Changes in maize silage fermentation products during aerobic deterioration and effects on dry matter intake by goats

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Chemical and microbiological changes occurring during aerobic exposure of maize silages and their influence on dry matter (DM) intake and preference by goats were evaluated. Eight maize silages differing in DM content, chopping length and compaction pressure were used for the study. After opening, silages were exposed to air for 8 days (d). In 2-d intervals, silage was stored anaerobically for use in preference trials. During the experimental phase, each possible two-way combination of the five silages (d0, d2, d4, d6 and d8) and one standard lucerne hay, was offered as free choice to six goats. Generally, a significant decline occurred in DM intake after 4 d of aerobic exposure. After 8 d, mean decrease in intake was 53% in comparison to the fresh silages. Preference when expressed as DM intake was negatively correlated to silage temperature (as difference to ambient), ethanol and ethyl lactate.

Key words: forage, preference trial, ruminant, volatile organic compound

Introduction

Maize is used as a major forage source for ruminants due to its high yields, nutritional value and good ensiling properties (Allen et al. 2003). Maize silage (like all silages) deteriorates on exposure to air, as a result of aerobic microbial activity (Jonsson 1989). Well preserved silages without butyric acid and low contents of acetic acid are particularly susceptible to aerobic deterioration (Cai et al. 1999).

Aerobic deterioration is a significant problem affecting profitability and feed quality throughout the world (Tabacco et al. 2011). Caused by the activities of bacteria, yeasts and moulds, there are changes in the chemical composition of the silage (Lindgren et al. 1985) with resulting loss of dry matter (DM) and nutritional components like residual sugars, lactic acid, acetic acid and ethanol that are used as substrates for oxidation. Additionally, there is an increasing risk of proliferation of potentially pathogenic or otherwise undesirable microorganisms. Mycotoxin-producing moulds, *Bacillus cereus* and *Listeria monocytogenes*, for example, can pose serious threats to the quality and safety of milk and animal health (Driehuis and Oude Elferink 2000).

It has long been believed that aerobic deterioration depresses DM intake (DMI), caused by an accumulation of degradation products (Lindgren et al. 1988) or changes in volatile compounds. Data on the effects of volatile compounds like alcohols, acids, esters, aldehydes and ketones on DMI or product quality (e.g. carry-over to milk) are insufficient (Kalač 2010). Silages that have been exposed to air for several days led to a decrease in roughage intake of about 10–20% in comparison to fresh silage (Wichert et al. 1998, Bolsen et al. 2002). However, in most studies no indication was given as to which substances or properties of the silages were responsible for selective feeding or at which point of deterioration the decline in DMI began.

The aim of the present study was to determine the changes taking place during eight days of aerobic exposure of maize silages and to characterize the effect on DMI and preference by goats.

Manuscript received August 2012

Materials and methods

Silage preparation and experimental design

Maize (*Zea mays,* dual-purpose hybrid 'Amadeo', KWS Saat AG, Einbeck, Germany) was planted on May 5, 2009, at a planting rate of 110,000 plants ha^{-1} at the research station Frankenforst of University of Bonn (Königswinter, Germany, 7°12´E and 50°42´N; 2009 average temperature 10.6 °C, annual precipitation 690 mm, average humidity 71.4%). Before planting, the soil was fertilized with about 25 m^3 ha^{-1} of swine manure, and at planting, 200 kg ha^{-1} of diammonium phosphate was applied. At May 29, 2009, 5 l ha^{-1} of Zintan Gold Pack (active components: terbuthylazine, metolachlor and mesotrione; Syngenta AG, Basel, Switzerland) was applied as herbicide. Maize was harvested as whole-crop (cutting height 20 cm) and chopped at two stages of maturity in 2009 (September 9 and 24). The study was arranged in a 2 × 2 × 2 factorial design consisting of DM content (33 and 40% DM), chopping length (10 and 21 mm) and packing density (compaction pressure 0.1 and 0.2 MPa) in the silo (Table 1).

Table 1. Details about the silage treatments used in the trials

Trial	DM (%)	Chopping length (mm)	Compaction pressure (Mpa)	Abbreviation of treatment	Month of opening	Temperature (°C)
1	33	10 (short)	0.2 (high)	S33hi	Feb 2010	13
2	40	10 (short)	0.2 (high)	S40hi	Feb 2010	13
3	33	10 (short)	0.1 (low)	S33lo	Apr 2010	17
4	40	10 (short)	0.1 (low)	S40lo	Apr 2010	17
5	33	21 (long)	0.2 (high)	L33hi	Jun 2010	22
6	40	21 (long)	0.2 (high)	L40hi	Jun 2010	22
7	33	21 (long)	0.1 (low)	L33lo	Aug 2010	22
8	40	21 (long)	0.1 (low)	L40lo	Aug 2010	22

DM = dry matter, temperature = mean ambient temperature during eight days of aerobic exposure

After harvesting, each crop was immediately ensiled in six 120-l plastic barrels (48 barrels in total; mean density at low and high compaction pressure 235 and 270 kg DM m^{-3}, respectively) and stored anaerobically for at least three months. In February 2010, the barrels containing the first two treatments were opened; the silages were taken out, silage from all six barrels was stirred completely for homogenization and stored aerobically on a heap (ground area 3 m × 3 m) for eight days. The aerobic exposure trials were conducted indoor with a continuous measurement of ambient temperature (data logger 175-T1, Testo AG, Lenzkirch, Germany). At the day of opening (d0) and at two-day intervals (d2, d4, d6 and d8 after opening), temperature of the silages was measured at three different points (middle, left, right) at a depth of 20 cm using a digital probe thermometer (TFA Dostmann GmbH & Co KG, Wertheim, Germany). Aerobic stability was defined as the number of days the silage remained stable before rising more than 3.0 K above the ambient temperature (Honig 1990). For chemical analyses, a composite sample (1000 g) of each homogenized silage was taken at the respective sampling days and frozen immediately (−18 °C).

For the preference trials with goats, silage samples from each day of the aerobic exposure (d0, d2, d4, d6 and d8) were stored anaerobically in polyethylene bags (170 µm, 400 mm x 600 mm, Innovapac GmbH, Durach, Germany) and sealed with a chamber vacuum-packing machine (MAX-F 46, Helmut Boss Verpackungsmaschinen KG, Bad Homburg, Germany). A single bag filled with about 1.5–2.0 kg silage was offered to each goat per meal. Bags were stored in a dark, dry and cool room (15 °C) until used in the preference trial. Storage time of the silages in the vacuum bags ranged from five to 26 days depending on the day when fed.

Preference trials

For each of the eight silage treatments, a preference trial was done at the Institute of Animal Science, University of Bonn, starting in February 2010 (trial 1 and 2), May 2010 (trial 3 and 4), June 2010 (trial 5 and 6) and August 2010 (trial 7 and 8). All trials were conducted with a total of twelve Saanen wethers (German Improved White Goat breed, mean (± SD) body weight 85.8 kg ± 13.9 kg), that were divided into two groups (six goats per group) to conduct two trials concurrently. Goats were allocated to groups such that average body weight was the same. Two animals shared an indoor pen of approximately 2 m × 3 m bedded with straw. They were tied up for the duration time of experimental feeding with the possibility of lying down and accessing water and salt-licks.

Preference trials were carried out according to Buntinx et al. (1997). Each trial started with an adaptation period (Kyriazakis et al. 1990), where single meals of each silage (d0–d8) and lucerne (*Medicago sativa* L.) hay as standard forage were offered to the animals to associate the silage with postingestive metabolic response, taste and smell produced by the forage. The adaptation period lasted six days and forages were offered in a randomized order. The standard forage was used to compare the different trials. During the experimental phase, each possible 2-way combination of the five aerobic stability treatments and the standard forage (n = 15) was presented to each of the six goats. Each forage was offered in a plastic feeding box and the silage pairs were presented side by side. The order of presentation of the pairs and the left-right position of the silages in the pair were randomized in all trials. The weight of the silages was determined before, 30 min after offering and after feeding to calculate the initial and total DMI after 3 h. During all trials, consumption of total amount of one preferred type was prevented; so there was always a choice between the two forages in the pair. This was guaranteed by offering additional material as soon as the silage fell below 300 g. Each trial lasted 21 days, consisting of six days for adaptation and 15 days for the experiment. Each day, the experimental meal was offered for 3 h, starting at 0730 h. Grass hay was offered for *ad libitum* consumption at 1530 h and removed the following morning at 0700 h.

For laboratory analyses, a subsample (1000 g) of each treatment and each stage of aerobic deterioration (d0–d8) was taken out of the polyethylene bag and frozen immediately at the end of each preference trial.

Laboratory analyses

General analyses

The silage samples were freeze-dried (Jumo Imago 500, Jumo GmbH & Co KG, Fulda, Germany) in triplicate replicates. The DM of the silages was then estimated by oven-drying of a duplicate subsample at 105 °C overnight. A correction of DM (DM_{cor}) for the loss of volatiles during drying was conducted according to Weißbach and Strubelt (2008) using the following equation:

DM_{cor} = DM + 0.95 × sum of fatty acids (C2−C6) + 0.08 × lactic acid + 0.77 × 1,2 propanediol + 1.00 × other alcohols (C2−C6 including butanediol) [g kg^{-1}].

Proximate analyses were done according to the German Handbook of Agricultural Research and Analytic Methods (VDLUFA 2012) and method numbers are given. Ash and crude lipids (CL) were analysed using methods 8.1 and 6.1.1., respectively. Crude protein (CP) was determined by Dumas combustion (4.1.2, FP328, Leco 8.1, Leco Instrumente GmbH, Mönchengladbach, Germany).

Neutral detergent fibre (aNDFom, 6.5.1; assayed with heat stable amylase), acid detergent fibre (ADFom; 6.5.2) and acid detergent lignin (ADL, 6.5.3) were analyzed using Ankom 2000 Fiber analyzer (Ankom Technology, Macedon, USA). The aNDFom and ADFom values are expressed exclusive of residual ash.

The Hohenheim gas test (VDLUFA 2012, method 25.1) was conducted for measuring the 24-h *in vitro* gas production (GP) and estimating the content of metabolizable energy (ME) using the equation of Menke and Steingass (1987):

ME (MJ kg^{-1} DM) = 0.136 × GP (ml 200 mg^{-1} DM) + 0.0057 × CP (g kg^{-1} DM) + 0.000286 × CL^2 (g kg^{-1} DM) + 2.20.

Starch was quantified after enzymatic hydrolysis of starch to glucose as described by Brandt et al. (1987).

Chemical analyses of fermentation products

A subsample (50.0 g) of each silage was used for determination of lactic acid, pH, volatile fatty acids, alcohols (methanol, ethanol, propanol, 1,2 propanediol, 2,3 butanediol), acetone, ammonia and water-soluble carbohydrates (WSC). Furthermore, silages were also analysed for two ethyl esters; ethyl lactate and ethyl acetate.

Cold-water extracts were prepared by blending the frozen samples with a mixture of 300 ml distilled water and 1 ml toluol, kept overnight in a refrigerator and afterwards filtered using a folded filter paper. Determination of pH in the extract was done potentiometrically by using a calibrated pH electrode. Lactic acid was analyzed by HPLC (RI-detector, Shimadzu Deutschland GmbH, Duisburg, Germany) according to Weiß and Kaiser (1995). Volatile fatty acids, alcohols and esters were determined by gas chromatography (flame ionisation detector, Shimadzu) as described by Weiß (2001). Ammonia concentration was analysed colorimetrically based on the Berthelot reaction using a continuous flow analysator (Skalar Analytical B.V., Breda, Netherlands). Concentration of WSC was determined by anthrone method according to von Lengerken and Zimmermann (1991).

Microbiological analyses

At the day of silage opening (d0) and at the fourth (d4) and eighth day (d8) of aerobic exposure, samples of each silage treatment were taken for determination of microbiological status. A composite sample (500 g) was taken using sterile gloves and polyethylene bags, then sealed anaerobically, cooled immediately and sent directly to a laboratory (Wessling Laboratorien GmbH, Altenberge, Germany), where all microbiological analyses were conducted the next morning. Aerobic mesophilic bacteria, yeasts and moulds were determined according to VDLUFA (2012, method 28.1.1-28.1.4). All microbial counts were log10-transformed to obtain log-normal distributed data and presented on a wet weight basis. The values below detection level were assigned as value corresponding to half of the detection level to calculate the averages (Tabacco et al. 2009).

Statistical analyses

All data were analyzed using SAS 9.2 (SAS Institute Inc., Cary, North Carolina, USA). The experimental design allowed statistical analysis by multidimensional scaling (Buntinx et al. 1997) and by traditional analyses. Multidimensional scaling (MDS) is used to develop a spatial arrangement representing the differences expressed as selective forage intake by the animals. For MDS, the difference in preference between a pair of silages was expressed by subtracting the amount of the least preferred forage from the most preferred forage and dividing the difference by the sum of both intakes. In this way, preference was expressed numerically as a relative difference or distance. If an animal consumed equal quantities in one pair, the difference ratio is equal to zero and no preference or distance between the silages was expressed. If only one of the pairs was consumed, the difference ratio is equal to one and the maximum difference in preference between forages is expressed (Buntinx et al. 1997). PROC MDS is an iterative fitting procedure for data with the aim to express distances or relative differences between stimuli (e.g., forages) in an unknown number of orthogonal dimensions, as described by Burns et al (2001). A least squares fit is approximated using an array of points representing the different stimuli. The coordinates of the points are adjusted iteratively until the reduction in residual sum of squares is below a specified level. The residual sum of squares is calculated by comparing the “distance” between the points representing the stimuli and the observed distances or differences between the stimuli. Subsequently, a map is developed with points representing each stimulus. (Burns et al. 2001). Forages with coordinates that are similar in the dimensional space are modelled as similar in preference and, conversely, coordinates being far-of from each other in the dimensional space indicate forages differing in preference (Buntinx et al. 1997). The order of fit is dimension one first, which will generally include the most important variables (most sums of squares), followed by dimension two (Burns et al. 2001).

Each preference trial was also tested by analysis of variance after averaging DMI of each forage (averaged across each combination, n = 6). The analysis of variance only included terms for animal and forage. Within the forage treatments, means were separated using the minimum significant difference (MSD) from the Waller-Duncan k-ratio t-test (Burns et al. 2001). Simple linear regression was used to examine the relationship between DMI and silage temperature during the days of aerobic exposure (expressed as difference to ambient temperature). Furthermore, correlation coefficients between silage composition and DMI were calculated. Significance was defined at $p<0.05$, whereas a trend towards a significant effect was noted when $0.05 \leq p \leq 0.10$.

Results

Composition of silages

The chemical composition of whole-crop maize before ensiling is shown in Table 2. Results of chemical composition ranged within expected values.

Table 2. Chemical composition of experimental maize crops before ensiling

Treatment	DM	Ash	CP	CL	aNDFom	ADFom	ADL	ME
	g kg^{-1}	g kg^{-1} DM						MJ kg^{-1} DM
S33	339	35	71	26	409	198	28	10.3
L33	341	37	79	30	379	218	21	10.5
S40	374	32	72	35	330	182	27	10.1
L40	367	39	78	33	329	173	26	10.3

DM = dry matter, S = short chopping length, L = long chopping length, 33 = 33% DM, 40 = 40% DM, CP = crude protein, CL = crude lipids, aNDFom = neutral detergent fibre assayed with heat stable amylase and expressed exclusive residual ash, ADFom = acid detergent fibre expressed exclusive residual ash, ADL = acid detergent lignin, ME = metabolizable energy

When the barrels were opened, all silages were free of visible moulds or signs of malfermentation. The chemical composition of the eight silages is given in Table 3.

Table 3. Chemical composition of silages (g kg^{-1} DM unless otherwise stated) at silo opening, lucerne hay (standard forage) and grass hay (fed for *ad libitum* intake in the afternoon)

	Silage								Lucerne	Grass
Variable	S33lo	S33hi	L33lo	L33hi	S40lo	S40hi	L40lo	L40hi	hay	hay
Dry matter (g kg^{-1})	317	330	315	340	392	379	398	391	908	909
Ash	37	36	35	32	37	33	34	36	91	76
Crude protein	78	76	75	78	72	71	71	77	153	93
Crude lipids	31	26	24	33	24	29	35	32	27	16
aNDFom	384	373	333	357	397	345	302	341	464	592
ADFom	206	214	198	209	231	201	173	194	346	352
ADL	25	27	30	26	35	35	24	27	83	52
24-h gasproduction (ml g^{-1} DM)	299	282	290	302	276	288	306	292	225	221
ME (MJ kg^{-1} DM)	11.1	10.5	10.7	11.2	10.3	10.7	11.3	10.9	9.4	8.3
Starch	351	392	374	383	433	421	362	426		
pH	3.9	3.9	3.9	3.9	4.0	4.0	4.0	4.0		
Lactic acid	59	63	68	56	57	51	54	54		
Acetic acid	16	15	17	14	11	11	13	9		
Butyric acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
Ethanol	7.4	6.4	7.6	5.5	4.7	6.0	8.1	5.9		
Ethyl acetate (mg kg^{-1} DM)	347	479	173	273	138	400	157	177		
Ethyl lactate (mg kg^{-1} DM)	138	157	180	116	176	184	181	161		
NH_3-N (g kg^{-1} of total N)	72	66	100	80	96	79	97	90		
WSC	18	17	20	27	13	9	8	18		
Yeasts (log10 cfu g^{-1})	4.5	3.8	4.3	4.5	5.3	4.6	3.8	5.5		
Moulds (log10 cfu g^{-1})	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4		
Aerobic mesophilic bacteria (log10 cfu g^{-1})	5.5	5.0	3.7	5.6	4.5	5.0	3.4	4.7		

S = Short chopping length, L = Long chopping length, 33 = 33% DM, 40 = 40% DM, lo = low packing density, hi = high packing density, n.d. = below detection limit (0.03% fresh matter), aNDFom = neutral detergent fibre assayed with heat stable amylase and expressed exclusive residual ash; ADFom = acid detergent fibre expressed exclusive residual ash, ADL = acid detergent lignin, ME = metabolizable energy, Butyric acid = iso-butyric acid + n-butyric acid, iso-valeric acid + n-valeric acid + n-caproic acid, WSC = water-soluble carbohydrates, cfu = colony-forming units

All silages were well fermented with lactic acid concentrations ranging between 51 and 68 g kg^{-1} DM, moderate levels of acetic acid and no butyric acid. Regarding proximate constituents and fibre fractions, all values were within expected ranges. Ethyl acetate and ethyl lactate could be detected in all silages at concentrations ranging from 138 to 479 mg kg^{-1} DM and 116 to 184 mg kg^{-1} DM, respectively.

Silage samples from each day of aerobic exposure (d0–d8) were analyzed and chemical composition is shown in Table 4. Regarding the concentration of fermentation variables, strong changes occurred during the aerobic exposure. Degradation of lactic acid and acetic acid ($p<0.001$) led to elevated pH value (3.9 to 5.8). Mean content of ethanol and WSC decreased during the eight days of aerobic exposure ($p<0.001$). In contrast, concentration of other compounds increased or emerged from below detection limit (propionic acid, iso-butyric acid, iso-valeric acid).

Microbiological analysis

At opening, all silages had low concentrations of yeasts, moulds and aerobic mesophilic bacteria (Table 4). Under aerobic conditions, a rapid development of yeasts occurred resulting in high concentrations at d4 and d8. The stagnation after d4 can be explained by the standard method of analysis that did not allow yeast counts exceeding 2×10^7 cfu g^{-1}. Growth of moulds started later and was limited to long cut silages. At d8 of aerobic exposure, it has passed over the orientation value of 5×10^3 cfu g^{-1}. Short cut silages did not contain numbers of moulds that exceeded orientation values. A similar development was noted in the numbers of aerobic mesophilic bacteria, that were also mainly restricted on the long cut silages.

Table 4. Composition of silages during eight days (d0–d8) of aerobic exposure, (g kg^{-1} DM unless otherwise stated; n = 8)

	d0	d2	d4	d6	d8	SE
Dry matter (g kg^{-1})	360	366	371	389	395	14
Ash	35	37	35	35	35	0.7
Crude protein	75	73	76	75	76	1.8
aNDFom	354	370	358	356	362	11.9
ADFom	203	209	217	208	206	10.3
WSC	17^a	18^a	15^a	9^b	11^b	1.6
Starch	387	399	408	438	434	16.6
24-h gasproduction (ml g^{-1} DM)	292	293	292	288	285	3.0
ME (MJ kg^{-1} DM)	10.8	10.8	10.9	10.7	10.6	0.1
Lactic acid	58^a	61^a	49^a	15^b	8^b	3.3
Acetic acid	13^a	12^a	9^b	6^b	3^b	1.1
iso-butyric acid	n.d.	n.d.	n.d.	0.4	0.4	0.1
n-butyric acid	n.d.	n.d.	n.d.	n.d.	n.d.	0
iso-valeric acid	n.d.	n.d.	n.d.	0.6	n.d.	0.1
n-valeric acid	n.d.	n.d.	n.d.	n.d.	n.d.	0
n-caproic acid	n.d.	n.d.	n.d.	n.d.	n.d.	0
Propionic acid	n.d.	n.d.	n.d.	0.1	0.5	0
Ethanol	6.2^a	5.5^a	4.3^b	0.6^c	0.1^c	0.4
Methanol	n.d.	n.d.	n.d.	n.d.	n.d.	0
Acetone	n.d.	n.d.	n.d.	n.d.	n.d.	0
NH_3-N (g kg^{-1} of total N)	83	99	73	62	55	7.8
Ethyl acetate (mg kg^{-1} DM)	284^a	221^a	114^b	7^c	n.d.^{c}	46
Ethyl lactate (mg kg^{-1} DM)	159^a	126^a	73^b	10^c	n.d.^{c}	10
pH	3.9^c	4.0^c	4.2^b	5.4^a	5.8^a	0.2
Yeasts (log10 cfu g^{-1})	4.6^b	n.a.	7.2^a	n.a.	7.3^a	0.9
Moulds (log10 cfu g^{-1})	2.4^b	n.a.	2.8^b	n.a.	4.2^a	0.5
Aerobic mesophilic bacteria (log10 cfu g^{-1})	4.7^c	n.a.	5.7^b	n.a.	6.7^a	0.7

^{a,b}Mean values within rows having different superscripts differ ($p<0.05$), n.d. = below detection limit (0.03% fresh matter), aNDFom = neutral detergent fibre assayed with heat stable amylase and expressed exclusive residual ash, ADFom = acid detergent fibre expressed exclusive residual ash, WSC = water-soluble carbohydrates, ME = metabolizable energy, n.a. = not analyzed.

Some differences were observed when comparing fresh silages and samples that had been stored in vacuum bags for use in preference trials. Vacuum-sealed silages contained more ethanol, ethyl lactate and ethyl acetate (p<0.01), possibly due to anaerobic yeast activity (data not shown). For calculation of correlation coefficients between silage characteristics and DMI in preference trials, data of vacuum-stored samples were used.

Temperature

Differences in silage temperature during aerobic exposure are shown in Table 5. Because a constant ambient temperature could not be provided exactly during all trials (see Table 1), silage temperature is expressed as difference to ambient temperature (ΔT). In most silages, a strong increase of ΔT was measured between d4 and d6 after opening, three of them heated between d2 and d4 in accordance to their high number of yeasts (long cut silages). Only one silage treatment (S33lo) kept a constant temperature for more than four days. All other silages were already aerobically instable at the fourth day of aerobic exposure, which means they showed an increase in temperature of more than 3.0 K above ambient temperature.

Table 5. Silage temperature (expressed as difference to ambient temperature TΔ, in K) during eight days (d0–d8) of aerobic exposure

Silage treatment	d0	d2	d4	d6	d8
S33lo	-1.5	-1.8	1.0	20.5	22.4
S33hi	0.3	1.3	4.3	22.7	22.7
L33hi	-2.0	0.6	13.7	12.4	28.2
L33lo	0.9	1.3	16.0	26.5	35.0
S40lo	-1.5	-1.6	4.7	21.2	33.2
S40hi	0.1	1.2	4.3	21.8	33.1
L40hi	-1.7	0.0	16.4	19.2	31.1
L40lo	0.9	0.5	6.6	15.3	23.7

S = Short chopping length, L = Long chopping length, 33 = 33% DM, 40 = 40% DM, lo = low packing density, hi = high packing density.

Animal preference and dry matter intake

The results of MDS showed that selection between forages was associated with two dimensions. The coordinates for the different silages from all preference trials are shown in Table 6.

Exemplarily, the results for one trial with 30-min and 3-h DMI are depicted in Figure 1. A forage with two positive coordinates is generally strongly preferred while two negative coordinates give evidence for strong avoidance (Burns et al. 2001). For the given trial, there was a strong preference for d0 (located in upper right sector in Figure 1), while lucerne hay and d8 were avoided (located in lower left-hand sector). The others (d2, d4 and d6) had one negative dimension and were generally of intermediate preference. The pattern for 30 min and 3 h is very similar, for most treatments values lying close together or at least within one quarter. During all trials, d0 was highly preferred in five and d2 in seven cases. d8-silages were highly avoided in four of eight trials and never preferred.

According to the MSD calculated with the Waller-Duncan k-ratio t-test, DMI did not differ between silages from d0, d2 and d4 but decreased at d6 (p<0.001) in six trials. In all trials, DMI was lowest for d8-silages (p<0.001). In the trial with silage L40hi, DMI decreased after two days of aerobic exposure (p<0.001). In contrast, DMI for S33lo was constant for silages d0–d6; only dropping significantly at d8 (p<0.001). Intake of lucerne hay was at the same level as fresh silages. All goats were of good health throughout the study.

Regression analysis showed that 3-h DMI by goats (y) was negatively related to ΔT during aerobic exposure, which is shown graphically in Figure 2.

Table 6. Dry matter intake and stimulus coordinates for the two-dimensional solution to the preference among goats, n = 40

Silage treatment		d0	d2	d4	d6	d8	Lucerne hay	Mean d0–d8	MSD
S33lo	Meal (3 h), g	651^{a}	657^{a}	650^{a}	625^{a}	464^{b}	575^{a,b}	609	118
	Meal (30 min), g	345$^{a, b}$	338$^{a, b}$	332$^{a, b}$	365^{a}	264^{b}	305$^{a, b}$	329	99
	Dimension 1	0.82	-0.49	1.38	0.60	-2.0	-0.31		
	Dimension 2	1.41	0.64	-0.09	-1.46	0.0	-0.50		
S33hi	Meal (3 h), g	650^{a}	610^{a}	633^{a}	380^{b}	136^{c}	680^{a}	481	128
	Meal (30 min), g	400^{a}	312^{b}	339$^{a, b}$	182^{c}	73^{d}	299^{b}	261	67
	Dimension 1	-0.54	0.85	0.57	-1.39	0.43	0.08		
	Dimension 2	0.81	0.20	1.53	-0.27	-2.33	0.06		
L33lo	Meal (3 h), g	580^{a}	597^{a}	641^{a}	373^{b}	223^{c}	602^{a}	483	92
	Meal (30 min), g	324^{a}	339^{a}	394^{a}	160^{b}	108^{b}	368^{a}	265	77
	Dimension 1	1.62	0.38	0.71	-1.06	-1.45	-0.20		
	Dimension 2	-0.28	-1.45	0.97	0.85	-0.95	0.86		
L33hi	Meal (3 h), g	609$^{b, c}$	700$^{a, b}$	720^{a}	585^{c}	284^{d}	732^{a}	580	104
	Meal (30 min), g	295^{a,b}	364^{a}	361^{a}	269^{b}	104^{c}	317^{a,b}	279	73
	Dimension 1	0.31	0.70	1.49	0.66	-2.3	-0.87		
	Dimension 2	-0.45	-1.14	0.44	0.61	-0.24	0.78		
S40lo	Meal (3 h), g	723^{a}	779^{a}	752^{a}	490^{b}	294^{c}	588^{b}	608	121
	Meal (30 min), g	370^{a,b}	425^{a}	437^{a}	215^{c}	123^{d}	326^{b}	314	83
	Dimension 1	-0.29	1.86	-0.03	0.56	-1.54	-0.55		
	Dimension 2	1.37	0.23	0.52	-1.66	-0.67	0.21		
S40hi	Meal (3 h), g	644^{a}	620^{a}	607^{a}	518^{b}	334^{c}	684^{a}	545	97
	Meal (30 min), g	358^{a,b}	384^{a}	301^{b,c}	272^{c}	156 ^{d}	314^{b,c}	294	66
	Dimension 1	0.66	0.54	1.37	-1.75	-1.00	0.19		
	Dimension 2	-0.56	0.61	0.23	1.19	-1.75	0.28		
L40lo	Meal (3 h), g	598^{a,b}	569^{a,b}	542^{b}	349^{c}	247^{d}	635^{a}	461	82
	Meal (30 min), g	291^{b}	295^{b}	318^{b}	215^{c}	119^{d}	392^{a}	248	73
	Dimension 1	-1.35	-0.28	-1.00	0.10	2.11	0.42		
	Dimension 2	-0.63	-1.12	0.55	1.54	-0.37	0.02		
L40hi	Meal (3 h), g	715^{b}	657^{b}	467^{c}	444^{c}	256^{d}	816^{a}	508	101
	Meal (30 min), g	364^{a}	342^{a}	245^{b}	186^{b}	114^{c}	344^{a}	250	66
	Dimension 1	0.80	0.77	-0.54	0.61	-2.17	0.52		
	Dimension 2	-0.2	-0.96	-1.29	1.37	0.44	0.64		

a-d = Means within a row with different superscripts differ, MSD = Minimum significant difference (Waller Duncan k-ratio t-test), d0–d8 = days of aerobic exposure after opening of the silo, S = short chopping length, L = long chopping length, 33 = 33% DM, 40 = 40% DM, lo = low packing density, hi = high packing density.

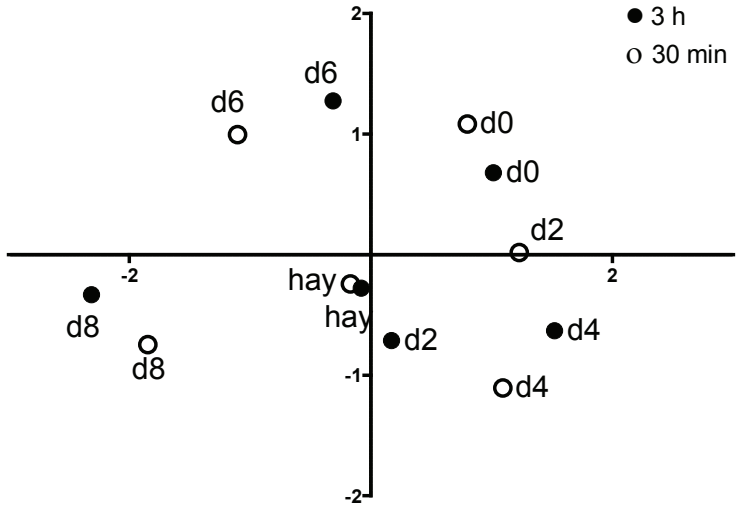

Fig. 1. Multidimensional scaling of the mean preference shown by six goats for five silages (d0–d8) and one lucerne hay (hay) in one preference trial (silage with short chopping length, 33% dry matter and high compaction pressure) after 30 min and 3 h (d = number of days of aerobic exposure).

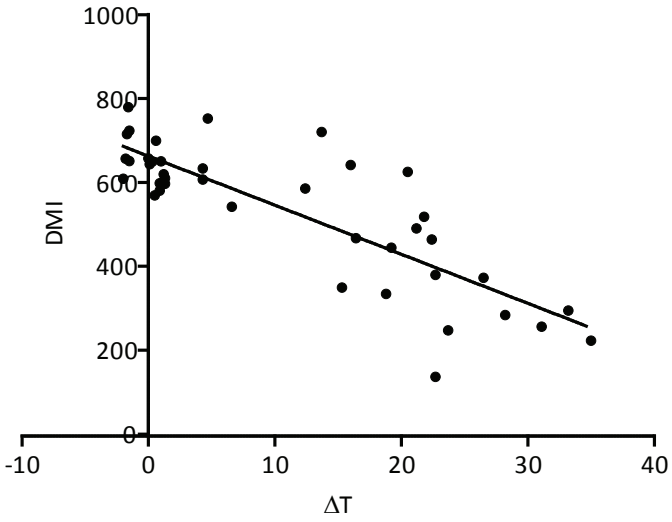

Fig. 2. Relationship between dry matter intake (DMI, g 3 h^{-1}) of goats and silage temperature during aerobic exposure (expressed as difference to ambient temperature, ΔT (K)); n = 40; y = 662 - 11.69 x; R^2 = 0.681; p<0.0001.

Silage characteristics influencing dry matter intake

Correlation coefficients were calculated between silage characteristics (of vacuum-stored samples used in preference trials) and DMI of goats (Table 7). A differentiation was made between data referring to silages at all stages of aerobic exposure and the corresponding DMI (n = 40) on one hand and only data connected with the fresh silages (d0) to disregard the spoilage process (n = 8) on the other hand. Across all silages, the strongest and negative correlation was between DMI and ΔT. The DMI had also a weak negative relationship with ethanol, ethyl lactate and pH. *In vitro* 24-h gas production and ME were positively associated with DMI.

When using only the fresh silages (d0) that had not undergone aerobic deterioration, DMI was negatively correlated with acetic acid. With an average of 12.9 g kg^{-1} DM, concentrations of acetic acid were generally low. The pH of these fresh silages showed a trend towards a positive relationship with DMI. Generally, fewer significant correlations were found, most likely due to the lower number of observations.

Table 7. Correlation coefficients between dry matter intake of goats (g 3 h^{-1}) and characteristics of eight maize silages at day 0–8 of aerobic exposure and the day of opening (d0) respectively.

Variable	r (d0–d8)	p	r (d0)	p
DM	-0.334	0.035	0.509	0.198
Ash	-0.181	0.264	0.352	0.393
Crude protein	-0.329	0.038	0.096	0.821
Crude lipids	-0.038	0.817	0.172	0.683
aNDFom	0.150	0.362	-0.175	0.679
ADFom	-0.248	0.123	-0.247	0.555
ADL	0.062	0.706	-0.044	0.917
ME	0.415	0.008	-0.063	0.882
24-h gas production	0.513	0.001	-0.114	0.789
Starch	-0.020	0.902	0.143	0.736
pH	-0.433	0.005	0.681	0.063
Lactic acid	0.387	0.014	-0.562	0.147
Acetic acid	-0.023	0.888	-0.723	0.043
Ethanol	-0.332	0.036	0.293	0.481
Propanol	-0.363	0.021	0.004	0.992
WSC	-0.072	0.658	-0.453	0.260
Ethyl acetate	-0.097	0.552	0.475	0.235
Ethyl lactate	-0.327	0.039	0.427	0.291
ΔT	-0.835	<0.0001		

Probabilities of r based on n of 40 (d0–d8) and 8 (d0)
aNDFom = neutral detergent fibre assayed with heat stable amylase and expressed exclusive residual ash,
ADFom = acid detergent fibre expressed exclusive residual ash, ADL = acid detergent lignin,
ME = metabolizable energy, WSC = water-soluble carbohydrates,
ΔT = silage temperature expressed as difference to ambient

Discussion

Composition of silages

This study was conducted to describe the changes occurring during aerobic exposure in maize silages and to evaluate their impact on preference and DMI. Strong shifts were observed in the composition of fermentation products, which is consistent with literature, as reviewed by Pahlow et al. (2003). The concentration of lactic acid decreased significantly in all maize silages during aerobic exposure being nearly depleted after eight days. This can be ascribed to the intense activity of lactate assimilating yeasts, whose population rose above target values within four days of aerobic exposure. A similar decline could be observed in the concentration of acetic acid and WSC. Lactic acid, acetic acid and WSC are the main energy sources for the microorganisms involved in the first phase of aerobic deterioration (McDonald et al. 1991). The microbiological results showed that deterioration was initiated by yeasts followed by moulds and aerobic mesophilic bacteria after the fourth day of aerobic exposure. Moulds have often been observed in advanced stages of aerobic deterioration (Woolford 1990, Pahlow et al. 2003). With reference to suggested target values (VDLUFA 2012), all silages were already spoiled after four days of aerobic exposure. The activity of these organisms leads to the oxidation of fermentation acids and is connected with production of carbon dioxide and water resulting in evolution of heat (McDonald et al. 1991). The ΔT was strongly correlated with pH, lactic acid, acetic acid and WSC (r = 0.804, -0.882, -0.796, -0.538, respectively; p<0.001) which is consistent with previous literature (McDonald et al. 1991).

Increase in silage temperature is seen as a convenient indicator for the extent and intensity of aerobic deterioration (Borreani and Tabacco 2010). In our experiment, ΔT rose drastically after two (long-cut silages) or four days (short-cut silages) of aerobic exposure, due to rapid colonization of lactate assimilating yeasts which have often

been shown to be capable of rapid growth and are initiators of aerobic deterioration. Maximum silage temperature was measured in silage L33lo with ΔT reaching 35.0 K at d8. It has to be considered that ambient temperature was 22 °C during this trial which gives good conditions to spoilage organisms like aerobic yeasts mostly being active at 20–30 °C (Ashbell et al. 2002). Due to different ambient temperatures during aerobic exposure periods, no further conclusions concerning the impact of different treatments (chopping length, DM, compaction pressure) on aerobic deterioration are drawn.

Dry matter intake and preference

The DMI of different maize silages decreased significantly after four days of aerobic exposure. After eight days it was more than halved, with reductions ranging between 29% and 79% in comparison to the fresh silages (d0). In the trial with silage L40hi, 3-h DMI decreased after two days of aerobic exposure. Long cut silages with higher contents of DM are especially prone to deterioration after opening, due to restricted fermentation and increased porosity and therefore movement of oxygen into the silage causing more rapid and extensive growth of aerobic microorganisms (Muck et al. 2003). The pH of L40hi rose from 4.0 to 4.5 within four days, giving evidence of a strong and fast spoilage process. This was also supported by an increase of temperature of 16.4 K during these four days. In contrast, DMI was constant for six days in the trial with silage S33lo. When looking at the ΔT in this treatment it can be assumed that the spoilage process started later, therefore temperature remained steady up to the sixth day after opening the silo. This prolonged aerobic stability might be due to the relatively high content of acetic acid in this treatment.

Few other studies dealing with the topic also reported a strong (Wichert et al. 1998) or slight decline (Bolsen et al. 2002) in feed intake after some days of aerobic exposure. Since oxygen can penetrate the silage for 1 to 2 m when still being in the silo (Weinberg and Ashbell 1994), air contact is not restricted on face and feed-out, thus days with air contact can easily exceed time interval of four days under field conditions. As DMI is one of the most important factors determining productivity in milk or beef production, care should be taken to avoid air contact and consequently aerobic deterioration in maize silages. Unfortunately, DM losses have not been calculated in these studies. With reference to literature (McGechan 1990, Bolsen et al. 1993, Tabacco et al. 2011), losses can account for up to 20% of the total stored DM and up to 70% in the peripheral areas and near the sidewalls of the bunkers. When adding these losses to the decline in DMI that occurred in the preference trials reported above, the negative consequences of aerobic deterioration are detrimental. However, data presented here are based on preference experiments. Keady and Murphy (1998) observed that differences in DMI were much stronger when cows were having the possibility to choose between two or more feedstuffs in comparison with single-choice experiments. Nevertheless, results give an impression of the potential in DMI that is lost when feeding spoiled silages in comparison to fresh ones. Low preference for deteriorated silages may probably result in greater feed sorting and lower intakes when animals have a choice of different feedstuffs. It might be interesting for studies to examine the impact of deterioration in single-choice experiments.

Silage characteristics influencing dry matter intake

The impact of deterioration on DMI and preference was strongly negative, but it was difficult to attribute the decline to a single compound. Some fermentation products (ethyl lactate, ethanol) were negatively related to silage intake, but correlation coefficients were weak.

With restriction to the fresh silages without aerobic deterioration, DMI was strongly negatively correlated to acetic acid, which is in agreement with the findings of Buchanan-Smith (1990) where concentrations of acetic acid were shown to be responsible for a decrease in DMI by sheep in a linear manner. In meta-analyses on the effect of fermentation quality on DMI by dairy cows (Eisner et al. 2006, Eisner 2007), acetic acid was the strongest single predictor of DMI when silages and concentrates were offered separately. Though, the dataset used was mainly based on grass silages or mixtures of grass and maize silages. New findings of Krizsan et al. (2012) showed that an addition of acetic acid to wilted grass silages fed to growing steers reduced silage DMI. However, the reduction equalled the amount provided by the added substances, so no differences in total DMI were observed. From our point of view, a lower DMI caused by slightly increased amounts of acetic acid in fresh silages is compensated by the better aerobic stability and therefore a smaller decline in DMI as a consequence of aerobic deterioration, as seen with silage S33lo.

Another component negatively related to DMI in this study was ethanol. Huhtanen et al. (2002) and Krizsan and Randby (2007) did not find a negative impact of ethanol on DMI, while results of Hetta et al. (2007) showed a positive effect that eventually could be an associative effect due to the negative correlation between concentrations of ethanol and ammonia-N in that study.

Correlation of pH with DMI was ambiguous, with different results for fresh and aerobically stored silages. For the fresh silages (that had not undergone aerobic deterioration), there was a positive relationship between pH and DMI. This is consistent with literature, which reported similar positive relationship for fresh silages (Erdman 1988, Dulphy and Van Os 1996, Eisner 2007). In well fermented silages with a low pH, DMI increases when silage pH increases. This positive effect implies a decrease in acidity caused by less fermentation without excessive formation of ammonia-N and fermentation acids (Dulphy and Van Os 1996). Otherwise, correlation of the complete silage dataset (fresh as well as spoiled silages) with DMI shows a negative relationship. Here, the effect of silage pH on intake seems to be a direct consequence of the spoilage processes. Steen et al. (1998) observed a quadratic relationship between pH and DMI with a slight positive value at low pH followed by a negative relationship at high pH. Huhtanen et al. (2002) proposed that this might be caused by the contrary influence of acidity at low pH and poor fermentation with high concentrations of ammonia N and volatile fatty acids at high pH.

Ethyl lactate had a weak negative influence on DMI. In other trials, esters were the most abundant class of volatile compounds in red clover silages (Figueiredo et al. 2007) as well as in grass silages (Mo et al. 2001) with ethyl esters being the predominant subclass of all esters (Figueiredo et al. 2007). Since esters are known to be odorant, they could have an effect on the taste of a silage and consequently on feed intake (Mo et al. 2001). Also Kristensen et al. (2010) expected them to contribute to the silage flavour due to their volatility. Many esters have low odour thresholds, so they can already be noticed in the parts per million ranges. To our knowledge, the effect of different ethyl esters in silages on voluntary feed intake by ruminants has not been studied previously. There may be need for further studies, since they have recently been observed in considerable amounts in fresh and well fermented silages (Weiß et al. 2011, Weiß and Auerbach 2012), where ethyl acetate and ethyl lactate showed a strong correlation with ethanol, which was also confirmed in our study ($r = 0.868$ and $r = 0.918$, $p<0.001$, data not shown).

By far the strongest correlation was between DMI and ΔT. The fact that temperature measured in the silage was a better predictor than any other analyzed constituent emphasizes the difficulty to identify chemical reactions being responsible for decreases in preference caused by aerobic spoilage. Nevertheless, it also underlines the suitability of temperature measurement for daily use, as recommended by Borreani and Tabacco (2010) to improve silage management. The target value of 5 °C for maximum ΔT given by Spiekers et al. (2009) for practical use was proven to be appropriate.

In the present study, goats were used as model animals for cattle. Strong evidence can be found in literature that their feeding and preference behaviour are very similar (Squires 1982, Burns et al. 2001). Nevertheless, continuative studies with dairy cows and beef cattle dealing with the topic presented here are needed to verify that assumption.

In conclusion, this study demonstrated that strong changes concerning the fermentation products of maize silage occurred during eight days of aerobic exposure. Counts of spoilage organisms, especially yeasts rose above target values within four days. There was a strong impact of deterioration on feed intake and preference by goats, marked by a decrease of DMI after four days of exposure shown by goats in choice situations. Temperature measured in the silage was the best predictor for DMI in comparison with any single silage constituent. It can be recommended to limit exposure of silages to oxygen during storage and feed-out as much as possible because of its detrimental effects on DMI.

Acknowledgements

This study was supported by the “Deutsche Forschungsgemeinschaft” (DFG, German Research Foundation, SU 124/22-1 and BU 1235/5-1). We thank Nadja Wahl and Petra Jacquemien, University of Bonn and the team of Central Analytical Laboratory, Humboldt University Berlin, for support in chemical analyses and Dr. Young Anele for language editing.

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