ^{B}	0.00
AWE (mg KOH/100g)	1352.70±12.18 ^{B}	48.37	1225.28±8.64 ^{A}	74.66	1215.85±7.92 ^{A}	62.75
TA (%)	0.131±0.00 ^{c}	2.59	0.098±0.00 ^{b}	2.89	0.069±0.00 ^{a}	2.41
LA (g/kg DM)	107.99±15.95 ^{B}	42.79	128.14±7.58 ^{C}	57.46	76.57±6.25 ^{A}	39.07
AA (g/kg DM)	29.44±0.61 ^{b}	5.05	25.38±5.04 ^{ab}	25.37	22.67±4.28 ^{a}	18.33
Σ acids (g/kg DM.)	137.42±1.87 ^{B}	3.33	153.52±12.24 ^{C}	1.49	99.24±10.47 ^{A}	1.09
LA/AA	3.67±0.05 ^{A}	3.45	5.17±0.74 ^{B}	0.55	3.44±0.41 ^{A}	0.17
Ethanol (g/kg DM)	4.61±0.19 ^{A}	9.83	14.01±1.46 ^{B}	2.13	5.13±0.62 ^{A}	0.38
NH_3 (mg/kg)	696.18±4.99 ^{C}	1.75	556.95±11.11 ^{B}	1.24	475.87±10.76 ^{A}	1.15

DM – dry matter; AWE – acidity of water extract; TA – titration acidity; LA – lactic acid; AA – acetic acid; vc – coefficient of variation; $^{a, b, c}$ significant differences at a significance level of 95 % (P< 0.05); $^{A, B, C}$ significant differences at a significance level of 99 % (P< 0.01)

The quality of fermentation process of the control and treated model lupine silages from wilted forage are shown in table 2.

Statistically significant differences in the values of individual fermentation quality parameters were established in the model experiment with the silages of moderately withered yellow lupine. Dry matter content of the model silages ranged from 187.99±0.68 g/kg in the control silage to 188.40±3.04g/kg in the experimental silage with a higher preservative dose. Significantly lower (P<0.01) dry matter content of the experimental silage was established in the silage treated with the mixture of acids at a dose of 3 L/ton. The coefficient of variation was low and ranged from 0.89 to 3.04 %. The pH value of the control lupine silage was significantly (P<0.01) higher (4.07±0.01) as compared with the values of experimental silages, while the lowest pH value (3.81±0.02) was found in the silage with the additive of 3 L/ton. Statistical difference (P<0.01) in the pH value was established among all variants. Lower pH value of the silage related to the lactic acid content and to the total acid content in the dry matter occurred in one case only. From the above results it may be stated that the rising concentration of the applied additive leads to the inhibition of available carbohydrates and starch in the ensiled lupine mass which resulted in a different rate of fermentation and level of acidification as well as in the form of created fermentation acids. Jones et al. (1999) also reported a lower pH value of inoculated silages made of wilted and unwilted lupines, compared with the control silages. Titration acidity of the water extract (AWE) corresponding usually with fermentation acid concentration, is a significant indicator of the quality of the fermentation process. From Table 2 and Fig. 1 it may further be interpreted that both the experimental silages are characterized by significantly lower (P<0.01) titration

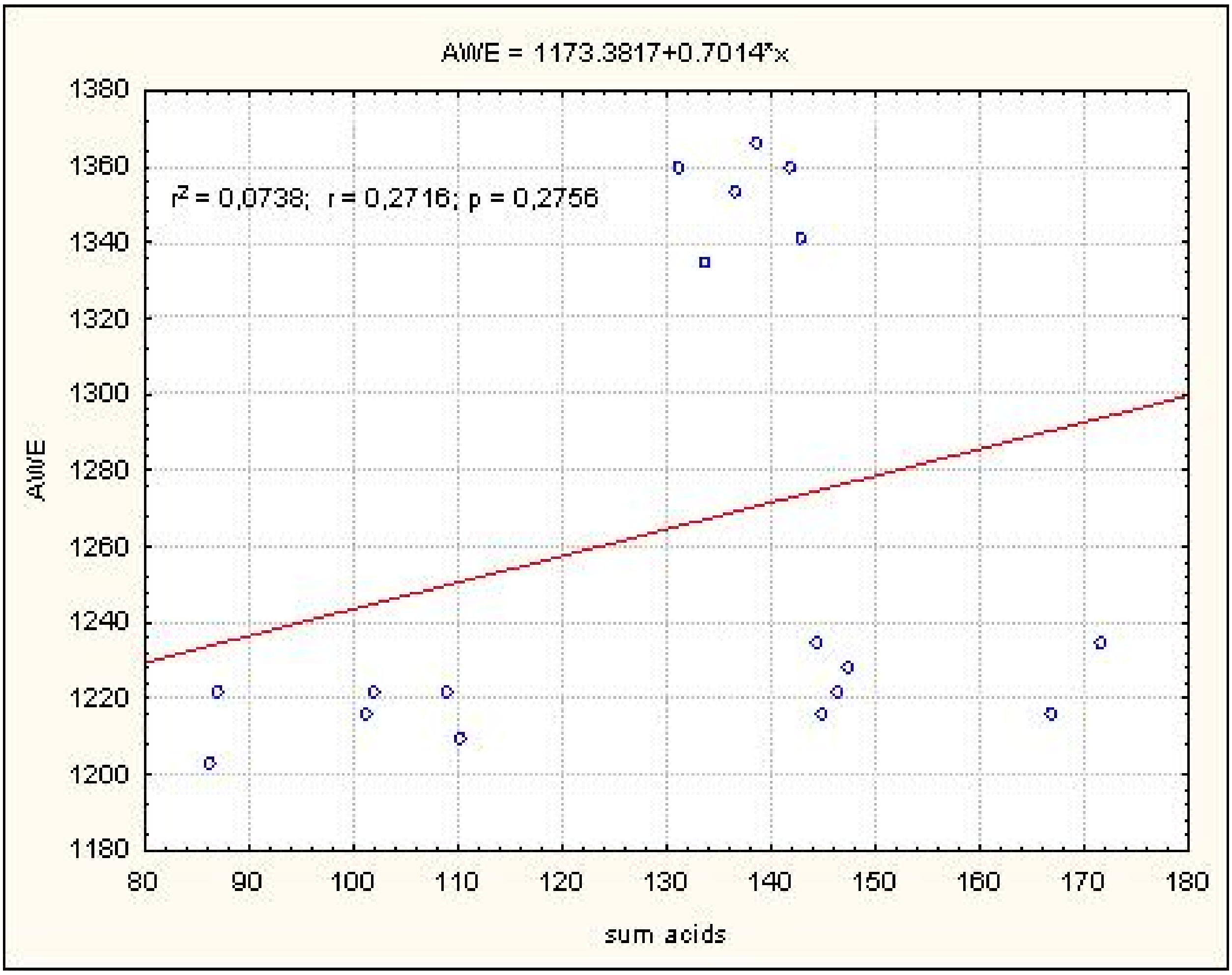

Fig. 1: Correlation between AWE and sum acids in lupine silages

acidity value, compared with the control untreated silage (1352.70±12.18 mg KOH/100 g of silage). Statistically significant ($P<0.01$) difference in the AWE value has not been found between the experimental silages mutually. Above findings clearly indicatet that there is a certain correlation, though not proved explicitly, between the pH and AWE values of the lupine silage. The correlation between the AWE value and the sum of acids in the silages is significantly low ($r^2 = 0.073$), see Fig. 1.

Significant changes during the fermentation itself took place not only in the content of individual fermentation acids and in their mutual ratio, but also in the total content. Concentration of the lactic acid ranks among important proteinaceous silage quality indicators. From Table 2 and Fig. 2 it is evident that the additive of the tested preparation in the 3 L/t concentration led to the statistically significant ($P<0.01$) rise of lactic acid content (128.14±7.58 g/kg DM), compared with the control silage (107.99±15.95 g/kg DM). On the contrary, the experimental lupine silage treated with the preparation 6 L/t resulted in reduction in not only the overall fermentation, but also in production of the lactic acid (76.57±6.25 g/kg DM). The reduction was statistically significant ($P<0.01$). The percentage share of LA was the highest among the sum of acids in the experimental silage with added 3 L/t (83.47 %), the lowest share was in the experimental silage with added 6 L/ t (77.16 %). A higher addition of the preparation to the ensilaged lupine led to the overall reduction in the percentage share of lactic acid in the total content of fermentation acids (Fig. 1). In the control silage, the lactic acid reached the 78.58% share of all fermentation acids. This finding is in accordance with the results obtained by Egorov and Myskov (2001), who also stated a good quality of the laboratory lupine silages. Their model silages had a mutually comparable pH value (4.1–4.3) and the lactic acid represented 56–62 % of the overall acid content. In biochemically treated crimped high moisture corn, Gálik et al. (2007) found lower content of LA, AA, and ammonia in comparison with untreated silage.

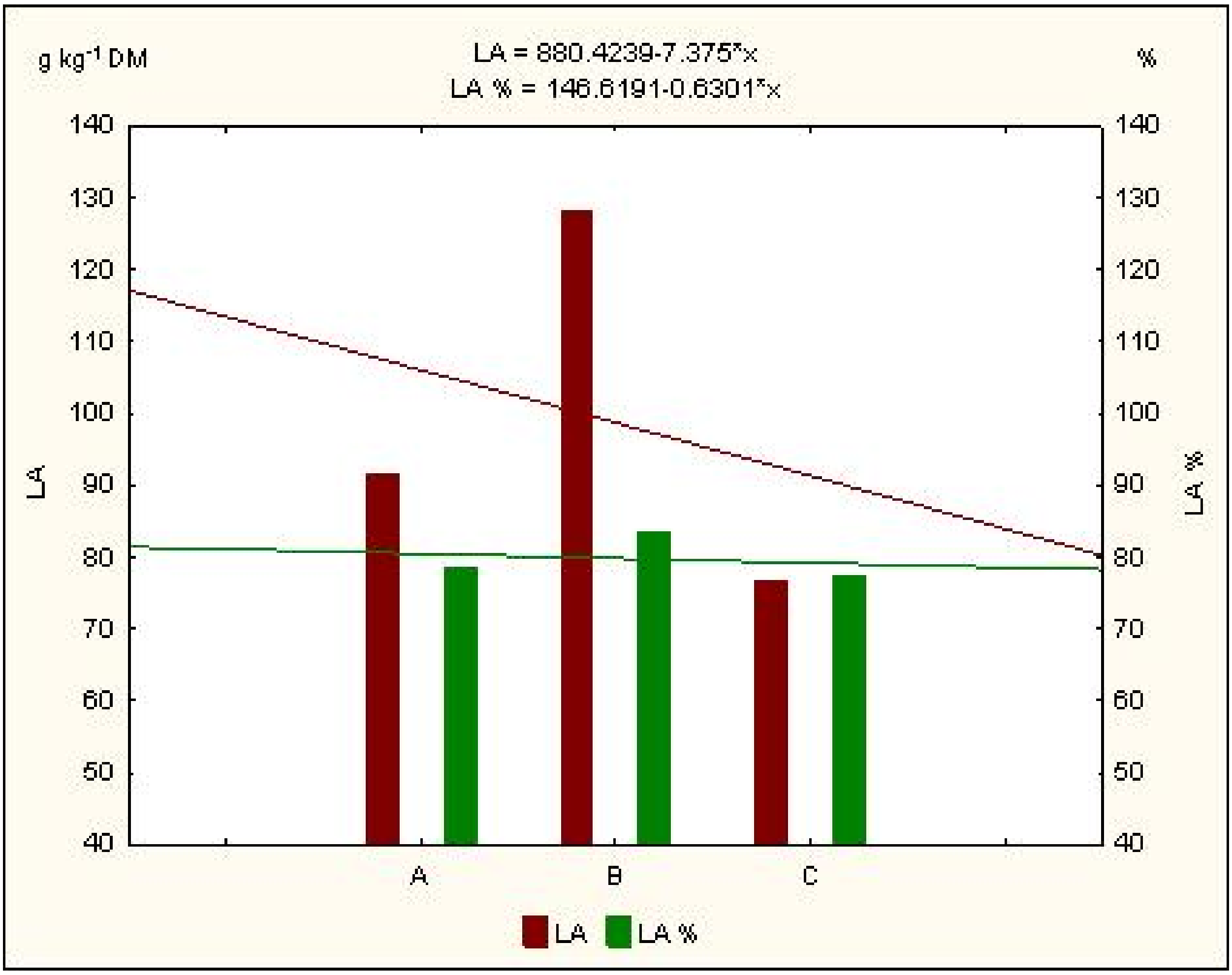

Fig. 2: Effect of additive supplementation of LA and the proportion of acids

The concentration of acetic acid in the experimental model lupine silages was significantly ($P<0.05$) lower, compared with the control silage (29.44±0.61 g/kg DM). Addition of the tested preservative solution to the ensilaged material led in general to a reduced production of acetate. A higher dose of this solution (6 L) limited the development of acetic acid in the silage significantly ($P<0.05$). This in turn led to increase in the LA:AA ratio, whilst the silage with lower addition of the preparation did not differ statistically significantly from the control silage by the production of acetic acid, though it contained a lower AA value. A higher LA:AA ratio (5.17±0.74) was caused in this experimental silage by the significantly ($P<0.01$) higher production of lactic acid. The acid ratio shown above was the highest one, compared with other silages and its value was statistically significant ($P<0.01$). It may therefore be stated that reduction of acetic acid production was to the benefit of the lactic acid, the production of which is likely to have been enhanced to a greater extent by the heterofermentative bacteria of lactic fermentation. According to Kalač and Pivničková (1987), these microorganisms are, besides yeast, important ethanol producers in silages. This hypothesis is also supported by significantly ($P<0.01$) higher levels of ethanol in this silage (14.01±1.46 g/kg DM). The content of alcohol in silages therefore belongs to important qualitative silage features. It is known (Randby et al., 1999) that forages with a high initial concentration of water-soluble carbohydrates are most susceptible to ethanol fermentation, especially if lactic acid fermentation is depressed (e.g., in silages treated with a small quantity of formic acid). This tendency of ethanol production in lupine silages differs from the results published by other authors in the past for the carbohydrate forages. The occurrence of alcohol as a minor product of fermentation in silages is often generally related to the factor of a higher DM content of the ensilaged mass - longer chop length in particular. In experimental silage with a higher dose of preservative lactic fermentation itself was found to be limited (Driehuis et al., 1999). Under conditions of our experiment, longer chop length (> 35 mm), in particular, appears to be the potential factor. Driehuis and van Wikselaar (1996) also stated that ethanol fermentation may occur in silage with low as well as with high DM concentrations. The results indicate that addition of the preparation in the dose of 6 L/t reduced fermentation in general, i.e. even the alcohol fermentation and ethanol content in the experimental lupine silage was ($P<0.01$) lower (5.13±0.62 g/kg DM). Contrary to expectations and in comparison with ethanol content in the silage with respect to the content of carbohydrates the fermentation process in the experimental silages can be evaluated as successful within certain limits only from the point of ethanol production. This difference in the production of ethanol between individual variants can be explained by a different degree of the fermentation process inhibition. It is evident that although the highest lactic acid amounts developed and acetic acid amounts were to some extent reduced in the experimental silages, the heterofermentative bacteria of lactic acid fermentation apparently participated in the production of ethanol in these silages largely, too. This model experiment with lupine ensiling failed to support the hypothesis of Kalač and Pivničková (1987) because the negative correlation between the development of lactic acid on the one side and the ethanol production on the other side could not be confirmed fully in all the silages treated. Driehuis and Van Wikselaar (1999) considered the prevention of alcohol fermentation in the inhibition of enterobacteria activity and namely in the stimulation of the fermentation process. On the other hand, Seija et al. (1999), Rammer et al. (1999 among others observed reduced production of not only alcohol, but also fermentation acids, including lactic acid, after application of effective chemical, organic acid

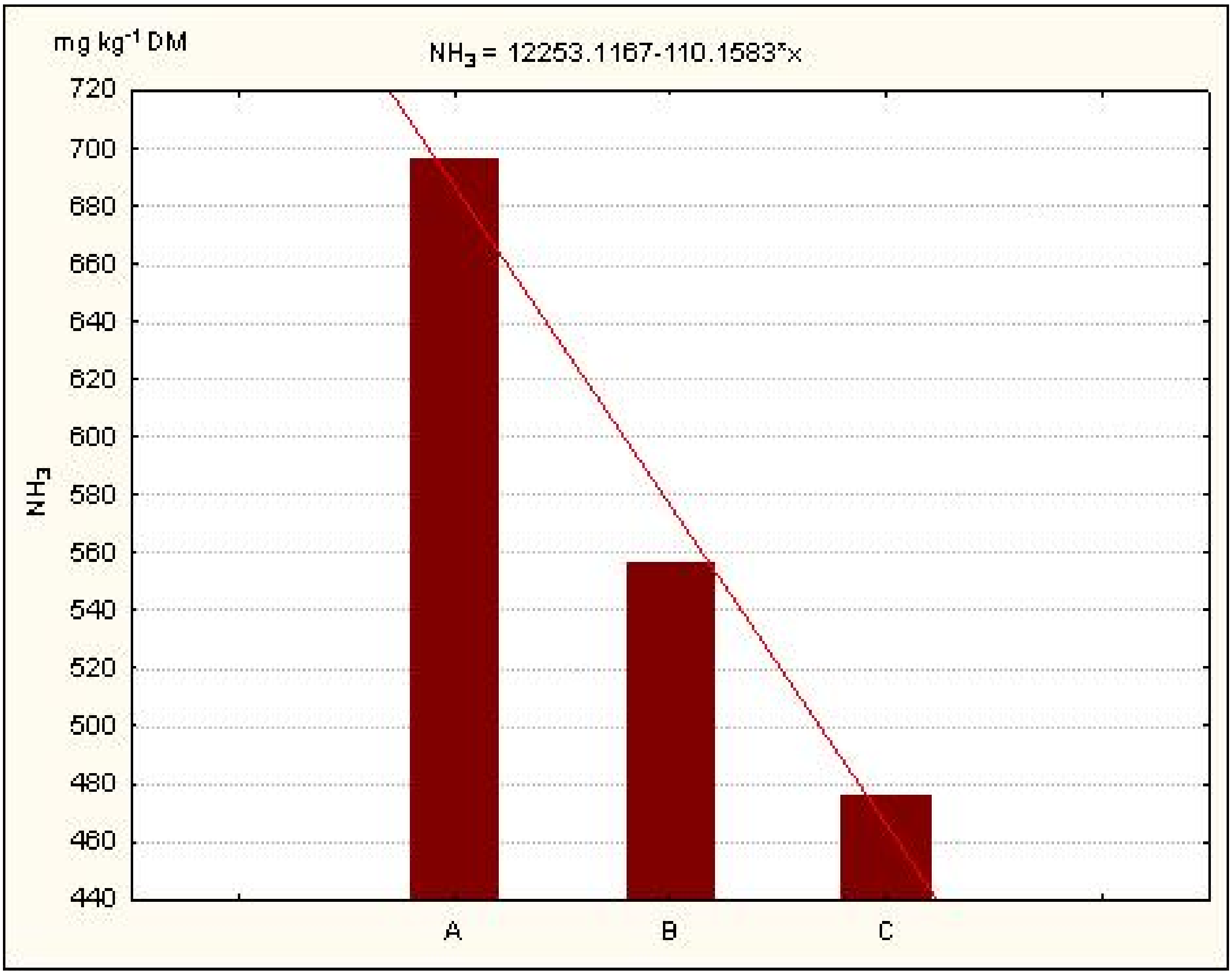

Fig. 3: Ammonia production in model lupine silages

based preservatives. Similarly, Juráček (2002) informed that the amount of alcohol in silages decreased after the use of microbial or chemical additives.

The results shown in Table 2 and Fig. 1 further indicate that an additition of the tested preparation at 3 L/t to the ensilaged lupine led to statistically significant ($P<0.01$) increase (153.52±12.24 g/kg DM) in the overall production of all fermentation acids in the model silages, compared with all other groups of silages. On the other hand, in case of the experimental silages with the preparation added at a dose of 6 L/t, statistically significant ($P<0.01$) reduction of the overall fermentation (99.24±10.47 g/kg DM) occurred, compared with the control untreated silage (137.42±1.87 g/kg DM), as measured by the production of all fermentation acids. The above mentioned conclusions have a similar tendency as of the results of other investigators who tested chemical preservatives in other kinds of forages (Rammer et al. (1999; Seija et al., 1999).

Advantages of chemical forage preservation lies in, but not limited to, in the limitation of proteolysis and in the reduced production of ammonia. The extensive protein degradation during the ensiling process is a major problem in silage production. Native proteases degrade 45 to 87 % of the plants' protein into ammonia, amino acids and small peptides. The results presented in Table 2 and Fig. 3 demonstrate that an addition of the tested chemical preparation led to a highly statistically significant ($P<0.01$) reduction in ammonia production in the silages (475.87±10.76, and/or 556.95±11.11 mg NH_3/kg of silage), compared with the untreated control silage (696.18±4.99 mg NH_3/kg). The findings are in accordance with those obtained by Jones et al. (1999) in lupine fermentation using a bacterial inoculant. A similar positive effect of silage additives on reduced production of ammonia in silages consisting of different forages has been reported by a number of authors (Wyss and Vogel, 1995; Driehuis et al., 1996; Heikkilä et al., 1999).

CONCLUSION

The results of our study showed that Lupine as a crop has usually a low DM content and insufficient amount of WSC, and therefore, it needs to be wilted before ensiling. Also, relatively low WSC content and the low buffering capacity of lupine crop necessitates the application of the chemical preservatives.

The best nutritive value was found in model silages with the supplement of acid mixtures dosed at 6 L/t since they showed not only a higher content of net energy (NEL) and CP but also a significantly ($P<0.01$) lower NH_3, LA and AA content as well as pH and TA value, a more favourable RDP content and a higher starch content than the control silage. The supplement of chemical preservatives resulted in the increase of DM content in stored silage as well as in the increased escape of silage effluents and had a positive effect on the quality of fermentation process of silages. The higher supplementation of chemical preservative (6 L/t) in the silage inhibited formation of lactic and acetic acids and ethanol, too. In these silages, a lower ratio of LA/AA was determined than in other silages. The positive effect on RDP and starch content was higher in silages treated with the preservative in level of 6L/t than the untreated silage.

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