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CHANGES IN AMINO ACID PROFILE OF ALFALFA SILAGE PRESERVED BY CHEMICAL AND BIOLOGICAL ADDITIVES DURING FERMENTATION

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Abstract

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Changes in amino acid profile of alfalfa silage preserved with chemical or biological additives were studied in fresh and wilted silage. The chemical additive was formic acid and the biological additive consisted of *Lactobacillus rhamnosus*, *L. plantarum*, *L. brevis*, *L. buchneri* and *Pediococcus pentosaceus*. Second cut alfalfa (*Medicago sativa* L.) was harvested at the bloom stage, ensiled in mini silos (15 dm^3) and fermented at 20–23 °C for 12 weeks. The dry matter of the fresh silage was 228 $g.kg^{-1}$ and 281.6 $g.kg^{-1}$ for the wilted before ensiling. The amino acid content was estimated by using an automatic amino acid analyzer AAA (INGOS Prague). The results of the experiments indicated that amino acid breakdown was inhibited by increased dry matter and the use of chemical and biological additive. Additionally, the content of amino acids was found to change in relation to the degree of wilting and formic acid treatment yielded the lowest amino acid breakdown. The amino acid breakdown was also reduced by biological preservative especially in the silage with a higher level of dry matter content.

alfalfa, amino acid, silage, silage additive

Alfalfa (*Medicago sativa* L.) is an important legume for ruminant agriculture widely grown under both tropical and temperate conditions. Unlike grasses, alfalfa does not possess large amounts of reserve polysacharides in the form of fructosans, but it does contain small amounts of starch and relatively large quantities of pectin. The protein content is comparatively high, and if the crop is cut in the early flowering stage the crude protein can be above 20% (Mc Donald *et al.*, 1966). Efficient utilization of crude protein in animal nutrition is paramount assessing the quantity and quality of silage is essential. Therefore it is necessary to monitor changes in content not only during the harvest of alfalfa, but also during the fermentation process. Protein degradation caused by plant proteases activity occurs after harvest and increases with prolongation of wilting time and air humidity (McDonald *et al.*, 1991). Proteolysis continues 24 hours after ensiling due to plant proteases (Kemble, 1956). According to Muck and Dickerson (1988) proteolysis speed in alfalfa silage decreases linearly when dry matter content increases and proteolytic activity ends when dry matter content reached 75%.

Silage additives have been developed to take some of the risk out of the ensilage process and to improve the nutritive value of silages. Ideally, a silage additive should be safe to handle, reduce DM losses, improve the hygiene of the silage, limit secondary fermentation and improve aerobic stability thereby increasing the nutritive value of the silage. Improving forage quality increases the efficiency of feed utilization and provides the farmer a return greater than the cost of additive (Merensalmi and Virkki, 1991). Both lactic acid bacterial inoculation (Doležal and Zeman, 2005) chemicals treatments (McDonald *et al.*, 1991; Henderson, 1993) have been used to protect proteins from microbial and plant enzyme hy-

drolysis. Lactic acid bacteria belong to the epiphytic microorganisms which occur on the lower parts of forage plants. The transition of hexoses by lactic acid bacteria to fermentation acids proceeds under anaerobic condition is of particular importance for ensiling. Lactic acid bacteria are capable to ferment a wide range of substances using different pathways. In the ensiling process, homo-fermentative lactic acid bacteria are preferred due to fast and abundant formation of lactic acid, which has a high acidifying potential. Homo-lactic fermentation also results in lower DM losses because of higher efficiency in the conversion of water soluble carbohydrates to acids compared with hetero-lactic fermentation (McDonald *et al*., 1991).

The purpose of the present experiments was to investigate the effect of wilting and chemical or biological additive on the change of amino acid profile in alfalfa silage.

MATERIAL AND METHODS

Silage preparation and treatments

Second cut of alfalfa (*Medicago sativa* L.) was harvested at the blooming stage without wilting at 228 g.kg^{-1} of dry matter (DM) (experiment No. 1). Alfalfa for wilted silage was allowed to wilt in the outside weather condition for 8 hours to 281.6 g.kg^{-1} of dry matter (experiment No. 2). Alfalfa was chopped by a Jaguar harvester to a length of cut 20 mm. After chopping, the forage was ensiled in 150 cm^3 laboratory silos (four repetitions of each variant) without preservatives (control: fresh and wilted matter), treated with biological additive at 1g.t^{-1} of silage matter (in both experiments) or treated with formic acid as positive control at 5.8 kg.t^{-1} of silage matter (experiment No. 1) and at 4 kg.t^{-1} of silage matter (experiment No. 2). Biological additive consists of *Lactobacillus rhamnosus*, *L. plantarum*, *L. brevis*, *L. buchneri* and *Pediococcus pentosaceus* and were applied at 10^4 cfu/g of silage matter. All laboratory silos were stored at the temperature of 20–23 °C and silos from each treatment were opened after 12 weeks of ensiling.

Analysis and Measurement

After opening each silo, samples of each treatment were immediately frozen in sealed plastic bags at –60 °C until analysis.

Firstly, 200 g of the wilted and unwilted silage were diluted to 2 dm^3 with distilled water and macerated at laboratory temperature (22 °C) during 24 hours. The silage was then filtered through two layers of filtrate paper and the pH of filtrate was measured immediately after filtration (Owens *et al*., 1999). Fermentation products were assessed by using of capillary isotachoforetic method and ammonia (NH_3-N) by titration colorimetric method. Ten ml of 25% (w/v) trichloroacetic acid was added to 40 ml of silage filtrate of each treatment (both experiment) to precipitate protein from the solution. The solution was allowed to stand at laboratory temperature (22 °C) for one hour. After it, the solution was centrifuged. Supernatant of silage was analyzed for non- protein N (NPN) using the micro–Kjeldahl method (AOAC, 2000).

After weighing, the remaining unmacerated material was dried in forced–air oven at 60 °C for determination of dry matter. Oven–dried samples were ground to pass a 1-mm screen. Total N (TN) was measured on oven–dried samples using the micro–Kjeldahl method. The oven-dried forage and silage samples were also used for amino acid analysis.

The amino acid content was determined by using of automatic amino acid analyzer AAA400 (INGOS Prague). Chromatographic estimation of hydrolysates of each sample was assessed by sodium-citrate buffer and post colon ninhydrin derivation. Samples were hydrolyzed by hydrogen chloride acid (6 mol.dm^{-3}) during 23 hours at 110 °C under an N_2 atmosphere. After filtering of the hydrolysed samples, the hydrolysates were neutralized with sodium hydroxide solution and completed to the volume of 100 cm^3 using sodium citrate buffer with a pH of 2.2. The hydrolysates were held at 4 °C to silage for 24 hours in fridge. Methionine content was estimated as methionine sulphon, cyst(e)ine was determined as cysteic acid after oxidation by performic acid and hydrolysis. The samples were hydrolysed for 16 hours at 110 °C. After the vaporization at vacuum rotary device and supplementation with sodium citrate buffer (pH = 2.2) to 50 cm^3 volume and following the dilution the analysis of amino acids was performed. The following amino acids were measured: Aspartic acid (Asp), Threonine (Thr), Serine (Ser), Glutamic acid (Glu), Proline (Pro), Glycine (Gly), Alanine (Ala), Cysteine (Cys), Valine (Val), Methionine (Met), Isoleucine (Ile), Leucine (Leu), Tyrosine (Tyr), Phenylalanine (Phe), Histidine (His), Lysine (Lys) and Arginine (Arg). Each analysis was performed in duplicate.

Calculations

The results of fermentation products, NPN, TN, NH_3-N and amino acid content were converted into 100% dry matter in both experiments. The rate of proteolysis was calculated as a percentage ratio of NH_3-N from amount of total nitrogen. Analysis of variance was used to test statistical significance of treatment (SAS Institute, 1985). Means were separated using Fischer's protected least significance difference LSD ($P < 0.05$) when F- tests were determined to be significant. The values in the tables with different superscripts are significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

Fermentation characteristics of unwilted and wilted silage

The pH of unwilted silage preserved with formic acid was the lowest among untreated silage and treated silage by biological additive (Table I). In

wilted silage, the lowest value of pH was also measured in silage treated by formic acid. The highest value of pH was observed in wilted silage preserved by biological additive ($P < 0.05$) (Table II). The well-fermented and stable silage should have the value of pH 4.2 or lower with 200 g.kg^{-1}DM and below 4.75 with 400 g.kg^{-1}DM according to Weissbach (2003). All silages had higher value of pH than mentioned results.

In experiment 1, the dry matter of the control silage was 232.64 g.kg^{-1}DM compared to silage treated with formic acid which was 245.45 g.kg^{-1}DM and silage preserved with biological additive which was 238.16 g.kg^{-1}DM. The total nitrogen content was the highest in the control silage which was also determined to have the highest amounts of non-protein nitrogen and NH_3-nitrogen. Concentration of lactic acid in biological treated silage was significantly higher than control silage (without additive) by 7 g.kg^{-1}DM. The amount of lactic acid in formic acid treated silage was significantly lower than control silage by 70.4 g.kg^{-1}DM. Wilkinson (2005) found that well-fermented silage should contain over 35 g.kg^{-1}DM of lactic acid, below 20 g.kg^{-1}DM of acetic acid and no butyric acid. Silage with formic acid had significantly lower concentration of acetic acid than the control and biological treated silage. The highest amount of acetic acid was found out in silage treated with biological additive. No difference ($P > 0.05$) was observed in concentrations of ethanol between the control silage and biological treated silage. The highest rate of proteolysis was established in the control silage (11.07%). Silage treated with formic acid had a significantly lower rate of proteolysis than the control silage (5.57%) and biological treated silage (6.28%).

In experiment 2, DM contents were 277.89 g.kg^{-1}, 293.64 g.kg^{-1}, 285.55 g.kg^{-1} for the control silage, formic acid treated silage and biological treated silage respectively (Table II). The TN content of silage preserved with formic acid was significantly higher than other silages and NPN was significantly lower. Olt *et al.* (2005) also reported an increase of total nitrogen and the lowest amount of NH_3-N. Silage without treatment yielded the highest amount of lactic acid among other treated silages. The contents of acetic acid and ethanol in silage with formic acid were significantly lower than those contents in the control si-

I: *Fermentation product of content (experiment No. 1) (mean ± S.D.)*

g.kg^{-1} DM	unwilted silage without additive			unwilted silage with formic acid			unwilted silage with biological additive		
	mean	S.D.	CV%	mean	S.D.	CV%	mean	S.D.	CV%
DM	232.64^a	1.309	0.56	245.45^b	4.379	1.78	238.16^c	1.510	0.63
TN	26.84^a	1.263	4.71	25.59^b	0.698	2.73	25.10^b	0.599	2.39
NPN	16.85^a	0.133	0.79	8.09^b	0.124	1.53	8.95^c	0.114	1.27
NH_3-N	2.97^a	0.023	0.79	1.43^b	0.022	1.53	1.58^c	0.020	1.27
pH	4.77^a	0.050	0.97	4.39^b	0.170	3.98	4.75^a	0.080	1.75
lactic acid	84.10^a	1.761	2.09	13.70^b	0.212	1.58	91.10^c	2.857	3.14
acetic acid	61.60^a	1.329	2.16	45.10^b	1.047	2.32	103.90^c	3.309	3.19
ethanol	6.60^a	0.311	4.71	4.70^b	0.212	4.51	6.70^a	0.198	2.96
RP (%)	11.07^a	0.487	4.40	5.57^b	0.168	3.01	6.28^c	0.200	3.19

DM – Dry matter; TN – Total nitrogen; NPN – Non-protein nitrogen; NH_3-N – ammonia nitrogen; RP – rate of proteolysis; ^{abc} – The values within the rows with different superscripts are significantly different at $P < 0.05$

II: *Fermentation product of content (experiment No. 2) (mean ± S.D.)*

g.kg^{-1} DM	wilted silage without additive			wilted silage with formic acid			wilted silage with biological additive		
	mean	S.D.	CV%	mean	S.D.	CV%	mean	S.D.	CV%
DM	277.89^a	2.772	1.00	293.64^b	1.102	0.38	285.55^c	1.341	0.47
TN	23.98^a	0.501	2.09	25.94^b	0.417	1.61	24.79^c	0.570	2.30
NPN	12.62^a	0.261	2.07	6.85^b	0.070	1.02	10.96^c	0.374	3.41
NH_3-N	2.33^a	0.048	2.07	1.27^b	0.013	1.02	2.02^c	0.069	3.41
pH	4.97^a	0.030	0.67	4.32^b	0.020	0.48	5.14^c	0.100	1.89
lactic acid	66.30^a	6.210	9.36	61.80^a	5.590	9.04	51.20^b	3.890	7.59
acetic acid	64.60^a	3.220	4.99	54.20^b	2.810	5.19	74.30^c	4.330	5.83
ethanol	5.10^a	0.020	0.40	4.40^b	0.020	0.50	5.30^c	0.020	0.40
RP (%)	9.73^a	0.262	2.69	4.88^b	0.097	1.98	8.16^c	0.128	1.57

DM – Dry matter; TN – Total nitrogen; NPN – Non-protein nitrogen; NH_3-N – ammonia nitrogen; RP – rate of proteolysis
^{abc} The values within the rows with different superscripts are significantly different at $P < 0.05$

lage and biological–treated silage. Silage treated with formic acid had the lowest rate of proteolysis (RP) and the rates of proteolysis in the experiment 2 were lower than the rates of RP in the experiment No. 1.

Changes of amino acid profile during fermentation

The change of amino acid profile during fermentation of fresh alfalfa is presented in Table III (experiment No. 1). During fermentation of fresh matter without additive, the amino acid profile was significantly decreased except for the amounts of Met, Ile, Leu and Tyr. Epiphytic microflora has been contributed to a decrease in amino acid content during fermentation (McDonald *et al*., 1991). Untreated silage had the lowest concentration of Arg and Ser than other silages. The same results were observed by Sneath *et al*. (1986) and Givens and Rulquin (2004). The Ser concentration decreased due to degradation to pyruvate by epiphytic microflora for a source of energy.

The amounts of amino acids (degradation) were lower in silage preserved with formic acid than amino acid content of untreated silage. We found that degradation of Arg was significantly lower with a formic acid treatment compared to untreated silage. Interestingly, there was not a significant reduction in Tyr. The observation could be associated with the rapid decline of pH during the early phase of fermentation with formic acid, thus depressing bacterial activity. We also investigated the breakdown of Glu, Thr, Ser, Asp, Met, Pro, Lys, Arg and Cys and observed that formic acid decreased the degradation of Ser, Glu, Arg and His, significantly at $P < 0.05$.

Givens and Rulquin (2004) established that amino acid content of silage treated formic acid has been the same as amino content of silage prepared from the fresh matter. Our observation was not in agreement with the findings of Givens and Rulquin (2004) as the fermentation with formic acid treatment caused a decline in amino acid profile.

In silage treated with biological additive, we found decreases in amino acid contents except for Pro, Gly, Ala, Ile, Leu, Val and Tyr. There was a non-significant increase in Gly. Arg was changed to ornithine, which reacted with glutamate to Pro. Delauney and Verma (1993) found out the same results. The decline content of Glu was associated with an increase in the amount of Ala. Ser was degraded to pyruvate for bacterial supply of energy. Accumulation of pyruvate under anaerobic conditions may shift towards Ala accumulation. The synthesis of Ala may occur at the expense of the acidic amino acids, glutamate and aspartate, and occurs concomitantly with the accumulation of 4-aminobutyrate or gamma-aminobutyrate (GABA) (Ratcliffe, 1995; Good and Muench, 1992). Givens and Rulquin (2004) established, that the content of Met has been increased during fermentation. This observation was not confirmed by our results for silage prepared from fresh matter. Ensiling of fresh matter treated by biological additive results in increased concentration of Ala, branched chain amino acids and decrease in basic amino acids.

The change of amino acid profile during fermentation of wilted alfalfa is shown in

Table IV (experiment No. 2). Amino acid content of untreated wilted silage was significantly decreased compared with amino acid content of wilted matter

III: *Amino acids of content in fresh matter and unwilted silage (experiment No. 1) (mean ± S.D.)*

treatment	without additive						formic acid						biological additive					
amino acid g.kg^{-1} DM	fresh matter			unwilted silage			fresh matter			unwilted silage			fresh matter			unwilted silage		
	mean	S.D.	CV%	mean	S.D.	CV%	mean	S.D.	CV%	mean	S.D.	CV%	mean	S.D.	CV%	mean	S.D.	CV%
Asp	16.49^{a}	0.693	4.20	15.58^{b}	0.137	0.88	15.16^{c}	0.410	2.71	14.27^{d}	0.211	1.48	13.83^{e}	0.396	2.86	13.41^{f}	0.040	0.30
Thr	5.96^{a}	0.339	5.70	4.79^{e}	0.093	1.95	5.13^{b}	0.156	3.03	4.84^{c}	0.125	2.58	5.18^{b}	0.099	1.91	4.81^{c}	0.059	1.27
Ser	6.58^{a}	0.283	4.30	2.66^{d}	0.031	1.17	5.52^{b}	0.141	2.56	4.93^{c}	0.152	3.08	5.70^{b}	0.042	0.74	2.91^{e}	0.049	1.67
Glu	13.45^{a}	0.481	3.58	6.25^{b}	0.071	1.14	10.54^{c}	0.170	1.61	9.91^{d}	0.108	1.09	11.72^{e}	0.226	1.93	6.56^{d}	0.074	1.13
Pro	6.41^{a}	0.184	2.87	5.47^{b}	0.043	0.78	5.61^{b}	0.099	1.77	5.33^{c}	0.060	1.11	6.17^{ab}	0.141	2.29	6.79^{d}	0.088	1.30
Gly	7.05^{a}	0.226	3.21	5.68^{b}	0.128	2.26	5.76^{b}	0.141	2.46	5.46^{b}	0.090	1.65	6.18^{c}	0.007	0.12	6.24^{c}	0.438	7.02
Ala	7.86^{a}	0.113	1.44	7.18^{b}	0.101	1.41	6.28^{c}	0.099	1.59	6.12^{c}	0.884	1.45	6.79^{d}	0.156	2.29	9.11^{e}	0.035	0.39
Val	7.89^{a}	0.113	1.43	6.77^{b}	0.128	1.90	6.22^{c}	0.007	0.11	5.99^{c}	0.177	2.96	6.60^{b}	0.071	1.07	7.03^{d}	0.127	1.81
Met	3.36^{a}	0.085	2.53	3.35^{a}	0.088	2.63	4.02^{b}	0.127	3.17	2.72^{c}	0.133	4.88	3.49^{d}	0.014	0.41	3.19^{e}	0.135	4.25
Ile	5.29^{a}	0.085	1.60	5.33^{a}	0.142	2.67	4.44^{b}	0.085	1.91	4.33^{b}	0.091	2.10	4.71^{c}	0.099	2.10	5.25^{d}	0.182	3.47
Leu	8.61^{a}	0.014	0.16	8.69^{a}	0.131	1.50	7.29^{c}	0.085	1.16	7.13^{c}	0.050	0.70	7.91^{b}	0.127	1.61	8.23^{e}	0.169	2.06
Tyr	3.90^{b}	0.226	5.80	8.32^{a}	0.097	1.17	3.65^{cb}	0.085	2.33	3.41^{b}	0.140	4.10	3.59^{e}	0.014	0.39	9.21^{d}	0.083	0.90
Phe	5.78^{a}	0.283	4.89	3.68^{b}	0.078	2.12	4.59^{c}	0.127	2.77	4.30^{d}	0.151	3.52	4.74^{c}	0.113	2.39	4.18^{cd}	0.136	3.26
His	4.41^{a}	0.099	2.25	2.75^{b}	0.095	3.45	3.65^{c}	0.057	1.55	3.41^{c}	0.156	4.57	3.50^{cd}	0.113	3.23	2.43^{e}	0.088	3.61
Lys	7.17^{a}	0.170	2.37	5.77^{b}	0.078	1.35	5.32^{c}	0.071	1.33	4.27^{b}	0.240	5.63	5.53^{c}	0.057	1.02	4.68^{d}	0.031	0.67
Arg	6.78^{a}	0.156	2.29	2.22^{b}	0.047	2.10	5.92^{c}	0.085	1.43	5.64^{d}	0.087	1.54	5.44^{e}	0.156	2.860	2.41^{e}	0.129	5.33
Cys	1.92^{a}	0.014	0.74	1.22^{b}	0.033	2.71	1.63^{c}	0.014	0.87	1.42^{d}	0.079	5.57	1.89^{a}	0.042	2.25	1.45^{d}	0.053	3.68

abcdef The values within the rows with different superscripts are significantly different at $P < 0.05$

IV: *The contents of amino acids in wilted matter and wilted silage (experiment No. 2) (mean ± S.D.)*

treatment	without additive						formic acid						biological additive					
amino acid g.kg^{-1} DM	wilted matter			wilted silage			wilted matter			wilted silage			wilted matter			wilted silage		
	mean	S.D.	CV%	mean	S.D.	CV%	mean	S.D.	CV%	mean	S.D.	CV%	mean	S.D.	CV%	mean	S.D.	CV%
Asp	16.11^{a}	0.368	2.28	5.82^{e}	0.215	3.70	15.92^{a}	0.255	1.60	15.57^{b}	0.117	0.76	15.43^{c}	0.113	0.73	6.08^{d}	0.057	0.93
Thr	5.23^{a}	0.042	0.81	2.71^{b}	0.156	5.77	5.21^{a}	0.028	0.54	5.12^{a}	0.093	1.82	5.37^{a}	0.071	1.32	2.78^{b}	0.079	2.85
Ser	5.64^{a}	0.113	2.01	2.68^{c}	0.145	5.42	5.44^{ab}	0.113	2.08	5.25^{b}	0.205	3.92	5.64^{a}	0.057	1.00	3.00^{d}	0.066	2.19
Glu	12.28^{a}	0.198	1.61	5.68^{b}	0.280	4.93	12.19^{a}	0.099	0.81	11.91^{c}	0.079	0.07	12.04^{a}	0.198	1,64	6.15^{d}	0.028	0.45
Pro	4.70^{a}	0.141	3.01	3.76^{b}	0.591	15.72	6.77^{c}	0.156	2.30	6.62^{c}	0.120	1.81	6.02^{d}	0.028	0.47	3.98^{b}	0.088	2.23
Gly	6.23^{a}	0.085	1.36	4.60^{b}	0.274	5.96	5.71^{c}	0.071	1.24	5.65^{c}	0.041	6.73	6.20^{a}	0.057	0.91	4.95^{d}	0.071	1.44
Ala	6.60^{b}	0.071	1.07	13.69^{c}	0.295	2.15	6.28^{a}	0.269	4.28	6.21^{a}	0.065	1.04	6.33^{ab}	0.099	1.56	15.38^{d}	0.093	0.63
Val	6.79^{a}	0.127	1.88	6.72^{ab}	0.121	1.79	6.55^{cb}	0.127	1.94	6.49^{c}	0.067	2.96	6.79^{a}	0.057	0.83	7.53^{d}	0.100	1.33
Met	3.94^{a}	0.057	1.44	2.13^{b}	0.099	4.66	3.88^{a}	0.099	2.55	2.92^{b}	0.113	3.88	3.94^{a}	0.028	0.72	3.34^{c}	0.081	2.42
Ile	4.90^{a}	0.071	1.44	4.84^{a}	0.053	1.10	4.79^{a}	0.057	1.18	4.71^{a}	0.061	1.29	4.74^{a}	0.071	1.49	5.63^{b}	0.053	0.94
Leu	8.37^{a}	0.127	1.52	7.48^{b}	0.106	1.42	8.11^{c}	0.410	5.06	7.47^{b}	0.068	0.90	8.34^{c}	0.085	1.02	8.61^{b}	0.062	0.72
Tyr	2.46^{a}	0.042	1.73	9.90^{b}	0.138	1.40	4.20^{c}	0.014	0.34	4.02^{c}	0.147	3.67	3.01^{d}	0.299	9.65	10.81^{e}	0.033	0.31
Phe	4.90^{ac}	0.028	0.58	4.33^{b}	0.161	3.71	4.95^{a}	0.099	2.00	4.80^{ca}	0.108	2.25	4.89^{ac}	0.170	3.47	4.13^{d}	0.058	1.41
His	3.39^{a}	0.042	1.25	4.07^{b}	0.075	1.84	3.35^{a}	0.113	3.38	3.21^{c}	0.099	3.07	3.39^{a}	0.085	2.50	3.34^{d}	0.037	1.11
Lys	5.91^{a}	0.071	1.20	2.95^{b}	0.181	6.14	5.26^{c}	0.071	1.34	5.16^{c}	0.100	1.93	5.90^{a}	0.255	4.32	3.47^{d}	0.056	1.61
Arg	6.26^{a}	0.057	0.90	2.87^{b}	0.186	6.50	5.75^{c}	0.085	1.48	5.54^{d}	0.186	3.35	6.23^{a}	0.269	4.31	3.03^{e}	0.043	1.41
Cys	1.78^{a}	0.219	5.35	1.22^{b}	0.046	3.74	1.63^{c}	0.014	0.87	1.29^{b}	0.042	3.27	1.65^{c}	0.028	1.71	1.43^{d}	0.056	3.91

^{abcde} – The values within the rows with different superscripts are significantly different at $P < 0.05$, s- standard deviation, v- coefficient of variation

without Val, Ile, Tyr and Ala ($P < 0.05$). In contrast, there were significant increases of Tyr and Ala. We observed losses of Glu, Met, Leu, His, Arg and Cys in silage preserved with formic acid significantly at $P < 0.05$. The amino acid content was significantly decreased in biological treated silage during fermentation out of the amounts of Ala, Val, Ile and Tyr.

Wilted silages had higher losses of Arg than unwilted silages. The significant decrease in Arg with wilting can also be explained by the fact that Arg is a storage amino acid that is readily mobilized by plant tissue during starvation periods. The observation of reduced Arg is in agreement with the findings of Makoni *et al.* (1993) and Givens and Rulquin (2004). A sharp decline in Arg concentration has been observed by Winters *et al.* (2000) during ensilage of grass. The decline of His content is connected with its transformation into histamine (Oshima and McDonald, 1978). The marked decrease of Lys in control silages was observed by Heron *et al.* (1986). Sneath *et al.* (1986) confirmed that lactic acid bacteria has generally limited ability to ferment amino acids.

Fairbairn *et al.* (1992) reported that the formic acid treatment had a positive effect on inhibition of amino acid degradation in ensiled alfalfa. Our results were in agreement with their survey.

SUMMARY

The goal of our paper was to found out the changes in amino acid profile, that are caused by wilting and supplement of chemical or biological additive. Experiments were carried out with fresh and wilted alfalfa, which were chopped to 20 mm length and ensiled in laboratory silos (150 cm^3). Fermentation process took 12 weeks at 20 – 23 °C. In both experiments, the second cut alfalfa (*Medicago sativa)* was ensiled as control variant (without preservative) and treated variants with biological additive consisted of *Lactobacillus rhamnosus*, *L. plantarum*, *L. brevis*, *L. buchneri* and *Pediococcus pentosaceus*, and chemical additive (formic acid as positive control). After opening each silo, silage samples were frozen at – 60 °C until analysis. Prepared silage filtrate was used for measure pH, assess fermentation products by capillary isotachoferetic method and ammonia (NH_3-N) by titration colorimetric method. The supernatant of centrifuged solution was analyzed after protein precipitation from silage filtrate on non-protein nitrogen (NPN) by micro-Kjeldahl method. The total nitrogen (TN) was also determined by using micro-Kjeldahl method and amino acid contents were estimated by chromatographic method in oven-dried forage and silage samples. The results of fermentation products, NPN, TN, NH_3-N and amino acid content were converted in to 100 % dry matter in both experiments. The rate of proteolysis was calculated as a percentage ratio of NH_3-N from amount of total nitrogen. Analysis of variance was used to test statistical significance of treatment. Means were separated using Fischer's protected least significance difference LSD ($P < 0.05$) when F- tests were determined to be significant. The va-

lues in the tables with different superscripts are significantly different at $P < 0.05$. During fermentation of fresh matter without additive, the amino acid profile was significantly decreased except for the amounts of Met, Ile, Leu and Tyr. Untreated silage had the lowest concentration of Arg and Ser than other silages. The Ser concentration decreased due to degradation to pyruvate by epiphytic microflora for a source of energy. The amounts of amino acids (degradation) were lower in silage preserved with formic acid than amino acid content of untreated silage. We found that degradation of Arg was significantly lower with a formic acid treatment compared to untreated silage. Interestingly, there was not a significant reduction in Tyr. The observation could be associated with the rapid decline of pH during the early phase of fermentation with formic acid, thus depressing bacterial activity. We observed that formic acid decreased the degradation of Ser, Glu, Arg and His, significantly at $P < 0.05$. Ensiling of fresh matter treated by biological additive results in increased concentration of Ala, branched chain amino acids and decrease in basic amino acids. Wilted silages had higher losses of Arg than unwilted silages. The significant decrease in Arg with wilting can also be explained by the fact that Arg is a storage amino acid that is readily mobilized by plant tissue during starvation periods. The results of the experiments indicated that amino acid breakdown was inhibited by increased dry matter and the use of chemical and biological additive. Additionally, the content of amino acids was found to change in relation to the degree of wilting and formic acid treatment yielded the lowest amino acid breakdown. The amino acid breakdown was also reduced by biological preservative especially in the silage with a higher level of dry matter content.

SÚHRN

Zmeny aminokyselinového spektra počas fermentačného procesu lucernových siláží konzervovaných chemickým a biologickým aditívom

V práci bol sledovaný vplyv chemického a biologického aditíva na zmeny aminokyselinového spektra lucerny siatej (*Medicago sativa*) v priebehu fermentačného procesu. Práca bola rozdelená do dvoch experimentov, kde bola silážovaná nezavädnutá a zavädnutá lucerna z druhej kosby s dĺžkou rezanky 20 mm. Silážna fermentácia trvala 12 týždňov v laboratórnom sile o objeme 150 cm^3 pri teplote 20–23 °C. Silážovali sa tri varianty v oboch pokusoch. Prvý variant bez použitia aditíva, druhý variant bol ošetrený kyselinou mravčou (chemické aditívum) a tretí variant s prídavkom biologického aditíva zloženého z *Lactobacillus rhamnosus*, *L. plantarum*, *L. brevis*, *L. buchneri* a *Pediococcus pentosaceus.* Po otvorení každého sila boli odobraté priemerné vzorky siláží a do realizácie ich rozboru boli uskladnené pri teplote – 60 °C. V pripravenom silážnom výluhu bolo merané pH a analyzovali sa fermentačné produkty izotachoforetickou metódou ako aj amoniak, ktorý bol zisťovaný titračnou kolorimetrickou metódou. Supernatant bol podrobený analýze na nebielkovinový dusík použitím mikro-Kjeldahlovej metódy, ktorý sa získal centifugáciou výluhu, z ktorého boli precipitáciou vyzrážané bielkoviny. Obsah dusíkatých látok bol stanovemý mikro- Kjeldalovou metódou z sušených vzoriek ako aj obsah aminokyselín. Stupeň proteolýzy bol vypočítaný ako percento amoniakálneho dusíka z obsahu dusíkatých látok. K štatistickej testácií rozdielov medzi jednotlivými variantmi bol použitý Fischerov LSD test. V priebehu fermentácie čerstvej lucerny bez ošetrenia, došlo k štatisticky preukaznému poklesu aminokyselín okrem Met, Ile, Leu a Tyr. Neošetrená siláž mala najnižšiu koncentráciu Arg a Ser v porovnaní s ostatnými silážami. Pokles koncentrácie Ser bol spôsobený jeho rozkladom na pyruvát epifytickou mikroflórou ako zdroj energie. Rozsah rozkladu aminokyselín bol nižší v siláži s prídavkom kyseliny mravčej ako v neošetrenej siláži. Rozklad Arg bol štatisticky preukazne nižší v siláži ošetrenej kyselinou mravčou v porovnaní so silážou bez ošetrenia. Navyše nebola pozorovaná štatisticky významná redukcia Tyr, čo môže byť spôsobené s prudkým poklesom pH v priebehu prvej fázy fermentačného procesu s kyselinou mravčou, ktorá znižuje bakteriálnu aktivitu. Kyselina mravčia znížila štatisticky preukazne straty Ser, Glu, Arg a His. Tieto aminokyseliny sú najčastejšie degradované bakteriálnou aktivitou. Pri silážovaní čerstvej hmoty ošetrenej s biologickým prípravkom došlo k nárastu obsahu Ala, rozvetvených aminokyselín a k poklesu bázických aminokyselín. V uvädnutej siláži boli pozorované vyššie straty Arg než v neuvädnutej siláži. Štatisticky preukazné zníženie obsahu Arg v priebehu uvädania je možné vysvetliť faktom, že Arg je zásobnou aminokyselinou, ktorá je k dispozícii rastlinnému telu v priebehu fázy hladovania. Výsledky experimentov naznačujú inhibíciu rozkladu aminokyselín vzrastom obsahu sušiny a použitím chemického a biologického aditíva. Zmeny v aminokyselinovom profile boli rôzne v závislosti od stupňa uvädania a ošetrenie kyselinou mravčou spôsobilo najnižší rozklad aminokyselín. Biologické aditívum napomohlo znížiť straty aminokyselín zvlášť v siláži s vyšším obsahom sušiny.

lucerna siata, siláž, silážne aditívum

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