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THE INFLUENCE OF INOCULATION ON CHEMICAL COMPOSITION, QUALITY AND PROTEOLYSIS IN SILAGES MADE FROM WHOLE MAIZE PLANT AND ALFALFA

SUMMARY

The paper presents the results for ensiling of the whole maize plant alone or in mixing with alfalfa. In this experiment maize FAO maturity group 600 and alfalfa at the end of butonization phase (wilted material with 390 gkg-1 dry matter), was used for ensiling. The experiment was organized as two-factorial (3□2), with three replications, where factor A was the different amount of alfalfa (A1=0%, A2=15%, A3=30%,) and factor B was inoculation (B1= no inoculant, B2= with inoculant). All silages after the treatment were compressed in plastic experimental siloses with volume of 60 dm^3. After 56 days the experimental siloses were opened and representative samples were taken for chemical analyses. Chemical composition and silage quality were analysed in the Laboratory for nutrition of domestic animals on the Faculty of Agriculture of the University of Belgrade. Statistical analysis was performed by software Statsoft (2006), where the analysis of variance examined the significance of the factors. Along with the increase of the share of alfalfa in silages, there was increase of crude proteins, lipids, cellulose, ash, pH value and production of ammonia, with the decreasing share of NFE ($p<0.05$). In treatments with inoculant increase in amount of lactic acid was noticed, as well as lower amount of ammonia nitrogen and acetic acid, which can be significant for aerobic stability of silage. All silages, with a slight variation in the point number, were ranked in the 1st quality class according to the Flieg method.

Keywords: maize plant, alfalfa, inoculants, silage, quality

INTRODUCTION

Maize and alfalfa are important for cattle nutrition throughout the world, and so are in Montenegro where area of their cultivation depends on climate, altitude and soil quality (Dubljević et al., 2013a, 2013b). High energy value of entire plant, cob and grain is being excellently supplemented with high protein content of alfalfa, why those two feeds are regularly combined in cattle diet (TMR – total mixed ratio), mainly in the form of silage of entire maize plant, alfalfa hay or haylage and maize grain flour (Dewhurst, 2013). This possibility

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can be used during the silaging of entire maize plant by adding certain amount of green or wilted alfalfa fourth or fifth cut. Technology of combining grains and legumes during silaging process was researched in number of experiments (Đorđević et.al, 2010a). Maize and alfalfa silaging enables better balancing of energy and protein but negative influence of alfalfa on non-degradable protein fractions is expressed (Slottner and Bertilsson, 2006). However total combined silage quality mainly stays unchanged which was determined by Koljajić et al. (1998) when 20 and 50% of wilted alfalfa was included in maize silage. Alfalfa share during silaging process mostly depends on available amounts which are low in fourth and fifth cut because of high temperatures and low precipitation during summer (Dinić et al., 2014). Therefore the choice of silaging time is chosen according to entire maize plant silaging optimal phase, and that is grain waxing phase (Đorđević et al., 2010b). In this case alfalfa nutritional value often can be lowered because it varies during growth (Peyraund et al., 2009; Božičković et al., 2015).

Inoculant usage became usual practice during silaging. Inoculants speed and direct fermentation in legume silage, while in maize silage they increase aerobic stability (Lynch et al., 2011; Đorđević et al., 2009a,b).

Goal of this paper was to research maize silaging possibility with addition of small amounts of wilted alfalfa (15 and 30%), in order to achieve better nutritional value of silage and maximal usage of all available feeds at the farm. In order to achieve better silage quality usage of inoculants was planned

MATERIAL AND METHODS

Whole maize plant (FAO maturity group 600) and alfalfa experiment was set as two-factorial (3×2; n=3), where A factor was amount of wilted alfalfa in silaging biomass (A1=0% A2=15% A3=30%), and inoculant was used as B factor (B1=without inoculant; B2=with inoculant). Inoculant used was BioStabil Plus and it contains homo-fermentative lactic acid bacteria (Enterococcus faecium and Bacillus plantarum) which intensifies and directs fermentation, as well as hetero-fermentative lactic acid bacteria (Bacillus brevis) whose products increase aerobic silage stability. For silaging 60 dm3 plastic containers closed with threaded lid were used.

Samples for laboratory analysis in order to determine the nutritional value and quality of silage were taken 56 days after ensiling. Chemical analysis of silage samples was performed in the laboratory of animal nutrition at the Agricultural faculty of the University of Belgrade. The parameters of the chemical composition were determined according to AOAC (2002) methods, the amount of lactic, acetic and butyric acids were determined by distillation method according to Wiegner (1926), the amount of ammonia nitrogen by a modified Kjeldahl's method (Dulphy and Demarquilly, 1981). The quality of silage was evaluated using the Flieg (Đorđević and Dinić, 2003) methods. Statistical analyse of obtained results was done with analysis of variance procedure with software package Statistica v.6. (Statsoft, 2006).

RESULTS AND DISCUSSION

Compared to starting material, whole maize plant silage (with and without inoculant) had couple of percent higher amount of moist, which can be explained by loss of volatile matters during drying of sample at 105ºC with the goal to determine amount of dry matter content (table 1). With the increase of alfalfa amount in silage, level of dry matter increased. In all silages determined amount of dry matter was higher than 350 gkg-1, while at the start extraction of juices was stopped and maximal control over butyric acid fermentation was achieved (Đorđević and Dinić, 2003).

Amount of crude protein was increased in silages along with the increase of alfalfa share ($p<0.05$). Silage containing 30% of wilted alfalfa had by 60% higher level of protein than silage made of whole maize plant. Silage that contained inoculant averagely contained significantly higher level of crude protein, which can be explained by lower level of proteolysis, e.g. low losses of volatile matters (NH3N), in lower pH value conditions.

Inoculation effect did not significantly influence the amount of fat, cellulose, nitrogen free extract (NFE) and mineral matters. However, with increase of alfalfa share, fat, cellulose and ashes level was significantly increased while NFE level dropped. Amount of crude fat in all silage was higher compared to starting sample, which was due to relative increase of same during the loss of volatile matters, also as result of lactic acid extraction (as non-volatile) by diethyl ether (Soxlet method) used for determining amount of crude fat (Đorđević et al., 2003).

Table 1. Chemical composition of starting material and silages, gkg^{-1} DM

Starting material	Inoculant	DM gkg	Proteins	Lipids	Cellulose	NFE	Ash
Whole maize plant (WMP)		358.26	74.52	60.71	168.35	649.58	46.84
Alfalfa (A)		388.30	216.47	61.04	252.18	345.10	125.29
Silages							
A_1 (100%WMP)	B_1 (-)	360.31	72.28	66.22	171.27	645.67	44.56
	B_2 (+)	363.07	76.56	57.27	164.42	656.51	45.24
A_2 (85%WMP+15%A)	B_1 (-)	365.35	95.31	71.32	186.22	589.82	57.33
	B_2 (+)	363.96	98.43	78.61	183.73	580.96	58.27
A_3 (70%WMP+30%A)	B_1 (-)	370.20	116.56	92.50	195.84	534.02	61.08
	B_2 (+)	375.11	120.23	80.18	198.11	540.93	60.55
Average for A_1		361.69c	74.42c	61.74c	167.84c	651.09a	44.90c
Average for A_2		364.66b	96.87b	74.96b	184.98b	585.39b	57.80b
Average for A_3		372.66a	118.40a	86.34a	196.98a	537.48c	60.82a
Average for B_1		365.29	94.72B	76.68	184.44	589.84	54.32
Average for B_2		367.38	98.47A	72.02	182.09	592.80	54.69
Significance for A		*	**	*	**	**	**
Significance for B		ns	*	ns	ns	ns	ns

ns – no significance; * ($p<0.05$); ** ($p<0.01$)

pH value varied in the interval 3,91 – 4,28, which secured stabile conditions for silage quality without additional (butyric) fermentation (table 2). According to McDonald (1991) for butyric fermentation high level of moist is needed (over 70%), as well as pH value over 4,5. With increase of alfalfa share amount of ammonia nitrogen increased. That can be explained by increase of alfalfa protein part whose characteristic is higher level of hydrolysis, which is one of the important specific traits of this species (Owens et al., 2002). Ammonia nitrogen in silage is main indicator of protein degradation; it is created as result of reaction between proteoyltic enzymes originating from plant cells and microorganisms, above all butyric clostridia. Ammonia presence in silage which does not contain butyric acid is result of plant enzyme reactions.

Increase of alfalfa share in silage did not significantly influence the production of lactic and acetic acid shown in absolute value. However, when alfalfa was added relative share of acetic acid increased which can be considered positive as it helps increasing the aerobic stability of silage. On the other hand, inoculant use influenced increase of lactic acid production and decrease of acetic acid production which helped achieve lower pH values.

To assess silage quality Flieg method was used, which takes relative share (percent) of lactic, acetic and butyric acid. This method is considered as most objective method for determining maize silage quality. According to used method all silages were appointed with highest (I) class, even with small variations in number of points (table 3).

Table 2. Parameters of biochemical changes in silages, gkg^{-1} DM

Silages	Inoculant	pH	NH3-N, gkg^{-1} N	Lactic acid	Acetic acid	Butyric acid
A_1 (100%WMP)	B_1 (-)	3.96	86.15	55.18	28.34	0.00
	B_2 (+)	3.91	87.42	54.87	18.46	0.00
A_2 (85%WMP+15%A)	B_1 (-)	4.14	103.11	52.75	42.57	0.00
	B_2 (+)	4.08	98.37	59.42	22.64	0.00
A_3 (70%WMP+30%A)	B_1 (-)	4.28	108.70	56.31	36.91	0.00
	B_2 (+)	4.25	105.18	55.06	24.35	0.00
Average for A_1		3.94c	86.78c	55.02	23.40	0.00
Average for A_2		4.11b	100.74b	56.08	32.60	0.00
Average for A_3		4.26c	106.94a	55.68	23.88	0.00
Average for B_1		4.13A	99.32A	54.75B	35.94A	0.00
Average for B_2		4.08B	96.99B	56.45A	21.82B	0.00
Significance for A		**	**	ns	ns	-
Significance for B		*	*	*	*	-

ns – no significance; * ($p<0.05$); ** ($p<0.01$)

Table 3. Relative ratio of acids (%) and silage quality by Flieg

Silages	Inoculant	Ratio of acids, %			Points	Quality class by Flieg
		Lactic	Acetic	Butyric		
A_1 (100%WMP)	B_1 (-)	66.07	33.93	0.00	49	I
	B_2 (+)	74.83	25.17	0.00	50	I
A_2 (85%WMP+15%A)	B_1 (-)	55.34	44.66	0.00	44	I
	B_2 (+)	72.41	27.59	0.00	50	I
A_3 (70%WMP+30%A)	B_1 (-)	60.41	39.59	0.00	46	I
	B_2 (+)	69.33	30.66	0.00	48	I

CONCLUSIONS

Whole maize plant in waxing grain phase represents good material for silaging as it contains a lot of fermenting carbohydrates which during fermentation give averagely 70% of lactic acid out of all acid amounts. When 30% of wilted alfalfa is added increase of crude protein share by 60% happens, while quality of the silage remains the same. When share of alfalfa is increased ammonia nitrogen amount increases which has to be taken in to consideration while balancing the diet for rumnivore as part of degradable proteins. Use of selected inoculants lead to increase of lactic acid and decrease of acetic acid amount which did not affect the silage quality.

Based on obtained facts it is recommended to add wilted alfalfa during maize silage (up to 30% of fresh mass), it will increase protein amount in silage, achieve better balance between energy and protein without significantly changing the silage quality. Use of inoculants for this type of silage is not significant for quality but can be significant for aerobic stability; therefore research should be continued in that direction.

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