Institut für Tierwissenschaften

The effect of air exposure before and after ensiling on maize silage quality and dietary choice by goats

Dissertation

zur

Erlangung des Grades

Doktor der Agrarwissenschaften
(Dr. agr.)

der

Landwirtschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität

zu

Bonn

vorgelegt von

Dave Brüning

aus Brandenburg an der Havel

Bonn 2018

Referent: **Prof. Dr. Karl-Heinz Südekum**

Korreferent: **Prof. Dr. Wolfgang Büscher**

Tag der mündlichen Prüfung: **20.04.2018**

Angefertigt mit Genehmigung der Landwirtschaftlichen Fakultät der Universität Bonn

Dedicated to my family

ABSTRACT

The effect of air exposure before and after ensiling on maize silage quality and dietary choice by goats

The overall aim of the thesis was to determine the effect of air exposure before and after ensiling on quality of whole-crop maize silage and to determine the influence of the resulting changes on dietary choice by goats. Three fixed factors (compaction, sealing and aerobic exposure post-opening) were selected and varied in order to ensure ingress of air in different stages of the conservation process and afterwards. Whole-crop maize (277 g kg^{-1} dry matter [DM]) was chopped and ensiled in 120-L plastic silos, which were either compacted to low (194 kg DM m^{-3}) or high (234 kg DM m^{-3}) density and sealed either immediately or with a delay at day 2 or at day 4 post-filling, making a total of six treatments. After sealing, all silos were stored for at least 175 days and then opened. After silo opening, silages were removed, sampled and exposed to air for 6 days. During aerobic exposure, samples were taken from the silages at 2-day intervals to determine silage composition and quality. Silage samples were also taken at the respective days of aerobic exposure (day 0, 2, 4 and 6) for subsequent preference trials and were then vacuum-stored in polyethylene bags for use as feed. A 15-day preference trial was carried out with goats (German Improved White Goat, n = 5) for each of the six treatments. During the experimental phase, each possible 2-way combination (n = 10) of the exposed silages and of a lucerne hay was offered for 3 hr to each goat. Delayed sealing after 2 and 4 days caused changes in both chemical and microbial composition before sealing. Sealing the silos after 4 days caused high DM losses of up to 11%. At silo opening, higher contents of ethyl acetate and ethyl lactate were found in silages sealed with a delay. A 4-day delay resulted in a shorter aerobic stability compared with immediate sealing (65 vs. 50 hr). Aerobic exposure post-opening led to considerable changes in silage composition, to a drastic loss in feed value and, finally, spoilage. This was mainly reflected in the increase in yeast counts, the strong rise in pH, the worsening of sensory properties and the rapid heating. Neither the different compaction nor the delay significantly influenced forage choice and short-term intake. Compared with this, prolonged aerobic exposure of more than 4 days had a detrimental effect. Exposing silages to air for 6 days resulted in strong avoidance and, across all treatments, a mean decrease in DM intake of 71% compared to silages at opening. Increasing contents of fibre fractions, a deteriorating microbial status and poor silage sensory properties, probably caused by a combination of different fermentation products can be considered for decrease in preference.

KURZFASSUNG

Einfluss von Luftzufuhr vor und nach der Silierung auf Maissilagequalität und Futterwahl von Ziegen

Übergeordnetes Ziel der Arbeit war, den Einfluss von Luft vor und nach der Silierung auf die Qualität von Maissilage und die sich daraus ergebenden Veränderungen auf die Futterwahl von Ziegen zu untersuchen. Drei fixe Prüffaktoren (Verdichtung, Verschluss und aerobe Exposition nach Öffnung) wurden ausgewählt und abgestuft, um die Zufuhr von Luft in den unterschiedlichen Stadien des Silierprozesses und danach zu gewährleisten. Silomais (277 g kg^{-1} Trockenmasse [TM]) wurde gehäckselt und in 120-L Plastiksilos einsiliert, die entweder niedrig (194 kg TM m^{-3}) oder hoch (234 kg TM m^{-3}) verdichtet und entweder jeweils sofort oder mit Verzögerung nach 2 oder 4 Tagen verschlossen wurden (sechs Varianten). Nach Verschluss wurden alle Silos für mindestens 175 Tage gelagert und dann geöffnet. Nach Öffnung wurden die Silagen entnommen, beprobt und 6 Tage an der Luft gelagert. Während der aeroben Exposition wurden im 2-tägigen Abstand Proben zur Bestimmung der Silagezusammensetzung und -qualität entnommen. Für die im Anschluss durchgeführten Präferenzversuche wurde ebenso Silage an den Tagen der aeroben Exposition (Tag 0, 2, 4 und Tag 6) entnommen und in Polyethylenbeuteln zur Verwendung als Futter vakuumverpackt. Anschließend wurde für jede der sechs Varianten ein 15-tägiger Präferenzversuch mit Ziegen (Weiße Deutsche Edelziege, n = 5) durchgeführt. Während der Versuchsphase wurde jeder Ziege jede mögliche Zweierkombination (n = 10) aus den aerob gelagerten Silagen und einem Luzerneheu für 3 h angeboten. Verzögertes Verschließen nach 2 und 4 Tagen verursachte Veränderungen in der chemischen und mikrobiellen Zusammensetzung vor Verschluss. Verschließen der Silos nach 4 Tagen führte zu hohen TM-Verlusten von bis zu 11 %. Bei Öffnung wurden in den verzögert verschlossenen Silagen höhere Gehalte an Ethylacetat und Ethyllactat gefunden. Eine 4-tägige Verzögerung führte zu einer geringeren aeroben Stabilität gegenüber dem sofortigen Verschluss (65 vs. 50 h). Aerobe Exposition führte zu erheblichen Veränderungen in der Zusammensetzung, zum drastischen Verlust an Futterwert und schließlich zum Verderb. Dies spiegelte sich vor allem im starken Anstieg des pH-Wertes und der Hefezahlen, in verschlechterten sensorischen Eigenschaften sowie in schneller Nacherwärmung wider. Weder die unterschiedliche Verdichtung noch die Verzögerung konnten die Futterwahl und die Kurzzeit-Futteraufnahme entscheidend beeinflussen. Dagegen wirkte sich die aerobe Exposition von mehr als 4 Tagen nachteilig aus. Aerobe Exposition von 6 Tagen führte zu starker Futtervermeidung und zum mittleren Rückgang der TM-Aufnahme von 71 % im Vergleich zu den Silagen bei Öffnung. Zunehmende Gehalte an Faserfraktionen, ein sich verschlechternder mikrobieller Status und schlechte sensorische Eigenschaften, die vermutlich durch eine Kombination verschiedener Fermentationsprodukte verursacht wurden, können für die Abnahme der Präferenz in Betracht gezogen werden.

TABLE OF CONTENTS

FIGURES

Chapter 3

Chapter 4

Chapter 5

Appendix

TABLES

Chapter 3

Chapter 4

Chapter 5

Appendix

ABBREVIATIONS

AA	Acetic acid
ADFom	Acid detergent fibre expressed exclusive residual ash
ADL	Acid detergent lignin
AE	Aerobic exposure
AIC	Akaike's information criterion
aNDFom	Neutral detergent fibre assayed with heat-stable amylase and expressed exclusive residual ash
ANOVA	Analysis of variance
ASTA	Aerobic stability
AT	Ambient temperature
BA	Butyric acid
BU	2-Butanol
BW	Body weight
C	Compaction
CF	Crude fibre
CFA	Continuous Flow Analyser
Cfu	Colony-forming units
CL	Crude fat
CP	Crude protein
DLG	Deutsche Landwirtschafts-Gesellschaft
DM	Dry matter
DMI	Dry matter intake
EA	Ethyl acetate
EL	Ethyl lactate
ETH	Ethanol
FFAP	Free fatty acid phase
FM	Fresh matter
GC	Gas chromatography
GP	Gas production in 24-h from 200 mg substrate
HPLC	High performance liquid chromatography

K	Kelvin
LA	Lactic acid
LUC	Lucerne hay
MDS	Multidimensional scaling
ME	Metabolizable energy
METH	Methanol
MSD	Minimum significant difference
N	Nitrogen
ND	Not detected (below detection limit)
NDF	Neutral detergent fibre
NH_3-N	Ammonia-N
NPN	Non-protein-N
NS	Not significant
PRO	*N*-Propanol
RI	Refractive index
S	Sealing
SEM	Standard error of the mean(s)
TN	Total nitrogen
TM	Trockenmasse
VDLUFA	Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten
VOC	Volatile organic compounds
WLKH	Wasserlösliche Kohlenhydrate
WSC	Water-soluble carbohydrates

Treatments

H0	High compaction, sealed immediately (day 0 post-filling)
H2	High compaction, sealed on day 2 post-filling
H4	High compaction, sealed on day 4 post-filling
L0	Low compaction, sealed immediately (day 0 post-filling)
L2	Low compaction, sealed on day 2 post-filling
L4	Low compaction, sealed on day 4 post-filling

CHAPTER 1 GENERAL INTRODUCTION

Maize silage is one of the most important forage used in ruminant nutrition. The good suitability for ensiling and the development of harvesting techniques have contributed equally to the spread of cultivation. Maize silage is among the most widely used component of dairy cow rations due to high nutritional value and energy content (Vogel, 1992; Driehuis et al., 2008; Dunière et al., 2013).

The success of making high-quality silage depends on many factors. Management factors that affect the time taken to achieve anaerobic conditions - the time taken to seal the silo and the degree of compaction - are very important, because they can significantly influence the extent of the aerobic phase at the beginning of the conservation and the volumes of trapped air.

A long aerobic phase at the beginning of the conservation is unfavourable since it can lead to respiration of water-soluble carbohydrates (WSC) by plant enzymes as well as to protein degradation. Respiration is undesirable, because it also results in a loss of dry matter (DM), metabolizable energy and available WSC for lactic acid fermentation. Additionally, a prolonged aerobic respiration phase due to poor compaction and a delay in sealing allows the growth of microorganisms such as yeasts, which can survive the anaerobic phase inactively. This can result in heating, visible spoilage and low aerobic stability during feed-out. Heating of silages can cause damages of protein and reduces silage digestibility (McDonald et al., 1991; Pahlow et al., 2003; Piltz and Kaiser 2009; Mickan et al., 2009).

The knowledge about the negative effects of inadequate compaction and delayed sealing is not new. For example, Vogel (1992) reported slightly higher DM losses of 0.5% in low compacted maize silage of 42% DM than in highly compacted silage (126 vs. 252 kg DM m^{-3}). The losses were attributed to increased respiration at the early stage of ensiling. Aerobic stability decreased by 1–2 days in the low compacted silage. Velho et al. (2007) compared highly with low compacted maize silage (30.8% DM, 170 vs. 137 kg DM m^{-3}) and found that the highly packed silage had higher contents of soluble sugars and non-structural carbohydrates, but lower contents of insoluble organic matter, neutral detergent fibre (NDF), acid detergent lignin (ADL) and ammonia-N, respectively. The authors stated that higher packing density improves preservation of soluble sugars, has lower effect on structural carbohydrates

and reduces proteolysis. Sucu et al. (2016) conducted a study to determine the effects of two ensiling densities (168 vs. 216 kg DM m^{-3}) on fermentation, aerobic stability, and nutritive value of maize silage. Increased packing density resulted in silages with lower acetate contents, ammonia-N levels, and fermentation losses but lactate contents did not differ. Tightly packed silages remained stable upon exposure to air. Tight packing increased nutrient digestibility and improved the silage energy content. The study shows that high density can limit air infiltration and reduces the oxidation loss during storage and feed-out.

Published data distinguish between delayed filling (ensiling), delayed sealing and duration of the delays. Exemplarily, Bolsen et al. (1985) found no effects in lactic and acetic acid contents, DM recovery, pH and aerobic stability between untreated maize silage remaining 12 hr loosely in forage wagons before ensiling and silage ensiled immediately. In contrast, Mills and Kung (2002) reported lower lactic and acetic acid and starch content, but higher pH, more butyric acid, ethanol, ammonia-N, ADF and NDF in barley silage that was stored 24 hr in loose piles before ensiling. Uriarte-Archundia et al. (2002) reported that maize silage sealed with a 48-h delay post-filling had higher yeast counts than immediately sealed silage and were 2 days less aerobically stable. Unfortunately, only few studies have been carried out in which silages have been sealed with a delay after they were compacted (Bolsen et al., 1993; Pauly et al., 2012) and in which compaction and sealing have been investigated together as influencing factors (Lancaster and McNaughton 1961; Miller et al., 1961). Therefore, more knowledge is needed on the effects of both factors and their interactions.

When silage is exposed to air after silo opening during feed-out, aerobic deterioration may occur as a result of undesirable microbial activity, particularly through yeasts. This activity inevitably leads to degradation of residual WSC, lactic acid, acetic acid and ethanol, raises pH and finally leads to spoilage (Woolford, 1990). Until spoilage of the exposed silo surface, few hours to several days can pass. Management factors can reduce the susceptibility to aerobic deterioration. These include, after harvesting the forage, sealing without a delay and a high degree of compaction to ensure low porosity (to minimize air penetration) and to reduce the availability of air for microorganisms responsible for deterioration (Piltz and Kaiser 2009; Wilkinson and Davies 2013).

Aerobically deteriorated silage is undesirable for feeding due to lower nutritive value and risk of negative effects on animal performance and health (Lindgren et al., 1988; Driehuis and Oude Elferink 2000). Additionally, those silages often have both poor sensory properties and poor hygienic status that can negatively influence feed intake, because of reduced palatability (Wichert et al., 1998). The extent of the decline in feed intake has been previously described by Gerlach et al. (2013) who found a mean decline in short-term dry matter intake (DMI, 3-h measurement period) by goats up to 79% when maize silage was exposed to air for 8 days compared with a silage that was fed without aerobic exposure after silo opening. However, there are very few studies in which aerobically deteriorated silages were fed to ruminants (Wichert et al., 1998; Bolsen et al., 2001). Against this background, it is still not fully understood, which silage characteristics lead to preference or avoidance of a specific silage.

Volatile organic compounds (VOC), such as ethanol and ethyl ester, formed in and emitting from silages have been a focus of interest for at least two reasons. First, ethanol and ester can contribute to air pollution by photochemical reaction with nitrogen oxides to form ozone (Howard et al., 2010; Hafner et al., 2013). Second, German and Danish farmers and local extension services have repeatedly reported on maize silages with unusual (atypical) odours (smelling of adhesive or acetone), which is associated with decreased feed intake and performance by dairy cows (Weiss et al., 2016). The affected silages had low pH and yeast counts, were highly compacted, well-fermented, stable upon exposure to air and had high concentrations of ethanol and odorous ethyl esters (Weiss et al., 2009; 2011). This suggests that ethyl esters are formed under optimal ensiling conditions, which can have a negative effect on feed intake by ruminants, as previously shown in preference trials with goats (Gerlach et al., 2013). Two ethyl esters, namely ethyl acetate and ethyl lactate, have been found in farm silages and in numerous ensiling experiments with maize and other substrates. Both ethyl ester strongly correlated with ethanol, highlighting the prominent role of ethanol in the esterification process (Weiss and Auerbach 2012; Weiss et al., 2016). The conditions of the occurrence of VOC and specifically of ethyl esters were recently described by Weiss et al., (2016). The immediate sealing of a silo leads to suppression of yeast growth. In contrast, a delay promotes the proliferation of yeasts. After sealing, the built-up population partly remains latent until

the silo is opened for feed-out (Pahlow et al., 2003). In between, yeasts are able to produce ethanol and other alcohols by changing their metabolism to anaerobic fermentation. This process is supported when high epiphytic yeast counts are already present in forage. However, the formation of ethanol and ethyl esters also depends on many other factors and reflects the complexity of the silage microbial ecosystem (Weiss et al., 2016). Little is known about the formation of ethyl esters and the impacts of various compactions on silage quality when silos are sealed with a delay. Studies on the influence of ethyl esters on forage choice and feed intake in ruminants are limited (Kristensen et al., 2007; Gerlach et al., 2013), so more detailed studies are warranted to clarify their influence.

REFERENCES

Bolsen, K. K., Hinds, M., Ilk, H. and Hoover, J. (1985). Effects of delayed filling and H/M inoculant on preservation and quality of corn silage, Cattlemen´s Day Conference, 1985. Kansas State University, Manhattan, KS: Agricultural Experimental Station and Cooperative Extension Service, *1985*, pp. 66–70.

Bolsen, K. K., Dickerson, J. T., Brent, B. E., Sonon, R. N., Dalke, B. S., Lin, C. and Boyer, J. E. (1993). Rate and extent of top spoilage losses in horizontal silos. *Journal of Dairy Science*, *76*, 2940–2962.

Bolsen, K. K., Huck, G. L., Siefers, M. K., Schmidt, T. E., Pope, R. V. and Uriarte, M. E. (2001). Silage Management: Five Key Factors, Kansas State University, Manhattan, Department of Animal Sciences and Industry, KS 66506. Available at: http://www.txanc.org/docs/silagemgt.pdf (accessed 10 December 2016).

Driehuis, F. and Oude Elferink, S. J. W. H. (2000). The impact of the quality of silage on animal health and food safety: A review. *Veterinary Quarterly*, *22*, 212–216.

Driehuis, F., Spanjer, M., Scholten, J. and Giffel, M. (2008). Occurrence of mycotoxins in maize, grass and wheat silage for dairy cattle in the Netherlands. *Food Additives and Contaminants*, *1*, 41–50.

Dunière, L., Sindou, J., Chaucheyras-Durand, F., Chevallier, I. and Thévenot-Sergentet, D. (2013). Silage processing and strategies to prevent persistence

of undesirable microorganisms. *Animal Feed Science and Technology*, *182*, 1–15.

Gerlach, K., Ross, F. H., Weiss, K., Büscher, W. and Südekum, K.-H. (2013). Changes in maize silage fermentation products during aerobic deterioration and its impact on feed intake by goats*. Agricultural and Food Science*, *22*, 168–181.

Hafner, S. D., Howard, C., Muck, R. E., Franco, R. B., Montes, F., Green, P. G., Mitloehner, F., Trabue, S. L. and Rotz, C. A. (2013). Emission of volatile organic compounds from silage: Compounds, sources, and implications. *Atmospheric Environment, 77*, 827–839.

Howard, C., Kumar, A., Malkina, I., Mitloehner, F., Green, P. G., Flocchini, R. G. and Kleeman, M. (2010). Reactive organic gas emissions from livestock feed contributes significantly to ozone production in Central California, *Environmental Science and Technology*, *44,* 2309–2314.

Kristensen, N. B., Storm, A., Raun, B. M. L., Røjen, B. A. and Harmon, D. L. (2007). Metabolism of silage alcohols in lactating dairy cows. *Journal of Dairy Science*, *90*, 1364–1377.

Lancaster, R. J. and McNaughton, M. (1961). Effects of initial consolidation on silage. *New Zealand Journal of Agriculture Research*, *4*, 504–515.

Lindgren, S., Pettersson, K., Jonsson, A., Lingvall, P. and Kaspersson, A. (1988). Silage inoculation: selected strains, temperature, wilting and practical application. *Swedish Journal of Agricultural Research*, *15*, 9–18.

McDonald, P., Henderson, A. R. and Heron, S. J. E. (1991). The biochemistry of Silage, 2nd ed. Marlow, UK: Chalcombe Publications.

Mickan, F. J., Martin, M. D. and Piltz, J. W. (2009). Chapter 9. Silage storage. In A. G. Kaiser, J. W. Piltz, H. M. Burns and N. W. Griffiths (Eds.), *Top fodder successful silage*. Dairy Australia and New South Wales Department of Primary Industries. Available at: http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0005/294053/successful-silage-topfodder-complete.pdf (accessed 10 August 2016).

Miller, W. J., Dalton, H. L. and Miler, J. K. (1961). Sudan grass silage at two stages of maturity versus rye grass and crimson clover with two filling procedures. *Journal of Dairy Science, 44*, 1921–1927.

Mills, J. A. and Kung, L. Jr. (2002). The effect of delayed ensiling and application of a propionic acid-based additive on the fermentation of barley silage. *Journal of Dairy Science*, *85*, 1969–1975.

Pahlow, G. and Muck, R. E. (2009). Managing for improved aerobic stability. In *Proceedings of the 15th International Silage Conference*, *Madison*, *Wisconsin*, *USA*, *2009* (pp. 77–90).

Pahlow, G., Muck, R. E., Driehuis, F., Oude Elferink, S. J. W. H. and Spoelstra, S. F. (2003). Microbiology of ensiling. In D. R. Buxton, R. E. Muck and J. H. Harrison (Eds.), *Silage Science and Technology* (pp. 31–93). Madison, USA: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America.

Pauly, T., Sundberg, M. and Spörndly, R. (2012). Effects of delayed sealing during silo filling-experiments with lab-scale silos. In *Proceedings of the 4th Nordic Feed Science Conference*, *Uppsala, Schweden, 2012* (pp. 30–33).

Piltz, J. W. and Kaiser, A. G. (2009). Chapter 2. Principles in silage preservation. In A. G. Kaiser, J. W. Piltz, H. M. Burns and N. W. Griffiths (Eds.), *Top fodder successful silage*. Dairy Australia and New South Wales Department of Primary Industries. Available at: http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0005/294053/successful-silage-topfodder-complete.pdf (accessed 10 August 2016).

Sucu, E., Kalkan, H., Canbolat, O. and Filya, I. (2016). Effects of ensiling density on nutritive value of maize and sorghum silages. *Revista Brasileira de Zootecnia*, *45*, 596–603.

Uriarte-Archundia, M. E., Bolsen, K. K. and Brent, B. E. (2002). A study of the chemical and microbial changes in whole-plant corn silage during exposure to air: Effects of a biological additive and sealing technique. In *Proceedings of the 13th International Silage Conference*, *Auchincruive*, *Scotland*, *2002* (pp. 172–173).

Velho, J. P., Mühlbach, P. R. F., Nörnberg, J. L., Velho, I. M. P. H., Genro, T. C. M. and Kessler, J. D. (2007). Composição bromatológica de silagens de milho produzidas com diferentes densidades de compactação [Chemical composition of maize silages with different packing densities]. *Revista Brasileira de Zootecnia*, 36, 1532–1538.

Vogel, R. (1992). Silierbedingungen für Silomais und Wirksamkeit der Impfung mit Milchsäurebakterien. *Landwirtschaft Schweiz, 5*, 161–168. (in German).

Weiss, K. and Auerbach, H. (2012). Occurrence of volatile organic compounds and ethanol in different types of silages. In *Proceedings of the 16th International Silage Conference*, *Hämeenlinna*, *Finland*, *2012* (pp. 128–129).

Weiss, K., Kalzendorf, C., Zittlau, J. and Auerbach, H. (2009). Novel results on the occurrence of volatile organic compounds in maize silage. In *Proceedings of the 15th International Silage Conference*, *Madison*, *USA*, *2009* (pp. 33–34).

Weiss, K., Gerlach, K. and Südekum, K.-H. (2011). Flüchtige Substanzen in Maissilagen in Abhängigkeit von Silierbedingungen und aerober Lagerungsdauer. *VDLUFA-Schriftenreihe*, *67*, 534−540. (in German).

Weiss, K., Kroschewski, B. and Auerbach, H. (2016). Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage. *Journal of Dairy Science*, *99*, 8053–8069.

Wichert, B., Kienzle, E. and Bauer, J. (1998). Palatability and intake of silage in dairy cows, in relation to hygienic quality. *Journal of Animal Physiology and Animal Nutrition, 80*, 253–259.

Wilkinson, J. M. and Davies, D. R. (2013). The aerobic stability of silage: Key findings and recent developments. *Grass and Forage Science, 68*, 1–19.

Woolford, M. K. (1990). A review. The detrimental effects of air on silage. *Journal of Applied Bacteriology, 68*, 101–116.

CHAPTER 2 SCOPE OF THE THESIS

This is a cumulative thesis composed of two manuscripts directly or indirectly addressing the problems mentioned in the general introduction. Chapters 3 and 4 compile the two manuscripts that are formatted according to the requirements of the journal chosen for submission. Chapter 5 provides a general discussion and overall conclusions. Furthermore, methodological aspects from chapter 3 and 4 will be critically discussed in order to be able to derive and suggest improvements for future studies.

The overall aim of the thesis was to determine the effect of air exposure before and after ensiling on quality of whole-crop maize silage and to determine the influence of the resulting changes on dietary choice by goats.

The thesis consisted of an ensiling experiment, which should address the aspect of silage quality. One aim was to determine the effects of compaction and delayed sealing on chemical composition, fermentation pattern and formation of VOC of maize silage before sealing and at silo opening. Whole-crop maize of 277 g kg^{-1} DM was either compacted to low (194 kg DM m^{-3}) or high (234 kg DM m^{-3}) density. The reason for the low compaction level was, in contrast to the higher one, to ensure higher gas exchange and air infiltration to show the effect of air at the early stage of conservation. Silos were sealed either immediately or with a delay at day 2 or at day 4 post-filling. The delays were chosen to show effects of air exposure and the immediate sealing was chosen to prevent it. The reason for choosing the rather small and unusual DM was to promote the formation of ethanol and ethyl ester by providing sufficient WSC for anaerobic fermentation to ethanol. Compaction and sealing times were also varied to promote growth of yeasts. Yeasts should provide ethanol for esterification. After opening the silos, silages were exposed to air for 6 days in order to deteriorate the silages. The aim of this experimental section was to determine the effect of aerobic exposure on chemical and microbial composition and on fermentation pattern. Before sealing, at silo opening and during aerobic exposure, a comprehensive sampling took place to characterise the status of silage and the changes that have taken place. Chapter 3 presents the results of the ensiling experiment.

The thesis also consisted of preference trials. The aim was to offer the silages prepared in the ensiling experiment in preference trials to goats to determine the effect of compaction, delayed sealing and aerobic exposure on forage choice and short-term DMI. By using short-term preference trials, preference behaviour when silages are offered in choice situations was described. In order to determine the determinants for preference, relationships between silage composition and DMI were tried to establish. The multidimensional scaling was used to present preference graphically. Chapter 4 deals with the results of the preference trials.

CHAPTER 3

EFFECT OF COMPACTION, DELAYED SEALING AND AEROBIC EXPOSURE ON MAIZE SILAGE QUALITY AND ON FORMATION OF VOLATILE ORGANIC COMPOUNDS

D. Brüning[1], K. Gerlach[1], K. Weiß[2], and K.-H. Südekum[1]

[1]Institute of Animal Science, University of Bonn, Bonn, Germany

[2]Albrecht Daniel Thaer-Institute of Agricultural and Horticultural Sciences, Humboldt-University, Berlin, Germany

Published in *Grass and Forage Science*, *73*, 2018, 53−66.

Correspondence: K. Weiß, Albrecht Daniel Thaer-Institute of Agricultural and Horticultural Sciences, Humboldt-University, Berlin, Germany. Email: kirsten.weiss@agrar.hu-berlin.de

ABSTRACT

Physical and management factors, such as compaction and sealing, greatly influence the outcome of conservation. This study aimed to determine the effects of compaction, delayed sealing and aerobic exposure after ensiling on maize silage quality and formation of volatile organic compounds (VOC). Whole-crop maize (277 g/kg dry matter [DM]) in 120-L plastic silos was compacted at either high or low density, and sealed immediately or with delay at 2 days or 4 days post-filling (six replicates each). After ensiling for at least 175 days, the silages were exposed to air for 6-day intervals and sampled at 2-day intervals. A delay in sealing caused an increase in yeast counts and a decline of up to 65% in water-soluble carbohydrates before ensiling. Sealing the silos after 4 days caused DM losses of up to 11%. Delayed sealing promoted the formation of ethyl esters at silo opening. A 4-day delay in sealing resulted in the lowest aerobic stability. Aerobic exposure led to considerable changes in silage composition, a loss in feed value and, finally, spoilage. This study indicates that maize silage quality is adversely affected by low compaction, delayed sealing and aerobic exposure.

Keywords: aerobic exposure, aerobic stability, delayed sealing, ethyl ester

INTRODUCTION

For several years, volatile organic compounds (VOC), such as ethanol and esters, formed in and emitting from silages have been a focus of interest. Ethanol can contribute to air pollution by photochemical reaction with nitrogen oxides to form ozone (Howard et al., 2010). Other VOC (e.g. aldehydes, esters and acids) may also contribute to poor air quality under certain conditions (Hafner et al., 2013). Furthermore, German and Danish farmers and local extension services have repeatedly reported on maize silages with unusual (atypical) odours (smelling of adhesive or acetone), which is associated with decreased feed intake and performance by dairy cows (Weiss, Kroschewski, & Auerbach, 2016). The affected silages had low pH and yeast counts, were highly compacted, well-fermented, stable upon exposure to air and had high concentrations of ethanol and odorous ethyl esters (Weiss, Gerlach, & Südekum, 2011; Weiss, Kalzendorf, Zittlau, & Auerbach, 2009). This suggests that ethyl esters are formed under optimal ensiling conditions,

which can have a negative effect on feed intake by ruminants, as partly shown in the study of Gerlach, Ross, Weiss, Büscher, and Südekum (2013). They reported the presence of ethyl acetate and ethyl lactate at up to 479 mg/kg dry matter (DM) in maize silages, which differed in cut length, DM concentration and compaction. When these silages were exposed to air after opening, both ethyl ester concentrations decreased during exposure. Also, both ethyl esters showed a strong correlation with ethanol, highlighting the prominent role of ethanol in esterification (Weiss & Auerbach, 2012; Weiss et al., 2016). The immediate sealing of a silo leads to suppression of yeast growth. In contrast, a delay in sealing promotes yeast proliferation and after sealing the built-up yeast population partly remains latent until the silo is opened for feed-out (Pahlow, Muck, Driehuis, Oude Elferink, & Spoelstra, 2003). In between, yeasts are able to produce ethanol and other alcohols by changing their metabolism to anaerobic fermentation. This process is supported when high epiphytic yeast counts are already present in forage. However, the formation of ethanol and ethyl esters depends on many factors and reflects the complexity of the silage microbial ecosystem (Weiss et al., 2016). Little is known about the formation of ethyl esters and the impacts of various compactions on silage quality when silos are sealed after a delay.

Therefore, this study aimed primarily to determine the effects of compaction and delayed sealing on maize silage quality regarding its chemical composition and fermentation pattern, with particular emphasis on VOC. It also determined the effect of aerobic exposure after opening (in accordance with a feed-out phase) on silage quality and VOC formation. The hypothesis was that maize silage quality is negatively affected by low compaction, delayed sealing and aerobic exposure, and that compaction and delayed sealing may influence the formation of ethyl esters by inducing ethanol production. In addition, the effect of diverging silage quality achieved by the different treatments (compaction, sealing, aerobic exposure) on short-time feed intake and preference by goats was also studied (D. Brüning, K. Gerlach, K. Weiß and K.-H. Südekum, unpublished).

MATERIALS AND METHODS

Silage preparation and treatments

Maize (*Zea mays*, var. Canon) (Maisadour Semences, France) was grown at the Frankenforst experimental station, Faculty of Agriculture, University of Bonn, Königswinter, Germany. Whole-crop maize was harvested at half-milk line stage on 2 September 2013 and chopped to a 6–8 mm theoretical cut length with a 1-row forage harvester (Mex GT; Pöttinger Maschinenfabrik, Grieskirchen, Austria). A total mass of 4 t from a homogenous field (based on visual inspection mainly considering plant height and colour) was used for the experiment. The chopped maize was thoroughly mixed with a wheel loader and shovels, sampled (about 1,000 g) from different points of the forage pile and filled in 120-L plastic barrels (80 cm height, 39 cm diameter). Maize in the barrels (hereafter referred to as silo) was either compacted to low (194 ± 4 kg DM m^{-3}) or high (234 ± 3 kg DM m^{-3}) density and sealed either immediately or with a delay at day 2 or day 4 post-filling. The six treatments (six silos per treatment) evaluated were as follows: L0—low compaction, sealed immediately; L2—low compaction, sealed on day 2 post-filling; L4—low compaction, sealed on day 4 post-filling; H0—high compaction, sealed immediately; H2—high compaction, sealed on day 2 post-filling; and H4—high compaction, sealed on day 4 post-filling. A forklift with a 1.8 or 3.6 t weight was used for low- and high-density compaction respectively. Approximately 10 kg of chopped maize was filled into the silo and compacted by the forklift; afterwards, the next layer (10 kg) was filled in and compacted. This procedure was repeated approximately ten times until the target density was achieved, which was based on the recommended minimum DM density for maize silage of 225 kg m^{-3} (Muck & Holmes, 2000).

During filling, two temperature data loggers (Tinytag Talk 2, TK-4014; Gemini Data Loggers, Chichester, UK) were placed at a depth of 20 and 60 cm into the maize (in three of six silos per treatment). Silage temperatures were recorded for the first 100 days. One of the six unsealed silos in each of the L2, L4, H2 and H4 treatments was sampled directly before sealing using a self-built sample drill (135 cm height, 30 cm sample chamber height, 4.5 cm diameter). The resulting sample (1,000 g) from the longitudinal section of the silo was mixed and subsampled, and the silos then sealed. All silos were sealed with plastic lids and stored in a barn at ambient

temperature (12 ± 2.9°C) for 175 (L0/H0), 217 (L2/H2) and 259 (L4/H4) days. The different storage times were chosen for experimental reasons.

Before opening the silos, the DM losses were determined as the difference between the initial and final weight of each silo and were calculated as DM loss [%] = 100 × (mass difference [g] / weight of DM [g]) + 2.5, based on Weissbach (2005).

Silos that were used for sampling before sealing were excluded from the calculation.

After opening, the silages were taken out of the silos, mixed thoroughly and exposed to air at room temperature (20 ± 1.5°C) for 6 days. All silages were stored separately on a concrete floor as a quadratic heap (1 x 1 m) with constant layer height (12 cm) and without any covering. The silage temperature of each heap was recorded every 2 hr by data loggers inserted at the geometric centre of each heap to determine aerobic stability, based on the method described by Honig (1990). Ambient temperature was recorded by two data loggers distributed across the room. Aerobic stability was defined as the number of hours that the silage remained stable before reaching 2°C (K) above ambient (Ranjit & Kung, 2000).

At silo opening (day 0) and at three times during the period of aerobic exposure (day 2, 4 and 6), each single heap was mixed and then sampled (1,000 g) for chemical analysis. Samples were frozen immediately (-18°C) after subsampling. A composite sample per treatment and day of aerobic exposure (100 g) was taken for microbial analysis and kept refrigerated until analysis. Furthermore, all treatments were evaluated for sensory properties (odour, texture, colour and visible moulds) using a point-based scheme (DLG, 2004). The heaps were then re-stored in quadratic form.

Laboratory analyses

General analyses

Samples were frozen immediately and then freeze-dried in triplicate (Gamma 1-16 LSC; Martin Christ, Osterode am Harz, Germany) and were ground prior to analysis (1 mm sieve, except samples for starch analysis at 0.2 mm) with a mill (SM 100; Retsch, Haan, Germany). The DM was determined in duplicate by oven drying 100 g of the fresh material overnight (60°C), then at 105°C for 3 hr (method 3.1; VDLUFA,

2012). The DM was corrected for the loss of volatiles during drying, using the following equation: DM_c [g/kg] = DM + 0.95 × sum of fatty acids (C_2–C_6) + 0.08 × lactic acid + 0.77 × 1,2 propanediol + 1.00 × other alcohols (C_2–C_6 including 2,3 butanediol) (Weissbach & Strubelt, 2008).

The oven-dried sample was subsequently discarded and was not used for further analysis. The following proximate analyses were carried out in duplicate on freeze-dried samples according to VDLUFA (2012): dry matter (method 3.1), crude ash (method 8.1), crude fibre (CF, method 6.1.2, using an ANKOM^{2000} fibre analyser; Ankom Technology, Macedon, NY, USA), crude fat (CL, method 5.1.1, using a Soxtec 2055; Foss Analytical Systems, Hillerød, Denmark), neutral detergent fibre (aNDFom, method 6.5.1, assayed with heat-stable amylase, Termamyl 120 L; Univar, Essen, Germany), acid detergent fibre (ADFom, method 6.5.2, using a Fibertec 1020; Foss Analytical Systems) and acid detergent lignin (ADL, method 6.5.3). The aNDFom and ADFom values were expressed exclusive of residual ash. Crude protein (CP = N x 6.25) was determined according to the Dumas method, using an automatic nitrogen analyser (vario MAX; Elementar Analysensysteme, Hanau, Germany). Non-protein nitrogen (NPN) was analysed as described by Licitra, Hernandez, and van Soest (1996) using the nitrogen analyser (vario MAX; Germany) after the filter was dried (30°C) overnight and the filter residue was transferred to a crucible before combustion. Starch was quantified after enzymatic hydrolysis of starch to glucose as described by Brandt, Schuldt, Mannerkorpi, and Vearasilp (1987).

The Hohenheim gas test (method 25.1; VDLUFA, 2012) was conducted to measure the 24 hr in vitro gas production (GP, [ml 200 mg^{-1} DM]) and estimate the metabolizable energy (ME) content using the equation of Menke and Steingass (1987), where ME [MJ kg^{-1} DM] = 0.136 × GP + 0.0057 × CP + 0.000286 × CL^2 + 2.20.

Chemical analyses of fermentation pattern

Forage and silage subsamples (50 g) were used to analyse the fermentation pattern. A cold-water extract was prepared by blending the frozen samples with 200 ml distilled water and 1 ml toluene and refrigerated overnight (4°C). The extracts were

then filtered using MN 615 filter paper (Macherey-Nagel, Düren, Germany) and followed by microfiltration (Minisart RC, 0.45 µm pore size; Sartorius, Göttingen, Germany).

The following fermentation variables were determined in duplicate: lactic acid, pH, volatile fatty acids, alcohols (methanol, ethanol, *n*-propanol, 1,2-propanediol, 2-butanol and 2,3-butanediol), acetone, ammonia-N (NH_3-N) and water-soluble carbohydrates (WSC). The samples were also analysed for ethyl lactate, ethyl acetate and propyl acetate in duplicate.

The pH of the extract was carried out potentiometrically by using a calibrated pH meter (827 pH lab; Metrohm, Herisau, Switzerland). Lactic acid was analysed by high-performance liquid chromatography (HPLC) with refractive index (RI) detection (LC-20 AB; Shimadzu Deutschland, Duisburg, Germany) according to Weiss and Kaiser (1995). The volatile fatty acids and alcohols were determined by gas chromatography (GC) with flame ionization detection (GC-2010; Shimadzu Deutschland, Duisburg, Germany) using a free fatty acid phase column (Permabond FFAP 0.25 µm; Macherey-Nagel, Düren, Germany) as described by Weiss (2001). The detection limit for each variable was 0.01%. Ethyl lactate, ethyl acetate, propyl acetate, *n*-propanol, 1,2-propanediol, 2-butanol, 2,3-butanediol, acetone and methanol were also determined by GC using a 0.25 µm Optima Wax column (Macherey-Nagel, Düren, Germany) (Weiss & Sommer, 2012). In this instance, the detection limit for each variable was 0.001%. The NH_3-N was analysed colourimetrically based on the Berthelot reaction using a continuous flow analyser (CFA, San^{++}, Skalar Analytical, Breda, Netherlands). The WSC concentration was determined by the anthrone method with the same CFA used for NH_3-N, according to von Lengerken and Zimmermann (1991).

Microbial analyses

Cooled samples (100 g) were sent directly to a laboratory (Wessling Laboratorien, Altenberge, Germany) for microbial analyses. Yeast, mould and aerobic mesophilic bacteria counts were determined according to VDLUFA (2012, methods 28.1.1–28.1.4).

Statistical analyses

The microbial data (and in one instance, the methanol data) were $\log_{10}$-transformed prior to analysis to obtain log-normal distribution and presented as log values. Statistical analyses were performed with SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Three fixed treatment factors (compaction, sealing and aerobic exposure) were tested. For treatment comparisons, ANOVA was used assuming a fixed effect model (Littell, Milliken, Stroup, Wolfinger, & Schabenberger, 2006) for a completely randomized design. Initially, residuals were tested for normal distribution by the Shapiro–Wilk test with the SAS UNIVARIATE procedure. For consideration of possible variance heterogeneity, different approaches were evaluated. Approach I assumed variance homogeneity (using one residual variance) and approach II, variance heterogeneity (using separate residual variances per level of only one factor or per factor level combination of two or three factors). The model fit of the different approaches was evaluated by Akaike's information criterion (AIC) and the likelihood ratio test. The ANOVA was performed with the SAS MIXED procedure using the restricted maximum likelihood algorithm (Schabenberger & Pierce, 2002). When significant treatment effects were detected by the global *F* test, pairwise comparisons were performed using Tukey's test, considering interactions between the treatment factors. In the instance of non-normal distribution, the data were transformed into ranks and analysed with an ANOVA-type statistical model for the global *F* test and pairwise comparisons among rank means (SAS MIXED procedure). Differences in all statistical analyses were considered significant at $p < .05$. Values were presented as least-squares means. The SAS CORR and REG procedures were used to evaluate the associations between individual silage fermentation characteristics at silo opening by the simple, multiple and partial coefficient of determination using regression analysis.

RESULTS

Characterization of and changes in the maize forage before sealing

The DM content of maize forage at harvest was 277 g/kg, while the WSC and starch contents were 160 and 285 g/kg DM respectively (Table 1). After 2 and 4 days, the concentrations of DM, NPN, NH_3-N, ethanol, ethyl acetate and the yeast counts

Table 1. Chemical composition and microbial counts of the harvested maize forage and of the compacted forages on day 2 and 4 post-filling (g kg^{-1} DM, unless stated otherwise)

		Status before sealing			
		On day 2 post-filling		On day 4 post-filling	
Item	Harvested maize forage	High compaction	Low compaction	High compaction	Low compaction
Dry matter (DM) (g/kg)	277	304	290	298	285
Crude protein (CP)	69.2	71.2	71.3	70.9	68.8
Crude fat	24.6	25.4	24.7	24.3	25.0
Starch	285	279	281	280	272
Crude fibre	213	227	220	216	242
aNDFom	457	466	486	470	492
ADFom	229	246	230	236	258
ADL	29.1	30.5	25.9	26.9	30.6
24-h gas production (ml 200 mg^{-1} DM)	60.2	57.8	58.9	58.7	56.7
Metabolizable energy (MJ kg^{-1} DM)	10.9	10.6	10.8	10.8	10.5

(Continues)

Table 1. Chemical composition and microbial counts of the harvested maize forage and of the compacted forages on day 2 and 4 post-filling (g kg^{-1} DM, unless stated otherwise) **(continued)**

		Status before sealing			
		On day 2 post-filling		On day 4 post-filling	
Item	Harvested maize forage	High compaction	Low compaction	High compaction	Low compaction
Non-protein nitrogen (g/kg of CP)	103	260	182	258	279
Ammonia-N (g/kg TN)	9.1	13.3	16.0	15.2	18.4
Water-soluble carbohydrates	160	72.2	72.3	57.0	55.9
pH	5.7	4.3	4.3	4.1	4.2
Lactic acid	ND	17.4	17.7	25.8	25.8
Acetic acid	1.7	9.1	8.5	11.8	12.6
Ethanol	0.7	5.1	7.4	5.0	5.1
Ethyl acetate (mg kg^{-1} DM)	ND	296	415	168	281
Ethyl lactate (mg kg^{-1} DM)	ND	ND	ND	ND	ND
Yeasts (log cfu g^{-1} FM)	6.04	7.08	7.20	6.70	6.57
Moulds (log cfu g^{-1} FM)	5.63	5.78	5.85	5.85	5.85
Aerobic mesophilic bacteria (log cfu g^{-1} FM)	>7.30	6.60	6.58	7.57	7.95

aNDFom = neutral detergent fibre assayed with heat-stable amylase and expressed exclusive of residual ash; ADFom = acid detergent fibre expressed exclusive of residual ash; ADL = acid detergent lignin; TN = total nitrogen; cfu = colony forming units; FM = fresh matter; ND = not detected (below detection limit); each *n* = 1 (based on at least two analytical replicates).

increased in the unsealed forages, regardless of compaction. After 4 days, the unsealed forages had decreased pH and increased lactic and acetic acid concentrations. Also, the WSC concentrations had markedly decreased by up to 65% compared with that of the harvested maize.

Influence of compaction and sealing on silage temperature

Table 2 shows the effect of compaction and sealing on the silage temperature during the first week of post-filling. The mean temperature difference between H2 and L2 at 20 cm depth until shortly before sealing on day 2 (48 hr) was 1.8°C, and the silage temperatures were higher in L2 than H2. After sealing, the temperature differences decreased continuously, so that the further away from the seal, the lower the differences were and the effect of low compaction decreased. The mean temperature difference between H4 and L4 until the silos were sealed on day 4 (96 hr) was 2.9°C and silage temperatures were higher in L4 than H4, which contributed to the effect of compaction after 72 and 108 hr. Until 60 hr post-filling, the temperatures between silos that were sealed on day 2 (H2 and L2) and day 4 (H4 and L4) were similar. Thereafter, the silage temperatures in H4 and L4 were higher than in H2 and L2 until 120 hr, which was particularly evident after 108 and 120 hr ($p = .01$ and $p < .01$ respectively). In comparison, L0 silos contained forages that were lower in temperature than all the others until 120 hr. After 156 hr, the silage temperatures were low in all silos and remained stable during conservation.

The silage temperatures were always higher at 20 cm depth than at 60 cm (data not shown). The mean temperature difference from top to bottom was 2.7°C, with a maximum value of 9.5°C (84 hr), regardless of compaction and sealing. No effect of compaction and sealing was observed at 60 cm depth during the first week of post-filling.

Table 2. Effect of compaction (C) and sealing (S) on silo temperature (°C) at 20 cm from the original surface of the forages that were sealed with delay during the first week post-filling

Hours (h) post-filling	Treatments evaluated									Effects		
	L0	Silo status	H2	L2	Silo status	H4	L4	Silo status	AT	SEM	C	S	C x S
24	21.5	sealed	22.3	23.6	unsealed	21.6	23.5	unsealed	24.1	0.1…1.0	*NS*	*NS*	*NS*
48	21.3	sealed	23.7	25.9	unsealed	22.5	25.3	unsealed	27.5	0.1…1.0	*0.02*	*NS*	*NS*
60	22.0	sealed	24.7	26.4	sealed	23.4	26.6	unsealed	17.4	0.1…1.0	*0.02*	*NS*	*NS*
72	22.2	sealed	23.7	25.1	sealed	23.7	26.8	unsealed	29.4	0.8…1.0	*0.04*	*NS*	*NS*
84	23.2	sealed	24.5	25.3	sealed	24.8	28.1	unsealed	20.4	0.3…1.5	*NS*	*NS*	*NS*
96	23.4	sealed	24.5	25.0	sealed	25.5	28.5	unsealed	30.4	0.2…1.4	*NS*	*NS*	*NS*
108	24.3	sealed	25.4	25.7	sealed	26.5	28.7	sealed	19.5	0.2…0.6	*0.04*	*0.01*	*NS*
120	23.9	sealed	24.5	24.9	sealed	25.9	26.8	sealed	21.4	0.2…0.3	*NS*	*<0.01*	*NS*
156	19.9	sealed	19.7	20.1	sealed	20.6	20.3	sealed	14.8	0.3…0.4	*NS*	*NS*	*NS*
168	18.5	sealed	18.4	18.5	sealed	19.2	18.7	sealed	14.9	0.3…0.4	*NS*	*NS*	*NS*

L0 = low compaction, sealed immediately (excluded from statistical analysis); H2 = high compaction, sealed on day 2 post-filling; L2 = low compaction, sealed on day 2 post-filling; H4 = high compaction, sealed on day 4 post-filling; L4 = low compaction, sealed on day 4 post-filling; AT = ambient temperature; SEM = standard error of the mean (min...max); NS = not significant ($p > .05$); each n = 3 (except L0, $n = 2$).

Chemical composition at silo opening and changes during aerobic exposure

At silo opening, the top surface layers (about 20 cm depth) of the silages that were sealed after a delay were discoloured (reddish-brown colour) without visible mould. The chemical composition of the silages at silo opening and during 6 days of aerobic exposure is presented in Table 3. At silo opening (day 0), the values of the chemical variables were within the range for maize silage values recommended by Kaiser and Piltz (2009). The concentrations of DM, crude ash, CP, CF, aNDFom, starch and NPN were either influenced by compaction, delayed sealing or both. Overall treatment effects on the chemical variables were small.

The chemical variables were influenced differently by aerobic exposure. The concentrations of DM, crude ash and CP increased after silo opening (day 0), while those of CF, aNDFom and ADFom increased after day 2 of exposure, regardless of compaction and sealing. The starch content remained constant during aerobic exposure, but the concentrations of NPN and CL decreased after day 2 and day 4 respectively. Silages that were exposed to air for 6 days had lower ($p < .01$) concentrations of ME (10.3 vs. 10.6 MJ/kg DM) than silages at silo opening.

Table 3. Effect of compaction (C), sealing (S) and aerobic exposure (AE) on chemical composition of the silages at silo opening and during aerobic exposure (g/kg dry matter, DM, unless stated otherwise)

Treatment	DM (g/kg)	Crude ash	Crude protein (CP)	Crude fat	Crude fibre	aNDFom	ADFom	ADL	Starch	NPN (g/kg CP)	GP (ml 200 mg^{-1} DM)	ME (MJ kg^{-1} DM)
Day 0 of AE (opening)												
H0	285	39.4	70.7	29.3	213	423	232	23.0	267	477	57.8	10.7
H2	278	40.9	71.1	29.8	227	445	238	24.6	259	516	59.1	10.9
H4	276	39.9	72.1	28.2	232	436	238	25.2	242	440	55.8	10.4
L0	279	39.6	68.8	28.6	219	420	237	25.4	276	448	57.4	10.6
L2	275	41.6	70.7	29.2	222	434	231	24.7	250	489	55.2	10.4
L4	272	41.0	70.0	28.8	232	432	244	25.6	247	456	57.5	10.7
SEM	1.4	0.5	0.6	0.5…1.5	2.8…6.5	6.0	4.2	1.0…1.9	2.1…5.6	10.0	55.2…59.1	0.1…0.2
p-value (global *F* test*)												
C	*<.01*	*NS*	*.01*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
S	*<.01*	*.01*	*NS*	*NS*	*<.01*	*.02*	*NS*	*NS*	*<.01*	*<.01*	*NS*	*NS*
C x S	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

(Continues)

Table 3. Effect of compaction (C), sealing (S) and aerobic exposure (AE) on chemical composition of the silages at silo opening and during aerobic exposure (g/kg dry matter, DM, unless stated otherwise) **(continued)**

Treatment	DM (g/kg)	Crude ash	Crude protein (CP)	Crude fat	Crude fibre	aNDFom	ADFom	ADL	Starch	NPN (g/kg CP)	GP (ml 200 mg^{-1} DM)	ME (MJ kg^{-1} DM)
Day 2 of AE												
H0	290	39.9	68.9	27.7	214	413	228	27.1	270	446	57.4	10.6
H2	286	42.4	73.2	29.6	220	441	237	23.2	256	492	58.5	10.8
H4	281	41.4	73.1	29.5	221	429	238	23.9	256	493	57.0	10.6
L0	286	39.9	67.7	28.3	217	426	236	28.2	260	429	56.6	10.5
L2	282	43.0	74.4	29.6	220	435	231	22.1	268	436	57.0	10.6
L4	275	42.4	72.8	30.5	232	445	238	25.0	242	434	55.0	10.4
Day 4 of AE												
H0	296	43.6	75.2	26.6	231	449	241	25.6	260	342	55.8	10.4
H2	285	45.4	77.2	29.5	228	456	247	25.6	268	342	58.0	10.8
H4	292	44.7	78.7	29.4	253	475	268	33.3	269	358	56.7	10.6
L0	291	42.8	72.8	26.1	240	450	247	24.9	248	293	55.9	10.4
L2	287	44.6	76.7	28.0	230	468	245	24.3	264	282	57.4	10.7
L4	285	45.1	75.5	25.5	251	483	271	30.6	263	287	55.9	10.4

(Continues)

Table 3. Effect of compaction (C), sealing (S) and aerobic exposure (AE) on chemical composition of the silages at silo opening and during aerobic exposure (g/kg dry matter, DM, unless stated otherwise) **(continued)**

Treatment	DM (g/kg)	Crude ash	Crude protein (CP)	Crude fat	Crude fibre	aNDFom	ADFom	ADL	Starch	NPN (g/kg CP)	GP (ml 200 mg^{-1} DM)	ME (MJ kg^{-1} DM)
Day 6 of AE												
H0	310	45.5	76.1	26.2	238	466	259	27.4	280	318	54.7	10.3
H2	298	46.4	79.7	21.0	238	487	265	28.4	269	335	56.9	10.5
H4	305	47.9	80.5	22.0	252	478	264	30.0	254	352	54.5	10.2
L0	314	44.0	76.7	24.7	240	474	256	25.9	256	317	54.3	10.2
L2	299	47.2	78.6	21.3	240	489	266	28.3	260	283	57.0	10.5
L4	310	47.9	77.2	20.8	254	492	277	33.0	242	327	53.2	10.0
SEM	1.4…2.3	0.3…1.2	0.6	0.6…2.4	2.0…7.2	6.2	4.1…5.9	1.2…2.5	3.2…9.3	11.0	0.6…1.1	0.1…0.2
p-value (global *F* test*)												
C	*<.01*	*NS*	*<.01*	*NS*	*NS*	*NS*	*NS*	*NS*	*.01*	*<.01*	*.03*	*.02*
S	*<.01*	*<.01*	*<.01*	*NS*	*<.01*	*<.01*	*<.01*	*<.01*	*<.01*	*NS*	*.01*	*<.01*
AE	*<.01*	*<.01*	*<.01*	*<.01*	*<.01*	*<.01*	*<.01*	*<.01*	*NS*	*<.01*	*<.01*	*<.01*
C x S	*NS*	*NS*	*.01*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
C x AE	*<.01*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*<.01*	*NS*	*NS*
S x AE	*<.01*	*NS*	*<.01*	*<.01*	*NS*	*NS*	*NS*	*<.01*	*<.01*	*<.01*	*NS*	*NS*
C x S x AE	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

aNDFom = neutral detergent fibre assayed with heat-stable amylase and expressed exclusive of residual ash; ADFom = acid detergent fibre expressed exclusive of residual ash; ADL = acid detergent lignin; NPN = non-protein nitrogen; GP = 24-h gas production; ME = metabolizable energy; H0 = high compaction, sealed immediately; H2 = high compaction, sealed on day 2 post-filling; H4 = high compaction, sealed on day 4 post-filling; L0 = low compaction, sealed immediately; L2 = low compaction, sealed on day 2 post-filling; L4 = low compaction, sealed on day 4 post-filling; Standard error of the mean (min…max); *based on Tukey's test ($p < .05$), NS = not significant ($p > .05$); n = 6 per treatment and day.

Fermentation pattern and volatile organic compounds at silo opening, and changes during aerobic exposure

The fermentation pattern at silo opening and during aerobic exposure is presented in Table 4. At silo opening (day 0), all silages were well-fermented with low acetic acid concentrations (<30 g/kg DM) and butyric acid contents below the detection limit. A delay in sealing slightly increased ($p < .01$) the silage pH at silo opening. The WSC concentration was highest in L0 (compaction x sealing interaction). Lactic acid content decreased ($p < .01$) when silages were sealed with delay. Ethanol content was increased ($p < .01$) by high compaction and depended on sealing (compaction x sealing interaction). Ethanol contents were high when compared with contents of maize silages from an on-farm evaluation of Gallo, Giuberti, Bruschi, Fortunati, and Masoero (2016) of 68 dairy farms in Italy. Ethyl lactate content was increased by high compaction and delayed sealing, but both effects interacted. Delayed sealing markedly increased ($p < .01$) ethyl acetate concentrations at silo opening. A 4-day delay in sealing caused a greater than two-fold increase in the ethyl acetate concentration compared with immediate sealing. Propyl acetate was not detected in any sample. The NH_3-N level was decreased by low compaction and delayed sealing ($p = .01$ and $p < .01$ respectively).

The fermentation variables were changed more strongly by aerobic exposure than by compaction or delayed sealing. The concentrations of WSC, acetic acid and NH_3-N decreased after silo opening, while lactic acid contents decreased after day 4 of exposure, regardless of compaction before ensiling and sealing. On day 4, the treated silages had pH ≥5. The concentrations of ethyl lactate and ethyl acetate had already decreased after 2 days of exposure, while ethanol content decreased when silages were exposed to air for 4 days.

Table 4. Effect of compaction (C), sealing (S) and aerobic exposure (AE) on fermentation pattern of the silages at silo opening and during aerobic exposure (g/kg dry matter, DM, unless stated otherwise)

Treatment	pH	WSC	LA	AA	BA	ETH	METH	PRO	BU	EL	EA	NH_3-N
		(g kg^{-1} DM)					(mg kg^{-1} DM)					(g kg^{-1} TN)
Day 0 of AE (opening)												
H0	3.63	35.6	68.1	13.2	ND	17.1	353	155	468	399	499	89.5
H2	3.82	27.7	54.9	10.3	ND	14.9	109	ND	ND	460	698	79.7
H4	3.71	27.2	66.1	11.7	ND	14.3	67	ND	ND	549	945	81.2
L0	3.65	53.5	67.0	11.7	ND	8.1	346	ND	448	245	398	85.2
L2	3.84	29.8	55.8	9.8	ND	12.8	70	6.1	ND	472	769	74.0
L4	3.72	31.6	62.9	11.3	ND	10.7	62	ND	ND	432	890	79.2
SEM	0.01	1.0…3.1	0.3…1.7	0.4	-	0.8	4…60	-	-	22	43	2.1…2.6
p-value (global *F* test*)												
C	*NS*	*<.01*	*NS*	*.01*	-	*<.01*	*NS*	-	-	*<.01*	*NS*	*.01*
S	*<.01*	*<.01*	*<.01*	*<.01*	-	*NS*	*<.01*	-	-	*<.01*	*<.01*	*<.01*
C x S	*NS*	*.01*	*NS*	*NS*	-	*<.01*	*.04*	-	-	*<.01*	*NS*	*NS*

(Continues)

Table 4. Effect of compaction (C), sealing (S) and aerobic exposure (AE) on fermentation pattern of the silages at silo opening and during aerobic exposure (g/kg dry matter, DM, unless stated otherwise) **(continued)**

Treatment	pH	WSC	LA	AA	BA	ETH	METH	PRO	BU	EL	EA	NH_3-N
		(g kg^{-1} DM)					(mg kg^{-1} DM)					(g kg^{-1} TN)
Day 2 of AE												
H0	3.69	34.2	66.6	9.3	ND	11.6	262	106	427	335	348	83.7
H2	3.82	25.3	60.6	8.3	ND	13.1	71	35	ND	358	555	73.5
H4	3.82	21.3	59.7	6.5	ND	14.5	44	ND	ND	371	423	60.0
L0	3.69	49.5	64.0	9.7	ND	7.6	233	58	495	216	338	83.0
L2	3.92	20.3	59.8	5.2	ND	15.4	55	12	ND	329	407	54.6
L4	3.90	19.5	51.2	3.3	ND	13.1	45	18	ND	331	308	43.4
Day 4 of AE												
H0	5.51	18.1	16.5	2.6	1.2	3.2	159	11	355	80	34	21.6
H2	5.90	12.9	0.6	1.9	1.7	ND	ND	ND	ND	ND	ND	18.6
H4	6.07	12.7	ND	1.6	1.7	ND	ND	ND	ND	3.36	ND	20.8
L0	6.15	20.5	5.3	1.7	1.6	0.7	103	ND	458	ND	ND	16.2
L2	6.12	12.2	ND	1.3	1.2	0.3	ND	ND	ND	ND	ND	14.2
L4	6.23	11.0	ND	1.1	1.2	0.3	ND	ND	ND	ND	ND	19.3

(Continues)

Table 4. Effect of compaction (C), sealing (S) and aerobic exposure (AE) on fermentation pattern of the silages at silo opening and during aerobic exposure (g/kg dry matter, DM, unless stated otherwise) **(continued)**

Treatment	pH	WSC	LA	AA	BA	ETH	METH	PRO	BU	EL	EA	NH_3-N
		(g kg^{-1} DM)					(mg kg^{-1} DM)					(g kg^{-1} TN)
Day 6 of AE												
H0	6.66	16.6	6.1	1.5	1.3	0.5	ND	ND	155	ND	ND	25.1
H2	6.61	7.8	ND	1.3	0.3	0.1	ND	ND	ND	ND	ND	20.5
H4	6.82	9.4	ND	1.6	0.2	0.2	ND	ND	ND	ND	ND	29.9
L0	6.46	16.2	4.9	1.6	1.4	1.0	ND	ND	169	ND	ND	15.2
L2	6.61	8.1	ND	1.1	0.2	0.1	ND	ND	ND	ND	ND	13.6
L4	6.96	8.8	ND	1.2	0.2	0.2	ND	ND	ND	ND	ND	22.9
SEM	0.1…0.4	0.2…3.6	-	-	-	-	-	-	-	-	-	0.3…7.1
p-value (global *F* test**)												
C	*NS*	*<.01*	*<.01*	*<.01*	*.01*	*.02*	*.01*	-	*.02*	*<.01*	*<.01*	*<.01*
S	*<.01*	*<.01*	*<.01*	*<.01*	*<.01*	*<.01*	*<.01*	-	*<.01*	*<.01*	*<.01*	*<.01*
AE	*<.01*	*<.01*	*<.01*	*<.01*	*<.01*	*<.01*	*<.01*	-	*<.01*	*<.01*	*<.01*	*<.01*
C x S	*NS*	*<.01*	*NS*	*<.01*	*.02*	*<.01*	*NS*	-	*.02*	*<.01*	*NS*	*NS*
C x AE	*NS*	*<.01*	*NS*	*.01*	*NS*	*<.01*	*NS*	-	*NS*	*.01*	*NS*	*NS*
S x AE	*<.01*	*<.01*	*<.01*	*<.01*	*<.01*	*<.01*	*<.01*	-	*<.01*	*<.01*	*<.01*	*<.01*
C x S x AE	*.01*	*<.01*	*NS*	*NS*	*NS*	*<.01*	*NS*	-	*NS*	*NS*	*NS*	*NS*

WSC = water-soluble carbohydrates; LA = lactic acid; AA = acetic acid; BA = butyric acid (sum of i-/n-butyric acid, i-/n-valerian acid and n-capronic acid); ETH = ethanol; METH = methanol; PRO = *n*-propanol; BU = 2-butanol; EL = ethyl lactate; EA = ethyl acetate; NH_3-N = ammonia-N; TN = total nitrogen; H0 = high compaction, sealed immediately; H2 = high compaction, sealed on day 2 post-filling; H4 = high compaction, sealed on day 4 post-filling; L0 = low compaction, sealed immediately; L2 = low compaction, sealed on day 2 post-filling; L4 = low compaction, sealed on day 4 post-filling; Standard error of the mean (min…max); ND = not detected (below detection limit); NS = not significant ($p > .05$); *based on Tukey's test ($p < .05$); **based on Tukey's test ($p < .05$, for pH, WSC, NH_3-N) and on global rank test (ANOVA-type statistics, $p < .05$, for variables LA, AA, BA, ETH, METH, BU, EL, EA); $n = 6$ per treatment and day.

Associations between fermentation characteristics and volatile organic compounds

Table 5 shows the squared, simple correlation and regression coefficients of the treatments at silo opening between the ethyl esters and the esterification reactants; i.e., the fermentation acids and ethanol. Generally, very few significant correlations occurred. The contents of acetic acid and ethyl acetate were correlated in H4 (r^2 = .68; p < .05), while ethanol and ethyl lactate concentrations were correlated in H0 and L2 (r^2 = .58; p < .05 and r^2 = .88; p < .01 respectively). The multiple regression analysis revealed higher r^2 in H0 for ethyl lactate (r^2 = .84; p < .05) when, in addition to ethanol, lactic acid was included in the model (data not shown). Nevertheless, the partial regression analysis showed that only ethanol was correlated with ethyl lactate (regression coefficient was 28.8 mg of ethyl lactate per 1 g of ethanol when lactic acid values were kept constant; p < .05) in H0. Acetic acid and ethanol explained the variation in ethyl acetate content in H4, but the effect was no better than acetic acid alone (r^2 = .56; p > .05). Including lactic acid in the multiple regression together with ethanol improved the fit (r^2 = .96; p < .01) for ethyl lactate in L2 compared with ethanol alone, but ethanol had a larger effect than lactic acid.

Dry-matter loss, aerobic stability, microbial composition and sensory properties

The DM losses and aerobic stability data are reported in Table 6. Sealing affected the DM losses that occurred during conservation and depended on compaction (compaction x sealing interaction). The highest DM losses were determined in L4 and H4. Aerobic stability was influenced by sealing. Silages that were sealed on day 4 post-filling showed lower (p = .01) aerobic stability (49 vs. 64 hr) than silages sealed immediately. Silages that were sealed on day 2 post-filling were aerobically less (p = .06) stable (52 vs. 64 hr) than silages that were sealed immediately.

Table 5. Squared simple correlation coefficients (r^2) and regression coefficients (in parentheses) of the treatments between ethyl esters, fermentation acids and ethanol at silo opening

	Adjusted simple r^2 (regression coefficient)							
	Variable		Treatment					
Model	Y (mg kg^{-1} DM)	X (g kg^{-1} DM)	H0	H2	H4	L0	L2	L4
1	Ethyl acetate	Acetic acid	-0.24^{NS}	-0.25^{NS}	0.68*	0.03^{NS}	-0.24^{NS}	-0.05^{NS}
			(-8.68)	(0.82)	(105)	(-35.9)	(29.5)	(-52.9)
		Ethanol	0.01^{NS}	-0.25^{NS}	-0.23^{NS}	0.22^{NS}	-0.07^{NS}	0.16^{NS}
			(14.4)	(0.52)	(-7.83)	(40.0)	(36.7)	(39.7)
2	Ethyl lactate	Lactic acid	0.12^{NS}	-0.17^{NS}	-0.21^{NS}	-0.24^{NS}	0.23^{NS}	0.53^{NS}
			(44.4)	(-4.06)	(2.56)	(1.63)	(7.86)	(77.8)
		Ethanol	0.58*	0.17^{NS}	0.40^{NS}	0.27^{NS}	0.88**	-0.10^{NS}
			(12.6)	(13.8)	(22.2)	(13.3)	(34.4)	(15.6)
3	Sum of ethyl acetate and ethyl lactate	Lactic acid	-0.10^{NS}	0.12^{NS}	-0.25^{NS}	-0.25^{NS}	0.46^{NS}	0.53^{NS}
			(82.9)	(-15.1)	(1.91)	(-3.01)	(31.9)	(193)
		Acetic acid	-0.25^{NS}	-0.07^{NS}	0.36^{NS}	0.09^{NS}	-0.23^{NS}	-0.07^{NS}
			(-2.63)	(-29.9)	(101)	(-51.2)	(-47.1)	(-79.3)
		Ethanol	0.19^{NS}	-0.10^{NS}	-0.21^{NS}	0.24^{NS}	0.18^{NS}	0.07^{NS}
			(26.9)	(14.3)	(14.3)	(53.4)	(71.1)	(55.2)

H0 = high compaction, sealed immediately; H2 = high compaction, sealed on day 2 post-filling; H4 = high compaction, sealed on day 4 post-filling; L0 = low compaction, sealed immediately; L2 = low compaction, sealed on day 2 post-filling; L4 = low compaction, sealed on day 4 post-filling; *$p < 0.05$, **$p < 0.01$, ^{NS} = not significant ($p > 0.05$); each $n = 6$.

Table 6. Effect of compaction (C) and sealing (S) on dry-matter (DM) losses and aerobic stability (ASTA)

	Treatment							Effects		
	H0	H2	H4	L0	L2	L4	SEM	C	S	C x S
DM loss (%)	5.5[a]	5.8[a]	9.0[b]	3.7[a]	5.7[a]	10.7[b]	0.3…0.7	*NS*	*<0.01*	*0.02*
n	*6*	*5*	*5*	*6*	*5*	*5*				
ASTA (h)	65	57	52	64	48	47	3.1…4.4	*NS*	*0.02*	*NS*
n	*6*	*4*	*5*	*3*	*5*	*6*				

H0 = high compaction, sealed immediately; H2 = high compaction, sealed on day 2 post-filling; H4 = high compaction, sealed on day 4 post-filling; L0 = low compaction, sealed immediately; L2 = low compaction, sealed on day 2 post-filling; L4 = low compaction, sealed on day 4 post-filling; SEM = standard error of the mean (min...max); [a-c]Means in rows with unlike superscripts differ ($p < .05$, Tukey's test); NS = not significant ($p > .05$).

The changes in silage temperature during aerobic exposure (Figure 1) revealed that on day 2 of exposure, the silage temperatures started to rise. Regardless of compaction, temperature increases were stronger in L2, H2, L4 and H4 than the other treatments. On day 3, all silage temperatures were more than 2 K above the ambient temperature. Thus, the silages were aerobically unstable. The L2, H2, L4 and H4 silages reached maximum temperatures of about 35°C or more (H4). In H0 and L0, the temperature rise was delayed and relatively low compared to the other treatments.

When the silages were exposed to air, yeast counts increased rapidly after 2 days of exposure (Table 7). Mould counts increased after day 4. The aerobic mesophilic bacteria counts determined on day 4 and day 6 were higher than those at silo opening and after day 2. At silo opening, no apparent differences in yeast counts among the treatments were visible (data not shown). On day 2 of exposure, H0 and L0 had lower ($p = .01$) yeast counts (6.53 vs. 7.63 log colony-forming units (cfu) g^{-1}) than the delayed sealed silages (L2, H2, L4 and H4).

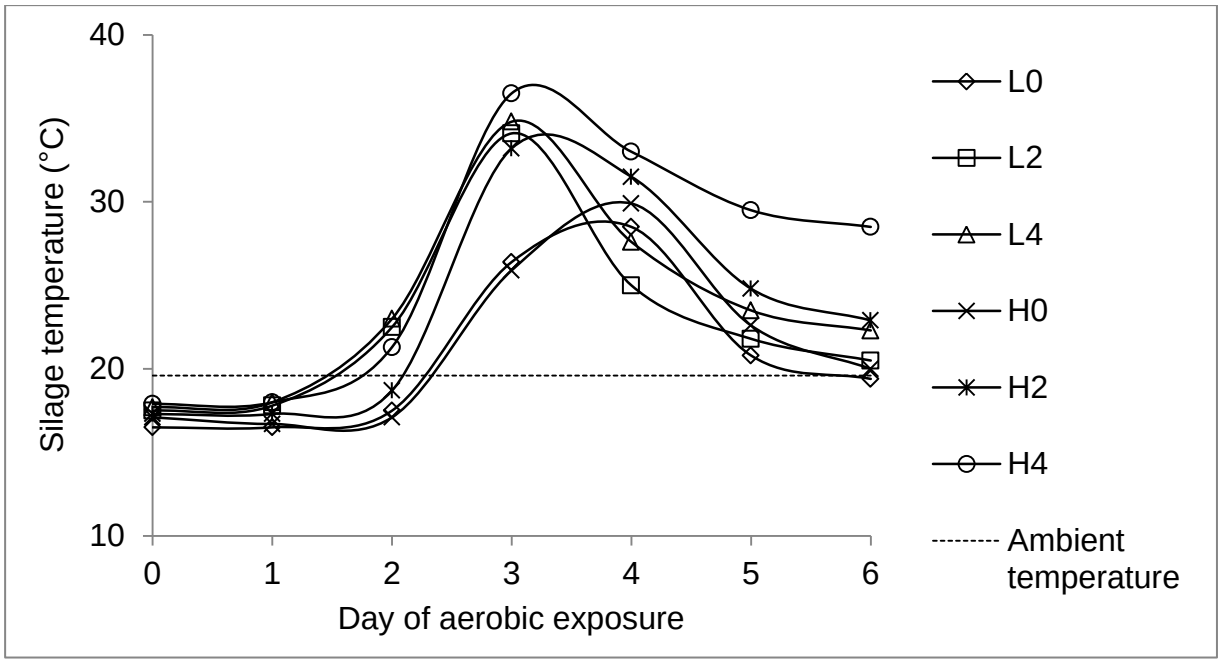

Figure 1. Effect of temperature changes in the treatments on aerobic exposure; H0 = high compaction, sealed immediately (*n* = 6); H2 = high compaction, sealed on day 2 post-filling (*n* = 4); H4 = high compaction, sealed on day 4 post-filling (*n* = 5); L0 = low compaction, sealed immediately (*n* = 3); L2 = low compaction, sealed on day 2 post-filling (*n* = 5); L4 = low compaction, sealed on day 4 post-filling (*n* = 6).

Table 7. Effect of aerobic exposure on microbial counts

	Day of aerobic exposure					
Microbial population[1]	0	2	4	6	SEM	*p-value*
Yeasts	5.16^{a}	7.26^{b}	8.26^{bc}	8.20^{c}	0.2	*<.01*
Moulds	3.13^{a}	2.70^{a}	2.70^{a}	4.84^{b}	0.4	*<.01*
Aerobic mesophilic bacteria	5.84^{a}	5.63^{a}	8.46^{b}	8.79^{b}	0.4	*<.01*

[1]log cfu g^{-1} FM; cfu = colony-forming units; FM = fresh matter; SEM = standard error of the mean; $^{a-c}$Mean values in rows with unlike superscripts differ (p < .05, Tukey's test); n = 6 per day.

The silages were evaluated for their sensory properties during aerobic exposure (data not shown). At silo opening, no treatments effects (p < .05) were observed. From day 2 of exposure, the sensory properties deteriorated. On day 6, all the silages were mouldy with a foul-smelling odour, a strongly offensive texture (greasy) and altered colour. Therefore, the silages were considered bad or very bad. The

worst odours emanated from L4 and H4 on day 6. Silages that were sealed on day 4 showed visible moulds on day 4 (L4) and day 6 (L4 and H4) of exposure.

DISCUSSION

Changes in maize forage composition during the pre-seal phase

Forages that were left unsealed for 2 and 4 days had increased NPN, NH_3-N, ethanol and ethyl acetate concentrations and increased yeast counts compared with the forage at harvest. The degradation of CP to NPN compounds, such as peptides and free amino acids, is a standard process during ensiling, indicating proteolysis activity by plant enzymes (Fairbairn, Alli, & Phillip, 1992). The further chemical reduction in the peptides and amino acids to NH_3-N and amines is caused largely by microbial activity during desmolysis that can deleteriously affect the feeding value of the nitrogen in silage (Muck, 1988). The increase in yeast counts was presumably caused by the aerobic conditions attained in the top surface layers, and this consequently encouraged yeast growth (Woolford, 1990). Ethanol concentrations are an indication of the metabolic activity of yeasts under anaerobic conditions (Mills & Kung, 2002). The ethanol produced could have been responsible for the increase in ethyl acetate concentration. Ethyl acetate can be formed by esterification of ethanol and acetic acid (Weiss & Auerbach, 2012). Exposing forage to air for 24 hr increased the pH in the study of Mills and Kung (2002) because a delay in ensiling postpones a pH decline (Muck, 1988). In contrast, in the present study the pH declined, and this was possibly because samples were taken from the longitudinal section of the silos and anaerobic conditions existed at the bottom, where lactic acid fermentation occurred. The WSC concentrations in the forages decreased to 35% of the original concentration on day 4 post-filling. Woolford (1984) noted that >50% of the WSC can be lost within 24 hr of filling if the silo is inadequately sealed. The results indicate that protein degradation by plant and microbial enzymes can occur after 2 days post-filling in unsealed silos and that ethyl acetate can already be formed from ethanol during this time. A gradual decline in WSC can occur, when sealing of the silos is delayed by up to 4 days.

Silo temperature as affected by compaction and sealing

Until the silos were sealed, an effect of low compaction on silage temperatures was observed. The disappearance of this effect when the silos were sealed suggests that as long as considerable air ingress occurs, higher silage temperatures will be measured in low compared with high compacted forages. Once the silo is sealed, and, thus, the air supply is interrupted, low compaction plays a subordinate role. The energy released by aerobic degradation of WSC in plant respiration is responsible for the temperature elevation (Ruxton, Clark, & McDonald, 1975). Honig (1991) stated that reduced silage density as a result of inadequate compaction is reflected in higher porosity, which allows more rapid air ingress. Thus, more air entered the silos within the first 20 cm depth, where more WSC were oxidized. The later the silos were sealed, the higher were the temperatures, regardless of compaction. This means that a delay in sealing can cause persistent high temperatures and that the positive effect of high compaction is nullified when the silo remains unsealed. Furthermore, this indicates that a 4-day delay in sealing results in a greater loss of WSC by plant respiration, which was reflected in high DM losses in these silages. The temperature increases may also have been caused by yeasts, which can oxidize WSC to carbon dioxide and water under heat evolution (Dunière, Sindou, Chaucheyras-Durand, Chevallier, & Thévenot-Sergentet, 2013). Silage temperatures were increased in unsealed silos on day 2 and day 4 post-filling. Immediate sealing and low compaction resulted in the lowest silage temperatures. Unfortunately, the data obtained from the highly compacted and immediately sealed silos could not be evaluated, because some loggers were broken. However, the results showed that immediate sealing is advantageous in reducing undesirable WSC oxidation in the top surface layers even if the compaction is inadequate.

Silage temperature was higher at a 20 than 60 cm depth, in agreement with Bolsen et al. (1993). According to McGuffey and Owens (1979), higher temperatures near the surface indicate less compaction. Thus, fewer WSC losses and a more desired fermentation can be expected lower down in the silo. Only silages that had delayed sealing were discoloured in the top surface layers. This was caused by overheating, as demonstrated by Vogel (1992), and indicates that only these silages underwent intensive WSC respiration in the top surface layers during the pre-seal phase.

Treatment effects on chemical composition and fermentation pattern at silo opening

All the silages were well-fermented, showed no signs of spoilage and had good sensory properties at silo opening. Moreover, the silages showed only marginal differences in chemical and in most fermentation variables, caused by compaction or sealing. This was induced by the low DM of the harvested maize forage, indicating a good fermentative efficiency by delivering sufficient amounts of WSC for fermentation (Muck, 1988). In the study of Henderson and McDonald (1975), delayed sealing had fewer effects on fermentation when the used forage had a high WSC content (28% of DM). In contrast, a delay in sealing resulted in higher pH, DM losses, butyric acid and volatile-N contents and lower lactic acid, when forage with a low WSC content (10% of DM) was used. Conversely, low DM forages with high WSC concentrations can result in silages that contain high ethanol and ethyl ester concentrations (Weiss et al., 2011) due to intensive anaerobic fermentation of WSC to ethanol. However, one aim of this study was to stimulate ethanol production and promote the formation of ethyl esters, not only by different compactions and sealing times. It has to be considered that the whole content of the silos was used and mixed together with the discoloured layers. Thus, the influence of low compaction and delayed sealing was mitigated, because their impact was mainly confined to the top layers and this accounted for only about a quarter of the whole silo. A separate analysis of the upper and lower layers would have yielded stronger adverse effects on the sides of the upper layers and weaker adverse effects on the sides of the lower layers, such as was shown in the study by Bolsen et al. (1993) and as indicated by the low temperatures at a 60 cm depth. The discoloured top layers represented about a quarter of the silo. According to Bolsen et al. (1993), more than 25% of the initial silage mass is within the top 1 m in a 1,000-t horizontal silo (32.0 m length x 12.0 m width x 3.7 m height), depending on silage density. Their results showed that the top layers (0–50 cm) can have elevated temperatures and reduced quality when the silo is sealed with delay. This means that about 6–12.5% of a farm silo is of reduced quality when it is sealed with delay.

Delayed sealing increased the CF and aNDFom concentrations at silo opening and decreased starch content. Similar observations were described by Mills and Kung (2002). They attributed the NDF increase to the loss of WSC before ensiling. Starch

degradation, in this study, could have been caused by continued plant enzyme hydrolytic activity before sealing, yielding additional carbohydrate substrates for lactic acid fermentation (Muck, 1988). About 45–52% of the CP was in the NPN form at silo opening. According to Hoedtke, Gabel, and Zeyner (2010), the NPN fraction can increase up to 50–70% during conservation by proteolysis in plants. The NPN values were similar across the treatments. Therefore, there was no indication that an increased proteolysis in the delayed sealed silages occurred, as might have been expected because protein degradation remains as long as the oxygen in the silo is not consumed and the pH is not lowered (Ohshima & McDonald, 1978). The NH_3-N concentrations were below the critical value of 100 g kg^{-1} N (Wyss, 2005) and were decreased in less compacted silages and silages that were sealed with a delay. Similar effect of delayed sealing on NH_3-N was shown by Kim and Adesogan (2006). The effect was compared with reduced fermentation and curtailed in-silo proteolysis. This could imply that only microbial desmolysis occurred in all the silages.

Delayed sealing marginally increased the pH at silo opening as a result of decreased lactic acid concentrations. A decrease in lactic acid content is associated with both impeded anaerobiosis and lactic acid fermentation caused by a delay in sealing (Weiss, 2001; Woolford, 1984). Additionally, continued respiration during the pre-sealing phase can cause a loss of WSC as substrates for lactic acid bacteria (Ruxton & McDonald, 1974). However, residual WSC were not higher in the delayed sealed silages, as stated by McDonald, Henderson, and Heron (1991).

Formation of VOC

High compaction before ensiling increased the ethanol and ethyl lactate concentrations at silo opening. Weiss, Olbrich, and Thaysen (2015) demonstrated that with increasing compaction (ranging from 100 to >300 kg DM m^{-3}), the concentration of ethanol, ethyl lactate and ethyl acetate increased. This was explained by the typically lower pH at the bottom compared to the top of a silo, and with the more compacted and less air-affected zones in the base. Esterification processes were shown to be stimulated by low pH (Weiss & Auerbach, 2013). Ethyl lactate and ethanol were correlated in silages that were highly compacted and immediately sealed. This suggests that ethanol that was formed from epiphytic yeast

was involved in the esterification processes occurring in these silages. Recently, Weiss et al. (2016) found strong correlations between ethyl lactate and ethanol levels, highlighting the prominent role of ethanol in ester formation. However, a correlation between ethyl lactate and ethanol contents was also found in silages that were low compacted and sealed on day 2 post-filling. No correlations between the levels of ethyl acetate and ethanol, or ethyl lactate and lactic acid were found in the treatments. Moreover, only one correlation between ethyl acetate and acetic acid concentrations was evident. Weiss et al. (2016) suggested that ethyl ester can also be directly produced by lactic acid bacteria (Liu & Siezen, 2006) or yeasts (Fredlund, Druvefors, Olstorpe, Passoth, & Schnürer, 2004; Nordström, 1966) and not only by the esterification of ethanol with a fermentation acid. They stated that it seems very likely that both chemical and biochemical processes are involved in forming esters. Delayed sealing increased both ethyl ester concentrations (ethyl acetate and ethyl lactate) at silo opening. This could have been caused by yeasts, which could multiply during delayed sealing and either directly form ethyl esters or supply ethanol for esterification. According to Weiss et al. (2009), low storage temperatures (as in the present study) can promote high ethyl ester concentrations. This could have contributed to the high concentrations of ethyl acetate and ethyl lactate at silo opening.

Changes in chemical composition, fermentation pattern and VOC during aerobic exposure

Silage DM was increased by aerobic exposure due to evaporation of moisture and VOC because the silages were not covered. During aerobic exposure, decreases were observed in the concentrations of WSC, and lactic and acetic acid. This led to corresponding increases in the concentrations of crude ash, CP, CF, aNDFom and to a lesser extent of ADFom (Honig & Woolford, 1980). The starch content remained unchanged when silages were exposed to air, which was consistent with the results of Tabacco, Righi, Quarantelli, and Borreani (2011). The CL decreased after day 4 and can be explained by oxidation processes in the fatty acids, as described by Khan, Cone, and Hendriks (2009), which caused a loss of ME. The NPN fraction decreased after day 2 of exposure, which was inconsistent with the results of Gerlach, Liao, and Südekum (2014), who observed no changes in NPN when lucerne

silages were exposed to air for 8 days. These silages, however, remained aerobically stable for 8 days. In the maize silages of the current study, shifts in the CP fractions may have occurred, driven by thermal processes, resulting in increases in neutral detergent insoluble nitrogen and acid detergent insoluble nitrogen fractions at the expense of the NPN fraction during aerobic exposure.

The degradation of the WSC and the fermentation acids can be attributed to an intense metabolic activity of yeasts in the early stage and moulds in the later stage (Wilkinson & Davies, 2013). The loss of the fermentation acids was accompanied by an increase in pH until day 4, which indicated spoilage. The NH_3-N content decreased after silo opening, possibly as a result of losses through volatilization (Honig & Woolford, 1980).

The decline in the levels of ethyl lactate and ethyl acetate after day 2, and of ethanol after day 4, was explained by their volatilities (Weiss et al., 2011) and is partly in accordance with the findings of Gerlach et al. (2013). However, the decline was promoted by mixing the silages at 2-day intervals, a lack of coverage and because the silages were stored loosely and not compacted in the feedstock during feed-out, as occurs in practice.

Treatment effects on DM loss, aerobic stability, microbial composition and sensory properties

Sealing the silos at 4 days after filling elevated the DM losses that occurred during conservation. Muck (1988) noted that DM loss can be caused by WSC respiration when a silo is filled slowly or imperfectly sealed. Henderson and McDonald (1975) reported three times higher DM losses in silages that were sealed after 3 days than those obtained from silos that were sealed immediately. Ruxton et al. (1975) stated that in highly compacted silages, the depth of penetration of air is somewhat limited and only prolonged exposure to air can cause high DM losses.

A 4-day delay in sealing the silos decreased their aerobic stability. Uriarte-Archundia, Bolsen, and Brent (2002) reported reduced aerobic stability when maize forage sealing was delayed by 2 day in comparison to immediate sealing. In contrast, Bolsen, Hinds, Ilg, and Hoover (1985) found no influence on aerobic stability when maize forage was filled 12 hr post-harvest. It might have been that delayed sealing,

i.e., a prolonged exposure to air in the pre-seal phase, allowed development of a greater critical yeast population. The developed yeasts partly survived the ensiling process during the resting stage. Re-exposing to air after opening would then have caused rapid yeast proliferation (Pahlow et al., 2003). This assumption was supported by the finding that on day 2 of exposure, silages sealed with delay had higher yeast counts than immediately sealed silages. This could explain the rapid rise in temperature, the lower aerobic stability and the rapid decline in sensory quality. Silage temperatures were higher in silages that were sealed following a delay (>30°C). This could be due to a higher yeast activity and possibly cause heat-damaged protein (Ruppel, Pitt, Chase, & Galton, 1995). Our method to determine aerobic stability was dissimilar to that described by Honig (1990), in that we used neither polystyrene boxes nor a covering. Additionally, the silages in our study were mixed every 2 day. Therefore, silage moisture was reduced (lost as a heat sink), further air was added and the silages warmed presumably faster than they would have done with a covering.

The longer the duration of aerobic exposure after opening, the more pronounced was the microbial development. Yeast counts responded faster to aerobic exposure than the moulds and bacteria numbers, in concurrence with Dolci, Tabacco, Cocolin, and Borreani (2011) and highlight the role of yeasts as initiators of aerobic deterioration.

CONCLUSIONS

The study shows that even in the instance of a silo with a small silo surface-to-volume ratio, detrimental effects of low compaction and delayed sealing on maize silage quality can occur before sealing and at silo opening. Furthermore, low compaction and delayed sealing can adversely impact on silage quality during aerobic exposure after opening (feed-out). Under the conditions of this study, the overall effects of sealing had a greater impact on silage quality than that of compaction. The longer the silo remains unsealed, the greater the silage quality deterioration. Hence, all measures should be taken to compact the silo as highly as possible and to seal as soon as feasible after filling, to minimize losses in feed value. Aerobic exposure after opening should be limited as much as possible to prevent steady deterioration of the silage.

ACKNOWLEGDMENTS

We sincerely acknowledge the expert technical support by the staff of the Corporate Analytical Laboratory, Humboldt-University of Berlin and the technical support by the staff of the Animal Nutrition Laboratory of the University of Bonn. The authors thank B. Kroschewski for statistical advice provided at the Humboldt University of Berlin.

REFERENCES

Bolsen, K. K., Dickerson, J. T., Brent, B. E., Sonon, R. N., Dalke, B. S., Lin, C., & Boyer, J. E. (1993). Rate and extent of top spoilage losses in horizontal silos. *Journal of Dairy Science*, *76*, 2940−2962.

Bolsen, K., Hinds, M., Ilg, H., & Hoover, J. (1985). Effects of delayed filling and H/M inoculant on preservation and quality of corn silage. Cattlemen´s Day Conference, 1985. Kansas State University, Manhattan, KS: Agricultural Experimental Station and Cooperative Extension Service, *1985*, pp. 66–70.

Brandt, M., Schuldt, A., Mannerkorpi, P., & Vearasilp, T. (1987). Zur enzymatischen Stärkebestimmung im Darminhalt und Kot von Kühen mit hitzestabiler Amylase. *Archives of Animal Nutrition*, *37*, 455. (Abstract, in German).

DLG. (2004). Grobfutterbewertung. Teil A - DLG-Schlüssel zur Bewertung von Grünfutter, Silage und Heu mit Hilfe der Sinnenprüfung. *DLG-Information 1/2004*. Deutsche Landwirtschafts-Gesellschaft, Frankfurt/Main, Germany. Available at: http://2015.dlg.org/fileadmin/downloads/fachinfos/futtermittel/grobfutterbewertung.pdf (in German) (accessed 18 May 2013).

Dolci, P., Tabacco, E., Cocolin, L., & Borreani, G. (2011). Microbial dynamics during aerobic exposure of corn silage stored under oxygen barrier polyethylene films. *Applied and Environmental Microbiology*, *77,* 7499–7507.

Dunière, L., Sindou, J., Chaucheyras-Durand, F., Chevallier, I., & Thévenot-Sergentet, D. (2013). Silage processing and strategies to prevent persistence of undesirable microorganisms. *Animal Feed Science and Technology*, *182*, 1–15.

Fairbairn, R. L., Alli, I., & Phillip, L. E. (1992). Proteolysis and amino acid degradation during ensilage of untreated or formic acid-treated lucerne and maize. *Grass and Forage Science*, *47*, 382–390.

Fredlund, E., Druvefors, U. Ä., Olstorpe, M. N., Passoth, V., & Schnürer J. (2004). Influence of ethyl acetate production and ploidy on the anti-mould activity of *Pichia anomala*. *FEMS Microbiology Letters*, *238*, 133–137.

Gallo, A., Giuberti, G., Bruschi, S., Fortunati, P., & Masoero, F. (2016). Use of principal factor analysis to generate a corn silage fermentative quality index to rank well- or poorly-preserved forages. *Journal of the Science of Food and Agriculture*, *96*, 1686–1696.

Gerlach, K., Liao, Y., & Südekum, K.-H. (2014). Aerobic exposure of lucerne silages and its impact on preference and dry matter intake by goats. *Small Ruminant Research*, *121*, 308–313.

Gerlach, K., Ross, F. H., Weiss, K., Büscher, W., & Südekum, K.-H. (2013). Changes in maize silage fermentation products during aerobic deterioration and its impact on feed intake by goats. *Agricultural and Food Science*, *22*, 168–181.

Hafner, S. D., Howard, C., Muck, R. E., Franco, R. B., Montes, F., Green, P. G., Mitloehner, F., Trabue, S. L., & Rotz, C. A. (2013). Emission of volatile organic compounds from silage: Compounds, sources, and implications. *Atmospheric Environment*, *77*, 827–839.

Henderson, A. R., & McDonald, P. (1975). The effect of delayed sealing on fermentation and losses during ensiling. *Journal of the Science of Food and Agriculture*, *26*, 653–667.

Hoedtke, S., Gabel, M., & Zeyner, A. (2010). Der Proteinabbau im Futter während der Silierung und Veränderungen in der Zusammensetzung der Rohproteinfraktion. *Übersichten zur Tierernährung*, *38*, 157–179. (in German).

Honig, H. (1990). Evaluation of aerobic stability. In *Grass and forage reports. Proceedings of the Eurobac conference*, *Uppsala*, *Schweden*, *1986*, *special issue 3,* pp. 76–82.

Honig, H. (1991). Reducing losses during storage and unloading of silage. In G. Pahlow, & H. Honig (Eds.), *Forage Conservation Towards 2000, Proceedings European Grassland Federation, Landbauforschung Völkenrode Sonderheft 123* (pp. 116–128). Braunschweig: Bundesforschungsanstalt für Landwirtschaft Braunschweig-Völkenrode.

Honig, H., & Woolford, M. K. (1980). Changes in silage on exposure to air. In: C. Thomas (Ed.), *Forage conservation in the 80's, BGS occasional symposium no. 11* (pp. 76–87). Reading, UK: British Grassland Society.

Howard, C., Kumar, A., Malkina, I., Mitloehner, F., Green, P. G., Flocchini, R. G., & Kleeman, M. (2010). Reactive organic gas emissions from livestock feed contribute significantly to ozone production in central California. *Environmental Science and Technology*, *44*, 2309–2314.

Kaiser, A. G., & Piltz, J. W. (2009). Chapter 12. Feed testing: Assessing silage quality. In A. G. Kaiser, J. W. Piltz, H. M. Burns & N. W. Griffiths (Eds.), *Top fodder successful silage*. Dairy Australia and New South Wales Department of Primary Industries. Available at: http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0005/294053/successful-silage-topfodder-complete.pdf (accessed 10 August 2016).

Khan, N. A., Cone, J. W., & Hendriks, W. H. (2009). Stability of fatty acids in grass and maize silages after exposure to air during the feed out period. *Animal Feed Science and Technology*, *154*, 183–192.

Kim, S. C., & Adesogan, A. T. (2006). Influence of ensiling temperature, simulated rainfall, and delayed sealing on fermentation characteristics and aerobic stability of corn silage. *Journal of Dairy Science*, *89*, 3122-3132.

von Lengerken, J., & Zimmermann, K. (1991). *Handbuch Futtermittelprüfung*. Berlin, Germany: Deutscher Landwirtschaftsverlag. (in German).

Licitra, G., Hernandez, T. M., & van Soest P. J. (1996). Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology*, *57*, 347–358.

Littell, R. C., Milliken, G. A., Stroup, W. W., Wolfinger, R. D., & Schabenberger O. (2006). *SAS for mixed models,* 2nd ed. Cary, NC, USA: SAS Institute Incorporation.

Liu, M., & Siezen, R. (2006). Comparative genomics of flavour-forming pathways in lactic acid bacteria. *Australian Journal of Dairy Technology*, *61*, 61–68.

McDonald, P., Henderson, A. R., & Heron, S. J. E. (1991). *The biochemistry of silage*, 2nd ed. Marlow, UK: Chalcombe Publications.

McGuffey, R. K., & Owens, M. J. (1979). Effect of covering and dry matter at ensiling on preservation of alfalfa in bunker silos. *Journal of Animal Science*, *49*, 298–305.

Menke, K. H., & Steingass, H. (1987). Schätzung des energetischen Futterwerts aus der in vitro mit Pansensaft bestimmten Gasbildung und der chemischen Analyse. *II. Regressionsgleichungen. Übersichten zur Tierernährung*, *15*, 59−94. (in German).

Mills, J. A., & Kung, L. Jr. (2002). The effect of delayed ensiling and application of a propionic acid-based additive on the fermentation of barley silage. *Journal of Dairy Science*, *85*, 1969–1975.

Muck, R. E. (1988). Factors influencing silage quality and their implications for management. *Journal of Dairy Science*, *71*, 2992–3002.

Muck, R. E., & Holmes, B. J. (2000). Factors affecting bunker silo densities. *Applied Engineering in Agriculture*, *16*, 613–619.

Nordström, K. (1966). Formation of esters from lower chain fatty acids by various yeast species. *Journal of the Institute of Brewing*, *72*, 38–40.

Ohshima, M., & McDonald, P. (1978). A review of the changes in nitrogenous compounds of herbage during ensilage. *Journal of the Science of Food and Agriculture*, *29*, 497–505.

Pahlow, G., Muck, R. E., Driehuis, F., Oude Elferink, S. J. W. H., & Spoelstra, S. F. (2003). Microbiology of ensiling. In D. R. Buxton, R. E. Muck, & J. H. Harrison (Eds.), *Silage science and technology (*pp. 31–93*).* Madison, USA: American

Society of Agronomy, Crop Science Society of America, Soil Science Society of America.

Ranjit, N. K., & Kung, L. Jr. (2000). The effect of *Lactobacillus buchneri, Lactobacillus plantarum*, or a chemical preservative on the fermentation and aerobic stability of corn silage. *Journal of Dairy Science*, *83*, 526−535.

Ruppel, K. A., Pitt, R. E., Chase, L. E., & Galton, D. M. (1995). Bunker silo management and its relationship to forage preservation on dairy farms. *Journal of Dairy Science*, *78*, 141–153.

Ruxton, I. B., Clark, B. J., & McDonald, P. (1975). A review of the effects of oxygen on ensilage. *Journal of the British Grassland Society*, *30*, 23–30.

Ruxton, I. B., & McDonald, P. (1974). The influence of oxygen on ensilage. I. Laboratory studies. *Journal of the Science of Food and Agriculture*, *25*, 107–115.

Schabenberger, O., & Pierce, F. J. (2002). *Contemporary statistical models for the plant and soil sciences*. Boca Raton, Florida, USA: CRC Press.

Tabacco, E., Righi, F., Quarantelli, A., & Borreani, G. (2011). Dry matter and nutritional losses during aerobic deterioration of corn and sorghum silages as influenced by different lactic acid bacteria inocula. *Journal of Dairy Science*, *94*, 1409−1419.

Uriarte-Archundia, M. E., Bolsen, K. K., & Brent, B. E. (2002). A study of the chemical and microbial changes in whole-plant corn silage during exposure to air: Effects of a biological additive and sealing technique. In *Proceedings of the 13th International Silage Conference, Auchincruive, Scotland, 2002* (pp. 172–173).

VDLUFA. (2012). Methodenbuch, Vol. III. In *Die chemische Untersuchung von Futtermitteln*. Darmstadt, Germany: VDLUFA-Verlag (in German).

Vogel, R. (1992). Silierbedingungen für Silomais und Wirksamkeit der Impfung mit Milchsäurebakterien. *Landwirtschaft Schweiz*, *5*, 161–168. (in German).

Weiss, K. (2001). Gärungsverlauf und Gärqualität von Silagen aus nitratarmen Grünfutter. Doctoral thesis. Humboldt-Universität zu Berlin, Germany (in German).

Weiss, K., & Auerbach, H. (2012). Occurrence of volatile organic compounds and ethanol in different types of silages. In *Proceedings of the 16th International Silage Conference, Hämeenlinna, Finland, 2012* (pp. 128–129).

Weiss, K., & Auerbach, H. (2013). Volatile organic compounds (VOC) in grass silages. In *Proceedings of the 22nd International Grassland Congress, Sydney, Australia, 2013* (pp. 721–722).

Weiss, K., Gerlach, K., & Südekum, K.-H. (2011). Flüchtige Substanzen in Maissilagen in Abhängigkeit von Silierbedingungen und aerober Lagerungsdauer. *VDLUFA-Schriftenreihe, 67*, 534−540. (in German).

Weiss, K., & Kaiser, E. (1995). Milchsäurebestimmung in Silageextrakten mit Hilfe der HPLC. *Wirtschaftseigene Futter*, *41*, 69–80. (in German).

Weiss, K., Kalzendorf, C., Zittlau, J., & Auerbach, H. (2009). Novel results on the occurrence of volatile organic compounds in maize silage. In *Proceedings of the 15th International Silage Conference, Madison, USA, 2009* (pp. 33–34).

Weiss, K., Kroschewski, B., & Auerbach, H. (2016). Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage. *Journal of Dairy Science*, *99*, 8053–8069.

Weiss, K., Olbrich, C., & Thaysen, J. (2015). Volatile organic compounds (VOC) in maize silages at German dairy farms. In *Proceedings of the 17th International Silage Conference, Piracicaba, São Paulo, Brazil, 2015* (pp. 98–99).

Weiss, K., & Sommer, G. (2012). Bestimmung von Estern und anderen flüchtigen organischen Substanzen (VOC) in Silageextrakten mit Hilfe der Gaschromatographie. *VDLUFA-Schriftenreihe, 68*, 561–569. (in German).

Weissbach, F. (2005). A simple method for the correction of fermentation losses measured in laboratory silos. In *Proceedings of the 14th International Silage Conference, Belfast, Northern Ireland, 2005* (p. 278). Wageningen: Wageningen Academic Publishers.

Weissbach, F., & Strubelt, C. (2008). Correcting the dry matter content of maize silages as a substrate for biogas production. *Landtechnik*, *63*, 82–83.

Wilkinson, J. M., & Davies, D. R. (2013). The aerobic stability of silage: Key findings and recent developments. *Grass and Forage Science*, *68*, 1–19.

Woolford, M. K. (1984). The silage fermentation (pp. 145–148). New York, USA: Marcel Dekker, Inc..

Woolford, M. K. (1990). A review: The detrimental effects of air on silage. *Journal of Applied Bacteriology*, *68*, 101–116.

Wyss, U. (2005). Beurteilung von Silagen. Merkblatt für die Praxis. *ALP aktuell, 18*, 1–4. (in German).

CHAPTER 4

EFFECT OF COMPACTION, DELAYED SEALING AND AEROBIC EXPOSURE ON FORAGE CHOICE AND SHORT-TERM INTAKE OF MAIZE SILAGE BY GOATS

D. Brüning[a], K. Gerlach[a], K. Weiß[b], and K.-H. Südekum[a]

[a]Institute of Animal Science, University of Bonn, 53115 Bonn, Germany

[b]Albrecht Daniel Thaer-Institute of Agricultural and Horticultural Sciences, Humboldt-University, 10115 Berlin, Germany

Published in *Grass and Forage Science*, *73*, 2018, 392−405.

Correspondence: K. Gerlach, Institute of Animal Science, University of Bonn, Bonn, Germany. Email: kger@itw.uni-bonn.de

ABSTRACT

Forage choice and intake by ruminants depend on various factors. This study aimed to determine the effects of compaction, delayed sealing and aerobic exposure on forage choice and short-term dry-matter intake (DMI) of maize silage by goats. Whole-crop maize (277 g/kg dry matter [DM]) in 120-L silos was compacted at either low (194 kg DM/m^{-3}) or high (234 kg DM/m^{-3}) density, and sealed immediately at day 0 or with a delay at day 2 or day 4 post-filling, making a total of six treatments. After ensiling for at least 175 days, silages were exposed to air for 6 days. In 2-day intervals, silages were sampled for chemical analyses and were vacuum-stored for use in preference trials. During experimental phase, each possible 2-way combination of the aerobically exposed silages (days 0, 2, 4 and day 6 post-opening) of the treatments and lucerne hay was offered as free choice to goats (n = 5) for 3 hr. Exposing silages to air for >4 days post-opening caused strong avoidance and lowest intakes. Under the conditions of the study, aerobic exposure after ensiling had a more pronounced effect on silage preference and short-time DMI than compaction and delayed sealing. Increasing fibre fractions, a deteriorating microbial status and poor silage sensory properties, probably caused by a combination of different fermentation products can be considered for decrease in preference.

Keywords: aerobic exposure, delayed sealing, diet preference, volatile organic compounds

INTRODUCTION

Physical and management factors, such as compaction and sealing, greatly influence the outcome of forage conservation. The main goal of packing maize silage densely is to generate an anaerobic environment in the silo. In this way, the amount of air that penetrates the silo is minimized and undesirable aerobic micro-organisms that lead to deterioration and quality losses during storage and feed-out are inhibited (Johnson et al., 2002). In a study of Sucu, Kalkan, Canbolat, and Filya (2016), increased packing density resulted in silages with lower acetate, ammonia-N and fermentation losses. Tightly packed silages remained stable upon exposure to air, showed increased nutrient digestibility and had higher energy contents than loosely packed silages. Although the effects of compaction on silage quality are widely known, there

is only a few published data on the impact on feed intake and preference by ruminants.

After chopping, fresh forage is very susceptible to aerobic deterioration, if not filled in silo, packed and sealed immediately. Poor management decisions, such as leaving the chopped and filled forage unsealed, can result in prolonged air exposure up to several days, which affects both the ensiling process and the quality of silage during storage and feeding (Brüning, Gerlach, Weiss, & Südekum, in press; Mills & Kung, 2002). Uriarte-Archundia, Bolsen, and Brent (2002) showed that maize silage sealed with a 48-hr delay was 2 days less stable on air after opening than immediately sealed silage. Coblentz, Coffey, and Chow (2016) reported declining lactic acid concentration in lucerne silage bales wrapped with a delay. To our best knowledge, there are no studies on the effect of delayed sealing on forage choice and intake. Therefore, more knowledge is needed, also with regard to possible interactions with compaction.

When silage is exposed to air during feed-out, aerobic deterioration may occur as a result of undesirable microbial activity, particularly through yeasts (Wilkinson & Davies, 2013). Yeast activity can cause degradation of residual water-soluble carbohydrates (WSC) and lactic acid and can lead to heating of silage. Maize silage with high contents of residual WSC and lactic acid is very susceptible to aerobic deterioration. Silage that had undergone aerobic deterioration is undesirable for feeding due to lower nutritive value and to risk of negative effects on animal performance and health (Driehuis & Oude Elferink, 2000). Recently, Gerlach, Ross, Weiss, Büscher, and Südekum (2013) observed a decrease in preference and dry-matter intake (DMI) when deteriorated maize silage was offered to goats in choice situations with fresh silage. The decrease was accompanied by changed silage composition and correlated with increased silage temperature. Silage DMI showed weak negative relationships to ethanol and ethyl lactate, some of the volatile organic compounds (VOC). German and Danish farmers and local extension services have repeatedly reported on odd-smelling (adhesive- or acetone-like odours) maize silages associated with decreased feed intake and performance by dairy cows (Weiss, Kroschewski, & Auerbach, 2016). Interestingly, those silages were highly compacted, well fermented (low pH, moderate acetic acid concentrations, no butyric acid), stable upon exposure to air, but had also high

contents of ethanol and odorous ethyl esters (Weiss, Gerlach, & Südekum, 2011; Weiss, Kalzendorf, Zittlau, & Auerbach, 2009). This suggests that these VOC are formed under optimal conditions and that they may negatively affect feed intake.

Recently, Brüning et al. (in press) determined the effects of compaction, delayed sealing and aerobic exposure on maize silage quality and on formation of VOC. The three factors were selected to promote or prevent ingress of air (oxygen) into the silo to produce different silage qualities. One hypothesis was that compaction and delayed sealing may influence the formation of ethyl esters by inducing ethanol production. Delayed sealing caused an increase in yeast counts and a strong decline in WSC before ensiling. The delay promoted the formation of ethyl lactate and ethyl acetate. Exposing silages to air for 6 days after silo opening led to degradation of residual WSC and lactic acid, microbial growth, heating and worsened sensory properties.

The present study aimed to offer the silages prepared in the first study in preference trials to goats to determine the effect of compaction, delayed sealing and aerobic exposure on forage choice and short-term DMI. It was hypothesized that low compaction, delayed sealing and aerobic exposure reduce silage preference and DMI. It should also be determined which silage characteristics have a strong relationship to silage short-time DMI.

MATERIALS AND METHODS

Silage preparation and treatments

Silage preparation was previously described in detail by Brüning et al. (in press). Maize (*Zea mays*, var. Canon) (Maisadour Semences, France) was grown at the Frankenforst experimental station, Faculty of Agriculture, University of Bonn, Königswinter, Germany. Whole-crop maize was harvested at half-milk line stage on 2 September 2013 and chopped to a 6-8 mm theoretical cut length with a 1-row forage harvester (Mex GT; Pöttinger Maschinenfabrik, Grieskirchen, Austria). The chopped maize was thoroughly mixed with a wheel loader and shovels, sampled (about 1,000 g) from different points of the forage pile and filled into 120-L plastic barrels. Maize in the barrels (hereafter referred to as silo) was either compacted to low (fresh weight 537 ± 11.1 kg/m^3; 194 ± 4 kg dry matter (DM)/m^3) or high

(fresh weight 648 ± 8.31 kg/m^3; 234 ± 3 kg DM/m^3) densities and sealed either at day 0 post-filling or with a delay at day 2 or at day 4 post-filling. The six treatments (six silos per treatment) evaluated were as follows: L0—low compaction, sealed on day 0 post-filling; L2—low compaction, sealed on day 2 post-filling; L4—low compaction, sealed on day 4 post-filling; H0—high compaction, sealed on day 0 post-filling; H2—high compaction, sealed on day 2 post-filling; H4—high compaction, sealed on day 4 post-filling. The target density was based on the recommended minimum DM density for maize silage of 225 kg/m^3 (Muck & Holmes, 2000).

All silos were sealed with plastic lids and stored in a barn at ambient temperature (12 ± 2.9°C) for 175 (L0/H0), 217 (L2/H2) and 259 (L4/H4) days. The storage times were different due to experimental reasons. After opening the silos, the silages were taken out of the silos, mixed thoroughly and exposed to air at room temperature (20 ± 1.5°C) for 6 days (in accordance with an extended feed-out phase). All silages were stored separately on a concrete floor as a quadratic heap (1 x 1 m) with constant layer height (12 cm) and without any covering.

Sampling of silages for feeding and for chemical analyses

At silo opening (day 0) and at three times during the period of aerobic exposure (days 2, 4 and day 6 post-opening), each single silage heap was homogenized. Then, a composite sample per treatment and day of aerobic exposure (about 100 g) was taken for microbial analysis and kept refrigerated until analysis. All silages were evaluated for their sensory properties (odour, texture, colour and visible moulds) at each day of aerobic exposure using a point-based scheme (DLG, 2004). For each preference trial, about 18 kg of fresh silage were taken from each of the six mixed silage heaps in 2-day intervals (days 0, 2, 4 and day 6 post-opening) and were each time collected on a pile. From each pile, 35 portions of 3 kg were taken and filled in single polyethylene bags (170 µm, 400 mm x 600 mm, Service-Verpackungen Frey, Dombühl, Germany) and were vacuum-sealed to preserve the actual stage of silage. This resulted in a total of 140 silage bags for each treatment (35 bags x 4 sampling days). After the silage for feeding had been taken from the heaps, the heaps were restored in quadratic form. The vacuum-sealed silage bags were stored in a dark, dry and cool (8°C) room until being used in preference trials as meals. Storage time ranged from 2 to 22 days depending on day when fed. During the experimental

phase, four of each 35 bags were chosen randomly, opened and sampled. The contents of the four bags were blended and then thoroughly mixed. Subsequently, a sample of approximately 1,000 g was taken and frozen immediately (–18°C) until analysis.

Hays

Baled grass and lucerne (*Medicago sativa* L.) hays were purchased from regional producers. Before feeding, bales were controlled for usability and homogeneity. Each bale was sampled on each day of the experimental phase by hand-plucking portions from multiple locations until approximately 300 g were collected. A composite sample of all bales of each hay was used for chemical analyses. Hay samples were stored dry and dark prior to milling.

Preference trials

For each of the six treatments, one preference trial (n = 6) was carried out in a barn at the Institute of Animal Science, University of Bonn. Each trial started 2 days after completion of the 6-day aerobic exposure phase. A total of ten Saanen-type wethers (German Improved White Goat breed, mean (± *SD*) body weight (BW) = 89.9 ± 0.1 kg) were used, which were divided into two groups (five goats per group/treatment). Goats were allocated to both groups such that average BW was equal between groups. Each group was assigned to a treatment. Two treatments were tested simultaneously in the same barn. The first two preference trials with the treatments H0 and L0 were conducted in March 2014, the next two trials (H2/L2) in April 2014 and the last (H4/L4) in May 2014. Due to experimental reasons, the six trials could not be conducted concurrently (high number of silage bags, limited refrigeration room space). All preference trials were carried out with the same ten goats. Two goats shared a pen of 2 × 3 m bedded with straw. The mean barn temperature across trials was 19.8 ± 1.7°C. Goats were examined regularly for internal parasites before beginning the trials. To measure individual feed intake, goats were tied up for duration of feeding with possibility of lying down and with access to water and salt licks (Höveler Spezialfutterwerke, Dormagen, Germany). Each kilogram contained 14.0% Ca, 5.0% P, 12.5% Na, 4.0% Mg, 6400 mg of Zn,

1300 mg of Mn, 100 mg of I, 24 mg of Se). Animal care and handling was done according to the official German regulations.

The preference trials were carried out as described by Buntinx, Pond, Fisher, and Burns (1997), Burns, Fisher, and Mayland (2001) and previously conducted on site by Gerlach et al. (2013) and consisted of two phases. During the first phase, a training phase (Kyriazakis, Emmans, & Whittemore, 1990), each of the five goats was only offered a single forage for 3 hr per day at 07:15, either one of the aerobically exposed silages (day 0, 2, 4 or day 6 post-opening) of the treatment or lucerne hay. This allowed each animal to experience and to associate the offered forages with post-ingestive metabolic response, taste and smell produced by the forages. Thus, each training phase lasted 5 days. The order in which the forages were offered to each goat was randomized. The lucerne hay (LUC) was used as standard forage and was fed without further processing. Its concentrations of crude ash, crude protein (CP), acid detergent fibre (ADFom), neutral detergent fibre (aNDFom) and acid detergent lignin (ADL) were 101, 160, 322, 423 and 74 g/kg DM respectively. For the remainder of the day during the adaptation period, each goat had free access to grass hay. During the experimental phase, the aerobically exposed silages (day 0, 2, 4 or day 6 post-opening) and the LUC were offered to each goat in combination of two, making a total of 10 combinations. The combinations were randomly assigned to each goat and provided to ensure free access to both forages. The left-right position of the forages in the pair was also randomized. Each pair combination was offered as one meal for one day over a period of 3 hr (total duration of the experimental phase = 10 days). Each forage was offered in a plastic box (400 × 340 × 250 mm) and the pair combination was presented side by side in front of each goat. Feeder positions were reversed daily. Weights of the forages were determined before offering as well as 30 min and 3 hr after beginning of the preference trials to calculate initial (g 30 min^{-1}) and 3-hr DMI (g 3 hr^{-1}). For calculation of intakes, the DM of the silages from vacuum-sealed bags was used. In this way, daily intakes for each combination and each goat were obtained. On each day of the preference trials, feeders were weighed at approximately 06:30 and the trials started at 07:15. The silages were allowed 30 min to warm up on ambient temperature before offering. At 14:30, grass hay was fed for 2 hr to each goat. Its concentrations of crude ash, CP, ADFom, aNDFom and ADL

were 94, 143, 295, 577 and 34 g/kg DM respectively. The grass hay was fed without further processing. During experimental phase, weight of the offered grass hay was determined before and after feeding to calculate total DMI (g 2 hr^{-1}).

Laboratory Analyses

Sample preparation

The frozen sample of the harvested maize and frozen silage samples from vacuum-sealed bags were each freeze-dried in triplicate (Gamma 1-16 LSC; Martin Christ, Osterode am Harz, Germany) and were then ground prior to analyses (1-mm sieve, except samples for starch analysis at 0.2 mm) with a mill (SM 100; Retsch, Haan, Germany). The dried hays were also ground prior to analyses (1-mm sieve) with the same mill.

General analyses

The DM was determined in duplicate after sampling by oven drying 100 g of the fresh material overnight (60°C), then at 105°C for 3 hr (method 3.1; VDLUFA, 2012). The DM was corrected for the loss of volatiles during drying according to Weissbach and Strubelt (2008). The oven-dried sample was subsequently discarded and was not used for further analysis. The following proximate analyses were carried out in duplicate on freeze-dried samples according to VDLUFA (2012): dry matter (method 3.1), crude ash (method 8.1), crude fibre (CF, method 6.1.2, using an ANKOM^{2000} Fiber Analyzer; Ankom Technology, Macedon, NY, USA), crude fat (CL, method 5.1.1, using a Soxtec 2055; Foss Analytical Systems, Hillerød, Denmark), neutral detergent fibre (aNDFom, method 6.5.1, assayed with heat-stable amylase, Termamyl 120 L; Univar, Essen, Germany), acid detergent fibre (ADFom, method 6.5.2, using a Fibertec 1020; Foss Analytical Systems) and acid detergent lignin (ADL, method 6.5.3). The aNDFom and ADFom values were expressed exclusive of residual ash. Crude protein (CP = N x 6.25) was determined using an automatic nitrogen analyser (vario MAX; Elementar Analysensysteme, Hanau, Germany). Non-protein nitrogen (NPN) was analysed as described by Licitra, Hernandez, and van Soest (1996) using the nitrogen analyser (vario MAX). Starch was quantified after

enzymatic hydrolysis of starch to glucose as described by Brandt, Schuldt, Mannerkorpi, and Vearasilp (1987).

The Hohenheim gas test (method 25.1; VDLUFA, 2012) was conducted to measure the 24-hr in vitro gas production (GP, [ml 200 mg^{-1} DM]) and estimate the metabolizable energy (ME) content according to Menke and Steingass (1987).

Chemical analyses of fermentation pattern

Silage subsamples (50 g) were used to analyse the fermentation pattern which is described in detail in Brüning et al. (in press). Shortly, following procedures and measurements were conducted: cold-water extracts were prepared by blending the frozen samples with 200 ml distilled water and 1 ml toluene and refrigerated overnight (4°C) afterwards filtered (Minisart RC, 0.45 µm pore size, Sartorius, Göttingen, Germany). The pH (potentiometrically), WSC and the following fermentation variables were determined in duplicate: lactic acid, pH, volatile fatty acids, alcohols (methanol, ethanol, n-propanol, 1,2-propanediol, 2-butanol and 2,3-butanediol), acetone, ammonia-N (NH_3-N), ethyl lactate, ethyl acetate and propyl acetate. Lactic acid was analysed by high-performance liquid chromatography (HPLC) according to Weiss and Kaiser (1995) and the volatile fatty acids, alcohols and ethyl esters were determined by gas chromatography (GC) with flame ionization detection as described by Weiss (2001) and Weiss and Sommer (2012). The NH_3-N and WSC (von Lengerken & Zimmermann, 1991) were analysed colourimetrically using a continuous flow analyser (CFA, San^{++}, Skalar Analytical, Breda, Netherlands).

Microbial analyses

Cooled samples were sent directly to a laboratory (Wessling Laboratorien, Altenberge, Germany) for microbial analyses. Yeast, mould and aerobic mesophilic bacteria counts were determined according to VDLUFA (2012, methods 28.1.1-28.1.4).

Statistical analyses

All statistical analyses were performed with SAS 9.4 (SAS Institute Inc., Cary, NC, USA). To analyse the effect of aerobic exposure after opening on silage composition, a mean value was calculated from individually observed values of the six treatments for each day of aerobic exposure (days 0, 2, 4 and day 6 post-opening). Then, an analysis of variance (ANOVA) was used to conduct pairwise comparisons between the aerobically exposed silages. Initially, residuals were tested for normal distribution by the Shapiro-Wilk test with the SAS UNIVARIATE procedure. For consideration of possible variance heterogeneity, two different approaches were evaluated as described by Brüning et al. (in press). ANOVA was performed with the SAS MIXED procedure using the restricted maximum likelihood algorithm (Schabenberger & Pierce, 2002). When significant effects were detected by the global *F* test, pairwise comparisons were performed using Tukey´s test.

The experimental design allowed statistical analysis by the multidimensional scaling (MDS) according to Buntinx et al. (1997). Application of MDS procedures requires an observation of the amount of perceived difference, or dissimilarity, between each pair of a set of stimuli. The goal of MDS is to estimate the positions in space (usually two- or three-dimensional space), or coordinates, for each of the stimuli (forages) such that the distances between them will correspond closely to their experimentally observed dissimilarities. Success is reflected by how large a proportion of the observed sum of squares in the dissimilarities are accounted for by the estimated distances between stimuli arranged in this dimensional space (Schiffman, Reynolds, & Young, 1981). The MDS was used to generate spatial maps representing the differences expressed as selective forage intake by the goats. For MDS, the difference in preference between two silages (or silage with LUC) was calculated from intake data obtained in each preference trial and the following ratio was generated: δ = (intake of preferred forage - intake of less-preferred forage)/total intake. Thus, delta (δ) values for each pair combination ranged from 0 to 1. In this way, preference was expressed as a difference ratio. If δ = 0 or close to 0, the goats had no preference for one forage over the other. If δ = 1 or close to 1, the goats had a strong preference, and the silages or the LUC were judged to be very different. According to Buntinx et al. (1997), the MDS procedure searches iteratively for a solution that minimizes residual sum of squares by arranging the forages by

estimating coordinates in a limited number of orthogonal dimensions to model the observed differences. Forages with coordinates that are similar in the dimensional space are modelled as similar in preference and, conversely, coordinates far apart in the dimensional space indicate forages that differ in preference. For each goat in each preference trial, the δ values were arranged in a matrix representing all possible pairwise combinations of the treatments. This results in a triangular matrix of the δ values for each goat. Two sets of δ matrices were obtained, one for the initial (30 min) DMI and one for the 3-hr DMI. The two sets of five matrices for each treatment were analysed only in two dimensions using the SAS MDS procedure. To apply the same procedure as with the SAS ALSCAL procedure described by Buntinx et al. (1997), which is no longer provided by SAS, the following statements were made with the SAS MDS procedure according to the SAS User's Guide (SAS Institute, 2008): fit = squared, formula = 1, coef = diagonal. Individual animal variation was accounted by running the MDS procedure in a combined analysis (multiple matrices) with weighting coefficients for each animal.

Each preference trial was also tested by ANOVA with the Waller-Duncan k-ratio *t* test (Burns et al., 2001) after averaging DMI of each forage (averaged across each pair combination, $n = 4$) by each animal. Within the treatments, means were separated using the minimal significant difference (*MSD*). The ANOVA only included terms for animal and forage. Within each day of aerobic exposure, means were analysed for the effect of compaction and sealing. The DMI of the pair combinations and of the grass hay were also tested for each treatment with the Waller-Duncan k-ratio *t* test.

Simple linear regression technique was used to examine the relationship of initial (30 min) and 3-hr DMI to silage composition from vacuum-sealed bags using the SAS procedure REG. Stepwise multiple regression analysis was used to associate the silage composition from vacuum-sealed bags ($p \leq .15$ for entry) with DMI within the treatments and for all silages.

RESULTS

Characterisation of silages at feeding

Table 8 gives an overview of the main chemical and fermentation variables of the harvested maize and of the silages from vacuum-sealed bags at feeding. The

harvested maize had a DM content of 277 g/kg and the WSC content was 160 g/kg DM. At silo opening (day 0 of aerobic exposure), the values of chemical variables ranged within the maize silage values recommended by Kaiser and Piltz (2009). On average, the ME content was 10.6 MJ/kg DM. At silo opening, all silages had moderate levels of acetic acid, butyric acid concentrations <2 g/kg DM as well as average pH values of 3.7. Ethanol contents ranged from 11.5 to 17.3 g/kg DM. Ethyl lactate and ethyl acetate contents ranged from 209 to 519 mg/kg DM and 371 to 939 mg/kg DM respectively.

The silage composition was altered by duration of aerobic exposure post-opening (Table 9). The concentrations of DM, fibre fractions (crude fibre, aNDFom and ADFom) and the pH increased with time of aerobic exposure (all $p < .01$). In contrast, the WSC, lactic acid, ethanol and both ethyl esters decreased (all $p < .01$) with prolonged aerobic exposure time.

Table 8. Chemical composition and fermentation pattern of the harvested maize and of the silages at feeding (g/kg DM, unless stated otherwise)

Treatment	DM	CP	aNDFom	ADFom	ADL	pH	WSC	LA	AA	BA	ETH	EL	EA	ME
	g/kg											mg/kg DM		MJ/kg DM
Maize at harvest	277	69.2	457	229	29.1	5.71	160	ND	1.7	ND	0.7	ND	ND	10.9
Day 0 of aerobic exposure post-opening														
H0	286	69.6	412	226	23.4	3.66	30.9	66.5	9.5	ND	11.9	377	494	10.7
H2	280	71.0	449	239	26.2	3.78	27.6	60.1	9.9	0.4	17.3	473	901	10.9
H4	277	71.5	462	255	29.1	3.71	25.5	63.9	10.5	ND	14.2	465	939	10.4
L0	267	68.7	413	228	22.4	3.67	36.6	71.8	12.3	ND	15.7	209	371	10.6
L2	277	72.8	450	231	26.2	3.80	27.3	55.2	6.3	1.8	13.4	519	619	10.4
L4	277	71.9	446	253	30.5	3.73	29.1	58.3	9.4	ND	11.5	397	939	10.7
Day 2 of aerobic exposure post-opening														
H0	291	65.8	417	252	32.3	3.69	29.3	68.7	9.6	ND	14.0	357	443	10.6
H2	286	74.3	430	232	23.1	3.82	21.1	61.3	9.2	0.4	16.6	434	729	10.8
H4	280	70.8	419	234	24.7	3.77	16.7	32.4	7.9	ND	13.9	374	588	10.6
L0	286	69.0	424	242	34.3	3.70	37.0	66.8	9.5	ND	12.8	257	515	10.5
L2	281	71.3	435	228	20.6	3.85	18.9	52.0	6.3	ND	17.1	466	646	10.6
L4	278	70.8	470	255	31.0	3.83	17.0	54.2	5.5	ND	15.5	363	539	10.4

(Continues)

Table 8. Chemical composition and fermentation pattern of the harvested maize and of the silages at feeding (g/kg DM, unless stated otherwise) **(continued)**

Treatment	DM	CP	aNDFom	ADFom	ADL	pH	WSC	LA	AA	BA	ETH	EL	EA	ME
	g/kg											mg/kg DM		MJ/kg DM
Day 4 of aerobic exposure post-opening														
H0	294	76.0	445	254	26.2	4.47	14.4	32.2	4.7	0.6	4.6	106	290	10.4
H2	287	73.8	441	233	21.4	4.81	9.1	17.1	8.0	1.2	ND	ND	ND	10.8
H4	286	75.6	489	280	32.1	4.74	8.0	20.7	5.8	1.8	ND	ND	ND	10.6
L0	293	69.5	485	262	30.8	4.70	12.1	21.2	2.7	1.0	1.2	68	106	10.4
L2	283	79.4	445	235	21.5	5.17	8.2	12.2	5.5	0.9	0.3	ND	ND	10.7
L4	289	76.5	505	258	28.1	4.91	7.3	17.5	5.7	1.3	0.3	ND	ND	10.4
Day 6 of aerobic exposure post-opening														
H0	313	70.1	435	244	46.6	5.30	11.9	13.9	5.5	1.3	1.7	ND	45	10.3
H2	305	79.0	512	268	26.0	5.38	8.0	9.5	7.5	0.6	ND	ND	ND	10.5
H4	304	77.4	505	279	32.0	4.79	8.3	19.4	7.6	0.3	ND	ND	ND	10.2
L0	319	76.6	467	291	47.7	5.33	11.0	15.0	4.8	1.2	1.2	ND	76	10.2
L2	313	80.1	499	265	27.3	5.61	5.8	5.2	6.4	0.2	0.2	ND	ND	10.5
L4	305	80.0	541	299	33.7	4.87	9.7	18.4	7.7	0.3	ND	ND	ND	10.0

DM, dry matter; CP, crude protein; aNDFom, neutral detergent fibre assayed with heat-stable amylase and expressed exclusive of residual ash; ADFom, acid detergent fibre expressed exclusive of residual ash; ADL, acid detergent lignin; WSC, water-soluble carbohydrates; LA, lactic acid; AA, acetic acid; BA, butyric acid (sum of i-/n-butyric acid, i-/n-valerian acid and n-capronic acid); ETH, ethanol; EL, ethyl lactate; EA, ethyl acetate; ME, metabolizable energy; H0, high compaction, sealed on day 0 post-filling; H2, high compaction, sealed on day 2 post-filling; H4, high compaction, sealed on day 4 post-filling; L0, low compaction, sealed on day 0 post-filling; L2, low compaction, sealed on day 2 post-filling; L4, low compaction, sealed on day 4 post-filling; ND, not detected (below detection limit); propyl acetate, 1,2-propanediol, 2,3-butanediol and acetone were below detection limit; *n* = 1 per treatment and day (based on at least two analytical replicates).

Table 9. Effect of the 6-day aerobic exposure phase post-opening on silage composition at feeding (g/kg DM, unless stated otherwise)

	Aerobic exposure post-opening					
	Day 0	Day 2	Day 4	Day 6	*SEM*	*p-value*
DM (g/kg)	277[a]	284[ab]	289[b]	309[c]	3.1	<.01
Crude ash	42.0[a]	42.4[a]	45.1[ab]	46.5[b]	0.9	<.01
Crude protein (CP)	70.9[ab]	70.3[a]	75.1b[c]	77.2[c]	1.2	<.01
Crude fibre	227[a]	228[a]	247[ab]	252[b]	5.4	<.01
Crude fat	29.4	30.6	29.1	27.3	1.1	.19
aNDFom	439[a]	432[a]	468[ab]	493[b]	11.1	<.01
ADFom	238[a]	240[a]	254[ab]	274[b]	6.4	<.01
ADL	26.3	27.7	26.7	35.4	2.5	.05
Starch	257	259	262	260	4.6	.87
Non-protein nitrogen (g/kg of CP)	481[b]	487[b]	334[a]	340[a]	16.4	<.01
Ammonia-N (g/kg total N)	78.5[b]	74.7[b]	34.8[a]	31.2[a]	5.3	<.01

(Continues)

Table 9. Effect of the 6-day aerobic exposure phase post-opening on silage composition at feeding (g/kg DM, unless stated otherwise) **(continued)**

	Aerobic exposure post-opening					
	Day 0	Day 2	Day 4	Day 6	*SEM*	*p-value*
24-hr gas production (ml 200 mg^{-1} DM)	57.1	56.9	56.6	55.1	0.5	.05
Metabolizable energy (MJ/kg DM)	10.6^{b}	10.6^{b}	10.6^{ab}	10.3^{a}	0.1	.01
pH	3.73^{a}	3.78^{a}	4.80^{b}	5.21^{c}	0.08	<.01
Water-soluble carbohydrates	29.5^{b}	23.3^{b}	9.8^{a}	9.1^{a}	2.0	<.01
Lactic acid	62.7^{b}	55.9^{b}	20.2^{a}	13.6^{a}	3.5	<.01
Acetic acid	9.6^{b}	8.0^{ab}	5.4^{a}	6.6^{a}	0.7	<.01
Butyric acid	0.4^{a}	0.1^{a}	1.1^{b}	0.7^{ab}	0.2	.01
Ethanol	14.0^{b}	15.0^{b}	1.1^{a}	0.5^{a}	0.7	<.01
2-Butanol (mg/kg DM)	106	102	108	68	59	.96
n-Propanol (mg/kg DM)	29	29	17	16	15	.86
Ethyl acetate (mg/kg DM)	710^{b}	577^{b}	66^{a}	20^{a}	60	<.01
Ethyl lactate (mg/kg DM)	407^{b}	375^{b}	29^{a}	ND^{a}	28	<.01
Yeasts (log_{10} cfu/g FM)	5.16^{a}	7.26^{b}	8.26^{bc}	8.20^{c}	0.2	<.01
Moulds (log_{10} cfu/g FM)	3.13^{a}	2.70^{a}	2.70^{a}	4.84^{b}	0.4	<.01
Aerobic mesophilic bacteria (log_{10} cfu/g FM)	5.84^{a}	5.63^{a}	8.46^{b}	8.79^{b}	0.4	<.01

aNDFom, neutral detergent fibre assayed with heat-stable amylase and expressed exclusive of residual ash; ADFom, acid detergent fibre expressed exclusive of residual ash; ADL, acid detergent lignin; cfu, colony forming units; FM, fresh matter; microbial data were obtained from fresh silages; *SEM*, standard error of the mean; ND, not detected (below detection limit); Means within row with different superscripts (a–c) differ ($p < .05$); $n = 6$.

Animal preference ranked by multidimensional scaling

Figures 2-6 show the graphical solutions of the MDS analysis for preferences determined after 3 hr. The full range in stimulus coordinates by the forages and the dimension weight given by the various goats are presented in Table 10. Generally, according to Burns et al. (2001), a positive rank of a forage in both dimensions, upper right sector in the figures, represents preference whereas a negative rank in both dimensions, lower left-hand sector in the figures, indicates avoidance. For example, H0-day 0 (Figure 2) had two positive coordinates and was therefore strongly preferred, while L0-day 6 (also Figure 2) had two negative coordinates and was avoided. Some forages had one negative and one positive coordinate and were generally of intermediate preference (e.g., H0-day 2, Figure 2).

Treatment effects on preference

When comparing the highly and the low compacted silage counterparts within silages sealed immediately (e.g., L0-day 4 vs. H0-day 4, Figure 2), silages were close together in dimensional space, suggesting a similar preference. Among silages that were sealed with a 2-day or a 4-day delay, some highly and low compacted silages were further apart in dimensional space (e.g., H2-day 2 vs. L2-day 2, Figure 3), which means a different preference. When only the highly compacted silages are considered (Figure 5), the variously sealed silages were sometimes preferred differently. For example, H0-day 0, H2-day 0 and H4-day 0 were strongly preferred. In contrast, H4-day 6 showed an intermediate preference while H0-day 6 and H2-day 6 were strongly avoided by goats. Within only the low compacted silages (Figure 6), there were greater differences between the variously sealed silages when the silages were exposed to air for 2 or 4 days post-opening. Across all six preference trials, day 0-silages (silages at silo opening) were strongly preferred four times and had two intermediate preferences. Silages of day 2 were twice strongly preferred and four times of intermediate preference. Day 4-silages were strongly preferred three times and three times of intermediate preference. Silages exposed to air for 6 days as well as LUC were avoided five times and showed both one intermediate preference. The graphical solutions for preferences determined after 30 min showed strong similarities with the above-described results and are not shown.

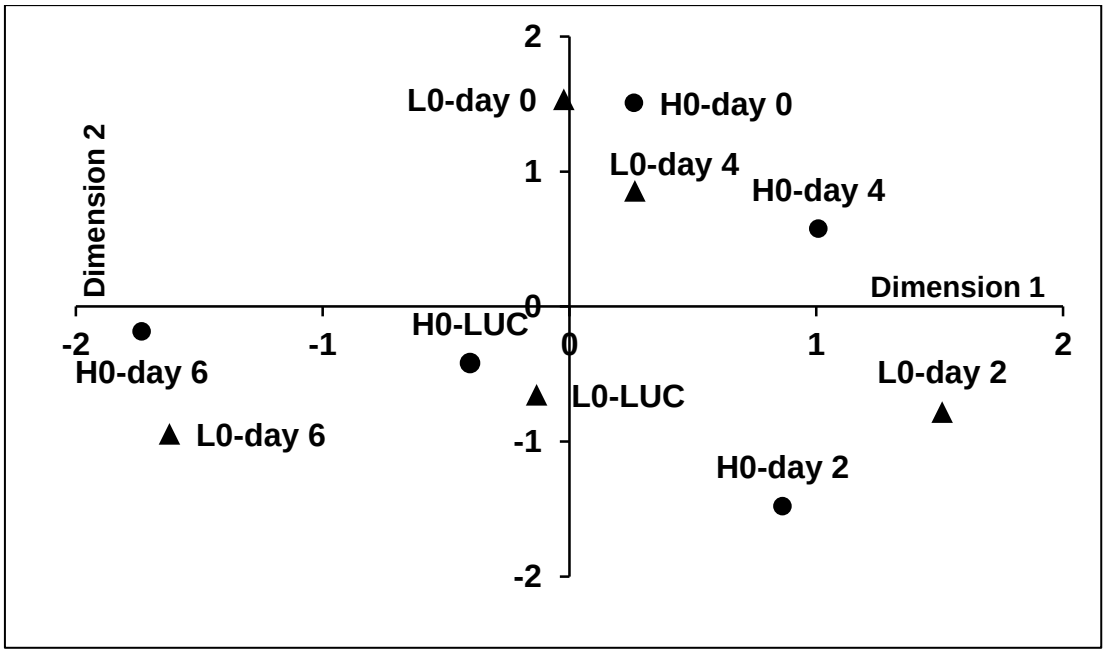

Figure 2. Multidimensional scaling of the mean preference shown by goats of the aerobically exposed silages (days 0, 2, 4 and 6 post-opening) and lucerne hay (LUC) within L0 (low compaction, sealed on day 0 post-filling) and H0 (high compaction, sealed on day 0 post-filling).

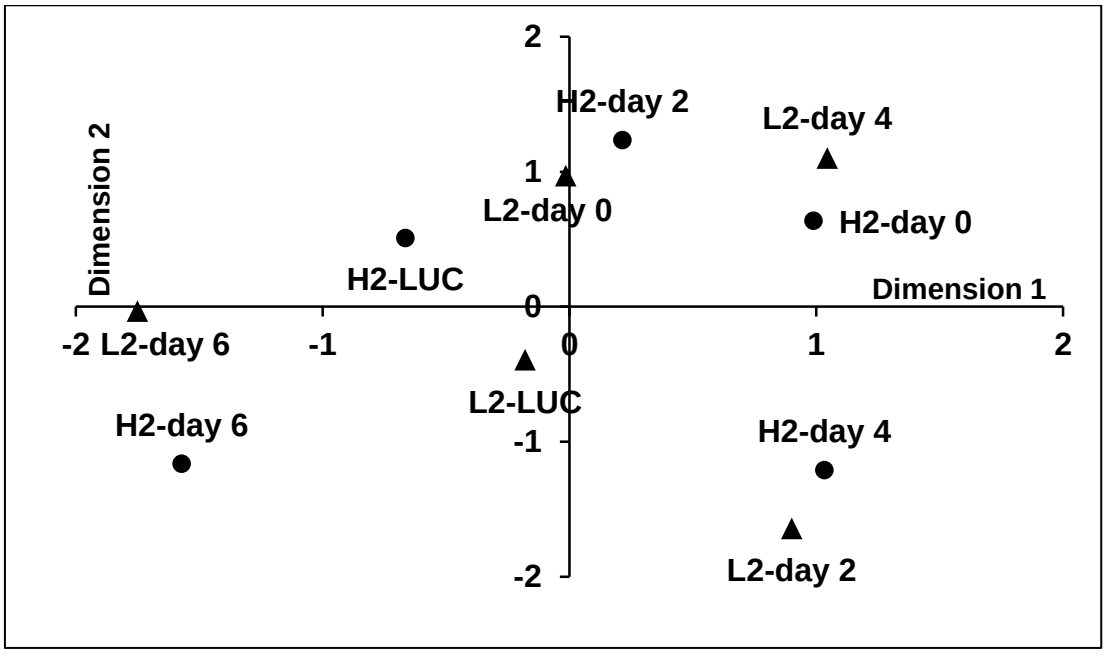

Figure 3. Multidimensional scaling of the mean preference shown by goats of the aerobically exposed silages (days 0, 2, 4 and 6 post-opening) and lucerne hay (LUC) within L2 (low compaction, sealed on day 2 post-filling) and H2 (high compaction, sealed on day 2 post-filling).

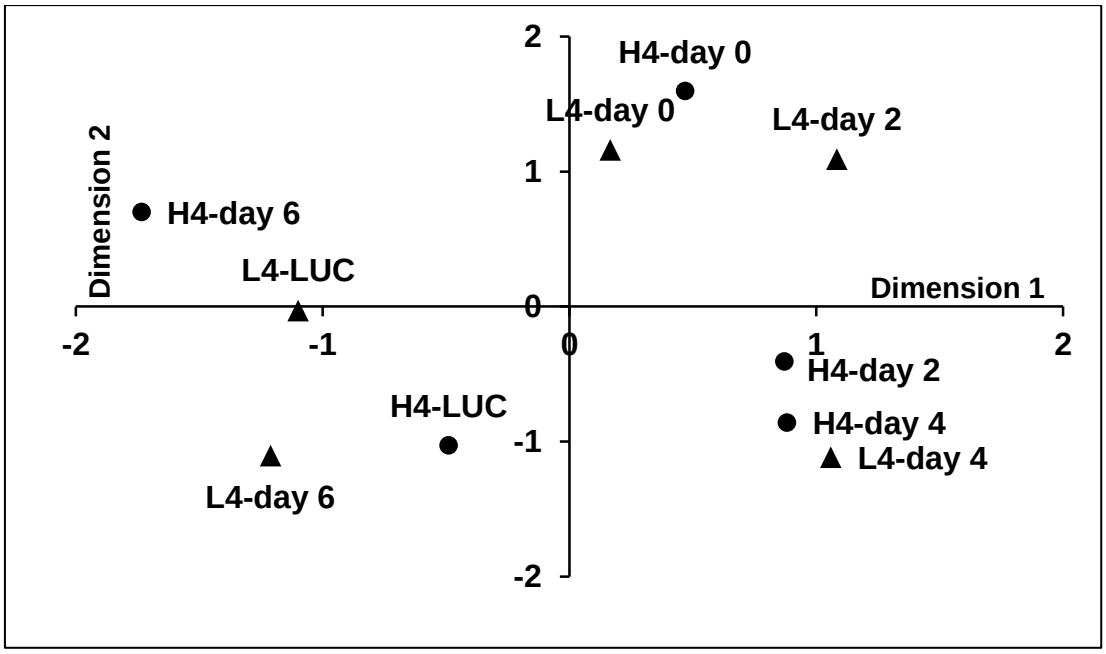

Figure 4. Multidimensional scaling of the mean preference shown by the goats of the aerobically exposed silages (days 0, 2, 4 and 6 post-opening) and lucerne hay (LUC) within L4 (low compaction, sealed on day 4 post-filling) and H4 (high compaction, sealed on day 4 post-filling).

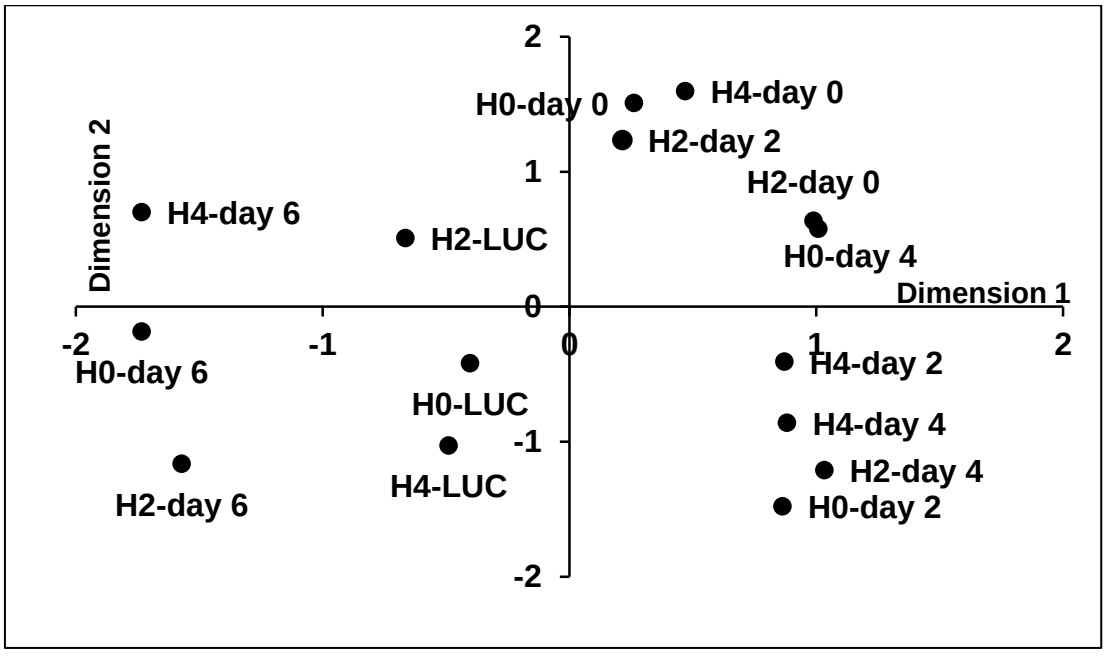

Figure 5. Multidimensional scaling of the mean preference shown by the goats of the aerobically exposed silages (days 0, 2, 4 and 6 post-opening) and lucerne hay (LUC) within H0, H2, and H4 (abbreviations see above).

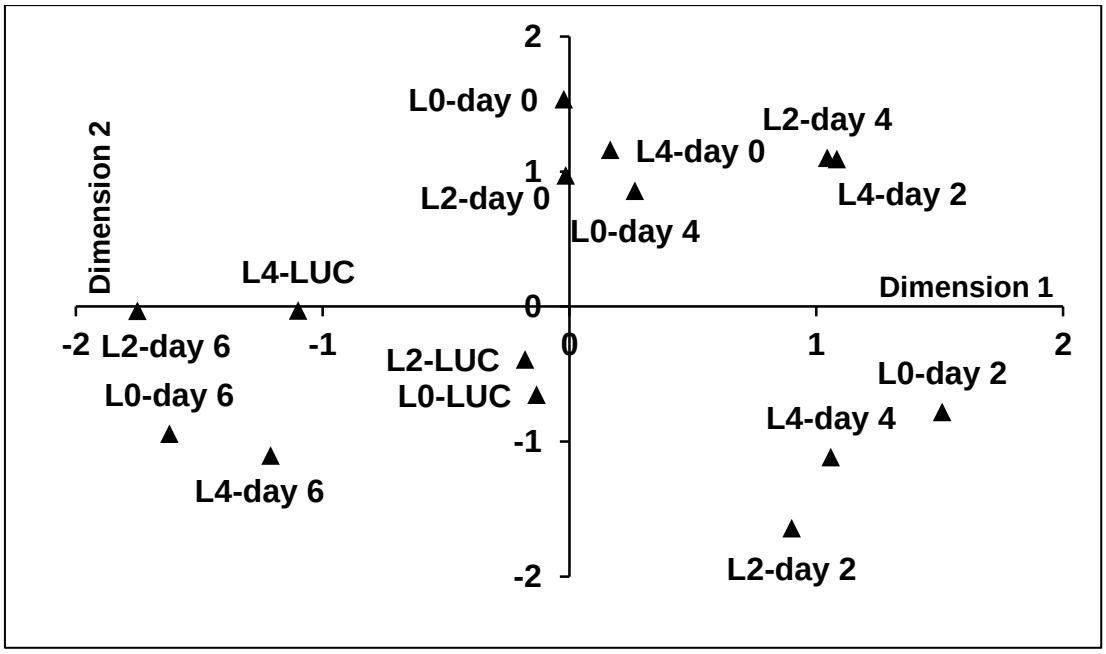

Figure 6. Multidimensional scaling of the mean preference shown by the goats of the aerobically exposed silages (days 0, 2, 4 and 6 post-opening) and lucerne hay (LUC) within L0, L2, and L4 (abbreviations see above).

Treatment effects on 30-min and 3-h dry-matter intake

Table 11 shows the preference when expressed as short-time DMI after 30 min and 3 hr. Compaction and sealing had no impact on DMI. A compaction x sealing interaction was observed on day 4 of aerobic exposure caused by the difference in 30 min-DMI between the treatments L0 and H4. Within the six treatments, DMI was influenced by aerobic exposure. For example, after 3 hr, DMI between L0-day 0 and L0-day 2 did not differ (613 vs. 718 g, p = .26), but both intakes differed from those obtained for L0-day 4 and L0-day 6 (p < .05). Generally, those silages that were 6-day exposed to air showed the lowest intakes among silages. Although not always significant, intakes of the forage pair combinations were high when the LUC was combined with day 0, day 2 or with day 4 and were low when day 4 was combined with day 6 (Table 12). Intakes were also low when day 6-silages were combined with other forages. When the combination day 4-day 6 was offered in the morning, goats consumed apparently the most grass hay in the afternoon.

Table 10. Forage stimulus coordinates and dimension weights by animal for the two-dimensional solution to the preference among goats

	Treatment											
	L0		H0		L2		H2		L4		H4	
	Dim 1	Dim 2	Dim 1	Dim 2	Dim 1	Dim 2	Dim 1	Dim 2	Dim 1	Dim 2	Dim 1	Dim 2
	Coordinates (3hr)											
Forage												
Day 0	-0.023	1.534	0.262	1.507	-0.015	0.970	0.989	0.636	0.166	1.159	0.469	1.596
Day 2	1.511	-0.782	0.864	-1.479	0.901	-1.642	0.215	1.233	1.084	1.091	0.871	-0.407
Day 4	0.266	0.856	1.009	0.577	1.045	1.099	1.033	-1.211	1.060	-1.116	0.882	-0.860
Day 6	-1.621	-0.943	-1.733	-0.186	-1.750	-0.034	-1.571	-1.164	-1.211	-1.104	-1.733	0.700
LUC	-0.133	-0.655	-0.402	-0.419	-0.180	-0.393	-0.665	0.506	-1.099	-0.030	-0.489	-1.028
Goat (Dimensions weights)												
1	0.843	1.136	1.007	0.993	1.255	0.651	0.836	1.141	1.016	0.984	0.935	1.061
2	1.265	0.631	1.091	0.900	1.320	0.507	1.266	0.631	1.046	0.951	1.403	0.175
3	0.882	1.105	1.298	0.561	1.337	0.460	1.414	0.000	1.065	0.930	1.084	0.909
4	0.875	1.111	1.107	0.880	1.166	0.800	1.146	0.829	0.208	1.399	1.025	0.975
5	1.414	0.000	1.290	0.580	1.030	0.969	0.296	1.383	1.234	0.691	1.319	0.510
R^2	0.94		0.96		0.97		0.94		0.90		0.96	

L0, low compaction, sealed on day 0 post-filling; H0, high compaction, sealed on day 0 post-filling; L2, low compaction, sealed on day 2 post-filling; H2, high compaction, sealed on day 2 post-filling; L4, low compaction, sealed on day 4 post-filling; H4, high compaction, sealed on day 4 post-filling; LUC, lucerne hay; Dim 1 and Dim 2, dimension one and dimension two.

Table 11. Dry-matter intake (g 30 min^{-1} and g 3 hr^{-1}) of the silages and lucerne hay (mean ± standard deviation) shown by five goats

		Forage						
		Aerobic exposure post-opening						
Treatment	Intake (g)	Day 0	Day 2	Day 4	Day 6	LUC	*MSD*	*p*-value
L0	30 min	$282^{ab} \pm 188$	$345^{a} \pm 164$	$243^{bA} \pm 175$	$51^{d} \pm 83$	$152^{c} \pm 79$	68	*<.01*
	3 hr	$613^{a} \pm 316$	$718^{a} \pm 315$	$432^{b} \pm 348$	$112^{c} \pm 141$	$431^{b} \pm 235$	145	*<.01*
H0	30 min	$336^{a} \pm 174$	$389^{a} \pm 196$	$367^{aAB} \pm 199$	$87^{c} \pm 123$	$187^{b} \pm 100$	88	*<.01*
	3 hr	$630^{ab} \pm 239$	$697^{a} \pm 232$	$649^{a} \pm 324$	$223^{c} \pm 237$	$505^{b} \pm 177$	138	*<.01*
L2	30 min	$346^{b} \pm 233$	$506^{a} \pm 165$	$385^{bAB} \pm 240$	$89^{d} \pm 115$	$201^{c} \pm 90$	92	*<.01*
	3 hr	$704^{ab} \pm 319$	$815^{a} \pm 210$	$639^{bc} \pm 394$	$169^{d} \pm 206$	$530^{c} \pm 204$	150	*<.01*
H2	30 min	$338^{a} \pm 218$	$394^{a} \pm 220$	$323^{aAB} \pm 262$	$93^{b} \pm 133$	$169^{b} \pm 91$	86	*<.01*
	3 hr	$733^{ab} \pm 291$	$790^{a} \pm 311$	$587^{bc} \pm 498$	$236^{d} \pm 308$	$411^{dc} \pm 181$	145	*<.01*
L4	30 min	$401^{a} \pm 221$	$423^{a} \pm 201$	$332^{aAB} \pm 247$	$144^{b} \pm 183$	$154^{b} \pm 108$	95	*<.01*
	3 hr	$817^{a} \pm 284$	$774^{ab} \pm 294$	$588^{bc} \pm 459$	$267^{d} \pm 339$	$470^{c} \pm 279$	175	*<.01*
H4	30 min	$351^{b} \pm 244$	$456^{a} \pm 165$	$527^{aB} \pm 219$	$87^{c} \pm 131$	$136^{c} \pm 80$	101	*<.01*
	3 hr	$748^{a} \pm 340$	$813^{a} \pm 265$	$853^{a} \pm 412$	$217^{c} \pm 310$	$433^{b} \pm 285$	192	*<.01*
MSD	30 min	211	147	150	119	78		
Compaction		0.96	0.74	0.04	0.83	0.75		
Sealing		0.38	0.10	0.05	0.29	0.15		
Compaction x Sealing		0.55	0.12	0.03	0.28	0.24		
MSD	3 hr	258	268	307	249	219		
Compaction		0.89	0.96	0.06	0.38	0.51		
Sealing		0.06	0.24	0.15	0.45	0.92		
Compaction x Sealing		0.73	0.84	0.18	0.38	0.18		

L0, low compaction, sealed on day 0 post-filling; H0, high compaction, sealed on day 0 post-filling; L2, low compaction, sealed on day 2 post-filling; H2, high compaction, sealed on day 2 post-filling; L4, low compaction, sealed on day 4 post-filling; H4, high compaction, sealed on day 4 post-filling; LUC, lucerne hay; MSD, minimum significant difference; Means within row with different superscripts (a–c) differ ($p < .05$); Means within column and time of intake with different superscripts (A–B) differ ($p < .05$); $n = 20$.

Table 12. Total dry matter intake (g) of the offered pair combination and of the grass hay within the treatments

	Treatment											
	L0		H0		L2		H2		L4		H4	
	Pair	Hay	Pair	Hay	Pair	Hay	Pair	Hay	Pair	Hay	Pair	Hay
Offered pair combination												
LUC-Day 0	1,081	616^{ab}	1,156^{abc}	836^{ab}	1,240	639^{b}	1,203	585	1,370	481	1,219^{ab}	729^{ab}
LUC-Day 2	966	619^{ab}	1,303^{ab}	629^{b}	1,268	720^{ab}	1,330	490	1,176	540	1,245^{ab}	765^{ab}
LUC-Day 4	1,064	694^{ab}	1,416^{a}	761^{ab}	1,314	669^{b}	1,262	697	1,423	615	1,543^{a}	529^{b}
LUC-Day 6	826	958^{a}	1,147^{abc}	1,011^{ab}	1,115	905^{ab}	952	643	1,139	754	1,184^{ab}	698^{ab}
Day 0-Day 2	932	710^{ab}	924^{cd}	1,017^{ab}	1,013	1,075^{a}	1,063	716	1,065	742	979^{b}	896^{ab}
Day 0-Day 4	1,010	750^{ab}	1,158^{abc}	1,036^{ab}	1,165	858^{ab}	1,144	684	1,135	541	1,370^{ab}	750^{ab}
Day 0-Day 6	878	716^{ab}	956^{cd}	930^{ab}	1,093	793^{ab}	952	880	1,089	752	1,074^{ab}	853^{ab}
Day 2-Day 4	1,037	383^{b}	1,056^{bcd}	851^{ab}	1,167	692^{b}	1,121	727	1,162	594	1,348^{ab}	863^{ab}
Day 2-Day 6	784	809^{ab}	776^{d}	904^{ab}	1,044	808^{ab}	1,212	725	1,171	716	1,128^{ab}	887^{ab}
Day 4-Day 6	651	1,014^{a}	920^{cd}	1,131^{a}	1,007	1,083^{a}	789	916	931	946	1,164^{ab}	927^{a}
MSD	894	524	327	476	468	366	868	740	583	553	555	390
p-value	.78	.03	<.01	.04	.41	.04	.60	.71	.36	. 32	.04	.04

L0, low compaction, sealed on day 0 post-filling; H0, high compaction, sealed on day 0 post-filling; L2, low compaction, sealed on day 2 post-filling; H2, high compaction, sealed on day 2 post-filling; L4, low compaction, sealed on day 4 post-filling; H4, high compaction, sealed on day 4 post-filling; LUC, lucerne hay; MSD, minimum significant difference; Means within column with different superscripts (a–d) differ ($p < .05$); $n = 50$.

Dry-matter intake and relationships to silage composition

One aim of the study was to determine which silage characteristics could be determinants for preference or avoidance. For this, composition variables of all silages from vacuum-sealed bags (listed in Table 9) were related to DMI (n = 480). In general, linear regression analysis generated very low R^2 values ($0 < R^2 < .28$) (data not shown). Stepwise multiple regression analysis showed the pH and starch in L0 accounted for 38% of the variation in DMI (Table 13). In H4, moulds accounted for 37% of the variation in DMI. Within all silages, five variables accounted only for 32% of the variation of intake. In this case, the pH, ADL and moulds were most important for DMI. Linear und stepwise regressions of silage composition on initial intake after 30 min showed similarities and are not shown.

Table 13. Stepwise regression analysis of silage composition (g/kg DM, unless stated otherwise) and DMI (g/3 hr) within the treatments and for all silages

Treatment	Intercept	Coefficient	Variable	p	R^2
L0	4,234.39	-393.02	pH	<.001	
		-7.91	Starch	.066	.38
H0	-2,368.82	-66.95	Acetic acid	.006	
		12.31	2-Butanol (mg/kg DM)	<.001	.34
L2	4,494.16	-16.32	ADFom	<.001	.42
H2	2,764.07	0.20	Ethyl acetate (mg/kg DM)	.084	
		-4.94	aNDFom	.002	.25
L4	4,532.96	-86.15	Crude ash	<.001	.28
H4	1,332.40	-195.64	Mould (log_{10} cfu/g FM)	<.001	.37
All silages	936.42	-249.70	pH	<.001	
		-0.21	2-Butanol (mg/kg DM)	.139	
		30.77	Crude ash	.011	
		-7.28	ADL	.008	
		-117.03	Mould (log_{10} cfu/g FM)	<.001	.32

L0, low compaction, sealed on day 0 post-filling; H0, high compaction, sealed on day 0 post-filling; L2, low compaction, sealed on day 2 post-filling; H2, high compaction, sealed on day 2 post-filling; L4, low compaction, sealed on day 4 post-filling; H4, high compaction, sealed on day 4 post-filling; aNDFom, neutral detergent fibre assayed with heat-stable amylase and expressed exclusive of residual ash; ADFom, acid detergent fibre expressed exclusive of residual ash; ADL, acid detergent lignin; cfu, colony forming units; FM, fresh matter; microbial data obtained from fresh silages; n = 80 per treatment; n = 480 (all silages).

DISCUSSION

Characterization of silages at feeding

At silo opening, values of chemical variables ranged within the maize silage values recommended and the silages showed moderate acetic acid contents, low pH values and only traces of butyric acid. Thus, the silages were classified as well fermented. Ethanol contents in all six silages were high in comparison with contents from an on-farm evaluation of Gallo, Giuberti, Bruschi, Fortunati, and Masoero (2016) in Italy (326 ± 67 g/kg DM). When using maize silage with relatively low DM concentrations like in the present study, Comino et al. (2014) observed similar high values for ethanol. High concentrations of ethanol are indicative of greater metabolic activity of yeasts under anaerobic conditions (Mills & Kung, 2002). The silages showed high ethyl lactate and ethyl acetate contents when compared with maize silages sealed with a 24-hr delay in the study of Weiss et al. (2016). According to Brüning et al. (in press), delayed sealing can promote the formation of both ethyl esters. During the time prior to sealing, air (oxygen) is available to microbes encouraging the growth of yeasts (Woolford, 1990). Yeasts can either directly form ethyl esters or supply ethanol for its esterification with lactic or acetic acid under anaerobic conditions (Weiss et al., 2016). The 6-day aerobic exposure phase post-opening caused extensive changes in silage composition. With prolonged aerobic exposure (>2 days), the WSC and lactic acid decreased. This could be ascribed to activity of lactate-utilizing yeasts (Wilkinson & Davies, 2013). The loss of lactic acid was accompanied by an increase in pH >4 on day 4 of aerobic exposure in all silages, which indicated the onset of aerobic deterioration. Ethanol and both ethyl esters decreased with aerobic exposure caused by volatility of these substances (Daniel et al., 2013).

Treatment effects on preference

Within immediately sealed silages, highly and low compacted silage counterparts showed similar preference. The results indicated that compaction did not affect preference when silos were immediately sealed. Among silages sealed with a 2-day or a 4-day delay, some of the highly and low compacted silages showed dissimilar preference. The results indicated that there were probably interactions between

compaction and sealing. Within highly and low compacted silages, the variously sealed silages were differently preferred, which depended on aerobic exposure. The hypothesis that preference decreases with delayed sealing could not be confirmed, because silages sealed with a delay were sometimes slightly stronger preferred by goats than silages sealed immediately. However, a direct comparison of silages with different densities and sealing times in the same preference trial could not be conducted under the experimental conditions of this study.

Day 0-, day 2- and also day-4 silages were strongly preferred or showed intermediate preferences while day 6-silages were mostly avoided. The results resemble those of Gerlach et al. (2013). The similarities between both studies suggest that prolonged aerobic exposure had a strong influence on preference. The effect of aerobic exposure on preference seems to be more pronounced than the effects of silage density and delayed sealing.

Treatment effects on short-time dry-matter intake

The intake data largely reflected the results of MDS analysis. Compaction had no effect on intakes with the exception of the interaction with sealing on day 4 of aerobic exposure. One reason could be that the range in compaction was not sufficiently large enough to achieve greater effects on silage quality as described by Brüning et al. (in press). Possibly, porosity and air infiltration were small and oxygen in the silos was quickly exhausted only a few hours post-filling. Sealing silos with delay had no effect on short-term intakes. This was unexpected as a negative effect was assumed. It should be noted that the whole content of the silos was used and the influence of delayed sealing and also of low compaction only affected the top layers, which, however, constituted only about a quarter of the whole silo. Thus, the effect of both factors on silage composition was mitigated. Bolsen et al. (2001) showed that when increasing the proportions of deteriorated silage (silage taken from top layers of unsealed silos that had undergone several months of exposure to air and rainfall, with foul odour, black colour and slimy, mud-like texture) in the ration, DMI and nutrient digestibility can be reduced. Miller, Dalton, and Miler (1961) indicated that slow silo filling, whose effects corresponds well with those of delayed

sealing, can have adverse effects on DMI of Sudan grass, rye grass and crimson clover.

Within treatments, forage choice was influenced by aerobic exposure and generally, those silages that were exposed to air for 6 days post-opening showed the lowest intakes among silages. The average DMI of the day 6-silages was only 29% of the value of the silages at opening. Similarly, Gerlach et al. (2013) reported a mean decrease of 53% in maize silage DMI at day 8 of aerobic exposure. Intakes of the forage pair combinations were high when the LUC was combined with day 0, day 2 or with day 4, but lower when day 0 was combined with day 2. One reason could be that the LUC served as a buffer against possible rumen acidification, because of the high fibre and CP content. Another explanation for this could be that the difference in composition between the LUC and the silages was greater than between the silages. It is known that feed diversity often leads to increased intakes (Baumont, Prache, Meuret, & Morand-Fehr, 2000). Intakes were low when day 4-silages were combined with day 6-silages and when day 6-silages were combined with other forages. Again, this showed the lower preference for the 6-day exposed silages. When the combination day 4-day 6 was offered, goats consumed at least numerically the most grass hay. The result gives evidence that the inadequate silage intake, which mainly resulted from low intake of day 6-silages, was balanced with intake of grass hay.

Dry-matter intake and relationships to silage composition

Linear regressions of silage composition on DMI showed very low R^2 values ≤ .28. Exemplarily, R^2 values for ethanol, ethyl lactate and ethyl acetate, although being significant, were only .17, .18 and .17 (all $p < .01$) respectively. Similarly, neither increase nor reduction in DMI could be observed in supplementation experiments of Gerlach, Scherer, Laudenbach, Weiss, and Südekum (2016) with increasing dosages of ethanol, ethyl lactate and ethyl acetate. Kristensen, Storm, Raun, Røjen, and Harmon (2007) fed a maize silage-based ration to dairy cows with ethanol and ethyl acetate contents close to this study and could not find any influence on DMI. The findings are also consistent with other studies, in which ethanol did not impair the voluntary feed intake (Daniel et al., 2013; Huhtanen et al., 2002; Krizsan & Randby, 2007; Randby, Selmer-Olsen, & Baevre, 1999). However, in a study of Hetta; Cone,

Bernes, Gustavsson, and Martinsson (2007), silage DMI was positively correlated with ethanol while Gerlach et al. (2013) reported weak negative relationship between DMI and ethanol and ethyl lactate in preference trials with goats. As a possible reason, the authors indicated the volatility of ethyl esters, which can contribute to influencing the taste and flavour of silages. However, direct comparisons between results of intake trials and those of a preference trial cannot be made. Intake studies evaluate only a single feed at a time, and, as a result, choice and selection are eliminated (Van Soest, 1982).

Stepwise multiple regression analysis slightly increased the R^2 values. Within both treatments and silages, DMI was negatively associated with silage composition variables that changed with time of aerobic exposure, which underlined its influence on DMI. The negative regression coefficients for fibre fractions (aNDFom, ADFom and ADL) are consistent with the ruminant's preference against those forages requiring increased chewing time because of elevated fibre constituents (McLeod & Smith, 1989). Studies with fibrous feeds have shown negative relationships between feed intake and its cell wall content (Jung & Allen, 1995).

The DMI was also negatively associated with moulds. Wichert, Kienzle, and Bauer (1998) offered a mixture of 85% deteriorated maize silage (produced by exposing silage to air) or 85% undeteriorated maize silage with 15% hay in a two-choice experiment to dairy cows. The deteriorated silages had higher pH values, higher contents of NDF and ADF, smelled of yeasts, moulds and ammonia and showed poorer sensory properties. Moreover, the silages were heated, were covered with moulds and had a loss of structure. Cows strongly preferred the undeteriorated silage. Those silages that were exposed to air for 6 days contained the highest mould counts. Aerobic deterioration negatively affects hygienic quality of silage, because of increased risk of proliferation of potentially pathogenic or otherwise undesirable micro-organisms, such as moulds, bacilli and *Listeria* (Lindgren, Pahlow, & Oldenburg, 2002). Fink-Gremmels (2008) summarized that various reports describe reduced feed intake associated with the consumption of mouldy feed (hay, silage) and feedstuffs contaminated with mycotoxins. Dairy calves showed less preference when lucerne hay with both high NDF and high fungal biomass was offered (Undi & Wittenberg, 1996). The authors concluded that low preference for moulded hay

would probably result in greater feed sorting and lower intakes when calves have a choice of feedstuffs.

Sensory properties of a feed per se can stimulate or depress intake (Baumont et al., 2000) and help animals to identify and discriminate among feeds (Provenza, 1995). According to the sensory evaluation of the fresh silages in 2-day intervals, all silages had good sensory properties (odour, colour and texture) at silo opening but from day 2 of aerobic exposure, the sensory properties began to deteriorate. Finally, on day 6, all the silages were mouldy with a foul-smelling odour, a strongly offensive texture (greasy) and altered colour. As a consequence, the changed sensory properties may have helped the goats to avoid the 6-day exposed silages. Interestingly, day 4-silages were well consumed although they showed high pH, high yeast counts and already poor sensory properties. Shaver, Erdman, and Vandersall (1984) have shown that pH of maize silages is a factor that affects voluntary consumption. Heifers decreased intake of silages with very low pH and ingested most silage when the pH was 5–6 (due to neutralization with sodium bicarbonate). After a critical point of 4 days of the aerobic exposure phase, preference and intakes decreased dramatically.

The DMI was negatively associated with pH. The effect of silage pH on intake seems to be a direct consequence of aerobic deterioration, because lactic acid was depleted by yeast activity throughout the aerobic exposure phase, which increased the pH.

CONCLUSIONS

Under the conditions of the study, it can be concluded that aerobic exposure after ensiling had a more pronounced effect on silage preference and short-time DMI than compaction and delayed sealing. Nevertheless, silos should be highly compacted and immediately sealed to avoid extensive losses. Exposing silages to air for more than 4 days post-opening caused gradual changes in silage composition, lead to aerobic deterioration and reduced feed value. A 6-day aerobic exposure post-opening caused strong avoidance and low DMI in choice situation. Increasing fibre fractions, a deteriorating microbial status and poor silage sensory properties, probably caused by a combination of different fermentation products can be considered for decrease in preference.

ACKNOWLEDGMENTS

We deeply acknowledge the expert technical support by the staff of the Common Laboratory of Analysis, Humboldt University of Berlin and the technical support by the staff of the Laboratory of the University of Bonn. The authors thank B. Kroschewski, Humboldt University of Berlin, for statistical advice.

REFERENCES

Baumont, R., Prache, S., Meuret, M., & Morand-Fehr, P. (2000). How forage characteristics influence behaviour and intake in small ruminants: A review. *Livestock Production Science*, *64*, 15–28. https://doi.org/10.1016/S0301-6226(00)00172-X

Bolsen, K. K., Huck, G. L., Siefers, M. K., Schmidt, T. E., Pope, R. V., & Uriarte, M. E. (2001). *Silage Management: Five Key Factors*. Kansas State University, Manhattan, Department of Animal Sciences and Industry, KS. Retrieved from http://www.txanc.org/docs/silagemgt.pdf

Brandt, M., Schuldt, A., Mannerkorpi, P., & Vearasilp, T. (1987). Zur enzymatischen Stärkebestimmung im Darminhalt und Kot von Kühen mit hitzestabiler Amylase. *Archives of Animal Nutrition*, *37*, 455. (Abstract, in German).

Brüning, D., Gerlach, K., Weiss, K., & Südekum, K.-H. (in press). Effect of compaction, delayed sealing and aerobic exposure on maize silage quality and on formation of volatile organic compounds. *Grass and Forage Science, 73,* 53–66, https://doi.org/10.1111/gfs.12288

Buntinx, S. E., Pond, K. R., Fisher, D. S., & Burns, J. C. (1997). The utilization of multidimensional scaling to identify forage characteristics associated with preference in sheep. *Journal of Animal Science*, *75*, 1641–1650. https://doi.org/10.2527/1997.7561641x

Burns, J. C., Fisher, D. S., & Mayland, H. F. (2001). Preference by sheep and goats among hay of eight tall fescue cultivars. *Journal of Animal Science*, *79*, 213–224. https://doi.org/10.2527/2001.791213x

Coblentz, W. K., Coffey, K. P., & Chow, E. A. (2016). Storage characteristics, nutritive value, and fermentation characteristics of alfalfa packaged in large-

round bales and wrapped in stretch film after extended time delays. *Journal of Dairy Science*, *99*, 3497–3511. https://doi.org/10.3168/jds.2015-10380

Comino, L., Tabacco, E., Righi, F., Revello-Chion, A., Quarantelli, A., & Borreani, G. (2014). Effects of an inoculant containing a Lactobacillus buchneri that produces ferulate-esterase on fermentation products, aerobic stability, and fibre digestibility of maize silage harvested at different stages of maturity. *Animal Feed Science and Technology*, *198*, 94–106. https://doi.org/10.1016/j.anifeedsci.2014.10.001

Daniel, J. L. P., Amaral, R. C., Sa Neto, A., Cabezas-Garcia, E. H., Bispo, A. W., Zopollatto, M., … Nussio, L. G. (2013). Performance of dairy cows fed high levels of acetic acid or ethanol. *Journal of Dairy Science*, *96*, 398–406. https://doi.org/10.3168/jds.2012-5451

DLG (2004). *Grobfutterbewertung. Teil A - DLG-Schlüssel zur Bewertung von Grünfutter, Silage und Heu mit Hilfe der Sinnenprüfung. DLG-Information 1/2004*. Frankfurt/Main, Germany: Deutsche Landwirtschafts-Gesellschaft. Retrieved from http://2015.dlg.org/fileadmin/downloads/fachinfos/futtermittel/grobfutterbewertung.pdf (in German)

Driehuis, F., & Oude Elferink, S. J. W. H. O. (2000). The impact of the quality of silage on animal health and food safety: A review. *Veterinary Quarterly*, *22*, 212–216. https://doi.org/10.1080/01652176.2000.9695061

Fink-Gremmels, J. (2008). Mycotoxins in cattle feeds and carry-over to dairy milk: A review. *Food Additives and Contaminants*, *25*, 172–180. https://doi.org/10.1080/02652030701823142

Gallo, A., Giuberto, G., Bruschi, S., Fortunati, P., & Masoero, F. (2016). Use of principal factor analysis to generate a corn silage fermentative quality index to rank well- or poorly preserved forages. *Journal of the Science of Food and Agriculture*, *96*, 1686–1696. https://doi.org/10.1002/jsfa.7272

Gerlach, K., Ross, F. H., Weiss, K., Büscher, W., & Südekum, K.-H. (2013). Changes in maize silage fermentation products during aerobic deterioration and its impact on feed intake by goats. *Agricultural and Food Science*, *22*, 168–181.

Gerlach, K., Scherer, R., Laudenberg, L., Weiss, K., & Südekum, K.-H. (2016). *Effects of ethyl esters on preference behaviour of goats*. Proceedings of the Society of Nutrition Physiology, *25,* 133*.*

Hetta, M., Cone, J. W., Bernes, G., Gustavsson, A. M., & Martinsson, K. (2007). Voluntary intake of silages in dairy cows depending on chemical composition and in vitro gas production characteristics. *Livestock Science*, *106*, 47−56. https://doi.org/10.1016/j.livsci.2006.06.013

Huhtanen, P., Khalili, H., Nousiainen, J. I., Rinne, M., Jaakkola, S., Heikkila, T., & Nousiainen, J. (2002). Prediction of the relative intake potential of grass silage by dairy cows. *Livestock Production Science*, *73*, 111–130. https://doi.org/10.1016/S0301-6226(01)00279-2

Johnson, L. M., Harrison, J. H., Davidson, D., Mahanna, W. C., Shinners, K., & Linder, D. (2002). Corn silage management: effects of maturity, inoculation, and mechanical processing on pack density and aerobic stability. *Journal of Dairy Science*, *85*, 434–444. https://doi.org/10.3168/jds.S0022-0302(02)74092-7

Jung, H. G., & Allen, M. S. (1995). Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. *Journal of Animal Science*, *73*, 2774–2790. https://doi.org/10.2527/1995.7392774x

Kaiser, A. G., & Piltz, J. W. (2009). Chapter 12. Feed testing: Assessing silage quality. In A. G. Kaiser, J. W. Piltz, H. M. Burns and N. W. Griffiths (Eds.), *Top fodder successful silage*. Dairy Australia and New South Wales Department of Primary Industries. Retrieved from http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0005/294053/successful-silage-topfodder-complete.pdf

Kristensen, N. B., Storm, A., Raun, B. M. L., Røjen, B. A., & Harmon, D. L. (2007). Metabolism of silage alcohols in lactating dairy cows. *Journal of Dairy Science*, *90*, 1364–1377. https://doi.org/10.3168/jds.S0022-0302(07)71623-5

Krizsan, S. J., & Randby, Å. T. (2007). The effect of fermentation quality on the voluntary intake of grass silage by growing cattle fed silage as the sole feed. *Journal of Animal Science*, *85*, 984−996. https://doi.org/10.2527/jas.2005-587

Kyriazakis, I., Emmans, G. C., & Whittemore, C. T. (1990). Diet selection in pigs: Choices made by growing pigs given foods of different protein concentrations. *Animal Science*, *51*, 189–199.

Licitra, G., Hernandez, T. M., & Van Soest, P. J. (1996). Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology*, *57*, 347–358. https://doi.org/10.1016/0377-8401(95)00837-3

Lindgren, S., Pahlow, G., & Oldenburg, E. (2002). *Influence of microbes and their metabolites on feed and food quality*. Proceedings of the 19th General Meeting of the European Grassland Federation, La Rochelle, France, 2002, pp. 503–511.

McLeod, M. N., & Smith, B. R. (1989). Eating and ruminating behaviour in cattle given forages differing in fibre content. *Animal Science*, *48*, 503–511.

Menke, K. H., & Steingass, H. (1987). Schätzung des energetischen Futterwerts aus der in vitro mit Pansensaft bestimmten Gasbildung und der chemischen Analyse. *II. Regressionsgleichungen. Übersichten zur Tierernährung*, *15*, 59–94. (in German).

Miller, W. J., Dalton, H. L., & Miler, J. K. (1961). Sudan grass silage at two stages of maturity versus rye grass and crimson clover with two filling procedures. *Journal of Dairy Science*, *44*, 1921–1927. https://doi.org/10.3168/jds.S0022-0302(61)89985-2

Mills, J. A., & Kung, J. R. L. (2002). The effect of delayed ensiling and application of a propionic acid-based additive on the fermentation of barley silage. *Journal of Dairy Science*, *85*, 1969–1975. https://doi.org/10.3168/jds.S0022-0302(02)74273-2

Muck, R. E., & Holmes, B. J. (2000). Factors affecting bunker silo densities. *Applied Engineering in Agriculture*, *16*, 613–619. https://doi.org/10.13031/2013.5374

Provenza, F. D. (1995). Postingestive feedback as an elementary determinant of food preference and intake in ruminants. *Journal of Range Management*, *48*, 2–17. https://doi.org/10.2307/4002498

Randby, Å. T., Selmer-Olson, I., & Baevre, L. (1999). Effect of ethanol in feed on milk flavour and chemical composition. *Journal of Dairy Science*, *82*, 420–428. https://doi.org/10.3168/jds.S0022-0302(99)75248-3

SAS Institute (2008). *SAS/STAT 9.3 User's Guide: The MDS Procedure (Book Excerpt)*. Cary, NC: SAS Institute Incorporation

Schabenberger, O., & Pierce, F. J. (2002). *Contemporary statistical models for the plant and soil sciences*. Boca Raton, FL: CRC Press.

Schiffman, S. S., Reynolds, M. L., & Young, F. W. (1981). *Introduction to Multidimensional Scaling*: *Theory*, *Methods and Application*. New York, NY: Academic Press.

Shaver, R. D., Erdman, R. A., & Vandersall, J. H. (1984). Effects of silage pH on voluntary intake of corn silage. *Journal of Dairy Science*, *67*, 2045-9. https://doi.org/10.3168/jds.S0022-0302(84)81542-8

Sucu, E., Kalkan, H., Canbolat, O., & Filya, I. (2016). Effects of ensiling density on nutritive value of maize and sorghum silages. *Revista Brasileira de Zootecnia*, *45*, 596–603. https://doi.org/10.1590/S1806-92902016001000003

Undi, M., & Wittenberg, K. M. (1996). Effect of fungal biomass in mouldy alfalfa hay on preference by dairy calves with no previous exposure to mouldy feeds. *Journal of Dairy Science*, *79*, 1250–1254. https://doi.org/10.3168/jds.S0022-0302(96)76478-0

Uriarte-Archundia, M. E., Bolsen, K. K., & Brent, B. E. (2002). *A study of the chemical and microbial changes in whole-plant corn silage during exposure to air: Effects of a biological additive and sealing technique*. Proceedings of the 13th International Silage Conference, Auchincruive, Scotland, pp. 172–173.

Van Soest, P. J. (1982). *Nutritional ecology of the ruminant: metabolism, nutritional strategies, the cellulolytic fermentation and the chemistry of forages and plant fibers*. 2nd ed. Corvallis, OR: O&B Books, Inc.

von Lengerken, J., & Zimmermann, K. (1991). *Handbuch Futtermittelprüfung*. Berlin, Germany: Deutscher Landwirtschaftsverlag. (in German).

VDLUFA (2012). *Methodenbuch, Vol. III*. *Die Chemische Untersuchung von Futtermitteln*. Darmstadt, Germany: VDLUFA-Verlag (in German).

Weiss, K. (2001). *Gärungsverlauf und Gärqualität von Silagen aus nitratarmen Grünfutter*. Doctoral Thesis. Humboldt-Universität zu Berlin, Germany (in German).

Weiss, K., Gerlach, K., & Südekum, K.-H. (2011). Flüchtige Substanzen in Maissilagen in Abhängigkeit von Silierbedingungen und aerober Lagerungsdauer. *VDLUFA-Schriftenreihe, 67*, 534–540 (in German).

Weiss, K., & Kaiser, E. (1995). Milchsäurebestimmung in Silageextrakten mit Hilfe der HPLC. *Wirtschaftseigene Futter*, *41*, 69–80. (in German).

Weiss, K., Kalzendorf, C., Zittlau, J., & Auerbach, H. (2009). *Novel results on the occurrence of volatile organic compounds in maize silage*. Proceedings of the 15th International Silage Conference, Madison, USA, 2009, pp. 33–34.

Weiss, K., Kroschewski, B., & Auerbach, H. (2016). Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage. *Journal of Dairy Science*, *99*, 8053–8069. https://doi.org/10.3168/jds.2015-10323

Weiss, K., & Sommer, G. (2012). Bestimmung von Estern und anderen flüchtigen organischen Substanzen (VOC) in Silageextrakten mit Hilfe der Gaschromatographie. *VDLUFA-Schriftenreihe, 68*, 561–569. (in German).

Weissbach, F., & Strubelt, C. (2008). Correcting the dry matter content of maize silages as a substrate for biogas production. *Landtechnik*, *63*, 82–83.

Wichert, B., Kienzle, E., & Bauer, J. (1998). Palatability and intake of silage in dairy cows, in relation to hygienic quality. *Journal of Animal Physiology and Animal Nutrition*, *80*, 253–259. https://doi.org/10.1111/j.1439-0396.1998.tb00538.x

Wilkinson, J. M., & Davies, D. R. (2013). The aerobic stability of silage: key findings and recent developments. *Grass and Forage Science*, *68*, 1–19. https://doi.org/10.1111/j.1365-2494.2012.00891.x

Woolford, M. K. (1990). A review: the detrimental effects of air on silage. *Journal of Applied Bacteriology*, *68*, 101–116. https://doi.org/10.1111/j.1365-2672.1990.tb02554.x

CHAPTER 5 GENERAL DISCUSSION AND CONCLUSIONS

The overall aim was to determine the effect of air exposure before and after ensiling on quality of whole-crop maize silage and to determine the influence of the resulting changes on dietary choice by goats. For this, an ensiling experiment and preference trials were carried out in which three fixed factors and their levels (compaction, sealing and aerobic exposure post-opening) were varied in order to ensure ingress of air.

As a general observation, the detrimental effect of air exposure was shown. Table 14 lists some of the effects of compaction, delayed sealing and aerobic exposure that were observed in the ensiling experiment and in the preference trials. Air exposure was directly or indirectly responsible for the adverse effects on silage quality and dietary choice that occurred at different times in the conservation process, before sealing, at silo opening, but also after silo opening. In the ensiling experiment, delaying sealing by 2 and 4 days led to unfavourable changes in chemical and microbial composition before sealing. Exemplarily, the WSC content, caused by continued respiration, decreased by 65% compared to the initial concentration. Consequently, high DM losses up to 11% occurred. Thus, it becomes clear that it is necessary to prevent air from entering the silo at an early stage of conservation. Silage temperatures at 20-cm depth were higher in lowly than in highly compacted silages until the silos were sealed. The higher temperatures were caused by energy released from aerobic degradation of WSC. This shows the disadvantageous effect of a low compaction, which results in higher porosity and higher gas exchange. As a result, air can exert its deleterious effect. Before sealing, ethyl acetate was formed in silages sealed with a delay. Further, ethyl acetate and ethyl lactate were analysed in high concentrations at silo opening in silages sealed with delays. It can be assumed that these VOC can be produced indirectly by yeasts by providing ethanol for esterification or directly by yeasts since both the yeast counts and the ethanol contents were increased by the delayed sealing and thus, by air exposure before sealing. Although the compaction and sealing were not of great importance for forage choice and short-term intake, maintaining a high degree of compaction and a fast seal is essential, because both factors warrant sufficient aerobic stability and slower deterioration after silage is opened for feed-out. As a consequence, negative effects on feed intake can be reducing. Suitable measures for achieving a high compaction

Table 14. Effects of compaction, delayed sealing and aerobic exposure on silage quality and dietary choice by goats

Factor	Affected variable	Effect	Time of observation	Possible explanation
Delayed sealing	WSC	↓↓	Before sealing	Plant respiration by continued air exposure, metabolising yeasts
	Yeast counts	↑		Stimulated growth by continued air exposure
	ETH	↑		Result of yeast activity (anaerobic)
	EA	↑		Result of yeast activity/ Result of esterification
Delayed sealing	DM losses	↑(↑↑)	At silo opening	WSC respiration by continued air exposure before sealing
Delayed sealing	LA	↓		Impeded anaerobiosis and lactic acid fermentation
	pH	↑		Result of reduced lactic acid fermentation
High compaction	ETH	↑↑		Result of yeast activity
High compaction, Delayed sealing	EL	↑↑		Result of yeast activity/ Result of esterification
Delayed sealing	EA	↑↑		Result of yeast activity/ Result of esterification
Aerobic exposure	WSC, LA	↓↓	After opening	Result of intensive metabolic yeast activity
	ETH, EL, EA	↓↓		Volatilization
	Yeast, Moulds, Bacteria	↑↑		Stimulated growth by continued air exposure
	Silage temperature	↑↑		Energy (Heat) released from oxidation of WSC and LA
	Aerobic stability	↓↓		Result of heating/ Result of yeast activity
	Sensory properties	↓↓		Spoilage signs by degradation processes
	Preference	↓(↓↓)		Result of silage aerobic deterioration
	Dry matter intake	↓(↓↓)		Result of reduced preference

WSC = water-soluble carbohydrates; ETH = ethanol; EA = ethyl acetate; EL = ethyl lactate; LA = lactic acid; (↓) = decrease; (↓↓) = strong decrease; (↑) = increase; (↑↑) = strong increase.

can be adjustment of tractor wheel pressure, rolling speed and layer thickness during storage into bunker silo (Mickan et al., 2009). If an immediate seal cannot be achieved, at least an intermediate covering with plastic seal should be executed (Mickan et al., 2009). Prolonged aerobic exposure after opening caused detrimental changes in silage composition, led to microbial growth, aerobic deterioration and to both strong avoidance and decline in DMI. Here, the impact of air exposure was stronger since air could affect the whole silage mass. In contrast, compaction and delayed sealing only affected the top surface layers of the silos. However, on farm, silage is not stored loosely on heaps, but compacted in feedstock during feed-out. Nevertheless, silage should be exposed to air as short as possible after silo opening. This can be achieved by adapting the size of the exposed silo face to livestock numbers and, thus, silage consumption (Ruppel et al., 1995; Martin et al., 2009). The use of silage additives which increase aerobic stability should be considered additionally. Silages exposed for more than 4 days to air were strongly avoided. Consequently, silage intakes were low. Such silage must be discarded in order to avoid the risk of reduced animal performance and health problems.

The results of this thesis clearly show that it is imperative to prevent ingress of air along the silage production chain, from harvesting to feed-out, to ensure both high feed value and high feed intake.

Methodological aspects regarding the ensiling experiment

Sampling before sealing

One aim of the ensiling experiment was to characterise the actual state of silage before sealing. For this purpose, one of the six silos of each silage treatment that were sealed with delay was sampled before sealing (on day 2 and day 4 post-filling). The results of the analyses of the samples were compared with the results of the harvested maize before ensiling. It must be noted that a statistical analysis could not be carried out due to the low number of observations (each $n = 1$). Further, no samples from silos sealed immediately were analysed. In future studies, these types of samples should be collected and analysed. For each treatment, additional silos could be prepared, which are then sampled after 2 and 4 days post-filling, without further use of the material.

Storage duration

The minimum storage duration was 175 days and was different between the treatments (longest for the silos sealed with 4-day delay), because the large number of silages could not be opened, stored and subsequently fed at the same time. The storage duration was not taken into account for statistical analysis, because silage normally remains stable after fermentation phase with no significant changes in composition when it is stored anaerobically for extended times. It has been generally accepted that most active metabolic processes in silo ceases after about 2 to 6 weeks of ensiling, as long as air is prevented from penetrating the mass during storage. This results in a stable silage (Pahlow et al., 2003). However, some microbial processes and changes in microbial composition can occur during prolonged storage. Kleinschmidt and Kung (2006) reported that *Lactobacillus buchneri* remained fairly active for prolonged periods of time in silage, which results in increased concentrations of 1.2-propanediol. Storm et al. (2010) found significant decline in yeast counts after 11 months of ensiling compared with 5 to 7 months. Similarly, Middelhoven and van Baalen (1988) showed decreasing yeast counts during anaerobiosis and stated that anaerobic silage is a hostile environment for yeasts. Other processes concerning chemical constituents also appear to continue after the main phase of fermentation. Kleinschmidt and Kung (2006) showed increased contents of ammonia-N in maize silage after 361 days of storage compared with silage stored for 282 days. Newbold et al. (2006) observed increased *in vitro* starch digestion and soluble-N concentrations in maize silage with longer storage duration. Against this background, it seems possible that the different storage durations could also have influenced the silage composition at opening. In further studies, it should be guaranteed that all silos are opened at the same time. If not, storage duration can be integrated as an influencing factor into the statistical model.

Sampling of silage at silo opening

At silo opening, the effects of compaction and delayed sealing on silage composition was marginal for most of the determined chemical and fermentation variables because sufficient WSC was available for satisfactory fermentation in the harvested

maize, and the silages were mixed together with the discoloured layers before sampling (and after removal from silo). Thus, the influence of low compaction and delayed sealing was mitigated, because their impact was mainly confined to the top layers and this accounted for about a quarter of whole silo. Figure 7 shows the drastic case of a completely missing seal, the effects of which are similar, but much stronger than those of a delayed sealing.

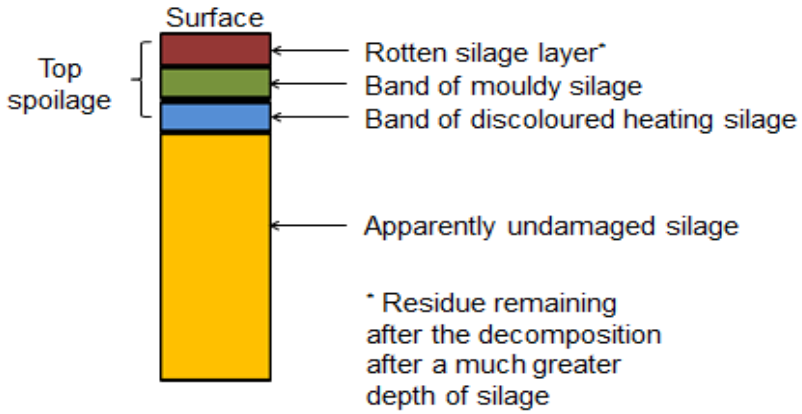

Figure 7. Layers observed near the surface of unsealed silage.
Source: Mickan et al. 2009

Figure 7 shows that the top surface layers are spoiled or of poor quality. Even the discoloured layers usually show reduced quality, although no visible moulds can be detected (Mickan et al., 2009). In the ensiling experiment, a separate analysis of the upper and lower layers would likely have yielded stronger adverse effects on sides of the upper layers and weaker adverse effects on sides of the lower layers, such as was shown in the study of Bolsen et al. (1993). As can be seen from Table 15, silos' top layers (25 cm from surface) can have higher DM losses and higher pH, but also lower lactic acid contents and higher temperatures when silos remain unsealed compared to silos sealed immediately. In future studies, it would be useful to carry out a separate sampling and analysis of single layers from top to bottom in order to make differentiated conclusions on the impact of compaction and delayed sealing depending on the depth.

Table 15. Effects of sealing treatment and depth from the original surface on maize silage quality stored in farm-scale silos

Sealing treatment	Depth	DM	DM-loss	pH	Lactic acid	Acetic acid	Ethanol	Ammonia-N	Temp
	(cm)	(%)	(%)		(% DM)				(°C)
1	25	23.0	80	6.69	0.15	0.42	0.20	0.03	57
	50	34.4	29	5.10	0.52	1.00	0.27	0.52	55
	75	30.3	19	3.78	3.51	3.43	1.64	0.14	38
2	25	29.5	23	4.46	1.31	2.19	0.37	0.11	42
	50	32.9	9	3.84	3.38	1.74	1.51	0.11	41
	75	31.9	12	3.86	3.26	1.62	1.52	0.10	38
3	25	30.3	32	4.71	0.83	1.23	1.09	0.09	49
	50	32.3	16	3.93	1.88	1.20	1.29	0.07	45
	75	32.0	12	3.81	3.03	2.03	1.63	0.10	34

Treatment 1 = unsealed; Treatment 2 = sealed immediately; Treatment 3 = sealed 7 days post-filling after application of a mould inhibitor; Temp = Maximum temperature from day 0 to day 42 post-filling.

Source: Bolsen et al. 1993, adapted

Lack of covering

The method to determine aerobic stability was dissimilar to that described by Honig (1990). No polystyrene boxes and no covering were used during time of aerobic exposure. Additionally, the silage heaps were mixed in 2-day intervals, therefore silage moisture evaporated (lost as a heat sink) and further air was added. The lack of covering caused an increase in DM, which is in contrast with results of the literature (Ohyama et al., 1975; Woolford et al., 1978; Chen and Weinberg, 2009). These authors reported decreasing DM contents when silage was exposed to air over an extended period of time (due to the degradation of DM and generation of oxidative H_2O). As a result of the increase in DM, however, microbial growth can also be slowed down. Therefore, in further studies, it would be preferable to use a covering, e.g., an aluminium foil or a cheese cloth, in order to prevent drying of the silage heaps and to exclude a potential bias of the results.

Assessment of silage during aerobic exposure

Silages were sampled during aerobic exposure to characterise the status and the changes that have taken place. In further studies, it would be helpful to determine the microbial composition of all silages on each day of aerobic exposure. Then, more targeted conclusions could be drawn about microbial dynamics depending on compaction and sealing.

Methodological aspects regarding the preference trials

Vacuum-sealing

For preference trials, silage was packaged and vacuum-sealed in polyethylene bags for further use as individual meal. The bags were sampled and analysed. Visual inspection of the data of the vacuum-sealed silages and of the fresh silages from the ensiling experiment revealed only minor differences in silage composition. It is assumed that these were caused by the low number of samples. To answer the question as to whether the vacuum-sealing changed the current silage status, more samples had to be taken. However, the comparisons cautiously point out that vacuum-sealing of silage seems to be a viable method to preserve silage in its current status and can be recommended for further application. According to Pippard et al. (1996), vacuum-sealing is a method suitable for preserving silage with considerable potential for use also in regular feeding trials.

Multidimensional scaling

The use of multidimensional scaling (MDS) was appropriate for the evaluation of the preference behaviour shown by the goats after 3 hr. The scaling helped systemising data by converting preference into coordinates in a two-dimensional coordinate system with four quadrants. The generated plots supported the evaluation for the preferences of the silages and the lucerne hay. The MDS also played a role in better understanding the results that were obtained by using the Waller-Duncan k-ratio *t*-test. Although the 30-min plots showed strong similarities with the 3-h plots, there were some silages, whose positions in dimensional space could not be explained and were not in agreement with the data on DMI. Thus, H2-day 2 and L4-day 4

showed negative signs in both dimensions, whereas L4-day 6 had positive signs (see Appendix Table 18), although the opposite was expected. Obviously the model fit was not adequate, which could be seen from the lower R^2 values (0.78 in H2 and 0.84 in L4). In any case, a check of the MDS statistics as well as of the generated plots should be carried out in further tests, and the intake data should be used as a basis for the evaluation.

3-h vs 30-min dry-matter intake

According to Provenza (1995), rate of intake, especially at the beginning of the meal, seems to be a key factor for understanding variations in voluntary intake between forages. The intake data of the silages determined after 30 min and 3 hr showed similarities. This indicates that preference was already predefined for a particular silage at the beginning of the preference trials and did not change essentially during the 3-h feeding. Presumably, this was already acquired during the adaptation phase in which silages were offered singly to allow animals to learn to associate a particular set of sensory properties with certain metabolic response (Forbes 1995). It is known that ruminants can identify and select preferred forages when they are offered in pairs on days after initial meal, as shown by Fisher et al. (1999; 2002).

Animals

Goats were used as model animals for larger ruminants (beef cattle and dairy cows), due to the experimental conditions (especially due to the amount and weight of the vacuum-sealed silages that served as meals) and for a better handling of the animals. This raises the question whether the results of the preference behaviour that were shown by the goats can also be applied to beef cattle or dairy cows. Since 20–25 years, it has been a common knowledge that digestion in cattle, sheep and goats is similar with moderate to high quality forage. However, goats are more efficient than sheep in ingesting forage rich in cell wall and low in nitrogen (Morand-Fehr, 2005). According to Hofmann (1989), cattle are grass/roughage eaters, while goats are intermediate types (between grass/roughage eaters and concentrate selectors). Recently, in a study of Ferreira et al. (2016), feed intake, preference, digestion and rumen function were compared between cattle, sheep and goats. The

ruminants grazed together on pastures with different vegetation. The sheep tended to prefer white clover (*Trifolium repens*), whereas the goats avoided the clover. The goats preferred perennial rye-grass (*Lolium perenne*) and natural herbaceous species (western gorse (*Ulex gallii*) and short heaths), which had higher contents of NDF and ADF, but also tannins and phenols. The cattle tended to select a diet (mainly *T. repens*) lower in cell wall components. The degree of overlap between the diets that were selected by the ruminants was greater between cattle and sheep than between sheep and goats or goats and cattle. According to Dulphy et al. (1997), differences in preference between cattle and small ruminants are larger when the animals are fed on low-quality feeds. Riaz et al. (2014) observed that goats appeared less responsive to increases in fibre fractions (NDF and ADF) than sheep, cattle and buffaloes such that these feed fractions had a less negative impact on their DMI. In the preference trials, the preference for a given silage appeared to decrease as the feed value decreased. For example, the preference was lower when a silage with high fibre fractions was offered. This was mainly the case with the day 6-silages. This could mean that when the day 0-silages had been fed to both goats and cattle, fewer differences between the two species in preference behaviour would have occurred. It would be conversely for the day 6-silages. Cattle would probably prefer these silages even less. On the other hand, these silages also had very poor sensory properties, which prevent a preference for these silages from the outset. However, it remains an open question whether the results can be transferred directly on cattle or, specifically, dairy cows.

Animal health

No clinical disorders, such as tympany or diarrhoea, were observed when the day 4- and day 6-silages, which had high yeast counts of more than 8 log cfu/g, were ingested by the goats. This fact may indicate that rumen function was not affected by intake. Generally, an influence on ruminal fermentation can not be excluded, if spoiled silage with high yeast levels is fed. For example, Santos et al. (2015) reported reduced fibre digestibility and increases in propionate and acetate concentrations in *in vitro* incubations with high yeast levels of *Issatchenkia orientalis* (formerly known as *Candida krusei*) of up to 8.40 log cfu/g from spoiled silage. For this reason, animals must be observed regularly during further trials.

REFERENCES

Bolsen, K. K., Dickerson, J. T., Brent, B. E., Sonon, R. N., Dalke, B. S., Lin, C. and Boyer, J. E. (1993). Rate and extent of top spoilage losses in horizontal silos. *Journal of Dairy Science*, *76*, 2940–2962.

Chen, Y. and Weinberg, Z. G. (2009). Changes during aerobic exposure of wheat silages, *Animal Feed Science and Technology*. *154*, 76–82.

Dulphy, J. P., Martin-Rosset, W., Dubroeucq, H., Ballet, J. M., Detour, A. and Jailler, M. (1997). Compared feeding patterns in ad libitum intake of dry forages by horses and sheep. *Livestock Production Science*, *52*, 49–56.

Ferreira, L. M. M., Hervás, G., Belenguer, A., Celaya, R., Rodrigues, M. A. M., Garcia, U., Frutos, P. and Osoro, K. (2016). Comparison of feed intake, digestion and rumen function among domestic ruminant species grazing in upland vegetation communities. *Journal of Animal Physiology and Animal Nutrition*, Available at: http://onlinelibrary.wiley.com/doi/10.1111/jpn.12474/abstract (accessed 22 November 2016).

Fisher, D. S., Mayland, H. F. and Burns, J. C. (1999). Variation in ruminants' preference for tall fescue hays cut either at sundown or at sunup. *Journal of Animal Science*, *77*, 762–768.

Fisher, D. S., Mayland, H. F. and Burns, J. C. (2002). Variation in ruminant preference for alfalfa hays cut at sunup and sundown. *Crop Science*, *42*, 231–237.

Forbes, J. M. (1995). Voluntary food intake and diet selection in farm animals. *CAB International*, Wallingford, UK, p. 532.

Hofmann, R. R. (1989). Evolutionary steps of ecophysiological adaption and diversification of ruminants: A comparative view of their digestive system. *Oecologia*, *78*, 443–457.

Honig, H. (1990). Evaluation of aerobic stability. In *Grass and Forage Reports*, *Proceedings of the Eurobac Conference, Uppsala, Schweden, 1986, special issue, 3* (pp. 76–82).

Kleinschmidt, D. H. and Kung, L. Jr. (2006). The effects of *Lactobacillus buchneri* 40788 and *Pediococcus pentosaccus* R1094 on the fermentation of corn silage during various stages of ensiling. *Journal of Dairy Science*, *89*, 3999–4004.

Martin, M. D., Kaiser, A. G. and Piltz, J. W. (2009). Chapter 10. Feeding silage. In A. G. Kaiser, J. W. Piltz, H. M. Burns and N. W. Griffiths (Eds.), *Top Fodder Successful Silage*, Dairy Australia and New South Wales Department of Primary Industries. Available at: http:// www.dpi.nsw.gov.au/__data/assets/pdf_file/0005/294053/successful-silage-topfodder-complete.pdf (accessed 10 August 2016).

Mickan, F. J., Martin, M. D. and Piltz, J. W. (2009). Chapter 9. Silage storage. In: In A. G. Kaiser, J. W. Piltz, H. M. Burns and N. W. Griffiths (Eds.), *Top Fodder Successful Silage*, Dairy Australia and New South Wales Department of Primary Industries. Available at: http:// www.dpi.nsw.gov.au/__data/assets/pdf_file/0005/294053/successful-silage-topfodder-complete.pdf (accessed 10 August 2016).

Middelhoven, W. J. and van Baalen, A. H. M. (1988). Development of the yeast flora of whole-crop maize during ensiling and during subsequent aerobiosis. *Journal of the Science of Food and Agriculture*, *42*, 199–207.

Morand-Fehr, P. (2005). Recent developments in goat nutrition and application: A review. *Small Ruminant Research*, *60*, 25–43.

Newbold, J. R., Lewis, E. A., Lavrijssen, L., Brand, H. J., Vedder, H. and Bakker, J. (2006). Effect of storage time on ruminal starch degradability in corn silage. *Journal of Dairy Science*, *89* (Suppl. 1): 190.

Ohyama, Y., Masaki, S. and Hara, S.-I. (1975). Factors influencing aerobic deterioration of silages and changes in chemical composition after opening silos. *Journal of the Science of Food and Agriculture*, *26*, 1137–1147.

Pahlow, G., Muck, R. E., Driehuis, F., Oude Elferink, S. J. W. H. and Spoelstra, S. F. (2003). Microbiology of ensiling. In D. R. Buxton, R. E. Muck and J. H. Harrison (Eds.), *Silage Science and Technology* (pp. 31–93). Madison, USA: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America.

Pippard, C. J., Porter, M. G., Steen, R. W. J., Gordon, F. J., Mayne, C. S., Poots, R. E., Unsworth, E. F. and Kilpatrick, D. J. (1996). A method for obtaining and storing uniform silage for feeding experiments. *Animal Feed Science and Technology*, *57*, 87–95.

Provenza, F. D. (1995). Postingestive feedback as an elementary determinant of food preference and intake in ruminants. *Journal of Range Management*, *48*, 2–17.

Riaz, M. Q., Südekum, K.-H., Clauss, M. and Jayanegara, A. (2014). Voluntary feed intake and digestibility of four domestic ruminant species as influenced by dietary constituents: A meta-analysis. *Livestock Science*, *162*, 76–85.

Ruppel, K. A., Pitt, R. E., Chase, L. E. and Galton, D. M. (1995). Bunker silo management and its relationship to forage preservation on dairy farms. *Journal of Dairy Science*, *78*, 141–153.

Santos, M. C., Lock, A. L., Mechor, G. D. and Kung, L. Jr. (2015). Effects of a spoilage yeast from silage on in vitro ruminal fermentation. *Journal of Dairy Science*, *98*, 2603–2610.

Storm, I. M. L. D., Kristensen, N. B., Raun, B. M. L., Smedsgaard, J. and Thrane, U. (2010). Dynamics in the microbiology of maize silage during whole-season storage. *Journal of Applied Microbiology*, *109*, 1017–1026.

Woolford, M. K., Honig, H. and Fenlon, J. S. (1978). Untersuchungen über den aeroben Abbau in Silage mit einer Labormethode. Teil 2: Mikrobiologische, physikalische und chemische Veränderungen während des aeroben Abbaus von Maissilage. *Das wirtschaftseigene Futter*, *24*, 125–135. (in German).

GENERAL SUMMARY

The effect of air exposure before and after ensiling on maize silage quality and dietary choice by goats

Management factors that affect the time to achieve anaerobic conditions in the silo are of utmost importance for production of high-quality silages. These factors include high compaction and rapid sealing. If this can only be achieved inadequately, a loss of dry matter (DM), nutrients and feed value threatens. The growth of microorganisms, in particular of yeasts, can be seen as particularly critical, since they can survive the anaerobic phase of conservation. This results in an increased risk of aerobic instability after opening the silo. In addition, yeasts are able to form ethanol from available water-soluble carbohydrates (WSC), from which, in turn, further volatile organic compounds (VOC), such as ethyl ester, can be formed during the anaerobic phase. These have been found in silages on farms and may have led to reduced feed intake of dairy cows and goats under experimental conditions. In extensive studies, a strong correlation was found between ethanol and the contents of ethyl esters, which were reported as indicator substances for other volatile organic compounds and which were considered together with the other VOC for the decrease in feed intake. When silage is exposed to air after opening, aerobic deterioration may occur as a result of undesirable microbial activity. Aerobically deteriorated silage is undesirable for feeding due to lower nutritive value and risk of negative effects on animal performance and health.

The overall aim of the thesis was to determine the effect of air exposure before and after ensiling on quality of whole-crop maize silages and to determine the influence of the resulting changes on dietary choice by goats. Therefore, an ensiling experiment and preference trials were conducted in which three fixed factors (compaction, sealing and aerobic exposure post-opening) were varied in order to ensure ingress of air in different stages of the conservation process and afterwards.

The aim of the ensiling experiment was to determine the effect of compaction and sealing on maize silage quality regarding chemical and microbial composition and fermentation pattern before sealing and at silo opening. Additionally, it should be determined how an aerobic exposure of 6 days affects the quality of silage after opening and what changes take place. Subsequently, the differently treated silages

were offered to goats in preference trials. The specific aim was to study the effects of the changes caused by treatments on forage choice and short-term feed intake. It should also be determined, which silage characteristics impact on preference or avoidance.

For this purpose, whole-crop maize (277 g kg^{-1} DM) was chopped, mixed, sampled and ensiled in 120-L plastic silos (6 replicates per treatment), which were either compacted to low (194 kg DM m^{-3}) or high (234 kg DM m^{-3}) density and sealed either immediately or with a delay at day 2 or at day 4 post-filling. One of the six silos of each treatment that were sealed with a delay was sampled immediately before sealing to determine the changes in chemical and microbial composition. After sealing, all silos were stored at ambient temperature of 12 ± 2.9°C for at least 175 days and then opened. After silo opening, the silages were removed, mixed, sampled and exposed to air for 6 days in form of a quadratic single heap. During aerobic exposure, samples were taken from the silages at 2-day intervals to determine chemical and microbial composition and fermentation pattern, as well as aerobic stability and sensory properties. Silage was removed from heaps at the respective days of exposure (day 0, 2, 4 and day 6) for subsequent preference trials and was then vacuum-stored in polyethylene bags for further use as feed. A 15-day preference trial consisting of an adaptation and an experimental phase was carried out with goats (German Improved White Goat, n = 5) for each of the six treatments (2 compaction x 3 sealing times). During the 10-day experimental phase, each possible two-way combination (n = 10) of the exposed silages (day 0, 2, 4 and day 6) and of a lucerne hay, which served as standard feed, was offered for 3 hr. During this time, each goat could freely choose between the two feeds present in combination. The amount of each feed, which was eaten, was determined after 30 min and after 3 hr. In addition, goats were offered grass hay in the afternoon for 2 hr and the intake was also determined.

Delayed sealing of the silos after 2 and 4 days caused changes in both chemical and microbial composition, which was particularly evident in the increase in yeast counts as well as in a decline of up to 65% in WSC before sealing. Sealing the silos after 4 days caused high DM losses of up to 11%. At silo opening, higher contents of ethyl acetate and ethyl lactate were found in silages sealed with a delay than in immediately sealed silages. A 4-day delay resulted in a shorter aerobic stability

compared with immediate sealing (65 vs. 50 hr). Aerobic exposure after opening led to considerable changes in silage composition, to a drastic loss in feed value and, finally, spoilage. This was mainly reflected in the increase in yeast counts, the strong rise in pH, the worsening of the sensory properties and the rapid heating. Neither the different compaction nor the delay significantly influenced forage choice and short-term feed intake. On the other hand, prolonged aerobic exposure of more than 4 days had a detrimental effect. Exposing silages to air for 6 days resulted in strong avoidance and, across all treatments, a mean decrease in DMI of 71% compared with silages at opening. Increasing fibre fractions, a deteriorating microbial status and poor silage sensory properties, probably caused by a combination of different fermentation products can be considered for decrease in preference.

The ensiling experiment shows that even in the instance of a silo with a small surface-to-volume ratio, detrimental effects of low compaction and delayed sealing on maize silage quality can occur before sealing and at silo opening. Furthermore, both factors can adversely impact on silage quality during aerobic exposure post-opening (feed-out). Under the conditions of the ensiling experiment, the overall effects of sealing had a greater impact on silage quality than that of compaction. The longer the silo remains unsealed, the greater the silage quality deterioration. Under the conditions of the preference trials, aerobic exposure after ensiling had a more pronounced effect on silage preference and short-time DMI than compaction and delayed sealing.

In conclusion, all measures should be taken to ensure a high degree of compaction and particularly an immediate seal in order to minimize losses in feed value. Aerobic exposure after opening the silo (feed-out) should be limited as much as possible to prevent steady deterioration of silage and reduction in feed intake.

Finally, it appears imperative to prevent ingress of air from harvesting to feed-out to ensure both high feed value and high feed intake.

ZUSAMMENFASSUNG

Einfluss von Luftzufuhr vor und nach der Silierung auf Maissilagequalität und Futterwahl von Ziegen

Managementfaktoren, die die Zeit zum Erreichen anaerober Bedingungen im Silo beeinflussen, sind äußerst wichtig, um qualitativ hochwertige Silage herzustellen. Zu diesen Faktoren gehören eine hohe Verdichtung und ein zügiger Verschluss. Können diese nur bedingt eingehalten werden, droht u. a. ein Verlust an Trockenmasse (TM), Nährstoffen und Futterwert. Besonders kritisch ist dabei die Vermehrung von Mikroorganismen zu sehen, insbesondere von Hefen, da diese die anaerobe Lagerungsphase überdauern können, so dass ein erhöhtes Risiko für aerobe Instabilität nach dem Öffnen des Silos besteht. Zudem sind Hefen in der Lage, während der anaeroben Lagerung aus den verfügbaren wasserlöslichen Kohlenhydraten (WLKH) Ethanol zu bilden, aus dem weitere flüchtige organische Verbindungen (VOC), wie Ethylester, entstehen können. Diese wurden in Praxissilagen nachgewiesen und könnten laut Praxisbeobachtungen zu verringerter Futteraufnahme von Milchkühen sowie von Ziegen unter Versuchsbedingungen geführt haben. In umfangreichen Untersuchungen konnte eine starke Korrelation zwischen Ethanol und den Gehalten an Ethylestern gezeigt werden, die als Indikatorsubstanzen für weitere flüchtige organische Verbindungen herausgestellt wurden und die zusammen mit den anderen VOC für die Verringerung der Futteraufnahme in Betracht kamen. Wird Silage nach dem Öffnen der Luft ausgesetzt, kann diese, als Folge unerwünschter mikrobieller Aktivität, verderben. Aerob verdorbene Silage ist für die Fütterung aufgrund des niedrigen Futterwertes und der Gefahr von negativen Auswirkungen auf Tierleistung und Tiergesundheit unerwünscht.

Das übergeordnete Ziel der Arbeit war, den Einfluss von Luft vor und nach der Silierung auf die Qualität von Maissilage und den Einfluss der sich daraus ergebenden Veränderungen auf die Futterwahl von Ziegen zu untersuchen. Zu diesem Zweck wurden ein Silierversuch sowie Präferenzversuche durchgeführt, in denen jeweils drei fixe Prüffaktoren (Verdichtung, Verschluss und aerobe Lagerung/Exposition nach Öffnung) so abgestuft wurden, dass die Zufuhr von Luft in

den unterschiedlichen Stadien des Silierprozesses und danach gewährleistet werden konnte.

Das Ziel des Silierversuches war es, die Wirkung von Verdichtung und Verschluss auf die Qualität von Maissilage bezüglich der chemischen und mikrobiellen Zusammensetzung und des Fermentationsmusters zum Zeitpunkt des Verschlusses und bei Siloöffnung, zu untersuchen. Zudem sollte untersucht werden, wie sich eine aerobe Lagerung (Exposition) von 6 Tagen auf die Qualität der Silagen nach Siloöffnung auswirkt und welche Veränderungen dabei stattfinden. Im Anschluss wurden die unterschiedlich behandelten Silagen an Ziegen in Präferenzversuchen geprüft, um die Auswirkungen der durch die Behandlungen hervorgerufenen Veränderungen auf die Futterwahl und die Kurzzeit-Futteraufnahme zu untersuchen. Dabei sollte herausgefunden werden, welche Silagecharakteristika für die Präferenz oder Vermeidung verantwortlich sein können.

Hierzu wurde Silomais mit dem Ende der Teigreife (277 g kg^{-1} TM) gehäckselt, vermischt, beprobt und in 120 L Plastiksilos (6 Wiederholungen pro Variante) einsiliert, die entweder niedrig (194 kg TM m^{-3}) oder hoch (234 kg TM m^{-3}) verdichtet und entweder sofort oder mit Verzögerung nach 2 oder 4 Tagen verschlossen wurden. Eines der jeweils sechs Silos der Varianten, die verzögert verschlossen wurden, wurde unmittelbar vor dem Verschluss beprobt, um die Veränderungen in der chemischen und mikrobiellen Zusammensetzung der Silagen zu untersuchen. Nach dem Verschluss lagerten alle Silos bei einer Umgebungstemperatur von 12 ± 2.9 °C für mindestens 175 Tage und wurden dann geöffnet. Nach dem Öffnen wurden die Silagen entnommen, durchmischt, beprobt und jeweils in Form eines quadratischen Einzelhaufens 6 Tage an der Luft gelagert. Während der aeroben Exposition wurden im zweitägigen Abstand Proben zur Bestimmung der chemischen und mikrobiellen Zusammensetzung und des Fermentationsmusters von den Silagen entnommen sowie aerobe Stabilität und sensorische Eigenschaften bestimmt. Für die im Anschluss durchgeführten Präferenzversuche wurde Silage an den jeweiligen Tagen der aeroben Exposition (Tag 0, 2, 4 und Tag 6) von den Haufen entnommen und in Polyethylenbeuteln zur weiteren Verwendung als Futter vakuumverpackt. Anschließend wurde für jede der sechs Varianten (2 Verdichtungsstufen x 3 Verschlusszeiten) ein 15-tägiger Präferenzversuch, bestehend aus einer Adaptations- und einer Versuchsphase, mit Ziegen (Weiße Deutsche Edelziege, n = 5) durch-

geführt. Während der 10-tägigen Versuchsphase wurde jeder Ziege jede mögliche Kombination (*n* = 10) aus zwei Futtermitteln, bestehend aus den aerob gelagerten Silagen (Tag 0, 2, 4 und Tag 6) und einem Luzerneheu, das als Standardfutter diente, für 3 h angeboten. Während dieser Zeit konnte jede Ziege zwischen den beiden in Kombination vorliegenden Futtern frei wählen. Die aufgenommene Menge an Einzelfutter wurde nach 30 min sowie nach 3 h bestimmt. Zusätzlich wurde den Ziegen am Nachmittag Grasheu zur freien Aufnahme für 2 h angeboten. Die aufgenommene Menge wurde ebenfalls bestimmt.

Das verzögerte Verschließen der Silos nach 2 und 4 Tagen verursachte sowohl Veränderungen in der chemischen als auch in der mikrobiellen Zusammensetzung, was besonders am Anstieg der Hefezahlen sowie an der starken Abnahme der WLKH um bis zu 65 % vor dem Verschluss deutlich wurde. Das Verschließen der Silos nach 4 Tagen führte außerdem zu hohen TM-Verlusten von bis zu 11 %. Bei Öffnung der Silos wurden in den verzögert verschlossenen Silagen höhere Gehalte an Ethylacetat und Ethyllactat als in den sofort verschlossenen Silagen gefunden. Eine Verzögerung von 4 Tagen führte zur geringsten aeroben Stabilität gegenüber dem sofortigen Verschluss (65 vs. 50 h). Die aerobe Exposition nach der Siloöffnung führte zu erheblichen Veränderungen in der Zusammensetzung, zu einem drastischen Verlust an Futterwert und schließlich zum Verderb. Dies spiegelte sich vor allem im starken Anstieg des pH-Wertes und der Hefezahlen, in der Verschlechterung der sensorischen Eigenschaften und in der schnellen Nacherwärmung wider. Weder die unterschiedliche Verdichtung noch der verzögerte Verschluss konnten die Futterwahl und die Kurzzeit-Futteraufnahme der Ziegen entscheidend beeinflussen. Demgegenüber wirkte sich die langanhaltende aerobe Exposition von mehr als 4 Tagen nachteilig aus. Die aerobe Exposition von 6 Tagen führte zur starken Futtervermeidung sowie zu einem mittleren Rückgang der TM-Aufnahme von 71 % (über alle Varianten) im Vergleich zu den Silagen bei Siloöffnung. Zunehmende Gehalte an Faserfraktionen, ein sich verschlechternder mikrobieller Status und eine verschlechterte Sensorik, die vermutlich durch eine Kombination verschiedener Fermentationsprodukte verursacht wurden, können für die Abnahme der Präferenz in Betracht gezogen werden.

Im Silierversuch konnte gezeigt werden, dass bereits im Falle eines kleinen Siloober-fläche-Volumen-Verhältnisses nachteilige Effekte auf die Maissilagequalität durch

geringe Verdichtung und verzögertem Verschluss vor der Einsilierung sowie bei Siloöffnung auftreten können. Darüber hinaus können beide Einflussfaktoren die Silagequalität während der aeroben Exposition nach der Öffnung (Futterentnahme) nachteilig beeinflussen. Unter den Bedingungen des Silierversuches war der Einfluss des Verschlusses von größerer Bedeutung für die Silagequalität als der der Verdichtung. Je länger ein Silo unverschlossen bleibt, desto größer ist die Minderung der Silagequalität. Unter den Bedingungen der Präferenzversuche hatte die aerobe Exposition einen stärkeren Einfluss auf die Präferenz und die Kurzzeit-Trockenmasseaufnahme als die Verdichtung und der verzögerte Verschluss.

Aus der Arbeit kann geschlussfolgert werden, dass sämtliche Maßnahmen ergriffen werden sollten, die eine hohe Verdichtung und insbesondere einen sofortigen Verschluss sicherstellen, um einen Verlust an Futterwert zu vermeiden. Die aerobe Exposition nach der Öffnung eines Silos (Futterentnahme) sollte so kurz wie möglich gehalten werden, um den stetigen Verderb der Silage und eine Reduzierung der Futteraufnahme zu verhindern.

Wie die Ergebnisse der Arbeit zeigen, ist es unerlässlich, den Einfluss von Luft von der Ernte bis zur Entnahme der Silage zu verhindern, um sowohl einen hohen Futterwert als auch eine hohe Futteraufnahme zu gewährleisten.

APPENDIX

Supplementary tables to Chapter 3

Table 16. Effect of compaction (C), sealing (S) and aerobic exposure (AE) on sensory properties at silo opening and during aerobic exposure (median (mean))

Treatment	Odour	Texture	Colour	Visible moulds	Total score	Status
Day 0 of AE (opening)						
H0	0 (0.0)	1 (1.0)	1 (1.0)	0 (0.0)	2 (2.0)	good
H2	0 (0.0)	1 (1.0)	1 (1.0)	0 (0.0)	2 (2.0)	good
H4	0 (0.0)	1 (1.0)	1 (1.0)	0 (0.0)	2 (2.0)	good
L0	0 (0.0)	1 (1.0)	1 (1.0)	0 (0.0)	2 (2.0)	good
L2	0 (0.0)	1 (1.0)	1 (1.0)	0 (0.0)	2 (2.0)	good
L4	0 (0.0)	1 (1.0)	1 (1.0)	0 (0.0)	2 (2.0)	good
			p-value (global *F* test*)			
C	NS	NS	NS	NS	NS	-
S	NS	NS	NS	NS	NS	-
C x S	NS	NS	NS	NS	NS	-
Day 2 of AE						
H0	1 (1.0)	1 (1.0)	1 (1.0)	0 (0.0)	3 (3.0)	improvable
H2	1 (1.7)	1 (1.0)	1 (1.0)	0 (0.0)	3 (3.7)	improvable
H4	2 (2.0)	1 (1.0)	1 (1.0)	0 (0.0)	4 (4.0)	bad
L0	0 (0.0)	1 (1.0)	1 (1.0)	0 (0.0)	2 (2.0)	good
L2	3 (3.0)	1 (1.0)	1 (1.0)	0 (0.0)	5 (5.0)	very bad
L4	3 (3.0)	1 (1.0)	1 (1.0)	0 (0.0)	5 (5.0)	very bad

(Continues)

Table 16. Effect of compaction (C), sealing (S) and aerobic exposure (AE) on sensory properties at silo opening and during aerobic exposure (median (mean)) **(continued)**

Treatment	Odour	Texture	Colour	Visible moulds	Total score	Status
Day 4 of AE						
H0	4 (4.0)	1.5 (1.5)	1.5 (1.5)	0 (0.0)	7 (7.0)	very bad
H2	5 (5.0)	1 (1.5)	2 (1.7)	0 (0.0)	8 (7.7)	very bad
H4	5 (4.7)	2 (2.0)	2 (2.0)	0 (0.0)	9 (8.7)	very bad
L0	1 (1.0)	1 (1.0)	2 (1.8)	0 (0.0)	4 (3.8)	bad
L2	5 (5.0)	1 (1.0)	2 (2.0)	0 (0.0)	8 (8.0)	very bad
L4	5 (5.0)	2 (2.0)	2 (2.0)	0 (1.2)	9 (10.2)	very bad
Day 6 of AE						
H0	5 (4.3)	1.5 (1.5)	2 (2.0)	0 (0.0)	8.5 (7.8)	very bad
H2	5 (5.0)	1 (1.5)	2 (2.0)	0 (0.0)	8 (8.0)	very bad
H4	7 (7.0)	2 (1.5)	2 (2.0)	0 (1.2)	11 (12.2)	very bad
L0	1 (1.0)	1 (1.0)	2 (2.0)	0 (0.0)	4 (4.0)	bad
L2	5 (5.0)	2 (2.0)	2 (2.0)	0 (0.0)	9 (9.0)	very bad
L4	7 (7.0)	2 (2.0)	2 (2.0)	0 (2.3)	11 (13.3)	very bad
	p-value (global *F* test*)					
C	<.01	NS	NS	NS	.01	-
S	<.01	<.01	NS	.05	<.01	-
AE	<.01	<.01	<.01	NS	<.01	-
C x S	<.01	<.01	NS	NS	<.01	-
C x AE	<.01	.02	NS	NS	.03	-
S x AE	<.01	<.01	NS	NS	<.01	-
C x S x AE	<.01	<.01	NS	NS	<.01	-

H0 = high compaction, sealed immediately; H2 = high compaction, sealed on day 2 post-filling; H4 = high compaction, sealed on day 4 post-filling; L0 = low compaction, sealed immediately; L2 = low compaction, sealed on day 2 post-filling; L4 = low compaction, sealed on day 4 post-filling; total score results from the sum of the individual evaluation and which characterizes the status of silage: 1 = very good, 2 = good, 3 = improvable, 4 = bad, ≥ 5 = very bad; *based on global rank test (ANOVA type statistics, $p < .05$); NS = not significant ($p > .05$); $n = 6$ per treatment and day.

Table 17. Scheme for the sensory evaluation of silages (DLG, 2004)

Sensory properties	Score
Odour	
Pleasantly acidic, aromatic, bread-like	0
Slightly alcoholic or slightly acetic acid odour	1
Strongly alcoholic or roasted smell	3
Musty or light butyric odour	5
Rank, putrid, sanious	7
Texture	
Unchanged (as the ensiled material)	0
Offended	1
Heavily offended, greasy, slimy	2
Rotted	4
Colour	
Similar to the ensiled material	0
Little changed	1
Greatly changed	2
Moulds	
Visible moulds	7

DLG = Deutsche Landwirtschafts-Gesellschaft.

Supplementary figures and tables to Chapter 4

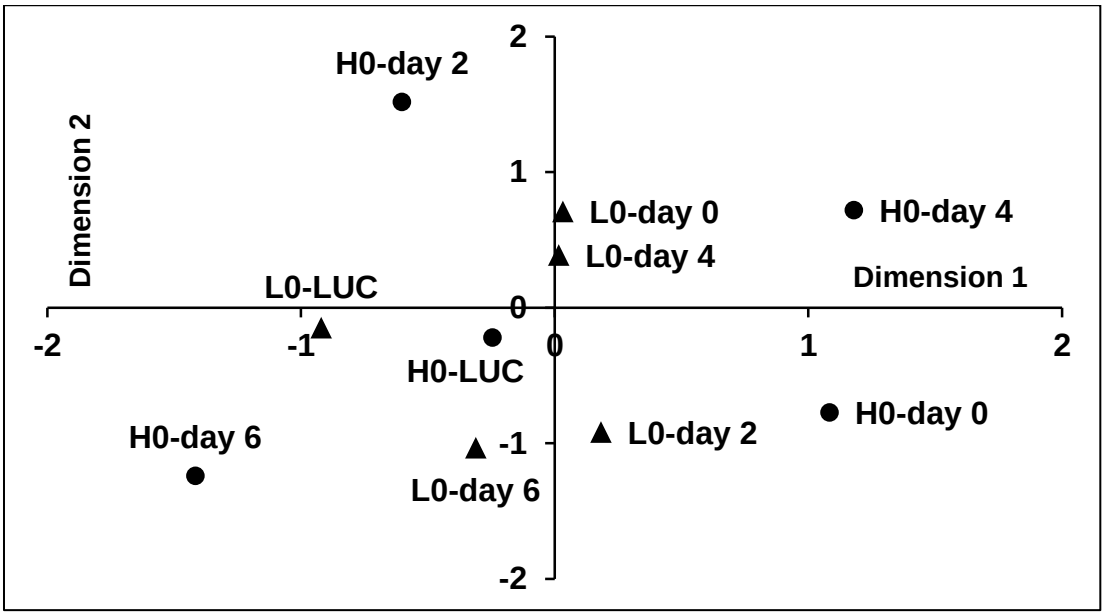

Figure 8. Multidimensional scaling of the mean preference shown by goats of the aerobically exposed silages (days 0, 2, 4 and 6 post-opening) and lucerne hay (LUC) within L0 (low compaction, sealed on day 0 post-filling) and H0 (high compaction, sealed on day 0 post-filling) after 30 min.

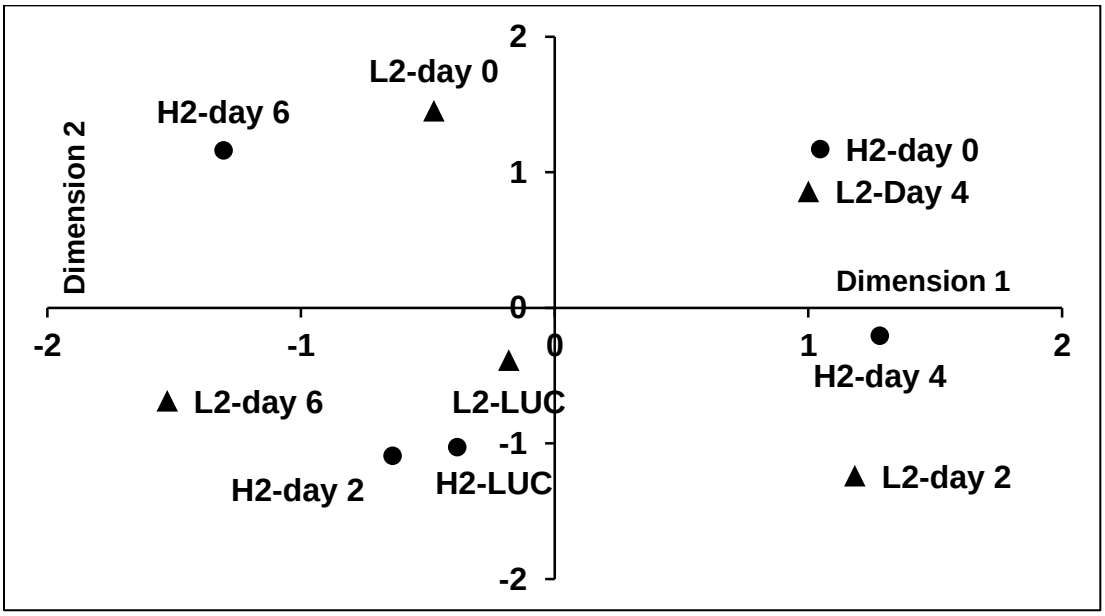

Figure 9. Multidimensional scaling of the mean preference shown by goats of the aerobically exposed silages (days 0, 2, 4 and 6 post-opening) and lucerne hay (LUC) within L2 (low compaction, sealed on day 2 post-filling) and H2 (high compaction, sealed on day 2 post-filling) after 30 min.

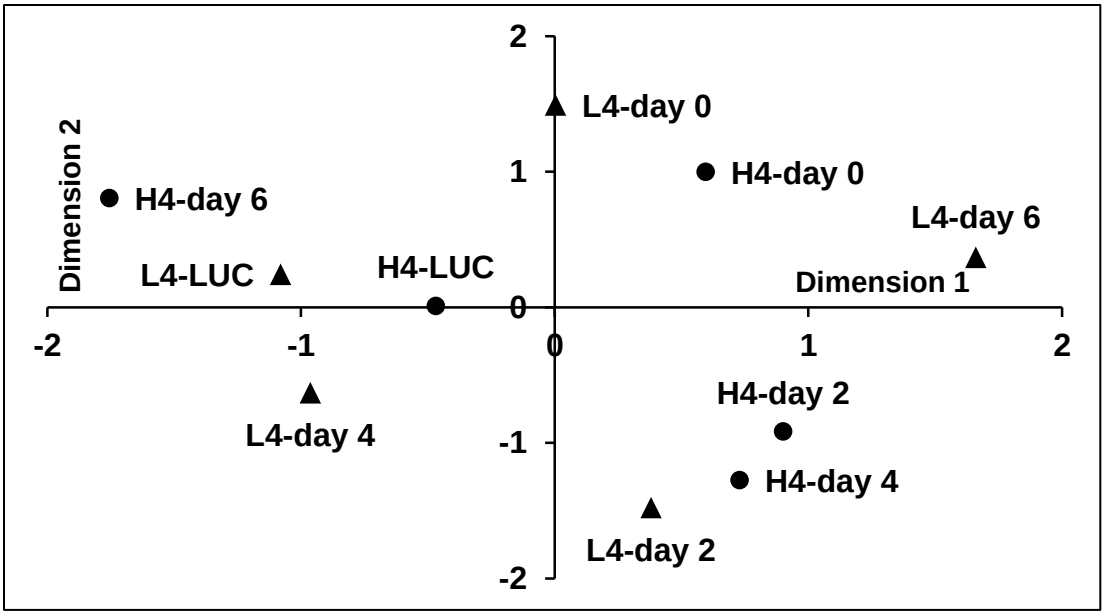

Figure 10. Multidimensional scaling of the mean preference shown by the goats of the aerobically exposed silages (days 0, 2, 4 and 6 post-opening) and lucerne hay (LUC) within L4 (low compaction, sealed on day 4 post-filling) and H4 (high compaction, sealed on day 4 post-filling) after 30 min.

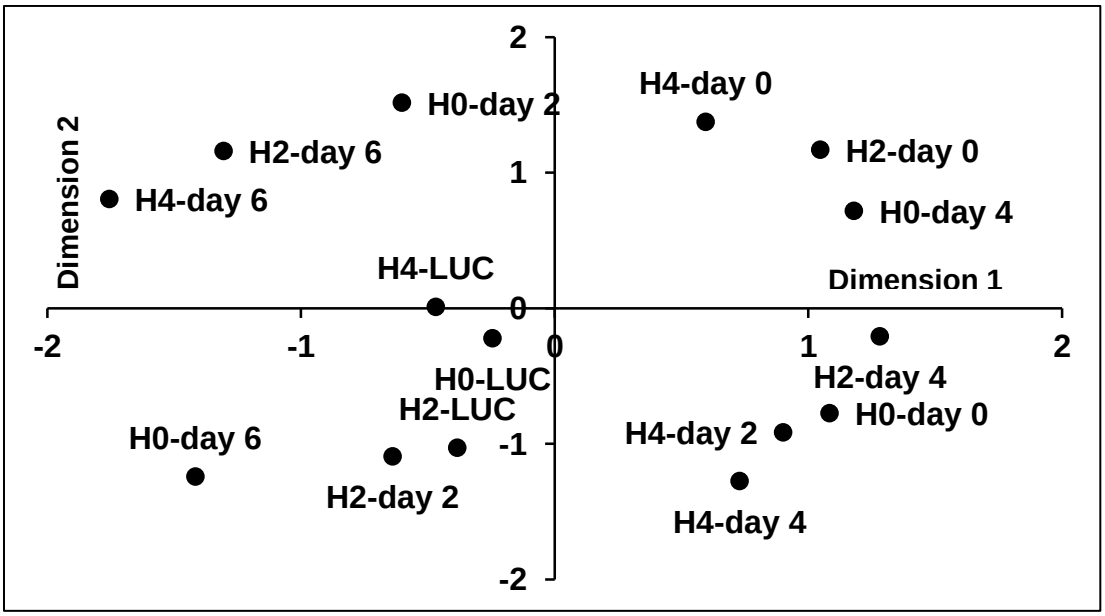

Figure 11. Multidimensional scaling of the mean preference shown by the goats of the aerobically exposed silages (days 0, 2, 4 and 6 post-opening) and lucerne hay (LUC) within H0, H2, and H4 (abbreviations see above) after 30 min.

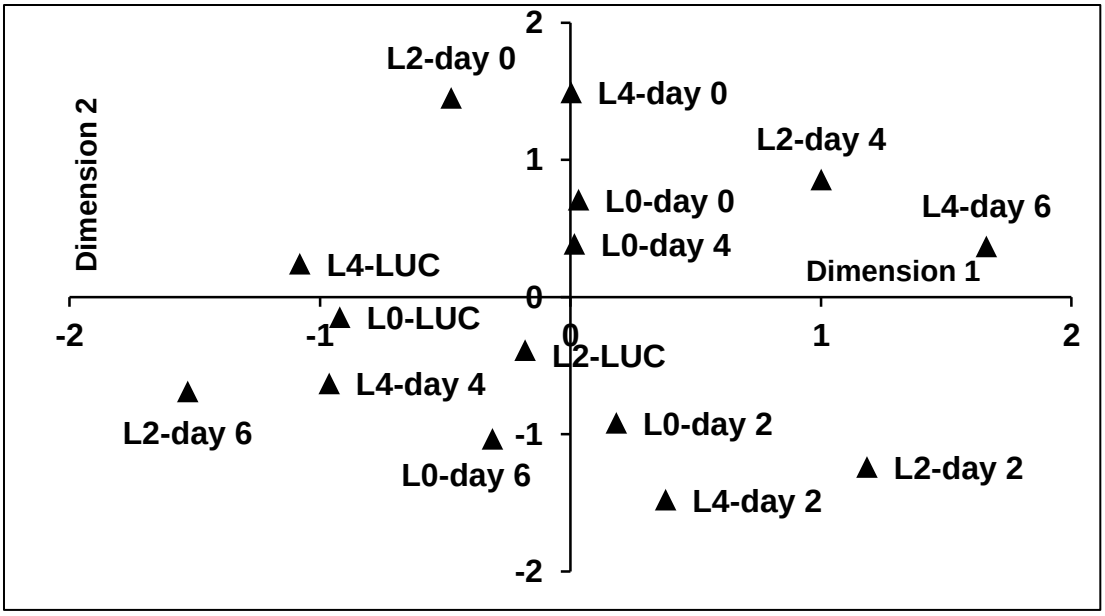

Figure 12. Multidimensional scaling of the mean preference shown by the goats of the aerobically exposed silages (days 0, 2, 4 and 6 post-opening) and lucerne hay (LUC) within L0, L2, and L4 (abbreviations see above) after 30 min.

Table 18. Forage stimulus coordinates and dimension weights by animal for the two-dimensional solution to the preference among goats (30 min)

	Treatment											
	L0		H0		L2		H2		L4		H4	
	Dim 1	Dim 2	Dim 1	Dim 2	Dim 1	Dim 2	Dim 1	Dim 2	Dim 1	Dim 2	Dim 1	Dim 2
						Coordinates (30 min)						
Forage												
Day 0	0.032	1.709	1.084	-0.773	-0.476	1.453	1.047	1.168	0.003	1.492	0.595	1.374
Day 2	1.183	-0.917	-0.602	1.516	1.184	-1.238	-0.639	-1.093	0.380	-1.477	0.901	-0.916
Day 4	1.016	0.388	1.180	0.719	1.001	0.858	1.282	-0.206	-0.963	-0.630	0.729	-1.273
Day 6	-1.311	-1.033	-1.417	-1.241	-1.528	-0.687	-1.306	1.159	1.661	0.369	-1.756	0.806
LUC	-0.921	-0.147	-0.245	-0.221	-0.181	-0.386	-0.384	-1.028	-1.081	0.244	-0.469	0.009
Goat (Dimensions weights)												
1	0.931	1.065	0.953	1.045	1.044	0.954	0.981	1.018	0.749	1.200	0.000	1.414
2	1.229	0.700	0.788	1.174	1.140	0.837	1.139	0.838	0.952	1.045	1.281	0.598
3	1.068	0.927	1.052	0.946	1.185	0.771	1.114	0.871	0.796	1.169	0.768	1.188
4	1.017	0.982	0.994	1.006	0.885	1.103	0.982	1.017	0.903	1.088	1.101	0.887
5	1.414	0.000	1.105	0.883	1.130	0.850	0.981	1.018	1.264	0.634	1.279	0.605
R^2	0.93		0.96		0.95		0.78		0.84		0.97	

L0 = low compaction, sealed on day 0 post-filling; H0 = high compaction, sealed on day 0 post-filling; L2 = low compaction, sealed on day 2 post-filling; H2 = high compaction, sealed on day 2 post-filling; L4 = low compaction, sealed on day 4 post-filling; H4 = high compaction, sealed on day 4 post-filling; LUC = lucerne hay; Dim 1 and Dim 2 = dimension one and dimension two.

Table 19. Linear relationships between silage composition (g/kg DM, unless stated otherwise) of all silages and DMI (g/3 hr)

	All silages			
Variable	Intercept	Coefficient	*p*	R^2
DM (g/kg)	5,164.56	-15.83	<.001	.28
Crude ash	2,322.25	-39.66	<.001	.08
Crude protein (CP)	2,760.09	-29.76	<.001	.09
Crude fibre	2,434.66	-7.80	<.001	.11
Crude fat	-731.14	44.91	<.001	.09
aNDFom	2,262.34	-3.68	<.001	.11
ADFom	2,281.18	-6.78	<.001	.12
ADL	1,093.19	-17.80	<.001	.09
Starch	1,066.99	-1.89	.269	.00
Non-protein nitrogen (g/kg of CP)	-256.01	2.03	<.001	.18
Ammonia-N (g/kg total N)	260.12	5.99	<.001	.14

(Continues)

Table 19. Linear relationships between silage composition (g/kg DM, unless stated otherwise) of all silages and DMI (g/3 hr) **(continued)**

	All silages			
Variable	Intercept	Coefficient	*p*	R^2
Metabolizable energy (MJ/kg DM)	-6,492.92	672.74	*<.001*	*.13*
pH	1,779.96	-274.98	*<.001*	*.22*
Water-soluble carbohydrates	348.57	12.67	*<.001*	*.10*
Lactic acid	314.18	6.87	*<.001*	*.16*
Acetic acid	325.67	33.81	*<.001*	*.04*
Butyric acid	621.05	-82.33	*<.007*	*.01*
Ethanol	400.23	22.98	*<.001*	*.17*
2-Butanol (mg/kg DM)	591.01	-0.16	*.237*	*.00*
n-Propanol (mg/kg DM)	569.02	0.30	*.566*	*.00*
Ethyl acetate (mg/kg DM)	409.84	0.48	*<.001*	*.17*
Ethyl lactate (mg/kg DM)	408.55	0.83	*<.001*	*.18*
Yeasts (log_{10} cfu/g FM)	1,177.88	-83.38	*<.001*	*.08*
Moulds (log_{10} cfu/g FM)	986.56	-122.96	*<.001*	*.14*
Aerobic mesophilic bacteria (log_{10} cfu/g FM)	1,318.05	-103.39	*<.001*	*.18*
Compaction	340.12	1.10	*.215*	*.00*
Sealing	513.27	31.31	*.004*	*.02*
Day of aerobic exposure	823.89	-82.67	*<.001*	*.22*

aNDFom = neutral detergent fibre assayed with heat-stable amylase and expressed exclusive of residual ash; ADFom = acid detergent fibre expressed exclusive of residual ash; ADL = acid detergent lignin; cfu = colony forming units; FM = fresh matter, microbial data obtained from fresh silages; *n* = 480.

Table 20. Stepwise regression analysis of silage composition (g/kg DM, unless stated otherwise) and DMI (g/30 min) within the treatments and for all silages

Treatment	Intercept	Coefficient	Variable	*p*	R^2
L0	1,025.66	1.56	2-Butanol	<.001	
		-29.33	Crude ash	.017	.32
H0	-1,600.25	-47.05	Acetic acid	.004	
		8.09	2-Butanol (mg/kg DM)	<.001	.32
L2	3,562.11	-13.89	Crude fibre	<.001	.37
H2	1,918.57	-3.56	aNDFom	<.001	.22
L4	2,282.09	-42.99	Crude ash	<.001	.20
H4	-14023.04	-36.99	Crude fat	.110	
		1,476.04	ME (MJ/kg DM)	<.001	.42
All silages	-1,078.91	-93.97	pH	.013	
		-25.23	Acetic acid	<.001	
		-0.96	*n*-Propanol (mg/kg DM)	.003	
		3.84	ADFom	<.001	
		-12.18	ADL	<.001	
		0.81	NPN (g/kg of CP)	.001	
		5.03	Starch	<.001	
		-73.03	Mould (log_{10} cfu/g FM)	<.001	.32

L0 = low compaction, sealed on day 0 post-filling; H0 = high compaction, sealed on day 0 post-filling; L2 = low compaction, sealed on day 2 post-filling; H2 = high compaction, sealed on day 2 post-filling; L4 = low compaction, sealed on day 4 post-filling; H4 = high compaction, sealed on day 4 post-filling; aNDFom = neutral detergent fibre assayed with heat-stable amylase and expressed exclusive of residual ash; ME = metabolizable energy; ADFom = acid detergent fibre expressed exclusive of residual ash; ADL = acid detergent lignin; NPN = Non-protein nitrogen; CP = crude protein; cfu = colony forming units; FM = fresh matter; microbial data obtained from fresh silages; *n* = 80 per treatment; *n* = 480.

ACKNOWLEDGEMENTS

At this point, I would like to thank everyone who supported me throughout the thesis.

A big thank you goes to Dr. Kirsten Weiß and Prof. Karl-Heinz Südekum for the impeccable support and cooperation. I would like to thank you for encouraging my research and for allowing me to grow as a research scientist. I would also like to thank Dr. Katrin Gerlach, who gave me a lot of help, advice and did a great groundwork.

I would like to thank Mr. Andreas Köster-Schmücker and his team for the help during maize harvest and the transport of the silos as well as Mr. Andreas Frenker and Mr. Horst Auerbach for their help.

Many thanks go to Mr. Walter Diefenthal, Mr. Viktor Braun and Mr. Heinz Biörnsen for the great cooperation in the preference trials and the keeping of the goats.

I would like to sincerely thank the whole team of the Corporate Analytical Laboratory of the Humboldt-University of Berlin for the support in analytics.

I would like to thank you Ms. Nadja Wahl and all student assistants of the Institute of Animal Science for the help in laboratory analysis and for the active support in the preference trials.

I would like to thank Ms. Rebecca Scherer and Ms. Annika Wille.

I would like to thank Dr. Bärbel Kroschewski for excellent statistical advice at all times. A special thank you goes to the family of Helmut and Monika Schmidt from Bergisch-Gladbach for the wonderful accommodation and the delicious food.

With all my heart, I would like to thank my beloved wife, who has always strengthened my back and demonstrated an immeasurable commitment. I especially thank my mum and dad for the great trust. I would like to sincerely thank my children, grandparents, siblings, relatives and friends for the great support during this time.

PUBLICATIONS

Brüning, D., Gerlach, K., Weiss, K., and Südekum, K.-H. (2015). Effect of aerobic exposure before and after ensiling on maize silage quality. In *Proceedings of the 17th International Silage Conference*, *Piracicaba*, *São Paulo*, *Brazil*, *2015* (p. 182).

Brüning, D., Gerlach, K., Weiss, K., and Südekum, K.-H. (2015). Einfluss aerober Lagerung vor und nach der Silierung auf die Qualität von Maissilage. *VDLUFA-Schriftenreihe*, 71, 561–569. (in German).

Weiss, K., Brüning, D., and Kalzendorf, C. (2015). The influence of air ingress and additives on fermentation pattern, volatile organic compounds (VOC) and aerobic stability of maize silage. In *Proceedings of the 17th International Silage Conference*, *Piracicaba*, *São Paulo*, *Brazil*, *2015* (p. 334).

Brüning, D., Gerlach, K., Weiss, K., and Südekum, K.-H. (2018). Effect of compaction, delayed sealing and aerobic exposure on maize silage quality and on formation of volatile organic compounds. *Grass and Forage Science*, 73, 53-66.

Brüning, D., Gerlach, K., Weiss, K., and Südekum, K.-H. (2018). Effect of compaction, delayed sealing and aerobic exposure on forage choice and short-term intake of maize silage by goats. *Grass and Forage Science*, 73, 392-405.