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Grass for biorefinery: effect of additive treatment on fermentation quality of ensiled intact grass and pulp

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Introduction

Novel business models are being developed to valorize green biomasses in multiple ways. The concept of green biorefinery involves mechanically separating grass into liquid and solid fractions. After the removal of soluble components by mechanical pressing, the pulp can be stabilized by ensiling which can be used *e.g*., as ruminant feed (Savonen *et al*., 2020). Furthermore, green biorefinery presents an alternative to utilizing grass with low dry matter (DM) content which can minimize the risk of effluent production and poor fermentation through increased DM concentration. The aim of the current experiment was to evaluate the preservation quality of ensiled pulp compared to intact grass, and how additives with different modes of action affect it.

Materials and Methods

The grass material was harvested on 15 June 2023 from a primary growth of pure timothy (*Phleum pratense*) stand grown in Jokioinen, Finland (60°48′N 023°29′E). The grass was mowed and precision chopped immediately after cutting. Growing conditions were cool and very dry during early summer. The fresh grass material was processed and separated into liquid and solid fractions (pulp) using a pilot scale single screw press (Smicon MAS SP300 filter press, Haderslev, Denmark). Water was added to the fresh grass material during the pressing process to "wash" out soluble components into the liquid due to its high DM content. The ensiling experiment was performed using both intact grass material (FR) and pulp obtained from the green biorefinery process which were ensiled using 3 additive treatments. Additive treatments consisted of: Control (without additive), a combination of homo- and heterofermentative lactic acid bacteria (LAB; Kofasil Duo, Addcon, Bitterfeld-Wolfen, Germany at 1 g/t [2×10^5 cfu / g fresh matter]) and a formic and propionic acid-based additive (FAPA; AIV Ässä Na, Eastman, Oulu, Finland at 5 l/t). Four replicates were used for each treatment, stored in plastic vacuum bags for 88 days. After opening, samples were analysed for fermentation quality, aerobic stability and chemical composition. The experimental data was analysed statistically using SAS MIXED procedure (SAS 9.4) with material type, additive and their interaction used as fixed effects and replicate as random effect. Tukey test was also done and statistical significance was considered at $P < 0.05$.

Table 1 Chemical composition of the intact grass and pulp materials before ensiling

Item	Fresh	Pulp
Dry matter (DM), g/kg	276	249
Buffering capacity, g lactic acid/100 g DM	4.89	3.90
Fermentation coefficient	55	58
In g/kg DM		
Ash	70	66
Crude protein	146	145
Water soluble carbohydrates	169	160
Nitrate-N	0.011	0.006
Neutral detergent fibre	492	549
Organic matter digestibility, g/g OM	0.837	0.834
D-value, g/kg DM	778	779

Table 2 Chemical composition and fermentation quality of ensiled intact grass and pulp treated without additive (Control), lactic acid bacteria (LAB) or formic and propionic acid based additive (FAPA)

Additive (Add)	Control		LAB		FAPA		SEM$^{1)}$	Statistical significance		
Material type (MT)	Fresh	Pulp	Fresh	Pulp	Fresh	Pulp		MT	Add	MT×Add
Dry matter (DM), g/kg	268^{b}	244^{c}	277^{ab}	242^{c}	279^{a}	249^{c}	2.2	<0.001	0.006	0.073
pH	4.05^{a}	3.74^{cd}	3.76^{cd}	3.92^{b}	3.81^{c}	3.71^{d}	0.018	<0.001	<0.001	<0.001
Ammonia N, g/kg total N	47.9^{a}	32.3^{bc}	33.6^{b}	33.4^{b}	28.3^{cd}	24.7^{d}	0.92	<0.001	<0.001	<0.001
In g/kg DM										
Ethanol	31.8^{b}	43.5^{a}	14.7^{c}	13.3^{cd}	9.6^{d}	8.9^{d}	1.12	0.002	<0.001	<0.001
Water soluble carbohydrate	50.4^{ab}	23.6^{c}	70.8^{a}	44.4^{bc}	43.8^{bc}	38.1^{bc}	5.75	<0.001	0.003	0.109
Lactic acid (LA)	66.5^{b}	97.3^{a}	105.5^{a}	65.3^{b}	63.1^{b}	59.9^{b}	2.65	0.069	<0.001	<0.001
Acetic acid (AA)	20.6^{bc}	15.8^{c}	25.3^{b}	47.1^{a}	26.8^{b}	27.0^{b}	1.53	<0.001	<0.001	<0.001
Propionic acid	0.06^{a}	0.04^{b}	0.10^{b}	0.04^{b}	0^{c}	0^{c}	0.008	0.002	<0.001	0.005
Butyric acid	2.46^{a}	0.05^{b}	0.04^{b}	0.04^{b}	0.06^{b}	0.05^{b}	0.053	<0.001	<0.001	<0.001
Total volatile fatty acids	23.2^{b}	15.9^{c}	25.4^{b}	47.2^{a}	26.8^{b}	27.0^{b}	1.52	0.001	<0.001	<0.001
Total fermentation acids	89.7^{c}	113.2^{b}	130.9^{a}	112.5^{b}	89.9^{c}	86.9^{c}	3.53	0.804	<0.001	<0.001
Total fermentation products	121.5^{b}	156.8^{a}	145.5^{a}	125.7^{b}	99.5^{c}	95.9^{c}	2.91	0.113	<0.001	<0.001
LA to AA ratio	3.42^{b}	6.16^{a}	4.20^{b}	1.40^{d}	2.36^{c}	2.23^{cd}	0.211	0.709	<0.001	<0.001
Aerobic stability, 2°C, h$^{2)}$	87.6^{b}	41.6^{b}	171.7^{a}	155.8^{a}	171.7^{a}	171.7^{a}	10.80	0.025	<0.001	0.112
Aerobic stability, 3°C, h$^{2)}$	101.0^{b}	92.0^{b}	171.7^{a}	171.7^{a}	171.7^{a}	171.7^{a}	15.47	0.815	<0.001	0.945

^{a,b,c,d,}Values within a row with different superscripts differ significantly at $P < 0.05$

$^{1)}$SEM, standard error of the mean

$^{2)}$The length of the observation period was 171.7 h.

Results and Discussion

The chemical composition of the fresh grass and pulp are in Table 1. Due to dry growing conditions, DM content of FR was relatively high while addition of water during the liquid-solid separation reduced DM in the pulp by 10% but with a similar crude protein (CP) concentration. We expected the pulp to be drier with lower CP than FR, but the press used was of low efficiency. Both materials were regarded as easy to ensile due to a high value of the fermentation coefficient which was above 45 (threshold for easy to ensile raw material). The high NDF concentration in the pulp was expected due to removal of solubles during mechanical processing (Franco *et al*., 2019) while the D-value was unaffected. The proportion of ammonia N in total N of all silages was below 50 g/kg N which indicates that they were all fermented. FR Control showed elevated butyric acid concentration, which was eliminated by additive treatments and pulping. Ethanol concentration was elevated in Control compared to other additive treatments, and even higher in pulp than FR. LAB decreased lactic acid production on pulp compared to Control while an opposite effect was observed in FR.

Conclusions

Ensiling of pulp was not markedly impaired compared to fresh grass indicating that ensiling is a viable approach to stabilize the solid fraction of green biomass after removal of solubles. In case of no additive use, pulp even improved fermentation quality as indicated by elimination of butyric acid formation. When treated with LAB, the pulp had a high acetic acid concentration. FAPA produced consistently good fermentation quality in both raw materials. Use of both additives improved the aerobic stability of the silages.

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