Small Ruminant Research 117 (2014) 131–141



Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

# Aerobic exposure of grass silages and its impact on dry matter intake and preference by goats

K. Gerlach[a,*], F. Roß[b], K. Weiß[c], W. Büscher[b], K.-H. Südekum[a]

[a] Institute of Animal Science, University of Bonn, Endenicher Allee 15, 53115 Bonn, Germany
[b] Institute for Agricultural Engineering, University of Bonn, Nußallee 5, 53115 Bonn, Germany
[c] Faculty of Agriculture and Horticulture, Humboldt University, Invalidenstraße 42, 10115 Berlin, Germany

ARTICLE INFO

*Article history:*
Received 10 September 2013
Received in revised form
23 December 2013
Accepted 24 December 2013
Available online 7 January 2014

*Keywords:*
Forage
Preference
Ruminant
Volatile organic compound

ABSTRACT

The effect of aerobic exposure of grass silages on short-term feed intake and preference by goats was studied. Eight grass silages differing in dry matter (DM) (25% and 33%), chop length (short and long) and compaction pressure at ensiling (0.1 MPa and 0.2 MPa) were exposed to air for eight days. Chemical analyses were conducted in 2-day (d) intervals (d0, d2, d4, d6 and d8 after silo opening) for proximate constituents, fermentation products and other volatile compounds as well as determination of microbiological status (yeasts, moulds and aerobic mesophilic bacteria). Furthermore, d0- to d8-silages were stored anaerobically in vacuum-sealed plastic bags for use in preference trials. After aerobic exposure, eight preference trials with Saanen-type wethers ($n = 5$) were carried out, where each possible two-way combination of silages and a standard hay ($n = 15$) was offered for 3 h. Data were analyzed using the SAS procedure Multidimensional Scaling, analysis of variance and correlation analysis between silage characteristics and DM intake (DMI). All silages were aerobically stable during the examination time. In trials with 33% DM-silages, DMI decreased at d6 or d8 (in each of two trials) of aerobic exposure. Silage that had been exposed to air for 8 d was avoided in each case with a reduction (mean ± standard deviation) of 50 ± 6.7% in comparison to the freshest silage. Low-DM silages showed signs of malfermentation with higher concentrations of butyric acid and ammonia-nitrogen ($NH_3$-N). Both DMI and the impact of aerobic exposure on DMI were lower. Mean decrease in DMI after 8 d of aerobic exposure was 20% (±11.0%). Products from protein and amino acid degradation ($NH_3$-N, butyric acid) were negatively correlated to DMI ($r = -0.55$ and $-0.59$; $P < 0.001$). It was concluded that in well-fermented silages, aerobic exposure for a length of time that is of practical relevance does have a negative impact on short-term DMI and preference by goats, even if silages are at an apparently low stage of deterioration. It is assumed that goats can detect subtle differences caused by aerobic exposure, sometimes even before an increase in temperature or changes in chemical composition occur. After a 1-d exposure of each variant, goats were able to differ between forages and showed preference or avoidance for different silages, with a high correlation between initial and total DMI. Therefore, results showed the potential for 30 min measurements in short-term preference trials, as goats remember post-ingestive feedback from the adaptation period.



* Corresponding author. Tel.: +49 228 73 2281; fax: +49 228 73 2295.
*E-mail address:* kger@itw.uni-bonn.de (K. Gerlach).

http://dx.doi.org/10.1016/j.smallrumres.2013.12.033
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## 1. Introduction

Grass silage is a major forage used in ruminant feeding, but due to the impact of crop management and weather, strong variations of the nutritional value and fermentation quality can occur (McDonald et al., 1991). Both of them can have a strong impact on feed intake (Forbes, 1995). During storage or feed-out, oxygen ingress into the silage can cause dry matter (DM) and nutritional losses and also increase the risk of proliferation of potentially pathogenic or otherwise undesirable microorganisms (Driehuis and Oude Elferink, 2000). The changes occurring during the aerobic feed-out phase are equally as important as those taking place in the anaerobic storage phase from the viewpoint of preserving nutrients and maintaining good quality until fed to the animal (Wilkinson and Davies, 2013). Furthermore, the activity of aerobic spoilage organisms may lead to changes in the composition of volatile compounds and therefore affect fermentation quality. Huhtanen et al. (2002) showed that variation in fermentation quality affects voluntary feed intake of cattle. However, it is difficult to attribute changes in DM intake (DMI) to a single fermentation product as some of them are strongly interrelated (e.g. ethanol and the ethyl esters of acetate and lactate (Weiß and Auerbach, 2012)). Mo et al. (2001) identified more than 50 different fermentation products in grass silages. Since a majority of them, especially esters, are known to be odorous, they all may have (to a greater or lesser extent), an effect on the smell and taste of feed and, consequently, feed intake. For unspoilt silages, attempts have been made to find a relationship between silage quality and intake (Huhtanen et al., 2003; Eisner et al., 2006; Krizsan and Randby, 2007). However, composition of volatile compounds may change as a result of aerobic spoilage, which often occurs after few days of oxygen ingress.

The aim of this study was to determine the effect of aerobic exposure of grass silages on short-term DMI and preference by goats.

## 2. Materials and methods

### 2.1. Preparation of silages

Italian ryegrass (*Lolium multiflorum* L.) was cultivated at the research station Frankenforst of the University of Bonn, Germany (7°12′ E and 50°42′ N; 2010: average temperature, 9.3 °C; annual precipitation, 635 mm; average humidity, 72.3%). Grass was cut in the morning (10:00) at June 20, 2010 and wilted on the field. To achieve two different levels of DM concentration, one part was harvested and ensiled the same day in the afternoon; the other part was wilted on the field and ensiled the next day at midday. Eight silage treatments (2 × 2 × 2-factorial design) were produced differing in DM concentration, chop length (short: chopped by knives in the self-loading wagon; long: unchopped) and compaction pressure at ensiling (0.1 and 0.2 MPa). Details about the treatments are presented in Table 1 and following abbreviations are used: S = short chopping length, L = long chopping length, 33 = 33% DM, 25 = 25% DM, lo = low packing density, hi = high packing density.

Grass of both DM stages before ensiling was sampled for laboratory analyses. Each treatment was ensiled in six 120-l plastic barrels using a forklift piler with two concrete weights (1.8 t and 3.6 t) for two different levels of compaction of the forage. Anaerobic storage time in the barrels ranged between 11 and 16 months. Before starting the first trial, packing density of all silages was determined by weighing the filled barrels in April 2011.

In May 2011, the barrels containing the first two treatments were opened; the silages were taken out, each treatment was thoroughly homogenized by mixing the content of all six barrels, and stored aerobically on a heap (3 m × 3 m) for 8 d. This aerobic exposure followed by a preference trial was done with each of the eight treatments during 2011, the last trial started in November, so that a maximum of six months was allowed between opening of first and last barrel. All the aerobic exposure trials were conducted indoor with a continuous measurement of ambient temperature (data logger 175-T1, Testo, Lenzkirch, Germany). At the day of opening (d0) and at 2-d intervals (d2, d4, d6 and d8 after opening) temperature of the material was measured in a depth of 20 cm at three different points in the silage heap (middle, left, right, 1 m distance between measurement points) using a digital probe thermometer (TFA Dostmann, Wertheim, Germany); afterwards the silage was homogenized completely. Furthermore, boxes ($n = 3$) for aerobic stability tests proposed by Honig (1990) were filled with 300 g of silage and the temperature was measured in the same interval. Aerobic stability was defined as the number of days the silage remained stable before rising more than 3 K above the ambient temperature (Honig, 1990). For chemical analyses, a composite sample (1000 g) of each silage heap was taken at the respective sampling days and frozen immediately (−18 °C). Another sample (50.0 g) for determination of fermentation variables was taken and also frozen.

For subsequent use in the preference trials with goats, samples of the homogenized silage heap from each day of the aerobic exposure (d0, d2, d4, d6 and d8) were stored anaerobically in polyethylene bags (170 μm, 400 mm × 600 mm; Innovapac, Durach, Germany), which were evacuated and sealed with a chamber vacuum-packing machine (MAX-F 46, Helmut Boss Verpackungsmaschinen, Bad Homburg, Germany). For each meal for each goat a single bag was used which was filled with 1.5–1.7 kg silage (requirements + reserve = 60 bags per day and treatment). 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 5 to 26 d depending on the day when fed. The time difference was randomly allocated to treatments, as each treatment was fed at each day of the preference trial.

### 2.2. Preference trials

For each of the eight silage treatments, a preference trial was done at the Institute of Animal Science, University of Bonn, starting directly after the aerobic storage period described above. A total of ten Saanen-type wethers (German Improved White Goat breed, mean (SD) body weight 90.8 (12.35) kg) were used which were divided into two

**Table 1**
Characteristics about the silage treatments used in the trials.

| Trial | Dry matter (%) | Chop length | Compaction pressure (MPa) | Abbreviation of treatment | Month of opening | Temperature[a] (°C) |
|---|---|---|---|---|---|---|
| 1 | 33 | Short | 0.2 (high) | S33hi | May 2011 | 22 |
| 2 | 33 | Long[b] | 0.2 (high) | L33hi | May 2011 | 22 |
| 3 | 25 | Short | 0.2 (high) | S25hi | June 2011 | 23 |
| 4 | 25 | Long | 0.2 (high) | L25hi | June 2011 | 23 |
| 5 | 33 | Short | 0.1 (low) | S33lo | August 2011 | 22 |
| 6 | 33 | Long | 0.1 (low) | L33lo | August 2011 | 22 |
| 7 | 25 | Short | 0.1 (low) | S25lo | November 2011 | 19 |
| 8 | 25 | Long | 0.1 (low) | L25lo | November 2011 | 19 |

[a] Mean ambient temperature during 8 d of aerobic exposure.
[b] Unchopped.

groups (five goats per group) to conduct two trials concurrently. Two animals shared an indoor pen of approximately 2 m × 3 m bedded with straw. To measure individual intake, goats were tied up for the duration of the experimental feeding, with the possibility of lying down and accessing water and salt licks. Animal care and handling was done according to official German regulations.

Preference trials were carried out as previously described by Burns et al. (2001). During an adaptation period prior to each experiment (Kyriazakis et al., 1990), single meals of each silage aerobic exposure stage (d0–d8) and lucerne (*Medicago sativa* L.) hay as standard forage were offered to allow the animal to associate the silage with postingestive metabolic response, taste and smell produced by the forage. For each initial treatment, the adaptation period lasted 6 d (silages from five stages of aerobic exposure and lucerne hay) and forages were offered in randomized order. Lucerne hay was compared as a standard to each of the eight silages. During the subsequent experimental phase, each possible 2-way combination of the five silages and the standard hay ($n = 15$) was presented. Each forage was offered in a plastic feeding box (400 mm × 340 mm × 250 mm) 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. Goats had free access to both feeding boxes so that free choice between both forages could be guaranteed. As proposed by Buntinx et al. (1997), the weight of the silages was then determined 30 min after offering and after 3 h to calculate the initial preference and total DMI. During the trials goats had a constant choice between two silages or a silage and lucerne hay. This was guaranteed by putting additional silage into the feeding box as soon as the weight fell below 300 g. Each trial lasted 21 d, consisting of 6 d for adaptation and 15 d for experimental measurements. Each day, the experimental meal was offered for 3 h, starting at 07:45. Grass hay was offered for ad libitum consumption at 15:30 and removed the following morning at 07:00. Its concentrations (g/kg DM) of ash, crude protein (CP), crude lipids (CL), acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) were 78, 98, 16, 360, 594 and 47, respectively, and concentration of metabolizable energy (ME, MJ/kg DM) was 8.4.

For laboratory analyses, a subsample (1000 g) of each silage 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.

### 2.3. General analyses

Silage samples were freeze-dried (Jumo Imago 500, Jumo, Fulda, Germany) in triplicate. The DM of the silages was then estimated by oven-drying 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: $DM_{cor} = DM + (1.05 - 0.059 \times pH) \times$ total volatile fatty acids (VFA, C2 – C6) + 0.08 × lactic acid + 0.77 × 1,2-propanediol + 0.87 × 2,3-butanediol + 1.00 × total of other alcohols (C2–C4). All concentrations are expressed as g/kg.

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 analyzed using methods 8.1 and 5.1. Crude protein was determined by Dumas combustion (4.1.2, FP328, Leco 8.1, Leco Instrumente, Mönchengladbach, Germany). The concentrations of NDF (6.5.1; assayed with heat stable amylase), ADF (6.5.2) and ADL (6.5.3) were analyzed using an Ankom 2000 Fiber Analyzer (Ankom Technology, Macedon, USA). The NDF and ADF values are expressed exclusive of residual ash.

The Hohenheim gas test (method 25.1, VDLUFA, 2012) was conducted for measuring the 24 h in vitro gas production (GP, ml/200 mg DM) and estimating the concentration of metabolizable energy (ME) of the forages using the equation of GfE (2008): ME (MJ/kg DM) = 7.81 + 0.07559 × GP – 0.00384 × ash + 0.00565 × CP + 0.01898 × CL – 0.00831 × ADF, where ash, CP, CL, and ADF are in g/kg DM and GP is in ml/200 mg DM.

### 2.4. Chemical analyses of fermentation variables

A subsample (50.0 g) of each silage with different aerobic exposure was used for determination of lactic acid, pH, volatile fatty acids, alcohols (methanol, ethanol, propanol, 1,2-propanediol, 2,3-butanediol, 1-butanol, 2-butanol), acetone, $NH_3$-N and water-soluble carbohydrates (WSC). Furthermore, silages were analyzed for two 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 (MN 615, Macherey-Nagel, Düren, Germany). Determination of pH in the extract was done potentiometrically using a calibrated pH electrode. The extract

was filtered through a Minisart syringe filter (pore size 0.45 μm; Sartorius, Göttingen, Germany) for lactic acid determination by HPLC (RI-detector, Shimadzu Deutschland, Duisburg, Germany) according to Weiß and Kaiser (1995). Volatile fatty acids and alcohols were determined by gas chromatography (flame ionization detector, Shimadzu Deutschland, Duisburg, Germany) as described by Weiß (2001). Analysis of ethyl esters as well as acetone, propanol, methanol, 1-butanol and 2-butanol was done according to Weiß and Sommer (2012). The lower detection limit for VFA and alcohols was at 0.01% and for ester at 0.001%. The $NH_3$-N concentration was analyzed colorimetrically based on the Berthelot reaction using a continuous flow analyzer (Skalar Analytical, Breda, Netherlands). Concentration of WSC was determined by anthrone method according to von Lengerken and Zimmermann (1991). Based on the concentrations of acetic acid, butyric acid and the pH, fermentation quality of the silages was assessed with the DLG scheme (DLG, 2006).

### 2.5. Microbiological analyses

Each silage was sampled at d0, d2, d4 and d8 of aerobic exposure 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, 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 log 10-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).

### 2.6. Statistics

All data were analyzed using SAS 9.2 (SAS®, 2002). The preference trials were analyzed by Multidimensional Scaling (MDS) as previously described by Buntinx et al. (1997), Burns et al. (2001) and Gerlach et al. (2013). This procedure was used to develop a spatial arrangement representing the differences expressed as selective forage intake by the animals. 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 trial was also tested by analysis of variance after averaging DMI of each forage (averaged across each combination, $n = 5$). 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 ($k = 100$) (Burns et al., 2001). 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 $0.05 \leq P \leq 0.10$.

## 3. Results

### 3.1. Chemical composition

Before ensiling, concentrations (g/kg DM) of ash, CP, CL, ADF, NDF and ADL were 88, 124, 29, 233, 410 and 21 for the low (26.1% DM) and 82, 121, 31, 245, 418 and 23 for the high DM grasses (33.3% DM), and 87, 143, 21, 323, 464 and 64 for the lucerne hay used as standard forage in the preference trials, respectively. Concentrations of ME (MJ/kg DM) were 11.5, 11.4 and 9.6 for the low and high DM grasses and the lucerne hay, respectively.

At opening (d0), silages did not show visible signs of moulding. Low-DM silages, especially the treatments used in the last trials (S25lo and L25lo) had a noticeable smell of butyric acid. Results of chemical analyses of silages at d0 are presented in Table 2. Lactic acid and acetic acid ranged within typical values for well-fermented grass silages. Butyric acid and ethanol were detected in all fresh silages; with high concentrations of butyric acid being restricted to low-DM silages. Water-soluble carbohydrates were found in low concentrations in only two treatments (S25hi and S25lo); the others had normal concentrations. Ethyl lactate concentrations ranged from 66 to 120 mg/kg DM, and ethyl acetate was not detected. Highest values of $NH_3$-N were found in S25hi and S25lo. No differences ($P > 0.05$) in the analyzed variables were found between fresh samples (taken directly during the aerobic exposure d0–d8) and vacuum-sealed samples used in the preference trials (data not shown). For calculation of correlation coefficients between silage characteristics and DMI in preference trials, data of vacuum-stored samples were used.

Results of chemical analyses of silages during 8 d of aerobic exposure are presented in Table 3.

**Table 2**
Chemical composition of grass silages at the day of opening (g/kg dry matter unless otherwise stated).

| Variable | Treatment[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | S33hi | L33hi | S25hi | L25hi | S33lo | L33lo | S25lo | L25lo |
| Density (kg DM/m³)[b] | 189 | 161 | 126 | 141 | 174 | 150 | 111 | 122 |
| Dry matter (g/kg fresh matter) | 330 | 325 | 231 | 271 | 328 | 330 | 234 | 268 |
| Ash | 89 | 97 | 113 | 90 | 90 | 98 | 105 | 91 |
| Crude protein | 120 | 130 | 135 | 125 | 126 | 129 | 141 | 130 |
| Crude lipids | 30 | 31 | 39 | 32 | 38 | 33 | 39 | 39 |
| Acid detergent fibre[c] | 272 | 278 | 314 | 285 | 274 | 274 | 289 | 277 |
| Neutral detergent fibre[d] | 450 | 448 | 442 | 435 | 439 | 465 | 449 | 470 |
| Acid detergent lignin | 18 | 17 | 19 | 17 | 21 | 18 | 18 | 18 |
| 24 h gas production (ml/g DM) | 280 | 284 | 263 | 271 | 296 | 290 | 262 | 278 |
| Metabolizable energy (MJ/kg DM) | 10.7 | 10.7 | 10.2 | 10.5 | 11.1 | 10.9 | 10.5 | 10.8 |
| pH | 4.5 | 4.6 | 4.5 | 4.2 | 4.5 | 4.6 | 4.4 | 4.5 |
| Lactic acid | 59 | 57 | 65 | 76 | 58 | 49 | 76 | 58 |
| Acetic acid | 23 | 16 | 23 | 30 | 30 | 21 | 27 | 26 |
| iso-Butyric acid | 0.4 | 0.3 | 2.8 | 0.9 | 0.7 | 0.5 | 3.0 | 1.9 |
| n-Butyric acid | 1.2 | 2.9 | 23.7 | 5.0 | 3.7 | 4.2 | 26.2 | 20.0 |
| iso-Valeric acid | –[e] | – | – | – | – | – | – | – |
| n-Valeric acid | – | – | – | – | – | – | – | – |
| n-Caproic acid | – | – | – | – | – | – | – | – |
| Propionic acid | – | – | 0.5 | – | – | – | 1.2 | – |
| 1,2-Propanediol | 1.8 | 1.7 | 2.5 | 2.5 | 5.8 | 2.6 | 2.5 | 3.1 |
| Ethanol | 9 | 9 | 14 | 11 | 13 | 16 | 20 | 15 |
| Methanol | 0.6 | 0.5 | 0.7 | 0.6 | 0.4 | 0.7 | 0.5 | 0.8 |
| Propanol | 0.6 | 0.4 | 1.9 | 1.8 | 1.6 | 0.7 | 3.5 | 1.3 |
| 1-Butanol (mg/kg DM) | – | – | 109 | – | – | – | 107 | 37 |
| 2-Butanol (mg/kg DM) | 224 | – | 829 | 549 | 275 | – | 2010 | 419 |
| Ethyl acetate (mg/kg DM) | – | – | – | – | – | – | – | – |
| Ethyl lactate (mg/kg DM) | 66 | 120 | 80 | 120 | 90 | 77 | 108 | 85 |
| Ammonia-N (g/kg of total N) | 111 | 93 | 130 | 129 | 112 | 100 | 150 | 130 |
| Water-soluble carbohydrates | 75 | 89 | 23 | 62 | 66 | 96 | 16 | 77 |

[a] Treatments: S = short chopping length, L = long chopping length, 33 = 33% DM, 25 = 25% DM, lo = low packing density, hi = high packing density.
[b] Mean density of silage in six barrels respectively, determined in April 2011.
[c] Expressed exclusive residual ash.
[d] Analyzed with heat-stable amylase and expressed exclusive residual ash.
[e] Below detection limit (0.01%).

### 3.2. Microbiological analyses and temperature

At the day of opening, all silages had low counts of spoilage organisms. Yeast and mould counts ranged from 2.4 to 2.7 log cfu/g, and concentration of aerobic mesophilic bacteria ranged from 3.7 and 4.2 log cfu/g. In two treatments (S25hi and L25hi), numbers of aerobic mesophilic bacteria increased during aerobic exposure and exceeded target values of 5.3 log cfu/g (VDLUFA, 2012) at d2, d4, d6, and d8. Critical counts of moulds (≥3.7 log cfu/g; VDLUFA, 2012) were detected in three silages (S25hi, S33lo and L33hi) at d8 of exposure, whereas yeasts rose above orientation values (5.3 log cfu/g; VDLUFA, 2012) only in one case (L25hi) at d8.

Temperature measured in the silages remained stable during the period of aerobic exposure. An increase of more than 3 K above ambient could only be detected in silage L25hi at d8 of aerobic exposure ($\Delta K + 3.4$, data not shown).

### 3.3. Animal preference and dry matter intake

Multidimensional Scaling revealed that selection between forages by goats was associated with two dimensions. Detailed results of eight preference trials consisting of 3 h- and 30 min-DMI and coordinates of both dimensions are presented in Table 4. The forage with the highest DMI in each trial was used as a positive control by assigning it positive coordinates (Burns et al., 2001). Consequently, a forage with two positive dimensions would represent preference while two negative dimensions indicate avoidance. Silage from d0 and d4 was preferred in four trials, lucerne hay three times, d2-silage two times and d6-silage in one case. Silage from d8 was never preferred and strongly avoided in six trials.

According to the MSD, DMI (g/3 h meal) was similar for d0–d6 in trial 1 (S33hi) and greater than DMI of d8 and standard hay ($P < 0.05$). In trial 2 (L33hi), DMI was highest for d0 and lowest for d8 and hay. In trial 3 (S25hi), DMI was highest for d4 and hay and lowest for d6 and d8. In trial 4 (L25hi), goats showed highest intake for d2, d4 and hay; d0 and d8 were avoided. In trial 5 (S33lo), intake was highest and similar for d2, d4, and d6 and lowest for hay and d8. In trial 6 (L33lo), d4 was the most and d8 together with d6 and lucerne hay the least consumed forage. According to the MSD, no differences between silages were observed in DMI for the last two trials (S25lo and L25lo); however, results of MDS indicated a preference for hay and d0 in both cases.

### 3.4. Silage characteristics influencing dry matter intake

Results of correlation analysis between silage characteristics, DM concentration and DMI are presented in Table 5.

**Table 3**
Chemical composition of silages during eight days (d0–d8) of aerobic exposure (g/kg dry matter unless otherwise stated).

| | Length of aerobic exposure (d) | | | | |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 |
| Dry matter (g/kg fresh matter) | 290 | 291 | 297 | 305 | 298 |
| Ash | 96 | 96 | 96 | 100 | 95 |
| Crude protein | 130 | 131 | 130 | 130 | 132 |
| Crude lipids | 35 | 35 | 35 | 33 | 34 |
| Acid detergent fibre[a] | 283 | 275 | 278 | 279 | 281 |
| Neutral detergent fibre[b] | 450 | 447 | 454 | 440 | 443 |
| 24 h gas production (ml/g DM) | 281 | 285 | 282 | 278 | 282 |
| Metabolizable energy (MJ/kg DM) | 10.7 | 10.9 | 10.8 | 10.7 | 10.8 |
| pH | 4.5 | 4.5 | 4.6 | 4.5 | 4.6 |
| Lactic acid | 62 | 61 | 57 | 57 | 68 |
| Acetic acid | 25 | 26 | 22 | 25 | 23 |
| iso-Butyric acid | 1.3 | 1.5 | 1.9 | 1.5 | 1.0 |
| n-Butyric acid | 11 | 12 | 14 | 12 | 14 |
| iso-Valeric acid | –[c] | – | – | – | – |
| n-Valeric acid | – | – | – | – | – |
| n-Caproic acid | – | – | – | – | – |
| Propionic acid | 0.2 | 0.2 | 0.1 | 0.5 | 0.1 |
| 1,2-Propanediol | 3 | 3 | 2 | 3 | 4 |
| Ethanol | 13 | 12 | 9 | 10 | 10 |
| Methanol | 0.6 | 0.7 | 0.7 | 0.6 | 0.6 |
| Propanol | 1.8 | 1.7 | 1.2 | 1.3 | 1.3 |
| 1-Butanol (mg/kg DM) | 32 | 27 | 23 | 10 | 28 |
| 2-Butanol (mg/kg DM) | 538 | 299 | 239 | 260 | 547 |
| Ethyl acetate (mg/kg DM) | – | – | – | – | – |
| Ethyl lactate (mg/kg DM) | 93 | 91 | 62 | 84 | 67 |
| Ammonia-N (g/kg of total N) | 122 | 129 | 138 | 140 | 134 |
| Water-soluble carbohydrates | 63 | 71 | 65 | 55 | 56 |
| Yeasts (log10 cfu/g) | 2.4 | 2.4 | 3.2 | n.a.[d] | 5.7 |
| Moulds (log 10 cfu/g) | 2.4 | 2.9 | 5.1 | n.a. | 6.1 |
| Aerobic mesophilic bacteria (log 10 cfu/g) | 4.0 | 5.5 | 5.6 | n.a. | 4.6 |

[a] Expressed exclusive residual ash.
[b] Analyzed with heat-stable amylase and expressed exclusive residual ash.
[c] Below detection limit (0.01% fresh matter).
[d] Not analyzed.
Each value represents the mean of eight experimental silages.

Most fermentation products were negatively correlated to silage DM concentration, with highest values for iso- and n-butyric acid, 1- and 2-butanol and $NH_3$-N. The same fermentation products were also negatively correlated to preference when expressed as DMI (g/3 h meal). Dry matter, acetic acid and ME influenced DMI positively ($P < 0.05$). Ethyl lactate was not correlated with DM or DMI.

## 4. Discussion

### 4.1. Silage quality

The goal of producing silages with two different DM concentrations was reached, but generally at a lower level than intended (30% and 40%). Consequently, DM of silages at the lower stage of wilting was below the recommendations of 30–40% DM for wilted grass silages (Thaysen, 2004). Concentrations of ADF were higher than given target values due to a relatively late harvest date; the other proximate constituents ranged within expected values (Thaysen, 2004). Silages at different levels of DM differed in fermentation quality. Concentration of acetic acid ranged from 16 to 30 g/kg DM, with the latter value being at the upper limit of recommendations of 30 g/kg DM (Kung and Shaver, 2001) that should not be exceeded as acetic acid influences DMI negatively (Buchanan-Smith, 1990; Eisner et al., 2006) even though in our study acetic acid is positively correlated to DMI. Silages S25hi, S25lo and L25lo contained considerable amounts of butyric acid, as well as relatively high concentrations of $NH_3$-N, which clearly exceeded the orientation value of 50 g/kg of total N for well-fermented grass silages reported by Huhtanen et al. (2002). Generally, the concentration of $NH_3$-N reflects the degree of protein and amino acid degradation; with high values having a depressing effect on utilization of nitrogen by ruminants (Driehuis, 2001). Both variables (i.e. butyric acid and $NH_3$-N) are evidence that clostridial fermentation had taken place in these silages (McDonald et al., 1991). Besides the low DM concentration, the long time interval between ensiling and opening could also have contributed to these considerable amounts of butyric acid, as described by Harrison et al. (2003), where higher concentrations of butyric acid and $NH_3$-N occurred after ten months of storage in comparison to six months.

Silages were analyzed for two ethyl esters, which were found in only low concentrations (ethyl lactate) or were not detected (ethyl acetate). For grass silages, there is a lack of information regarding ethyl esters, factors influencing their development and their impact on silage quality. A large study including data from laboratory experiments as well as on-farm data with whole-crop maize and sorghum silages found a wide range of ethyl ester concentrations

**Table 4**
The dry matter intake (DMI) and stimulus coordinates for the two-dimensional solution to the preference among five goats, $n = 25$.

| Silage treatment[A] | | Length of aerobic exposure (d) | | | | | Lucerne hay | MSD[B] |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | | |
| S33hi | DMI (3 h) | 566a | 561a | 630a | 509a | 272b | 289b | 124 |
| | DMI (30 min) | 333a,b | 357a,b | 380a | 290b,c | 108d | 264c | 75 |
| | Dimension 1 | −0.60 | 0.69 | 0.63 | −0.94 | −0.66 | 0.88 | |
| | Dimension 2 | −0.18 | 0.55 | 1.21 | 1.46 | −1.80 | −1.23 | |
| L33hi | DMI (3 h) | 658a | 549a,b | 550a,b | 492b,c | 354d | 412c,d | 129 |
| | DMI (30 min) | 418a | 341a,b | 326b | 311b,c | 199c | 202c | 79 |
| | Dimension 1 | 2.05 | −0.68 | −0.08 | −0.31 | −0.60 | −0.38 | |
| | Dimension 2 | 0.43 | 1.21 | −1.00 | −1.10 | 1.41 | −0.94 | |
| S25hi | DMI (3 h) | 418b,c | 479a,b | 537a | 396c | 379c | 536a,b | 118 |
| | DMI (30 min) | 260a | 224a | 271a | 243a | 232a | 283a | 86 |
| | Dimension 1 | 0.18 | −1.97 | 1.99 | −0.03 | −0.21 | 0.05 | |
| | Dimension 2 | 1.47 | 0.46 | 0.25 | −0.93 | −0.70 | −0.54 | |
| L25hi | DMI (3 h) | 312b | 493a | 513a | 409a,b | 322b | 473a | 130 |
| | DMI (30 min) | 220a,b | 295a | 300a | 258a,b | 230a,b | 207b | 84 |
| | Dimension 1 | 0.77 | 0.77 | 0.14 | −0.28 | −1.63 | 0.24 | |
| | Dimension 2 | −2.04 | 0.74 | 1.57 | 0.61 | −0.62 | −0.26 | |
| S33lo | DMI (3 h) | 496a,b | 558a | 545a | 564a | 399b,c | 359c | 126 |
| | DMI (30 min) | 297a,b | 291a,b | 351a | 291a,b | 236b | 215b | 91 |
| | Dimension 1 | 0.14 | −0.53 | −1.53 | 0.08 | 0.14 | 1.69 | |
| | Dimension 2 | 1.80 | 0.00 | 0.00 | 0.00 | −1.80 | 0.00 | |
| L33lo | DMI (3 h) | 492a,b | 492a,b | 587a | 406b | 378b | 433b | 130 |
| | DMI (30 min) | 302a,b | 231b | 361a | 239b | 253b | 229b | 85 |
| | Dimension 1 | 1.05 | −0.75 | 0.56 | 0.98 | −0.40 | −1.43 | |
| | Dimension 2 | −0.21 | 1.57 | 1.36 | −0.91 | −1.04 | −0.77 | |
| S25lo | DMI (3 h) | 305 | 312 | 333 | 354 | 323 | 379 | 151 |
| | DMI (30 min) | 168 | 212 | 189 | 243 | 191 | 240 | 98 |
| | Dimension 1 | 0.29 | 0.67 | −1.08 | 0.40 | −0.80 | 0.52 | |
| | Dimension 2 | 1.93 | −1.92 | 0.47 | 0.39 | −1.16 | 0.29 | |
| L25lo | DMI (3 h) | 343 | 388 | 401 | 377 | 324 | 409 | 131 |
| | DMI (30 min) | 173 | 193 | 233 | 199 | 206 | 249 | 79 |
| | Dimension 1 | 1.07 | 1.08 | −1.59 | −0.39 | −0.37 | 0.21 | |
| | Dimension 2 | 1.60 | −1.60 | −0.02 | 0.93 | −0.92 | 0.01 | |

Means with different alphabets (a, b, c and d) between columns differ significantly ($P < 0.05$).
[A] Treatments: S = short chopping length, L = long chopping length, 33 = 33% DM, 25 = 25% DM, lo = low packing density, hi = high packing density.
[B] Minimum significant difference (Waller–Duncan $k$-ratio $t$-test).

with maximum values reaching 1109 and 1305 mg/kg DM for ethyl acetate and ethyl lactate, respectively (Weiß and Auerbach, 2012). It was assumed that the formation of ethyl esters in silages is a straight chemical reaction, whose magnitude is determined by the concentration of ethanol. This was not confirmed by this study because no relationship was found between ethyl lactate and ethanol ($r = 0.137$, $P = 0.397$). Another study with whole-crop maize silages (Gerlach et al., 2013) found considerable amounts of both compounds with evidence of negative effects on short-time DMI, especially for ethyl lactate ($r = -0.33$, $P < 0.05$). Based on the data presented here, these two ethyl esters may not have the same importance in grass silages, especially in low DM grass silages at a relatively high pH. This is supported by the results of correlation analysis which did not show a relationship to DMI.

### 4.2. Changes during aerobic exposure

During aerobic exposure, silage temperature did not rise more than 3 K above ambient, which means all silages (except L25hi at d8) were aerobically stable during the 8 d period. This is due to the microbiological status of the silages during aerobic exposure; only few samples slightly exceeded target values. Aerobic mesophilic bacteria and moulds were more often detected at critical concentrations than yeasts (L25hi at d8). This is consistent with a previous report of Lindgren et al. (1985), who noted that initiation of spoilage was caused by bacteria or moulds instead of yeasts. Moulds have been described to be of particular importance in grass silages (McDonald et al., 1991). Yeasts did not develop despite ambient temperatures being close to their optimum range of 20–30 °C (Ashbell et al., 2002). The relatively high amounts of acetic and butyric acids measured in the eight silages at opening might be responsible for the stability of the silages in the trials due to their inhibitory effect on spoilage organisms (Kung et al., 1998; Danner et al., 2003; Wilkinson and Davies, 2013). Due to the fact that growth of spoilage organisms was limited during aerobic exposure, concentration of fermentation products did not change appreciably. Silages did not show typical signs of deterioration, such as growth of spoilage organisms resulting in an increase in temperature and pH, and the degradation of fermentation acids (Courtin and Spoelstra,

**Table 5**
Correlation (Pearson coefficients) between silage characteristics, dry matter (DM) concentration of silages and DM intake (DMI) of goats, $n = 25$.

| | DM | DMI |
|---|---|---|
| DM | 1 | 0.45** |
| DMI | 0.45** | 1 |
| Ash | −0.46** | −0.27 |
| Crude protein | −0.75*** | −0.54*** |
| Crude lipids | −0.49** | −0.38* |
| Acid detergent fibre[a] | 0.59*** | 0.38* |
| Neutral detergent fibre[b] | 0.39* | 0.23 |
| Acid detergent lignin | −0.10 | −0.08 |
| 24 h gas production | 0.20 | 0.20 |
| Metabolizable energy | 0.34* | 0.30† |
| pH | 0.40* | −0.20 |
| Lactic acid | −0.38* | −0.10 |
| Acetic acid | 0.29† | 0.53*** |
| Propionic acid | −0.46** | −0.38* |
| iso-Butyric acid | −0.85*** | −0.59*** |
| n-Butyric acid | −0.84*** | −0.55*** |
| Total acids | −0.69*** | −0.22 |
| Ethanol | −0.42** | −0.21 |
| Methanol | −0.37* | 0.17 |
| Propanol | −0.42** | −0.04 |
| 1,2-Propanediol | 0.26 | 0.17 |
| 1-Butanol | −0.63*** | −0.50*** |
| 2-Butanol | −0.69*** | −0.51*** |
| Ethyl lactate | 0.15 | −0.03 |
| Ammonia-N | −0.82*** | −0.55*** |
| Water-soluble carbohydrates | 0.09 | −0.11 |

[a] Expressed exclusive residual ash.
[b] Analyzed with heat-stable amylase and expressed exclusive residual ash.
† $P < 0.10$.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

1990; McDonald et al., 1991). One silage had yeast counts exceeding target values (L25hi at d8); this was the only silage with an increase in temperature of more than 3 K above ambient.

### 4.3. Changes in preference and dry matter intake

Regarding goats' preference and the impact of aerobic exposure, noticeable differences were observed between low- and high-DM silages. The 3 h-intake of low-DM silages was numerically lower (386 g vs. 503 g DM), but the decrease in DMI due to the aerobic exposure for 8 d was relatively low (20 ± 5.5%). As postulated by Gill et al. (1986), silage intake, especially in short-time experiments, seems to be controlled mainly by oropharyngeal factors (taste, smell) and chemostatic regulation and not by rumen fill. The lower intake might therefore be a result of both sensory characteristics, especially smell and a negative post-ingestive feedback eventually derived from higher amine concentrations or other unidentified silage characteristics. Low-DM forages were classified as badly fermented but, as stated above, aerobically stable. Consequently, feed intake was lower than for well-fermented silages but did not change as a consequence of aerobic exposure. Only in S25hi, the DMI of d6 and d8 was lower in comparison to fresher silages ($P < 0.05$). This might be due to the microbiological status of that treatment, where orientation values for moulds and aerobic mesophilic bacteria (VDLUFA, 2012) were exceeded at d4 and d8 of aerobic exposure. The impact of hygienic quality of forages on feed intake and preference by dairy calves has been studied by Undi and Wittenberg (1996) using different qualities of lucerne hay. For treatments similar in NDF, a decrease ($P < 0.05$) in preference occurred as amount of fungal biomass in hay increased. As other variables did not change during aerobic exposure, the decrease in microbiological quality might have caused the avoidance of d6 and d8 silages.

The 3 h-DMI of high-DM silages was greater and more differences occurred between silages with different lengths of aerobic exposure. For these silages, d8 was consumed less in all of the four trials ($P < 0.05$). There was a decrease in DMI between d0 and d8 of 50% (±3.3%), with 8 d of aerobic exposure representing a time interval that will be easily reached under practical conditions (Wilkinson and Davies, 2013). For aerobic stability and its impact on preference and DMI, we suggest focusing on these high-DM silages, as they are closer to recommendations and consequently have a major relevance for practical work.

For a comparison with literature, there is not much data available dealing with the impact of aerobic deterioration of silages on preference and DMI by ruminants. Whitlock et al. (2000) found that the addition of surface-spoiled maize silages to rations for crossbred steers had large negative associative effects on DMI and organic matter, NDF and ADF digestibility. Gerlach et al. (2013) conducted preference trials with goats and found a decrease in DMI after 4 d of aerobic exposure, with reductions reaching values between 29% and 79% after 8 d of exposure in comparison to fresh silages. Counts of yeasts and moulds showed rapid increase within 4 d of air ingress, causing severe changes in fermentation products and temperature. It is assumed that the fast deterioration process is responsible for the stronger impact on DMI in comparison to the grass silages in the present study.

Our results are consistent with a previous study by Wichert et al. (1998); who used single- and two-choice experiments to examine the effect of hygienic quality of different combinations of grass and maize silages and hay on DMI of dairy cows. Aerobic deterioration of silage led to a decrease in DMI of about 10–20% in single-choice experiments; and the difference was greater, when cows had the option to choose between two qualities. This was also observed by Keady and Murphy (1998), where differences in feed intake were much stronger when cows were having the possibility to choose between two or more feedstuffs. One of our aims was to identify feed characteristics responsible for preference or avoidance. Because feeding behaviour is more sensitive to feed characteristics in choice situations (Baumont, 1996), the design was judged as being appropriate for reaching these aims. Eight trials with different grass silages have shown that aerobic exposure does have a negative effect on short-term feed intake and preference by goats, especially with well-fermented silages; the extent, however, was much less than reported for maize silage (Gerlach et al., 2013). Nevertheless, results gave an insight into decreased DMI when feeding spoiled silages in comparison to fresh ones. It will be interesting for future studies to examine the impact of deterioration in single-choice experiments or in a TMR.

One-day adaptation for each silage variant, as used in different studies (e.g. Buntinx et al., 1997; Burns et al., 2001; Fisher et al., 2005; Gerlach et al., 2013) has proven to be a sufficient time interval for the animals to associate each forage (sensory characteristics) with the post-ingestive signals and to remember it some days afterwards in the experimental period. These reports indicated differences in goats' preference between variants. Although goats like testing new feedstuffs (Provenza et al., 1996) there were few shifts in preference during the 3 h presentation. Within each experimental day, goats were consistent in their preference behaviour and did not change their initial choice, which is underlined by a strong correlation of $r = 0.91$ ($P < 0.001$) between initial and total DMI. During the first 30 min, goats consumed 58.8% (±3.09%) of the total 3 h-DMI, with only a small variation between trials. We suggest that after an adaptation period, the initial preference behaviour has some potential to be used as an indicator for total DMI and preference in short-term preference experiments.

Only in both poorly fermented silages (S25lo and L25lo) more "testing" was observed and no difference between variants was found in initial or 3 h-DMI. The smell of butyric acid as major substance of malfermentation per se does not lead to avoidance or a reduced intake and sometimes even increases DMI (Arnold et al., 1980). More likely, intake of silages S25lo and L25lo during the adaptation period may have resulted in a negative post-ingestive feedback, eventually due to higher concentrations of $NH_3$-N or amines. As described by Burritt and Provenza (2000) lambs that were offered meals containing different toxins ate less food than lambs offered rations without toxins. Their results indicated that in some cases ruminants can increase intake of toxic foods by consuming foods containing different toxins. Also in our case, animals could try to reduce the negative feedback derived from the poorly fermented forages (e.g. with elevated amine concentrations) by eating and testing both variants available during the experimental feeding.

### 4.4. Silage characteristics influencing dry matter intake

When using data from all trials, correlation between silage DM and DMI was strongly positive. This is in agreement with previous work (Forbes, 1995; Steen et al., 1998; Eisner et al., 2006) but likely more silage quality (especially fermentation quality resulting from DM concentration) than DM concentration per se might be responsible. In case of the low-DM silages fed in these trials, the negative impact of DM on feed intake may also be a side-effect of the components having developed during malfermentation. Some of the fermentation products, especially those indicating poor fermentation qualities, were consequently negatively correlated to DMI. These were especially butyric acid, 1-butanol and 2-butanol and $NH_3$-N with correlation coefficients ranging between −0.50 and −0.59. The $NH_3$-N had limited intake in other studies, either directly or indirectly due to correlation with some other end-products of silage proteolysis or amino acid degradation (Huhtanen et al., 2002; Hetta et al., 2007). Steen et al. (1998) proposed that the $NH_3$-N concentration was not directly responsible for reduced intake, but a possible relationship between ammonia and other products. Also Buchanan-Smith and Phillip (1986) concluded from their experiments that soluble constituents in silages can inhibit intake but no single component was primarily responsible. It seems that goats can detect subtle differences between silages and have also shown in other studies to prefer forages based on small changes in plant chemistry that are difficult to detect in chemical analyses (Burritt et al., 2005). In a meta-analysis on the relationship between silage characteristics and feed intake of dairy cows, Eisner et al. (2006) did not find any influence of $NH_3$-N on intake; instead the importance of protein quality was emphasized. Protein degradation products like biogenic amines were suggested to limit silage intake (Buchanan-Smith and Phillip, 1986; Dulphy and Van Os, 1996). As concentrations of $NH_3$-N and butyric acids represent good indicators for biogenic amines ($r = 0.67$ and $r = 0.80$, $P < 0.05$; Richardt et al., 2011), there might have been high concentrations of biogenic amines in the low-DM silages used in the preference trials, which may have negatively affected DMI. This assumption can only be confirmed with further studies.

The positive effect of acetic acid on DMI contradicts Eisner et al. (2006) who concluded from a large meta-analysis that acetic acid had a strong negative impact on feed intake when offering silages and concentrates separately. This might be due to the fact that the level of acetic acid in the eight trials was generally lower than in studies where intake reductions took place. For example, silage DMI was depressed at acetic acid concentrations of 54 g/kg DM that were added to a basal silage containing 21 g/kg DM (Krizsan et al., 2012), therefore exceeding our values threefold. Concentrations of 10–30 g/kg DM that are typically found in well-fermented grass silages (Kung and Shaver, 2001) were not exceeded, consequently the negative impact on feed intake might only be relevant at higher levels.

## 5. Conclusions

During 8 d of aerobic exposure, grass silages did not show strong signs of spoilage. Nevertheless, for well-fermented silages, short-term DMI and preference by goats decreased at the sixth and eighth day of exposure, also at apparently low levels of deterioration. It is assumed that goats can detect subtle differences caused by oxygen ingress, sometimes even before an increase in temperature or changes in chemical composition occur. It underlines the assumption of different authors that unidentified volatile compounds might affect preference and feed intake. In low-DM silages with higher concentrations of butyric acid, therefore giving evidence for malfermentation, DMI was generally lower and fewer differences between silages with different lengths of aerobic exposure were observed. Results highlighted the importance of fermentation quality as well as aerobic stability in order to achieve high levels of DMI. The study pointed out the potential for 30 min experiments to measure short-term preference, due to a high correlation between initial and total DMI after a 1-d adaptation period for each variant.

## Acknowledgments

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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