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The aerobic deterioration of silages as estimated from chemical composition and dietary choice by goats

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Meiner Familie

SUMMARY

The aerobic deterioration of silages as estimated from chemical composition and dietary choice by goats

Conserved forage as silage provides an important source of energy and nutrients for livestock in many countries of the world. After opening the silo for the feed-out phase or due to damages of the covering, oxygen ingresses into the silage and abolishes anaerobic conditions. The activity of aerobic spoilage organisms can cause dry matter (DM) and nutrient losses as well as negatively affect both fermentation and hygienic quality of the silage. There is a lack of information regarding the changes taking place after opening and especially their impact on feed intake and preference by ruminants. Furthermore, it is difficult to objectively assess silage quality and its usability as feedstuff once aerobic deterioration has started.

This study uses an interdisciplinary approach for comprehensively measuring and evaluating fermentation quality and aerobic stability of grass and maize silages by means of different analytical methods. It focuses on characterizing chemical and microbiological changes caused by aerobic exposure and evaluating the effect on short-time feed intake and preference behaviour by goats. For these purposes, a 2 x 2 x 2 x 2-factorial design was used to evaluate 16 silages differing in substrate (maize and grass), DM content (maize silages 34% and 40%, grass silages 25% and 33%), chopping/cut length (short and long) and compaction pressure at ensiling (0.1 MPa and 0.2 MPa). After anaerobic storage for at least 90 days (d), silages were exposed to air for 8 d. In 2-d intervals (d0, 2, 4, 6 and 8 after silo opening), silages were sampled for chemical analyses, and the microbiological status was determined by enumeration of yeasts, moulds, aerobic mesophilic bacteria and lactic acid bacteria. Furthermore, silage from these days was stored anaerobically in vacuum-sealed plastic bags for use in preference trials. After aerobic exposure, for each silage a preference trial was done with Saanen-type goats (maize silage trials, n = 6; grass silage trials, n = 5), each one lasting 21 days. During the experimental phase, each possible two-way combination of the five silages (d0, d2, d4, d6, and d8) and one standard lucerne hay, which was used for comparison of different trials, was offered as free choice for 3 h.

At opening, maize silages had a high fermentation quality with a low pH and an absence of butyric acid, but during aerobic exposure, strong changes concerning fermentation products and microbiological quality occurred. Lactic and acetic acids were degraded, resulting in an increase of the mean pH of the experimental silages from 3.9 (d0) to 5.8 (d8). Counts of

yeasts rose drastically during the first 4 d of aerobic exposure causing an increase of mean silage temperature of 29 °C above ambient. In the preference trials, a significant decline occurred in DM intake (DMI) after 4 d of aerobic exposure with a mean decrease in DMI of 53% at d8 in comparison to the fresh silages. Preference when expressed as DMI was negatively correlated to silage temperature (as difference to ambient) and the concentrations of ethanol and ethyl lactate.

All grass silages were aerobically stable during the examination time showing neither an increase in temperature (> 3.0 K above ambient) nor changes in the composition of fermentation products. The DMI of 33% DM-silages decreased after 4 or 6 d of aerobic exposure and silage that had been exposed to air for 8 d was avoided in each case with a mean reduction of 50% in comparison to d0-silages. Low-DM silages showed signs of malfermentation with higher concentrations of butyric acid and ammonia-N. The DMI was lower and fewer differences between silages with different lengths of aerobic exposure occurred. Mean decrease in DMI after 8 d of storage was 20%. Especially products from protein and amino acid degradation (ammonia-N, iso- and n-butyric acid) were negatively correlated to DMI.

It was concluded that, in well-fermented silages, aerobic exposure for a length of time that is of practical relevance negatively impacts short-time 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 before an increase in temperature or changes in chemical composition occur. Still unidentified degradation products might therefore have an impact on feeding behaviour of goats when silages are offered in choice situations. The results highlight the importance of fermentation quality and aerobic stability in the preference behaviour of ruminants, but identifying single silage characteristics being responsible for preference or avoidance was difficult. At the moment measurement of silage temperature seems to be the best suitable single indicator for predicting preference behaviour and DMI at farm level.

ZUSAMMENFASSUNG

Die Bewertung des aeroben Verderbs von Silagen anhand von Veränderungen der chemischen Zusammensetzung und des Präferenzverhaltens von Ziegen

Die Konservierung von Grobfutter als Silage spielt weltweit eine bedeutende Rolle in der Ernährung landwirtschaftlicher Nutztiere. Nach Öffnen des Silos zur Entnahme oder bedingt durch Beschädigungen der Abdeckung kommt es zu Sauerstoffzutritt und damit zur Aufhebung der anaeroben Verhältnisse. Die hiermit einhergehenden Umsetzungsprozesse aerober Verderborganismen können zu Trockenmasse-(TM)- und Nährstoff-Verlusten sowie negativen Auswirkungen auf die Silagequalität führen. Der Kenntnisstand über die aeroben Umsetzungsprozesse und die damit verbundenen Auswirkungen auf das Futteraufnahmeverhalten von Wiederkäuern ist unzureichend; weiterhin mangelt es an Methoden zur objektiven Bewertung von Silagequalität und Gebrauchsfähigkeit nach Einsetzen aerober Verderbsprozesse. In einem interdisziplinären Ansatz wurden die Gärqualität und aerobe Stabilität von Silagen mit Hilfe verschiedener analytischer Methoden gemessen und bewertet. Die durch Lufteinfluss ausgelösten Veränderungen an Gärfuttern wurden dazu durch chemische und mikrobiologische Untersuchungen umfangreich charakterisiert, weiterhin wurden die Auswirkungen dieser Veränderungen auf das Präferenzverhalten sowie die kurzzeitige TM-Aufnahme von Ziegen untersucht. Für diese Vorhaben wurden in einem 2 x 2 x 2 x 2-faktoriellen Versuchsdesign insgesamt 16 verschiedene Silagen verwendet, welche sich hinsichtlich Substrat (Mais und Gras), TM-Gehalt (Maissilagen 34 % und 40 %, Grassilagen 25 % und 33 %), Häcksel- bzw. Schnittlänge (kurz und lang) und Verdichtungsdruck bei der Einlagerung (0,1 MPa und 0,2 MPa) unterschieden. Nach mindestens 90-tägiger Silierdauer wurden die Silagen über einen Zeitraum von acht Tagen aerob gelagert, wobei im zweitägigen Intervall (Tag (T) 0, 2, 4, 6, 8 nach Öffnung) die Probennahme für die chemische Analytik sowie die Bestimmung des mikrobiologischen Status anhand des Besatzes an Hefen, Schimmelpilzen, aerober mesophiler Bakterien und Milchsäurebakterien erfolgte. Für den späteren Einsatz im Präferenzversuch wurde Silage der jeweiligen aeroben Lagertage in Polyethylenbeuteln vakuumverpackt. Anschließend wurde für jede der 16 Silagen ein 21-tägiger Präferenzversuch mit Ziegen (Weiße Deutsche Edelziege, n = 6 bzw. n = 5) durchgeführt. Das Design der Versuchsphase sah vor, jeder Ziege jede mögliche Kombination zweier Silagen (T0, 2, 4, 6, 8) und eines Luzerneheus, welches als Standardfutter zur Vergleichbarkeit in allen Durchgängen diente, für 3 h zur

freien Wahl anzubieten. Die Maissilagen wiesen zum Zeitpunkt des Öffnens bei niedrigem pH-Wert eine sehr gute Gärqualität auf; die aerobe Stabilität betrug jedoch nur wenige Tage. Während der aeroben Lagerung nahmen die Konzentrationen an Milch- und Essigsäure deutlich ab, was zu einem Anstieg des mittleren pH-Wertes von 3,9 (T0) auf 5,8 (T8) führte. Bereits innerhalb der ersten vier Tage kam es zu einem starken Anstieg des Keimgehaltes an Hefen, deren Aktivität einen mittleren Temperaturanstieg von 29 °C über Umgebungstemperatur verursachte. In den Präferenzversuchen nahm die mittlere TM-Aufnahme nach vier Tagen aerober Lagerung signifikant ab; an Tag 8 betrug der durchschnittliche Rückgang 53 % gegenüber dem Material vom Tag des Öffnens. Die TM-Aufnahme wies eine stark negative Korrelation zur Silagetemperatur (ausgedrückt als Differenz zur Umgebungstemperatur) sowie schwach negative Korrelationen zu den Gehalten an Ethanol und Ethyllactat auf. Die Grassilagen waren im Beobachtungszeitraum aerob stabil, es wurden weder ein Temperaturanstieg (> 3,0 K über Umgebungstemperatur) noch Veränderungen im Gärsäuremuster festgestellt. Die Futteraufnahme bei 33 % TM-Silagen nahm nach vier bzw. sechs Tagen aerober Lagerung signifikant ab, und T8-Silagen wurden mit einem mittleren Rückgang von 50 % gegenüber der frischesten Variante in allen Durchgängen gemieden. Die Silagen mit niedrigeren TM-Gehalten wiesen höhere Gehalte an Buttersäure und Ammoniak-Stickstoff und damit Anzeichen von Fehlgärungen auf. Die TM-Aufnahmen an frischer Silage sowie der Einfluss der aeroben Lagerung auf die TM-Aufnahme waren mit einem mittleren Rückgang von 20 % nach acht Tagen geringer. Vor allem Produkte aus dem Protein- und Aminosäurenabbau waren negativ mit der Futteraufnahme korreliert. Eine aerobe Lagerung über einen praxisrelevanten Zeitraum führte in Silagen mit hoher Gärqualität zu einem deutlichen Rückgang der kurzfristigen TM-Aufnahme in Wahlsituationen; im Falle der Grassilagen sogar bei nur leichtem Verderbsstatus. Es wurde geschlussfolgert, dass Ziegen minimale verderbsbedingte Unterschiede wahrnehmen können, teilweise bevor Veränderungen in Temperatur oder chemischer Zusammensetzung messbar sind. Möglichweise haben bisher nicht identifizierte Abbauprodukte einen Einfluss auf die sensorischen Silageeigenschaften oder postingestive Vorgänge. Die Bedeutung von Gärqualität und aerober Stabilität zur Realisierung hoher TM-Aufnahmen wurde in allen Versuchsdurchgängen verdeutlicht, wobei es schwierig ist, einzelne, für Präferenz oder Ablehnung verantwortliche Silagecharakteristika im Wahlversuch zu identifizieren. Die Messung der Silagetemperatur ist zum derzeitigen Kenntnisstand die am besten geeignete Methode zur Bestimmung der Gebrauchsfähigkeit der Silage für den Praxiseinsatz.

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ABBREVIATIONS

AA	Amino acid(s)
ADFom	Acid detergent fibre expressed exclusive residual ash
ADL	Acid detergent lignin
BW	Body weight
cfu	Colony-forming unit
CL	Crude lipids
CP	Crude protein
d	Day
DM	Dry matter
DMcor	Dry matter corrected for the loss of volatiles during drying
DMI	Dry matter intake
GC	Gas chromatography
GP	Gas production in 24 hours from 200 mg (DM) substrate
ME	Metabolizable energy
mo	Month
MSD	Minimum significant difference
MDS	Multidimensional scaling
MS	Mass spectrometry
n.a.	Not analyzed
n.d.	Not detected (below detection limit)
NDFom	Neutral detergent fibre expressed exclusive residual ash
NH_3-N	Ammonia-Nitrogen
SD	Standard deviation
SE	Standard error

Silage treatments

L/S	Long/short chop/cut length
25/33/34/40	25%/33%/34%/40% DM
hi/lo	High/low compaction pressure at ensiling
d0-d8	Length of aerobic exposure (0-8 days)

ΔT	Silage temperature expressed as difference to ambient (K)
TMR	Total mixed ration
VFA	Volatile fatty acid(s)
WSC	Water-soluble carbohydrates

CHAPTER 1

General introduction

Ensiling of forages is a common method of conservation in many parts of the world, because it makes crops available for feeding throughout the year or in periods of restricted seasonal availability of pastures for the grazing animal (Wilkinson and Davies 2012). It is based on the natural fermentation under anaerobic conditions with epiphytic lactic acid bacteria converting water-soluble carbohydrates (WSC) into organic acids, mainly lactic acid (Ashbell et al. 2002). As a consequence, pH decreases and forages are preserved from spoilage organisms. Crops used for ensiling should contain adequate levels of fermentable substrate, a relatively low buffering capacity and dry matter (DM) contents above 20% (McDonald et al. 1991). The quotient of WSC and buffering capacity produces a minimum DM content for stable silage in relation to crop ensilability, which differs considerably between forages. For wilted silages, DM contents between 30% and 40% are recommended, due to positive effects on the ensiling process and related factors like DM intake (DMI) (Thaysen 2004).

In European countries, silage maize, grasses, whole-crop cereals and legumes are the major crops used as fresh or conserved forage for the nutrition of ruminants. Maize (*Zea mays* L.), harvested as whole-plant, is a high energy, low protein forage commonly fed to beef cattle, growing dairy heifers and lactating dairy cows in climatic regions where maize is moderately to well adapted (Allen et al. 2003). Grass is one of the commonest crops to be conserved by ensiling; it traditionally forms a basic component of ruminant diets in many parts of Europe, North America, New Zealand and Australia (Keady et al. 2012). There are both single species of grass like Italian ryegrass (*Lolium multiflorum L.*) or perennial ryegrass (*Lolium perenne L.*) and mixed species of grasses and legumes that are grown as silage crops (McDonald et al. 1991).

The overall quality of a forage is defined as its ability to meet the nutritional needs of the animal consuming it. It is therefore a function of nutritive value, which includes digestibility, concentration and quality of crude protein, mineral and vitamin concentration and forage intake level, which can be influenced by availability, acceptability and forage behaviour in the digestive tract (Harrison et al. 2003). When crops are conserved by ensilage, the fermentation quality, mainly consisting of silage pH, concentrations of lactic acid and short-chain fatty acids, WSC and products resulting from degradation of protein and amino acids,

adds a new source of variation to the forage quality (Randby et al. 2012). This description points out that several and often interrelated factors may influence silage quality. As silage is a part of the food chain and quality insufficiencies affect feed intake and animal health, food safety and quality and also environmental aspects (Borreani et al. 2008), an adequate assessment of forage quality is an important but also complex challenge. Determination of silage quality is normally conducted at the moment of silo opening and it is made difficult by the fact that it may change as consequence of aerobic deterioration. When oxygen penetrates into the silage during storage or feed-out, acid-tolerant (facultative) aerobic microorganisms, mainly yeasts and moulds, start to proliferate, oxidizing substrates such as residual sugars, lactic acid, acetic acid and ethanol. With progression of this process, silage pH starts to rise caused by the decreasing concentration of the preserving acids, and the heat released from oxidation gives rise to a measurable increase of temperature (Pahlow et al. 2003). As a consequence, all silages sooner or later deteriorate in the presence of oxygen, but with considerable variation concerning their length of stability upon exposure to air. The microbial dynamics taking place during the spoilage process are still not fully understood, but it is suggested that aerobic stability is a matter of microbial interactions and competitiveness (Martens 2006). It is the result of crop, environmental and management factors interacting at harvest and filling, during storage and at feed-out (Wilkinson and Davies 2012). This complex connection becomes apparent in Figure 1.

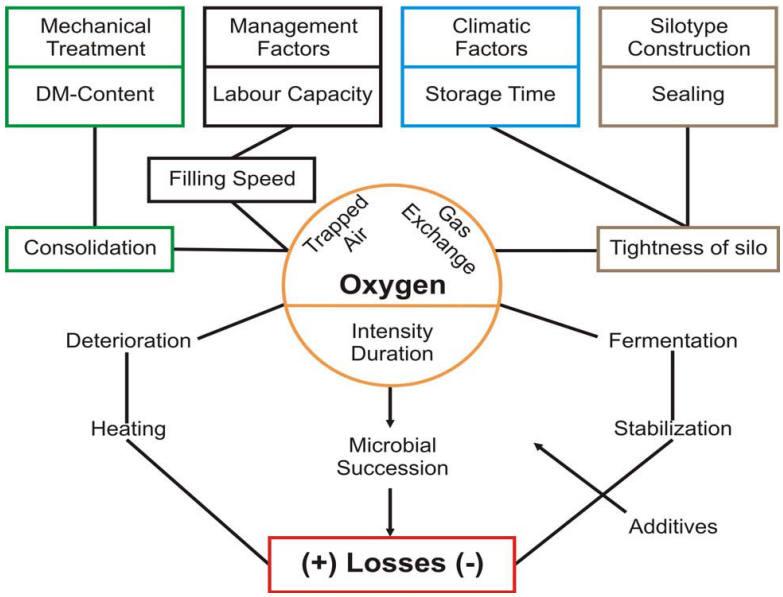

Figure 1. Oxygen-related factors causing losses in silages (Pahlow and Muck 2009)

The nutritional value of silages as a consequence of aerobic deterioration can be reduced by degradation of fermentation acids and cell-wall carbohydrates and catabolism of protein to ammonia-N (Rooke and Hatfield 2003), furthermore, there are changes in the composition of volatile compounds and intake of deteriorated material is often restricted or even rejected completely (McDonald et al. 1991). The extent of the decline in feed intake however is unclear, and additionally, it is still not fully understood which silage characteristics lead to preference or avoidance by ruminants.

Food selection and preference behaviour involve interactions between the senses of taste and smell and mechanisms to sense the consequences of food ingestion, such as satiety and malaise, the so-called postingestive feedback, which is based on effects like release of saliva and digestive enzymes, release of most gastrointestinal and pancreatic hormones and release of neurotransmitters involved in satiety after ingestion of the feedstuff (Provenza 1995). Aversive feedback, for example, caused a decrease in DMI of poor-quality silage by sheep (Buchanan-Smith 1990). It is believed that fermentation end-products do have an influence on silage intake, probably by influencing both sensory properties and postingestive feedback. Some attempts have been made to find a relationship between fermentation characteristics and DMI using large datasets from production trials in meta-analyses (Huhtanen et al. 2002; Eisner et al. 2006), but only fresh, unspoilt silages were considered, therefore neglecting changes occurring after infiltration of oxygen.

Well-fermented, energy-rich forages with low concentrations of acetic acid and an absence of butyric acid are prone to spoilage as soon as oxygen becomes available. These changes which may occur during the feed-out phase are equally as important as those in the anaerobic storage phases from the viewpoint of preserving nutrients and maintaining good hygienic quality to the point of silage consumption by the animal (Wilkinson and Davies 2012). Consequently, the aerobic stability is a major component in the general assessment of silage quality. However, in contrast to fermentation quality of fresh silages, where orientation values help interpreting results of chemical analyses, it is difficult to objectively determine silage quality and usability once the silo has been opened and spoilage processes have started.

In addition to proximate constituents and fibre fractions, which are commonly determined for use in the feeding management, silages are analyzed for fermentation products to assess fermentation quality by means of contents of the undesired degradation products acetic acid and butyric acid (DLG 2006). However, this evaluation of silage quality during the feed-out phase requires many samples, expensive labour and equipment, qualified personnel and time-

consuming laboratory analyses. As results are not available directly, they can hardly be used for the daily control of the silage working face.

For a fast and cost-effective assessment of forage quality, a sensory evaluation key (DLG 2004) can be used; here, smell, colour, structure and DM content are directly determined by human senses. However, this method is subjective and also requires experienced staff. Furthermore, the evaluation sheet does not consider any oxygen-related changes that may occur after opening the silo. The aerobic stability is normally determined under laboratory conditions by continuously measuring temperature of silages exposed to air for several days at constant ambient temperature (Honig 1990). Concerning the increase in temperature where aerobic instability begins, there are controversial orientation values in literature ranging from 1.7 °C (Johnson et al. 2002) to 10 °C (Spiekers et al. 2009) above ambient. It underlines the difficulty of implementing objective control points for the silage management under practical conditions. This demand has been intensified by implementation of the "Regulation of the European Parliament and of the council laying down requirements for feed hygiene" (Anonymus 2005), which prescribes the traceability of compliance with hygienic regulations at all stages of food production. This regulation also includes forages produced as feedstuff for animals that are used for milk or meat production and emphasizes the need for objective control instruments in the silage management providing directly available and reliable results.

One possibility for an objective assessment of forage quality is seen in the application of sensor array systems. In contrast to analytical procedures like gas chromatography (GC), mass spectrometry (MS) or their combination (GC-MS), chemosensor arrays do not measure single volatile compounds but gas compositions by using different sensors. For the measurement, both odorous substances but also other volatile compounds are considered, therefore characterizing the mixture of all volatile components (Boeker 2001). Different gas compositions create diverse signal patterns produced by several single sensor combined in a sensor array, and the responses given by the sensors are proportional to the concentration of the volatile compound being sensed. Arrays of sensors produce signal patterns that can be used as descriptors for discriminating complex odours (Persaud et al. 1996). The use of chemosensor arrays is especially popular in industries where aroma is an important component of the product quality, such as perfume industry and food industry for regularly monitoring product quality and safety by continuously comparing samples with standards (Boeker 2001; Sankaran et al. 2012). Sensor array systems have been developed and previously used for different purposes, like as an electronic odour measurement system at a

waste incineration plant (Haas et al. 2008) and for detection of odour emissions from a composting facility (Yuwono et al. 2003). As reviewed by Sankaran et al. (2012), they are also used in the quality management of food-processing plants, like for the detection of beef spoilage or microbial contamination of processed tomatoes or the evaluation of cheddar cheese quality. Such systems are also applied for environmental air quality monitoring and toxic vapour detection. In contrast to the subjective human sensory evaluation, an olfactory sensing system is able to give a qualitative as well as quantitative estimate of odour perception without any bias (Sankaran et al. 2012).

Chemosensor systems were also tested and used for agricultural applications like an on-line assessment of milk quality in automatic milking systems or for selectively predicting ammonia at varying humidity concentrations in agricultural emissions after application of multivariate regression models (Boeker et al. 2000). Another example of use is the monitoring of plant diseases and toxins, like the detection of fungal contamination of maize as presented by Falasconi et al. (2005). This sensor system may represent a valid method of screening the maize bulk in order to prevent the entry of mycotoxins into the food chain.

By developing and adjusting such a chemosensor system for the application on silages it might be possible to differentiate between silage qualities with the help of changes in the resulting signal pattern and finally to objectively assess silage quality and stage of deterioration.

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CHAPTER 2

Scope of the thesis

As there is a lack of studies dealing with the interplay between chemical composition of different silages and the effects on preference behaviour by ruminants, an interdisciplinary approach was chosen for the following study.

The aim was to present detailed descriptions of silage quality and shifts in chemical composition as well as in the microbiological status during aerobic exposure. In this context the impact of different management factors (substrate, DM content, cut length and density) on fermentation quality and aerobic stability was evaluated.

By using short-time feeding trials, the preference behaviour of goats when forages are offered in choice situations was described. For both grass and maize silages attempts were made to find silage characteristics being responsible for preference or avoidance in DMI. Furthermore, quantification of the resulting decrease in feed intake and identification of points during the deterioration process where avoidance starts was attempted.

Further, an attempt was made to link results on forage quality as measured by means of chemical composition, microbiological status and signals given by a chemosensor system with the preference behaviour of goats. The chemosensor system is the main objective of a second part in this project conducted by a group at the Institute for Agricultural Engineering, University of Bonn. An existing chemosensor array should be improved, adjusted and used for assessment of silages by measuring volatile components. The aim was to evaluate the applicability of a chemosensor system for the assessment of silage variables that were studied in this thesis (fermentation quality, hygienic status, stage of deterioration, preference by ruminants). In both studies (animal nutrition and agricultural engineering) analyses were conducted using identical substrates to guarantee comparability.

The results of different analytical methods were compared and the overall aim was to find correlations between preference behaviour of goats, results of classical chemical analyses and changes in the signal pattern measured by the chemosensor system in silages with different lengths of aerobic exposure. The scope is to differentiate between silage qualities with the help of changes in the resulting signal pattern. If possible, critical control points could be implemented after establishing the sensor system for an objective measurement and assessment of silage quality. When used as a control instrument in the silage management, a

sensor array system could provide an important contribution to the traceability of food safety. The overall aim is to reduce the risk of quality losses caused by malfermentation or aerobic deterioration, therefore improving forage and product quality and contributing to both animal and consumer protection.

The third and fourth chapters as the main parts of this cumulative thesis are manuscripts which are formatted according to the instructions of the journal chosen for submission.

CHAPTER 3

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

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ABSTRACT

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.

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).

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.

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

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):

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

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.

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.

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

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)

Variable	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.

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.

Exemplarily, the results for one trial with 30-min and 3-h DMI are depicted in Figure 2. 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 2), 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.

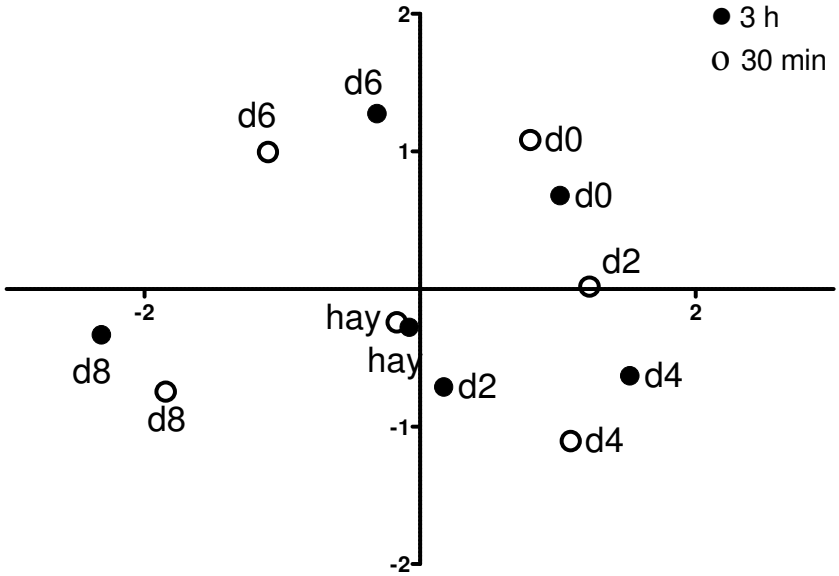

Figure 2. Multidimensional scaling of the mean preference shown by six goats for five silages (d0–d8) and one lucerne hay (hay) in one preference trial (silage with short chopping length, 33% dry matter and high compaction pressure) after 30 min and 3 h (d = number of days of aerobic exposure)

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

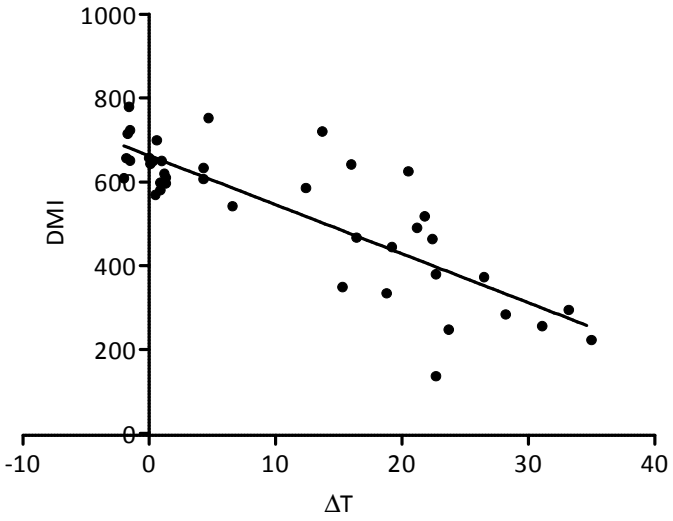

Figure 3. Relationship between dry matter intake (DMI, g 3 h^{-1}) of goats and silage temperature during aerobic exposure (expressed as difference to ambient temperature, ΔT (K)); n = 40; y = 662 - 11.69 x; R^2 = 0.681; p<0.0001

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.

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CHAPTER 4

Changes in grass silage fermentation products during aerobic exposure and its impact on dry matter intake by goats

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Interpretive summary

Changes in grass silage fermentation products during aerobic exposure and its impact on dry matter intake by goats. *By Gerlach et al.* The effect of aerobic exposure on grass silage quality and the impact on DMI by goats was studied. Different grass silages were exposed to air for eight days and chemically analyzed in 2-d intervals, afterwards they were used in preference trials conducted with goats. For well-fermented silages, DMI started to decrease at d6 of aerobic exposure, whereas poorly fermented silages were generally less consumed with a smaller influence of aerobic exposure.

ABSTRACT

The effect of aerobic exposure of grass silages on short-time feed intake and preference by goats was studied. Eight grass silages differing in dry matter (DM) (25% and 33%), cut 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-d intervals (d 0, 2, 4, 6, and 8 after silo opening) for proximate constituents, fermentation products and other volatile compounds as well as determination of microbiological status (yeasts, molds, 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-tpye 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 analysed 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-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 AA degradation (ammonia-N, butyric acid) were negatively correlated to DMI. 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 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.

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

INTRODUCTION

Grass silage is a major forage used in the nutrition of ruminants, but due to the impact of crop management and weather, strong variations concerning nutritional value and fermentation quality can occur (McDonald et al., 1991). Both of them may have a strong impact on feed intake (Forbes, 1995). During storage or feed-out, oxygen ingress into the silage can cause 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, 2012). 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 does have an influence on voluntary feed intake of cattle. However, it is difficult to attribute changes in 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)). In grass silages, Mo et al. (2001) could identify more than 50 different fermentation products. 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 (Eisner et al., 2006; Huhtanen et al., 2003; 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.

In this study, eight different grass silages were used to describe the changes occurring in chemical composition and microbiological status during aerobic exposure. The aim was to evaluate the effect of these variations on short-time DMI and preference by goats and to identify silage characteristics being responsible for preference or avoidance.

MATERIALS AND METHODS

Silage Preparation

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 at June 20, 2010 and wilted on the field. One part was harvested and ensiled at the same day in the afternoon; the other part was ensiled at the next day at midday. Eight silage treatments (2 x 2 x 2-factorial design) were produced differing in DM content (target value 30 and 40%), cut length (short and long/unchopped) and compaction pressure at ensiling (0.1 and 0.2 MPa). Details about the treatments are presented in Table 8. 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 mo. Before starting the first trial, packing density of all silages was determined by weighing the filled barrels in April 2011.

Table 8. Details about the silage treatments used in the trials

Trial	DM[1] (%)	Cut length	Compaction pressure (MPa)	Abbreviation of treatment	Month of opening	Temperature[2] (°C)
1	33	short	0.2 (high)	S33hi	May 2011	22
2	33	long[3]	0.2 (high)	L33hi	May 2011	22
3	25	short	0.2 (high)	S25hi	Jun 2011	23
4	25	long	0.2 (high)	L25hi	Jun 2011	23
5	33	short	0.1 (low)	S33lo	Aug 2011	22
6	33	long	0.1 (low)	L33lo	Aug 2011	22
7	25	short	0.1 (low)	S25lo	Nov 2011	19
8	25	long	0.1 (low)	L25lo	Nov 2011	19

[1]Target values for DM were 30 and 40%.

[2]Temperature = Mean ambient temperature during 8 d of aerobic exposure.

[3]long = unchopped.

In May 2011, the barrels containing the first two treatments were opened; the silages were taken out, each treatment was thoroughly homogenized, and stored aerobically on a heap (3 m × 3 m) for 8 d. 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 silages (middle, left, right) using a digital probe thermometer (TFA Dostmann, Wertheim, Germany); afterwards the silage was homogenized. 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 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 experiments 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 x 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 to 1.7 kg silage. 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.

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. All trials were conducted with a total of ten Saanen-type wethers (German Improved White Goat breed, mean (SD) BW 90.8 (12.35) kg) that were divided into two 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. Goats were tied up for the duration of the 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). During an adaptation period prior to each experiment (Kyriazakis et al., 1990), single meals of each silage (d0-d8)

and alfalfa (*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. The adaptation period lasted 6 d and forages were offered in randomized order. The standard hay was used to compare the different trials. 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 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 then determined 30 min after offering, and after feeding to calculate the initial and total DMI after 3 h. During all trials care was taken to always offer a choice between the two forages in the pair. This was guaranteed by putting additional silage into the feeding box as soon as the weight fell below 300 g. Each trial lasted 21 days, consisting of 6 d for adaptation and 15 d for experimental measurements. Each day, the experimental meal was offered for 3 h, starting at 0745 h. Grass hay was offered for ad libitum consumption at 1530 h and removed the following morning at 0700 h. For laboratory analysis, 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 Analysis

General Analysis. 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 × pH) × total 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 analysed using methods 8.1 and 5.1. The CP 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, NY). 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 ME-content 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.

Chemical Analysis of Fermentation Variables. 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, 1-butanol, 2-butanol), acetone, ammonia-N, and water-soluble carbohydrates (**WSC**). Furthermore, silages were 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 (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 ionisation 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 ammonia-N concentration was analysed 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).

Microbiological Analysis. 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 molds 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 Analysis

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) and Burns et al. (2001). This procedure was 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-off 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 (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 $0.05 \leq P \leq 0.10$.

RESULTS

Chemical composition

Chemical composition of grasses before ensiling is shown in Table 9.

Table 9. Chemical composition of grasses before ensiling (n = 4) and of alfalfa hay (standard forage in preference trials), and grass hay (fed for ad libitum intake in the afternoon)

	DM	Ash	CP	CL[1]	ADF[2]	NDF[3]	ADL	ME[4]
	g/kg	g/kg DM						MJ/kg DM
Grass (low DM)	261	88	124	29	233	410	21	11.5
Grass (high DM)	333	82	121	31	245	418	23	11.4
Alfalfa hay	908	87	143	21	323	464	64	9.6
Grass hay	911	78	98	16	360	596	47	8.4

[1] CL = Crude lipids.

[2]expressed exclusive residual ash.

[3]analyzed with heat-stable amylase and expressed exclusive residual ash.

[4]ME (estimated according to GfE, 2008, see Materials and Methods).

At opening (d0), silages did not show visible sign of molding. Low-DM silages, especially the treatments used in the last trials (S25lo and L25lo) had a noticeable smell of butyric acid. Results of chemical analysis of silages at d0 are presented in Table 10.

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 ammonia-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.

Table 10. Chemical composition of grass silages at the day of opening (g/kg DM unless otherwise stated)

	Treatment[1]							
Variable	S33hi	L33hi	S25hi	L25hi	S33lo	L33lo	S25lo	L25lo
Density (kg DM/m³)[2]	189	161	126	141	174	150	111	122
DM (g/kg)	330	325	231	271	328	330	234	268
Ash	89	97	113	90	90	98	105	91
CP	120	130	135	125	126	129	141	130
CL[3]	30	31	39	32	38	33	39	39
ADF[4]	272	278	314	285	274	274	289	277
NDF[5]	450	448	442	435	439	465	449	470
ADL	18	17	19	17	21	18	18	18
GP[6] (ml/g DM)	280	284	263	271	296	290	262	278
ME[7] (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	n.d.[8]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
n-Valeric acid	n.d	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
n-Caproic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Propionic acid	n.d.	n.d.	0.05	n.d.	n.d.	n.d.	0.12	n.d.
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)	n.d.	n.d.	109	n.d.	n.d.	n.d.	107	37
2-butanol (mg/kg DM)	224	n.d.	829	549	275	n.d.	2010	419
Ethyl acetate (mg/kg DM)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
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

[1]Treatments: S = Short chopping length, L = Long chopping length, 33 = 33% DM, 25 = 25% DM, lo = low packing density, hi = high packing density.

[2]Density = Mean density of silage in six barrels respectively, determined in April 2011.

[3]CL = crude lipids.

[4]expressed exclusive residual ash.

[5]analyzed with heat-stable amylase and expressed exclusive residual ash.

[6]GP = 24 h-gasproduction.

[7]ME (estimated according to GfE, 2008, see materials and methods)

[8]n.d. = below detection limit (0.01%).

Results of chemical analysis of silages during eight days of aerobic exposure are presented in Table 11. Concentrations of proximate constituents as well as fermentation variables did not change ($P > 0.05$) within 8 d.

Microbiological Analysis and Temperature

At the day of opening, all silages had low counts of spoilage organisms. Yeast and mold counts ranged from 2.4 to 2.7 log cfu/g, and content 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 (VDLUFA, 2012) at d2, d4, d6, and d8. Critical counts of molds were detected in three silages (S25hi, S33lo, and L33hi) at d8 of exposure, whereas yeasts rose above orientation values 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 (ΔK + 3.4, data not shown).

Table 11. Chemical composition of silages during eight days (d0-d8) of aerobic exposure (g/kg DM unless otherwise stated), n = 8

	Length of aerobic exposure (d)					
	0	2	4	6	8	SEM[1]
DM	290	291	297	305	298	7.1
Ash	96	96	96	100	95	1.5
CP	130	131	130	130	132	1.2
CL[2]	35	35	35	33	34	0.7
ADF[3]	283	275	278	279	281	1.6
NFD[4]	450	447	454	440	443	2.5
GP (ml/g DM)[5]	281	285	282	278	282	1.9
ME (MJ/kg DM)[6]	10.7	10.9	10.8	10.7	10.8	0.04
pH	4.5	4.5	4.6	4.5	4.6	0.02
Lactic acid	62	61	57	57	68	2.1
Acetic acid	25	26	22	25	23	0.9
iso-Butyric acid	1.3	1.5	1.9	1.5	1.0	0.24
n-Butyric acid	11	12	14	12	14	1.8
iso-Valeric acid	n.d.[7]	n.d.	n.d.	n.d.	n.d.	0
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	0.2	0.2	0.1	0.5	0.1	0.07
1,2-propanediol	3	3	2	3	4	0.3
Ethanol	13	12	9	10	10	0.7
Methanol	0.6	0.7	0.7	0.6	0.6	0.003
Propanol	1.8	1.7	1.2	1.3	1.3	0.20
1-butanol (mg/kg DM)	32	27	23	10	28	7.0
2-butanol (mg/kg DM)	538	299	239	260	547	68.4
Ethyl acetate (mg/kg DM)	n.d.	n.d.	n.d.	n.d.	n.d.	0
Ethyl lactate (mg/kg DM)	93	91	62	84	67	6.3
Ammonia-N (g/kg of total N)	122	129	138	140	134	3.3
Water-soluble carbohydrates	63	71	65	55	56	3.1
Yeasts (log10 cfu/g)	2.4	2.4	3.2	n.a.[8]	5.7	0.2
Molds (log 10 cfu/g)	2.4	2.9	5.1	n.a.	6.1	0.2
Aerobic mesophilic bacteria (log 10 cfu/g)	4.0	5.5	5.6	n.a.	4.6	0.2

[1]SEM = standard error of the mean.

[2]CL = crude lipids.

[3]expressed exclusive residual ash.

[4]analyzed with heat-stable amylase and expressed exclusive residual ash.

[5]GP = 24 h-gasproduction.

[6]ME (estimated according to GfE, 2008, see materials and methods).

[7]n.d. = not detected = below detection limit (0.01% fresh matter).

[8]n.a.= not analyzed.

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 12.

The forage with the highest DMI 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 respectively, alfalfa 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 d8. In trial 4 (L25hi), goats showed highest intake for 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 the least consumed silage. 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. Over all trials, mean 3 h-intake was greatest in L33hi and lowest in S25lo. During the first 30 min, goats consumed between 55% and 64% of the total 3 h-DMI.

Table 12. The DMI and stimulus coordinates for the two-dimensional solution to the preference among five goats, n = 25

Silage treatment[1]		Length of aerobic exposure (d)					Alfalfa hay	Mean d0-d8	MSD[2]
		0	2	4	6	8			
S33hi	DMI (3h)	566[a]	561[a]	630[a]	509[a]	272[b]	289[b]	508	124
	DMI (30min)	333[a,b,c]	357[a,b]	380[a]	290[b,c]	108[d]	264[c]	294	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 (3h)	658[a]	549[a,b]	550[a,b]	492[b,c]	354[d]	412[c,d]	521	129
	DMI (30min)	418[a]	341a,b	326 [b]	311[b,c]	199[c]	202[c]	319	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 (3h)	418[b,c]	479[a,b]	537[a]	396[c]	379[c]	536[a,b]	442	118
	DMI (30min)	260[a]	224[a]	271[a]	243[a]	232[a]	283[a]	246	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 (3h)	312[b]	493[a]	513[a]	409[a,b]	322[b]	473[a]	410	130
	DMI (30min)	220[a,b]	295[a]	300[a]	258[a,b]	230[a,b]	207[b]	260	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 (3h)	496[a,b]	558[a]	545[a]	564[a]	399[b,c]	359[c]	513	126
	DMI (30min)	297[a,b]	291[a,b]	351[a]	291[a,b]	236[b]	215[b]	293	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 (3h)	492[a,b]	492[a,b]	587[a]	406[b]	378[b]	433[b]	471	130
	DMI (30min)	302[a,b]	231[b]	361[a]	239[b]	253[b]	229[b]	277	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 (3h)	305[a]	312[a]	333[a]	354[a]	323[a]	379[a]	325	151
	DMI (30min)	168[a]	212[a]	189[a]	243[a]	191[a]	240[a]	200	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 (3h)	343[a]	388[a]	401[a]	377[a]	324[a]	409[a]	367	131
	DMI (30min)	173[a]	193[a]	233[a]	199[a]	206[a]	249[a]	201	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		

[a-d] = Means within a row with different superscripts differ ($P < 0.05$).

[1]Treatments: S = Short chopping length, L = Long chopping length, 33 = 33% DM, 25 = 25% DM, lo = low packing density, hi = high packing density.

[2]MSD = Minimum significant difference (Waller Duncan k-ratio t-test).

Silage Characteristics Influencing Dry Matter Intake

Results of correlation analysis between silage characteristics (of vacuum-stored samples used in the preference trials), DM content, and DMI are presented in Table 13.

Table 13. Correlation coefficients between silage characteristics, DM and DMI of goats, n = 25

	DM	DMI
DM	1	0.45**
DMI	0.45**	1
Ash	-0.46**	-0.27
CP	-0.75***	-0.54***
CL[1]	-0.49**	-0.38*
ADF[2]	0.59***	0.38*
NDF[3]	0.39*	0.23
ADL	-0.10	-0.08
GP[4]	0.20	0.20
ME	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***
WSC[5]	0.09	-0.11

[1]CL = crude lipids.

[2]expressed exclusive residual ash.

[3]analyzed with heat-stable amylase and expressed exclusive residual ash.

[4]GP = 24 h-gasproduction.

[5]WSC = water-soluble carbohydrates.

†$P < 0.10$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Most fermentation products were negatively correlated to silage DM content, with highest values for iso- and n-butyric acid, 1- and 2-butanol, and ammonia-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 to DM or DMI.

DISCUSSION

Silage Quality

The aim to produce silages at two stages of DM could be reached, but generally at a lower level than intended (30% and 40%). Consequently, DM of silages at the lower stage was below the recommendations of 30 to 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 remarkably in fermentation quality. Content 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). Silages S25hi, S25lo, and L25lo contained considerable amounts of butyric acid, as well as relatively high concentrations of ammonia-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 ammonia-N reflects the degree of protein and AA degradation; with high values having a depressing effect on utilization of nitrogen by ruminants (Driehuis, 2001). Both variables (i.e. butyric acid and ammonia-N) are evidence that clostridial fermentation had taken place in these silages (McDonald et al., 1991). This is typical for wet silages with low concentrations of WSC (Kung and Shaver, 2001; Pahlow et al., 2003) or silages produced from grasses low in nitrate (Weiß, 2001). In low-DM ensiling material, a direct Clostridia inhibitor like nitrate is necessary in addition to acidification (Kaiser et al., 2009). When lactic acid production is too low to decrease silage pH enough or nitrate concentration in the forage is insufficient for a permanent inhibition of Clostridia, AA and amides are catabolised with ammonia-N and butyric acid as some of the major end products of clostridial deamination and oxidation-reduction reactions (McDonald, 1991; Weiß, 2001). Beside the low DM content, 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 ammonia-N occurred after 10 mo of storage in comparison to 6 mo.

Low-DM silages were therefore classified as badly fermented. This is supported by the results of the DLG scheme (DLG, 2006), which objectively assesses silage quality by means of contents of butyric acid, acetic acid and pH using a points-based system. According to that scheme, fermentation quality was ranked as "very bad" for S25lo, S25hi, and L25lo and "in need for improvement" for L25hi. The other, high-DM silages were classified as "well-fermented" or "very well-fermented". Consequently, silages strongly differing in fermentation quality were used as basis for the aerobic exposure and preference trials.

The two esters the silages were analysed for 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 corn and sorghum silages found a wide range of ethyl ester concentrations with maximum values reaching 1,109 and 1,305 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 content 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 corn 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.

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 molds 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 molds instead of yeasts. Molds 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 to 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 (Danner et al., 2003; Kung et al., 1998; Wilkinson and Davies, 2012). Clostridial silages are more stable during aerobic exposure than well-preserved lactate silages, which is attributed to the presence of higher volatile fatty acids like butyric, iso-valeric and caproic acids, as reviewed by McDonald et al. (1991). Due to the fact that growth of spoilage organisms was limited during aerobic exposure, concentration of fermentation products did not change. 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, 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 3K above ambient.

Changes in Preference and Dry Matter Intake

Noticeable differences were observed between low- and high-DM silages. Intake of low-DM silages was 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%). These 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, was the DMI of d6 and d8 lower in comparison to fresher silages ($P < 0.05$). This might be due to the microbiological status of that treatment, where orientation values for molds 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 alfalfa hay. A decrease in preference occurred as amount of fungal biomass in hay increased. As other variables did not change significantly 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 significantly 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, 2012). For aerobic stability

and its impact on preference and DMI, we suggest focussing 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. For corn silages, which are especially prone to spoilage after opening, Gerlach et al. (2013) conducted preference trials with goats and found a significant 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 molds 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 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 corn silages, and hay on DMI of dairy cows. Aerobic deterioration of silage led to a decrease in DMI of about 10 to 20% in single-choice experiments; and the difference was greater, when cows had the choice to select 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 significant negative effect on feed intake and preference, especially with well-fermented silages; the extent, however, was much less than reported for corn 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.

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 (Eisner et al., 2006; Forbes, 1995; Steen et al., 1998) but likely more silage quality (especially fermentation quality resulting from DM

content) than DM content 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 ammonia-N with correlation coefficients ranging between -0.50 and -0.59. Also ammonia-N had limited intake in other studies, either directly or indirectly due to correlation with some other end-products of silage proteolysis or AA degradation (Hetta et al., 2007; Huhtanen et al., 2002). Steen et al. (1998) proposed that the ammonia-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. 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 ammonia-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 ammonia-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 contents 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.

Correlation analysis also gave evidence of a negative impact of propionic acid on DMI which has previously been observed by Krizsan et al. (2012) where propionic acid decreased silage DMI more than other organic acids. However, Huhtanen et al. (2002) questioned the direct effect on DMI due to the small concentrations of propionic acid usually found in silages. The same might be relevant for 2-butanol, which was also negatively related to DMI. It was found in considerable amounts, especially in low-DM silages. Detected values clearly exceeded concentrations reported by Krizsan et al. (2007). Mean concentration found in 24 grass silages was 18 mg/kg DM with a maximum of 84 mg/kg DM. Data on low-molecular weight alcohols in silages are limited, especially regarding their influence on feed intake and potential carry-over to milk (Kalač, 2010). It might therefore be an area requiring further research.

The positive effect of acetic acid on DMI was unexpected; as Eisner et al. (2006) 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 to 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.

CONCLUSIONS

During 8 d of aerobic exposure, grass silages did not show strong signs of spoilage. Nevertheless, for well-fermented silages, short-time 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 evidence for malfermentation, DMI was generally lower and fewer differences between silages with different lengths of aerobic exposure were observed. Some fermentation products, especially butyric acid and ammonia-N influenced DMI negatively. Results highlighted the importance of fermentation quality as well as aerobic stability in order to achieve high levels of DMI.

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CHAPTER 5

General conclusions

The main focus of this thesis was to characterize the quality of different silages and changes occurring after penetration of oxygen, which normally takes place after opening the silo for feed-out. In an interdisciplinary approach, forages were studied using chemical and microbiological analyses as well as a newly developed chemosensor system and methods for measuring the preference and short-time feeding behaviour of goats.

For both maize and grass silages the impact of fermentation quality as influenced by both ensiling and aerobic exposure on preference and short-time dry matter intake (DMI) by goats was pointed out. It was also shown that, especially well-fermented and energy-rich silages are prone to aerobic deterioration after opening, as seen with maize silages (Chapter 3). At opening, all maize silages were of high quality as assessed by sensory and microbiological evaluation as well as by analyses of fermentation products. After air infiltration, drastic shifts concerning silage temperature and both microbiological and chemical variables were observed within eight days. These changes during the course of deterioration strongly influenced DMI and preference when silages were offered in choice situations**,** marked by a significant decrease in DMI after four or six days of aerobic exposure. This length of oxygen ingress is often reached and even exceeded under practical conditions (Wilkinson and Davies 2012). These findings support the importance of aerobic stability in the context of forage quality, and for the silage management it can therefore be recommended to restrict air contact as much as possible because of its strong impact on preference behaviour. Drastic decreases in intake of aerobically unstable silages cancel out a big part of the efforts made to produce high yields of high quality maize silages.

Grass silages were stable for the time of aerobic exposure, due to higher concentrations of acetic and butyric acid and lower concentrations of water-soluble carbohydrates (WSC) (Chapter 4). Nevertheless, there was a decline in preference and DMI at the sixth (d6) or eighth day (d8) of aerobic exposure in six out of eight trials. As the decrease took place at silages with few signs of aerobic spoilage it was concluded that goats could detect even subtle differences in silage quality, eventually perceiving still unidentified volatile compounds or mixtures of fermentation products. This assumption of small differences in forage quality influencing preference behaviour of ruminants was also described by Fisher et

al. (2002). Grass silages showing evidence for malfermentation with higher concentrations of products from protein and amino acid (AA) degradation were less consumed which emphasized the significance of high fermentation and protein quality.

After these general conclusions, some parts of the study warranting further comments are mentioned and shortly discussed.

Fermentation products

Comparison of silage characteristics and DMI resulted in some significant correlations to fermentation products while relationship to proximate constituents and fibre fractions was poor. In maize silages, preference when expressed as DMI was weakly negatively correlated to the concentrations of ethanol and ethyl lactate, and in fresh silages to acetic acid. Concentrations of ethanol and the ethyl esters of acetate and lactate are strongly and positively correlated in whole-crop wheat, maize and sorghum silages (Weiß and Auerbach 2012). As ethanol in grass silages or when added to a TMR (+ 5%, DM basis) has recently shown to influence DMI and milk yield in dairy cows positively (Hetta et al. 2007; Daniel et al. 2013), the negative correlation in our study could also be a side-effect of the impact of ethyl lactate. However, to our knowledge, other data concerning the impact of ethyl esters on DMI are not available; therefore further studies are needed to confirm the assumption of ethyl lactate negatively influencing preference in ruminants.

When thinking about improvements in aerobic stability, the negative effect of acetic acid on DMI shown in this study and a previously conducted meta-analysis (Eisner et al. 2006) should be kept in mind; however, the positive effect on aerobic stability might compensate for the small decline in DMI caused by acetic acid in fresh silages. In grass silage trials, there were more products resulting from malfermentation than spoilage-related products that were negatively related to DMI. As these were especially products from protein and AA degradation, the importance of fermentation quality was stressed as mentioned before.

However, none of the fermentation products showed a strong relation to DMI, i.e. a correlation coefficient higher than 0.6. Consequently, it was concluded from both experiments that assigning a decline in feed intake to single constituents is difficult; often their impact on preference is still unclear. Huhtanen et al. (2002) proposed that eventually a complex of fermentation products or still unidentified compounds might be responsible, which would also explain the decrease in DMI of grass silages showing no measurable signs

of deterioration. The difficulty of identifying single constituents or understanding chemical reactions being responsible for preference or avoidance is emphasized by the fact that silage temperature expressed as difference to ambient (ΔT) was by far the best single predictor in maize silages. The strong negative correlation between ΔT and DMI underlines its utilisability for the practical silage management and suitability of recommendations as given by Spiekers et al. (2009).

Another aspect that needs to be considered is that fermentation products occurring during the deterioration process may also have a negative influence on product quality after ingestion of the forage by the animal. Information on carry-over of fermentation products to milk and meat with an impact on sensory properties is very limited but may be possible, as reviewed by Martin et al. (2005) and Kalač (2010). Ethanol and propanol that were mixed into silage before feeding significantly reduced the organoleptic quality of milk (Randby et al. 1999; Randby 2007). Yet this possible carry-over has not been studied with aerobically deteriorated silages and may therefore be an interesting approach for further experiments.

Microbiological quality

Concerning the microbiological quality, all maize silages had counts of yeasts and partly moulds exceeding orientation values (VDLUFA 2012) at d4 of aerobic exposure, sometimes even before an increase in temperature, conspicuities in sensory evaluation or a decrease in DMI were detected. The impact of high counts of spoilage organisms in forages on animal health (e.g. blood metabolites) or productivity has not been investigated in this study, but might be an area deserving further research.

Due to opening times of the lab and the fact that microbiological analyses have to be carried out on fresh material, sampling of silages for enumeration of yeasts and moulds could not be conducted at each day of aerobic exposure. As a consequence, results of d2 and d6 (maize silages) and d6 (grass silages) are not available and the development of counts of spoilage organisms cannot be presented in the same time intervals like the other variables. Nonetheless, the microbiological status at the day of opening and after eight days of aerobic exposure provides important information for the broad description of silage quality that was planned to give.

Vacuum-storage of silages

One of the difficulties in the experiment was to keep silage samples at specific stages of deterioration for their use in preference trials some days after the period of aerobic exposure. For this purpose, silages were stored completely airtight in vacuum-sealed polyethylene-bags, as previously proposed by Pippard et al. (1996) as a method for obtaining and preserving uniform silages for feeding experiments. When storing different grass silages in evacuated bags for 18 days, the authors did not observe aerobic spoilage related changes and evaluated the methodology as suitable for preserving silages with considerable potential for use in feeding trials. The use of vacuum-packed silages has also shown to be applicable for lab-scale ensiling experiments using perennial ryegrass and red clover (Johnson et al. 2005) or different silage maize hybrids (Cherney et al. 2004). Vacuum bag ensiling of perennial ryegrass, festulolium, red and white clover and lucerne resulted in high quality silages and a high repeatability of duplicate bags (Weisbjerg et al. 2012). It offered a high throughput of samples, consistent packing and the possibility of easily adjusting packing density at ensiling, therefore representing a flexible and cost-effective alternative to fixed glass jars (Johnson et al. 2005).

In our study, the method of vacuum-sealing has been proven to be suitable for fresh silages. The vacuum-storage of grass silages and fresh maize silages (d0-d4 after silo opening) did not influence the analysed variables and was therefore evaluated as an appropriate method for storing silages for a later use in feeding trials.

However, when using samples with strong signs of spoilage (maize silages after six days of aerobic exposure) the process of deterioration could not be inhibited completely by the airtight storage (Gerlach et al. 2011). This resulted in higher concentrations of ethanol and ethyl esters in some vacuum-sealed maize silage samples, possibly due to anaerobic yeast activity. As data of fresh silages (unsealed) was used for description of the deterioration process and data of vacuum-sealed samples for calculation of correlation coefficients between silage characteristics and DMI, this problem could be handled satisfactorily. For the measurement with the chemosensor system, also vacuum-stored samples were used, therefore providing the same material as fed to the animals and guaranteeing comparability.

Ambient temperature during aerobic exposure

One of the aims was to study the impact of different forage characteristics like substrate, DM content, cut length and compaction pressure on aerobic stability. Unfortunately it was not possible to maintain identical ambient temperatures during all 16 periods of aerobic exposure. As there is a big influence of temperature on aerobic stability, which was also shown by Ashbell et al. (2002) and Koc et al. (2009), where spoilage was strongest at elevated temperatures (30-37 °C), no further conclusions are drawn concerning the impact of these management factors. Storing silages at constant temperatures is clearly one possible point of improvement for following studies. However, despite variations in ambient temperatures, the big number of silage treatments helped to get a detailed impression of aerobic deterioration of different silages.

Preference behaviour

The food preference shown by goats, as expressed by the proportions eaten when a choice is given (Forbes 1995), was used for the description of feeding behaviour when offering different silages in short-time feeding trials to goats. The design, based on the principle of presenting each possible combination of silages to the animals, allowed a detailed description of feeding behaviour with the possibility of detecting even small differences between forages. It has previously been used for identification of ruminants' preference for tall fescue hays (Fisher et al 1999; Burns et al. 2001), for switchgrass hays (Fisher et al. 2005) and for lucerne hays (Fisher et al. 2002) as well as for identification of forage characteristics associated with preference in sheep (Buntinx et al. 1997).

Each trial started with an adaptation period, in which foods were offered singly in turn to allow the animal to learn to associate a particular set of sensory properties with certain metabolic 'feelings' (Forbes 1995). It is known that ruminants can identify and select the preferred forages when they are offered in pairs on days after the initial meal, as shown by Fisher et al. (1999) and Fisher et al. (2002) feeding tall fescue hay and lucerne hay cut either at sunup or sundown to sheep, goats and cattle. Farm animals can select a diet appropriate to their metabolic needs as long as the foods offered are clearly differentiated by sensory properties and the animals have the opportunity to learn the nutritional difference between the foods (Forbes 1995), which emphasizes the need for an adaptation period prior to the experimental feeding. As described by Forbes (2007), the study of choice or preference

behaviour is often seen as a sequel to single food intake studies, although the former is normal in wild ruminants and in the ancestors of domesticated animals. It is therefore postulated to handle the control of intake of a single food as a special case of the more general situation in which animals can select between different feedstuffs.

As presented in Chapter 3 and Chapter 4, mean decrease in 3 h-DMI between d0- and d8-silages was 53% and 35% for maize and grass silages, respectively. Differences between forages are smaller when animals do not have the possibility to choose (Keady and Murphy 1998). However, as one of the aims of the study was to describe the effect of deterioration on feed intake in detail and to detect subtle differences in preference, the experimental design is seen as being appropriate for this topic. It was helpful in identifying points during the deterioration process where a decline in DMI began and identifying silage characteristics influencing preference behaviour. Nevertheless, for subsequent studies it might be interesting to test the impact of spoilage also in single-choice experiments. It has to be determined whether the short-term preference for forages is reflected in daily DMI and DM digestion when fed in conventional intake and digestion trials, because the extent to which animal short-term preference relates to potential animal performance is not known (Burns et al. 2005).

The intermediate determination of DMI after 30 minutes showed that the initial feed intake gave a good impression of total DMI after 3 h, underlining that there are few shifts in preference during presentation of two forages in a choice experiment with grass and maize silages for some hours. These findings might indicate the potential and usability for short-time trials.

The analysis of multidimensional scaling (MDS) was a valuable tool in the investigation of preferences, as also previously reported by Buntinx et al. (1997). It helped systematizing data by converting preferences for forages into coordinates in a two-dimensional space. The graphical solution supported in getting an impression of preference and avoidance for different forages, especially in the grass-silage trials, where differences between silages were less pronounced. It is helpful to conduct MDS together with an analysis of variance to separate means.

Animals

In all trials, goats were used as model animals also for larger ruminants (particularly beef cattle and dairy cows), most likely due to the extent of the experiment (especially amount and weight of vacuum-sealed silage samples needed in preference trials) and a better handling of the experimental animals.

There is a lack of studies comparing the intake and preference behaviour of cattle and goats. Most of the studies available were conducted on extensively grown grass or shrub land in arid or semi-arid regions and can therefore not be transferred directly to intensively grown, energy-richer forages in European countries. On marginal heathland areas, Celaya et al. (2007) found a low dietary overlap between cattle and goats, especially during the grazing season. Differences in the feeding behaviour between these species grazing on a shrub and tree savannah in West Africa were also observed by Ouédraogo-Koné et al. (2006) and their selection of browse species on natural pasture in a Sahelian area differed as described by Sanon et al. (2007). The bipedal stance which allows grazing in both horizontal and vertical direction is seen as one possible cause for the differing feeding behaviour of goats from other ruminants under range conditions (GfE 2003).

On the other hand, studies using high-quality forages have described a good coincidence in the preference behaviour of both species (GfE 2003). Goats, lambs and cattle were found to ingest largely identical material, when grazing on a good quality ryegrass/white clover sward (Hughes et al. 1984). The degree of dietary overlap, and hence potential competition, was greatest between goats and cattle when grazing on semi-arid rangelands in Australia (Squires 1982). Beside these grazing studies, there are some few experiments using harvested forages. Tall fescue hay (Fisher et al. 1999) and lucerne hay (Fisher et al. 2002) cut either at sundown or sunup was fed to sheep, goats and cattle in preference experiments, using six animals in each case. Hays cut in the afternoon were of higher nutritive value and were preferred by all species, therefore showing similar preference behaviour. In two consecutive short-time feeding experiments, goats preferred hays of the same cultivars that were also most preferred by heifers when grazing the same cultivars in the previous study (Burns et al. 2001). The authors concluded that such general agreement between studies indicates that the cues which cattle used for preference were apparently perceived similarly by goats. When offering fresh and aerobically spoiled silages to dairy cows, Wichert et al. (1998) found a similar decrease in DMI and preference as reported in this thesis.

Based on the studies above, the presented results should be transferable to larger ruminants as well, however, the assumption needs to be confirmed in studies feeding silages to goats and cattle simultaneously.

Chemosensor system

To link both parts of the study, data of silage characteristics and preference behaviour when expressed as DMI were analyzed together with results of different sensors used in the chemosensor array. After adjusting variables like temperature, flow and time in the different measuring phases (adsorption, reference, desorption 1, desorption 2 and cooling), the experimental design proved to be an efficient gas sampling method, and for some treatments differences between silage qualities as consequence of aerobic deterioration could be detected under the given laboratory conditions.

Data of the chemosensor array were transformed using a principal component analysis and presented in a two- and three-dimensional way. The relative relationship of the sensor signal (order) generally represents a pattern dependent on the composition of silage gases, while the intensity of the signal (signal height) reflects the concentration of the measured gases. When strong changes in DMI occurred, especially with maize silages, there was also a change in the signal pattern (Roß et al. 2012). Figure 4 and Figure 5 combine results of the sensor array with the DMI in two preference trials using aerobically stored maize and grass silages. For a simplified illustration only the signal height is considered, therefore neglecting the relative relationship of the sensor signals. At the fourth day of aerobic exposure, DMI of the maize silage decreased significantly, concurrently there was a strong increase in signal height of all sensors (Figure 4). However, the strong decrease in DMI at the eight day after silo opening was not accompanied by a shift in the sensor signal. This might be negligible, as for use as a control instrument the starting point of the decrease in DMI would be of paramount interest and importance.

For grass silages, which showed a smaller degree of deterioration, differentiation between silages with different lengths of aerobic exposure was not possible in most cases, and changes in DMI did not come along with a change in signal height, as depicted in Figure 5. Sensor signals were generally lower and did not vary as a consequence of the aerobic exposure period.

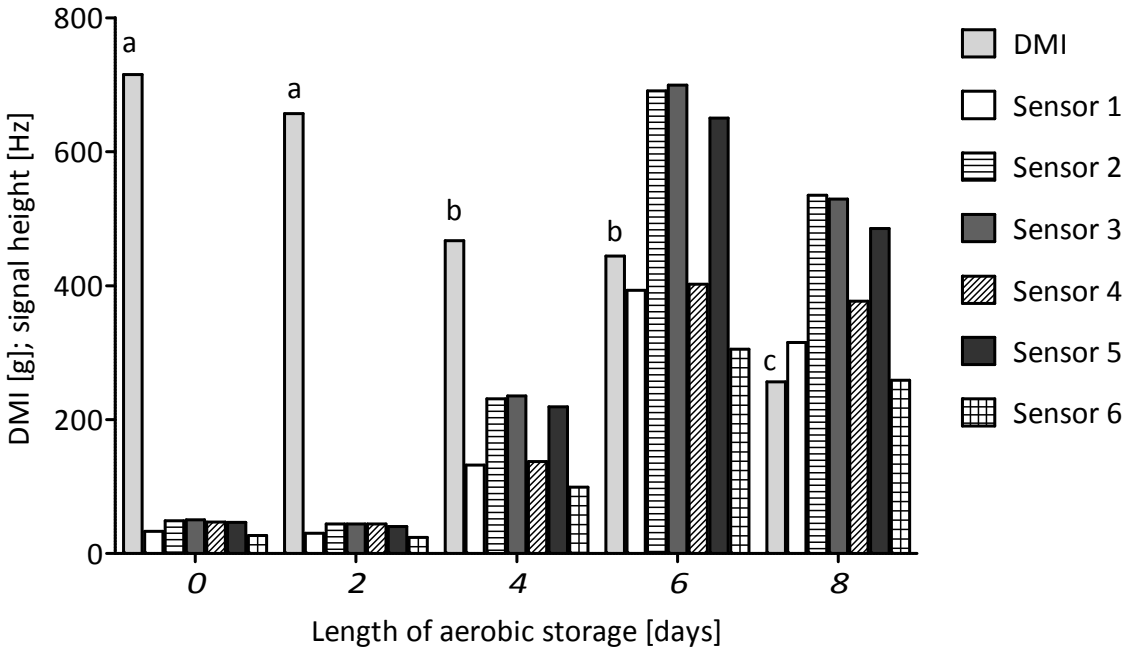

Figure 4. Signal heights of six sensors (n = 30) and dry matter intake (DMI; n = 40) shown by six goats for a maize silage (long chop length, 40% DM, high compaction pressure at ensiling) during eight days of aerobic exposure.

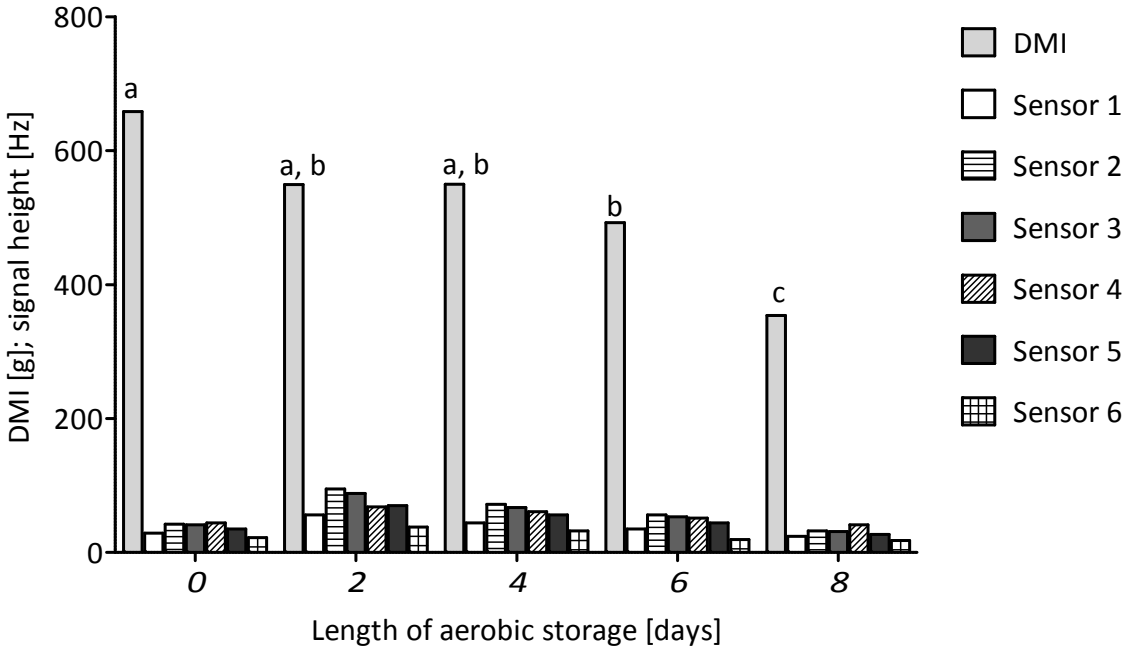

Figure 5. Signal heights of six sensors (n = 15) and dry matter intake (DMI; n = 25) shown by five goats for a grass silage (long cut length, 33% DM, high compaction pressure at ensiling) during eight days of aerobic exposure.

Additionally, correlation analyses with data of both parts were conducted. For maize silages, a significant relationship was found between DMI and signal height with highest correlation coefficients for sensors 1-3 in desorption level 2 (r = -0.50, -0.50 and -0.53 respectively, $p < 0.05$). Despite these significant correlations, temperature measured in the silage was a much better predictor than a single or the sum of sensor signals (r = 0.89, $p < 0.0001$). For the grass silages, only a weak relationship existed between DMI and signal height (desorption level 1) with correlation coefficients of sensors 1-6 ranging between 0.33 and 0.36 ($p < 0.05$).

These results demonstrate the possibility of objectively measuring forage quality with the help of chemosensor systems. Nevertheless, it was difficult to guarantee constant measurement conditions and to clearly differentiate between silages. Furthermore, sensor array systems are known to have a high sensitivity to temperature and humidity changes, signal drift over time, moderate reproducibility and high costs (Sankaran et al. 2012), which might impede the use of sensor arrays directly at the silo face. The fact that temperature showed a much higher correlation to DMI underlines the need for further modifications concerning the suitability for daily use.

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TAGUNGSBEITRÄGE

XVI International Silage Conference, 01.-04.07.2012, Hämeenlinna, Finnland

Gerlach, K., Weiß, K., Roß, F., Büscher, W. & Südekum, K.-H.:

Changes in maize silage fermentation products during aerobic deterioration and its impact on feed intake by goats. (Vortrag)

3rd Nordic Feed Science Conference, 28.-29.06.2012, Uppsala, Schweden

Gerlach, K., Roß, F., Büscher, W. & Südekum, K.-H.:

The use of multidimensional scaling to evaluate preferences of goats for grass silages after different times of aerobic exposure. (Poster)

123.VDLUFA-Kongress, 13.-16.09.2011, Speyer, Deutschland

Gerlach, K., Hewicker, I.E., Weiß, K., Roß, F., Büscher, W. & Südekum, K.-H.:

Veränderungen in der chemischen Zusammensetzung von Maissilagen unter Sauerstoffeinfluss. (Vortrag)

Joint Annual Meeting American Dairy Science Association and American Society of Animal Science 10.-14.07.2011, New Orleans, Louisiana, USA

Gerlach, K., Roß, F., Büscher, W. & Südekum, K.-H.:

The impact of aerobic deterioration of corn silage on feed intake by goats. (Vortrag)

122.VDLUFA-Kongress, 21.-24.09.2010, Kiel, Deutschland

Gerlach, K., Roß, F., Büscher, W. & Südekum, K.-H.:

Untersuchungen zum aeroben Verderb von Maissilagen. (Vortrag)

VERÖFFENTLICHUNGEN

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