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The effect of nitrate content in forage on quality of silage fermentation

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Introduction

Nitrate content in fresh herbage is one of the factors affecting fermentation in silage. Hein (1970) observed that ensiling of forages with low nitrate content often results in silages with high butyric acid contents. Butyric acid is an undesirable product of clostridia in silages indicating low silage nutritional quality (Pahlow et al., 2003). The effect of nitrate on butyric acid formation is derived from its degradation products. Nitrate undergoes reduction to nitrite which can be further converted to nitric oxide which is considered to be toxic for clostridia (Spoelstra, 1983). Therefore, crops high in nitrate decreases clostridial activity and, hence, butyric acid formation. The effect of nitrate content in fresh crops on butyric acid formation was summarized by Weissbach (1996). The summary shows high occurrence (78%) of butyric acid in silages made from crops low ($<10^5$) in epiphytic lactic acid bacteria (LAB) while containing <0.5 g NO_3 per kg dry matter (DM). In contrast, incidence of butyric acid in silages from crops with similarly low LAB count but containing >1 g NO_3 per kg DM was only 26%. Since it is common to use silage additives to improve or secure a proper ensiling process, it is interesting to study how different nitrate contents in fresh crops influence efficiency of silage additives. The objective of the study was, therefore, to study the effect of nitrite containing silage additives on silage quality with crops differing in nitrate content.

Material and Methods

Two types of crops were used representing high (Crop 1) and low (Crop 2) nitrate levels. Crop 1 which represented a mixture of perennial ryegrass (50%, vegetative stage), and red clover (vegetative stage, 50%) was fertilized with a manure slurry and harvested as a third cut on 16th of October. Crop 2 consisted of timothy (15%, head visible), perennial ryegrass (30%, vegetative stage), meadow fescue (16%, head visible), and red clover (vegetative stage, 39%). Crop 2 was cultivated without fertilizer and harvested as a first cut on 10th of June. Both crops were directly chopped in a stationary cutter to approx. 2 cm particle length. After chopping, both forages were mixed with a suspension of *Clostridium tyrobutyricum* spores at the rate of 10^5 per g fresh matter (FM) and partitioned into fractions. One forage fraction was left untreated and served as control and another fraction was treated with an additive mixture of 20% sodium benzoate, 10% potassium sorbate and 5% sodium nitrite at the rate of 3 L/t (fresh matter). The silage additive was applied by hand with a spray bottle on the forage which was spread out on a sheet of plastic film and mixed thoroughly. Forages from each fraction were then ensiled in lab-silos (1.7 L volume with water locks). Crops were ensiled according to the DLG design for testing efficiency of silage additives WR1 (DLG, 2009) with a compaction density of 100 kg DM per m^3. Each treatment consisted of 3 replicates. Silos were stored for 98 days in room temperature of 20˚C. Two samples of fresh crop prior to additive application were collected. Each sample was mixed and divided into 3 sub-samples; microbiological sample, chemical sample and reserve sample. Microbiological samples were analyzed for homofermentative and heterofermentative lactic acid bacteria (LAB), yeasts, moulds, enterobacteria and clostridia spores. Chemical analyses

determined DM, ash, total N, water soluble carbohydrates (WSC), metabolizable energy (ME), nitrate+nitrite, and buffering capacity. In addition, botanical composition of harvested crop and growing stage of plant were assessed.

At the end of storage, silo contents were emptied into separate plastic bags and mixed thoroughly. Extracted silage samples were analyzed for DM, volatile fatty acids, lactic acid, ethanol, pH, WSC, LAB, clostridia spores, yeasts and for aerobic stability by standard methods described by Knicky & Spörndly (2009).

Results and Discussion

Chemical and microbiological composition of the forages, prior to ensiling, are in Table 1. The application of slurry (Crop 1) resulted in high nitrate and CP contents, whereas absence of fertilization caused low nitrate and CP contents in Crop (2). The calculated fermentation coefficient (FC) of 26 indicates that the Crop (1) should be difficult to successfully ensile whereas the FC of Crop (2) of 38 characterized it as intermediate for ensiling purposes (Weissbach et al., 1974).

Table 1. Chemical and microbiological compositions of fresh forages (n=2)

Analyses	Unit	Crop (1)	Crop (2)
DM	%	18.5	19.9
Ash	%	11.8	9.5
CP	%	24.4	11.6
WSC	%	7.3	15.7
NDF	%	41.1	44.8
Nitrate-N	mg/kg DM	1467.6	2.1
Nitrite-N	mg/kg DM	1.9	2.1
ME	MJ/kg DM	10.9	11.1
Ammonia-N	% TN	-	1.2
Buffering capacity	g LA/100 g DM	7.5	7.1
LAB-homofermentative	log cfu/g FM	5.8	6.2
LAB-heterofermentative	log cfu/g FM	5.6	3.9
Clostridia spores	log cfu/g FM	3.8	3.8
pH		6.0	5.8
Fermentation coefficient		26	38

DM-dry matter; FM-fresh matter; CP-crude protein; WSC-water soluble carbohydrates; NDF-neutral detergent fiber; ME-metabolizable energy; TN-total nitrogen; LAB-lactic acid bacteria; cfu-colony-forming unit.

Table 2. Chemical composition of silages after 98 days of storage (n=3)

Treatment	DM	pH	NH_3-N*	NO_3-N	Lactic acid	Acetic acid	Butyric acid	2.3-butanediol	Ethanol	WSC
	%		% of TN	mg/kg DM	% of DM					
Crop (1)										
Control	18.1	4.2	6.3	868.4	9.8	2.6	0.1	0.05	0.7	0.10
Additive	18.6	4.1	5.4	1224.8	10.1	2.1	0.0	0.04	0.5	0.03
$LSD_{0.05}$		**0.05**	**0.91**	**170.0**	**1.54**	**0.31**	**0.09**	**0.01**	**0.03**	**0.17**
P-value		0.02	0.05	0.004	0.7	0.01	0.6	0.1	0.001	0.4
Crop (2)										
Control	18.1	4.5	10.9	1.0	9.3	2.6	1.7	2.9	2.0	0.7
Additive	19.4	4.1	4.9	18.8	11.6	1.4	0.0	0.1	0.4	6.4
$LSD_{0.05}$		**0.07**	**0.38**	**6.11**	**1.28**	**0.47**	**0.29**	**0.53**	**0.35**	**0.20**
P-value		0.001	0.001	0.001	0.01	0.002	0.001	0.001	0.001	0.001

* N.S. – Not significant. DM-dry matter; FM-fresh matter; TN-total nitrogen; WSC-water-soluble carbohydrates.

Table 3. Microbiological composition and aerobic stability of silages after 98 days of storage (n=3)

Treatment	Yeasts	Clostr. spores	LAB Homoferm.	Heteroferm.	Weight loss	Time (hours) until temp. aerated silages increased 3˚C	Max-temp	Max. temp-increase	pH after stability
	log cfu/g				% DM		(˚C)	(˚C)	
Crop (1)									
Control	-	2.4	5.1	7.9	2.8	210.5	31.6	11.0	5.0
Additive	-	2.5	5.3	7.7	2.0	262.0	21.9	0.9	4.5
$LSD_{0.05}$		**0.72**	**0.68**	**0.53**	**0.36**	**13.6**			**0.56**
P-value		0.9	0.6	0.4	0.01	0.001			0.6
Crop (2)									
Control	<1.7	4.6	<4.7	7.4	14.7	216.0	20.5	0.0	4.5
Additive	<1.7	1.7	<4.7	6.2	2.4	216.0	20.7	0.2	4.1
$LSD_{0.05}$	-	**0.19**	-	**0.45**	**0.76**	-			**0.07**
P-value	n.s.	0.001	n.s.	0.002	0.001	n.s.			0.001

* N.S. – Not significant. DM-dry matter; LAB-lactic acid bacteria.

Results from chemical and microbiological analyses of the silages are in Tables 2 and 3. As expected, low DM contents of the crops caused extensive fermentation. This was evidenced by low silage pH and high levels of fermentation products associated with a high depletion of WSC.

Additive treated silages had lower pH, lower concentration of acetic acid, ethanol and ammonia than control silages. Concentration of butyric acid was near the detection limit in all additive treated silages which confirms the efficiency of the present additive composition to eliminate clostridial activity in silage shown in previous studies (Knicky & Spörndly, 2009, 2011). Reduced formation of undesirable ensiling products such as butyric and acetic acid, ethanol and 2.3-butanediol were probably the reasons for lower silage losses in the additive treatments as compared to the control.

However, differences between additive and control treatments were not obvious in Crop (1). According to the fermentation coefficient, Crop (1) should be more difficult to ensile successfully and would, therefore, be expected, at least in the untreated control silage, to show signs of undesirable processes in comparison with Crop (2). However, results of the control silage from Crop (1) were not different for several silage parameters in comparison with the additive treated silage. This situation was likely associated with an abundance of nitrate in the fresh crop, resulting in nitric oxide production which eliminated clostridial activity and, hence, butyric acid formation (Spoelstra, 1983). In contrast, lack of nitrate in Crop (2) was reflected in a high clostridial activity in the control silage and a pronounced butyric acid and ammonia formation, and consequently high silage losses. Presence of butyric acid stabilized the control silage in Crop (2), whereas lack of butyric acid reduced aerobic stability of the control silage in Crop (1), compared to the additive treatment. A minor increase in concentration of nitrate in additive treated silages was probably the consequence of $NaNO_2$ addition, a component of the silage additive. It is assumed that the nitrate concentration increase was caused by conversion of added $NaNO_2$ to nitrate (McDonald et al., 1991).

Conclusions

Ensiling of the nitrate rich forage resulted in a good fermentation process, similar to treatment with the additive. In contrast, the quality of fermentation in the low nitrate forage was poor and lower ($P<0.001$) than the additive treated silage. Results verify earlier observations about the importance of appropriate nitrate content in forages for successful ensiling.

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