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Contents

Ensiling total mixed rations - an innovative procedure

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Introduction

Total mixed ration (TMR) is composed of fiber-rich foods (silage, hay, fresh grass, fibrous waste, etc.) and concentrates (cereals, industrial co products, minerals, supplements and additives). Typically, these foods are mixed in a balanced way on the farm and immediately provided to animals as complete diets for a uniform intake. As it consists of quickly damageable moist food, TMR is prepared several times every day.

TMR use is hampered on farms with a limited workforce, machinery, purchase scale of inputs and even technical expertise to balance rations. In some cases, the producer may be faced with high costs for a poorly made and inadequately prepared ration.

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A recent alternative to meet the needs of these producers is TMR silage, balanced to the desired production levels of cows. For that purpose, this feed is produced in processes that involve various techniques, and ensiling in small units (bundles or bags) is the most viable (Wang & Nishino, 2008; Hu et al., 2015). Ensiled TMR is preserved for long periods and can be marketed, transported and stored on the farm.

Although at first, costs of ready diet seem a limiting factor when compared to preparation costs of on the farm, indirect advantages can be important factors in the decision-making process to outsource production, such as convenience, reduced workforce and implements, and greater ease in feed handling.

The Centro de Pesquisas em Forragicultura (CPFOR) of the Federal University of Paraná has evaluated this technology in terms of diet quality and conservation of nutrients during storage. In this study, we present partial data and reflections on the adoption of this technology.

Ensiling TMR

Ensiling TMR is not a recent practice (Nishino et al., 2003); however, it has been little studied in Brazil, despite the growing interest of the industry and rural producers.

In Japan (Wang & Nishino, 2008), Iran (Abdollahzadeh et al., 2010), Israel (Weinberg et al., 2011), Finland (Seppälä et al., 2012) and China (Hu et al., 2015), this practice has been evaluated to optimize the use of humid waste and coproducts in ruminant rations. In Brazil, TMR ensiling allows marketing ready-to-ruminant feed, in addition to a better use of moist coproducts. In some cases, outsourcing feed production allows the cattle breeder a better management of the farm, maximizing time for other activities.

Ensiled TMR can be formulated specifically for each animal category (dairy cows, dry cows, heifers, calves, cattle, sheep, goats, etc.) and includes many ingredients in the diet, such as moist fiber-rich products and coproducts (Weinberg et al., 2011), as well as concentrates, minerals and additives. This technology allows feed formulation of excellent quality, with less variability in composition and better stability in nutrient consumption. Additionally, it decreases costs of harvesting and transporting fresh forage, and it requires less investment in infrastructure, machinery, and labor force for ration preparation.

At first, Brazilian producers have two possibilities to use this technique:

1. Farm production: Ensiling TMR on the farm allows optimizing the use of moist coproducts (brewery residue, bagasse from the juice industry, distillery waste, etc.), concentrating activities and labor force, ensuring better food standardization. This allows associating products with different fermentation potential, correcting levels of moisture and soluble carbohydrates, and favoring the fermentation process, in addition to the nutritional balance of TMR. TMR can be made in bunker silos, with overlapping layers of different ingredients, most homogeneously possible. Studies are needed to construct technical recommendations to apply this technology on farms.
2. Industrial production of TMR: TMR has already been produced on a commercial scale in Brazil, and the market is growing markedly. Investments in large mixers and structure of packing, storage and transportation are required. Similarly, permanent consulting of a nutritionist is required to ensure nutrient uniformity and adequate levels, even with the use of different coproducts, in highly variable storage time.

Outsourcing feed production for ruminants (dairy and beef) aims to reduce the need for labor force and investments in the farm while offering products with standardized quality, and, in many cases, reduce production costs on farms with management difficulties.

The CPFOR-UFPR has worked to obtain technical data to optimize ensiled TMR production at commercial units (50 to 1000 kg), as well as on the perception of producers regarding the adoption of this technology.

TMR shelf life: additives and ensiling

First, tests evaluated preservation strategies of TMR in aerobic environments, compared to ensiling, in order to determine the maximum period for its use without compromising quality, investigating the real need of the ensiling process (Table 1). Doses of additives natamycin and buffered propionic acid were assessed. TMR was composed of sugarcane bagasse (bulky), ground corn, high moisture corn gluten feed (GoldenMill®), soybean meal, molasses, urea and minerals (18.7% CP; 80% TDN) and formulated to meet nutritional requirements of dairy cows producing 25 L of milk per day (NRC, 2001).

The TMR was prepared manually and placed in plastic buckets (5 kg per bucket, with 5 replicates) with dataloggers inside to collect temperature every 30 min for up to 10 d. The tests were carried out in a room with controlled temperature (25 ± 1 °C).

The results showed a technical and economic superiority of ensiling to maximize TMR stability exposed to air. The use of additives, preservatives, such as buffered propionic acid (BPA), showed reasonable potential to improve TMR stability; however, below the values recorded for ensiled TMR, which did not display heat even after 140 h of exposure. The addition of BPA generated a strong sulphur smell from the second day onward, probably due to the sulfurous amino acids breakage.

Table 1. Aerobic stability (shelf life) of TMR[1] with preservatives or ensiled with *Lactobacillus buchneri*

	Treatments[1]								
Variables	Control	N8	N12	PA5	PA10	PA15	LB	SEM	P[4]
Dry matter (%)	59.6[a]	58.9[a]	58.6[a]	43.4[b]	44.0[b]	43.9[b]	56.8[a]	0.42	P<0.01
Initial pH	4.80	4.92	4.96	4.42	4.42	4.48	4.19	0.18	NS
Stability[3] (hours)	42[c]	46[c]	44[c]	77[bc]	95[b]	106[b]	>140[a]	1.77	P<0.01
Highest temperature (°C)	49.9[b]	50.2[b]	49.5[b]	43.9[b]	42.1[b]	41.5[b]	24.5[a]	1.12	P<0.01
Accumulated temp. (°C)	183.1[b]	173.1[b]	173.8[b]	107.2[b]	77.4[b]	67.6[b]	0[a]	2.13	P<0.01
DM losses (%)	12.6	14.1	14.2	16.2	15.9	14.1	0.42	2.77	NS

[1]TMR composed by sugarcane bagasse, corn grain, high moisture corn gluten feed, soybean meal, molasses, urea, and minerals.

[2]Control – no additives; N8 – natamycin, 8 g t^{-1} wet basis (WB); N12 – natamycin, 12 g t^{-1} WB; PA5 – buffered propionic acid (Profresh Plus TMR & Feed Stabilizer - Micron Bio-Systems Ltd – UK - BPA), 5 g t^{-1} WB; PA10 – BPA, 10 g t^{-1} WB; PA15 - BPA, 15 g t^{-1} WB; LB – TMR ensiled with *Lactobacillus buchneri* ($1x10^5$ CFU g^{-1} WB).

[3]Stability – hours to reach 2°C above room temperature.

[4]Non significant.

Additives and storage time of ensiled TMR

From the decision to ensile TMR as the storage procedure, a new test (Table 2) was developed to assess microbial inoculants and storage period on quality parameters, microbiology and stability of ensiled TMR.

Liu et al. (2016) evaluated the addition of L. plantarum and fibrolytic enzymes on the fermentation profile of ensiled TMR and observed that all treatments, including the control, presented proper fermentation, although additives allowed a lower pH and higher lactic acid concentration. Nishino and Hattori (2007) assessed the inoculation of lactic acid bacteria on TMR stability with moist coproducts, ensiled and exposed to air. The authors observed good resistance to deterioration even without the use of lactic acid bacteria. For our local conditions, the evaluation of additives is necessary to measure cost-benefit for recovery and stability of ensiled TMR.

TMR was formulated for dairy cows producing 25 L of milk per day (NRC, 2001) and was composed of corn silage, wilted ryegrass silage, ground corn, soybean meal, corn gluten meal (Promill®), urea, mineral core and sodium bicarbonate (15.5% PB; 82.6% TDN). The ingredients were homogenized for 7 min in a vertical mixer and immediately ensiled in experimental silos (8.8 L). Inoculants based on Lactobacillus plantarum or L. buchneri were applied at the dosage $1x10^5$ CFU g^{-1} WB. After 15 or 60 days of storage, the silos were opened and TMR exposed to air for 216 h in a room with controlled temperature (25 ± 1°C), with temperature monitoring every 30 min, and the pH every 24 h. Samples were collected at different stages of the process to evaluate bromatological composition, fermentation end-products, microbiology, and digestibility.

The TMR based on corn (45% DM) and wilted ryegrass silages (19.5% DM) showed good fermentation pattern, regardless of the use of additives. The longer storage (60 d) led to increased gas production during fermentation, more pronounced pH reduction and marked increase in ration stability after opening the silos. No heat was observed for TMR stored for 60 d, during 9 d of aerobic exposure, remaining with smell and color typical of good quality feed. Similarly, the pH of TMR stored for 60 d remained stable throughout the storage period (Figure 1).

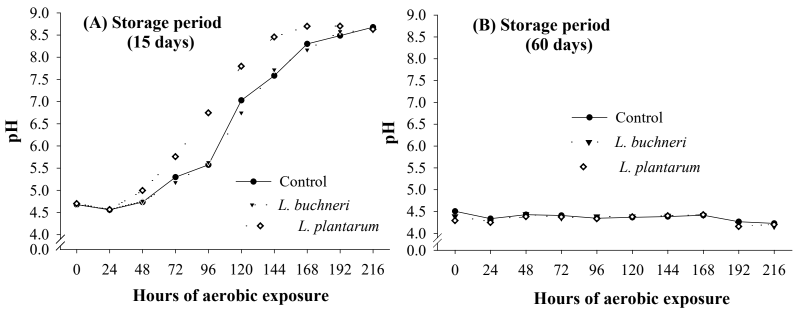

Figure 1. Daily pH evaluation of ensiled TMR (15 or 60 days of storage) after aerobic exposure

TMR stored for 15 d showed lower levels of lactic acid (25.0 g kg^{-1} DM) and acetic acid (23.7 g kg^{-1} DM) when compared to TMR stored for 60 d (37.4 and 26.8 g kg^{-1} DM, respectively). There was no effect of additives on the concentration of fermentation products. Contrary to expectations, ensiled TMR did not influence DM digestibility nor the ammoniacal nitrogen content (Table 2).

The data show that the minimum fermentation time of 15 d is insufficient to ensure good TMR stability, and industries interested in marketing this technology should consider this fact according to the profile of TMR purchasers. Many times, TMR is produced, packed and transported to the customer on the same day, and is more prone to deterioration if the unit is opened and not fully used on the same day. Companies that sell TMR must think of a stock-flow that allows longer fermentation or refermentation, before transporting it to the consumer.

Effects of sealing holes

For logistic and economic viability of ensiled TMR, packing should provide low cost in relation to the amount of feed packed. Thus, the use of cylindrical bales of up to 1100 kg, wrapped with layers of plastic film, appears to better cost-effective than packing in smaller units. Eventually, during transportation and storage, holes in the plastic film can occur, which is undesirable, but relatively common and often overlooked.

In this sense, a trial was conducted to evaluate the effect of holes in the plastic film on quality, temperature and microbiology of ensiled TMR in cylindrical bales. Eight cylindrical bales (1000 kg each) were made with the same blend of TMR described in the previous test (Orkel – MP 2000 Compactor). In the center of every bale, two programmed data loggers were inserted to collect temperature every 15 min. The bales were stored in an open place, subjected to weather factors, for 60 d.

On the tenth day after the preparation, two holes of 25 cm^2 each were made in four of the eight bales, with the removal of the plastic film (Silotite 100 μm – 7 layers), on opposite sides of the bales. The silos remained in the same location. After 50 d, the bales were weighed and manually cut in half, leaving the surface clear for temperature measurement through thermographic camera images (Fluke Ti25). The data loggers were removed and samples were collected to evaluate DM content, pH, bromatological and microbiological composition (Table 3).

Table 3. Variables related to TMR[1] ensiled in round bale silos with or without holes in the plastic film

Variables[2]	Holes	No holes	Mean	SEM	P-value
Dry matter recovery (%)	96.5	98.5	97.5	0.97	0.129
DM (%)	41.4	41.8	41.5	0.08	0.063
IVDMD (%)	84.6	84.5	84.5	0.36	0.968
$N\text{-}NH_3$ (% TN)	17.6	17.5	17.5	0.10	0.489
Lactic acid bacteria (log CFU g^{-1})	6.9	6.9	6.9	0.23	0.8825
Yeasts (log CFU g^{-1})	2.5	2.5	2.5	0.26	0.9380
Molds (log CFU g^{-1})	2.5	1.8	2.1	0.86	0.3076
pH	4.41	4.23	4.32	0.03	0.016
Average border temperature (°C)	23.9	22.4	23.1	-	-
Average center temperature (°C)	23.4	21.9	22.6	-	-

[1]TMR composed by corn silage, wilted ryegrass silage, grounded corn, soybean meal, corn gluten feed, urea, minerals, sodium bicarbonate.

[2] IVDMD - *in vitro* dry matter digestibility; TN – total nitrogen; DML – dry matter losses.

Unlike expected, the presence of two holes (25 cm² each) did not cause significant damage to TMR quality. Of all the variables studied, there was only a slight reduction in the DM amount and increased pH. DM recovery was similar among the silos with and without holes.

The infrared thermography was unable to identify the heating zones at the edge of the bales where the holes were located. Average temperatures of central areas and edges were very similar at the time of silos opening.

Similarly, the monitoring of the internal temperature of silos (Figure 2) did not show any sign of heat due to the holes in the plastic film.

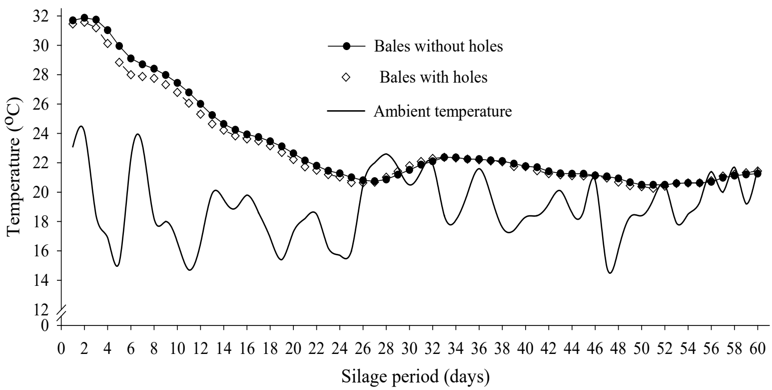

Figure 2. Room temperature and average temperature inside round bale TMR silos with or without holes in the plastic film

Although air imput in ensiled materials should be extensively countered, the presence of small holes in the plastic film of cylindrical bales of TMR does not seem to cause great impact on the overall quality of the material. This problem is unlikely to affect the performance of the animals, probably due to the high density of the silage material (370 kg DM m^{-3}) in this system.

Consumer perception

In addition to research work, the CPFOR team conducted surveys with farmers in order to describe the perception of potential and effective users on the adoption of this technology.

Initially, an online questionnaire was made available in a portal for milk producers (www.milkpoint.com.br) about the possibility of acquiring ready-to-use TMR. We obtained 175 responses of 16 Brazilian states. We highlight the following points:

- 47% of the respondents never heard about TMR marketing;

22% heard something or were unsure; 31% acknowledged knowing this possibility.

- 51% of the respondents admitted that the technology could be used on their farms; 10% would not use it, and 39% said they might use it.
- Of those who showed some interest in acquiring TMR (n = 147), 28% said they would use it for up to 3 months; 43% said they could use it between 3 and 6 months, and 29% said they would use it for more than 6 months in the year.

When asked about how much they were willing to pay for 1000 kg of balanced TMR for 25-L cows (without freight), the respondents reported the following (converted to L of milk per average values for the period – CEPEA/USP):

- up to 134 kg of milk – 36%
- up to 268 kg of milk – 42%
- up to 401 kg of milk – 15%
- above 401 kg of milk – 7%.

The results show that the marketing of ready-to-use TMR is still little known in Brazil and that there is good potential to expand the market of diets. Depending on the final cost of the product, the interest in outsourcing part of animal feed production is quite significant.

In a second survey, the CPFOR team visited 12 farms in the states of Paraná (n = 9) and São Paulo (n = 3) that are already buyers of ensiled TMR for feeding dairy cows. A questionnaire with 19 questions was applied to each property, followed by an interview to collect opinions of owners/managers of the farm.

The properties visited were quite variable, with herds between 19 and 160 lactation cows. Of the 12 properties, five used less than a bale (1000 kg) per day; three used up to two bales; and four used more than two bales per day. Seven of the 12 properties kept cows in the pasture, in addition to receiving TMR. Table 4 presents the data compiled on some of the questions.

Table 4. Mean, standard deviations (SD), maximum (Max.) and minimum (Min.) values of on-farm survey data

	Mean	SD	Max.	Mín.
For what level of production (kg of milk per day) the TMR you have bought is balanced?	30	6.7	45	25
How many kg of TMR are offered per animal per day?	33.3	12.3	53	15
What is the average productivity of the cows (kg of milk/day) fed with TMR silage?	23.5	6.7	35	13
How many months of the year do your animals consume TMR silage?	10.0	3.8	12	03

All producers reported checking the integrity of plastic film at the reception time of TMR. Eight of 12 producers have already had problems with holes in the plastic film. Producers fix the bales with adhesive tapes and give priority in the use of these bales.

When asked about the advantages of buying ready-to-use TMR, the main arguments presented were: 1) decrease of labor force; 2) convenience; 3) less investment in equipment (tractor, mixer wagon, silo) and fuel; 4) constant feed quality the whole year; 5) absence of losses; 6) use of farmlands for other activities. Some producers reported knowing the real diet costs when buying TMR and the focus is on milk production. Moreover, there are no risks regarding the weather, and risks are transferred to third parties.

About disadvantages of purchasing TMR, producers reported lack of competition in the market, with few suppliers and little chance of negotiating values. They argue that the distance between the farm and the TMR production company raises freights and complain about the lack of uniformity in rations, with an eventual decrease of milk consumption and production with changes of TMR batches.

Finally, 11 of 12 producers declared the interest to continue acquiring ensiled TMR for animal nutrition.

Altogether, these perceptions show a great potential for expansion of the market of ready-to-use diets. Brazilian and multinational companies are watchful for this potential market and new suppliers will be arriving in the next few years. Certainly, ensiled TMR will be the conservation option for most of these enterprises.

Conclusion

In order to be marketed, total mixed rations need great stability period, and ensiling is the technique with greater viability. The use of additives seems to change slightly quality and preservation of these ensiled feeds. Marketing of ensiled TMR in Brazil is still recent, but with a great market potential to meet different profiles of producers.

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An update of particle size and effective NDF in maize silage-based rations

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Abstract

Optimization of particle size and effective neutral detergent fiber are key aspects in dairy cattle nutrition. While long particle size provides sufficient physical effectiveness to maintain chewing activity and physiological rumen function, the thereof resulting limitations of feed intake can prevent modern dairy cows from covering their energy requirements. Maize silage is a major forage crop in ruminant diets due to the high content of digestible fibre and starch. To improve dry matter intake, preservation characteristics and avoid selective feed intake, maize silage is typically chopped finely. However, besides positive effects on feed intake and milk yield, decreasing the particle size is generally associated with reduced chewing activity and salivary secretion, thereby impairing ruminal fermentation

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conditions, which finally can lead to reduced ruminal pH, fiber digestibility, and milk fat percentage. Therefore, it is important to determine the optimal theoretical chop length of maize silage, to improve ensiling characteristics and animal performance without adversely affecting ruminal digestion and animal health. Efforts have been made to investigate the effects of forage particle size on animal health and productivity. This review attempts to characterize the effect of forage particle size in maize silage based diets of cattle on feed intake, nutrient digestibility, ruminal fermentation, and health as well as productivity.

Introduction

Maize silage (MS) is ideal forage in the diets of dairy and beef cattle due to its high starch, high-yielding and –ensiling properties as well as relatively high content in potentially digestible fibre. The high starch content coupled with the high content of potentially digestible fibre enable MS reaching energy contents of about 6.5-7 MJ NEL/kg dry matter (DM). Moreover, including MS in total mixed rations (TMRs) adds moisture and consistency, thereby reducing selective feed consumption (Martin et al. 2008). On the other hand, however, besides having low contents of protein and minerals, intensive chopping of MS leads also in reduced physically effective neutral detergent fiber (peNDF). In the practice, the MS is typically chopped at 5-10 mm theoretical chop length (TCL) with additional kernel processor of 2 mm to allow extensive degradation of kernel starch. Lower TCL decreases the contents of peNDF, as well as an increased degradation of starch, might be useful in enhancing feed intake and energy output but negatively affects rumen health and functioning (Nasrollahi et al. 2015, 2016). On the other hand, excessive TCL leads to increased sorting behaviour and decreases feed intake (Leonardi and Armentano 2003). In addition, MS is commonly not fed as single forage in the diet. Thus, it is highly important to take this into account because increased sorting leads to important shifts in the nutrient intake (Kononoff and Heinrichs 2003). The aim of this review article is to provide an overview of the effects of particle size of MS in the feeding of dairy cattle.

Particle size of maize silage and peNDF

In general, a high range in the chemical composition and physical characteristics of MS exists. Depending on the cultivar, time of harvest, applied technique (TCL, kernel processing, packing, tight-closed storage) and ensiling properties, a wide range of silage qualities can occur.

Figure 1. Shows the chemical composition of different qualities of MS. Overall, the fiber content is inversely related to the starch content, with a range from 36 to 54% for neutral detergent fiber (NDF) and 15 to 31% for starch, respectively.

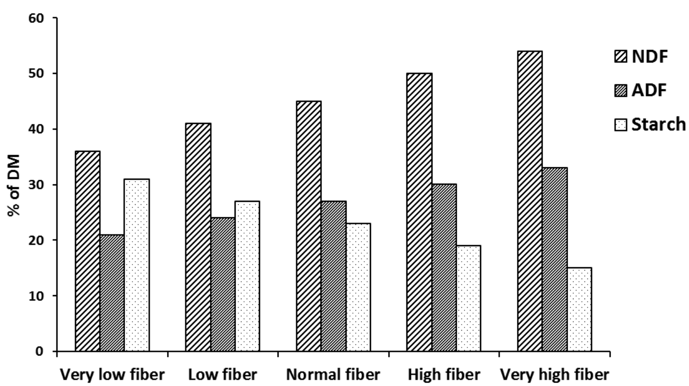

Figure 1. Chemical Composition of MS varying in the fiber content (adapted from Mertens 2002 and Martin et al. 2008)

Besides providing sufficient NDF, it is well-known that cattle require a minimum amount of fiber that is of adequate particle size to stimulate chewing and to obtain a healthy ruminal environment that mitigates the risk of digestive upsets and optimizes fermentation (Beauchemin and Yang 2005). In this regard, the concept of peNDF has been widely accepted as an appropriate measure to take the physical (i.e., the particle size) as well as chemical properties of the fiber (i.e., the NDF content) into account (Zebeli et al. 2008). The particle size can be determined with the Penn State Particle Separator which commonly contains three sieves to determine four fractions: large particles (> 19 mm), medium particles (8-19 mm), fine particles (1.18-8 mm) and very fine particles (<1.18 mm; Kononoff et al 2003a). The peNDF can be determined as $peNDF_{>8}$ (proportion of particles >8 mm multiplied with the NDF content) or $peNDF_{>1.18}$ (proportion of particles >1.18 mm multiplied with the NDF content). Adequate peNDF in diets is essential for proper digestion of the diet and maintenance of healthy rumen conditions and milk fat production (Zebeli et al. 2008).

In general, the recommendation of $peNDF_{>8}$ in dairy rations ranges from 16 to 20% of DM, depending on starch content and DMI level (GfE, 2014). However, as the concentrate does not contribute to this fraction, the peNDF content of the forage portion has to be proportionally higher.

Because MS is often chopped finely or processed through rollers, its $peNDF_{>8}$ is typically 70% of NDF; however, this can vary from 30 to 90% (Kononoff et al. 2003b, Kononoff and Heinrichs 2003, Soita et al. 2005, Yang and Beauchemin 2006, Bhandari et al. 2007, Zebeli et al. 2009). Table 1 summarizes the particle size distribution, NDF content and resulting peNDF values obtained from feeding trials with high and low TCL of MS. Enhancing the TCL from on average 6.6 ± 2.27 mm to 20.9

± 4.43 mm caused a more than 3-fold increase in the proportion of large particles, mainly at the expense of fine particles, while no change in the proportion of medium and very fine particles occurred. In result, the $peNDF_{>8}$ was enhanced due to the coarser chopping technique, while the $peNDF_{>1.18}$ remained similar.

Table 1. Particle size distribution, NDF and peNDF of finely or coarsely chopped maize silage (obtained from the studies of Kononoff et al. 2003b, Kononoff and Heinrichs 2003, Soita et al. 2005, Yang and Beauchemin 2006, Bhandari et al. 2007, Zebeli et al. 2009)

Item	Fine	Coarse
Particle fraction, %		
>19 mm	5.5 ± 4.44	18.6 ± 9.30
8-19 mm	54.2 ± 15.14	57.6 ± 3.5
1.18-8 mm	36.8 ± 15.05	21.3 ± 7.9
<1.18 mm	3.5 ± 2.75	2.5 ± 1.74
NDF, %	44.3 ± 6.12	45.3 ± 7.03
$peNDF_{>8}$, %	26.1 ± 8.27	34.6 ± 7.06
$peNDF_{>1.18}$, %	42.7 ± 5.78	44.2 ± 6.81

The general recommendation for particle size distribution of MS is that the main fractions should be retained on the 8 and 1.18 mm sieves (i.e., 45-65 and 30-40%, respectively), while only about 3-8% of the particles should be large (>19 mm), and less than 5% should be very fine (<1.18 mm, Heinrichs and Kononoff 2002). Thus, enhancing the TCL led to an unfavourable shift in the particle size distribution, which might enhance feed sorting by the cows.

In general, the amount of peNDF required to maintain rumen health also depends on the amount of rapidly degradable carbohydrates (mainly starch) in the rumen (GfE, 2014). The high starch content of MS is one of the most important characteristics compared to other forages, such as alfalfa silage, grass silage or hay. If the kernels are immature and adequately processed the starch in MS can be readily fermented in the rumen (Martin et al. 2008). While this enhances the energy intake, the excess of rapidly fermentable starch may lead to a decline in ruminal pH, thereby reducing DMI and fiber digestion (Nasrollahi et al 2015). Thus, a reduced TCL concomitant with an increased ruminal degradability of starch might predispose causes to digestive upsets. Therefore, it is not recommended to offer MS as the sole forage in dairy rations, then rather comprising between 1/3 and 2/3 of the forage portion (Martin et al 2008).

Effects on production performance

A recently conducted comprehensive meta-analysis of 46 studies on the effect of forage particle size (FPS) on production performance and nutrient digestibility revealed that decreasing the FPS enhanced the DMI (>0.5 kg/d) and NDF intake (Nasrollahi et al. 2015). This indicates a less rumen-filling effect of fine particles and/or enhanced palatability of the feed (Nasrollahi et al. 2015; Zom et al. 2012). In general, the increased DMI positively affects energy intake in high-producing dairy cows and therefore might help to decrease the energy deficit at the onset and peak of lactation. However, it has to be stated that the enhancing effect of decreased FPS on DMI was only pronounced in diets containing high levels of forage (>50% of DM), whereas the opposite effect was noticed at lower levels of forage in the diet. Thus, in diets containing high concentrate levels it cannot be recommended to further decrease the TCL as a decreasing rumen pH, resulting from the lower peNDF content, might even cause a decline in DMI (Zebeli et al. 2008).

Further meta-regression analysis revealed that in rations containing mainly MS as forage source, decreasing the FPS had no effect on the intake of NDF. Table 2 summarizes studies conducted only with diets based on MS as forage. When the TCL was lowered from on average 23.3 ± 8.22 mm to 10.1 ± 6.78 mm, the DMI was increased by on average 0.6 kg/d, with a range from -1.1 kg to +2.3 kg DMI/d.

Table 2. Effect of feeding maize silage with shorter theoretical chop length (TCL) on dry matter intake (DMI), milk yield as well as milk fat and protein concentration (differences are expressed as absolute differences of means measured in cows receiving the shortest TCL vs. the longest TCL)

DMI, kg	Milk yield, kg	Milk Fat %	Milk Protein %	Reference
-0.8[1]	*+0.6*	*+0.05*	*+0.04*	Clark and Armentano 1999, year 1
+0.7	+1	*+0*	*+0,03*	Clark and Armentano 1999, year 2
+0.1	*+0.4*	*-0.01*	*-0.02*	Bal et al. 2000
+1.1	*-0.3*	*-0.04*	*-0.01*	Schwab et al. 2002
-0.7	*+0.6*	*-0.07*	*-0*	Johnson et al. 2003, year 1
+1	*-0.5*	-0.25	*+0.03*	Johnson et al. 2003, year 2
+0.6	*+1*	-0.09	+0.03	Kononoff and Heinrichs 2003
+2.3	*+0.2*	*-0*	*-0*	Kononoff et al. 2003b
+0.7	*+0.7*	*-0.06*	*-0*	Onetti et al. 2003
+0.6	*+1.4*	*-0.13*	*-0.01*	Fernandez et al. 2004
-1.1	–	–	–	Beauchemin and Yang 2005
+0.4	*-1.4*	*+0.12*	*-0.05*	Soita et al. 2005
-0.8	*-1.3*	*+0.14*	*+0.27*	Yang and Beauchemin 2005
+1.3	*+1.3*	*+0*	*+0*	Couderec et al. 2006
+1.1	-0.6	*-0*	*-0.06*	Yang and Beauchemin 2006
+1	*+0.4*	*-0.05*	*-0.01*	Bhandari et al. 2007
+1.5	*+0.5*	*+0.02*	*+0.01*	Zebeli et al. 2009
+2	*+0.4*	*+0.05*	+0.03	Maulfair and Heinrichs 2013

[1]the values in italics refer to no-significant differences according to the statistical methods applied in the respective studies

The meta-analysis revealed an increased milk production of 0.5 kg milk/d in cows fed finer particles, which went along with a higher percentage of protein, which might be attributed to the increased energy supply to the cows. On the other hand, the percentage of fat decreased by 0.06% in cows fed finer particles which resulted in an unaffected production of fat and fat-corrected milk, respectively (Nasrollahi et al. 2015). The decreasing effect on fat content is likely due to a reduced chewing activity that finally lowers ruminal pH when finer forage is fed (Zebeli et al. 2008). A decrease in ruminal pH is known to enhance the production of trans fatty acids, which act as inhibitors of de novo milk fat synthesis in the mammary gland (Baumann and Griinari 2001).

However, when specifically looking at diets containing MS as forage source, varying effects of reducing the TCL on milk yield result with a range from -1.4 to +1.4 kg/d (Table 2). Also, changes in milk constituents such as an increase in protein and a decrease in fat were only found to be significant in two out of 17 studies in each case. Averaged among studies decreasing the TCL led to an increase of 0.26 kg milk/d, while fat percentage decreased by 0.02 percentage units and protein increased by 0.02 percentage units. Considering the higher DMI and only minor changes in milk yield, the efficiency of milk production might even be reduced when reducing the TCL of MS, as observed previously (Maulfair and Heinrichs 2007, Zebeli et al. 2009). However, it has also to be considered that the additional feed intake might have been rather used for body mass increase than for milk production, especially when taking into account the short-term duration of the respective studies (Al-Trad et al. 2009).

According to the meta-analysis, the reduction of the FPS did not affect digestibility of DM, but went along with an increase in starch digestibility by on average 0.10% and a decrease in NDF digestibility by on average 1.6% (Nasrollahi et al 2015).

As summarized in Table 3, the apparent total tract digestibility (ATTD) of NDF was decreased with lowered TCL in the majority of the studies conducted on cows that were fed MS-based, with a range from -0.3 to -9.3 percentage units. Effects on the ATTD of DM and starch were less pronounced and not significant in most studies, although an overall tendency towards higher starch digestibility (on average +0.53%) and lower DM digestibility (on average -1.09%) can be seen. In this regard, it has to be stated that the starch digestibility measured on the total tract level does not appropriately reflect the starch digestion in the rumen, as a lower ruminal starch degradation can be partly of fully compensated by increased intestinal starch digestion (Beauchemin et al. 2001). Indeed, a shift of starch digestion from the rumen to the intestine with increasing FPL has been reported previously (Yang et al. 2002). Thus, it can be assumed that a decreased TCL of MS likely enhances ruminal starch degradability, as supported by Johnson et al (2003).

Table 3. Effect of feeding maize silage with shorter theoretical chop length (TCL) on apparent total tract digestibility (ATTD) of DM, starch and NDF (differences are expressed as absolute differences of means measured in cows receiving the shortest TCL vs. the longest TCL)

ATTD DM	ATTD Starch	ATTD NDF	Reference
-0.1[1]	*+0.1*	-5.3	Bal et al. 2000
+0.8	*-0.8*	*-0.3*	Schwab et al. 2002
-1.9	-0.4	-3.6	Johnson et al. 2003, year 1
+0.7	+0.7	-1.8	Johnson et al. 2003, year 2
-3	–	*-2.6*	Kononoff and Heinrichs 2003
-0.7	*+3.7*	*-2.8*	Fernandez et al. 2004
-2.6	*-1.1*	-9.3	Yang and Beauchemin 2005
-1.9	*+1.5*	-4.8	Yang and Beauchemin 2006

[1]the values in italics refer to no-significant differences according to the statistical methods applied in the respective studies

Effects on chewing and rumen health

A recent meta-analysis including 42 studies with the change of FPS in the diet of dairy cattle (Nasrollahi et al. 2016) indicates a shortened chewing behaviour when the FPS was reduced from about 10.0 ± 4.9 mm to 6.7 ± 4.11mm.

Although an overall decreasing effect of feeding finer particles on chewing activity is a common observation (e.g., Zebeli et al. 2006, Tafaj et al. 2007), this decrease is especially important in MS-based diets, as MS contains higher amounts of fermentable carbohydrates, more specifically degradable starch (up to 350-450 g/kg starch per kg DM), compared to other forages (Nasrollahi et al. 2016).

Table 4 summarizes the effect of reduced TCL of MS conducted in cows fed MS-based diets. The time spent eating was reduced by on average 6.2 min/d, the time spent ruminating by 10.8 min/d and the total chewing time by 20.1 min/d when the MS was finer chopped. When expressed in relation to DMI, no overall depressing effect on the time spent eating was found, while the time spent ruminating per kg DM was lowered by on average 0.81 min/kg DMI. Finally, the total chewing time per kg DMI was on average 0.64 min shorter in cows fed finer MS.

Table 4. Effect of feeding maize silage with shorter theoretical chop length (TCL) on chewing activity (differences are expressed as absolute differences of means measured in cows receiving the shortest TCL vs. the longest TCL)

Eat	Ruminate	Total Chew	Eat	Ruminate	Total Chew	Reference
(min/d)			(min/kg DM)			
-5[1]	*-25*	*-30*	–	–	–	Clark and Armentano, 1999
-8	*-13*	*-23*	-0.6	-1	-1.6	Schwab et al. 2002
-9	*+51*	*+4*	*-0.4*	*-0.1*	*-0.25*	Kononoff and Heinrichs 2003

-11	*-8*	*-13*	*-1.1*	*-1.6*	-3	Kononoff et al. 2003b
+15	*+34*	*+47*	*+0.5*	*-0.5*	*+0.5*	Fernandez et al. 2004
-29	-52	-82	*-0.2*	*-1*	*-1.2*	Beauchemin and Yang 2005
+27	*+17*	*+53*	*+1.7*	*+0.8*	*+2.9*	Couderec et al. 2006
-9	-97	-106	*+0.5*	-2.3	*-1.8*	Yang and Beauchemin 2006
-27	*-4*	*-31*	–	–	–	Maulfair and Heinrichs 2013

[1]the values in italics refer to no-significant differences according to the statistical methods applied in the respective studies

Thus, decreasing the TCL in MS-based diets is expected to increase its degradation rate, while facing less neutralization of VFA (Allen 1997, Zebeli et al 2012). In this regard, it has been reported that large particles in MS are more effective in stimulating chewing activity than those in alfalfa silage (Krause and Combs 2003). In result, higher amounts of neutralizing saliva buffers are needed in diets based on MS compared to other forages to counterbalance the higher generation of VFA in MS-based diets. Therefore, decreasing the TCL in MS-diets poses higher risks to impair rumen health compared to hay- or grass-silage based diets (Nasrollahi et al. 2016).

In accordance with the chewing and DMI data, reducing the TCL was also associated with a decrease in rumen pH; however, this was only significant when the forage level of the diet was low (Nasrollahi et al. 2016). As mentioned above, the greater contents of degradable starch in MS-based diets compared with non-maize diets, causes a relatively stronger drop of pH in MS-based diets compared to diets based on other forages, as revealed by further meta-regression analysis taken different forage sources into account.

Also, an increase in the total amount of VFA with decreasing TCL was found; however, this effect was only pronounced when a high level of forage was fed. The VFA profile changed towards a higher proportion of propionate and butyrate as well as a trend towards decreased acetate to propionate ratio (Nasrollahi et al 2016).

As summarized in Table 5, effects of reducing the TCL in MS-based diets caused only minor and often insignificant changes in pH. One explanation might be that enhanced chewing activity is not necessarily related to the improved ruminal environment in high-performing cattle (Mertens, 1997). Furthermore, the only minor improvement in pH might also be due to inadequate measurement methods, as in most studies no continuous pH monitoring was conducted. The latter is supported by Yang and Beauchemin (2006), who observed unchanged average pH values when the TCL of MS was reduced from 28.6 to 4.8 mm, while the time the pH fell below 5.5 was 72 min/d shorter in cows fed the coarsely chopped MS.

A pH-decreasing effect of finer TCL of MS is supported by the higher content of volatile fatty acids (VFA) of up to 10 mM; however, changes in the VFA composition were not a general finding and mainly the molar proportion of butyrate was affected by the TCL of MS (Table 5).

Table 5. Effect of feeding maize silage with shorter theoretical chop length (TCL) on ruminal pH, concentration of volatile fatty acids (VFA) and molar proportion of individual VFA (differences are expressed as absolute differences of means measured in cows receiving the shortest TCL vs. the longest TCL)

pH	VFA, mM	VFA composition	Reference
+0.03[1]	*-4.4*	+1.7% butyrate	Bal et al. 2000
-0.03	*+3*	n.s.[2]	Schwab et al. 2002
-0.01	*-1.5*	n.s.	Kononoff and Heinrichs 2003
-0.1	+4.5	n.s.	Kononoff et al. 2003b
-0.1	*+4.4*	n.s.	Onetti et al. 2003
+0.17	*+1.9*	n.s.	Fernandez et al. 2004
0	+10.1	+3.8% propionate, -1.8% butyrate	Beauchemin and Yang 2005
-0.18	*+2.1*	n.s.	Couderec et al. 2006

-0.09	*+0.3*	n.s.	Yang and Beauchemin 2006
+0.08	*+0.7*	n.s.	Bhandari et al. 2007
-0.12	*+1.6*	+0.55% butyrate	Maulfair and Heinrichs 2013

[1]the values in italics refer to no-significant differences according to the statistical methods applied in the respective studies
[2]non-significant differences in the composition of VFA according to the statistical methods applied in the respective studies

A further aspect that has to be taken into account is the sorting behaviour of the cows that might change due to changes in the TCL of MS. As mentioned above a shift in the particle distribution from the particles in the middle sieves towards large particles when increasing the TCL was observed (Table 1). Thus, feeding coarser chopped MS might favour selection against longer particles, which results in a lower intake of peNDF than scheduled. Therefore, an increase in the TCL does not necessarily guarantee improved ruminal pH and fermentation characteristics. Indeed, Yang and Beauchemin (2006) observed that relatively less particles >19.0 and >8.0 mm were consumed for cows fed the diet that included the coarsest MS. Therefore, an increase in diet uniformity with decreasing TCL might even prevent feed sorting and increase the absolute amount of fiber intake (Nasrollahi et al. 2015).

Shredlage vs. Maize silage

In recent years, a new method of harvesting whole-plant MS has become popular as a method to enhance kernel processing, while increasing the peNDF content. The so called shredlage is MS produced with a harvester that is set for a longer TCL, ranging from 26 to 30 mm. The harvester is fitted with cross-grooved processing rolls that run at a greater speed than conventional rolls to enhance starch digestibility through increased kernel processing (Seglar and Shaver 2014). Due to the increased TCL a higher proportion of stover particles in the MS is achieved. However, as this causes an almost 4-fold enhancement of the proportion of large particles (i.e., >19 mm) at the expense of medium particles (i.e., 8-19 mm), similar amounts of $peNDF_{>8}$ and $peNDF_{>1.18}$ result (Table 6).

Table 6. Particle size distribution, NDF and peNDF of maize silage or shredlage (obtained from the studies of Ferraretto and Shaver 2012, Vanderwerff et al. 2015 and Ferraretto et al. 2016)

Item	Maize Silage	Shredlage
Particle fraction, %		
>19 mm	5.8 ± 0.95	22.6 ± 6.29
8-19 mm	65.3 ± 9.71	48.0 ± 5.31
1.18-8 mm	27.3 ± 9.91	27.3 ± 2.61
<1.18 mm	1.6 ± 0.87	2.1 ± 0.93
NDF, %	37.7 ± 4.66	34.2 ± 8.36
$peNDF_{>8}$, %	27.2 ± 6.30	24.4 ± 6.79
$peNDF_{>1.18}$, %	37.1 ± 4.45	33.6 ± 8.29

In general, enhancing the portion of very large particles likely enhances feed sorting against long particles, thereby leading to a reduced intake of peNDF than scheduled. Additionally, the enhanced ruminal starch degradability due to the greater kernel breakage during harvesting (Ferraretto and Shaver 2012) has to be considered. Previous research found no improvement of rumination activity or milk fat content in cows fed TMR containing shredlage compared to conventionally processed MS. Nevertheless, an improved starch digestibility and trend towards higher milk yield was found (Ferraretto and Shaver 2012, Vanderwerff et al. 2015).

Overall, care has to be taken to formulate diets that provide optimal levels of peNDF, depending on the amount of rapidly degradable starch in the diet and the DMI level. Moreover, preparing a homogeneous TMR with a favourable distribution of the particle size in the TMR, to prevent cows from discriminating against longer forage components and selecting for small concentrate particles out of the diet, is of paramount importance. The general recommendation is that only a small amount of particles longer than 19 mm or finer than 1.18 mm should be contained in the TMR. The majority (80-90%) of particles should be in-between, with medium and fine particles being equally represented (Heinrichs and Kononoff 2002). In this regard also providing enough eating space, reducing stress, and enhancing the frequency of feed delivery and push-up are important managerial factors to reduce feed sorting while improving eating patterns towards more evenly distributed meals throughout the day, thereby enabling a uniform feeding with a balanced nutrient intake and improved rumen fermentation conditions (De Vries et al. 2005, Macmillan et al. 2017). To sum up, feeding shorter but uniform MS could be as important as the feeding of long particles to stimulate fiber intake and chewing activity in cattle (Nasrollahi et al. 2016).

Conclusions

The optimization of particle size and effective NDF of MS is important to maintain rumen health and productivity. On the one hand, chopping MS too finely causes an enhanced ruminal starch degradation, which together with lowered chewing activity might impair rumen health and production efficiency. On the other hand, enhancing the TCL provides higher amounts of peNDF, but this can cause a decline in DMI and might lead to unfavourable changes in particle size distribution, which might enable feed sorting. Overall, several factors like the maize cultivar and stage of maturity, the diet formulation (including the composition of the forage portion), the performance level and several managerial factors modulate the effect of TCL of MS on production performance and animal health. All these variables have to be taken into account when choosing the TCL of MS.

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Fibre quality of grass silages and its effects on ruminant performance

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Introduction

Various grass species are grown all over the world, where they are grazed by ruminant animals or harvested for silage or hay to be fed to livestock during the winter season. Warm-season grasses are grown in the tropical and subtropical regions and some semi-arid areas of central and western United States. Warm-season grasses, termed C_4 grasses, initially produce a 4-carbon molecule during their photosynthesis, whereas the cool-season grasses, termed C_3 grasses, produce a 3-carbon molecule. Because of a more efficient use of oxygen, the C_4 grasses have higher growth rates, higher water, and nitrogen use efficiencies but lower

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nutritive value compared to C_3 grasses (Hanna and Sollenberger, 2007). There seems to be a growing interest for C_3 grasses in some areas, where C_4 grasses dominate as forage for ruminants. This partly depends on differences in optimal temperatures for growth, which is near 20ºC for C_3 grasses and 30-35ºC for C_4 grasses, which would result in more biomass over the growing season under optimal conditions. Another reason for increased interest in C_3 grasses is the higher nutritive value in form of lower contents of neutral detergent fibre (NDF), which is more digestible and higher crude protein content, which result in higher dry-matter (DM) digestibility of C_3 compared to C_4 grasses (Minson, 1980; Coleman et al., 2004). There is a balance between DM yield and feed value when choosing types of grasses on a farm depending on the acreages of farm land and types of ruminants (low- or high producing) to be fed. The feed value of grasses varies considerably with climate as well as species and growth stage of the plants. It is more challenging to manage ration formulations based on perennial grasses, which can have great variations in feed value between harvests, compared to corn, which is cut only once during the growing season. Management systems must consider the whole production scheme of land use and match this with animal production on the farm.

As the cell wall components constitute a significant part of the DM of grasses and are only partly digested by the ruminants, they play a key role in managing intake predictions for maximum feed efficiency in ruminants. However, fibre digestion kinetics cannot easily be analysed at a reasonable cost for the farmer, which leaves the feed ration manager with table values or values predicted from near-infrared scanning, which normally varies from the actual value of the grass silage. The aim of this paper is to describe some key structural fibre characteristics of C_3 grasses as affected by management and its effects on intake and performance by ruminants.

Cell-wall structure

Lignification of the cell wall during secondary growth is well known to limit digestion of forages (Jung and Deetz, 1993). All cell wall polysaccharides are completely digestible when removed from the cell wall matrix, but the degradation occurs slowly (Weimer, 1996). Cellulose, which is an unbranched polymer of glucose units, is the most abundant polysaccharide in forage cell walls. Individual cellulose molecules form microfibrils that are held together by hydrogen bonds (Hatfield, 1993). The hemicellulose fraction of the cell walls is composed of a range of polysaccharides of which xylan is by far the most abundant. Xylans of grasses are complex, containing substitutions of arabinose, glucuronic acid or both. The arabinoxylan chains are cross linked by diferulate bridges, and the esterified ferulates and diferulates of the arabinoxylans act as a nucleation site, where lignin polymerization begins (Ralph et al., 1994; Ralph et al., 1995). Ferulates of arabinoxylans interfere with the digestion of the polysaccharides to which they are esterified by hindering the alignment of xylanase with its substrate, which is necessary for hydrolysis to occur (Jung and Deetz, 1993; Jung and Allen, 1995). Another hydroxycinnamic acid, the p – coumaric acid, is in its ester form to a major extent linked to lignin without forming bridges to polysaccharides and is, therefore, unlikely to directly affect cell-wall digestion (Jung and Deetz, 1993).

Cell division starts with forming the middle lamella, which is the outer wall of the cell. The primary wall, which is formed next to the middle lamella during cell elongation, is mainly composed of polysaccharides, including cellulose, hemicellulose, pectin, and glycoproteins. When the cell has reached full size, the secondary cell wall is deposited by the formation of microfibrils of cellulose molecules by hydrogen bonds and lignification (Fales and Fritz, 2007). Lignin deposition starts in the middle lamella and proceeds through the primary wall into the secondary wall. This process of the thickening of the cell wall explains why the most recently deposited polysaccharides of the secondary wall are not lignified and the most lignified region is the middle lamella/primary cell wall (Jung and Allen, 1995). The degree of cross-linking between lignin and arabinoxylan by ferulate esters in the primary cell wall and increased syringyl lignin content in the secondary cell wall have been related to decreased cell wall digestibility as forages advance in maturity (Grabber et al., 1998; Jung and Engels, 2002). Lignin acts as a physical barrier for the rumen microbes to degrade cell walls and may be a reason why the microbes start degrading plant cell walls from the lumen of the cell outward (Engels, 1989).

In grasses, deposition of a thick secondary cell wall in all tissues of both stems and leaves, except for mesophyll and phloem, causes increased cell wall concentration. Xylem, which provides conduction, sclerenchyma tissue, which provides mechanical support and epidermis, which provides protection, are lignified and, consequently, poorly digested in contrast to collenchyma and mesophyll tissues, which do not lignify and are completely digestible even in mature forages (Wilson, 1993). Proportions of xylem and sclerenchyma are similar in C_3 and C_4 grasses but proportions of parenchyma bundle sheath and epidermis cells are higher and proportions of mesophyll cells are lower in C_4 grasses. Stem anatomy of C_3 and C_4 grasses is similar with high proportions of sclerenchyma and xylem that are highly lignified and undegradable. Degradability of stem parenchyma varies with tissue age and species (Akin, 1989).

Considerations regarding fibre analyses of cell walls

In ruminant nutrition, the cell-wall components are classified as fibre. Fibre is analysed as NDF, which contains hemicellulose, cellulose, lignin and cell-wall bound protein. However, if sodium sulphite is added to neutral detergent (ND) solution, much of the protein is removed (Mertens, 2002). Pectin is solubilized in the ND solution and thus not found either in NDF, but this is less of a problem in grasses than in legumes as there is much less pectin in grasses (Jung and Allen, 1995). However, in grasses, especially at immature stages of growth, highly substituted arabinoxylans in the hemicellulose fraction can be solubilized in the ND solution (Hatfield et al., 2007). Therefore, the NDF concentration is not equal to the total cell-wall concentration of forages but is highly relevant to use for nutritional purposes as it regulates intake by ruminants in high-forage diets (Mertens, 1994) and ND solubles are an ideal fraction in the analysis of digestibility according to Lukas test (Nousiainen et al., 2009). Cellulose and lignin plus protein, cutin and some minerals bound to these compounds constitute the acid detergent fibre (ADF), which is the remaining cell wall after treatment with an acid detergent (AD) solution (Van Soest et al., 1991). Lignin can be determined as the residue after treatment with 72% sulphuric acid and defined as acid detergent lignin (ADL; Van Soest et al., 1991).

The ADL method underestimates the lignin content compared to the Klason lignin using the Seaman hydrolysis, which is the standard method for cell wall analysis (Jung et al., 1999; Hatfield et al., 2007), however, Klason lignin includes soluble phenolic compounds which may not inhibit digestion. Therefore, the ADL has been shown to be a more nutritionally uniform measure of lignin than the Klason lignin in grass silage of primary growth and regrowth of a mixture of timothy and meadow fescue and completely recovered in faecal samples (Krizsan et al., 2013). Relatively high NDF content of the grass silage from the primary growth of 637 (s.d. 31) g/kg DM and harvest dates from June 18 to July 8 indicate relatively mature grasses for half of the grass samples in that report, which could have contributed to the total recovery of the samples as lignin in less mature grasses appears to be more prone to solubilization in the rumen than late harvested grass (Van Soest, 1994).

Lignin and digestibility

Lignin is principally indigestive, and could theoretically be used as a laboratory method for prediction of the in vivo organic matter digestibility (OMD). However, using lignin, when analysed as ADL, Klason lignin and Permanganate lignin, to predict in vivo OMD across both grass and red clover silages was much less precise than using the 288-h indigestible NDF (iNDF) in situ as a predictor ($R^2 = 0.52$ vs. 0.89 and RMSE = 37.6 vs. 18.5, n = 25; Krizsan et al., 2013). Using a larger data set, Krizsan et al. (2015) showed a strong negative linear relationship between ash-free indigestible NDF (iNDFom) in situ and in vivo OMD of fresh and ensiled grass (fresh grass: $R^2 = 0.93$ and RMSE = 15.4 g kg^{-1}, n = 34; grass silage: $R^2 = 0.95$ and RMSE = 12.1 g kg^{-1}, n = 34). Furthermore, results by Krämer et al. (2012) indicated that prediction of iNDF exclusively from ADL did not give strong equations within plant type and predictions across plant types were not possible. The iNDF/ADL ratio, which indicates the inhibitory effect of lignin on digestion per unit of lignin, varied between cut numbers over the growing season in Denmark from 3.4 in primary growth to 2.3, 2.8 and 2.7 in the first, second and third regrowth of perennial ryegrass, festulolium, hybrid ryegrass and orchardgrass. When the same grass species were harvested at different maturity stages in the primary growth from May 19 to June 9, the iNDF/ADL ratio increased from 2.4 to 3.7. There were also species differences, where perennial ryegrass differed from the other grass species in iNDF/ADL ratios (Krämer et al., 2012). Likewise, Rinne et al. (2002) showed increasing iNDF/ADL ratios from 1.7 to 2.4 with advanced maturity in the primary growth of a timothy/meadow fescue silage harvested from June 13 to July 4. Results by Nadeau et al. (2016) showed even a larger effect of maturity on the iNDF/ADL ratio ranging from 1.8 to 3.3 in silages from the primary growth of mixed leys of timothy/meadow fescue/perennial ryegrass silage harvested from June 2 to June 21 in Sweden.

In an experiment at Rådde Experimental Station, The Rural Economy and Agricultural Society Sjuhärad, Sweden, monocultures of tall fescue × Italian ryegrass, tall fescue, meadow fescue and timothy were harvested at two maturity stages in the primary growth (May 29 and June 4) and in the first regrowth (July 7 and July 14) followed by one harvest in the second regrowth (August 31) in 2015 (Nadeau and Hallin, 2016). Figure 1 shows that the grasses differ in their relationships between iNDF in situ and ADL with no or even negative (tall fescue) relationships for the fescues and their hybrid but a strong linear relationship for timothy for increasing iNDF with increasing lignification of the cell walls (iNDF = 6.8*ADL - 126, $R^2 = 0.87$, n = 5). This finding confirms previous results by Krämer et al. (2012) and highlights that the lignin structure, such as the syringyl/guaiacyl-unit ratio and cross linkings between lignin and the xylans, for example through the esterified ferulates in the cell wall, can differ between forage species, maturity (Jung and Deetz, 1993; Jung and Allen, 1995; Hatfield et al., 2007) and cut number (Krämer et al., 2012). These differences in the chemical structure of the lignified cell walls could possibly be a reason why fescues have lignin that is more inhibitory to digestion per unit of lignin than the lignin of timothy but more research is needed to explain the differences in inhibitory effects of lignin on digestion. The average iNDF/ADL ratios were 4.1, 4.5, 5.3 and 3.3 for tall fescue × Italian ryegrass, tall fescue, meadow fescue and timothy, respectively. These values are higher than found previously, which indicates that the lignification of the cell walls of C_3 grasses is affected by the climate in addition to forage characteristics and management. Consequently, the use of a standard factor to predict iNDF from lignin in ration formulations creates a large source of error.

Nearly no relationship exists between ADL and the in vitro rumen organic matter digestibility (OMD), which agrees with results by Krizsan et al. (2013), and further indicates that ADL content alone is not sufficient to use for predictions of digestibility, at least not for the fescues and their hybrid in this study. The data for timothy is more promising, and it seems to be a clear difference between timothy and the fescues used in this study (In vitro rumen OMD (g/kg OM) of timothy = – 5.0 * ADL (g/kg DM) + 969, R^2 = 0.75, n = 10; Figure 2).

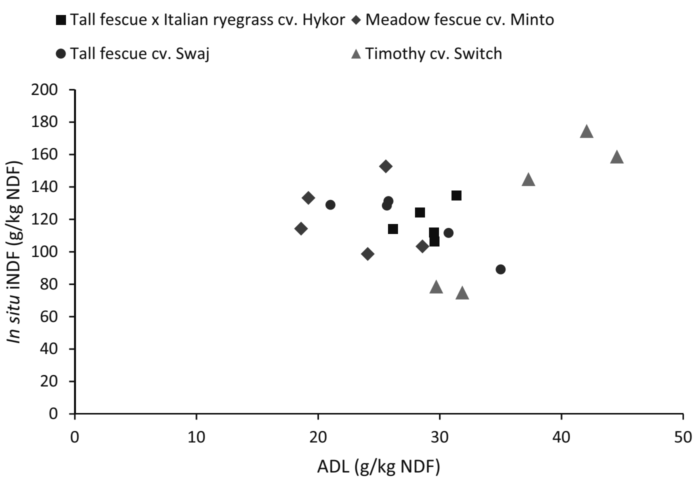

Figure 1. Relationship between acid detergent lignin (ADL) and in situ indigestible NDF (iNDF) in grasses grown at Rådde Experimental Station, Sweden, 2015 (n = 5 per species/hybrid).

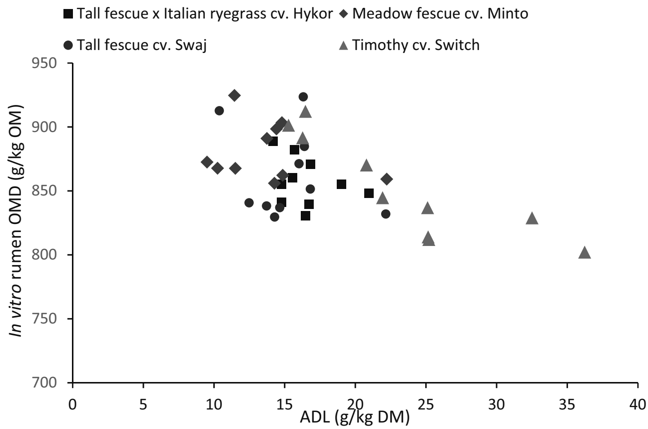

Figure 2. Relationship between acid detergent lignin (ADL) and in vitro rumen organic matter digestibility (OMD) in grasses grown at Rådde Experimental Station, Sweden, 2015 (n = 10 per species/hybrid). In vivo OMD = -2.0 + 0.90 * in vitro rumen OMD (Lindgren, 1983).

Our hypothesis that both lignin content and its structure and degree of cross-linking with the cell-wall polysaccharides, most likely arabionxylans, play a role in forage digestibility is confirmed by the improved relationship when iNDF in situ replaces ADL as a predictor for in vitro rumen OMD (Figure 3), which is in agreement with results on in vivo OMD by Krizsan et al. (2013, 2015). By using the iNDF in situ, the relationship with in vitro rumen OMD is improved for tall fescue (in vitro rumen OMD (g/kg OM) = – 2.2 * in situ iNDF (g/kg NDF) + 1123, R^2 = 0.97, n = 5) and meadow fescue (in vitro rumen OMD (g/kg OM) = – 1.0 * in situ iNDF (g/kg NDF) + 1009, R^2 = 0.71, n = 5) and is even stronger for timothy (in vitro rumen OMD (g/kg OM) = – 0.95 * in situ iNDF (g/kg NDF) + 975, R^2 = 0.95, n = 5).

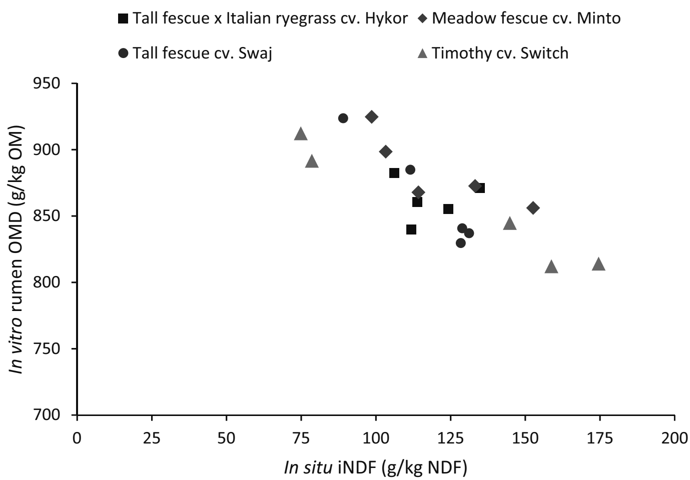

Figure 3. Relationship between in situ indigestible NDF (iNDF) and in vitro rumen organic matter digestibility (OMD) in grasses grown at Rådde Experimental Station, Sweden, 2015 (n = 5 per species/hybrid). In vivo OMD = -2.0 + 0.90 * in vitro rumen OMD (Lindgren, 1983).

Figure 4 shows that the iNDF/ADL ratio is closely related to in vitro rumen OMD for tall fescue (in vitro rumen OMD (g/kg OM) = -26.3 * iNDF/ADL + 981, R^2 = 0.84, n = 5) and timothy (in vitro rumen OMD (g/kg OM) = -53.0 * iNDF/ADL + 1031, R^2 = 0.84, n = 5) and intermediate for meadow fescue (in vitro rumen OMD (g/kg OM) = -15.3 * iNDF/ADL + 966, R^2 = 0.62, n = 5) , whereas no relationship was found for tall fescue x Italian ryegrass (R^2 = 0.03). These negative relationships indicate that an increased iNDF/ADL ratio has an inhibitory effect on in vitro OMD within the grass species in this study but not for the tall fescue hybrid Hykor, which agrees with the absence of a relationship between in situ iNDF and in vitro rumen OMD for the tall fescue hybrid in figure 3 (R^2 = 0.00).

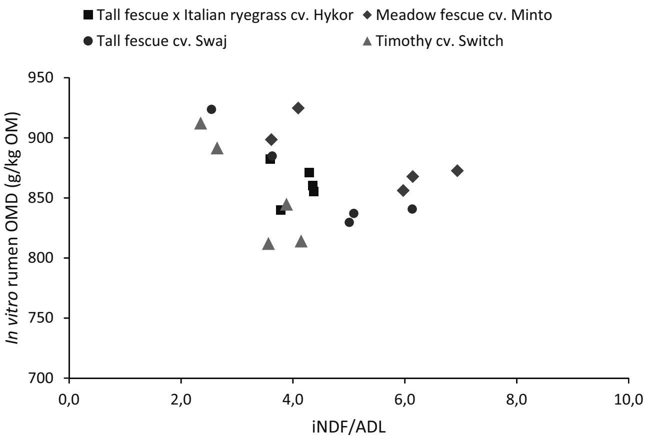

Figure 4. Relationship between iNDF/ADL ratio and in vitro rumen organic matter digestibility (OMD) in grasses grown at Rådde Experimental Station, Sweden, 2015 (n = 5 per species/hybrid). In vivo OMD = -2.0 + 0.90 * in vitro rumen OMD (Lindgren, 1983).

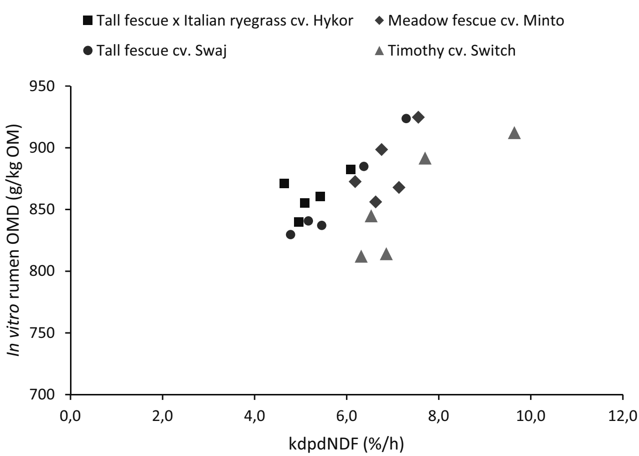

Figure 5. Relationship between fractional degradation rate of potentially digestible NDF (kdpdNDF) predicted from in situ incubation and in vitro rumen organic matter digestibility (OMD) in grasses grown at Rådde Experimental Station, Sweden, 2015 (n = 5 per species/hybrid). In vivo OMD = -2.0 + 0.90 * in vitro rumen OMD (Lindgren, 1983).

The relationship between the digestion rate of potentially digestible NDF (kdpdNDF) calculated from in situ incubation and in vitro rumen OMD is not as strong as between iNDF in situ and in vitro rumen OMD for meadow fescue (R^2 = 0.41) and timothy (R^2 = 0.78) but is still strong for tall fescue (in vitro rumen OMD (g/kg OM) = 29.6 * kdpdNDF (%/h) + 636, R^2 = 0.97, n = 5; Figure 5). However, the kdpdNDF probably plays a greater role than iNDF in predicting feed intake and production of high-producing ruminants, where a high rate of passage limits rumen retention time and, consequently, OMD (Mertens, 2007).

As the analysis of 288-h in situ iNDF is considerably less expensive than in situ digestion kinetics of kdpdNDF it would be beneficial to find strong relations between in situ iNDF and kdpdNDF. As shown in figure 6, this small data set only showed low to intermediate relationships between iNDF and kdpdNDF for meadow fescue (R^2 = 0.45), tall fescue × Italian ryegrass (R^2 = 0.65) and timothy (R^2 = 0.68) but strong for tall fescue (R^2 = 0.90). Collecting samples from various environments to create robust relationships between inexpensive and quick laboratory feed analyses with more expensive and time-consuming feed analyses that have high nutritive value is of major importance for efficient formulations of feed rations for more accurate predictions of milk yield responses, which improves profitability for farmers.

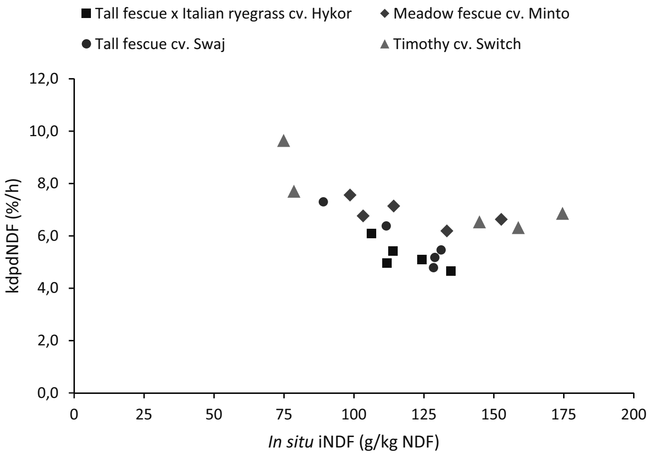

Figure 6. Relationship between in situ indigestible NDF (iNDF) and fractional degradation rate of potentially digestible NDF (kdpdNDF) predicted from in situ incubation in grasses grown at Rådde Experimental Station, Sweden, 2015 (n = 5 per species/hybrid).

In the experiment at Rådde, Sweden, the kdpdNDF was lower for tall fescue × Italian ryegrass cv. Hykor than for tall fescue in the primary growth but the kdpdNDF of tall fescue decreased to a level similar to that of the hybrid in the regrowths. Timothy had higher kdpdNDF than the tall fescue and its hybrid in all five harvests and of that of meadow fescue in the primary growth (Nadeau and Hallin, 2016). Østrem et al. (2014) presented similar kdpdNDF values for the tall fescue × Italian ryegrass cv. Hykor as in this study but a lower kdpdNDF of timothy, especially in primary growth, compared to this study, which probably is a combined effect of differences in cultivars (Grindstad vs. Switch) and environments.

Physically effective fibre

A certain amount of physically effective NDF in rations for ruminants is needed as it is the NDF that stimulates chewing and, consequently, important for rumination, salivation and rumen motility (Mertens, 1997; Nørgaard et al., 2011). Forage, such as perennial grasses, is the main source of physically effective NDF in a ration. The daily time spent eating and ruminating, which sums to total chewing time, is closely related to forage NDF intake ($NDFI_f$; Table 1) and particle size. Mertens (1997) defined physically effective NDF (peNDF) as the NDF in feed particles retained on a sieve with a pore size of 1.18 mm.

The chewing time per kg $NDFI_f$ decreases with increasing body weight (BW) from lamb, ewes, growing cattle to mature dairy cows and beef cows. A standard dairy cow of 625 kg body weight, at an intake of 20 kg DM/day and an NDF intake of 0.7% of BW spends 50 minutes eating and 100 minutes ruminating per kg NDF intake of unchopped forage according to the Nordic Chewing Index system (Nørgaard et al., 2010, 2011). However, the time spent ruminating per kg $NDFI_f$ decreases at increasing $NDFI_f$ expressed as percentage of BW (Nørgaard et al., 2010; Schulze et al., 2015). In addition, increasing lignification of NDF and increasing iNDF/NDF ratio of forages are considered to increase rumination time per kg $NDFI_f$ according to the Nordic Chewing index system (Nørgaard et al., 2010; Nørgaard et al., 2011), and this effect has been supported by the observation by Schulze et al. (2015). Furthermore, time spent eating per kg DM intake and per kg $NDFI_f$ appears to increase at increasing feeding level up to ad libitum intake (Schulze et al 2014a).

The chewing index has recently been used in models to predict energy intake of dairy cows (Jensen et al., 2016) and ewes (Nielsen et al., 2015, 2017), where the energy intake decreases with increasing chewing index. The modelling work is based on the original model presented by Nørgaard and Mølbak (2001), who observed a decreasing net energy intake

(NEI) at increasing dietary chewing index value (min/NEI) of lactating dairy cows, dry cows and growing cattle. The intercept value (NE_0), predicted as the chewing index approaching zero has been interpreted as the theoretical maximum metabolic energy intake capacity of NEI when feed intake is unconstrained by rumen fill. Furthermore, the decrease in NEI at increasing chewing index values has been found to be proportional with the squared intercept.

When using a ruminating monitoring system (RuminAct-Milkline, Gariga di Podenzano, Italy) on lactating dairy cows, variation in daily rumination time was to a lesser extent explained by variations in intakes of dietary fractions, such as NDF_f, starch, and sugar, than to the individual variations between cows. Furthermore, rumination time in minutes per kg of DM intake was negatively related to milk yield and milk protein content but positively related to milk fat content (Byskov et al., 2015).

Table 1. Intake, chewing activity and faeces characteristics in pregnant ewes fed long (300 mm) grass silage harvested at three maturity stages (Early, Medium, Late) during spring growth cycle 2006 at The Swedish University of Agricultural Sciences, Skara, Sweden (Jalali et al., 2012).

	Maturity stage[1]			
	Early	Medium	Late	*P* - value
Intake				
DM (kg/day)	2.40^a	2.00^b	1.73^c	< 0.001
NDF (% of BW[2])	1.13^a	1.27^b	1.33^b	< 0.05
iNDF[3] (% of BW)	0.09^a	0.22^b	0.38^c	< 0.001
Chewing				
Eating time (min/kg DMI[4])	146^b	208^a	226^a	< 0.01
Eating time (min/kg NDFI[5])	325	359	357	NS
Ruminating time (min/kg DMI)	154^b	187^b	228^a	< 0.01
Ruminating time (min/kg NDFI)	343	323	360	NS

Total time (min/kg DMI)	300[b]	394[a]	455[a]	< 0.01
Total time (min/kg NDFI)	668	682	717	NS
Faeces characteristics				
DM (%)	26.9[c]	30.7[b]	34.6[a]	< 0.01
PDM[6] (%)	41.3[c]	60.1[b]	67.9[a]	< 0.001
Sieving particle DM				
Mean particle size (mm)	0.18[b]	0.20[b]	0.23[a]	< 0.01
Large particles (> 1 mm)	2.9[a]	2.4[ab]	1.8[b]	< 0.05
Image analysis of particles				
Most frequent particle length (mm)	0.37	0.31	0.34	NS
Most frequent particle width (mm)	0.071	0.064	0.074	NS
Mean particle length (mm)	0.82	0.78	0.88	NS
Mean particle width (mm)	0.11[b]	0.13[b]	0.16[a]	< 0.01
95 percentile length[7] (mm)	3.79	3.20	3.46	NS

[1]Early = June 2, leaf stage; Medium = June 12, early heading stage; Late = June 21, late heading stage.
[2]Body weight; [3]Indigestible NDF determined after 288-h rumen incubation in situ. [4]Dry matter intake. [5]NDF intake. [6]Particle dry matter relative to total DM measured as residuals after washing procedure in nylon bags. [7]Fractile value, which defines the minimum length of the 5% longest particles. NS = none significance P > 0.05.

Faeces characteristics

Faeces characteristics in cattle, sheep, and goats are strongly affected by the quality of forage fibre. Plant species, stage of maturity at harvest, NDF content, lignification of NDF, digestibility and physical form of forages affect faeces characteristics, which can be used to assess the effects of diet and management on digestion. Faeces have been characterized by the content of DM, the proportion of particle dry matter in DM (PDM), particle size and distribution of particle size dimensions in the PDM fraction (Table 1). The PDM values of faeces from cattle and sheep generally increase at increasing stage of maturity at harvest, increasing ADL/NDF ratio of forage (Jalali et al., 2015), increasing NDF content and at decreasing apparent digestibility of NDF (Schulze et al., 2014a,b; Schulze et al., 2015).

The dimension size of the faeces particles in the PDM fraction has been characterized by sorting of PDM in different sieving fractions and by density and accumulated distribution functions of particle length and width values (Jalali et al., 2012). The length and width distributions of particles in faeces from ruminants are characterized by many short and thin particles and a few long and wide particles and faeces particles from mainly forage fed ruminants are typically about 5 times longer than their width value (Table 1). The critical particle size (CPS) has been defined as the 95 percentile value of large particles (LP) in faeces retained on a sieve with a pore size of 1.18 mm (Poppi et al., 1980). Likewise, the critical particle length (CPL) value of 3-5 mm has been predicted as the 95 percentile value from the accumulated distribution of faeces particle length values measured by image analysis (Nørgaard, 2006). The CPS is not a constant and can vary with the interactive effects of the physiological state of the animal and intake of peNDF, which is related to the NDF content and particle size of the forage and to the forage:concentrate ratio of the diet. Forage particles, which are larger than the CPS or the CPL are selectively retained in the reticulo-rumen by their ability to retain gas during rumen fermentation, regurgitated and masticated by rumination to smaller particles with an increasing density and lower capacity to retain gas. The high-density small particles, which are found in the ventral rumen, pass through the reticulo-omasal orifice during rumen contraction (Allen, 1996). This process is continuous and digestion of various plant tissues vary dramatically with non-lignified mesophyll and phloem being degraded before lignified tissues (Akin, 1989; Wilson, 1991). The mean particle size and the mean particle length of faeces particles from ruminant animals increase with increasing BW, increasing lignification of NDF and increasing intake of forage NDF relative to BW (Jalali et al., 2015). In addition, the proportion of particles larger than the CPS value of 1.18 mm appears to increase with increasing BW and increasing $NDFI_f$ relative to BW (Jalali et al., 2015), which raises the question on the use of a fixed CPS value when modelling NDF_f digestibility in rations for dairy cows.

Intake and performance by ruminants

When feeding forages to ruminants, the degree of degradation of potentially digestible NDF (pdNDF) is affected by the DM intake, which influences rate of passage of the digesta from the rumen. The higher the digestion rate of pdNDF (kdpdNDF), the greater proportion of the pdNDF will be degraded at a given rate of passage (Mertens, 2007). As mentioned in the previous section, potentially digestible NDF is selectively retained in the rumen compared to iNDF because of differences in particle density or buoyancy (Lund et al., 2007). Intake of NDF and degradation of pdNDF in the rumen contribute to a major extent to the energy intake from grass silage, when fed to ruminants (Huhtanen et al., 2009). As a combination of animal factors, such as physiological state and size of the animal, and forage factors, such as species, maturity stage and particle size, affect energy intake and, consequently, production by ruminants, the effect of fibre content and quality of grass silage on intake and performance by ruminants is highly relevant to investigate.

In a feeding trial on dairy cows at Lantmännen Research Station Nötcenter Viken, Sweden, forty dairy cows, 110 days in milk (DIM), were fed silages of tall fescue cv. Swaj (Lantmännen) and timothy cv. Switch (Lantmännen) harvested at two different dates during the spring growth cycle in 2015. The grass species, which were grown in monoculture, were harvested at a very early maturity stage (leaf-to-stem elongation stage) on May 25 and at an early maturity stage (leaf-to-stem elongation-to-flag leaf stage) on May 31, 2015. The grass was wilted to 30-35% DM before being ensiled in bales.

Table 2. Effect of crop (tall fescue cv. Swaj vs. timothy cv. Switch) averaged over harvests (Harvest 1, May 25 and Harvest 2, May 31) and effect of harvest date averaged over crop on fibre content and in vitro rumen organic matter digestibility (OMD) of the silage (n=6; Murphy et al., 2017).

	Crop			Harvest			*P*- value[1]	
	Swaj	Switch	SEM	1	2	SEM	Crop	Harvest
aNDFom (g/kg DM)	407	403	7.9	381	429	7.9	NS	< 0.01
ADF (g/kg DM)	224	232	3.9	216	241	3.9	NS	< 0.01
ADL (g/kg DM)	15.1	16.6	0.68	14.4	17.3	0.68	NS	< 0.05
In vitro OMD[2] (%)	89.3	93.3	0.29	92.1	90.4	0.29	< 0.001	< 0.001

[1]No crop × harvest interaction. NS = non-significance P > 0.05. [2]In vitro rumen organic matter digestibility (Lindgren, 1979, 1983, 1988). In vivo OMD = -2.0 + 0.90 * in vitro rumen OMD (Lindgren, 1983).

Despite no differences between the crops in fibre content, the in vitro rumen OMD was greater for Switch than for Swaj (Table 2). The in vitro rumen OMD and the effective fibre degradation (EFD) were positively related to the kdpdNDF estimated in situ (in vitro OMD (%) = 2.1 * kdpdNDF (%/h) + 77, R^2 = 0.99, n = 4; EFD (% of NDF) = 3.7 * kdpdNDF (%/h) + 36, R^2 = 0.99, n = 4). Furthermore, kdpdNDF was negatively related to iNDF in situ (kdpdNDF (% / h) = -0.085 * iNDF (g/kg NDF) + 15, R^2 = 0.80, n = 4) and to in situ iNDF/ADL ratio (R^2 = 0.78). The higher iNDF/ADL ratio for Swaj indicates that Swaj contains lignin that is more inhibitory to digestion per unit of lignin than the lignin of Switch. There was an effect of harvest date on the fibre content and in vitro rumen OMD and it appeared to be an effect of harvest date on the kdpdNDF (Tables 2 and 3). The NDF content of the wilted herbage before ensiling ranged from 461 to 517 g/kg DM, which was considerably higher than the NDF content of the silage (Table 2). This indicates acidic hydrolysis of the NDF, especially of the hemicellulose fraction, during the fermentation process and occurs easier in immature than in mature grasses (Nadeau et al., 2000).

Table 3. Fibre digestion kinetics and the iNDF/ADL ratio of the ensiled crops (n = 1; Murphy et al., 2017).

	Harvest 1 May 25		Harvest 2 May 31	
	Swaj	Switch	Swaj	Switch
EFD[1] (% NDF)	59	66	56	64
In situ iNDF[2] (g/kg DM)	39	33	47	36
In situ iNDF[2] (g/kg NDF)	99	89	112	82
pdNDF[3] (g/kg NDF)	901	911	888	918
kdpdNDF[4] (% / h)	6.4	8.2	5.4	7.5
iNDF/ADL	2.8	2.2	2.9	2.0

[1]Effective fibre degradation in rumen at 3 % passage rate (Lindgren, 1991). [2]Indigestible NDF determined after 288-h rumen incubation in situ (Åkerlind et al., 2011). [3]Potentially digestible NDF (g/kg NDF) = 1000 – iNDF (g/kg NDF) in situ (Åkerlind et al., 2011). [4]Fractional degradation rate of potentially digestible NDF from curve fitting of in situ values (Ørskov and McDonald, 1979; Åkerlind et al., 2011).

Cows of Holstein and Swedish Red breeds were fed a total mixed ration (TMR) containing 49% of one of the four grass silages (10 cows/silage), 49% of a grain-based complementary feed and 2% of a salt/mineral/vitamin mixture during the trial that lasted for 6 weeks. The rations for Swaj harvest 1, Switch harvest 1, Swaj harvest 2 and Switch harvest 2 contained 300, 288, 314 and 322 g NDF and 173, 170, 171 and 166 g crude protein per kg DMI, respectively. The NDF concentrations of the rations were lower than planned as the rations were based on the NDF content of the herbage before ensiling. The dietary contents of starch and crude fat were 164 and 40 g/kg DM intake, respectively. Cows fed silage of timothy Switch had a higher feed efficiency (P = 0.010) as a result of a tendency to increased energy-corrected milk (ECM) yield by 1.8 kg/day (P = 0.058) compared to cows fed silage of tall fescue Swaj, without affecting DM intake (Table 4). This higher feed efficiency of cows fed the timothy silage results in improved profitability for the farmer but the magnitude of the improvement varies with relative differences in DM yields between the crops and milk price.

Table 4. Intake, milk yield and feed efficiency (ECM/DM intake) in cows fed silage of tall fescue cv. Swaj and timothy cv. Switch, when averaged across harvests (n = 20; Murphy et al., 2017).[1]

	Swaj	Switch	SEM	*P* - value
DM intake (kg/day)	24.4	24.5	0.68	NS[2]
NDF intake (kg/day)	7.5	7.5	0.21	NS
NDF intake (% BW)	1.26	1.21	0.038	NS
Silage NDF intake (% BW)	0.82	0.78	0.025	NS
Milk (kg/day)	35.1	36.2	1.24	NS
ECM[3] (kg/day)	34.1	35.9	1.03	0.058
ECM/DM intake (kg/kg)	1.40	1.51	0.034	0.010

[1]No significant main effect of harvest and no crop × harvest interaction, [2]non-significance (P > 0.05), [3]energy-corrected milk.

Concentrations of fat and protein in milk did not differ between the crops but the milk protein yield was higher for Switch than for Swaj (1.31 vs. 1.22 kg; P < 0.05). No effects of harvest date and its interaction with crop were found. Cows fed silage of tall fescue Swaj lost body weight whereas cows fed silage of timothy Switch gained body weight during the experiment (-3.1 vs. +8.6 kg, $P < 0.05$), which indicate differences in nutrient partitioning between the cows fed the different grass silages. The more efficient production by the cows fed the silage of timothy Switch seems to be related to a more efficient fibre degradation in terms of higher digestion rate, which might be related to a less inhibitory structure of the lignin-polysaccharide matrix to cell-wall digestion in timothy Switch than in tall fescue Swaj as indicated by a lower iNDF/ADL ratio for timothy Switch (Jung and Deetz, 1993; Krämer et al., 2012). This warrants more research in cell wall chemistry and rumen microbiology to explain these differences in kdpdNDF between the crops.

As tall fescue and timothy differ in regrowth capacity and drought tolerance, which can affect feed quality of these grasses, the same monocultures of tall fescue cv. Swaj and timothy cv. Switch, were harvested at two occasions during the first regrowth cycle 2016 and fed in TMR to 48 dairy cows at Lantmännen Research Station Nötcenter Viken, Sweden. There were dry weather conditions in June of 2016, which made large differences in growth rate after the spring harvest between the species in favour of tall fescue. To achieve comparable developments of the regrowths, the crops were harvested at different dates. Swaj was harvested 32 and 43 days after the spring growth on June 27 (early harvest) and July 8 (late harvest), respectively, whereas Switch was harvested 43 and 60 days after the spring growth on July 8 (early harvest) and July 25 (late harvest), respectively. Tall fescue was at the leaf stage of maturity at both harvests as it normally does not head in the regrowth, whereas timothy was at the leaf-stem elongation-flag leaf stage of maturity at the early harvest date and at the stem elongation- to-flag leaf stage at the late harvest date. The grass was wilted to 26-31% DM before being ensiled in bales. Switch had lower content of NDF but higher content of ADL than Swaj in the early harvest. In the late harvest, Switch had higher contents of NDF, ADF and ADL and lower in vitro rumen OMD compared to Swaj (Table 5). Fibre contents increased while the in vitro rumen OMD decreased with later harvest of Switch, whereas there was no effect of harvest date on fibre contents and in vitro rumen OMD of Swaj.

Table 5. Effects of crop and harvest date on fibre content and in vitro rumen organic matter digestibility (OMD) of the silage (n= 3).

	Early harvest		Late harvest			*P* - value		
	Swaj	Switch	Swaj	Switch	SEM	Crop × Harvest	Crop	Harvest
NDF (g/kg DM)	484[b]	448[c]	478[b]	518[a]	7.1	< 0.001	NS	< 0.01
ADF (g/kg DM)	276[b]	262[b]	276[b]	307[a]	3.6	< 0.001	< 0.05	< 0.001
ADL (g/kg DM)	14.1[c]	22.9[b]	12.9[c]	34.5[a]	1.07	< 0.001	< 0.001	< 0.01
In vitro OMD[1] (%)	83.4[ab]	85.4[a]	82.0[b]	78.9[c]	0.56	< 0.01	NS	< 0.001

[1]According to Lindgren (1979, 1983, 1988). In vivo OMD = -2.0 + 0.90 * in vitro rumen OMD (Lindgren, 1983). NS = non-significance P > 0.05.

The digestion rate of pdNDF was higher for silage of timothy Switch compared to silage of tall fescue Swaj in the early harvest but the digestion rate of Switch decreased to a level close to the digestion rate of Swaj with delayed harvest (Table 6). The in situ iNDF content of Switch was slightly higher than the iNDF content of Swaj in the early harvest and the iNDF content of Switch increased by 47% with delayed harvest, whereas the iNDF of Swaj did not increase much. However, the iNDF/ADL ratio of Swaj increased more with delayed harvest than the iNDF/ADL ratio of Switch. The higher iNDF/ADL ratio for Swaj than for Switch (4.7 vs. 3.5), when averaged across harvests indicates that Swaj contains lignin that is more inhibitory to digestion per unit of lignin compared to the lignin of Switch (Table 6). There was no clear relationship between kdpdNDF and in vitro rumen OMD and between kdpdNDF and EFD of these regrowth grasses as it was in the grasses in the spring growth cycle from the previous trial.

Table 6. Fibre digestion kinetics and the iNDF/ADL ratio of the ensiled crops (n = 1).

	Early harvest		Late harvest	
	Swaj	Switch	Swaj	Switch
EFD[1] (% NDF)	55	57	53	47
in situ iNDF[2] (g/kg DM)	59	75	66	127
in situ iNDF[2] (g/kg NDF)	123	167	138	245
pdNDF[3] (g/kg NDF)	877	833	862	755
kdpdNDF[4] (%/h)	5.4	6.8	5.1	5.4
iNDF/ADL	4.2	3.3	5.1	3.7

[1]Effective fibre degradation in rumen at 3 % passage rate (Lindgren, 1991). [2]Indigestible NDF determined after 288-h rumen incubation in situ (Åkerlind et al., 2011). [3]Potentially digestible NDF (g/kg NDF) = 1000 – iNDF (g/kg NDF) in situ (Åkerlind et al., 2011). [4]Fractional degradation rate of potentially digestible NDF from curve fitting of in situ values (Ørskov and McDonald, 1979; Åkerlind et al., 2011)

Forty-eight cows of Holstein and Swedish Red breeds (109 DIM) were fed a TMR containing 46% of one of the four grass silages (12 cows/silage), 46% grain-based complementary feed, 7.6% dried sugar beet pulp and 0.4% mineral/vitamin mixture of DM intake during the trial that lasted for 7 weeks. The rations for early harvested Swaj, early harvested Switch, late harvested Swaj and late harvested Switch contained 321, 305, 318 and 337 g NDF and 189, 188, 184 and 167 g crude protein per kg of DMI, respectively. The contents of starch and crude fat in the ration were 167 and 42 g/kg DMI, respectively.

The DM intake of the ration containing timothy Switch silage from the early harvest was higher compared to the DM intake of the ration containing tall fescue Swaj silage from the same harvest (Table 7). As the NDF content increased while the rate and extent of NDF digestion decreased with later maturity of the timothy silage, the dietary DM intake decreased to similar levels as for the rations containing tall fescue Swaj silage, which did not differ between early and late harvests. The dietary NDF intake of the timothy silage was higher than the dietary NDF intake of the tall fescue silage at the early harvest, which probably was related to the higher digestion rate of pdNDF of the timothy silage allowing more NDF to be digested in the rumen per unit of time compared to the tall fescue silage. The dietary DM intake of late harvested timothy silage was partly limited by rumen fill as a result of a higher iNDF content and a lower rate of NDF digestion compared to timothy silage harvested early (Tables 6 and 7).

Table 7. Intake, milk yield and feed efficiency (ECM/DM intake) in cows fed silage of tall fescue cv. Swaj and timothy cv. Switch (n = 12).

	Early harvest		Late harvest			*P* - value		
	Swaj	Switch	Swaj	Switch	SEM	Crop × Harvest	Crop	Harvest
DM intake (kg/day)	20.0^{b}	23.8^{a}	21.4^{ab}	19.9^{b}	0.70	< 0.001	0.080	0.057
NDF intake (kg/day)	6.4^{b}	7.3^{a}	6.8^{ab}	6.7^{ab}	0.23	< 0.05	NS	NS
NDF intake (% BW)	1.08	1.17	1.13	1.06	0.037	< 0.05	NS	NS
Silage NDF intake (% BW)	0.75	0.80	0.78	0.75	0.026	NS	NS	NS
Milk (kg/day)	32.5	37.2	32.5	33.9	1.25	NS	< 0.01	NS
ECM[1] (kg/day)	33.5$^{(b)}$	37.5$^{(a)}$	34.2$^{(ab)}$	34.8$^{(ab)}$	1.09	0.063	< 0.05	NS
ECM/DM intake (kg/kg)	1.77	1.60	1.69	1.84	0.071	< 0.05	NS	NS

[1]energy-corrected milk.

Averaged over harvests, the milk yield was 3.0 kg higher (35.5 vs. 32.5 kg, $P < 0.01$) and the ECM yield was 2.3 kg higher (36.2 vs. 33.9 kg, $P < 0.05$) for cows fed rations containing timothy silage compared to cows fed rations containing tall fescue silage and the difference was mostly driven by the high milk yield of cows fed the early harvested timothy silage (Table 7). Concentrations of fat and protein in the milk did not differ between silages, but the yield of milk protein was higher for the timothy silage than for the tall fescue silage at the early harvest (1.32 vs. 1.15 kg, $P < 0.05$), but not at the late harvest.

Although the cows tended to decrease their DM intake with later harvest date (20.6 vs. 21.9 kg, $P = 0.057$), there was no significant effect of delayed harvest on milk yield. Cows did not lose body condition score during the time of the trial and there were no differences in body condition between treatments with an average score of 3.0. Results on milk yield from this trial confirm results from the previous trial; timothy silage results in higher yield of ECM than tall fescue silage, which seems to be related to a higher rate of potentially digestible NDF in timothy silage and a lignin that is less inhibitory to digestion than the lignin of tall fescue. The time interval between harvests of the grasses was shorter in the previous trial and occurred very early with low fibre contents of the silages and no rumen fill constraints on intake, resulting in no differences in DM intake between harvests. However, in this trial, the time interval between harvests was longer, resulting in differences in DM intake for the timothy but not for the tall fescue silage, which already had lower intake than timothy at the early harvest. In practice, tall fescue needs to be combined with a concentrate of high fibre digestibility in a dairy cow ration to compensate for its low kdpdNDF value and high iNDF/ADL ratio.

Increased NDF content that is less digestible and has a reduced rate of digestion with delayed harvest of grass silage (timothy/meadow fescue/perennial ryegrass) has in our earlier work shown significant decreases in DM intake by lactating ewes, which resulted in loss of body condition. Furthermore, the live weight gain of the nursing lambs decreased, while the intake of concentrate by the lambs increased with decreasing digestibility of the grass silage fed to their mother ewes (Nadeau et al., 2016). When studying the effect of chopping grass silage (perennial ryegrass/timothy/meadow fescue), when supplemented with 0.8 kg concentrate, Helander et al. (2014b) showed decreased dietary selection, daily eating time and eating to rumination ratio but increased daily rumination time in pregnant and lactating ewes with high production potential. Chopping of the grass silage did not affect feed intake, probably because of dietary selection of the unchopped silage by the ewes. However, chopping of the silage increased live weight gain from 373 to 450 g/day of the weaned lambs without affecting intake (Helander et al., 2014a). From late pregnancy to early lactation, feed intake and proportion of large particles in faeces increased, whereas eating, rumination and total chewing time per kg DM intake decreased, suggesting less efficient fibre degradation due to increased passage rate of feed particles through the digestive tract (Helander et al., 2014b).

Conclusions

Good management in choice of grass species and cultivars, maturity stage at harvest and cutting length is the key factor to achieve physically effective fibre of high digestibility of C_3 grasses, which results in increased ruminant performance. Differences in fibre digestibility between C_3 grass species are closely connected to the cell wall structure and more research is warranted where we can investigate relations between the cross-linking of lignin and polysaccharides in the cell wall to digestibility and physical effectiveness of the fibre.

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Compact Total Mixed Rations for Dairy Cattle (Compact TMR)

NIELS BASTIAN KRISTENSEN [1]

Summary

The Compact TMR concept was developed in Denmark where feeding of dairy cows generally is based on both grass and maize silage. The Compact TMR concept originated from research demonstrating industry over-emphasis on physical structure and has become a holistic nutritional school with recommendations related to ration formulation, feed mixing, feed bunk management, analytical monitoring of TMR, and inline NIR for dynamic adjustments of mixer loading.

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Introduction

The use of feed mixers for preparation of total mixed rations (TMR) or partial mixed rations (PMR, supplemented with separate feeding of concentrate or grazing) has completely changed the presentation of feed for dairy cattle over the past 30 years. Before 2012 most advisors recommended farmers to be very careful not to destroy physical structure of forages during feed mixing. The general recommendation was to mix for a short time using a mixer that was able to blend ingredients with a minimum of physical impact on fiber structure and length of particles. For decades the dairy industry has suffered from sorting problems and excessive dilution of diets with straw and other fibrous feedstuffs as a result of the structure dogma in dairy cattle nutrition. The structure dogma has under the Northwest European conditions led to unnecessary stress on cows related to feeding and undernutrition. Undernutrition and feeding induced stress have severe consequences especially for high genetic merit cows that appear to be less resilient to inadequate nutrition and stressful environments compared with low merit and dual purpose cattle. This paper presents the Compact TMR concept which is a dairy feeding concept containing several elements: mixing protocol that includes a soaking phase to avoid particle loss from TMR to avoid sorting, high impact mixing that reduce physical structure of high fiber diets, feed bunk and orts management, analytical feedback loop and in-line NIR (near infrared spectroscopy) mounted on feed mixers.

Initial steps toward the Compact TMR concept

The TMR concept was developed by SEGES in on-farm R&D

projects running from 2012 to 2013 and was primarily based on research out of Aarhus University (Research Centre Foulum) in combinations with on-farm observations. Studies using both calves and dairy cows suggested that the recommendations for minimum requirements of physical structure based on the chewing time system might severely overestimate the need for physical structure in moderate and high yielding cows (Storm and Kristensen, 2010). The dominating argument in the industry for recommending short mixing times for TMR in mixers with a minimum number of blades/knives was that higher impact mixing would destroy physical structure of the TMR and it was claimed that mixer degradation of physical structure would cause problems with subacute ruminal acidosis (SARA), displaced abomasum, milk fat depression, and low feed efficiency. Studies of the author and co-workers at Aarhus University were not consistent with all these negative effects of high impact mixing on the contrary, high impact mixing and particle reduction in standard Danish diets indicated that rumen health and feed efficiency were maintained accompanied by reduced fill of forage based diets. In the initial phase of the development of Compact TMR several issues were considered:

- TMR should not be susceptible for sorting by cows, neither when fed or after drying in the feed bunk.
- In high filling rations (especially grass based rations) physical breakage of particles in the mixer is advantageous and not dangerous to rumen health.
- Sorting by cows is probably induced by a combination of taste, olfactory and tactile stimuli and therefore a TMR needs to be homogenous with respect to all three characteristics. A TMR should appear to the cow as a single homogenous feedstuff.
- The full benefit of Compact TMR was hypothesized to require control of feed allowance so that the feed bunk will

never be empty and that cows will find a ration of constant composition any time they go to the feed bunk.

- Mixing of high-density rations is a challenge to many vertical auger mixers, and it's absolutely necessary to monitor mixer function and intervene immediately if the mixer does not maintain sufficient material flow.
- High impact mixing of TMR that result in a homogenous mix that cannot be sorted by cows enable sampling of TMR on-farm to be used for monitoring mixing quality of TMR, and when combined with on-farm feeding and production data an industry wide nutritional learning loop can be established.
- Systematic loading of mixers according to the Compact TMR protocol and mixing times needed for high impact mixing might be ideal conditions for implementing analytical devices on mixers such as NIR to adjust for variation in dry matter of silages during loading (dynamic recipe adjustment) and to monitor batch to batch variation in chemical composition.

Compact TMR is a holistic feed management concept for dairy cattle that aims to address elements of rations formulation, mixing protocol, feed mixer surveillance, feed bunk management, cow behavior, in-line NIR applications, and nutritional feedback loops based on laboratory analysis of TMR combined with performance data from dairy farms.

Soaking to prevent sorting

In the original Northwest European version of Compact TMR soaking of dry ingredients before loading of silages is an important element of the Compact TMR protocol. In the soaking phase, dry ingredients absorb moisture from added water or other moist ingredients (sugar beets, potato pulp, wet waste products, etc.). The moistening of dry ingredients decrease dry matter of mix and is expected to aid in hiding and trapping small particles in the matrix of fibrous ingredients to be loaded after the soaking phase. Data from scoring of TMR and PMR samples showed that the mean dry matter of samples evaluated as not having any loss of particles (score = 1) was lower (360 ± 26 g/kg) compared with samples evaluated as suffering from particle loss (increasing loss from score 2 to 5; dry matter from 378 ± 28 to 418 ± 47 g/kg).

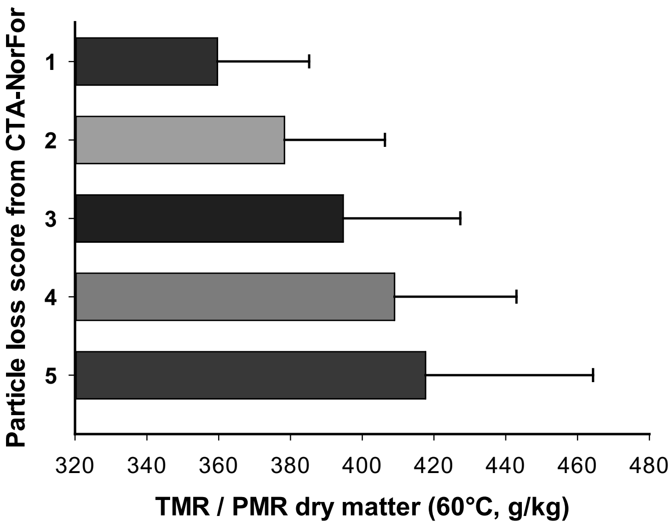

Figure 1. Average dry matter (stack indicate SD) of total mixed rations (TMR) and partial mixed rations (PMR) analyzed at KFL, SEGES, Denmark. Scores for particle loss were evaluated upon arrival of samples at the lab and are from 1 = no particle loss to 5 = heavy particle loss and loss of intact pellets from the mix. The dataset contained 3454 samples of TMR and PMR for lactating cows.

Shredding and cutting of fibrous ingredients

Several properties of the feed mixer are important for the shedding of materials during mixing, including mixer principle, the geometry of mixing chamber and augers, blade/knife configuration, the speed of augers, shear bar/counter knife configuration and the eventual use of restrictor plates. Many of these features are locked by make and model of a mixer, and therefore it's of major importance to provide a thorough analysis of the need for the mixer to impact the physical structure of the material during mixing before a new mixer is acquired. The need for shredding of material during mixing is dependent on the production level of the animals to be fed, the source of the fibers and the extent of particle reduction during harvest. In situations where rations are based on corn silage as main or sole source of forage or situations where more filling forages like grass silage is chopped short (4 – 5 mm) there will be limited need for shredding of fibers in the mixer. A paddle mixer and all vertical auger mixers that can maintain good flow when mixing heavy and finely chopped ration can be used when there is no need for shredding in the mixer. When more fibrous ingredients are included in the ration, when the length of fibers increases and when the ration is intended for feeding of high producing cows or growing calves it becomes of increasing importance that fibers are shredded during mixing. In Northwest Europe, ensiled grass is an important forage, and grass silage is characterized as being much more variable in digestibility compared with maize silage. In many rations based on grass silage, the grass silage is a determining factor for the ability of the ration to support the production potential of the cows. The need for shredding in the mixer increases substantially when decreasing digestibility and grass with increasing particle length is included in the rations. Mixers vary considerably in their impact on fiber particles. The primary impact of the mixers on fibrous particles is shredding and reduction of the length of long particles. In paddle mixers material gets cut more than shredded, in the vertical auger mixes material is more shredded and to a lesser extent cut, and in horizontal auger mixers there is both shredding and cutting of fibers. It's strongly recommended that farmers consult other that sellers of feed mixers or at least invite sellers of different mixing principles and makes before deciding on which type of mixer and mixer size to acquire. In the standard mixing protocol for Compact TMR shredding and cutting is done in phase 2, the structuring mix. In the CTA NorFor analytical program, the homogeneity, as well as the length of particles, are evaluated and reported as the TMR score. The TMR score is given on a scale from 1 to 5 where score 1 is given for mixing that are homogeneous and totally dominated by particles below 30 mm, score 5 is for a heterogeneous mix containing many long particles above 70 mm.

Homogenizing and compacting of TMR

The transformation of a mix of ingredients into Compact TMR that's so uniform in taste, olfactory and sensory properties that no cow will attempt to sort the mix require either fully preprocessed feedstuff that can be blended into a ration behaving as Compact TMR or increasing capabilities of the mixer to impact the structure and compactness of the ration during mixing. Figure 2 gives an overview of the capabilities of different mixer principles and their ability of impact the ration being mixed. The paddle and tumble mixers are able to cut particles if equipped with enough cutting knives and the mixer is operated for long enough time / enough rotations. Among vertical auger mixers, some manufacturers make mixers with stronger capabilities for cutting and shredding compared to others. The geometry of the mixing chamber, as well as the augers, have an impact on these properties of the mixer. The full effect of cutting, shredding and compacting is obtained using horizontal auger mixers that are able to impact the mix with higher pressure compared with the other types of mixers. The pressure and knives effect that can be put on a mix have increasing importance with rations containing more ingredients that either is susceptible for sorting as they are added, e.g., beets, potatoes, etc. or because the ingredients are clumping during mixing. Clumping of ingredients during mixing might be caused by mixing on a soaking phase that is too dry so that it forms clumps on its own or because of long fibers that roll up into grass balls or long fibers that roll up on clumps of soaking phase in the mix.

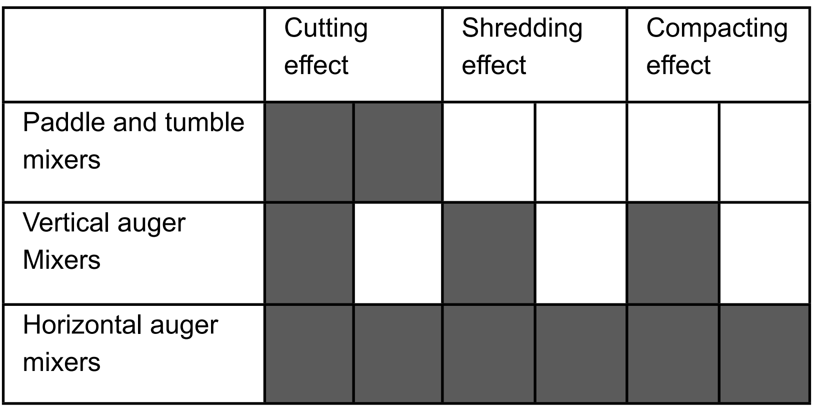

Figure 2. Schematic overview of the effectiveness of different mixers types to cut, shred and compact the mix during mixing of TMR. No filling indicate no or very limited effect, and half filling indicates intermediate effect and full green filling indicate full effect. Be aware that capabilities of mixers to mix and impact the mix during mixing depend on the proper maintenance of the mixer and sufficient auger speed.

Feed bunk management

Feed bunk management is of paramount importance to meet the strategic aims of the Compact TMR concept. The strategic aims are to improve access to feed (especially for subordinate cows), minimize the time cows spent at the feed bunk, minimize aggressive behavior at the feed bunk, and prevent turmoil in the herd related to feeding. For a large part time wasting at the feed bunk is reduced by providing Compact rations because this minimizes the time spent sorting. Also, the need for push up of feed is affected by mixing quality because cows largely stop manipulating the ration with a compact mix and don't push the feed out of reach. Another important aspect of good feed bunk management is the control of left overs and the distribution of left overs during the last hours before the daily rinsing of the feed bunk. It's a fundamental requirement in the Compact TMR concept that left overs are at least 2% of feed allowance and that the leftovers are evenly distributed along the feed bunk. Distribution obviously increasingly important with decreasing amount of TMR left. Control of the amount of leftover is important to reduce herd turmoil associated with feeding. Slick bunk management practices cause stress on herds at feed out and even the highest mixing quality cannot compensate for insufficient feed allowance, and therefore the Compact TMR concept depends on the combined effects of TMR mixing quality, feed allowance, and managing the position as well as the distribution of feed in the bunk.

Optimizing feed mixers for Compact TMR

Mixing Compact TMR is a challenge for many vertical auger mixers because they are unable to maintain flow during mixing a ration that's so heavy and finely chopped as a typical Compact TMR. The fundamental problem is that the mixers fail to clean up the mixing chamber leaving mix standing on the floor along the sides of the mixer (Photo 1). This "foot" of material will prevent the downward flow of material between auger and side of mixing chamber, and therefore the flow in the mixer is blocked, and the mixing process stops.

Photo 1. Material blocking flow in vertical auger mixer because of the inability of the auger to clean the base of the mixing chamber. This problem is common among vertical auger mixers and is solved by adding an effect plate, auger share or auger shoe on the augers. The example shows a Kongskilde mixer (Kongskilde, Sorø, Denmark) without effect plate mounted on the augers. Tuning of the mixer with a combination of effect plate and 2 long blades enable the mixer to mix a Compact TMR.

Photo 2. Auger share mounted on auger of vertical mixer helping the auger to clean the base of the mixing chamber which enables the mixer to safely mix a Compact TMR. The example shows a BVL (BvL Group, Emsbüren, Germany) vertical auger mixer with a factory designed auger share.

It's of paramount importance that the flow in vertical auger mixers is carefully monitored if it's attempted to implement Compact TMR. It's especially important to look for downwards movement of the mix along the sides of the mixing chamber, and it's especially important to inspect the flow during the final mixing phase where corn silage is mixed with the structuring phase. Most manufacturers of feed mixers marketing mixer in Denmark have factory kits available for tuning mixer to mix a Compact TMR (Photo 2).

TMR based learning loop in dairy production

With a high mixing quality of TMR new possibilities arise for monitoring nutrition of cows through a TMR surveillance program. When cows are fed according to the principles laid out in the Compact TMR concept then the mixed, the fed and the ingested ration is one entity, and that one entity can be sampled and analyzed (Photo 3). The absolute requirement for ad lib feeding means that the composition and characteristics of the TMR contain all the input information to be used for solving the cow performance puzzle in combination with information on barn system, climate, breed and management variables.

Photo 3. With the mixing protocol of Compact TMR, the ration is mixed so completely that the mixed, the fed, and the ingested ration are of the same composition. With a systematic sampling protocol and the analytical program CTA-NorFor, a big nutritional learning loop has been established. The photo shows the sampling of TMR at approximately 50 % unload. The sample is collected in a 65 L bucket, and sample reduction is made by coning and quartering. The TMR was mixed in a Storti Husky 19 m^3 (Storti S.p.A., Belfiore, Italy) horizontal auger mixer.

The analytical program named Compact TMR Analyzer – NorFor (CTA-NorFor) is an analytical service for dairy farmers with the sampling of TMR 11 times per year. The TMR sampling is done parallel to milk testing and data from TMR testing are evaluated with reference to rations formulated in the NorFor feed evaluation system (https://www.norfor.info) and performance data from the herds including milk yield, milk quality, reproduction, veterinary treatments, clinical assessments, forage quality, weather, etc. TMR samples are scored as shown above followed by drying, milling, and NIR scanning. A full set of calibrations have been developed for Bruker MPA full spectre FT-NIR instruments (Bruker Optik GmbH, Ettlingen, Germany) and includes major analytical entities such as crude protein, NDF, starch, crude fat, ash, sugars as well as calibration based on NorFor calculated parameters such as NEL20 and metabolizable protein (NorFor AAT20).

In-line NIR for dynamic adjustment of silage loading

The Compact TMR protocol ties the mixed, the fed, and the ingested ration together so that it becomes one and the same ration. However, the Compact TMR protocol in combination with in-line NIR will take TMR feeding one step further in that it helps close the potential gap between the formulated and the mixed ration. In silage based TMR, it's often so that the silage dry matter vary due imperfect filling of the bunker and exposure of bunker face to weather and silage dry matter will decrease with rain and dry up during dry weather. The ultimate control of the number of left overs requires frequent adjustments of the amount of silage to be loaded depending on the variable dry matter of the silage. The Compact TMR mixing protocol is a good basis for implementing in-line NIR systems for monitoring the dry matter as well as chemical composition of the mix.

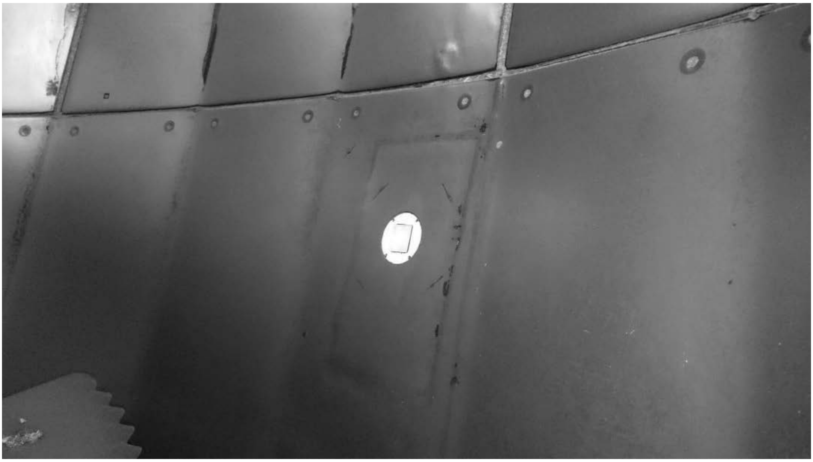

Photo 4. NIR sensor mounted on mixing chamber of feed mixer and scanning the mix during the mixing process. Predicted dry matter of mix is used to adjust loading of silages to meet targeted total dry matter content in the batch. Adjusting for silage dry matter variability is important to control the chemical composition of the individual batch as well as controlling the number of leftovers.

Based on dry matter measurements in the mix and the expected dry matter of each component as well as the amount loaded the weighing computer calculate adjustments of the amount of silage to be loaded. Usually, the loading of each silage type, i.e., grass silage and corn silage is split into two parts. First, is loaded 80 % of expected target weight, then NIR measurements are performed, and the difference between expected and measure dry matter is calculated. The second loading is based on recalculated target weight taking into account if more or less dry matter actually was loaded with the first 80 % of the expected target weight. The Compact TMR mixing protocol, the high impact mixing, and the applied mixing times contribute to the Compact TMR being an ideal basis for implementing analyzers like NIR instruments directly on the mixing chamber.

Some of the presumed advantages of installing NIR instruments on the feed mixer are that the sample is very well presented to the analyzer and with long mixing times there is plenty of time for repetitive measurements. With high impact mixing the mix is being compacted which means that air pockets in the mix are minimized and therefore have minimal negative impact on the quality of spectra collected. Feed mixer NIR applications are currently being developed in collaboration between Dinamica Generale (Poggio Rusco, Italy), HiSpec Denmark Aps (Vodskov, Denmark), and SEGES (Aarhus, Denmark). The calibration models to be used with the inline NIR applications are under development, however, at present, it seems that the prediction error for dry matter with high impact mixing is approximately 1 % (Figure 3). Data indicate that the performance of the NIR application depends on the mixing protocol with improved performance of inline NIR with increasing compacting of the mix.

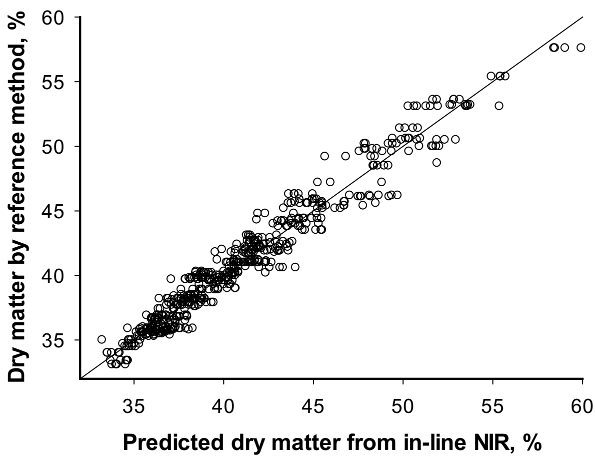

Figure 3. Cross validation of feed mixer in-line NIR predictions of dry matter in structuring and final phase of mixing. The dataset contains 110 batches with 5 repetitions per batch. The calculated standard error of prediction was 1.1% DM.

Conclusion

The present paper presented the Compact TMR concept and its multiple prerequisites and targets. The Compact TMR concept was developed in Denmark where feeding of dairy cows generally is based on both grass and maize silage. The Compact TMR concept originated from research demonstrating industry over-emphasis on physical structure and has become a holistic dairy nutrition school with recommendations related to ration formulation, feed mixing, feed bunk management, analytical monitoring of TMR, and inline NIR for dynamic adjustments of mixer loading.

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Selection of maize silage hybrids - agronomic and nutritional traits

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Msc. MARYON S. D. CARBONARE [1]

1. What is ABC Foundation?

ABC Foundation was the first foundation (a private non-profit institution) based on agricultural research in Brazil, founded in October 23, 1984. ABC Foundation carries out applied research to develop and adapt new technologies in agribusiness, with the objective to supply farmers and technicians with technological solutions for the current problems. The company is supported by 4.500 farmers affiliated to the Cooperatives Capal (Arapoti-PR), Frísia (before Batavo in Carambeí-PR), Castrolanda (Castro-PR) and other groups of farmers in South and Centre-West regions in Brazil.

The quest for production with excellence has always been present in the ideals of the Dutch immigrants, founders of the 3 cooperatives. These cooperatives are marked by specialized

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technical assistance, their farmers were pioneers in the developing of the no-till system and are reference in productivity of cereals (soybeans, beans, corn and wheat), pork and milk. The farmers associated to the cooperatives cultivate an area of 454.000 hectares and produce 1.4 million liters of milk per day.

ABC Foundation also carries out research projects in cooperation with private companies and public research companies like IAPAR, EMBRAPA and Universities.

Actually ABC has 210 coworkers, 23 of these are researchers working at 7 different areas (phytopathology, entomology, mechanization and precision agriculture, crops science, agrometeoroly, herbology and forage crops).

The headquarter and laboratories are located on highway PR 151, Km 288, in the city of Castro-PR, Brazil, post code 84165-700. Besides the main office ABC Foundation has 6 experimental fields located in Paraná (4), São Paulo (1) and Goiás (1), with 215 hectares of experimental area in total.

2. What should we expect in a corn hybrid for silage?

The seed companies offer a wide range of corn hybrids to the farmers. There are hybrids for many purposes like grain production, silage or special uses like starch industries, human consume and others. For each purpose, there are a range in genetics that aims to attempt the requirements of every region, according to the environmental conditions, pest and disease tolerance, cycle for harvest, soil and crop management (Fernandes, 2014), all these characteristics given to the fields a range in yield and quality indexes (Paziani et al., 2009).

The corn hybrid choosing for silage must be considered not just by the cost of the seed or the dry matter production, but as well for the nutritional benefits that each hybrid brings itself (Reis et al., 2014). Due the complex interaction between genetics and environment (Shioga et al., 2015), the process of choosing a corn hybrid for silage should to take account several factors of analysis:

2.1 Dry matter production:

This is one of the most important parameters in a selection of corn for silage. Investments in the corn field are high and for these reason high dry matter production is mandatory to reduce the cost of silage. Besides this, normally the farms in Brazil have area limitations for feed production, than we have to aim to produce the most feed as possible in the available area to feed more cows.

The fresh matter production has been the first factor at corn hybrid choosing by the farmers in Brazil. This is important like it was mentioned before, but we must to think ever in dry matter production and not fresh matter, and these dry matter must have the maximum concentration of digestible nutrients.

In Brazil fresh matter (FM) production of corn has ranged from 26 until 87 T ha^{-1}, and the dry matter (DM) production from 9 until 29 T ha^{-1}, that is a large variation (Neumann et. al., 2017; Moraes et. al., 2013). The factors affecting the DM yield are the weather and soil conditions, the crop management and the genetics of the hybrid (Mittelmann et. al., 2005). Crop management, fertility of soil and genetics can be are controlled by the farmers. The weather not, but is possible to have a better understanding about the historical climate and try to schedule the field operations in the best seasons to minimize the risks.

2.2 Nutricional value:

In the last decades there was a large improvement in animal breeding and management of the herds that resulted in expressive gain in animal production. But without better quality of feed, is not possible to guarantee the expression of the animal potential. Then the nutritional value of the feed must be improved at least in the same proportion as the new animal requirements (Paziani et al., 2009), aiming to achieve the needs of high-production dairy cows.

Unfortunately, in Brazil, most of corn breeding programs are focused at grain production and not at silage purpose. Usually the hybrids with higher grain yield are desired for silage making (Gomes et al., 2002) once the grains contain most of energy in the silage. However, it is important to evaluate the fiber content (NDF, NDA and Lignin) and the digestibility of these fractions (Reis et al., 2014). At a work with a large variety of hybrids, Nussio et. al. (2001) didn't find relation between digestibility of the whole plant or of the fibers with the grain production. Then, we can conclude that the grain content is important but it must be accompanied with low fiber content and high digestibility of the plant components. After the hybrid chosen, then we need to do all the best for the crop to express its potential of grain production, once the grains can provide until 2/3 of the silage energy content.

There are a lot of nutritional parameters to evaluate in a corn hybrid for silage, but some of the most important are Starch, NFD, IVDNDF, Fat, Ash and Crude Protein contents. 30% of variation on these parameters can reflect in 18%, 14%, 12%, 3%, 2% and 2% of variation in milk production, respectively (Shaver et. al., 2006). A meta-analysis from Ferraretto and Shaver (2015) showed that the variation in total tract fiber digestibility is much higher than total tract starch digestibility (258% versus 24%). The same meta-analysis found differences for fiber digestibility between four different kind of stalk of corn and didn't found differences for starch digestibility between four different types of kernels.

In overall, in Brazil we use more tropical hybrids, with higher fiber contents and lower digestibility. Comparing Brazilian winners (ABC farmers) with American winners at their corn silage contests, we can realize lower IVDNDF for the Brazilian hybrids (Figure 1, ABC Foundation, 2017a and World Forage Superbowl, 2016). In another comparison between corn silage of many countries and Brazil (ABC farmers), we found the same, Brazilian hybrids presented lower fiber digestibility (ABC Foundation, 2017a).

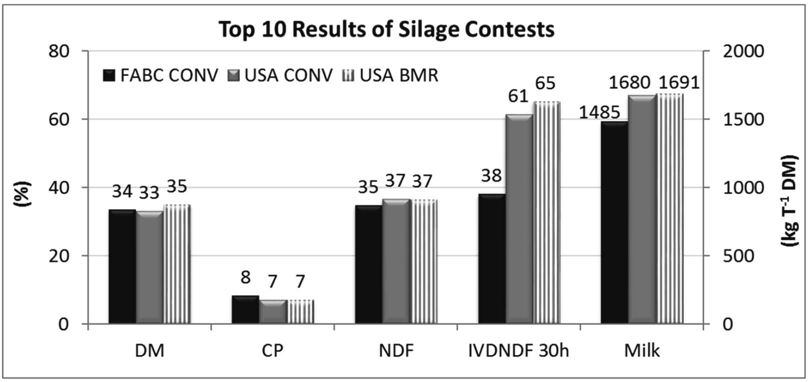

Figure 1 – Average results of the top 10 winners of the silage contests from ABC Foundation and USA.

Thus, the Brazilian corn breeders could develop better hybrids for silage with focus not just at grain production but for lower fiber content and better digestibility as well. BMR (brown midrib) corn hybrids shall be considered in breeding programs for silage, once they present higher fiber digestibility, that results in higher dry matter intake (DMI) and higher milk production (Ferrareto & Shaver, 2015).

2.3 Health

Nowadays we are using corn hybrids with high grain yields potential but more requisite in crop management, like more fertilizers rates and more pesticides spraying. Usually the breeding for increased grain production work in the opposite way of diseases and pest resistance. If we don't care about the leaf fungal diseases in corn in Brazil, until 80% of the grain production can be lost, affecting the corn silage quality (Schipanski, 2011).

However we can observe that some corn hybrids can afford better the leaf fungal diseases even with good grain and mass production. These kinds of hybrids are desired. To combine both production and stresses tolerance will be the challenge for the breeders in the next years. Biotechnology can be a great tool to hurry this process.

2.4 Biotechnology

GMO (genetically modified organism) corn hybrids started to be used by USA farmers at 1996 and by Brazilian farmers at 2007. Since then, many traits have been developed and launched to the marked. Most of the traits in corn have been developed for pest and herbicides tolerance. These technologies are helping the farmers to better manage the crops. In some regions of Brazil insect control have becoming very difficult just with pesticides, and for these reason the biotechnology is taking an important place for the farmers in these regions, mainly for the small ones that don't have appropriated equipment.

It is good to consider that there is difference in feeding preference by the worms for some hybrids. And there are differences between the traits for pest control as well. Some traits that were working well in the past don't work more. The reason is the resistance evolution, one of the major limitations to continued use of Bt crops (GMO for pest control). The main goal of resistance management is to delay or prevent the occurrence of field failures (Andow, 2008) and the main strategies to achieve these are work with high-dose of protein expression and refuge areas (Shelton et al., 2000).

It is important that the companies continue to develop new and better traits and the research institutes do local experiments to evaluate the efficiency of the traits throughout the years and advice the farmers about this.

2.5 "Stay Green"

The "stay green" character is desired for corn hybrids cause is possible to harvest in a more mature stage with the plants green yet. The later harvest allow to the plant accumulate more starch in the grains and the forage part of the plants (stem, cob leafs, cob and bracts) remain green with suitable DM and sugar content for the proper chop, compaction and fermentation of the silage.

The traditional recommendation of 30 until 35% of DM for harvest can be reviewed for these type of corn hybrids. A research carried out in ABC Foundation last season with one stay green hybrid found a lightly decrease in fiber digestibility with the plant maturity, but there was a remarkable gain in starch accumulation until 45% of DM. The estimated milk production per ton of silage increased until 38% of DM and then this hybrid could be harvested at this DM instead of 30% until 35% (ABC Foundation, 2017b).

2.6 Earlyness

A short cycle hybrid is important for farmers who need to do a second crop at the autumn season. At the experiments of ABC Foundation, the cycle from seeding until silage harvest, have ranged from 110 days until 151 days depending on the hybrid, year and location. The difference between the earliest and the latest hybrids have been varied from 18 to 27 days. To know the cycle of each hybrid at a certain location is very important to help the farmers to select the correct hybrid for the first season that allow seeding the second crop in time to achieve a good result. Every day in the seeding date of the second crop is important to assure better production and quality for soybean, bean, sorghum or even corn again. In January, the delay in the seeding date of corn decrease 125 kg ha^{-1} of dry matter and 91 kg ha^{-1} of grain per day (ABC Foundation, 2017c).

2.7 Stability and adaptability

Other important attributes to a corn hybrid are the stability and adaptability. Some hybrids perform very well in one year but not in the next year. These means lack of stability between different years. In other cases, one hybrid performs very well at one place but bad at other close fields. This phenomenon means lack of adaptability for different kinds of soil, management or weather conditions.

We should choose hybrids with broad stability and adaptation, at least for the region surrounding the farm. The ABC does this comparison and calculates a frequency of success of every hybrid considering all the experiments where that hybrid was present (Figure 3).

2.8 Seed cost

Lately the difference of seed price between corn hybrids has increased in a fast way. While old and conventional hybrids has kept the price in the last years, the new ones, more productive and with biotechnological traits have the price increased a lot. Differences between hybrids seed price can reach more than 300%. In the cooperatives of the ABC region it is possible to find corn seeds with prices ranging from US$ 72 up to 276 per bag with 60,000 seeds. This factor should not be the main concern when choosing a hybrid for silage, but most of farmers in Brazil look first for the price, despising all the other marks talked before. We will comment more about this issue at the end of next chapter.

3. How do we conduct the trials at ABC Foundation?

The corn hybrids respond differently according to the environmental conditions to which they are subjected (genotype-environment interactions). The productive, morphological and nutritional characteristics of the genotypes can switch when exposed to different climate and soil conditions. Thus, the recommendation of hybrids for silage cannot be generalized (Mittelmann et. al., 2005).

The Forage Research Sector of ABC Foundation annually performs competition trials of corn hybrids for silage in the main producing milk regions of the ABC Group. Two trials are carried out in the hot region of the group, in the cities of Arapoti-PR and Itaberá-SP and two trials are performed in the cold region in the cities of Ponta Grossa-PR and Castro-PR. In this way, it is possible to select the most suitable hybrids according to the edaphoclimatic region that the farm is located.

Seeding date of the hybrids competition trials is carried out at the preferential period for each region. But to attend the need of some farmers, other trials are also carried out with anticipated seeding (one month before) and in the second season that is called “safrinha” in Brazil.

The crop managements such as fertilization, pest control, diseases and weeds are carried out as recommended by the other research sectors of the ABC Foundation.

The plots are formed by four lines of plants with five meters length. The rows between lines are spaced by 80 cm, making a plot with 16 m^2 of area. The useful harvest area is formed by two central lines with 4m long (6.4 m^2). The dry matter content of each hybrid is monitored twice a week and the cut is performed when the plants reach from 30 until 35% dry matter (Nussio et. al., 2001). Soon after cut, all the plants of the useful area are weighed to estimate the production of fresh matter. Five plants with average weight of the plot are selected, chopped in a small forage machine, and a sample of the forage is taken and sent to the laboratory of the ABC Foundation for the bromatological analyzes.

To know the nutritional value of the hybrids, bromatological (chemical) analyses are performed at the four replicates of the trial. In the 2016/2017 season, 456 analyses were carried out with an investment of more than US$ 22,000.00.

The bromatological analyses are performed by the technique of the infrared reflectance spectrophotometry (NIRS), with a FOSS® equipment and calibration from the Dutch laboratory BLGG.

The main parameters analyzed are: Dry matter content (DM), crude protein (CP), insoluble acid detergent fiber (ADF), neutral detergent insoluble fiber (NDF), in vitro digestibility of neutral detergent fiber (IVDNDF), lignin, total digestible nutrients (TDN), relative forage value (RFV), starch, in vitro digestibility of organic matter (IVDOM), ash and fat.

Through the production of the dry mass and the nutritional value of each hybrid is estimated the milk production per ton of silage and per hectare using the Milk 2006 worksheet (Shaver et. al., 2006). Statistical analyses are performed for production and quality parameters every place every year. Subsequently, the pondered average is calculated between the years for milk estimated per hectare and per ton of DM. Then quadrant charts are created for each location (Figure 2). Hybrids with above-average of dry mass production and quality are positioned in the upper right quadrant and these are the most suitable for silage.

The best hybrids will result until 16% more liters of milk per ton of silage (quality) and 30% more liters of milk per hectare compared to the worst hybrids. A farmer that currently has a production of 20,000 L/ha of milk can produce 26,000 L/ha in the next season, just by choosing the correct hybrid for silage making.

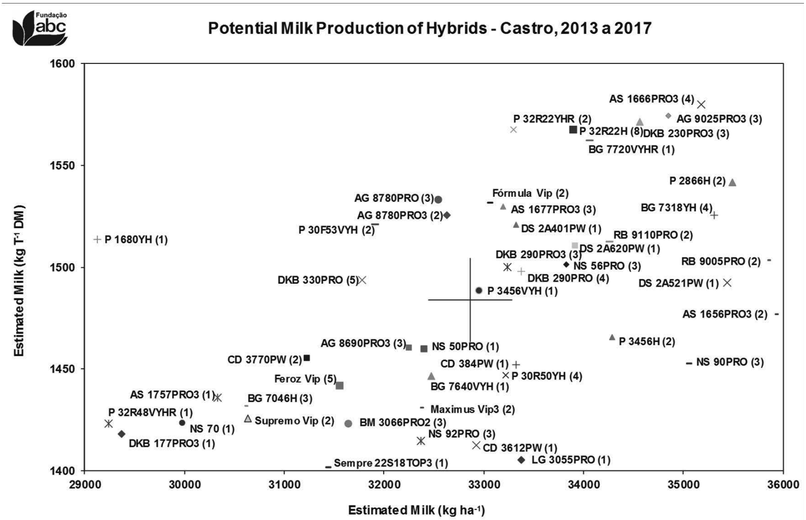

Figure 2 – Potential of milk production between several corn hybrids in Castro.

Through these quadrant graphs, the best hybrids are selected (upper right position) and a list is made summarizing the main characteristics of each hybrid (Figure 3). The characteristics given in the list are the average of the years for (i) cycle, (ii) dry matter production, (iii) estimated milk production per ton of DM, (iv) estimated milk production per hectare, (v) reaction to the main foliar diseases and (vi) the frequency where the hybrid had been success between all the trails.

In addition, the indicated hybrids are divided into three categories according to the maturity and the technological management level. With all these information, the consultants and the farmers can make a better decision according to the status of each farm.

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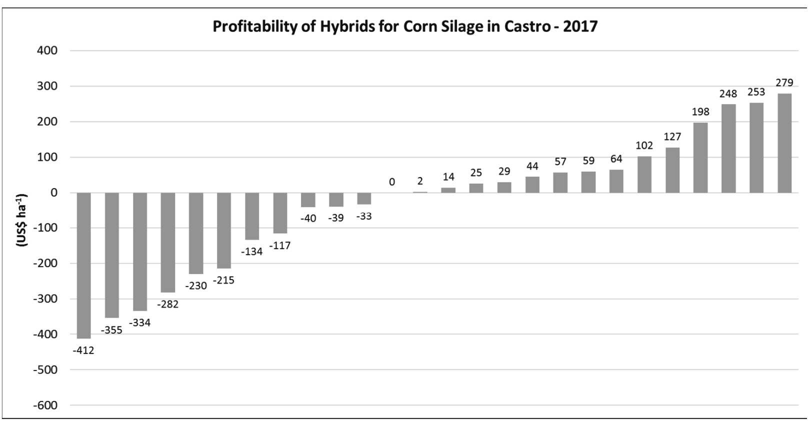
Corn Hybrids Indicated for Silage in Castro - 2017

Hybrids	Cycle (days)	DM ($kg\ ha^{-1}$)	Estimated Milk ($kg\ T^{-1}$ DM)	Estimated Milk ($kg\ ha^{-1}$)	Diseases Score[2] PS	PM	CZ	ET	Freq.[3] (%)
High Technology - Early Cycle									
P 2866H (2)[1]	131	23011	1542	35488					100
BG 7318YH (4)	131	23096	1526	35311					69
AS 1666PRO3 (4)	131	22257	1580	35178					85
DKB 230PRO3 (3)	128	21986	1572	34564					55
P 32R22YHR (2)	131	21263	1568	33296					33
AS 1677PRO3 (3)	129	21680	1530	33192					70
Fórmula Vip (2)	128	21589	1532	33067					50
BG 7720VYHR (1)	131	21810	1562	34066					100
DS 2A401PW (1)	131	21911	1521	33325					75
High Technology - Normal Cycle									
RB 9005PRO (2)	140	23831	1503	35847					71
AG 9025PRO3 (3)	135	22132	1575	34853					80
RB 9110PRO (2)	140	22654	1513	34269					50
NS 56PRO (3)	139	22506	1501	33826					33
DKB 290PRO (4)	134	22241	1498	33377					29
DKB 290PRO3 (3)	135	22153	1500	33237					73
DS 2A521PW (1)	132	23747	1493	35442					100
DS 2A620PW (1)	141	22445	1511	33910					75
P 3456VYH (1)	137	22144	1489	32957					100
Medium Technology - Normal Cycle[4]									
NS 90PRO (3)	144	24126	1453	35062					70
P 30R50YH (4)	141	22941	1447	33223					38
CD 384PW (1)	142	22944	1452	33325					50

[1]Number of years in trials.
[2]PS: *Puccinia sorgui*; PM: *Phaeosphaeria maydis*; CZ: *Cercospora zeae-maydis*; ET: *Exserohilum turcicum*.
White blocks: good health, Grey blocks: medium health, black blocks: poor health.
[3]Frequency of all trials where the hybrid had milk production above the average.
[4]Inexpensive seed, good health, dry matter and milk estimated ($kg\ ha^{-1}$) above average (slightly below average quality).

Figure 3 – Corn hybrids indicated for silage in Castro.

Even with all these information, hybrids that result in higher milk production are not always more profitable. The prices of corn seeds varies widely. In addition, hybrids distinguish in biotechnology traits for pests and weed control and also in terms of tolerance to diseases. A corn hybrid seed can be cheaper, but it can demand several spraying of insecticides, fungicides and herbicides, increasing the cost of production. So, an innovation created by the ABC Foundation this year was the calculation of profitability for each hybrid. The developed worksheet considers the technology cost for each hybrid, which includes the cost of seed and the inputs involved in control of pest, diseases and voluntary corn plants that germinate after harvesting. The benefit was calculated by the contribution margin of the estimated milk yield of each hybrid (net return per liter of milk). Finally, the balance is the difference between the cost/benefit for every hybrid, compared to the most popular hybrid at the region (Graphic 1). Some hybrids considered "expensive" may be economically viable depending on the return they give in milk production. And some hybrids that are not so good in milk production but have low cost of production can be also interesting financially.



Graphic 1 – Difference in profitability of corn hybrids for silage in Castro.

4. Final considerations

- In Brazil the technology level adopted by farmers to grow corn is very broad and the variation in corn silage production is significative. Many farmers are small, do poor investments at the crop and have low production. Then, first of all we should think about how to help these farmers to manage better the corn fields and just at the second moment we should recommend them better quality hybrids;
- Several factors must be considered when selecting a corn hybrid for silage, like dry matter production, nutritional value, tolerance to diseases, biotechnology, stay green, earliness and price of the seeds;
- Besides the characteristics listed above, the seed companies in Brazil have the opportunity to develop special corn hybrids for silage, aiming lower fibers content and higher fiber digestibility;
- Due the genetic-environment interaction, corn hybrids trials for silage must be done locally aiming evaluation and recommendation to the farmers.

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Shredlage technology: a key to expanding grain sorghum silage

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NICOLAS DILORENZO [2]

Introduction

Beef and dairy production around the world constantly compete with agriculture for land use, and there are many situations in which limited rainfall or unfavorable soil conditions make corn silage production uncertain. Because of its drought tolerance and high forage production, sorghum is an alternative (Andewakun et al., 1989), thereby providing the opportunity to increase the land area where ensiled crops can be produced. The most important factors that define sorghum silage quality are grain content (Young et al., 1996) and stage of maturity (Bolsen, 2004). The major potential limitation of sorghum silage is the lower digestibility of the grain due to the dense proteinaceous matrix in the peripheral endosperm layer of

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the kernel (Gutierrez et al., 1982), which renders starch granules inaccessible to ruminal digestion. However, this limitation can be overcome by rolling the silage to crack the grains, resulting in similar digestion levels than those of corn silage (Havilah and Kaiser, 1992). When silage was rolled properly, no differences were reported when comparing grain sorghum silage with corn silage, when used as a supplement for beef steers grazing high-quality pastures (Abdelhadi and Santini, 2006).

Previous generations of kernel processors were designed for each crop; thus, forage harvesting contractors have to change processors depending on the crop they are going to harvest. Because around the world, almost 70% of silage is made from corn, the corn cracker is the most commonly found cracker in harvesters, and this type of processor was not designed to crack sorghum berries.

Recently, a new generation of multi-crop cracker (Shredlage®) was developed for corn silage to achieve a good balance between long fiber and better grain processing, and as shown by Ferrareto and Shaver (2012), and Vanderwerff et al. (2015), cows fed diets based on corn silage processed with Shredlage® had improved starch digestion and milk production when compared with cows fed diets based on corn silage conventionally processed.

Although Shredlage® was developed for corn, a great contact surface with 9.8” diameter rolls, a 50% speed difference, 110 and 145 teeth per roll, and the possibility to work as closely as 1 mm of roll gap, led us to suspect that crops as sorghum with smaller grains could be processed.

Because little information has been generated using Shredlage® for grain sorghum silage, our objective was to conduct a trial to evaluate the effect of Shredlage® when compared with a conventional sorghum cracker or “no cracker” at all, on particle size and in vitro organic matter and in situ dry matter and starch digestion.

Materials and Methods

1. Crop and treatments

Grain sorghum (8006T, Pannar hybrid, Venado Tuerto, Argentina) was non-till seeded at 52cm between rows on November 25, 2015, at Los Pinares beef farm (35^{0}03'29"S-58^{0}02'11"W) located in nearby La Plata, in Buenos Aires province, Argentina. Seeded occurred after 45 days of herbicide application (3 lts/ha of Glyphosate plus 0.6 lts/ha of 2-4D) in a soil complex consisting of 900g/kg fine, mixed, thermic Typic Argiudol and 100g/kg fine, illitic, thermic Petrocalcic Paleudoll (pH 6.5; organic matter = 50g/kg, NO_3–N = 4.0 ppm and P = 7.8 ppm) at a rate of 170,000 seeds/ha to achieve 150,000 plants/ha at harvest, according to seed manufacturer recommendations. In addition, 50kg/ha of diammonium phosphate (i.e., 180g/kg N, 460g/kg P) were included at seeding and no-urea was applied because of mud due to frequent rainfalls which, from seeding to harvest, were 470mm plus 100mm from the time of herbicide application.

Sorghum was harvested at ground level on May 23, 2016, at the hard dough stage of grain maturity (Vanderlip and Reeves, 1972) using a precision chop-harvester (Claas Jaguar 980, Harsewinkel, Germany) equipped with 9 meters Orbis header, V-Max 28 knife drum and two types of grain crackers: 1) Sorghum cracker with 250mm rolls (125 and 150 teeth's) working at 40% speed difference and 2) Multi-crop Shredlage® with 250mm rolls (110 and 140 teeth's) working at 50% speed difference.

When harvesting three treatments were applied over the crop, set to a theoretical cut length of 15mm: NC – no cracker, SC – sorghum cracker and MS – multi-crop Shredlage®.

Rolls gap spacing for SC and MS was manually set to 1mm.

2. Sampling

At harvest time, carton boxes were fixed at ground level using iron staples in the line over which the discharge spout was going to leave the chopped sorghum. When the harvest occurred, all the chopped sorghum inside a box was constituted one sample. We had 3 blocks over which we randomized one of the treatments (NC, SC, MS) and within treatments, 3 samples were taken, to finally had 27 samples (3 blocks x 3 treatments x 3 samples). Each sample was layered over a plastic sheet, homogeneously mixed and separated in 4 sub-samples that were frozen for analyses.

3. Laboratory and physical analyses

Frozen sorghum samples (totally 108) were oven-dried at the forage laboratory of the Faculty of Agronomic Sciences of the National University of La Plata (Buenos Aires, Argentina) and analyzed for DM (60ºC for 48h).

One sub-sample was baled in Ziploc plastic bags and sent to the forage laboratory of North Florida Research and Education Center of the University of Florida (UF-IFAS) to study in vitro organic matter digestibility as Ciriaco et al. (2015) and Henry et al. (2015). Samples used for in vitro and in situ studies were ground through a 6 mm screen using a Wiley Mill (Arthur Thomas Co., Philadelphia, PA, USA) to simulate rumination to reduce the level of interference with the main effect evaluated (processors effects). Another sub-sample was used at the forage laboratory of the Faculty of Agronomic Sciences of the National University of La Plata (Buenos Aires, Argentina) to study particle size as described by Heinrichs (2013).

Ruminal in situ digestibility of dry matter and starch was performed as described in Ciriaco et al. (2015). Briefly, two ruminally cannulated Angus crossbred steers consuming a 50% concentrate diet were used to incubate 10 × 20 cm Dacron polyester bags (R1020, Ankom Technology Corp., Macedon, NY) containing approximately 5 g of dried material ground through a 6 mm sieve. Samples were incubated for 24 and 48h, and DM remaining and starch concentration were analyzed as described by DiLorenzo et al. (2011) using an enzymatic digestion assay.

4. Statistics

Data were analyzed as a randomized block design with a total of nine replicates per treatment. Three separate rows (blocks) were harvested for each treatment, and within each block, three samples were taken (beginning, middle, and end of the row). Each one of the samples collected in the field was the experimental unit, and for the in situ digestibility analyses, the average of the measurements from both steers was used. Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) as a randomized block design with repeated measures. Field sample was the subject, and the covariance structure used for all the parameters was first-order autoregressive based on the smallest Akaike information criterion values. The model included the fixed effect of treatment, ruminal incubation time, and their interaction. For the analysis of in situ starch digestibility, initial starch concentration in the incubated sample was used as a covariate. Significance was declared at $P \leq 0.05$ and tendencies were discussed when $0.05 < P \leq 0.10$.

Results and discussion

Crop evaluation at harvest time shown 144230 ± 6850 plants/ha (7.5 plants by square meter), closed to the objective at seeding time. Average yield, considering a cutting height of 20cm, was 24952 kg organic matter (OM)/ha of which 9750 kg OM/ha was the head (39%) and 15202 kg OM/ha was stover (61%).

DM content of 108 samples taken at harvest time was of 53.8 ± 3.5%, so average DM yield was 13224 kg/ha.

Results of physical characteristics are shown in Table 1. When multi-crop Shredlage® (MS) was used, an increase in upper particles was detected (P<0.01) when compared to no-cracker (NC) and sorghum cracker (SC) treatments, implying that at the same theoretical length of cut (TLC), MS was effective to give longer particles as detected by Vanderwerff et al.(2015) for corn. Besides, looking at the bottom sieve, we found a big proportion of fine particles in both crackers treatments when compared to NC one (P<0.01).

Table 1. Particle size of grain sorghum dried plant harvested with no-cracker (NC), sorghum cracker (SC) or multi-crop Shredlage® (MS).

	% grain sorghum dried plant				
Treatments*	**NC**	**SC**	**MS**	***SEM***	***P<***
Upper sieve, >1,9cm	2,37 [c]	5,10 [b]	7,89 [a]	0,57	0,01
Middle sieve, 1.89 - 0.79cm	32,59	33,08	31,98	1,49	0,87
Lower sieve, 0.78 - 0.18cm	62,34 [a]	57,19 [b]	52,83 [c]	1,45	0,05
Bottom sieve, <0.17cm	2,71 [b]	5,74 [a]	6,21 [a]	0,21	0,01

[abc] Means within row follow by unlike superscripts differs

* NC= no cracker, SC= sorghum cracker and MS = multicrop Shredlage®, all at 15cm theoretical length of cut

As established by Johnson et al. (2016), the amount of starch passing through 1.7 mm screen in sorghum silage must be considered for a berry processing score, compared with 4.75 screen proposed by Ferreira and Mertens (2005) for corn silage. Thus, an increase in fine particles collected in the bottom sieve (P < 0.01, Table 2) when both crackers were used, indicates that more digestible starch will be available when compared with NC.

Results of the in vitro organic matter (OM) digestibility are presented in Figure 1. Significant differences among treatments (P < 0.001) were detected at 24 h of incubation, where NC reduce OM digestibility when compared to SC or MS, without differences between crackers. It is interesting to know that in vitro incubation of different types of sorghum silages (grain, sweet and bmr) for 24 h was an accurate predictor of in vivo DM digestibility (Di Marco et al., 2009), so increases on digestibility found when crackers were used, could be achieved in vivo.

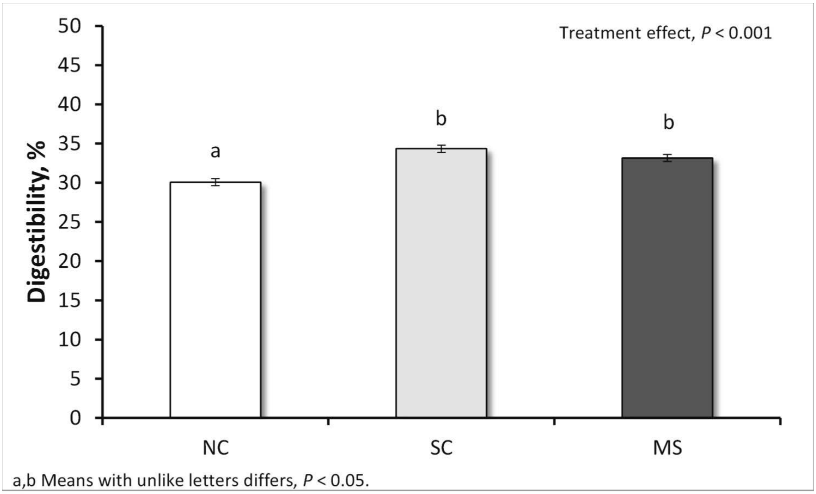

Figure 1. In vitro OM digestibility at 24 h for grain sorghum plant harvested at 15mm TLC with no-cracker (NC), sorghum cracker (SC) or multi-crop Shredlage® (MS).

Although samples were dried and ground at 6mm to be in a cow-relevant particle size for digestion measurements (as proposed by Sapienza 2008), which could balance processor effects, differences among treatments were detected (Figure 1). Visual differences we saw before grinding samples (Figure 2), disappeared after grinding; therefore we suspect that 6mm in sorghum could alter treatment effects. Although studies for corn silage showed that 6 mm provides results more consistent with in vivo values and a particle size consistent with rumen digesta (Johnson et al., 2002; Leonardi et al., 2005); it is unbelievable to think that whole sorghum grains entered into the rumen are going to be ruminated, because they are small, weighted and hard, so in the rumen fluid goes down and surely pass. So the question for future research is: Does grinding samples to 6 mm really represent what is going to happen when grain sorghum silage is fed to cattle?

Figure 2. Grain fraction collected in the lower sieve (0.78-0.18cm) of the Penn State Separator before grinding samples.

In situ ruminal DM and starch digestibility was studied and results are presented in Table 2. A first analysis of in situ ruminal DM digestibility shows a tendency for a treatment effect (P = 0.09) and treatment × time interaction (P = 0.09). However no differences between treatments were detected when separating means.

Table 2. In situ ruminal DM and starch digestibility of grain sorghum harvested at 15 mm TLC with no-cracker (NC), sorghum cracker (SC) or multi-crop Shredlage® (MS).

	Treatments*				***P value***		
***In situ* incubation**	**NC**	**SC**	**MS**	***SEM*** (TRT x INC)	**Treatment** (TRT)	**Incubation time** (INC)	*TRT x INC*
DM digestibility							
time 24 hs	39.33	42.62	41.46	0.995	0.09	0.01	0.09
time 48 hs	46.14	47.75	44.94				
Starch digestibility							
time 24 hs	40.64 ^{B}	47.85 ^{B}	39.93 ^{B}	4.609	0.23	0.01	0.76
time 48 hs	61.19 ^{A}	71.98 ^{A}	62.86 ^{A}				

^{AB}Means within column with unlike superscripts differs.

* NC= no cracker, SC= sorghum cracker and MS = multicrop Shredlage®, all at 15cm theoretical length of cut

As expected, a significant effect of incubation time (P < 0.01) was observed for ruminal starch digestibility in situ, which increase with the incubation time; nevertheless, no effect of treatments was detected (P = 0.23).

Differences in the initial concentration of starch in the samples ground through a 6 mm sieve, before ruminal incubation, were detected among treatments (Table 3); thus initial starch concentration was included as a covariate for the ruminal in situ starch digestibility, and results are shown in Figure 3.

Table 3. DM and starch content before in situ incubation in grain sorghum plant harvested at 15mm TLC with no-cracker (NC), sorghum cracker (SC) or multi-crop Shredlage® (MS).

Initial composition, %	*Treatments**			*SEM*	*P<*
	NC	SC	MS		
DM	54.45 ^{a}	53.90 ^{a}	51.03 ^{b}	1.012	0.05
Starch *(on DM basis)*	22.17 ^{a}	19.96 ^{a}	16.24 ^{b}	1.247	0.01

^{abc}Means within row follow by unlike superscripts differs

* NC= no cracker, SC= sorghum cracker and MS = multicrop Shredlage®, all at 15cm theoretical length of cut

No interaction treatments x incubation time was detected (P = 0.76) for in situ ruminal starch digestion, so results are presented as a mean between 24 and 48 h of incubation. Ruminal incubation time effects were detected for all treatments (P < 0.01), being 36.2, 47.5 and 45.3 starch digestion at 24 h and 56.8, 71.7 and 68.2 starch digestion at 48 h for NC, SC, and MS, respectively. No differences at a mean incubation time of 24 and 48 h of incubation were detected when compared MS and SC, but decreased starch digestion was observed for NC treatment when compared to SC, and not differing with MS (P < 0.04).

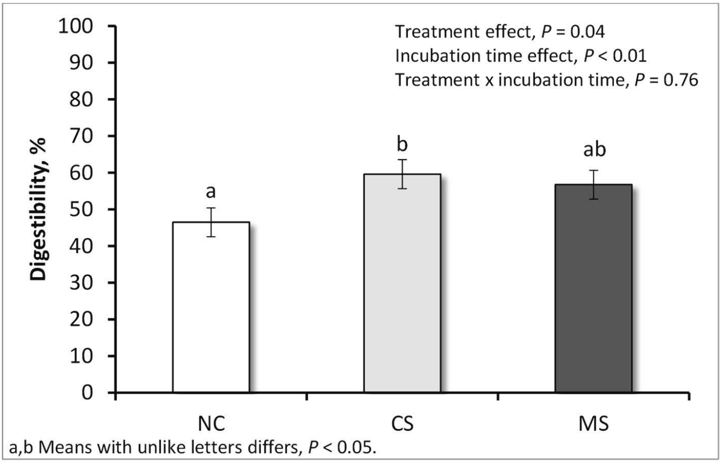

Figure 3. In situ ruminal starch digestibility (mean between 24 and 48 h) for grain sorghum plant harvested at 15mm TLC with no-cracker (NC), sorghum cracker (SC) or multi-crop Shredlage® (MS), considering initial starch concentration (before ruminal incubation) as a covariate.

Treatment differences reported in this trial working with ground samples of grain sorghum crop led us to suspect that feeding trials working with unground silage may increase differences because as said in our opinion no possibility of re-chewing and rumination is waited for a whole sorghum berries entered in the rumen. Besides, 6mm sieve could increase kernel damage and hence starch hydrolysis (Hall et al., 2000) overestimating starch digestion in no-cracker grain sorghum silage. This study is still ongoing so further results will become available in the future.

Implications

As the result of physical evaluations, the Shredlage® processor was effective at achieving a longer particle size when compared with the sorghum processor or no-cracker, at 15 mm of TLC, increasing a number of fine particles (<0.17 cm) as with sorghum cracker when compared with no-cracker usage.

Harvesting grain sorghum crop without processor reduces in vitro OM digestibility, with no differences between sorghum cracker and multicrop Shredlage®.

When the initial starch concentration was considered as a covariate in the analysis, in situ ruminal starch digestibility increased when using sorghum cracker, when compared with no-cracker, not differing with multi-crop Shredlage®.

Although in vitro and in situ evaluations are going to continue with in vivo digestibility and feeding trials, we can conclude that multi-crop Shredlage® could be the only processor needed to give long fiber and processed grain when grain sorghum or corn crops are going to be harvest for silage.

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Dairy cows rations based on grain silages

MARCOS NEVES PEREIRA [1]

Introduction

Starch in cereal grains, such as corn and sorghum, is important to maximize ruminal microbial yield (Hall and Herejk, 2001) and is a major contributor to the energy required for lactation by dairy cattle. Silage is an effective and relatively inexpensive storage method for grains in the farm. Compared with mature ground grain, silage concentrates the grinding operation and reduce labor, requires less investment in equipment and facilities for grain storage, reduces transportation, drying, and storage costs, and can reduce storage and handling losses. Silage of immature grain also has the advantage of allowing for earlier harvesting than mature grain, reducing field losses and allowing for earlier seedling of a successive crop.

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Types of grain silages

Silages of high-moisture corn (**HMC**) and rehydrated and ensiled corn (**REC**) or sorghum (**RES**) have been used for dairy cattle. Other small grains, such as wheat and oats, can also be stored as silage. In HMC, corn grain around the black layer maturity stage (25 to 40 % moisture concentration) is coarsely ground or rolled before ensiling. High-moisture corn may be harvested as shelled corn, ear corn (seeds + cobs), or snapped ear corn (seeds + cobs + husks). High-moisture snapped ear corn is considerably higher in fiber and has lower energy value than high-moisture ear corn or shelled corn.

Silages of REC and RES are the product of homogeneous addition of water to ground mature kernels in order to obtain more than 30 % moisture concentration for ensiling (Andrade Filho et al., 2010b; Pereira et al., 2013). Compared with the traditional storage as silage of HMC, REC has the advantage of allowing for a wider harvesting window of the crop or for strategic purchasing of mature dry grain. Since moisture concentration in mature grain is usually below 15 %, fine grinding can be adopted, allowing for extensive damage of sorghum seeds at ensiling. The silage of REC has been shown to be as effective as HMC silage in reducing the prolamin content of corn (Fernandes, 2014).

Ruminal in situ degradation data suggests that RES is at least as digestible in the rumen as REC (Fernandes et al. 2017). These authors rehydrated ground mature sorghum and corn to obtain 35 % moisture concentration for ensiling in laboratory silos for 30 and 180 d. Both grains were ground with the same 3 mm mesh diameter screen in a stationary hammer mill. Sorghum had smaller geometrical mean particle size than corn (129 vs. 211 μm) and similar prolamin content after ensiling (9.5 % of starch). The effective ruminal degradation (**ERD**) was estimated with a 2-pool model (Fractions A and B), data from ruminal incubations for 3, 6, 12, 18, and 48 h, and a fractional passage rate (**kp**) of 6 %/h. Silage of RES had a larger instantaneously degradable A fraction (39.8 vs. 32.5 % of DM) and slower fractional degradation rate (**kd**. 3.14 vs. 3.33 %/h) than REC. The ERD [(A + B × kd / (kd + kp)] was 60.3 % of DM for RES and 56.5 % of DM for REC. Ensiled sorghum is an opportunity to reduce the cost of grain in the diet compared to corn, without penalizing ruminal and total tract starch digestibility.

Starch digestion

Grain ensiling can affect the proportion of starch digested in the rumen or in the lower digestive tract (Oba and Allen, 2003b). During ensiling, zein protein (prolamin) cross-linked to starch granules in the endosperm is degraded by microbial and plant proteolytic enzymes (Heron et al., 1986; Hoffman et al., 2011), thereby increasing ruminal and intestinal starch digestibility (Knowlton et al., 1998). Based on data from 6131 samples of HMC from a commercial laboratory and assuming that the month of sample submittal was associated with the duration of the ensiling period, Ferraretto et al. (2014) observed that the gain in starch digestibility was dependent on the length of storage and up to 10 months may be needed to reach maximum starch digestibility. The effect of ensiling on starch digestibility may be exacerbated in areas where the dairy industry uses sorghum and hard endosperm corn hybrids that contain greater proportion of vitreous endosperm than floury corn (Andrade Filho et al., 2010a) or when coarse grinding or rolling is adopted as a strategy to increase the processing rate.

In vivo estimates of ruminal starch digestibility obtained with cannulated lactating cows suggests that the proportion of starch digested in the rumen or intestine can have large variation in lactating cows. The ruminal digestibility of starch was 61.5 % of intake for a diet containing silage of floury corn and 29.0 % for a flint corn diet (Taylor and Allen, 2005). Rémond et al. (2004) observed that the change in ruminal digestibility of starch in cows fed mature corn differing in particle size ranged from 35.5 to 69.8 % of intake. Oba and Allen (2003b) observed that ruminal starch digestibility ranged from 45.9 to 71.1 % of intake when diets differed in concentrations of HMC or ground corn. Callison et al. (2001) detected a variation in ruminal starch digestibility of 35.5 to 70.1 % of intake in response to ground corn differing in particle size. Knowlton et al. (1998) observed ruminal starch digestibility of coarsely and finely ground mature corn or HMC of 60.9 to 86.8 % of intake. Fredin et al. (2015) detected variation in ruminal starch digestibility of 75.9 to 85.2 % of intake for ground corn differing in particle size and inclusion in the diet.

Increased ruminal starch availability may increase ruminal microbial yield, amino acid supply to the animal, and feed efficiency by intake depression induced by increased liver oxidation of propionate (Allen et al. 2009) at similar milk yield (Bitencourt, 2012). However, increased ruminal starch degradation can undesirably induce ruminal acidosis, leading to reduction in ruminal microbial synthesis, milk solids secretion, and cow longevity. Ration formulation with ensiled grain needs to consider the relationship between ruminal starch availability and the requirement for physically effective NDF in the diet.

Effect of grain silages on digestibility and performance of dairy cows

The effect of increased ruminal grain digestibility on lactating cow performance and intake seems to be dependent on the concentration of starch in the diet. When compared to finely ground corn (**FGC**), HMC silage (63 % DM) reduced short-term intake of a 32 % starch diet and did not affect intake of a 21 % starch diet formulated by replacement of corn with a 50:50 blend of alfalfa silage and corn silage (Oba and Allen, 2003a). The high starch diet increased solids corrected milk yield compared to the low starch diet for cows fed FGC (+ 3.3 kg/d), but not for cows fed HMC (+ 0.7 kg/d). High starch reduced milk fat % with HMC and had no effect on fat % with FGC. Oba and Allen (2003b) observed that the difference in ruminal starch digestibility between HMC and FGC in high and low starch diets were 24.2 %-units and 12.6 %-units, respectively, but the change in total tract starch digestibility was 1.6 %-units and 0.3 %-units. The compensatory action of the intestines on starch digestibility reduces the difference in ruminal digestibility between diets varying in corn processing and content. Minor change in total tract starch digestibility may occur between diets with large difference in site of starch digestion in the digestive tract (Nozière et al., 2014; Fredin et al., 2015). The effect of grain ensiling on total tract starch digestibility is minor compared to the effect of grain processing on the partition of starch digestion between rumen and intestines.

Bitencourt (2012) evaluated the effect of REC on lactation performance and nutrient digestibility by dairy cows. Treatments were FGC, REC with 43.7 % moisture concentration ensiled for 358 d, and extruded corn (**EC**). Dietary corn content was 17 % of DM and starch concentration was 27.2 % in all treatments. Fifteen Holstein cows received the treatments in 3 x 3 Latin squares with 22-d periods. Treatments had no effect on milk yield (33.3 kg/d), but the daily starch intake was reduced in REC and EC compared to FGC. The milk to feed ratio was increased by EC and REC. The negative impact of EC on DMI and milk fat and energy secretions suggested that processing corn by extrusion increased ruminal fermentable starch more than processing by grinding and ensiling. The REC tended to increase OM and NDF digestibilities and rumen microbial yield compared to FGC. Treatments had no effect on total tract starch digestibility (mean of 95 % of intake). Corn stored as REC reduced chewing behavior, meal size, and milk urea-N and increased the milk to feed ratio compared to FGC.

Arcari et al. (2016) evaluated the effect of the replacement of FGC with REC on performance and diet digestibility of late-lactation dairy cows. These authors fed sugarcane based diets (46 % of DM) and replaced 0, 33, 66, and 100 % of FGC with REC in a 4 × 4 Latin square experiment with 21-d periods. The REC was ensiled for 90 d before the start of the experiment and diets contained 34 % of corn and 25 % of starch in DM. The replacement of FGC with REC induced a linear increase in intake (18.3 to 18.8 kg/d) and quadratic increases in starch digestibility (91.0 to 99.0 % of intake) and milk yield (21.3 to 23.4 kg/d). When sugarcane was the only forage in the diet, REC increased starch digestibility, intake, and milk yield relative to FGC.

Variation in particle size may be a strategy to manipulate ruminal starch fermentability of REC, as suggested by the minor increase in in vitro digestibility of fine compared to coarse ground REC ensiled for more than 30 d (Lopes, 2017). We recently evaluated the effect of REC particle size and dietary starch concentration on performance and digestibility of dairy cows (Castro, in press). Mature kernels from a high vitreous endosperm corn hybrid were ground with a 3 mm (Fine) or a 9 mm (Coarse) mesh screen for rehydration rehydration to 40 % moisture and ensiling in 200 L buckets for 247 ± 24 d (205 to 289). The grinding rate was 3.9 ton/h for Fine and 11.7 ton/h for Coarse, demonstrating the beneficial effect of larger particle size on labor and energy usage of REC silage. The ERD estimated by in situ incubations for 0, 3, 6, 18, and 48 h and assuming a kp of 6.5 %/h was 34.2 % of DM for FGC and 63.8 % for REC. Ensiling increased the A fraction (52.0 vs. 12.5 % of DM) and tended to reduce the kd (2.03 vs 2.15 %/h) of corn. Although FGC was more degradable than Coarse FGC, the ruminal in situ degradations of Fine and Coarse REC were similar. Diets were formulated to contain 22.1 or 14.3 % of DM of Fine or Coarse REC and were fed to 16 lactating Holstein cows in 4 x 4 Latin squares with 21-d periods. Dietary starch was reduced from 29.1 to 23.5 % of DM by replacement of REC with citrus pulp. Treatments had no effect on milk yield (31.0 kg/d), but intake was reduced when Fine replaced Coarse in the high starch diet. Feed efficiency tended to be increased by Fine only when the high starch diet was fed, associated to increased plasma D-lactate concentration, lower ruminal pH, and reduced total tract NDF digestibility than Coarse with high starch. When the low starch diet was fed, the particle size of REC stored for more than 205 d did not affect digestion, lactation performance, intake, and feed efficiency. The data suggests that coarse grinding of REC reduced the ruminal acidogenic capacity of the diet and may be desirable when dietary starch concentration is high.

The effect of REC particle size on digestion, intake, and performance of grazing dairy cows was evaluated by Batalha (2015). Twenty Holstein-Jersey crossbred cows were fed on napier grass pasture and concentrates (2 x/d, 4.3 kg of DM/d) containing 82 % of FGC, fine REC (2 mm mesh screen), coarse REC (6 mm mesh screen), or flaked corn in 4 × 4 Latin squares with 16-d periods. The REC had 35 % moisture and was ensiled for 177 d. Coarse REC reduced the total tract non-fiber carbohydrates digestibility compared to fine REC, but ensiling did not increase the total tract digestibility compared to FGC. Corn processing had no effect on intake (11.1 kg/d) and milk yield (13.4 kg/d). Milk urea-N was increased by coarse grinding of REC. Although coarse grinding apparently reduced the ruminal starch degradation of REC, corn processing did not affect the performance of low producing cows on pasture.

Conclusions

The ensiling of grain increases ruminal starch digestibility, but intestinal starch digestion compensates for the lower ruminal degradation of more resistant starch sources, resulting in lower variation in total tract starch digestibility than in the partitioning of starch digestion between the rumen and the lower digestive tract.

The increased fermentability of grains due to ensiling may induce ruminal acidosis. When high starch diets are fed, ensiled corn may reduce intake at similar or increased milk yield than ground corn. Long-term effects of grain silages on feed efficiency and cow health have not been sufficiently evaluated.

Diets based on highly fermentable grain silage may require increased physically effective NDF concentration in the diet and feed management practices capable of spreading concentrate intake throughout the day. Concentrate feeding to grazing cows may be particularly challenging, since the frequency of concentrate feeding is usually low in pasture based systems.

Particle size and duration of storage can manipulate ensiled grain ruminal digestibility, but the negative effect of increased particle size on ruminal degradation seems to be reduced when duration of storage is elongated.

Grain storage as silage allows for strategic buying or cultivation of grain in the farm, potentially reducing feed costs relative to the storage and processing of ground grain. Coarse grinding of mature corn for storage as REC can reduce the labor and energy usage of grain processing, but the effect of coarse grinding on cow digestion, performance, and intake when the duration of silage storage is less than 30 d needs evaluation.

Nutrition should aim at maximizing feed efficiency of dairy cows in farms taking advantage of grain storage as silage as a tool to increase profitability.

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Effects of silage additives on ruminal and intestinal microbiology

KEVIN PANKE-BUISSE [1]

Abstract:

Ensiling is the preservation of forage for livestock through microbial fermentation. Although ensiling of plant material by its associated surface microbial community alone is possible, the cost of an uncontrolled fermentation can include dry matter loss, decreased quality, and spoilage. While proper harvesting, handling, and storage play a critical role in promoting optimal fermentation conditions (anaerobic, low pH), the use of additives to further optimize ensiling and silage quality is common. Some of these additives, most notably inoculants, have been linked to increases in animal performance. The mechanisms underlying these increases are not fully understood and may be due in part to microbial shifts in the gut. Until recently, our knowledge of the microbial communities in the rumen was measured indirectly

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using evidence of their activity or relied heavily on the ability to cultivate organisms of interest. Advances in high-throughput next-generation sequencing technologies offer insight into the effects of silage additives on complex ruminal microbiomes and a potential tool for linking silage characteristics to animal performance via the ruminal microbiome. This discussion seeks to connect silage additives to the ruminal microbiome, identify gaps in knowledge, and inform future directions in silage research.

Background:

Silage

Silage production offers distinct advantages as a forage conservation method, particularly in terms of dry-matter (DM) preservation, nutrient content, and tolerance of sub-optimal weather.[1] At its simplest, ensiling is a technique for preserving a forage crop by preparation and storage of harvested, chopped forage to exclude oxygen and allow naturally-occurring bacteria on the surface of the plant material to ferment sugars into organic acids. From this basic template, several alterations can be implemented to manipulate the properties of the resulting silage. Ensiling consists of four distinct phases: Initial aerobic phase, main fermentation phase, stable phase, feedout phase.[2]

The initial aerobic phase can be a source of significant losses both initially and later in ensiling. Slow silo filling, poor packing, and insufficient protection from external air can all increase the length of time the crop is exposed to oxygen. The consequences of an extended aerobic phase include accumulation of spoilage organisms like wild yeasts and continued plant respiration that reduce forage nutritional quality and ensilability as carbohydrates are lost as CO_2 and protein is degraded.[3] Best practices aim to limit aerobic contamination/infiltration, spoilage organism growth, and leaching through efficient packing of wilted forage.[4]

The second phase is characterized by the anaerobic fermentation of sugars. The acids produced during this phase lower pH in the silo. The lack of oxygen and low pH together preserve the crop by inhibiting the growth of yeasts, molds, and most bacteria. This results in the third, anaerobically stable, phase of ensiling. In most cases, lactic acid bacteria (LAB) are the desired fermentation microorganisms for ensiling. Primarily consisting of members of the genera *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Lactococcus* and *Streptococcus*, LAB are divided into two fermentative classes, homofermentative and heterofermentative, based on the products produced during fermentation of hexose. Homofermentation yields primarily lactic acid, while heterofermentation yields additional products including CO_2, ethanol, and acetic acid. In terms of energy and DM preservation, homofermentative LAB are the most efficient due to their ability to convert a single hexose to two lactic acid molecules. This pathway preserves energy in a form readily available to an animal, avoids loss of CO_2, and lowers pH quickly.[5] However, the presence of other fermentation products, such as acetic acid, in silage confers benefits for anaerobic and aerobic stability.[6,7]

The feedout stage of ensiling occurs when the silage is re-exposed to oxygen as the silo is unsealed. Aerobic stability is measured as the length of time a silage can resist microbial degradation following oxygen exposure. Oxygen infiltration into the silage and rate of feedout determine how long silage has been exposed to oxygen by the time it is fed out.[6,8] When aerobic stability is insufficient, this phase is characterized by consumption of lactic acid and remaining carbohydrates by aerobic yeasts, molds, and bacteria. Mycotoxins present in fungally-contaminated silage can result in additional animal health impacts and mycotoxin contamination of milk.[9,10] The loss of DM and heating during aerobic decay can represent significant reduction in silage quality and can also reduce feed intake. In addition, volatile compounds like ethanol and acetic acid produced during fermentation can be lost through volatilization which reduce silage DM and energy.[11]

Ruminant Gut Microbiology

The rumen is a digestive fermentation chamber that is critical to the ability of ruminants to efficiently degrade typically indigestible plant structural carbohydrates into volatile fatty acids (VFA) and cultivate microbial cells as a protein source.[12] Ease of access via cannulation, the size, and the importance of the rumen have made it the focus of most microbial investigations into the ruminant gut. The ruminal microbial community is a complex mix of primarily bacteria and protozoa, but also methanogenic archaea and fungi. Until the recent advent of sequencing and computational technologies for microbial ecology, inquiries into the rumen were limited to the culturable fraction of the ruminal community. Estimates of the fraction of cultivable ruminal microorganisms, however, remained low (<10%), even with advances in culturing methods.[13–15] As a result, investigations into the ruminal microbiome largely focused on fermentation products and animal performance. Now, however, the insights gained through molecular and computational biology offer new strategies for detecting organisms and patterns of interest within these complex communities.

Cultivation-independent investigations based on next-generation sequencing (NGS) of ruminal microbial communities have focused mostly on the prokaryotic component via the 16S rRNA gene. This method is weakened by discrepancies between methodologies and extraction methods, which limit the strength of microbial abundance comparisons across studies.[16] Despite this, amplicon-based sequencing gives us the most accessible insight into ruminal microbial ecology and will likely remain the standard until the technological, financial, and infrastructural barriers to metagenomic and metatranscriptomic methods shrink. Estimates of bacterial and archaeal diversity in the rumen are in the thousands.[17,18] Eukaryotic members of the ruminal community have been subject to less intensive study by 18S rDNA NGS due to difficulties in primer design, bias, and copy number variation, but current estimates place fungal and protozoal operational taxonomic units (OTU), a proxy for species, at 21-40[18] and 24,[19] respectively.

The trans-domain, high-diversity microbiome of the rumen is problematically complex and displays a remarkable resistance to change in individual animals and the ability to restore itself even after near-total ruminal content switching with another animal.[20,21] As a result, manipulating the ruminal microbiome can be a challenge and even when a microbial response to treatment is observed, persistence and a clear delineation between cause and effect are rare.[22] These difficulties are compounded by individual differences between animals[21,23–25] and fluctuations over time[26] Though, age-related shifts in the microbiome may be useful in understanding age-related changes in efficiency and productivity.[27,28] Despite these obstacles, the ruminal microbiome can be experimentally altered, and induced changes can be quantified.

Manipulation of ruminal fermentation is an area of intense interest owing to the implications for milk/meat production efficiency and agricultural greenhouse gas emissions. Early attempts at manipulating ruminal fermentation focused on chemical[29] and enzyme[30,31] feed additives. The newest strategies for inducing shifts in the ruminal microbiome, concomitant with the rapid expansion of our knowledge of microbial ecology, focus on biological additives including probiotics and plant extracts. These strategies are paralleled in silage and present a potential synergy in which bovine probiotics or modifiers of the ruminal microbial community could be packaged in the silo. Many of the direct-fed additives of interest are already used as silage additives to alter ensiling, but their effects on the ruminal microbiome alone or in comparison to direct-feeding of similar additives are largely unknown.

Silage additive effects on the ruminal microbiome:

Ensiling is a complex process with many interacting components that make it a challenge to achieve controlled, consistent preservation of forages across environments, management practices, and year-to-year. Most often, additives are used to enhance final silage quality by improving performance in one or more of the ensiling phases. To be effective, they must demonstrate an improvement in animal performance (milk yield/quality, gain, composition, reproduction) and/or reduce waste through forage preservation (DM-recovery, nutritional quality). For their impact on animal performance, it is likely that many silage additives are also potential modifiers of ruminal fermentation. Additives can be divided into groups based on type or intended function. Traditionally, reviews of silage additives have focused on intended function. However, as additive combinations and new inoculants for multiple purposes are introduced, delineating by function becomes less tractable.[32–34] For the purpose of this discussion, additives fall into 5 type-groups: 1) Chemical, 2) Biological Inoculant, 3) Microbial-intended Nutrient, 4) Animal-intended Nutrient, and 5) Biological Non-inoculant.

Chemical Additives

Chemical additives are not biological in origin and are used primarily as preservatives. Acid additives are the most common and widely used in this group, but can also include fungicides and mycotoxin binding agents.

Acids are added to silage in order to ensure a rapid decline in pH to preserve forage and to inhibit spoilage organism growth throughout ensiling. Acidification is especially useful as a means of salvaging forage exposed to marginal conditions that increase risk for spoilage, e.g. ensiling at higher than desired moisture or suboptimal silo conditions. Propionic acid is the most toxic to fungi of the common silage acids but can also inhibit primary fermentation.[35–37] Formic acid is also used with similar results to propionic acid, but is a more potent inhibitor of lactic acid fermentation.[35] Application rates of either are recommended at levels that inhibit spoilage organisms without inhibiting LAB. Both formic acid and propionic acid are present naturally in the rumen and there are no indications that either silage treatment or direct feeding would have a remarkable effect on the ruminal microbial community.[38,39]

Additives for the remediation of toxigenic fungi and their products include mycotoxin binding agents and fungicides. Despite the resistance to mycotoxins conferred by microbial action of the rumen, toxigenic fungi represent an animal health hazard[40] as well as a human health hazard because mycotoxins and their metabolic derivatives can be found in milk.[9] Occurrence and mitigation of toxigenic fungi were the subject of recent and thorough review.[41] One remediation strategy, mycotoxin binders, are employed to reduce transfer to animals of fungal mycotoxins present on a crop or as a result of spoilage during ensiling. Recent work evaluating commercially available binders suggests that they can be effective, but that there is variability between products and formulations.[42,43] At this time, the effects of mycotoxins and mycotoxin binders on the ruminal microbiome are unknown.

Biological Inoculant Additives

Microbial inoculants are the most consistently and widely used and studied silage additives and are the subject of several excellent reviews.[5,32,33,44,45] Members of the LAB group (genera *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Lactococcus*, and *Streptococcus)* and propionic acid bacteria (genus *Propionibacteria*) have all been studied as potential silage inoculants. Reported success of inoculants is generally favorable, with inoculation of homofermentative LAB increasing rate and overall acid production and inoculation of heterofermentative LAB, particularly Lactobacillus buchneri, reducing. The degree of improvement and effects on digestibility and nutritional quality are variable, however. This is likely due to inconsistencies in the intractable number of combinations of crop ensiled, inoculant used, additive combination, and environmental conditions. Most recently, the use of "third-generation" inoculants have been evaluated. These inoculants are formulated as multi-purpose consortia containing the highest efficiency strains and species. One example of this is a *L. buchneri* strain (LN4017) that produces an enzyme called ferulic acid esterase (FAE) that degrades a recalcitrant cell wall phenolic acid. In practice, FAE inoculants exhibit positive ensiling qualities[46–48] and increased feed efficiency in feedlot steers.[49] Despite these developments in microbial inoculants, however, linking positive silage inoculant effects back to animal performance and the ruminal microbiome remains difficult.[50]

The greatest challenge of inoculation into a previously occupied environment is persistence. This is true of the rumen and the silo. Even under optimal ensiling conditions, there is no guarantee that an inoculated LAB will dominate the fermentation.[51] In the rumen, inoculated organisms are almost guaranteed to be flushed from the animal rapidly, even when the organism inoculated was isolated from the same animal.[21,22,52] The only reports of successful introduction of an organism into the rumen are in the case of a diet-induced niche in the rumen. In one of the most dramatic cases, the presence of a toxic amino acid, mimosine, in the diet was mitigated by transferring ruminal contents of mimosine-resistant goats into mimosine-sensitive goats.[53] Formerly sensitive goats remained mimosine-resistant following the treatment without additional inoculation and the protective bacterial species, *Synergistes jonesii*, could be isolated from their rumens[54]. This is likely for this reason that microbial rumen additives have come to focus on a "probiotic" model. Rather than count on stable integration of an inoculant, probiotics offer an alternative; induction of a desired effect at the cost of continuous supplementation.

Probiotic supplements for ruminants are an area of continued and growing interest that have been the subject of recent review.[55] Fungal probiotics show promise in buffalo and sheep for enhancing fiber digestibility and reducing protein degradation.[56–58] Although variable in the consistency of performance promotion, yeast cultures are also popular direct-fed probiotic preparations that are associated with increases in propionate and decreases in methane emissions and protein degradation.[59,60] *Megasphaera elsdenii* inoculation has been suggested as a means of mitigating ruminal acidosis through utilization of lactic acid.[61] However, the most common and widely studied bovine probiotic is silage itself. Silage can contain members of genera *Lactobacillus*, *Bacillus*, *Pediococcus*, *Bifidobacterium*, *Corynebacterium*, *Sphingobacterium*, *Enterococcus*, *Leuconostoc*, and more in numbers exceeding billions of cells per gram silage.[62] The development and refinement of a consistent ruminal probiotic that can be delivered to the animal in silage is a highly desirable advancement. This is especially true if the probiotic is also fermenting the forage because its numbers will grow during ensiling. LAB have been shown to be capable of growing in rumen fluid[63] and several studies have recorded beneficial effects on animal performance when fed inoculated silage even in the absence of quality differences between inoculated and uninoculated silages.[2] The mechanism(s) behind these performance benefits remain unclear, and next generation sequencing tools just starting to be applied to this question.[50]

Microbial-intended Nutrient Additives

Microbial nutrient additives are used to bolster the ensiling process. These are distinct from animal nutrient additives because they are intended for use by the ensiling microbiome even though nutritive value to an animal may increase. The oldest additives for the promotion of fermentation are substrates for LAB to ferment or use to support growth. Fermentation-promoting additives provide a simple carbohydrate source and/or non-protein nitrogen to enhance fermentation. Common sources of nutrients include ammonia and food manufacturing byproducts like molasses, whey, and fruit pomace. For the sake of brevity, only molasses and ammonia will be discussed as case examples of supplemental carbon and nitrogen respectively.

Molasses is a by-product of sugar manufacturing and is among the longest-used silage additives. It is a viscous solution of water-soluble carbohydrates containing approximately 40% sucrose. A 1996 review of molasses-treated grass silages observed consistent improvement of ensiling and animal dry-matter intake (DMI), but no effects on digestibility or overall animal performance.[64] The effects of molasses supplementation, alone or in conjunction with other additives, on silo fermentation and ruminal fermentation, seem to depend heavily on the forage ensiled and the target ruminant.[65–68] Specific to effects on the ruminal microbiome, molasses likely does not have effects outside of its nutritional value. However, there is potential for synergy between molasses and silage probiotics.

Ammonia is applied in either its anhydrous form or in solution, sometimes with molasses. Ammoniation of forages for ensiling has been shown to enhance aerobic stability,[69] increase crude protein,[70–72] and inhibit protein degradation.[73,74] These benefits are contrasted by slower fermentation rates and higher stable phase pH due to the higher starting pH induced by ammoniation. This practice is discouraged in alfalfa silages due to potential increases in clostridial fermentation[75]. In the rumen, ammoniated silage shows increases in ammonia, propionate, and bypass protein, but no data on ruminal microbial shifts are available. Given the consistent presence of ammonia within the rumen, it is unlikely that ammoniated silage would have strong effects on the ruminal community.

Animal-intended Nutrient Additives

Animal nutrient additives are included to provide nutrients to an animal and contribute little or not at all to the microbial fermentation community. Additives in this group are not distinguished by direct animal uptake or microbial transformation/incorporation followed by subsequent animal uptake of nutrients. Valorization of food industry byproducts that would otherwise be wasted is growing in popularity in this category including distillers grain, bean curd, and defatted oil crops. These byproducts represent a cost-effective nutrient source that can displace more costly alternatives and reduce environmental impacts.[76,77] A logical next step from byproduct mixed silages has been ensiled total mixed rations (TMR). Practically, this extends the preservation method used on green forages to also preserve the whole animal ration. TMR silages regularly exhibit adequate ensiling, perform equivalently to un-ensiled TMR or better, and mitigate TMR spoilage.[78–81] The nutrient content and suitability of common byproducts for ruminal fermentation show sufficient in vitro digestibility[82] and a recent review of several Asian agricultural byproducts suggests complex modulation of the ruminal community through direct feeding of saponin- and anacardic acid-containing byproducts.[83] While anacardic acid containing-components (cashew, ginkgo, mango) show promise for tropical production, saponin-containing byproducts (many fruit seeds and peels) are more ubiquitous geographically. Each appears to have distinct effects on the ruminal microbiome. Anacardic acid exhibits an inhibitory effect on methanogens and promotes succinate/propionate production[84] and saponins also shift VFA production toward succinate/propionate and inhibit protozoa and fungi.[85] The effects of such byproducts on ensiling and ruminal fermentation post-ensiling are unknown, but are worth investigating. One potential limitation may be the degradation of active components during ensiling, as results for saponin have been mixed.[86,87]

Biological Non-Inoculant Additives

These additives are the products of plant or microbial action including fibrolytic enzymes, tannins, and essential oils.

Fibrolytic enzyme treatment of silages is intended to provide carbohydrates to fuel fermentation and also to enhance fiber digestibility in the rumen. In practice, the success of this method has been highly variable in reviews,[32] with some studies even reporting negative effects including decreased aerobic stability and silo efflux,[88,89] reduced DM recovery,[90] and decreased digestibility.[91,92] A meta-analysis of 16 recent studies on enzyme inclusion at ensiling confirmed a decrease in total acid production, neutral, and acid detergent fiber, but also improvements in DM recovery and water-soluble carbohydrates available for ensiling.[93] There are no indications that enzyme additives affect the ruminal microbial community beyond nutritional modification of ruminal substrates.

Tannins are plant phenolic compounds that can be added to silage in the forms of tannin-rich plant material or isolated tannins. Interest in tannins stems from their ability to complex with proteins and inhibit proteolysis. In the silo, this consistently translates to better protein-nitrogen preservation, especially in leguminous forages.[94–98] However, the effects of tannins can vary with dose,[98] tannin molecular qualities,[99] and forage ensiled. In the rumen, tannins have been associated with both positive (increased bypass protein, improved production, intestinal parasite suppression) and negative effects (decreased production, nutritional deficiency) and are the subject of multiple reviews[94,100,101]. The distinguishing factor between these appears to be related to dose, tannin structure, and ruminal microbiome. Tannins will bind to protein indiscriminately and free tannin in the rumen or intestines can inhibit fibrolytic and digestive enzymes. Too-large, direct-fed doses can exhibit anti-nutrient depression of digestion. Even when dosed appropriately, tannin-binding must be reversible or protein cannot be absorbed by the animal. Effective use of tannins in the diet of ruminants relies on a fine balance of tannin dose, tannin forms susceptible to decomplexation or degradation, and tannin-tolerant ruminal microorganisms. Tannin-containing silage presents an ideal buffer from the anti-nutrient qualities of tannins by protecting forage protein from degradation in the silo and rumen, but without introducing free tannin into the rumen.[95] In addition, tannin-tolerant members of the genus Streptococcus have been identified and may represent new targets for tannin-containing silage inoculants.[102–104]

Essential oils (EO) are plant volatile compounds that are extracted or distilled from plant matter. The effects of essential oils on silage fermentation are variable with some studies reporting no effects,[105] others reporting improvements in aerobic stability[106,107] and reductions in protein degradation.[107] There is insufficient evidence that essential oils added to silage can also have downstream effects on ruminal fermentation.[105,106] Similarly, direct feeding of essential oils to ruminants shows variable effects on ruminal fermentation, with no or negative effects in some cases.[108–110] In studies reporting beneficial effects, in vitro and in situ ruminal fermentations containing essential oils show changes in VFA profile and ammonia production.[111,112] One study of sheep, in particular, isolated inhibition of the ruminal microbial community as the likely mechanism, but no molecular data were collected.[112] A likely explanation for the lack of effect on the ruminal microbiome of EO-treated silage is the loss through volatilization and dilution of the active ingredient during ensiling and feedout. These factors suggest EO treatment may be effective as a modifier of either silo or ruminal fermentation separately. Further work characterizing dosage-dependent and specific EO effects are necessary and may explain variability in results. In addition, genomic or transcriptomic data from EO-altered fermentations is needed to determine if the effects of EO target specific microbial groups or function ubiquitously.

Conclusion:

Ensiling is the primary method of forage preservation in many parts of the world and relies on microbial fermentation of sugars in the silo to produce organic acids. The use of additives in this process can allow for greater control over the ensiling process. Many of these additives have effects in the silo, e.g., acid production, aerobic stability, protein quality. Other additives are included to increase animal performance, often with consequences for the ruminal microbiome. The proportion of animal response due to shifts in the ruminal microbiome have been largely obscured by the perplexity of variables involved in ruminant feeding studies including environment, ruminant species, diet formulation, and additives used. Developments in molecular and computational techniques have provided a means of disentangling these variables, but silage and silage additives have not yet been robustly probed using these techniques. However, it is clear that there is still potential for improvement of ruminant production and sustainability through optimization of ruminal fermentation. Several silage additives show promise toward that goal, most notably byproducts and microbial inoculants. In particular, byproducts containing complex plant secondary metabolites such as saponins, tannins, and other phenolics and multi-member microbial inoculants with effects in both the silo and the rumen warrant further research.

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Bacterial silage additives and their influence on animal performance

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Introduction

This paper will review the impact of bacterial silage additives on the performance of dairy and beef cattle with special attention to factors associated with silage that can impair animal performance such as anti-nutritional compounds; specifically, the presence of yeasts, molds, mycotoxins and pathogenic bacteria which are collectively referred to as 'silage hygiene'.

1. The impact of homofermentative and facultative heterofermentative lactic acid bacteria on the performance of dairy cattle.

Homofermentative bacteria are used to stimulate fermentation and promote acidification due to their capacity to transform soluble sugars into lactic acid, via the Embden-Meyerhof pathway,

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which yields recoveries of energy approaching 99.3% and DM at almost 100% (Kung et al., 2003b; White, 2007). Some of most commonly used bacteria in commercial silage inoculants belong to this group, such as *Pediococcus pentosaceus*, *Enterococcus faecium* and *Lactobacillus plantarum*. It is important to note that *Lactobacillus plantarum* is now classified as a facultative heterofermentative bacteria because when glucose is lacking, it shifts fermentation away from the exclusive production of lactic acid to fermentation of pentose to lactic acid, CO_2 and acetic acid (Holzer et al., 2003).

The effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation were analyzed through a meta-analysis by Oliveria et al. (2017). According to the authors, silage inoculation with LAB at rates of 10^5 or 10^6 cfu/g of forage was associated with increased milk yield (0.37 kg/d; P<0.01) and tended to increase DMI (0.26 kg/d; P=0.08). Inoculation did not increase feed efficiency (0.03; P=0.18), probably because DM digestibility was not affected by the use of LAB (0.42%; P=0.31). The only explanation for the improvement in milk yield relies on the increase of DM intake, which based on the results of silage quality are due to the reduction of hypophagic compounds such as butyrate, ammonia and biogenic amines. The reduction in lignin concentration (0.25%; P<0.01) and the improvement in silage protein preservation (NH3-N=1.31%; P<0.01), could be related to the increase in DMI and consequently milk production. Silage inoculation with LAB tended to increase milk fat content of cows fed treated silage (0.04%; P=0.08) and milk protein concentration (0.02%; P=0.06). The mechanisms to explain the positive effect of inoculation on milk composition are unclear (Oliveira et al., 2017).

The meta-analysis published by Oliveira et al. (2017) consisted of 130 peer-reviewed papers used to evaluate the impact of LAB inoculation on silage quality and 31 peer-reviewed papers to evaluate the impact on animal performance and milk composition. That work covered the last 20 years of publication on the subject. Previous to the meta-analysis, two important reviews were published on the effect of inoculation with homofermentative LAB on silage quality and animal performance (Muck, 1993; Kung and Muck, 1997). Muck reviewed 32 studies published from 1985 through 1992 and found positive results in milk production in 40% of the trials, with a 5% increase in milk when silages were treated with LAB. In the review from Kung and Muck (1997), 36 trials were evaluated and 47% of the trials had a positive response in milk production (1.37 kg/d). The articles published between 1985 and 1992, showed that animal performance was improved in 56% of studies where inoculation improved DM digestibility (DMD), but only 13% of studies where inoculation did not affect DMD (Muck, 1993). Kung and Muck (1997) also reported that in studies published between 1990 and 1995, at least one aspect of animal performance was increased in 69% of studies where inoculation improved DMD, but only in 36% of studies where inoculation did not affect DMD. These reports illustrate that in many cases, inoculant-mediated improvements in animal performance were attributable to improved digestibility.

The effect of homofermentative inoculants on silage nutrient digestibility was reviewed by Adesogan et al. (2009). The authors used 38 trials with exclusive homofermentative/facultative heterofermentative treatments published between 1989 and 2009, with many crops such as alfalfa, corn, and grass. Inoculation increased digestibility of DM, organic matter (OMD), neutral detergent fiber (NDFD), and acid detergent fiber (ADFD) in 36, 47, 31, and 36% of studies, respectively. In such studies, average increases in the respective measures of digestion were +4.3, +3.3, +6.3, and +9.0%. The reason why NDFD was less frequently improved than ADFD is unclear because ADF contains more recalcitrant components than NDF. When considering specifically corn silages, inoculation increased DMD, OMD, NDFD, and ADFD in 37, 40, 25 and 40%, of the studies respectively. The average change for DMD, OMD and NDFD in corn silages were +4.1, +5.2, and +7% points. The same parameters, DMO, OMD and NDFD were also positive for grasses +4, +1.1, and +5.3%. Nevertheless, inoculation had no positive effect on alfalfa digestibility. The meta-analysis covering publications after 1996 did not find any effect of inoculation with LAB on DMD when evaluating the performance trials (0.42%; P=0.31; n=6) or laboratory silos (0.24%; P=0.24; n=36). Apparently, the meta-analysis from Oliveira et al. (2017) did not confirm the importance of LAB inoculation on digestibility. According to Adesogan et al. (2009), homofermentative inoculation had a forage-species specific effect on nutrient digestibility. Bacterial strain and inoculation rate are other possible sources of variation in the results observed in that review.

The fact that improvements in animal performance were observed in studies where no improvement in digestibility or fermentation parameters were obtained, suggests DMD is not the only determinant of animal performance and that other parameters are being overlooked (Kung and Muck, 1997). Weinberg and Muck (1996) suggested that silage microbial inoculants might provide a probiotic effect by inhibiting detrimental microorganisms in the silage and rumen or by producing beneficial substances that promote specific rumen microbial populations. Subsequent *in vitro* research supports these claims (Weinberg et al., 2003; 2004; 2007) but more *in vivo* animal work is needed to validate these theories. A recent study (Ma et al., 2107) also attributes mycotoxin binding capabilities to specific strains of commercial silage inoculants (described later).

2. The impact of heterofermentative lactic acid bacteria on the performance of dairy cattle.

Heterofermentative bacteria produce lactic acid and additional products such as ethanol, CO_2, and acetic acid during hexose fermentation (Oude Elferink et al., 2001). This is because these bacteria lack fructose diphosphate aldolase; thus glucose 6-phosphate is fermented to 6- phosphogluconate rather than fructose-6-phosphate (Kung et al., 2003b). The heterofermentative pathway is attractive because it generates acetate or propionate (Oude Elferink et al., 2001; Krooneman, 2002), which are powerful antifungal agents that have increased the aerobic stability of corn, sorghum, and ryegrass silages inoculated with heterofermentative inoculants (Kung and Ranjit, 2001; Tabacco et al., 2011; Driehuis et al., 2001; Huisden et al., 2009). Acetate produced by heterofermentative bacteria can also curtail yeast-induced ethanolic fermentation by inhibiting fungal growth in forages with high sugar concentrations.

Lactobacillus buchneri is a gram positive, rod-shaped, non-spore forming, anaerobic bacterium that is the most commonly used heterofermentative bacteria in silage inoculants. Oude Elferink et al. (2001) described the pathway used by *L. buchneri* to convert lactic acid to acetic acid, 1,2-propanediol, and traces of ethanol under anoxic conditions. They also reported that the conversion of lactate to these products is dependent on environmental conditions, such as pH (4.3-3.8) and temperature (15-25°C). Effects of *L. buchneri* on corn silage were also dose-dependent with doses $>10^5$ cfu/g being more effective than $<10^5$ cfu/g of forage (Kleinschmit and Kung, 2006).

The effect of inoculation with *L. buchneri* on dairy cattle performance was evaluated with barley, corn, ryegrass, alfalfa, sugarcane and other silages (Taylor et al., 2002; Kung et al., 2003a; Pedroso et al., 2010). Despite being difficult to compare results from different trials, and different crops, it is important to review a broad number of articles to identify possible trends on the impact of an inoculant on dairy cattle performance. Following similar inclusion criteria, as described by Oliveira et al. (2017), eight articles were selected from 2002-2016. The articles had to be published in English language peer-reviewed journals, concurrently examine uninoculated (control) forages and those inoculated with *L. buchneri*, attain at least 30 days of ensiling to ensure that silage was properly preserved, and the search was done by using the Web of Science software. The use of *L. buchneri* improved milk production in only 1 out of 8 trials, had a negative impact in 2 out of 8 trials, and no effect in 62.5% of the trials examined. It is important to note that the negative results were associated with sugarcane silage, inoculated with only $5x10^4$ cfu/g and corn silages with low DM content (29.7%). Kristensen et al. (2010) observed that corn silage made at approximately 35% DM with *L. buchneri* had no negative effect on predicted energy-corrected milk production (30.1 vs. 30.6 kg/d ECM, P=0.49) when compared to uninoculated silage. Andrade et al. (2016) observed no difference in milk production when comparing cows fed sugarcane silage with or without *L. buchneri* at the inoculation rate of $5x10^4$ cfu/g (18.3 vs. 19.1 kg/d, P= 0.59) respectively. The contradictory results obtained by Pedroso et al. (2010) and Andrade et al. (2016), could be partially explained by the low inoculation rate ($5x10^4$ cfu/g) of the *L. buchneri* used in those trials. Feed efficiency was improved in 25% of the trials; however, in one of the trials the improvement was caused by the reduction in DMI of cows fed the inoculated silage (0.88 kg/d; P<0.05) without a proportional decrease in FCM (0.51 kg/d; P<0.05; Pedroso et al. 2010). Kung et al. (2003a) noted that cows fed alfalfa silage inoculated with *L. buchneri* at a rate of $4x10^5$ cfu/g had a higher FCM production (40.0 vs. 38.9 kg/d) and feed efficiency (1.62 vs. 1.60, P<0.05) compared to cows fed control silage.

Aerobic stability was reported in 5 of the 8 articles used in this review and it was increased when silages were inoculated with 100,000 cfu/g or more. The average increase in aerobic stability observed for treated silage compared with control was 75%. Based on the articles reviewed, it appears that *L. buchneri* has less of an impact on milk production and performance compared with those articles reporting results for homofermentative bacteria. The fact that milk yield was not reduced by the use of *L. buchneri* suggests that the belief that fermentation acids produced by heterofermentative bacteria would impair performance is probably inaccurate. Inoculation rates of *L. buchneri* greater than 100,000 cfu/g successfully increased aerobic stability in commercial scale silos.

3. The impact of lactic acid bacteria silage inoculants on the performance of growing beef cattle.

Two previous reviews of the published literature have summarized the relative paucity of data describing the effects of silage inoculants on various measures of performance of growing and finishing beef cattle (Muck, 1993; Kung and Muck, 1997). Muck reviewed 32 studies published from 1985 through 1992 and found positive results in the gain in 25% of the trials when silages were treated with LAB. Subsequently, Kung and Muck (1997) reviewed 36 trials published from 1990-1995 and found positive responses in gain when inoculated silages were fed in 8 out of 15 studies (53%). Similarly, studies were summarized from a single research station (Bolsen et al., 1992) on various cattle growth performance measurements for inoculated versus control corn and forage sorghum silages. For both corn and forage sorghum silage, treatment with an inoculant improved DM recovery (1.3-2.1%, P=0.01) and reduced acetic acid (0.2-0.5%, P<0.05), without significantly altering pH, lactic acid and ethanol content. Both inoculated crops improved gain per ton of crop ensiled compared with controls (*P*=0.01), in the absence of any detectable difference in average daily gain (ADG) of beef cattle. Feed efficiency (F/G, DM basis) improved when inoculated forage sorghum was fed to beef cattle compared to control sorghum (*P*<0.05), but only tended to improve feed efficiency when the comparison was made for inoculated versus control corn silage (*P*=0.11).

More recently, McAllister et al. (1998) demonstrated that alfalfa silage inoculated with *Lactobacillus plantarum* and *Enterococcus faecium* increased DM intake (6.88 vs. 6.37 kg/d) and ADG (1016 vs. 892 g/d) of feedlot cattle (Simmental x Charolais steers, 238 kg) during an 84-day feeding trial (all *P*<0.05). In the same study, alfalfa silage inoculated with *Lactobacillus plantarum* alone did not alter any measure of performance of growing steers (McAllister et al., 1998). Acosta et al. (2012) reported that a combination of *Enterococcus faecium, Lactobacillus plantarum* and *Lactobacillus brevis* applied to whole-plant corn silage resulted in higher DM intake (~6%), and improved weight gain (8%) and feed efficiency (3.4%) when fed to beef cattle compared with control silage (all *P*<0.01). Conversely, feed efficiency and ADG were not affected by chop length, inoculation or chop length x inoculation interaction of barley silage fed to finishing steers (Addah et al., 2015), and inoculated whole-plant corn silage and inoculated corn stalk silage did not exert any direct effect on any measure of beef cattle performance (He et al., 2017). Clearly, the growth performance response of growing beef cattle to inoculated silages is inconsistent across a variety of reported trials.

It is important to note that numerous researchers and reviewers, and we concur, have pointed to potentially significant differences in research methodology (e.g., nutritional background of cattle fed, maturity and moisture of forage inoculated, storage conditions, forage:concentrate ratio of the experimental diets, etc.) and silage inoculants (species and strains of inoculant LAB and their various combinations, application rate, etc.) in order to explain differences in efficacy and response to inoculated forages fed to growing and finishing beef cattle.

4. Impact of bacterial inoculants to improve silage hygiene.

As mentioned previously in this article, the improvements in animal performance observed in studies where no improvement in digestibility or fermentation parameters were obtained, suggests that there are other parameters that may affect animal performance and are currently being overlooked (Kung and Muck, 1997). Some of those parameters were suggested by Weinberg and Muck (1996) as a possible probiotic effect of silage inoculants inside the rumen. The authors also suggested that bacterial inoculants could have the capacity to produce bacteriocins capable of controlling undesirable microorganisms. Recent studies have confirmed some of those ideas that were published more than 20 years ago. Studies on pathogenic bacteria have shown that commercial bacterial strains used in silage inoculants have the potential to produce bacteriocin-like substances (Gollop et al., 2005; Pedroso et al., 2010, Ogunade et al., 2016). Commercial bacterial inoculants containing *Lactococcus lactis*, capable of producing nisin, have been successfully used to control the growth of *Clostridia* in silages. Ma et al. (2017) demonstrated that strains commonly used as silage inoculants have the ability to bind to mycotoxins which could also affect animal performance if that binding is confirmed in future performance trials.

4.1 Use of bacterial inoculants to bind to mycotoxins

Mycotoxins are deleterious secondary metabolites that can reduce feed intake, growth, and milk production and can also cause diseases, reproductive problems, and death in livestock.

The most common and most problematic mycotoxins are those produced by *Penicillium* (PR toxin, mycophenolic acid, roquefortine C, patulin), *Fusarium* (deoxynivalenol, zearalenone, T-2 toxin), and *Aspergillus* (aflatoxin, gliotoxin, fumitremorgens, fumigaclavines), but others may also be present (Whitlow and Hagler, 2005).

Among the mycotoxins, aflatoxin is the one that causes more concern due to its carcinogenic effect and transfer from diet to milk. On average, milk aflatoxin M1 concentrations are approximately 1.7% of the aflatoxin B1 concentration in the total ration dry matter (Whitlow and Hagler, 2005). Dietary levels of aflatoxin B1 above 100 ppb can compromise the performance of dairy cattle, and cause kidney damage in beef cattle (Garett et al., 1968; Whitlow and Hagler, 2005). Feeding aflatoxin-contaminated diets to lactating cows compromise their health and performance and also causes transfer of the toxin to milk and dairy products (Diaz et al., 2004).

The use of bacterial inoculants has been studied as a form of food detoxification for many years (CAST, 2003). To our knowledge, the first work focused on the use of silage inoculants to mitigate mycotoxins in silages was published by Ma et al. (2017). The authors evaluated the capacity of silage inoculant bacteria to bind aflatoxin B1 *in vitro* and in artificially contaminated corn silage. Ten different LAB strains were tested on aflatoxin B1 binding effects in five different experiments and conditions (two doses: 10^6 and 10^9 cfu/mL); evaluation of binding capacity of viable and non-viable forms of LAB at different pH, the impact of viable vs. non-viable LAB on the quality of silage and the ability to bind aflatoxin B1 (corn silage artificially inoculated with the toxin). The commercial strains used were: three *Lactobacillus plantarum*; one *Lactobacillus buchneri*, two *Pediococcus acidilactici*; two *Pediococcus pentosaceus*, one *Propionibacterium jensenii* and one *Propionibacterium acidipropionici*. At the lowest dose (10^6 cfu/mL) the binding capability was low for all strains evaluated and ranged from 0 up to 4.27% for one strain of *Lactobacillus plantarum*. When the applied LAB dose was 10^6 cfu/mL, all ten strains were able to bind aflatoxin B1 but the responses were strain specific ($P<0.001$). Among the different LAB, two *Lactobacillus plantarum*; one *Lactobacillus buchneri* and one *Pediococcus acidilactici* showed the highest propensity to bind the toxin (ranged from 23.9% to 33% bound).

Viable and non-viable LAB were evaluated on their capacity to bind mycotoxins. Non-viable cells were obtained after treatment with 2 M HCl; whereas, viable cells were incubated and maintained at 37°C in PBS. The results showed that even at different pH, simulating the rumen, abomasum and small intestine of dairy cows, the non-viable forms of *Lactobacillus plantarum* and *Lactobacillus buchneri* were able to bind the mycotoxin. Alternatively, *Pediococcus acidilactici* was able to bind more of the toxin when present in the viable form (21.92%) vs. the non-viable form (2.44%).

In the experiment focused on the quality of silage, non-viable LAB were not able to improve fermentation during the silage process, as they were not able to ferment substrate and produce acids. Acid treatment (HCl) or heat treatment (85°C) reduced viability by nearly 45%, and at a higher temperature (100°C) reduced viability by more than 70%.

When the same LAB was evaluated for the first time in silage artificially inoculated with toxins, the mycotoxins seemed to have a negative impact on the nutritive value of silage. Desirable nutrients were reduced resulting in more fibrous silage. No changes were observed in acetic acid level and the decrease in pH was slower in the toxin-inoculated silage compared to the control.

4.2 Use of bacterial inoculants to reduce the load of undesirable/pathogenic bacteria in silages

Clostridia are gram positive, mostly obligate anaerobic, sporulating bacteria that grow in silages with low sugar, high moisture (<30% DM) and high buffering capacity. Saccharolytic *Clostridia* can metabolize and reduce lactate while proteolytic *Clostridia* deaminate proteins and catabolize amino acids (McDonald et al., 1991). The formation of butyric acid, ammonia and biogenic amines such as putrescine, cadaverine, tyramine, and glucosamine are unpalatable and cause a reduction in DMI by livestock (Fusi et al., 2004). Histamine can be lethal since it causes reduction of rumen motility and eructation while putrescine is associated with ketonemia and reduction in milk yield (Dain et al., 1955; Lingass and Tveit, 1992). *Clostridia* and other pathogenic bacteria are considered part of the normal microflora of ruminants; however, they may cause abdominal pain, diarrhea, ulceration and death of stressed or injured animals (McGuirk, 2008). *Clostridium perfringens* type A has frequently been associated with hemorrhagic bowel syndrome in cows.

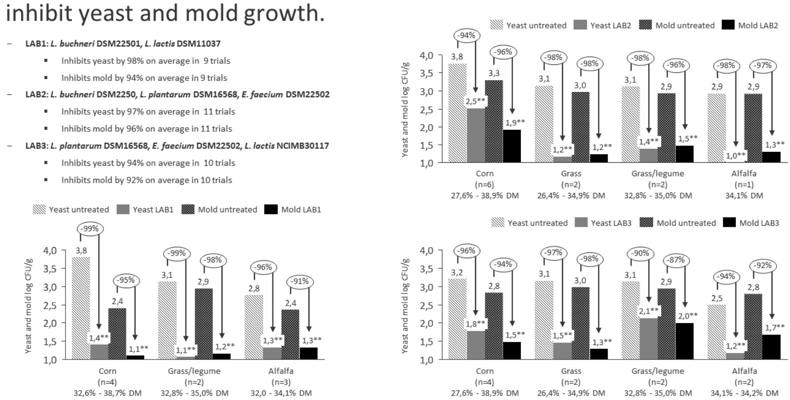
Bacterial inoculants and chemical additives have been tested to control C*lostridia* contamination in silages (Spoelstra, 1985; Kaiser and Weiss, 1997). Since *Clostridia* are intolerant of low pH, homofermentative lactic acid bacteria can inhibit the activity of *Clostridia* in silage by promoting fermentation and rapid decline of pH. Chemical additives can also reduce spores of *Clostridia* in silage. Application of a chemical mixture of sodium benzoate, sodium nitrite, hexamine, and sodium propionate inhibited clostridial growth in low-wilted forages (Lättemäe and Lingvall, 1996).

In addition to pH-mediated inhibition, silage inoculants may also contain metabolites, such as bacteriocins, which are small proteins that inhibit or kill closely related species of bacteria or even different strains of the same species (Yildirim, 2001). Bacteriocins synthesized by one bacterial strain can bind to specific receptors on the membrane of susceptible cells. The receptors are proteins whose normal function is to transport substances, growth factors, or micronutrients from the outer membrane (Madigan et al., 2009). Bacteriocins also can have deleterious effects on DNA and RNA of target cells. For example, *Lactobacillus buchneri* produces buchnericin, a bacteriocin that inhibits the growth of select species of *Listeria*, *Bacillus*, *Micrococcus*, *Enterococcus*, *Streptococcus*, *Lactobacillus*, *Leuconostoc*, and *Pediococcus* genera (Yildirim, 2001). Several trials have demonstrated the effects of bacteriocin-like substances on the control of pathogenic bacteria (Gollop et al., 2005; Pedroso et al., 2010). In the case of *Clostridia*, the use of nisin-producing *Lactococcus lactis* in conjunction with homofermentative LAB in commercial silage inoculants reduced ammonia and butyrate production by *Clostridia* in barley silages (Jatkauskas, 2015). Queiroz et al. (2013) evaluated the effect of feeding corn silage inoculated with *L. lactis*, *E. faecium* and *L. plantarum* at a total inoculation rate of 150,000 cfu/g to high producing cows. According to the authors, cows fed the inoculated silage showed an increase in energy corrected milk (38.2 vs. 37.3 kg/d, $P<0.05$) and feed efficiency (1.72 vs. 1.54, $P<0.05$) when compared to cows fed untreated silage.

4.3 Use of bacterial inoculants to reduce fungi (yeast and molds) in silage.

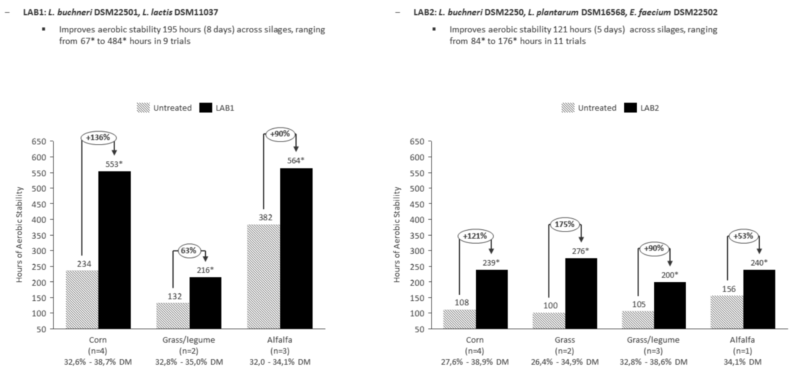
The concern about the presence of fungi in silage extends beyond the potential risk of mycotoxin contamination. Santos et al. (2014) demonstrated that medium and high levels of *Issatchenkia orientalis* (6.4 and 8.4 log10cfu of yeast/ mL of rumen fluid), a common yeast in spoiled silage, can decrease the FDN digestibility when compared with the control treatment (no addition of the yeast). After 12h of fermentation, the high treatment showed a decrease of 10% in NDF digestibility (as % of NDF) and a reduction in rumen fluid pH (6.39 vs. 6.35, $P<0.01$). Those data suggest that depending on the concentration of a specific yeast, rumen fermentation can be affected and nutrients losses increased. Yeasts are also the primary organisms responsible for initiating the aerobic spoilage of silages (Kung et al., 2003b). Yeasts belonging to the *Candida* and *Hansenula* genera can initiate aerobic deterioration of silage by using lactate as a substrate during feedout, and also have the capacity to cause secondary fermentation under anaerobic conditions. When present in the epiphytic population of crops with high WSC concentrations such as sugarcane, yeasts ferment hexoses to ethanol primarily via an alcoholic fermentation. Co-existence of yeasts and heterofermentative LAB cause considerable DM losses and result in silages of poor quality with high ethanol concentrations (Pedroso et al., 2005; Avila et al., 2009). The capacity of silage inoculants to reduce yeast or minimize the presence of yeast is well documented in a variety of silages, such as: corn, sorghum, and ryegrass (Kung and Ranjit, 2001; Driehuis et al., 2001; Huisden et al., 2009; Tabacco et al., 2011). Studies conducted by Chr. Hansen have shown how the use of different combinations of homo- and heterofermentative lactic acid bacteria, also known as dual-purpose or combination inoculants, were able to significantly inhibit total yeast and mold growth for different crop types with varying DM content. Additionally, the aerobic stability of the inoculated silages was improved (Figures 1 and 2).

Figure 1. Different combinations of lactic acid bacteria and their ability to inhibit yeast and mold growth.



**P<0,01 significantly different from untreated.

Figure 2. Different combinations of lactic acid bacteria and their ability to improve aerobic stability of a variety of silages.



*P<0,05 significantly different from untreated.

Conclusions

Bacterial silage inoculants, especially homofermentative lactic acid bacteria, are associated with improvement in animal performance. This partially can be explained by improvements in digestibility of nutrients and reduction of anti-nutritional compounds. Nevertheless, results from numerous studies suggest that improvements in animal performance cannot always be explained by differences in traditional nutrient analyses. Silage hygiene or other anti-nutritional factors may play more significant roles in explaining some of these resultant differences in performance. More studies on mycotoxin binding and pathogen mitigating properties by LAB in silage inoculants should provide a better understanding of how the inoculants can enhance animal performance even when fermentation parameters are not altered. Heterofermentative bacteria seem to be less directly linked to performance; however, it is important to note that the significant impact of this type of inoculant is realized when aerobic stability is challenged, which normally does not occur in controlled academic trials.

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Volatile organic compounds (VOC) in silages - Effects of silo management factors on its formation.

KIRSTEN WEISS

Introduction

The occurrence of volatile organic compounds (VOC) in grass and corn silages has only recently attracted significant attention (Howard et al., 2010; Malkina et al., 2011; Hafner et al., 2010, 2012) due to their significant contribution to air pollution. Hafner et al. (2013) associated the highest VOC emissions from corn and the resulting ozone formation to various alcohols. Bonifacio et al. (2017) postulated that silage on dairy farms can emit large amounts of VOC and developed a model for predicting VOC emissions from silage. There has been anecdotal evidence provided by farmers in Germany (Weiss et al., 2009a), Denmark (Raun and Kristensen, 2010) and the US (Richard Muck, personal communication) on negative effects of odd (atypically) smelling corn silages on feed intake and performance by dairy cows. The analyses of maize silage samples with GC (Gas Chromatography) - MS (Mass Spectrometry) revealed that over 200 volatile compounds in maize silage were found. Weiss et al. (2009a) has identified in commercial and laboratory ensiled maize silages methanol, ethanol, propanol, butanol, iso-butanol, 2-butanol, allyl alcohol, 2-methylbutanol, 3-methylbutanol, hexanol, 2-phenylethanol, 1.2-propanediol, methyl acetate, ethyl acetate, propyl acetate, ethyl lactate and propyl lactate besides acetic, lactic and propionic acids. It may be concluded from the results of this study that composition of total volatiles and concentration of each individual substance cause atypical smell and its intensity in maize silages. In particular silages with markedly increased ethanol content were also shown to contain significant concentrations of other volatile organic substances, especially esters. These ethyl esters were considered as indicator substances for the total VOC´s production in silage (Weiss et al., 2009a) and can be determined by routine analytical procedures, such as GC. However, the knowledge on the effects of specifical esters on feed intake by ruminants is still very limited, and conflicting. Krizsan et al. (2007) and Gerlach et al. (2013) observed negative correlations between some VOC and feed intake. In contrast, Daniel et al. (2013c) reported no difference when fresh sugar cane silages were compared with oven-dried material resulting in the loss of volatiles, which was reconstituted with water before feeding. In preference trials (Gerlach et al., 2016), ethyl esters added to different forages have not altered feeding behavior and short-time dry matter intake of goats. On-farm observations of reduced intake when silage high in ester concentration is fed may, therefore, indicate that not esters alone but, more likely, a combination of VOC are responsible.

As even high concentrations of added acetic acid (Daniel et al., 2013a) or ethanol (Randby et al., 1999, Daniel et al., 2013a, Daniel et al. 2013c) and also increased acetic acid levels (≥4% of DM) by inoculation with heterofermentative inoculants do not appear to adversely affect feed intake (Ranjit et al., 2002; Kleinschmit et al., 2013), other VOC (e.g. ethyl and propyl esters) may be considered to explain the observations on dairy farms.

Volatile organic compounds in maize silages at German dairy farms

In a farm survey (Weiss et al., 2016) the fermentation pattern of the 11 silages was within the typical range for lactate, acetate, and ethanol despite the fact that they were characterized by atypical smell and were believed to have contributed to feed intake depression. The large variation, especially in lactate, acetate, and ethanol concentrations, supported previous findings from farms in the US, Denmark, and Italy (Mari et al., 2009; Borreani and Tobacco, 2010, Kristensen et al., 2010). In our study, n-propanol was detected less frequently and at lower concentrations compared to previous investigations, which reported up to 19.1 g/kg DM (Kalač and Pivničkova, 1987; Kristensen et al., 2010; Weiss et al., 2009a; Weiss et al., 2015). The concentration of ethyl lactate in the farm silages was higher than that of ethyl acetate, thereby confirming previous results (Weiss et al., 2009a). Ethyl and propyl esters of lactate and acetate, respectively, have also been found in farm silages in Denmark (Kristensen et al. 2010; Raun and Kristensen, 2010). Concentrations of ethyl acetate in our farm survey were very similar to results by Weiss et al. (2015) but about 10 times lower than the values reported by Raun and Kristensen (2010).

A survey in Schleswig-Holstein (Northern part of Germany) has been carried out to investigate the incidence of VOC in maize silages from dairy farms and to monitor the concentrations of ethanol, n-propanol and the corresponding esters ethyl acetate, ethyl lactate and propyl acetate, depending on the sampling site in the silo and the compaction of silages (Weiss et al., 2015). The survey included a detailed examination of silages stored in bunker silos on 52 dairy farms. Most silages were produced without silage additives (n=43), whereas 9 farms had used biological additives. The highest contents of fermentation acids (acetic, lactic and propionic acids) and alcohols (methanol, ethanol, n-propanol) in maize silages were found in the bottom, highly compacted core and to some extent in middle core (Table 1), which supports empirical observations by Weiss et al. (2009a) and therefore highest contents of esters produced from them. This is in line with findings of Borreani and Tabacco (2010) that sampling site in farm silos may also play a role with regard to the concentrations of fermentation end-products. Bonifacio et al. (2017) reported greater emissions and, therefore greater concentrations of ethanol and methanol for conventional piles in comparison to silage bags, which typically have better packing than bags.

Table 1. Fermentation characteristics of maize silages on 52 German dairy farms in different sections of bunker silos (mean ± SEM, g/kg DM unless otherwise stated) (Weiss et al., 2015)

characteristics	BC[3]		MC[4]		TE[5]		*P-Value*
DM (%)	34.1	± 0.5	33.4	± 0.4	34.0	± 0.5	0.950
pH	3.85[a,b]	± 0.18	3.83[a]	± 0.02	3.89[b]	± 0.03	0.036
Lactic acid	49.3[b]	± 2.6	51.4[b]	± 1.9	41.8[a]	± 2.1	0.001
Acetic acid	23.0[b]	± 1.2	19.5[a]	± 0.9	19.6[a]	± 1.0	0.009
Propionic acid	0.8[b]	± 0.2	0.4[a]	± 0.1	0.6[a,b]	± 0.1	0.028
Methanol	0.3[b]	± 0.0	0.2[a]	± 0.0	0.3[b]	± 0.0	0.008
Ethanol	6.9[b]	± 0.5	5.9[a,b]	± 0.4	5.1[a]	± 0.4	0.001
2-Butanol	0.2[b]	± 0.1	0.2[a,b]	± 0.1	0.1[a]	± 0.0	0.015
n-Propanol	4.4[b]	± 0.7	2.7[a]	± 0.5	2.1[a]	± 0.4	0.001
Ethyl acetate[1]	51[a,b]	± 4	40[a]	± 3	59[b]	± 5	0.007
Ethyl lactate[1]	210[b]	± 17	176[a,b]	± 15	150[a]	± 14	0.003
Propyl acetate[1]	44	± 17	30	± 7	46	± 16	0.626
Total esters[1]	305	± 24	246	± 18	255	± 24	0.080

Ammonia	1.3[b]	± 0.0	1.1[a]	± 0	1.1[a]	± 0.0	<0.001
WSC[2]	8.2[a]	± 0.7	10.5[b]	± 1.0	9.9[a]	± 0.7	0.001
ASTA (d)	7.2	± 4.8	6.6	± 4.1	6.3	± 4.2	0.2613
Yeasts (log cfu /g FM)	4.7[a]	± 4.6	6.2[b]	± 5.9	6.1[b]	± 5.8	<0.001
Compaction (kg/m³)	256	± 5.6	226	± 5.8	217	± 5.9	<0.001

[1]mg/kg DM, [2]water-soluble carbohydrates; [3]Bottom core; [4] Middle core; [5]Top edge; means in rows with unlike superscripts differ at *P<0.05* (Tukey's test).

Ethanol was detected in the German study of 2015 at up to 17.8 g/kg DM and the highest n-propanol level was 20.2 g/kg DM (Figure 1a). In agreement with data by Weiss et al. (2009a), ethyl lactate (EL) concentrations in maize silages were higher than the levels of ethyl acetate (EA) and propyl acetate (PA) (Figure 1b). The contents of total esters (up to 925 mg/kg DM) were higher than in silages from ensiling laboratory trials (Weiss et al., 2009a). With increasing compaction, the concentrations of n-propanol and ethanol as well as those of the ethyl esters EA and EL and aerobic stability ($R^2 = 0.920$, *P<0.001*) increased (data not shown). This may be explained by the usually lower pH in the bottom, more compacted and less air-affected zones in farm silos. Esterification processes were shown to be stimulated by low pH (Weiss and Auerbach, 2013).

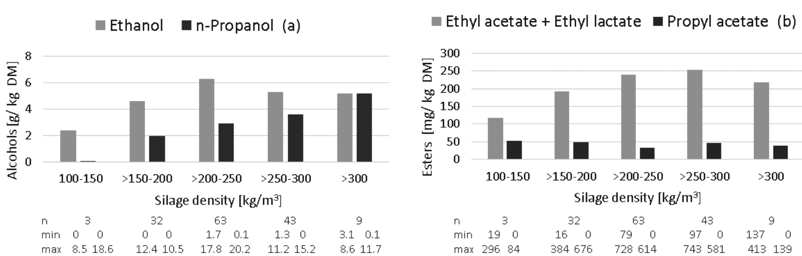


Figure 1. Average and range concentrations of ethanol and n-propanol (a) and the esters ethyl acetate, ethyl lactate and propyl acetate (b) as affected by silage density (Weiss et al., 2015).

A farm study in Lower Saxony/Germany (Weiss et al., 2017) aimed to investigate the fermentation quality and yeast count in short and long chopped maize silages (Table 2). Four samples per farm were taken from the upper and lower core and upper and lower edge (left or right).

Table 2. Fermentation quality and yeasts in short (S) and long (L) chopped maize silages

S: Ø 7. 4 mm, n=13 farms, L:Ø 23.7 mm, n=15 farms (Weiss et al., 2017)

	Compaction (kg/m^3)							
	< 200		>200 - 250		> 250 - 300		> 300	
	Chop							
Characteristics	S	L	S	L	S	L	S	L
N	4	12	27	17	16	27	5	4
DM (%)	39.2	36.1	38.2	35.3	37.5	36.1	37.9	37.6
Acetic acid (g/kg DM)	13.6	26.1	14.9	19.9	16.7	16.4	20.0	10.1
Ethanol (g/kg DM)	6.2	11.9	6.7	9.8	8.7	9.8	6.4	7.9
Esters, total[1)] (mg/kg DM)	55	231	197	238	198	236	164	274
Yeasts (log cfu/g FM)	5.9	3.5	4.7	4.2	4.4	3.7	4.0	3.5
ASTA (days)	3.8	5.7	4.2	3.5	4.7	5.0	4.8	5.0

[1)] Sum of ethyl acetate, ethyl lactate, propyl acetate

The dry matter contents are very high. Only 29% of short samples and 42% of long samples were in the range of 30 to 35 % dry matter that we recommend for a good silage quality. The content of ethanol and esters is in long silages higher than in short silages. The highest ester contents were found in long chopped silages until 663 mg/kg DM. The content of propanol with the maximum of 1.4 g/kg DM is lower than in the study in Schleswig-Holstein. The yeast counts, over 3.5 log cfu/g FM, were higher with lower compaction in short chopped silages. The ASTA is in comparison with the study from SH (Weiss et al., 2015) significantly lower and not different between short and long maize silages. The results demonstrate that the maize silage quality, independent of shredding system, could still be improved. High contents of ethanol and a high risk of heating occur with both systems that are associated with yeast activity and the occurrence of ethanol and esters.

Occurrence of volatile compounds in different types of silages

VOC in maize silages

Elevated levels of ethanol, ethyl acetate (EA) and ethyl lactate (EL) were detected in maize silages from laboratory ensiling trails (Table 3). Ester and ethanol levels were highest in silages stored under strict anaerobic conditions. It was also shown that esters remain detectable in silages for a few days after the opening of the silos under aerobic conditions (Weiss et al., 2011).

Table 3. Contents (range) of volatile organic compounds (VOC), especially esters and their correlation to ethanol, in different maize silages

Reference		Lactic acid	Acetic acid	Ethanol	Ethyl acetate (EA)	Ethyl lactate (EL)	Regression EA+EL(y), Ethanol (x)	
	n		(g/kg DM)		(mg/kg DM)		y = ax + b	R^2
Weiss et al., 2009a	60	6.9–74.8	5.8–79.4	0.9–51.7	12–284	16–379	12.50x+ 91.2	0.70
Weiss et al., 2009b	30	32.5–119.8	8.6–25.8	3.2–28.3	55–343	30–683	26.47x+121.5	0.65
Weiss et al., 2012b	12	73.8–124.6	5.3–29.2	6.2–50.8	116–262	156–661	11.55x+266.0	0.93
Gerlach et al., 2013	79	0–75.5	0–36.6	0–36.9	0–1109	0– 986	52.51x+ 0.2	0.88
Weiss et al., 2016	72	13.7–67.4	0.5–26.7	3.3–20,1	38–639	0–224	18.10x+ 91.7	0.20

Results of ensiling experiments concerning the effect of storage period on fermentation pattern indicate that concentration of ethanol strongly affected formation of esters during the fermentation process. Weiss et al. (2009b) found increasing contents of ethanol and especially lactic acid over 90 days, whereas the corresponding ethyl esters increased during the first 30 days. These findings are in line with results from Gerlach et al. (2015) who investigated the effect of storage length of different maize silage varieties.

VOC in grass and legume silages

The extensive literature search yielded only one study by Krizsan et al. (2007), who detected variable concentrations of esters in grass silage, but the mean content never exceeded 30 mg/kg DM. Therefore, the aim of investigations with grass silages (Weiss and Auerbach, 2013) was to determine the incidence of VOC in grass silages, particularly ethanol and the ethyl esters of lactic and acetic acids. Grass silages (Lengyel et al.,2011) originated from three different semi-natural grassland sites of Estonia, Germany, and Wales, contained high ethanol and ester concentrations, particularly those from trials 1 and 2 (Table 4). The detected ester levels in this two grass silages were extremely high compared with those for maize silages. This may be attributed to the lower storage temperature of 15°C, which promotes ester formation. Weiss et al. (2009a) observed that maize silages stored at 20 °C had higher ester contents than were detected at 35 °C. Kim and Adesogan (2006) presented no data on ester contents, but observed lower contents of the reactants for ester formation, lactate and acetate, in corn silage by storage at higher temperature.

Table 4. Fermentation products, pH, and ester concentrations in grass silages (n= 620) (Weiss and Auerbach, 2013)

Trial	n	pH	Lactic acid	Acetic acid	Ethanol	Total esters[1]	Correlation[2]	
			(g/kg DM)	(g/kg DM)	(g/kg DM)	(mg/kg DM)	r_s	*P* value
1	213	3.7 - 6.7	0 - 99.5	1.5 - 62.8	0.7 - 39.6	0 - 3540	0.35	<0.001
2	209	3.6 - 5.8	0 - 89.8	2.0 - 46.7	0 - 35.3	0 - 3995	0.37	<0.001
3	49	4.0 - 4.5	60.6 - 117.5	11.1 - 36.5	2.2 - 18.7	0 - 359	0.91	<0.001
4	12	3.8 - 4.2	42.7 - 81.8	13.2 - 35.4	6.7 - 12.0	216 - 455	0.52	ns
5	45	3.8 - 4.5	32.3 - 89.2	14.2 - 76.7	1.6 - 13.1	73 - 378	0.64	<0.001
6	17	4.2 - 4.9	30.0 - 116.7	19.7 - 49.3	2.4 - 7.8	0		-
7	12	4.3 - 4.7	36.6 - 86.5	7.5 - 13.3	2.1 - 19.9	0 - 161	0.65	<0.05

8	21	3.8 - 4.2	42.6 - 105.1	8.4 - 19.9	0.9 - 15.1	0 - 378	0.84	<0.001
9	21	3.9 - 4.3	49.9 - 110.6	1.6 - 13.9	1.0 - 14.1	0 - 189	0.85	<0.001
10	21	4.0 - 4.7	24.0 - 76.2	14.0 - 31.5	3.9 - 12.3	62 - 272	0.85	<0.001

1) Sum of ethyl acetate and ethyl lactate, 2) correlation between ethanol and total ester concentrations, r_s Spearman rank correlation coefficient, ns not significant; Trial 1Lengyel, et al.; 2012; Trial 2 Lengyel unpublished; Trial 3,4,6,7 Nadeau, unpublished data; Trial 5,8,9,10 unpublished data Kalzendorf

The correlation coefficients between ethanol and total ester concentrations, presented in Table 4, varied widely between 0.35 and 0.85, depending on the trial. The pH of the silages had a pronounced effect on ester levels (Table 5). Strong relationships (r_s>0.50) were mostly observed when the pH of the silages did not exceed the value of 4.25. This is in line with observations by Hangx et al. (2001) who found ester reactions be stimulated by low pH in the environment. According to Peter and Vollhardt (1987), the ester formation depends on pH and is an acid-catalysed reaction.

Table 5. Relationship between ethanol and ester contents in grass silages (n= 620) as affected by pH (Weiss and Auerbach, 2013)

pH class	n	Total esters 1) (mg/kg DM)	Ethanol (g/kg DM)	Correlation 2) r_s	*P* value
> 3.50 - 3.75	19	482 - 3995	0 - 35	0.60	<0.01
> 3.75 - 4.00	126	0 -1856	1 - 40	0.72	<0.001
> 4.00 - 4.25	176	0 - 920	1 - 25	0.55	<0.001
> 4.25 - 4.50	131	0 - 762	1 - 18	0.21	<0.05
> 4.50 - 4.75	81	0 - 550	1 - 24	0.26	<0.05
> 4.75 - 5.00	42	0 - 384	0 - 38	0.49	<0.001
> 5.00 - 5.25	26	0 - 255	1 - 37	0.49	<0.05
> 5.25 - 5.50	10	63 - 211	4 - 28	-0.35	ns
> 5.50	9	0 - 171	3 - 24	0.25	ns

1) Sum of ethyl acetate and ethyl lactate, 2) correlation between ethanol and total ester concentrations, r_s Spearman rank correlation coefficient, ns not significant

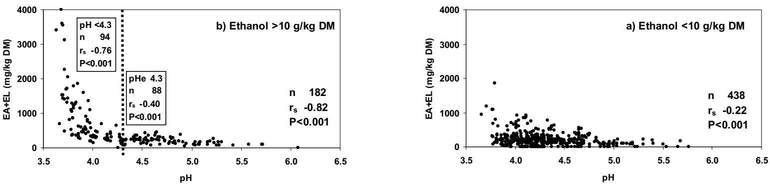
The allocation of the grass silages to different ethanol classes showed clear effects of ethanol content on the relationship between pH and total ester concentration (Table 6). Within each ethanol class, a great variation in ester concentration was observed.

Table 6. Relationship between pH and ester content in grass silages (n= 620) as affected by ethanol (Weiss and Auerbach, 2013)

Ethanol class	n	Total esters[1)]	pH	Correlation[2)]	
(g/kg DM)		(mg/kg DM)	range	r_s	*P* value
≤ 5	257	0 - 1180	3.7 - 5.8	-0.12	ns
> 5 - 10	181	0 - 1856	3.8 - 6.7	-0.46	<0.001
> 10 - 15	100	0 - 1147	3.7 - 5.7	-0.66	<0.001
> 15 - 20	39	87 - 3116	3.7 - 5.7	-0.88	<0.001
> 20 - 25	21	0 - 3540	3.6 - 6.1	-0.93	<0.001
> 25 - 30	12	63 - 3589	3.7 - 5.3	-0.83	<0.001
> 30 - 35	5	274 - 2054	3.8 - 4.8	-0.60	ns
> 35 - 40	5	182 - 3995	3.7 - 5.2	-0.90	<0.05

[1)] Sum of ethyl acetate and ethyl lactate, [2)] correlation between pH and total ester concentration, r_s Spearman rank correlation coefficient, ns not significant

As shown in figure 2a, the correlation between total ester content and pH in grass silages was very weak (r_S=-0.22; *P*<0.001) up to an ethanol content of 10 g/kg DM, whereas a very strong negative relationship was found (r_S=-0.82; *P*<0.001) at higher ethanol levels (Figure 2b). The least correlation existed if silage pH exceeded the threshold value of pH 4.3.



a b

Figure 2. Total ester concentrations as affected by ethanol class a) ≤10 g/kg DM, b) >10 g/kg DM, depending on pH-value (Weiss and Auerbach, 2013).

In summary, it can be stated that grass silages may also contain ethyl esters. However, the relationship between ethanol and ethyl esters in grass silages seems to be not as close as that for maize silages. This can be explained by the fact that the intensity of ester reactions is affected by the pH of the silage and grass silages that often show pH values above 4.0. As a consequence, the correlation coefficients decrease with increasing pH. In conclusion, it can be stated that the ester concentrations are strongly correlated with the ethanol concentration and the silage pH.

Investigations from Weiss and Kalzendorf (2016) concerning the effect of wilting of ensiling material on silage quality of lucerne, red clover and grass mixtures demonstrate the occurrence of VOC in **legume silages**. In this studies, ester contents are comparable with contents in grass silage (Weiß and Auerbach, 2013) considering the pH level between 4.0 and 6.3. The total esters ranged between 124 and 197 mg kg^{-1} DM in untreated silages and consisted of only ethyl lactate. Elevated levels of alcohols and esters occur particularly in the lower range of DM. However, yeast counts were high and increased during the wilting period. In accordance to the fact that under anaerobic conditions yeasts are responsible for ethanol formation, the ethanol content in silages without any additives was between 4.8 and 10.9 g kg^{-1} DM with a strong negative correlation to DM content (R^2= 0.81) and positive correlation to ester content (R^2= 0.65).

Laboratory ensiling trials with **white lupin-wheat silages** (König et al., 2017) demonstrated the occurrence of volatile compounds, also esters, in this special ensiling material. The authors found that increased proportion of lupin correlated positively with the concentration of VOC.

VOC in sugar cane silages

In tropical areas ensiled sugarcane are an important forage with more than 400 g/kg DM water-soluble carbohydrates, which are substrates for intensive fermentation (Daniel et al., 2013a). Ethanol is the main fermentation end product in sugar cane silages (Kung and Stanley, 1982). Daniel et al. (2013b) found in silages from farm-scale (n=25) and laboratory-scale silos (n=8) that the volatile organic compounds comprised up to 22% of the sugarcane dry matter. Table 7 contains data concerning the occurrence of VOC in sugarcane silages, without additives, with sodium benzoate and *Lactobacillus buchneri*. In addition to high contents of ethanol, acetic acid, and lactic acid, 1,2-propanediol, ethyl lactate, acetone, 2,3-butanediol, propionic acid, n-butyric acid, ethyl acetate, 2-butanol, methanol, propanol, and iso-butyric acid were found (Daniel et al., 2013b).

Table 7. Concentrations of fermentation products in sugarcane silages (Daniel et al., 2013b)

Characteristics	Mean[1)]	SD [2)]	Min.	Max.
DM oven[3)] (g/kg)	28.3	4.0	22.2	34.9
DM corr[4)] (g/kg)	31.1	3.1	26.7	36.5
Ethanol (g/kg DM)	54.2	48.1	5.0	154.5
Acetic acid (g/kg DM)	32.8	11.5	14.3	53.5
Lactic acid (g/kg DM)	26.0	20.9	6.5	60.4
Propane-1,2-diol (mg/kg DM)	1532	2348	<100	12186

Ethyl Lactate (mg/kg DM)	697	799	132	2401
Acetone (mg/kg DM)	573	527	<5	2072
Butane-2,3-diol (mg/kg DM)	358	250	<100	905
Propionic acid (mg/kg DM)	284	350	<100	1107
n-Butyric acid (mg/kg DM)	273	369	<100	1383
Ethyl Acetate (mg/kg DM)	167	174	<5	597
2-Butanol (mg/kg DM)	135	194	<5	538
Methanol (mg/kg DM)	133	359	<100	1555
Propanol (mg/kg DM)	123	81	<5	290
iso-Butyric acid (mg/kg DM) [5]	<100	55	<100	274

[1] N= 33 [2] Standard deviation. [3] Dry matter determined by oven drying (pre-drying at 55 °C for 72 h followed by drying at 105 °C for 12 h). [4] Dry matter corrected for volatile compounds (Weissbach, 2009). [5] iso-valeric acid, n-valeric acid, and caproic acid were below the limit of detection of 100 mg/kg DM; 1-butanol was below the limit of detection of 5 mg/kg DM.

Daniel et al. (2013b) performed a statistical calculation with principal component analysis using PRINCOMP procedure of SAS (Figure 3). They postulated some functional relationships among the fermentation end-products in sugarcane silages. Ethanol was negatively associated with acetic acid and 2,3-butanediol, but positively correlated with lactic acid and esters.

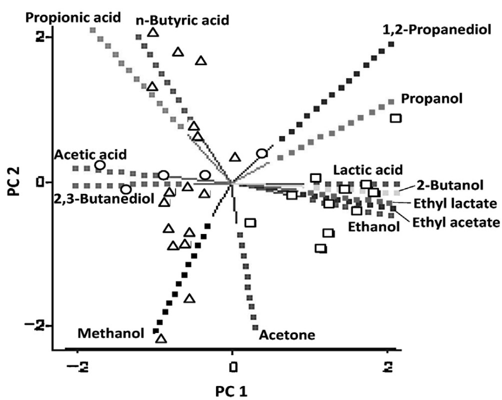


Figure 3. Principal components analysis of volatile organic compounds in sugarcane silages. PC 1, first principal component (0.48); PC 2, second

principal component (0.15). Silages were untreated (□), treated with sodium benzoate (△) and inoculated with *Lactobacillus buchneri* (○) (Daniel et al., 2013b)

The study of Cardoso et al. (2016) examined the chemical composition, fermentation pattern and microorganism of **sugar cane** silages without and with chemical additives and inoculants (Figure 4) and showed a strong correlation between ethanol and ethyl esters.

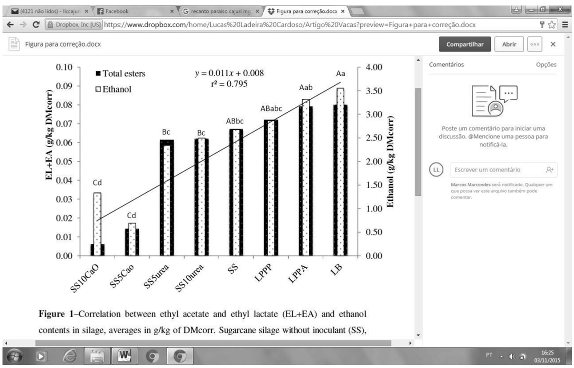


Figure 4. Correlation between ethyl acetate and ethyl lactate (EL+EA) and ethanol contents in silage, averages in g/kg of DMcorr. Sugarcane silage without inoculant (SS), SS with *Lactobacillus buchneri* (LB), SS with *Lactobacillus plantarum* and *Pediococcus pentosaceus* (LPPP), SS with *Lactobacillus plantarum* and *Propionibacterium acidipropionici* (LPPA), SS with 5 g/kg CaO (SS5CaO), SS with 10 g/kg CaO (SS10CaO), SS with 5 g/kg urea (SS5urea), and SS with 10 g/kg urea (SS10urea) (Cardoso et al., 2016)

Summary

Based on a total of 1148 data sets from grass silages as well as from silages from whole-crop maize, whole-crop wheat, sorghum, high- moisture corn, a regression model was used to describe the relationship between total ester and ethanol concentrations. As shown in figure 5, each incremental increase in ethanol content by 5 g/kg DM resulted in increased total ester concentration by 114 mg/kg DM (R^2= 0.76). Therefore, the following equation can be applied to calculate ester concentration in silages based on the ethanol content: predicted total ester concentration [mg/kg DM] = ethanol concentration [g/kg DM] x 114/5. Validation of estimated equation for ester content is ongoing and taking into account the pH value.

The use of this predictive model offers the possibility to avoid laborious and expensive chemical ester analyses.

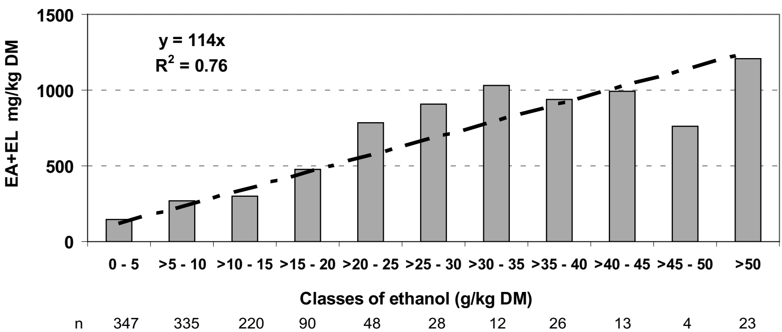


Figure 5. Average total content of esters (ethyl acetate and ethyl lactate) in classes of ethanol in silages from whole-crop maize, whole-crop wheat, sorghum, high- moisture corn and grass (n=1148) (Weiss and Auerbach, 2013)

Management factors on formation of VOC

The effects of ensiling conditions (e.g., delayed sealing and air infiltration during the ensiling process and storage temperature) and silage additive type on the formation of VOCs, especially on the formation of esters, are still not well investigated.

Esters can be formed by abiotic esterification process via the protonation of the oxygen atom of the carboxylic group and reaction with nucleophile oxygen atom of the alcohol (Peter and Vollhardt, 1987) and stimulated by low pH (Hangx et al., 2001; Raun and Kristensen, 2010; Weiss and Auerbach, 2013). Furthermore, esters can be directly produced by lactic acid bacteria expressing the required enzymes for this reaction (Liu and Siezen, 2006). Moreover, certain yeast species, which also occur in silages (Pahlow et al., 2003), are capable of forming esters (Nordström, 1966; Fredlund et al., 2004). Thus, it seems very likely that both chemical and biochemical processes are involved.

Effect of oxygen by delayed sealing and during aerobic exposure

In previous studies, air infiltration resulted in a decline of lactic acid concentration (Mills and Kung, 2002; Moshtagi Nia and Wittenberg, 2000; Bolsen et al. 1993). Conflicting results were described on the effects of delayed sealing and air infiltration on acetic acid and production of VOC, especially ethanol (Moshtagi Nia and Wittenberg, 2000; Mills and Kung, 2002; Kim and Adesogan, 2006).

The effect of oxygen by delayed sealing is described in the literature primarily with lower lactate contents (Bolsen et a., 1993, Kim and Adesogan, 2006) and a stimulation of acetic acid formation (Mills and Kung, 2002). Brüning et al. (2017) have carried out a trial with whole –crop maize (28%DM) for the evaluation of the impact of aerobic exposure before and after ensiling. They filled 120l plastic silos which were either compacted low or high and sealed immediately or delayed on day 2 or 4 post filling. After anaerobic storage over more than 175 days under constant ambient temperatures and opening the silos, silages were exposed to air for 6 d. At silo opening ethanol was increased by high compaction with concentration under 20 g/kg DM. Ethyl lactate and ethyl acetate formation were correlated with ethanol content. Ethyl lactate content was significantly higher in high compacted silages compared with low compacted silages on day 0 and 2 of aerobic exposure on air. In spite of high volatility, ethanol and esters were found in maize silages until 4 days after the opening of silos; this is in line with results from Weiss et al., 2011. The longer aerobic exposure, the fewer contents of VOC.

The ethanol content is after 2 or 4 days of delayed sealing in high and low compacted silages not significantly reduced, but ester contents increased in tendency. This could have been caused by yeasts, which most probably are increased by delayed sealing and either directly formed ethyl esters or supplied ethanol for esterification. The content of ethyl acetate is high (up to 900 mg/kg DM) and in tendency increased with delayed sealing. The ethyl lactate content differs not significantly depending on sealing time which is in line with the ethanol content. Weiss et al. (2016) designed 3 ensiling laboratory experiments with immediately or delayed ensiled, however, the ensiling material left loosely piled at approximately 16 to 18°C ambient temperature before being filling in the silos. They reported that delayed sealing enhanced aerobic stability that can be explained by lower yeast counts and higher contents of acetic acid. The delayed sealing had no effect on ethanol and therefore ester concentrations, but it reduced the concentrations of n-propanol. Delayed sealing may have affected the metabolic activity of n-propanol producers like yeasts and certain lactic acid bacteria (McDonald et al., 1991; Krooneman et al., 2002 and Amin et al. 2013).

Effects of silage additives on formation of VOC

The findings that volatile organic compounds are frequently found in silages and may detrimentally affect feed intake by dairy cattle (Weiss et al., 2009a) have initiated more research with the focus on the use of silage additives to reduce VOC, especially ethanol, and ester formation. In consideration of increasing emissions of VOC with increasing concentrations in silages (Bonifacio et al., 2017), it is important to determine additive treatment effects on VOC concentrations and emissions.

It is well known that silage additives can alter ethanol contents thereby exerting an effect on ethyl ester production. Silage additives affect fermentation pattern, and aerobic stability, in different ways according to their specific mode of action (Kung et al., 2003).

Chemical additives containing **sodium benzoate and potassium sorbate** had in the most cases no effects on lactate and acetate levels (Bernardes et al., 2014; Da Silva et al., 2014, Hafner et al., 2014), but decreased the ethanol concentration. This is a result of the strong inhibitory effect of sodium benzoate and potassium sorbate on yeasts as producers of ethanol (McDonald et al., 1991). Weiss and Auerbach (2012) tested the effects of silage additives containing sodium benzoate/potassium sorbate on fermentation pattern, production of VOC and aerobic stability of maize silage. They found that treatment had significant effects on all parameters tested. The concentration of ethyl esters was clearly affected by the concentration of ethanol and the respective organic acids. Lactate content was high, and sodium benzoate/ potassium sorbate increased the concentration of this fermentation acid. Acetic acid, ethanol, ethyl lactate (EL) and ethyl acetate (EA) were reduced by the used additive with benzoate/sorbate. Hafner et al. (2014, 2015) postulated that especially potassium sorbate is an effective additive for reducing production of ethanol and ethyl esters in corn silage. Bernardes et al. (2014) and Da Silva et al. (2014) reported that the application of sodium benzoate and potassium sorbate to corn and sorghum at ensiling, alone or in combination, has consistently reduced ethanol contents in silages. When reported, concentrations of ethyl esters have also been reduced (Auerbach and Weiss, 2012; Hafner et al., 2015). Evidently, the added amount of those active substances is crucial in the inhibition of VOC formation. Too low application rates may result in higher VOC concentration (Hafner et al., 2014).

Interestingly, buffered acid mixtures, mainly containing **formic and propionic acids** stimulated ethanol and ethyl lactate (Auerbach et al., 2012; Weiss and Auerbach, 2012). DM losses were highest in acid treatments, whereas a significant reduction in DM loss was found by the liquid mixture of sodium benzoate and potassium sorbate. These observations can be explained by differences in ethanol concentrations, whose formation always results in the CO_2 release, which escapes from the silo.

Brüning et al. (unpublished) tested chemical additives in whole-crop maize (277 g/kg DM), ensiled in 120-L plastic silos, high compacted (silage density approx. 239 kg m^{-3}) and sealed immediately. As seen in table 8 a mixture of sodium benzoate and potassium sorbate reduced ethanol and esters (EA and EL) significantly. Contrary, the silage additive with formic acid increased the ethanol content drastically.

Table 8. Effects of silage additives on DM losses, fermentation pattern, VOC`s and aerobic stability of whole crop maize silage (DM 277 g kg^{-1})[1]; Brüning et al., unpublished

Feature	Unit	Silage treatment [1] Control	SBPS	FAFS	SEM[2]	Significance
pH		3,63 [a]	3,59 [a]	3,73 [b]	0.02	<.001
DM loss	%	5,5 [a]	5,2 [a]	7,4 [b]	0.3	<.001
WSC[3]	g/kg TM	35,6 [a]	49,9 [a]	72,8 [b]	4.8	<.001
Lactic acid	g/kg TM	68,1 [b]	72,4 [b]	43,2 [a]	0.3…2.1	<.001
Acetic acid	g/kg TM	13,2 [a]	23,0 [b]	12,5 [a]	0.4…2.2	0.012
Ethanol	g/kg TM	17,1 [b]	6,9 [a]	36,2 [c]	0.2…1.7	<.001
Ethyl lactate	mg/kg TM	399 [c]	199 [a]	306 [b]	4…21	<.001
Ethyl acetate	mg/kg TM	499 [c]	59 [a]	91 [b]	2…44	<.001
n-Propanol	mg/kg TM	155 [a]	469 [a]	n.A. [b]	37…116	0.014
Yeasts	log_{10} KBE/ g	5,2	2,7	4,15		
Moulds	log_{10} KBE/ g	2,7	3,6	3,48		
ASTA[4]	Hours	65 [a]	152 [b]	118 [b]	7.6…9,3	<.001

[1]SBPS = maize treated with an additive based on sodium benzoate and potassium sorbate; FASF = maize treated with an additive based on formic acid and sodium formate; [2]SEM = Standard error mean (Min…Max); [3] WSC = water soluble carbohydrates; [4]defined as the number of hours the silage remained stable before a temperature rise by ≥ 2 °C above ambient temperature; [a-b]means in rows bearing unlike superscripts differ ($P < 0.05$); n=6

Further investigations of Weiss et al. (2015b, 2016) with **corn** confirmed that silage additives containing sodium benzoate, calcium propionate, and potassium sorbate were superior to other treatments regarding suppression of ethanol and ester formation as well as improvement of aerobic stability, with and without air ingress.

In investigations with sugarcane silages (Cardoso et al. (2016) and calcium oxide (CaO) as an additive, this chemical product even inhibited ethanol and ester formation.

A study of Auerbach and Weiss (2012) with **sorghum** silages has been implemented to evaluate the effect of **inoculants**. Sorghum was chosen as silage type because it represents an important forage source for ruminants in semi-arid regions, and its production often bears the risk of excessive ethanol fermentation so that high concentrations of VOC are expected.

Lactic and acetic acids, as well as ethanol, were affected by variety and treatment, and an interaction was determined between the two factors for lactic acid. Ethanol and ethyl esters were reduced by *Lactobacillus buchneri* at all inoculation rates, but the lowest levels were consistently found in a mixture of sodium benzoate and potassium sorbate were used. On the contrary, Hafner et al. (2014) and Savage et al. (2014) detected elevated concentrations of ethanol and ethyl acetate, and Kristensen et al. (2010) found an increase in n-propanol and propyl acetate in corn silages treated with *Lactobacillus buchneri*.

The use of *Lactobacillus plantarum* alone or in combination with *Lactobacillus buchneri* did not affect ethanol and ester production when compared with control silages. This effects of different additives on VOC in sorghum silages support earlier results by Weiss and Auerbach (2011). Hafner et al. (2014) observed increased ethanol and ethyl acetate accumulation by the use of homofermentative lactic acid bacteria (LAB).

The above-described results with sorghum were confirmed in the ensiling laboratory experiment with legumes (Weiss and Kalzendorf, 2016). Silage additives with LAB did mainly not affect the contents of ethanol, the same applies for the contents of esters. The additive salts containing benzoate, nitrite and hexamine strongly reduced the ethanol and ester contents. According to Woolford (1975), these substances be able to inhibit yeasts and possibly heterofermentative LAB which also produce ethanol. Trials (König et al., 2016) with different mixtures of white lupin and spring wheat, treated with formic acid (FA), sodium nitrite-hexamine mixture (NaHe) or homofermentative lactic acid bacteria, resulted in higher ethanol and ethyl ester contents in inoculated silages compared with FA- and NaHe-treated silages, regardless of the mixture used.

In a study with fourth-cut natural grassland (Weiss and Auerbach, 2015), wilted to 26,8% DM, forages received the treatment with 21 commercial additives which were obtained from the German marketplace and used according to the instructions of the manufacturers. Grass silages were well fermented as reflected by low pH and the absence of butyric acid. The production of lactic acid was stimulated by some additives of the types of homofermentative LAB, a combination of homo- and heterofermentative LAB and a combination of homofermentative LAB and antimycotic chemicals, whereas the pure heterofermentative LAB inoculant as well as two chemicals reduced it. The treatment with homofermentative LAB, either applied alone or in combination with antimycotic chemicals, always resulted in lower acetate levels. The lowest ethanol and ethyl ester contents were detected in silages that received chemical additives. There was a strong positive linear correlation between these two parameters (R^2=0.72, $P<0.001$). The production of 1-propanol was highest in silages treated with the heterofermentative inoculant, similar with results of grass ensiling in Weiss and Auerbach (2015). In the study with corn (Weiss et al. 2016), n-propanol was detected less frequently and at lower concentrations compared with previous investigations, which reported up to 19.1 g/kg of DM (Kalač, P. and L. Pivničkova, 1987; Kristensen et al., 2010). Although n-propanol represents a typical minor end product of anaerobic metabolism by yeasts (McDonald et al., 1991), it can also be formed by *Lactobacillus diolivorans* in conjunction with propionic acid via conversion of 1,2-propanediol (Krooneman et al., 2002).

Summary

Results from ensiling experiments on the effects of different types of silage additives on ethanol and ester formation in different ensiling materials clearly indicated that chemical products containing active ingredients with specific antifungal effects can significantly reduce ethanol and ester concentration. Salts of sorbic, benzoic or propionic acids or mixture are an effective treatment for reducing VOC production.

Conclusions

Delayed sealing, aerobic exposure, temperature, and silage additives use largely affected fermentation pattern and especially the contents of volatile organic compounds. The production of ethyl esters of lactic acid and, to a lesser extent, of acetic acid is correlated with the concentration of ethanol highlighting the prominent role of the alcohol in the formation of esters in silage. These ethyl esters were considered as indicator substances for the total VOC´s production in silage and feed intake-reducing substances. Esters occur in all different silage types, depending on ensiling conditions. Any measures that reduce ethanol accumulation will restrict ester formation. Salts of sorbic, benzoic, or propionic acids or a mixture thereof applied at sufficient quantities appear to be the most promising additives to control VOC production in silages, whereas formic acid-based additives and homofermentative inoculants do not have this potential.

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Quantification of the emission reduction benefits of mitigation strategies for dairy silage

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Introduction

Our previous work has shown that dairy silages are a major emission source of volatile organic compounds (VOCs) and to some unknown degree of oxides of nitrogen (NOx), both contributing to the San Joaquin Valley's (SJV) ozone challenges. In general, emission of VOCs from silage can be mitigated by either 1) reducing VOC production in the liquid/solid phase of the silage pile, or 2) reducing relative emission from the face of the silage pile or the feedlane. While NOx mainly forms in the initial phase of ensiling, the current research focused on the later stages of storage and feedout of silages; therefore, the NOx picture of the present report is incomplete and requires further research. The focus of the present research was on monitoring

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and modeling of VOC production using silage additives (Chapter 2), as well as emissions mitigation via various silage storage methods, defacing practices, and feed management approaches (Chapters 3 & 4). Microbial and chemical silage additives were investigated using bucket silos, to reduce the production and emissions of volatile organic compounds in corn silage. The VOC concentrations were measured using headspace gas chromatography method. For the field monitoring of emissions from different silage storage and defacing methods, we used flux chambers and wind tunnels that were attached vertically on the silage face, immediately after defacing. These sampling devices were attached to a fully equipped mobile air quality lab, in which concentrations of all relevant gases were analyzed in situ. This set-up allowed us to compare different storage methods (i.e., conventional standard pile vs. silage bag), and defacing methods (e.g., perpendicular, lateral, and rake extraction), as well as various water inclusion rates for the feed all aiming at reducing emissions. The monitoring data were used to inform and validate a new VOC process-based model that was developed to predict VOC emissions from silage sources on farms using theoretical relationships of mass transfer and parameters determined through our earlier (published) laboratory experiments and numerical modeling. The results of the silage additive studies showed that most microbial and chemical additives actually increase VOC production and emissions. Only one chemical additive used at one particular concentration reduced VOCs. The results for silage storage indicated that silage bags vs. conventional silage piles emit considerably fewer emissions. Furthermore, lateral defacing versus perpendicular- and rake defacing reduced emissions of most gases. Finally, reducing emissions in the feed lane seems to be possible via inclusion of water to the TMR. Simulations of all relevant silage mitigation options that were studied on the commercial dairies were conducted using the

VOC modeling tool. These simulations clearly showed that most of the reactive VOC emissions on a California dairy occur from feed lying in feed lanes during feeding as opposed to the silage storage pile or bag. In conclusion, regulations aimed at reducing VOC emission could be ineffective or even increase emission if they promote silage additives without recognition of different types of additives. The monitoring results of the storage and defacing study results point to certain practices as being advantageous. However, one shall not view those monitoring results in isolation, because only the integration of other parts of the feed's life cycle, using whole farm modeling, explains not just the relative- but also the absolute effectiveness of mitigation techniques in reducing VOCs and NOx on the entire dairy. The whole farm modeling clearly showed that mitigation efforts should be applied to reducing emissions from feeding rather than focusing solely on those from the exposed face of silage piles.