Contribution of silage volatile compounds for the animal nutrition

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1. Introduction

Silages are produced by fermentation of forage which contains enough moisture. The anaerobiosis, which is the most important condition to the process, allows the microbial growth and formation of organic acids from soluble carbohydrates oxidation. It is the most common way to inhibit undesirable microorganisms and to preserve the ensiled substrate (McDonald et al. 1991; Pahlow et al., 2003).

Under normal condition, lactic acid is the major fermentation end-product in silages. In the homolactic pathway, lactic acid is the single product and the microorganisms obtain 2 ATP per hexose fermented, therefore, dry matter and gross energy are almost entirely preserved. On the other hand, microorganisms which produce volatile fatty acids extract 4 ATP per hexose, however, the substrate is partially converted to gases and emitted into the atmosphere, mainly as CO_2, which usually represents energy loss (McDonald et al., 1991, Van Soest, 1994; Pahlow et al., 2003). From a microbial point of view, more glucose should be broken down by homolactic fermentation in comparison with VFA pathway to obtain the equivalent amount of energy, due to the differences in ATP yield. Nevertheless, lactic acid is stronger (pKa ~ 3.8) than VFA (pKa ~ 4.8), so silage pH drop faster through homofermentative pathway, which decreases the microbial activity and anticipates the stability phase where losses become lower (McDonald et al., 1991).

In addition to the carboxylic acids, other end-products such as alcohols, aldehydes, esters, and ketones have been identified in silages (Morgan and Pereira, 1962; Weiß et al. 2009; Chmelová, 2010) (Tables 1 and 2). In sugarcane silages, alcohols are the major fermentation end-products (Zopollatto et al., 2009) (Table 3). This pathway is characterized by high loss of dry matter, while the gross energy is preserved due to the high heat of combustion of the ethanol.

In general, silage fermentations lead to losses, but the end-products are essential to the preservation. Moreover, these compounds have high energy content and they are significant sources of nutrient to the animal.

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Table 1. Concentration of fermentation end-products in corn silages (Weiß et al., 2009)

Compound	Minimum	Maximum
Lactic acid, g/kg DM	6.9	78.9
Acetic acid, g/kg DM	5.8	79.4
Propionic acid, g/kg DM	<0.1	11.2
Ethanol, g/kg DM	0.9	64.0
Methanol, mg/kg DM	114	1030
Propanol, mg/kg DM	14	19096
Butanol, mg/kg DM	<3	9
iso-Butanol, mg/kg DM	<3	23
2-Butanol, mg/kg DM	12	1314
Allyl alcohol, mg/kg DM	<4	28
2-Methylbutanol, mg/kg DM	<3	10
3-Methylbutanol, mg/kg DM	<3	73
Hexanol, mg/kg DM	<3	21
2-Phenylethanol, mg/kg DM	<4	44
1,2-Propanediol, mg/kg DM	<5	13630
Methyl acetate, mg/kg DM	<3	154
Ethyl acetate, mg/kg DM	12	789
Propyl acetate, mg/kg DM	<5	379
Ethyl lactate, mg/kg DM	16	1263
Propyl lactate, mg/kg DM	9	77

Table 2. Aldehydes in farm-scale silages (Chmelová, 2010)

Aldehyde	Corn silages (n = 8)			Grass silages (n = 13)		
(mg/kg FM)	Mean	S.D.	Range	Mean	S.D.	Range
Ethanal	25.8	16.1	9.4-48.2	29.8	14.2	9.9-49.4
Propanal	70.2	17.8	51.3-99.1	76.6	12.0	54.4-97.6
Butanal	77.9	16.9	52.2-100.0	71.9	17.2	46.1-98.7
2-Methylpropanal	28.9	15.1	10.4-49.0	25.7	15.8	9.2-50.4
Pentanal	150.0	37.8	97.8-200.0	147.0	30.3	105.0-189.0
3-Methylbutanal	78.3	17.4	50.6-97.3	76.0	18.5	46.7-100.0
Hexanal	153.0	38.9	97.1-196.0	150.0	30.3	104.0-190.0
Heptanal	24.4	17.6	7.9-50.3	23.1	11.8	10.3-46.5

Table 3 – Fermentation end-products in sugarcane silage (adapted from Zopollatto et al.,2009)

Compound (% DM)	n	Mean	Minimum	Maximum
Lactic acid	12	3.3	0.8	6.3
Acetic acid	14	4.0	1.6	9.3
Lactic/Acetic	-	0.83	-	-
Propionic acid	9	0.7	0.2	1.9
Butyric acid	6	0.1	0.0	0.1
Ethanol	19	7.8	0.3	21.8†
Total		15.9		

† *L. plantarum* was used as additive (Freitas et al., 2006)

2. Methods to determine dry matter content in silages

In conventional chemical analysis, feed samples are dried in a forced air oven. The weight loss is assumed as water and, in turn, the residue is called dry matter. Thus, this gravimetric method is not accurate to achieve the dry matter content of samples containing volatile compounds other than the water itself. In silages, much of fermentation products are lost by volatilization during heating (Colovos et al. 1957; McDonald and Dewar, 1960; Fox and Fenderson, 1978). It leads to deviations on nutrient and energy contents of silages, dry matter intake, diet digestibility and energy efficiency (Stone et al., 1960). The determination of dry matter by using forced air oven also brings on errors for calculations of dry matter losses during silage storage (Figure 1).

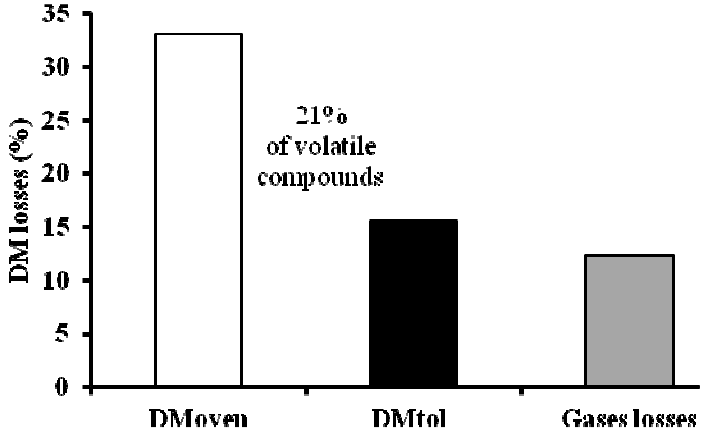

Figure 1. Dry matter and gases losses in sugarcane silage without any additive. Dry matter contents were determined by oven (DMoven) or toluene distillation (DMtol) (Daniel, 2011).

For long time it was known that silage had volatile substances that could be lost during the drying process. Until early 1940s, most studies had ignored this fact. Therefore, Perkins (1943) carried out a trial to compare the traditional method (oven drying at 100°C) with a new method of volumetric determination of water content in fodder samples. The alternative method was based on sample distillation with excess of solvent, the toluene (boiling point 110°C). Although the volumetric methods were less accurate than that gravimetric, there were no deviations relative to volatile substances. Perkins (1943) showed that both methods achieved exactly the same content of water in green fresh plants. On the other hand, the water content was always higher when silage samples were dried at 100°C. Then, the authors suggested that the simultaneous application of both methods to allow quantifying the volatiles fraction of silages.

Ever since, it was suggested that in the toluene's method, part of the volatile fraction could be found in the distilled water and increase its volume. Although measurable, the amount of volatile compounds represented less than the lower scale interval of the device, and therefore it can be negligible (Perkins, 1943). In addition, the volume of volatile components found in the aqueous phase from distillate could numerically compensate the small amount of residual water in the sample (Undersander et al., 1993). Thus, the dry matter determined by toluene distillation with no correction can be considered more appropriate.

In 1960s, McDonald and Dewar (1960) studied the nature of the volatilized fraction from silages at heating. The volatility averaged 87.9% for acetic acid and 89.4% for butyric acid. In all samples, lactic acid also evaporated, with volatility ranging from 1.4 to 16.4%. Nitrogen losses occurred mainly in the silages with higher pH. At the same decade, Wilson et al. (1964) to compared the oven drying (DMoven) to the toluene distillation (DMtol). The DMoven was up to 22% lower than the DMtol.

Haigh and Hopkins (1977) determined DMoven and DMtol content of 205 silage samples. The relationship between both methods was described as: DMtol (%) = 0.96 * DMoven (%) + 2.2 (r^2 = 0.98, P<0.01). DMtol averaged 11% higher than the DMoven. In sugarcane silage, Miranda (2006) noted that the DMoven content at 100°C averaged 80% of the value achieved by toluene distillation. Thus, 20% of the dry matter from sugarcane silages was composed of volatile compounds. This value was recently confirmed by Daniel (2011) (Figure 1).

Table 4. Percentages of dry matter lost estimated by various methods, using the toluene distillation as the reference method (Aerts et al., 1974)

Sample	Freeze drying	Microwave	Oven 70ºC	Oven 100ºC
Corn silage	6.71	-	6.21	7.47
Grass silage	9.73	6.84	8.58	10.72
High moisture corn silage	3.59	3.56	3.42	4.35
Fodder-beet	12.71	-	11.39	13.79
Mean	*7.58*	*5.37*	*6.89*	*8.42*
(Range)	*(1.14 – 28.38)*	*(1.14 – 20.30)*	*(1.41 – 22.77)*	*(0.65 – 23.70)*

Freeze drying is an alternative to drying silage samples. Aerts et al. (1974) compared several methods for determining dry matter in comparison to the toluene distillation. They observed 7.58% of dry matter lost during freeze-drying (1.14 to 28.38%) (Table 4). Alomar et al. (1999) determined the chemical composition and gross energy of silage samples after freeze-drying or drying in an oven at 65°C. There was no effect of drying method on the dry

matter and energy content, because both methods led to losses. It was not surprise to the authors, since they could smell the aroma of volatiles compounds during freeze-drying. In fact, it happens due to the lower pressure during freeze drying, which decreases the boiling point of compounds (Atkins, 1994).

Currently, there are other methods to determine the volume of water in feed samples, such as gas chromatography and Karl Fisher titration. In both, water is extracted from the sample with organic solvents, usually alcohols. In the former, the extract is injected into chromatograph coupled to a thermal conductivity detector (Fenton et al., 1981). In the Karl Fisher method, the extracted water is titrated with iodine in the presence of pyridine and methanol (Galletti and Piccaglia 1988). These methods led to results similar to the toluene distillation (Table 6). Chromatography, although a quick and accurate method, requires access to a gas chromatograph coupled to thermal conductivity detector, which has high cost. On the other hand, the reagents used in the Karl Fisher titration are potentially harmful to the health, as well as toluene.

One promising method to correct the dry matter content obtained by dehydration is to determine the concentrations of fermentation end-products in fresh silage and input those values into the equation containing the respective volatility coefficients (Weissbach, 2009). In corn silage, whose pH is usually low, the volatility coefficient of VFA could be assumed as 95%. In grass silage, however, this coefficient was defined as: Volatility (%) = 105 - 5.9 * pH. The volatility of lactic acid averaged 8%. Alcohols with one hydroxyl group evaporated almost completely at oven drying. Other alcohols such as 1,2-propanediol and 2,3-butanediol showed volatility coefficients of 77% and 87%, respectively (Table 5). The following equations were proposed for the dry matter correction (Weissbach, 2009):

Corn silage

DM = DMoven + 0.95*VFA + 0.08*lactic acid + 0.77*1,2-propanediol + 1*other alcohols (g/kg FM)

Grass silage

DM = DMoven + (1.05 – 0.059*pH)*VFA + 0.08*lactic acid + 0.77*1,2-propanediol + 0.87*2,3-butanediol + 1*other alcohols (g/kg FM)

Table 5. Incidence, content and volatility of fermentation end-products in corn silages (Weissbach, 2009)

	Corn silages			Grass silages		
	Incidence (%)	Content (g/kg FM)	Volatility (%)	Incidence (%)	Content (g/kg FM)	Volatility (%)
Acetic acid	100	9.98	95	100	8.27	78
Propionic acid	100	0.28	97	100	0.45	78
iso-Butyric acid	16	0.01	100	63	0.19	84
Butyric acid	62	0.09	100	91	2.06	88
iso-Valeric acid	78	0.06	100	98	0.36	71
Valeric acid	9	0.01	100	55	0.10	93
Caproic acid	21	0.01	100	68	0.19	92
Lactic acid	100	15.20	8	100	14.63	10
Ethanol	100	5.80	100	100	2.50	99
Propanol	50	0.25	100	49	0.20	100
Butanol	1	0	100	14	0.01	100
1,2-Propanediol	92	0.70	77	70	0.60	77
2,3-Butanediol	52	0.08	100	80	0.26	87

3. Losses of volatile compounds from silages

During storage and especially at silage feed-out, part of fermentation end-products can be either emitted into the atmosphere (Mitloehner et al., 2009) or oxidized by aerobic microorganisms (Spoelstra et al. 1988; Driehuis et al., 1999). However, when they are actually consumed by the animals, these compounds have nutritional significance (Daniel et al., 2011a).

The loss of volatile compounds occurs because all liquid substances and some solid evaporate continuously. The rate of evaporation or volatilization is defined by the vapor pressure, which depends on the temperature and type of substance. Materials with higher vapor pressure releases more molecules to the vapor phase and volatilized faster than that under lower vapor pressure (Atkins, 1994).

Temperature affects the kinetic energy of molecules and hence the vapor pressure. As higher is the temperature, more particles may evaporate. Moreover, substances with different polarities have dissimilar intermolecular forces. Polar molecules are strongly attracted to their neighbors because of the positive and negative ends, which increase the resistance to vapor loss. The opposite is applicable for nonpolar molecules (Atkins, 1994). A good example is the comparison between lactic and propionic acids, which differ just by the presence of a

hydroxyl group bonded to the alpha carbon. The hydroxy acid is more polar and therefore less volatile, even at higher temperatures.

In the trial carried out by Weissbach (2009), the volatility of lactic acid was 8% compared to 95% for the propionic acid in corn silage exposed to 105^{o}C. In the same study, the authors confirmed the influence of pH on the volatility index of silage compounds. The pH and pka established the ratio of dissociated and non-dissociated molecules, with the last being volatile component of the equilibrium. Therefore, as lower is the pH, as higher is the rate of acids volatilization and as lower is the alkali volatilization (Atkins, 1994).

Another factor contributing to the volatilization is the silage moisture. Porter and Murray (2001) found higher rates of evaporation of alcohols within drier silages, probably due to the higher gradient concentration between the sample and environment. In practice, mixing silage and concentrates increases the dry matter content, which might favor the volatilization of silage fermentation end-products, although there are no published data on this issue.

When organic compounds volatilize, they play a central role in atmospheric chemistry, because: 1) react with OH radicals, 2) form ozone by interaction with nitrogen oxides in the presence of sunlight, and 3) form secondary organic aerosols (Andreae and Crutzen, 1997). While the ozone layer in the upper atmosphere is beneficial, tropospheric ozone is extremely pollutant and proven risk to the human health. Respiratory problems, nervous damage, liver and kidneys injure, allergies and cancer are some of deleterious effects of pollutants related to the volatile organic compounds (Mendell, 2007, Bernstein et al., 2008).

The difficulty of linking health problems to these pollutants is because the clinical symptoms appear slowly and after chronic exposure (NRC, 2008). Currently, high levels of ozone are found not only in areas with high population density, but also in areas with intense agricultural activity (U.S. Climate Change Science Program, 2008). The control of this pollutant has been difficult since it is not emitted directly into the atmosphere, but formed by photochemical process. In this way, volatile organic compounds play a central role and its control has been advocated as an effective means to reduce levels of ozone in the troposphere (EPA[2]).

In the north hemisphere there are some studies about emissions of volatile organic compounds (VOC) from silages. Mitloehner et al. (2009) identified 24 VOC from silages and total mixed rations (TMR), being 6 alcohols, 5 VFA, and 13 esters. Alcohols, especially ethanol, predominated in all silages and TMR. The highest emissions of ethanol and propanol

[2] Environmental Protection Agency – United States of America, www.epa.gov

were detected in corn silages. The second most abundant group of VOC were VFA, being acetic acid the main component due to the high concentration in silages. Montes et al. (2009) measured the emission of VOC from silages under controlled conditions. The emission rates were higher at beginning of the tests, declined faster within 2 hours and then declined slowly over a 24 h period (first-order kinetic). The range of emissions, however, differed across the VOC. Ethanol was the main VOC from corn silage, while the alfalfa silage had the highest emissions from methanol. The VOC concentrations explained the differences between the rates of emissions in different forages, but not among the compounds. Regardless of the higher concentration, acetic acid had lower emission rates than that of ethanol, due to the lower volatility.

Krauter and Blake (2009) conducted a survey of VOC emissions on dairy farms in San Joaquin Valley, California, USA. The TMR based on silages were the main source of VOC (60% of total). In that study the authors showed that the emission rate of VOC was 4.5 times higher in disturbed silage piles compared to the intact piles, due to the higher mass porosity. Stored undisturbed silages represented 7% of total VOC emission.

Despite the exhausting search, no publications were found addressing VOC emissions from tropics silages. The research efforts to characterizing VOC from agricultural operations might be useful for proposing mitigation strategies, reducing environmental pollution and nutrients losses.

4. Usage of organic acids and ethanol on animal nutrition

While some part of fermentation end-products can be lost, the fraction consumed by the animals have nutritional significance (Daniel et al. 2001a), since they have high energy content per unit mass (Table 6). In the reticulo-rumen, silage VFA are incorporated into the rumen pool, removed by absorption and passage (Peters et al. 1990; Resende Júnior et al., 2006), and thereafter used by tissues as metabolizable energy (MacLeod and Orskov, 1984; Bergman, 1990; Orskov, 1995). Across major VFA, only butyrate is significantly metabolized by the rumen epithelium (Kristensen et al., 2000, Kristensen and Harmon, 2004). Such high quality silages have low butyric acid levels, and, in general, silage VFA must contribute significantly to the nutrient pool in the portal blood.

Table 6. Heat of combustion of major fermentation end-products in silages (Clymer, 2004)

Compound	Heat of combustion (kcal/g)	Compound	Heat of combustion (kcal/g)
Lactic acid	3.7	Ethyl lactate	6.5
Acetic acid	3.7	2,3-Butanediol	6.6
Propionic acid	5.1	Ethanol	7.1
Methanol	5.4	Propanol	8.1
Ethyl acetate	5.6	Pentanal	8.5
1,2-Propanediol	5.8	2-Butanol	8.7
Butyric acid	6.1	Hexanal	8.9

Additionally to the VFA, silages are rich in lactic acid. In the rumen, lactate can be absorbed or converted to VFA (Waldo and Schultz, 1956, Hueter et al., 1956). If absorbed, lactate is a substrate for hepatic gluconeogenesis (Reynolds et al., 1988). However, about 80% of the lactate is converted to propionate via acrylate pathway, mostly by *Megasphaera elsdenii* (Counotte et al. 1983; Stewart and Bryant, 1988). In this pathway, lactate is esterified by coenzyme-A, dehydrated and subsequently reduced by a flavoprotein. This path does not include steps of carboxylation or decarboxylation, and ATP synthesis has not been demonstrated (Russell and Wallace, 1988). So, duodenal flow of non-ammonia nitrogen (NAN) might decrease when rumen fermentable carbohydrates are replaced with lactic acid (Jaakkola et al., 2006, Jaakkola and Huhtanen, 1989) (Table 7).

Table 7. Effects of replacement of sucrose with lactic acid (adapted from Jaakkola and Huhtanen, 1989)

	Sucrose	Suc.+Lactic acid	Lactic acid	*P* linear
Rumen				
pH	5.93	6.15	6.44	<0.05
NH_3, mM	9.64	11.21	9.29	NS
Lactic acid, mM	6.05	1.37	0.55	<0.05
Total VFA, mM	111.1	118.3	108.5	NS
Acetic acid, mmol/mol	593	599	609	NS
Propionic acid, mmol/mol	179	225	226	<0.05
Butyric acid, mmol/mol	189	144	134	<0.05
Protozoa, 10^5/mL	6.8	5.8	4.1	<0.10
Duodenum				
NAN flux, g/d	97.9	96.0	90.9	<0.05
Microbial N / RFOM, g/kg	29.5	30.1	28.9	NS

On the other hand, the rumen environment spend two electrons per mole of lactate converted to propionate, leading to a product more energetic (propionate, 378 kcal/mol) than its substrate (lactate, 336 kcal/mol) and contribute to the rumen pH buffering (Table 7). The lowest lactate concentration in the rumen fluid from animals infused with lactic acid (Jaakkola and Huhtanen, 1989) occurs because of the microbial adaptation and the increased rate of metabolism of this intermediary (Huntington and Britton, 1979).

Rinne et al. (2009) carried out a meta-analysis to review the need of including new elements in feed protein evaluation models relative to the silage fermentation end-products, which provide little energy to the rumen microbes. The application of a discount factor leads to higher prediction error for milk protein production. Authors justified that partial loss of rumen microbial synthesis due to the partial offset by sparing glycogenic amino acids, because of the increasing of propionate flux as the main end-product from ruminal lactic acid fermentation. Thus, including new elements theoretically correct may not improve the precision of protein evaluation systems. In a recent trial (Daniel, 2011), the presence of volatile compounds from sugarcane silage did not depress the rumen microbial synthesis and unaffected the efficiency of protein synthesis (g/kg of TDN).

Ethanol is also an end-product from silages (Morgan and Pereira, 1962; Zopollatto et al., 2009) and had high gross energy content per gram (Table 6). When consumed by animals, ethanol is absorbed or metabolized by the rumen microorganisms (Jean-Blain et al., 1992; Moomaw and Hungate, 1963).

Durix et al. (1991) infusing ^{14}C-ethanol into the semi-continuous fermentor (Rusitec). Ethanol had a negligible effect on the digestibility of solid feedstuffs, although the microbial protein synthesis was decreased. VFA production was enhanced by 40% and acetate represented 80% of VFA formed from ethanol. The largest part of reducing equivalents (electrons) from ethanol oxidation was used to reduce CO_2 to CH_4.

Although some species of rumen microorganisms are negatively affected by ethanol (Caldwell and Murray, 1986), this alcohol could trigger positive effects in the rumen. In the experiment carried out by Chalupa et al. (1964), the in vitro digestibility of cellulose increased 6% when 2% ethanol was added (DM basis). This improvement was attributed to ethanol serving as a source of readily available energy to microorganisms. Moreover, the additionally supply of electrons by oxidation of ethanol to acetate could decrease the redox potential (Eh), shorten the lag phase and accelerates the microbial growth. Otsuki et al. (1991) studied the effects of ethanol on rumen metabolism of steers fed high-concentrate diets. There

were no effects on pH and Eh, while the concentrations of acetate and ammonia were increased and rumen protozoa counts were markedly reduced by alcohol intake.

In addition to the acetate flow enhanced by 2-carbon compounds, rumen biohydrogenation might be related with the higher milk fat content in ruminants fed ethanol. Gould (2000) observed that ethanol decreased the proportion of biohydrogenation intermediates in the fatty acids flow in the duodenum. The additional electrons supply by the oxidation of ethanol to acetate may have stimulated the rumen biohydrogenation. Randby et al. (1999) verified lower proportion of C18:1 and C18:2 fatty acids and higher fat content in milk from cows fed ethanol. Plascencia et al. (1999) suggested that ruminal biohydrogenation was positive correlated with methane production, which is often observed with ethanol supplementation (Table 8).

Table 8. Effects of ethanol on rumen VFA and methane production (in vitro) (adapted from Yoshii et al., 2005)

	Ethanol suplemented (mmol)	
	0	34
Ethanol metabolized in 24 h (mmol)	-	13.4
Ethanol metabolized (%)	-	39.4
Methane (mmol)	14.4	21.3*
Acetate (mmol)	32.2	45.2*
Increase of acetato (mmol)	-	13.0
Propionate (mmol)	13.6	13.2
Butyrate (mmol)	3.8	4.1
VFA (mmol)	49.6	62.5*

* $P < 0.05$

Kristensen et al. (2007) evaluated the metabolism of silage alcohols in dairy cows. Alcohols were partially metabolized by rumen microorganisms and ruminal epithelium and the remainder reached the portal blood. Most of absorbed ethanol was metabolized in the liver (Table 9). In another study by the same research group (Raun and Kristensen, 2009), ethanol metabolism was evaluated in fresh cows. Dry matter and ethanol intakes increased while the portal recovery of ethanol decreased with the advance of the period of lactation, due to the rumen adaptation (epithelium and microorganisms). Pradham and Hemken (1970) also found higher rates of ethanol metabolism in rumen fluid from adapted cows. Moreover, high concentrate diets leads to higher rates of ethanol oxidation.

In the Danish studies and in the recently work published by Daniel et al. (2011b), ruminal and hepatic metabolisms resulted in low arterial concentrations of ethanol. In both

trials there was no evidence for saturation of hepatic metabolism, since the low arterial concentrations. Considering Michaelis-Menten kinetics (Vmax and Km) and the dose of ethanol potentially fed to ruminants by silage intake, Jean-Blain et al. (1992) concluded that the enzymatic systems would not be saturated and the plasma concentration of ethanol would predominantly be low.

Table 9. Ruminal and splanchnic metabolism of ethanol in dairy cows

	Kristensen et al. (2007)	Raun e Kristensen (2009)		
Days in milk	257	4	15	29
Dry matter intake (kg/d)	15.8	14.7	16.5	19.7
Dietary content of ethanol (% MS)	1.42	1.9	1.9	1.9
Ethanol concentration in the rumen (mM)	2.86	3.2	3.3	2.8
Portal recovery (%)	-	39%	28%	24%
Net portal flux (mmol/h)	113	71	97	114
Net hepatic flux (mmol/h)	-131	-	-	-
Net splanchnic flux (mmol/h)	-18	-	-	-
Arterial concentration (mM)	0.165	0.046	0.073	0.074

As the fact of ethanol is converted to acetate, the Table 10 shows theoretical values of metabolizable energy (ME) and net energy for lactation (NEl) of ethanol and acetate mixtures absorbed by ruminants. For simulation, acetic acid was assumed the only product from ethanol oxidation. Considering that the portal recovery of ethanol is lower than 25% (Raun and Kristensen, 2009), it is expected that NEl of ethanol is close to 2.9 Mcal/kg. Considering NEl conservation over silage fermentation process, 1 kg of ethanol is produced in the silo at the expense of about 2 kg of sucrose (McDonald et al., 1991), containing 4.5 Mcal of NEl (2 x 2.26 Mcal/kg, NRC, 2001). Thus, alcoholic fermentation led to loss of NEl of approximately 35% [(4.5 - 2.9) / 4.5]. This proportion may be even greater if the volatilization is taking into account.

Although ethanol is metabolized by rumen microorganisms and at the epithelium, a considerable amount of it reaches the liver. In hepatocytes, three major metabolic pathways are described. In the cytoplasm, ethanol is oxidized to acetaldehyde by alcohol dehydrogenase, and requires the oxidized form of coenzyme nicotinamide adenine dinucleotide (NAD^+) to carry the electrons. In peroxisomes, ethanol is oxidized by catalase and hydrogen peroxide is the electron acceptor. When ethanol reaches high concentrations in the liver, microssomal cytochrome P4502E1 (CYP2E1) plays an important role in metabolism. Acetaldehyde generated in all pathways is metabolized to acetate by

acetaldehyde dehydrogenase in mitochondria. Coenzyme NAD^+ is also the electron acceptor to this step (Lieber and Abittan, 1999).

Table 10. Theoretical energy content of ethanol and acetic acid mixtures absorbed by ruminants

	Mixtures				
Ethanol, %	*100*	*75*	*50*	*25*	*0*
Acetic acid, %	*0*	*25*	*50*	*75*	*100*
ME, Mcal/kg	7.10	6.25	5.40	4.55	3.70
NEl[1], Mcal/kg	4.54	4.00	3.46	2.91	2.37
NEl / Heat of combustion of ethanol, %	64%	56%	49%	41%	33%

[1] NEl was assumed as 0.64*ME (Moe et al.,1972; NRC, 2001)

As a result, the hepatic oxidation of ethanol generates an excess of reducing equivalents. The large amount of NADH, the production of acetaldehyde and, the activity of microsomal enzymes capable to generating reactive oxygen species (ROS) are involved in the ethanol hepatotoxicity (Lieber and Abittan, 1999). The plasma activity of the enzyme gamma-glutamyl transferase has been used as a marker of liver damage caused by ethanol (Souza et al. 2004; Tennant and Center, 2008).

Moreover, the amount of reducing equivalents could exceed the liver capacity to keep the intracellular redox homeostasis, resulting in metabolic disorders. Most of reducing equivalents are transferred to the mitochondria and decrease the activity of Krebs cycle and lipid oxidation, stimulating the synthesis of fatty acids. As the supply of ethanol also promotes lipolysis in adipocytes, alcohol consumption can lead to liver fat accumulation (Lieber and Abittan, 1999).

The lower plasma glucose concentrations observed by Pradham and Hemken (1970) after rumen infusion of ethanol, may be due to the inhibitory effect of ethanol on the conversion of propionate to glucose, with concomitant intracellular accumulation of malate and depletion of phosphoenolpyruvate (Demigné et al ., 1991). The inhibitory effect of ethanol on gluconeogenesis seems mediated by increasing of NADH:NAD^+ ratio (Demigné et al., 1991). Thus, the consumption of high doses of ethanol would be extremely undesirable in fresh cows, which naturally are prone to ketosis and fatty liver.

5. Effects of fermentation end-products from silages on voluntary feed intake and animal performance

The nutritional value of silages differs from its fresh or dried forage source. Physical and chemical characteristics of silages may interfere on dry matter intake, microbial and visceral metabolism, and in turn on animal performance (Weiss et al., 2003). High concentrations of organic acids, alcohols, and other volatile organic compounds instead of water soluble carbohydrates is the main nutritional differences between well preserved silages and their original crops.

The performance of ruminants fed silage based diets depends mainly on feed intake and diet nutritive value, usually the digestibility. Across the variation on digestible energy intake, 60% to 90% is related to feed intake and only 10% to 40% to digestibility (Mertens, 1994). Unlike fresh or dried fodders, the relationship between intake and digestibility of silages is poor, probably due to the fermentation end-products (Huhtanen, 2002).

Several meta-analysis were published by Scandinavian researchers in attempt of understanding the relationship involving silage composition, feed intake and animal performance. Huhtanen et al. (2002, 2003 and 2007) and Krizsan and Randby (2007) showed that both silage intake and animal performance were negatively correlated to the silage fermentation extension (ammonia, lactic acid, VFA and total acids concentrations). Hetta et al. (2007) established a negative correlation between silage intake and acetic acid concentration, but found a positive relation between feed intake and ethanol content. The authors discussed the opposite association between ethanol and ammonia concentrations ($r = -0.67$), since sugar-rich ensiled crops usually present high ethanol and low ammonia concentrations (Driehuis and Van Wikselaar, 2000).

It is remarkable that correlations obtained by meta-analysis can leads to misleading conclusions due to the collinearity across variables. The relationship between silage pH and intake is a good example of confounding. Although many studies did not show strong correlation between these variables (Offer et al. 1998; Steen et al. 1998; Huhtanen et al., 2002), pH tends to be negatively correlated to silage consumption, because high pH silages have higher concentrations of ammonia and higher VFA/lactic acid ratio. Furthermore, high concentrations of ammonia do not seem a causative factor per se, but a marker of proteolysis end-products (Rook et al., 1990). For this reason, several studies were carried out in order to isolate effects of silage fermentation end-products on voluntary feed intake.

Hutchinson and Wilkins (1971) carried out a well-known study about the effect of acetic acid on silage intake. Ryegrass silage was treated with increasing doses of acetic acid (2%, 5% and 8.8% of DM) before feeding adult sheep. The pH and moisture content of silages were kept by adding of NaOH and water. Daily dry matter intake was similar across

treatments, but ingestive behavior pattern was altered by acetic acid. Ingestion time was lower during first 4 hours after feeding, but the opposite behavior occurred between 16 to 20 hours after feed offering. The deviation on ingestive pattern with maintenance of feed intake was also found by Daniel (2011) in high producing dairy cows (Figure 1). Feed refusal in initial period after morning feeding may be related to the higher volatilization rate of fermentation products (Figure 2).

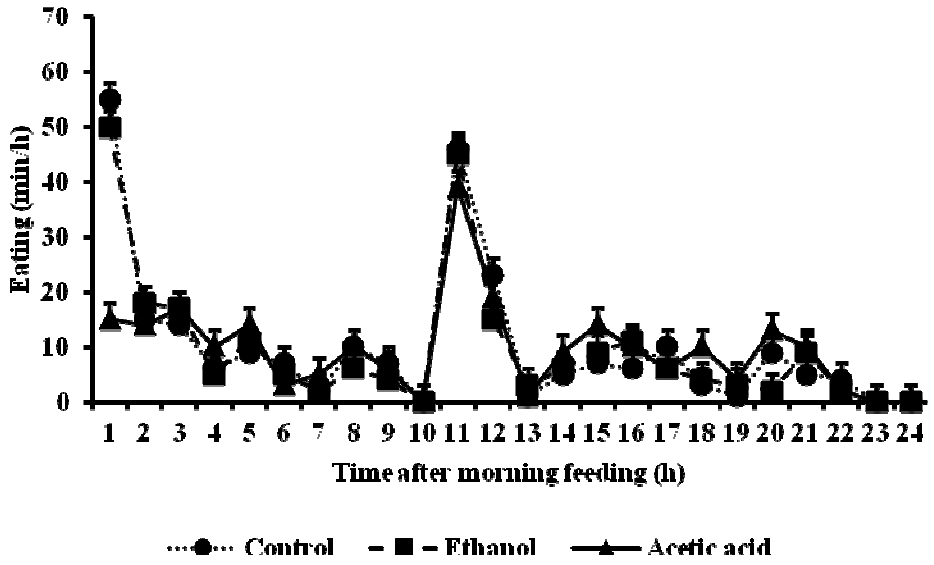

Figure 1. Eating pattern of dairy cows supplemented with ethanol or acetic acid (Daniel, 2011)

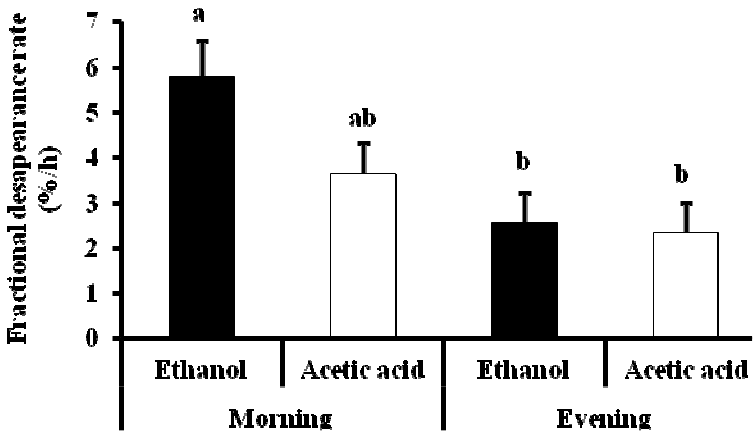

Figure 2. Fractional desapearance rates of ethanol and acetic acid addded onto the total mixed rations (Daniel, 2011)

Wilkinson et al. (1976) studied the performance of steers fed corn silage or fresh plants added or not with a mixture of acetic and lactic acids. Sucrose was used to balance the

energy content across treatments. The ensiling process did not affect the dry matter intake, but decreased the daily weight gain. On the other hand, the acid mixture reduced the feed intake without affecting the daily weight gain. The lower value for daily intake may have been caused by the adopted method to determine the dry matter content (oven drying at 70^{o}C).

Krizsan et al. (2006) supplemented steers with acetic acid, caproic acid, tryptamine or a mixture of substances. Acetic acid or its combination reduced the silage intake. Though, when the compounds mass were taken into account, daily intake was similar across treatments. So, it is possible to suggest that the method used to measure the dry matter content was the main determinant of the decrease on silage dry matter intake when it was compared to unfermented crop.

Especially in 1990s, the use of by-products as ruminant feed ingredients was increased around the world. From these sources, wet distillers grains emerged due to the alcohol production from corn through industrial fermentation. At that time, the distillation process was less efficient and the by-product contained as much as 10% of ethanol (Larson et al., 1993). This fact trigged an increase interest among researchers, who investigated the nutritional value of ethanol (Ham et al., 1994). Partial replacement of 5% or 10% of corn with ethanol did not affect the performance of lambs (Table 11). In view of high levels of ethanol in sugarcane silages, Daniel (2011) studied the performance of high producing Holstein cows fed ethylic alcohol. Energy efficiency of cows supplemented with ethanol was similar to the control group (Figure 3). The net energy for lactation (NEl) of ethanol was estimated as 2.6 Mcal/kg.

In both studies the higher theoretical energy value of ethanol compared to carbohydrate was not demonstrated. Rumen conversion of ethanol to acetate and concomitant increase of methane production might be reasonable explanation to the deviation on the predicted energetic value based on heat of combustion. With NEl of ethanol, the authors speculated that approximately 90% of the ethanol was converted to acetate, which is in agreement to the absence of ethanol in the peripheral blood (Daniel et al., 2011b). From forage conservation point of view, it was possible to extend the traditional knowledge regarding energy recovery through this metabolic pathways operating in silages (Table 12).

Table 11. Effects of replacement of ground corn with ethanol on the performance of lambs (Ham et al., 1994)

Ethanol (% MS)	0	5	10	EPM
DM intake (kg/d)	1.92	2.03	2.03	0.14
Weight daily gain (kg/d)	0.34	0.34	0.34	0.04

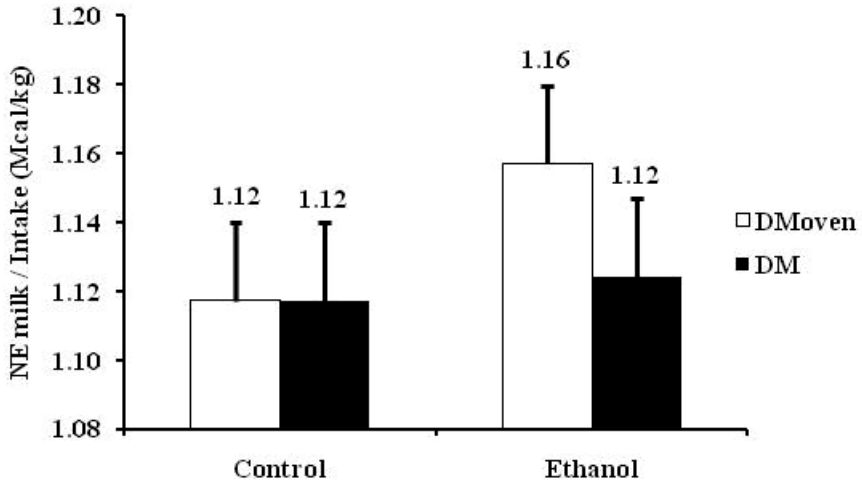

Figure 3. Energy efficiency of dairy cows fed ethanol (Daniel, 2011)

Table 12. Energy recovery by fermentation of glucose into alcohol in silages

		McDonald et al. (1991)	McDonald et al. (1991)	
		DM recovery	GE recovery[1]	NEl recovery[2]
Pathway	Products	(%)	(%)	(%)
Yeast	Ethanol and CO_2	51	99	69[†]

[1] GE = gross energy; [2] NEl = net energy for lactation

[†] Heats of combustion considered: glucose 676 kcal/mol, ethanol 329 kcal/mol and, acetic acid 222 kcal/mol. Assume that 90% of ethanol is converted to acetate

Randby et al. (1999) fed 600 g/d of ethanol to Norwegian Red cows, in a crossover design with 9 days-periods. When ethanol was take in to account in their calculations, the dry matter intake and energy correct milk yield were higher than that of control, due to the higher milk content of fat and protein. Organoleptic milk quality was reduced with ethanol intake. Probably, the trial length did not allow the complete adaptation of enzymatic systems related to ethanol metabolism, resulting on milk with lower sensory quality. In the trial of Daniel et al. (2011a), ethanol supplementation induces to higher daily yields of milk and protein. As the milk fat content was slightly decreased by ethanol treatment (not significant), 3.5% fat corrected milk was similar across treatments (Table 13). Unlike the results of Randby et al.

(1999), sensory milk quality was unaffected by diets, probably due to the trial length, and in turn, tissue metabolic adaptation to the ethanol (Daniel et al., 2011c).

Table 13. Performance of dairy cows fed ethanol or acetic acid (Daniel, 2011)

Parameter	Control	Ethanol	Acetic acid	SEM	*P*
Dry matter intake, kg/d	22.16[ab]	22.72[a]	21.42[b]	0.58	<0.01
Milk yield, kg/d	35.50[b]	37.82[a]	35.70[b]	1.38	<0.01
3.5% fat corrected milk, kg/d	35.79	36.93	36.60	1.14	0.39
Fat, %	3.55	3.37	3.69	0.11	0.12
Protein, %	3.32	3.33	3.29	0.03	0.56
Fat, kg	1.25	1.28	1.31	0.05	0.73
Protein, kg	1.19[ab]	1.26[a]	1.15[b]	0.03	0.03

[a, b] Means with different superscripts differ (Tukey; $\alpha = 0.05$)

6. Take home messages

- ✓ Volatile compounds represent up to 20% of silage dry matter;
- ✓ Lactic acid, acetic acid and ethanol are major fermentation end-products, however, several novel compounds has been found;
- ✓ Due to the volatile compounds, the method adopted to determine dry matter of silages can alter the nutrient and energy contents, dry matter intake, digestibility and energy efficiency of diets. Moreover, it may brings on errors for calculations of dry matter losses during silage storage;
- ✓ During storage and especially at silage feed-out, part of the volatile compounds can be either emitted into the atmosphere or oxidized by aerobic microorganisms. However when they are actually consumed by the animals, these compounds have nutritional significance;
- ✓ Under optimal silage management, approximately 80% of silage volatile compounds may be ingested by the animals. This value might differ depending on the silage feed-out and feeding frequencies;
- ✓ Alcoholic fermentation is the major pathway in sugarcane silages which might lead to high recovery of gross energy with low dry matter recovery rate;
- ✓ The net energy for lactation from ethanol estimated based on animal performance is lower than the predicted values traditionally available in the literature;
- ✓ Animal performance data suggest that silages volatile compounds might be responsible for additional 10% over the energy content predicted based on chemical analysis.

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