Ensiling total mixed ration for ruminants

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1. Introduction

Complete or total mixed rations (TMR) are produced by mixing forages, byproducts, concentrates, minerals, vitamins, and additives. From this mix, animals consume the nutrients necessary to meet the requirements of maintenance and production (Schingoethe, 2017).

Ensiling TMR is not a recent practice (Owen e Howard, 1965); meanwhile, the ruminant production industry has shown a renewed interest in TMR silage in a number of countries (e.g., Japan, China, South Korea, Vietnam, Thailand, Indonesia, Nepal, Israel, Iran, South Africa, and Argentina), including Brazil (see Schmidt et al., 2017 in the proceedings of the V Intl. Symp. Forage Qual. Conserv.).

Several benefits have been associated with TMR silages, such as a reduced requirement for labor and machinery (if TMR silage is purchased), uniform composition during storage under farm conditions, potential for incorporating unpalatable byproducts (if their flavors and odors are altered by fermentation), and high aerobic stability after feedout (Nishino et al., 2003; Weinberg et al., 2011; Schmidt et al., 2017). Additionally, TMR silages, similar to other silages, have the potential for commercialization, especially if stored in smaller structures (e.g., bales, bags, and pouches).

However, in our view, the main motivation for resuming the use of TMR silages has been the great availability of wet residues coproduced by the agroindustry. Byproducts from the industrialization of cereal grains (including breweries and distilleries), soybean (including the biodiesel industry), sugarcane, cotton, peanut, cassava, vegetables, legumes and fruits (including citrus growers and wineries) are among the valuable residues available in Brazil. Therefore, marketing TMR silages (containing byproducts) has been a promising business opportunity for agroindustry. In

small farms, TMR silage has also been proposed as a strategy to improve the conservation of wet forages (Gusmão et al., 2018).

Feeding a conventional TMR (prepared daily) allows the nutritional composition of the diet to be adjusted by using different feedstuffs and supplements [e.g., sources of rumen undegraded protein (RUP) and vitamins] in addition to conserved feeds (hays and whole-crop or grain silages). On the other hand, ensiling a TMR directs all nutrients to fermentation, which ultimately modifies the nutritive value of the diet by altering nutrient content and availability.

This review attempts to nutrient changes during the ensiling of complete rations and their feeding value for ruminants. In many cases in which studies investigating TMR silage were not available (to the best of our knowledge), we discussed data from other silage types (whole-crop or grain silages). Partial mixed ration (PMR) silage has also gained prominence in ruminant production systems (e.g., dairy cows receiving concentrates in feed stations or automatic milking systems) but is beyond the scope of this paper.

2. Nutrient changes during silage fermentation

2.1. Carbohydrates

2.1.1. Soluble carbohydrates and fermentation end-products

Water-soluble carbohydrates (primarily sucrose, glucose and fructose) are the main substrates for microbial growth during silage fermentation (Rooke and Hatfield, 2003). After ensiling, a reduction in soluble carbohydrates is expected at the expense of the formation of fermentation end-products.

In TMR ensiled in round bales, Weinberg et al. (2011) reported a continuous decrease in soluble carbohydrates during 144 d of storage. Wang et al. (2016) reported a marked decrease of soluble carbohydrates in a TMR silage after 7 or 56 d of storage compared with the fresh TMR (3.0 vs. 6.3% of DM, respectively). Ning et al. (2017) observed losses of soluble carbohydrates by 57% and 54% TMR silages containing alfalfa hay or *Leymus chinensis* hay stored for 56 d (11.1 to 4.93% and 11.7 to 5.58%, respectively). Compared with fresh TMR, Kondo et al. (2016) found lower concentrations of soluble carbohydrates in TMR silage (5.3 vs. 0.7% of DM, respectively), regardless of the length (30 d or 90 d) and temperature (15ºC or 30ºC) of storage. Hence, the final content of soluble carbohydrates in TMR silages will depend

on their initial contents and the course of fermentation, but most soluble carbohydrates are consumed during the first weeks of fermentation.

The fermentation end-products are essential for the preservation of TMR silages during storage and feedout. Moreover, these compounds may contribute to animal nutrition by supplying nutrients, altering feed intake and changing rumen and host metabolism. As in conventional silages, LAB dominate the fermentation, and lactic acid is the main end-product in TMR silages; whereas several other compounds may be formed during fermentation (e.g., volatile fatty acids, alcohols, esters, aldehydes and ketones). Of course, the final profile of fermentation products will depend on ration ingredients, DM content, storage length and temperature and application of additives (Wang and Nishino, 2013; Chen et al., 2014; Hao et al., 2015). Not rarely, TMR silages present high concentrations of lactic acid (e.g., >8% DM) (Weinberg et al., 2011; Chen et al., 2014; Chen et al., 2017; Chen et al., 2019; Restelatto et al., 2019). Although this finding is seldomly discussed, the inclusion of urea, mineral mixtures, limestone and buffers (e.g., sodium bicarbonate) certainly increases the buffering capacity and stimulates the formation of lactic acid in TMR silages, as previously reported for corn and sugarcane silages (Klosterman et al., 1961; Byers et al. 1964; Custódio et al., 2016). The intake of lactic acid has been associated with a greater proportion of propionic acid and higher pH (*in vivo*) (Jaakkola and Huhtanen, 1989; Daniel et al., 2013) and lower methane production (*in vitro*) (Wagner et al., 2018) in the rumen fluid, which might be beneficial to animal performance.

2.1.2. Starch

Once most bacteria responsible for silage fermentation have not been associated with amylolytic activity, a decrease in starch content is generally not expected due to ensiling (Rooke and Hatfield, 2003; Der Bedrosian, 2012; Ferrareto et al., 2015). However, Ning et al. (2017) observed that amylase activity from silage microorganisms decreased by 75% and 57% of the original activity in TMR silages containing alfalfa hay or *Leymus chinensis* hay, respectively, and that starch losses occurred until the end (56 d) of storage (by 20.5.% for alfalfa hay TMR and by 17.1% for *Leymus chinensis* hay TMR). Miyaji et al. 2017 reported a starch loss by 22.8% (on average) in TMR silages containing steam-flaked corn or brown rice and stored for 210 d. Nevertheless, in TMR silage, the starch concentration tends to be similar to the fresh TMR due to the consumption of other nutrients during fermentation.

In addition to starch loss, ensiling often increases starch digestibility, especially in cereals with a higher content of prolamins in their endosperm (e.g., flint corn and sorghum) (Benton et al., 2005; Hoffman et al., 2011). Prolamins are hydrophobic proteins that surround the starch granules, impairing starch digestion. Protease activity during ensiling reduces prolamin concentration and increases starch availability (Hoffman et al., 2011; Der Bedrosian et al., 2012; Junges et al., 2017). In TMR silages, Miyaji et al. (2017) reported an increase in ruminal starch degradation with a greater benefit in a TMR silage containing steam-flaked corn (6.83%) than brown rice (1.57%). A pronounced enhancement in starch digestibility is expected for dry ground or dry rolled corn (or sorghum) when ensiled as part of a TMR, especially for flint hybrids. Such an increase in starch digestibility has been associated with higher feed efficiency in animals fed TMR silages (Hibbs and Conrad, 1976; Lazzari et al., unpublished data from the State University of Maringá).

2.1.3. Cell wall polysaccharides

The plant cell wall is a complex matrix of polysaccharides, mainly consisting of cellulose, hemicellulose and pectin. Although soluble carbohydrates from the cell content are the main fuel for microbes during ensiling, in a minor proportion, the constituents of the cell wall may also be cleaved and used as substrate for fermentation (Rooke and Hatfield, 2003). Such partial breakage of cell wall polymers is likely favored in silages with higher moisture content. For instance, in alfalfa silage, Jones et al. (1992) reported a decrease in constituents of cell wall carbohydrates (e.g., uronics, rhamnose, arabinose and galactose) in low DM silage (29% DM), whereas in high DM silage (40% DM), no changes were observed.

Weinberg et al. (2011) reported NDF loss (recovery of 88%) and a lower content of NDF (38% vs. 34%) in a TMR ensiled for 140 d compared with the fresh TMR (both with 50% of DM). The same authors did not find loss of NDF or alterations in NDF concentration (38% of NDF on average) in a similar TMR ensiled with higher DM content (64.8% of DM).

Ning et al. (2017) examined the occurrence of carbohydrate losses and hemicellulase activity in TMR silages. They did not observe hemicellulase activity after 14 d of storage, but hemicellulose loss continuously increased in both silages up to 56 d of storage, probably due to acid hydrolysis (by 19.9% in alfalfa hay TMR and by 23.5% in *Leymus chinensis* hay TMR). Compared to the fresh TMR, Wang et al. (2016)

observed a reduction in NDF content of ensiled TMR formulated with corn silage during 56 d of storage (46% vs. 43% of NDF), whereas ADF concentration (27% on average) did not change during storage. Kondo et al. (2016), on the other hand, did not observe changes in NDF concentration after ensiling a TMR, regardless of the length (30 d or 90 d) and temperature (15°C or 30°C) of storage.

In alfalfa and orchardgrass silages, Yahaya et al. (2001) observed that ensiling slightly reduced the concentrations of hemicellulose and pectin, whereas changes in cellulose concentrations were inconsistent. Compared to fresh forage, hemicellulose and pectin decreased by 2.9% and 1.5% in alfalfa silage and by 4.1% and 1.2% in orchardgrass silage, respectively. In Napier grass silage, Desta et al. (2016) reported a slight decrease of fiber entities during 90 d of storage (NDF by 2.2%, ADF by 1.2%, hemicellulose by 3% and cellulose by 1.9%).

Although lignin is not a carbohydrate, it is closely related to polysaccharides in the cell wall. Classically, lignin has been assumed to be undegradable under anaerobic conditions. In fact, most silages have higher lignin concentrations than their fresh crops due to the disappearance of soluble nutrients. However, lignin is not completely recovered after ensiling. Recently, Machado et al. (unpublished data from the State University of Maringá) examined the effects of an enzymatic complex on the recovery of nutrients in whole-plant corn and sugarcane silages. For lignin (assayed as acetyl bromide lignin), these researchers reported recoveries of 84% and 81% for untreated corn and sugarcane silages, respectively. In both silages, lignin recovery linearly decreased with enzyme dose (from 84 to 54% and from 81 to 69%, respectively), while the contents of flavonoids and polyphenols steeply increased, resulting in silages with greater antioxidant capacity. Most likely, those molecules formed during silage fermentation would promote health in animals and, perhaps, consumers of animal products.

2.2. Proteins

During silage fermentation, N losses may occur; however, they are normally less expressive than other soluble fractions with minor effects on the total content of crude protein (CP) (Rooke and Hatfield, 2003). Silages with slightly higher CP contents than their crops are not rare due to the consumption of other nutrients, primarily soluble carbohydrates. On the other hand, protein breakdown undergoes extensively during fermentation. The partial transformation of true protein into nonprotein compounds due

to ensiling is an inevitable process performed by plant and microbial enzymes (McDonald et al., 1991).

The occurrence of proteolysis in high-CP forages (e.g., legumes, temperate grasses) is undesirable and leads to poorer N use efficiency (NUE) (Huhtanen et al., 2008; Hymes-Fecht et al., 2013). However, in corn and sorghum silages (whole plant or grain silages), proteolysis has been positively associated with starch digestibility due to the degradation of hydrophobic proteins (prolamins) that surround the starch granules (Hoffman et al., 2011). Such an increase in starch digestibility often results in greater synthesis of ruminal microbial protein and, in turn, higher NUE (Wilkerson et al., 1997; Valadares et al., 1999; San Emeterio et al., 2000). Nevertheless, in conventional (non-ensiled) TMR, protein sources (e.g., heat-treated soybean meal) are incorporated into the ration to balance any loss of true protein during the storage of ensiled ingredients and, in turn, meet the requirements of metabolizable protein.

In TMR silages, however, all ingredients are ensiled. Therefore, all protein sources are exposed to proteolysis. During ensiling, protein degradation occurs in two phases. Primarily, protein hydrolysis occurs due to plant and microbial proteases, resulting in peptides and free amino acids (AA) (Klembe, 1956; Heron et al., 1986; McDonald, 1991; Rooke and Hatfield, 2003). Afterwards, the decarboxylation of AA leads to the formation of biogenic amines and carbon dioxide, whereas deamination of AA results in NH_3 and organic acids (Oshima and McDonald, 1978; Scherer et al., 2015).

Heron et al. (1986) and Klembe (1956) observed that ryegrass and timothy silages sterilized before ensiling still present high amounts of free AA, indicating a large contribution of plant enzymes to proteolysis. In wilted alfalfa silage, Ding et al. (2013) reported that ensiling increased the proportion of nonprotein N (NPN) from 26% to 73% of total N and that plant enzymes contributed approximately 2/3 of the proteolysis, whereas microorganisms contributed 1/3. Interestingly, most NPN remained as peptides (~70% of NPN), which is more efficiently used by both ruminal microorganisms and animal than NH_3-N (Broderick et al., 2013). In rehydrated corn grain silage, however, Junges et al. (2017) reported that bacterial enzymes were the major responsible for proteolysis (60%) followed by plant proteases (30%). Fungi enzymes and fermentation products contributed approximately 5% of the proteolysis during fermentation. In TMR silages, which are mixtures of ingredients, we did not find any report that accounted for the contribution of microorganisms and plant proteases to proteolysis. Meanwhile,

enterobacteria, clostridia and bacilli are the main candidates involved in protein breakdown during silage storage (McDonald et al., 1991; Pahlow et al., 2003).

The extent of protein transformation during ensiling is determined by factors capable of altering enzyme and microbial activities, such as pH, moisture, temperature, and storage length. In general, a rapid pH decrease below 4 notably reduces protein breakdown (Virtanen, 1933; McKersie, 1985; Heron et al., 1986; Heron and Philips 1989). However, protein degradation may continue during extended storage (Hoffman et al., 2011).In TMR silages, ingredient sources may also affect proteolysis.

Hao et al. (2015) evaluated moisture contents (40%, 45% and 50% moisture) and storage times (from 0 to 56 d) and reported no differences in total N content in TMR silages. Nevertheless, higher moisture content increased the contents of NPN, free AA and NH_3-N during fermentation. No effect was observed on the content of peptides. Although proteolysis continuously increased during storage, major transformations in N fractions were observed within the first week of storage. According to the authors, on d 56 of storage, 43% of N was NPN. Kondo et al. (2016) observed that storage length and temperature influenced the concentrations of soluble protein (SP) and NH_3-N in TMR silage. The authors reported no differences of SP in ensiled TMR stored for 30 d compared with fresh TMR, whereas extended storage (90 d) increased SP concentration. In addition, silage stored at 30°C had a higher content of SP than silage stored at 15°C. The NH_3-N increased with temperature and length of storage.

Nishino et al. (2007) reported lower synthesis of biogenic amines (histamine, cadaverine, tyramine and putrescine) in TMR silage compared with whole plant corn and wilted festulolium silages, possibly because of the ingredients used to formulate TMR (dry grains and heat-treated byproducts) were less susceptible to proteolysis, reducing substrates (free AA) for biogenic amine synthesis.

Recently, we compared the N fractionation in TMR silages containing different sources of CP (urea, soybean grain and soybean meal) for finishing beef cattle (Lazzari, unpublished). As expected, ensiled TMR had higher proportions of the A1 fraction (NH_3-N) than fresh TMR, but the values remained within acceptable levels (<10%), except for the TMR silage formulated with urea. The proportion of the A2 fraction (non-ammonia SP, including soluble true protein) also increased in ensiled TMR due to the proteolytic process. On the other hand, the insoluble N fractions [insoluble true protein (B1), fiber-bound protein (B2) and indigestible protein (C)] decreased during ensiling (Figure 1).
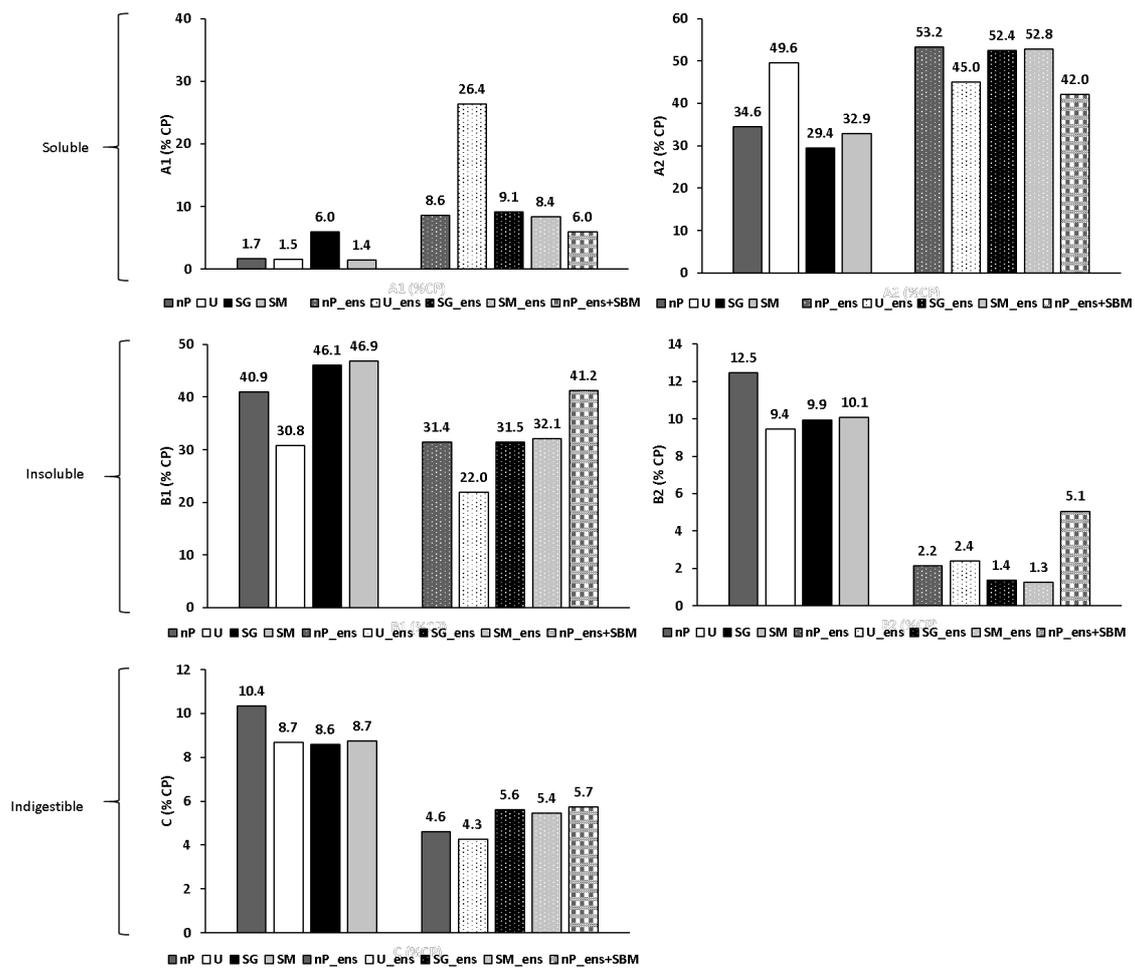
Figure 1. Nitrogen fractionation (CNCPS; Higgs et al., 2015) in fresh and ensiled TMR with difference sources of CP. nP: fresh TMR without a protein supplement; U: fresh TMR with urea; SG: fresh TMR with soybean grain; SM: fresh TMR with soybean meal; nP_ens: ensiled TMR without a protein supplement; U_ens: ensiled TMR with urea; SG: ensiled TMR with soybean grain; SM: ensiled TMR with soybean meal; nP_ens+SBM: nP_ens supplemented with soybean meal before feeding.

Source: Lazzari (unpublished).

Combining N fractionation outcomes enabled the content of rumen-degraded protein (RDP) of each ration to be estimated (Figure 2). Except for the TMR silage containing urea, all ensiled rations contained similar concentrations of RDP, including the ensiled ration supplemented with soybean meal before each feeding. The estimated values of RDP were relatively close to the estimated requirements of RDP (TDN × 0.13) with a slightly higher difference for the TMR formulated with soybean grain due to the greater TDN value of this fat-rich feedstuff (although fat is not fuel for ruminal

fermentation). Higher values of TDN were assumed for ensiled TMR (than fresh TMR) due to the improved starch digestibility in ensiled corn grain. Supplying (nonfermented) soybean meal at feeding or ensiling it with other ingredients altered only 0.2%-units the content of RDP or RUP.
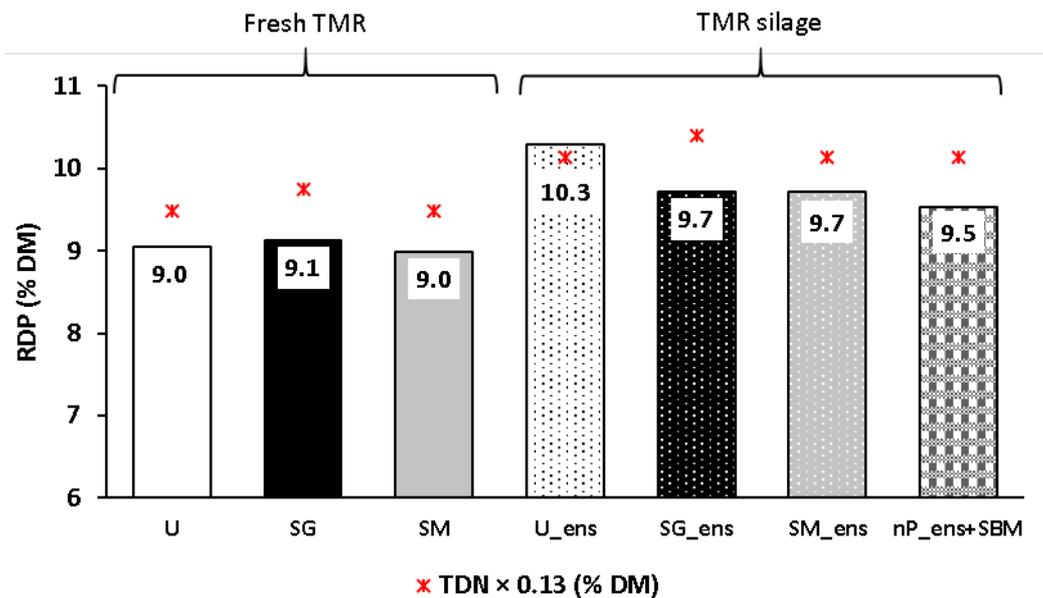
Figure 2. Estimates of rumen-degraded protein (RDP) from N fractionation, for finishing beef cattle. Ruminal degradability was calculated using the first-order approach [kd / (kd + kp)] (CNCPS; Van Amburgh et al., 2015). Fractional passage rates (liquid, concentrate and forage) were estimated using actual values of DM intake, dietary forage level and shrunk BW (Tylutki et al., 2008; NRC, 2016). Requirements of RDP are indicated as TDN × 0.13 (NRC, 2016).
Source: Lazzari (unpublished).

In brief, proteolysis extension seems to be lower in TMR silages (except for urea-containing TMR) than we usually see in high-CP forages (e.g., alfalfa and temperate grasses), mainly due to the higher DM content. Additionally, the use of byproducts that have undergone washing and/or heating during food processing (at agroindustry) might also decrease protein fractions that are more susceptible to proteolysis, both during ensiling and rumen fermentation. Of course, the content of RUP in TMR silage is lower than in fresh TMR, but it does not mean that RDP/RUP cannot be balanced in the diet. From our perspective, more research is merited to define and predict patterns of proteolysis for a given ingredient or a combination of ingredients

and, ultimately, allow the supply of adequate amounts of metabolizable protein to meet animal requirements.

2.3. Lipids

A vast group of molecules with different biological functions sharing a common characteristic, 'insolubility in water', are called lipids. Fatty acids (FA), for instance, are synthesized as a form to store energy, whereas galactolipids and phospholipids are the main components of biological membranes in forages (Nelson and Cox, 2015). Overall, linolenic acid (C18:3 n-3) is the main FA in forages, whereas cereals and oilseeds are rich in linoleic acid (C18:2n-6). Palmitic acid (C16:0), stearic (C18:0) and oleic (C18:1) acids are also representative FA in forages and concentrates (Alves et al., 2011; Liu et al., 2018; Liu et al., 2019). In complete rations, grains, oilseeds and byproducts contribute most to lipid content.

Overall, FA are not usual fuels for fermentation (Mackie et al., 1991). In well-sealed silos, the lack of oxygen generates an excess of reducing equivalents (e.g., NADH), which constrains the use of reduced molecules to produce energy, such as FA (Nelson and Cox, 2015). In this way, well-conserved silages will present similar content of total fat as in fresh material (Alves et al., 2011; Liu et al., 2019). However, changes in proportions of individual FA have been observed in silages (Liu et al., 2018; Liu et al., 2019).

A higher proportion of free (non-esterified) FA has been reported for silages in comparison with the corresponding fresh crop. Elgersma et al. (2003) reported that only 2% of the FA were in their free form in fresh ryegrass, whereas after ensiling, this level increased to 50% in ryegrass silage. Lipases and lipoxygenases are associated with lipolytic activity during ensiling. Lipases (carboxylic ester hydrolase) are present in fresh forages and grains (Barros et al., 2011, Liu et al., 2018; Liu et al., 2019), and they can cleave ester bonds of triglycerides, thereby releasing glycerol and free FA (Gadge et al., 2011). Under favorable conditions, the free FA may undergo lipoxygenase action. Lipoxygenases can oxidize free FA, mainly linoleic and linolenic acids, initially producing hydroperoxides that are later cleaved in aldehydes and ketones (Feussner and Wasternack, 2002; Senger et al., 2005). Lipase and lipoxygenase activities are elevated soon after harvesting or in poorly fermented silages. However, lipase and lipoxygenase activities are altered by pH or temperature variation. Optimum conditions for lipase activity are near 24ºC and pH 8 (Gadge et al., 2011). For lipoxygenase, optimal activity

occurs in neutral to basic pH conditions (6.5 to 8.0) (Malekian et al., 1999). This finding means that rapid acidification might preclude the cleavage of FA and the formation of some aldehydes in silages.

Although plant enzymes seem to be primarily responsible for lipolysis, some strains of lactic acid bacteria (LAB) found in silage possess biohydrogenation activity, reducing the content of unsaturated FA (Liu et al., 2019). Ding et al. (2013) observed a decrease in unsaturated FA (C18:2n-6 and C18:3n-6) in control (43%) or sterile silages (28%), whereas autoclave-treated silages (to inhibit plant enzymes and microbial activity) had a similar profile of FA compared to fresh forage; however, control silage presented a higher level of FA C16:0 compared to fresh forage (alfalfa). Liu et al. 2019 also reported more FA C16:0 in oat silage compared to fresh herbage. Han and Zhou (2013) observed alterations in the main FA present in corn silage (C16:0, C18:0, C18:1, C18:2 and C18:3) during the first 2 d after ensiling. According to the authors, saturated FA increased in favor of a decrease of unsaturated FA. These results were attributed to lipoxygenase activity, which benefited from the high pH observed during the first hours after ensiling.

Liu et al. (2018) evaluated changes in the FA composition of alfalfa silage in relation to temperature (15ºC, 30ºC and 45ºC) and length of storage (0 to 65 d). The decrease observed in the total FA content, as well as in C18:1 and C18:3n3, was larger on the first day of fermentation than on the remaining 65 d of storage. In addition, losses of FA were higher at 45ºC than at lower temperatures. The authors attributed those results to the thermolability of FA, as well as an increase in LAB development at higher temperatures. However, on d 65 of storage silages kept at 15°C had the lowest amount of total FA. The authors attributed this result to an enhanced activity of aerobic bacteria, yeasts and plant lipoxygenase.

In brief, in well-fermented silages, the content of total FA is not expected to change expressively, once FA are not usual fuels for fermentation. However, an increase in free FA and changes in the concentration of specific FA, such as a decrease in unsaturated FA (e.g., linoleic and linolenic) and an increase in the proportion of saturated FA, are expected.

2.4. Minerals

Minerals are highly important to the maintenance of vital processes, as well as animal productivity, despite the lower requirements compared to other nutrients. In

general, well-preserved silages have slightly higher ash contents than their fresh crops (Meschy et al., 2005; Baumont et al., 2011). However, the contents of specific minerals may change during ensiling.

Schlegel et al. (2018) observed higher concentrations of Mn (65%), Na (33%), Se (27%), Zn (13%) and Mg (12%) in grass and legume silages compared with their respective herbages. No changes were observed in the Ca, P, K, Cl, S and Cu contents. Overall, macro- and micromineral concentrations increased by an average of 6% and 31%, respectively, explained by the loss of fermentable nutrients.

Microbial development and low pH conditions have been related to higher availability of minerals in silage. Lee et al. (2019) reported that some LAB strains possess the ability to increase the bioavailability of inorganic selenium during silage fermentation by converting sodium selenite into organic selenium. Hansen and Spears (2009) observed that ensiling increased Fe availability in whole-plant corn silage, probably because of the acidic conditions in silage. Ibrahim et al. (1990) evaluated the ruminal solubility of minerals in feedstuffs used in ruminant nutrition. They reported higher ruminal availability of minerals (Ca, Mg, P, Na, K, Cu, Zn) in corn silage, despite the lower content of ash in corn silage compared to other tested feeds (4.9% DM vs. 10.7% DM on average). Overall, the ruminal availability of minerals in corn silage was above 80% (from 70% to 100%), which was also attributed to the effect of low silage pH (3.7 in the mentioned study). Rooke et al. (1983) also reported high availability of minerals (K, Ca, Na, P, Mg and Cu) in grass silage (average above 93%).

Although there is lack of information on the fate of minerals in ensiled TMR, one would expect minor changes in their concentrations but an increase in their availability, as previously reported for other silages.

2.5. Vitamins

Vitamins are complex molecules essential for different metabolic pathways, the immune system and gene expression (NRC, 2001). Vitamins are classified as fat-soluble (A, D, E and K) and water-soluble (B and C) (McDowell, 2000). Adult ruminants are less dependent on exogenous sources of vitamins than nonruminants. Vitamins from B and K complexes are synthesized within the rumen by microorganisms; vitamin D is produced from steroids present in skin after sunlight exposure; glucose and galactose are converted in vitamin C when required (NRC, 2001).

In this way, fat-soluble vitamins, mainly A and E, are more required from exogenous sources. The carotenes (provitamin A) and tocopherols (vitamin E) present in fresh forages are the main sources of those vitamins, although grains may also contribute (Kalac, 2012; Nozière et al., 2006; Lindquisvist et al., 2011; Liu et al., 2016).

Forage conservation (haying and ensiling) has been generically linked to losses of carotenoids and tocopherols, but losses related to ensiling are much smaller than for wilted forages and hay (Carter, 1960). Carotenes and tocopherols are prone to oxidation after harvesting. Exposition to ultraviolet radiation (even in the dark), oxygen, high temperature and lipoxygenase activity are the primary cause of carotene destruction (Kalac and McDonald, 1981; Cardinault et al., 2004; Nozière et al., 2006; Kalac, 2013). Tocopherols are natural antioxidants protecting other molecules from oxidation; however, due to this protective activity, tocopherol may also be degraded (McDowell, 2000).

Kalac and Kyzlink (1979) reported that carotenoids are rapidly degraded when exposed to oxygen under low pH conditions (pH 3.7 to 4.2). In the same study, carotenoid degradation was greater within the temperature range of 30 to 40ºC. On the other hand, a rapid lactic acid synthesis in the absence of oxygen had a positive correlation with β-carotene and α-tocopherol concentration, which means that a rapid pH drop under anoxic conditions is beneficial to the conservation of (pro)vitamins as found in well-preserved silages (Muller et al., 2006; Lindqvist, Nadeau and Jensen, 2011). In well-fermented crops, Nozière et al. (2006) and Liu et al. (2019) reported losses of carotenes during ensiling by 20% (for silages in general) and 25.5% (for oat silage). Carotene loss during ensiling was attributed to lipoxygenase activity (Liu et al., 2019). Tocopherol, on the other hand, was less degraded (11.6%) than carotene (Liu et al., 2019). This phenomenon has been attributed to a higher stability of tocopherol in anoxic conditions (Liu et al., 2019), as well as a possible activity of tocopherol-producing bacteria (Tani and Tsumura, 1989).

Silo structure, length of storage, additives and temperature during storage are also important factors related to vitamin conservation. Nadeau et al. (2004) observed a reduction of vitamins in grass-legume silage stored in round bales. α-Tocopherol and β-carotene decreased by 49% (from 35 to 18 mg/kg DM) and 37% (from 19 to 12 mg/kg DM), respectively, during 3 months of storage. However, vitamin contents in silages stored in bunker and tower silos were similar to vitamin contents in the fresh herbage.

These results were attributed to a greater risk of oxygen infiltration and oxidation of vitamins in round-bale silos.

Jares (2018) evaluated the concentrations of tocopherols (α, γ and δ) in rehydrated corn grain silages (32% moisture) during storage (0, 21, 38, 63 100 and 185 d) and observed a decrease over time (e.g., α-tocopherol decreased from 9.7 μg/g at ensiling to 6.91 μg/g at 185 d of storage). Most of the reduction occurred during the first 21 d of fermentation. A reduction of tocopherols was also observed in dry grains, but ensiled grains had lower concentrations of tocopherols than dry grains.

Liu et al. (2016) compared the effects of additives (fibrolytic enzyme, tert-butylhydroquinone and tea polyphenols) and temperature (15, 30 and 45ºC) on vitamin concentrations in Napier grass silage. They found that silage stored at 30°C had more tocopherol than fresh forage or silage kept at 15°C. Ensiling reduced the content of carotenes independent of temperature, but carotene was higher in silage kept at 30ºC than at 15 or 45ºC. According to the authors, silage stored at 30ºC presented more tocopherol because of the probable activity of microorganisms capable of producing this molecule. Additives had no beneficial effect, except for tert-butylhydroquinone, which is an antioxidant. Lindqvist et al. (2011) reported that bacterial inoculation (*Lactobacillus plantarum* and *Pediococcus acidilactici*) improved the tocopherol concentration in red clover silage (50.1 vs 34.2 mg/kg DM). In contrast, Liu et al. (2019) observed a reduction in carotene due to *Lactobacillus plantarum* inoculation in oat silage. The use of propionic acid (4 kg/t as fed), however, was effective in controlling carotene loss because carotene concentration in treated silage did not differ from fresh forage.

In brief, losses of carotene and tocopherols are expected during ensiling; however, tocopherol seems more stable than carotenes in silages. The TMR silages often contain exogenous sources of vitamins which, in terms of stability, may differ from natural sources of vitamins (or provitamins) present in feeds. To the best of our knowledge, the fate of vitamins in TMR silage is unknown.

2.6. Feed additives

Knowledge on the course of feed additives during ensiling is rare (e.g., ionophores, essential oils, tannins, probiotics, prebiotics, sodium bicarbonate, β-adrenergic agonists, etc.). Some studies have examined the effects of feed additives on silage conservation, but the fate of those additives is unknown.

Hoon and Meeske (2011) investigated the effect of lasalocid (an ionophore antibiotic) on the conservation of corn silage. It is worth noting that LAB are gram-positive and ionophore-sensitive. Compared with untreated silage, lasalocid sodium (0.15 g/kg as fed) decreased lactic acid concentration and increased fermentative losses, resulting in silage with poorer *in vitro* organic matter digestibility. The treated silage also had a more intense aerobic deterioration, as indicated by the higher production of CO_2 during air exposure. No information was provided on the concentration/activity of lasalocid at silage feedout.

Kung et al. (2008) examined the effect of a commercial blend of essential oils (40 or 80 mg/kg as fed) on the conservation of corn silage. The blend of essential oils did not affect the populations of yeasts, molds, lactic acid bacteria, or enterobacteria, the fermentation end-products or the aerobic stability of the corn silage. Foskolos et al. (2016) investigated the effects of essential oils on protein degradation during ensiling. Five essential oil compounds (thymol, eugenol, cinnamaldehyde, capsaicin and carvacrol) in four doses (from 0 to 2 g/kg as fed) were sprayed on ryegrass forage before ensiling. Thymol, eugenol, and cinnamaldehyde at 2 g and carvacrol at 0.5 and 2 g/kg inhibited deamination. Cinnamaldehyde at 2 g/kg resulted in silages with approximately 10% more true protein than other silages. However, the highest dose of the essential oils (2 g/kg) negatively affected the ensiling process by decreasing LAB counts and lactic acid concentration and increasing silage pH.

Recently, Pereira (2018) studied the effects of essential oils (thymol and carvacrol) as additives in corn and sugarcane silages. In sugarcane silages, the addition of thymol, carvacrol or a combination of thymol and carvacrol led to higher concentrations of soluble carbohydrates. The combination of thymol and carvacrol decreased the lactic acid concentration. Carvacrol alone decreased the DM loss during fermentation, whereas thymol alone decreased the counts of LAB and ethanol concentration, whereas it increased the aerobic stability of sugarcane silage. In corn silage, LAB counts were reduced by thymol alone or combined with carvacrol. Carvacrol alone or in combination with thymol decreased the lactic acid concentration. All treated silages had less ethanol, lower DM loss during fermentation, and slightly higher aerobic stability upon air exposure. As in the aforementioned studies, no information was provided on the concentration/activity of essential oils at silage feedout.

Even as discussed for nutrients in previous topics, feed additives may be altered during fermentation. In the meantime, the capacity of the additives to act in the rumen and/or host metabolism after undergoing fermentation warrants further investigation.

3. Nutritive value of TMR silages for ruminants
3.1 Ensiled TMR for dairy cows

During the 1960s, TMR increased in dairy operations in the US, as milk production per cow increased, herds became larger, freestall and large-group handling of cows became more common, and milking parlors became more prevalent (McCoy et al. 1966; Schingoethe, 2017). At nearly the same time, the first studies on TMR silages appeared in the literature.

Owen and Howard (1965) evaluated the effects of ensiled TMR with different moisture levels (68%, 53% and 47%), obtained by varying the period of alfalfa wilting, on the performance of dairy cows. The rations were composed of 50% alfalfa and 50% cracked corn (DM basis) and contained approximately 17% CP. Cows fed low- and medium-moisture rations (47% and 53%) had higher DM intake than cows fed high-moisture rations. However, milk fat content was higher for the high-moisture ratio, which counterbalanced the differences in milk yield, resulting in similar yield of fat-corrected milk (FCM) among treatments. The authors concluded that the use of TMR silage is a good alternative to simplify feeding management.

Marshall and Voigt (1975) compared the nutritive value of an ensiled TMR formulated with whole plant corn and a fresh TMR formulated with corn silage and moistened concentrate mix. The rations contained 61% corn silage and 39% concentrate mix (citrus pulp, cottonseed meal, urea and minerals). The authors found no difference among treatments for cow performance (20.2 kg of FCM, 4.8% milk fat, DM intake of 3.2% of BW).

Since the pioneering studies, ensiling TMR formulated with cereal grain was associated with higher feed efficiency. Hibbs and Conrad (1976) compared fresh with ensiled TMR for dairy cows. In both diets, a rate of 0.45 kg of concentrate was mixed with 3.18 kg of corn silage (as fed basis). The concentrate mix contained 29.1% CP and was composed of 57.32% corn, 30.00% soybean meal, 5.00% dehydrated alfalfa meal, 3.52% urea, 3.20% bone meal and 0.96% salt. Both diets contained 14% CP. Total-tract DM digestibility increased (76.7% vs. 79.1%) and DM intake decreased (18.1 vs. 16.3 kg/d) for cows fed TMR silage, whereas there was no difference in milk yield (18.8 vs.

19.0 kg/d). There was no difference in milk fat content and FCM among treatments (18.3 vs. 18.7 kg/d). Therefore, feed efficiency was higher for cows fed TMR silage (1.01 vs. 1.15 FCM/DM intake for fresh and ensiled TMR, respectively).

In a second trial, Hibbs and Conrad (1976) used the same proportion of corn silage and concentrate as in the previous trial to compare fresh and ensiled TMR, but they altered the concentrate composition (40.80% of corn, 50.00% of soybean meal, 5.00% of dehydrated alfalfa, 3.20% of bone meal and 1.00% of salt). Diet CP was similar to the first trial (14.00%). As in the first trial, DM intake was lower (17.1 vs. 15.6 kg/d), and feed efficiency was greater (FCM/DM intake 1.17 vs. 1.32) for cows fed ensiled TMR without differences in FCM (19.9 vs. 20.4 kg/d, for fresh and ensiled TMR, respectively).

Pardue et al. (1975) evaluated the effect of ensiling on the nutritive value of a TMR for dairy cows. Two groups of ten Holstein cows (approximately 84 d postpartum) were assigned to a switch-back design with three 28-d periods to compare the ensiled TMR with the same fresh ingredients (silage and concentrate) but fed separately at each milking (Ctrl). Although the performance was similar, the ruminal concentration of volatile fatty acids was greater in cows fed the ensiled TMR (85.4 vs. 95.2 mM, for Ctrl and TMR silage, respectively), suggesting a higher ruminal degradability of the TMR silage.

After a temporal gap, the ruminant production industry has shown renewed interest in TMR silage, probably due to the availability of moisture byproducts. In 2009, Wongnen et al. evaluated the nutritive value of fresh or ensiled TMR, containing whole or cracked cottonseed, for dairy cows. Four multiparous Holstein Friesian crossbred cows (48 ± 12 DIM and 450 ± 13 kg BW) were assigned to a 4×4 Latin square design (21-d periods) with 4 dietary treatments in a 2×2 factorial arrangement (fresh or ensiled TMR $\times$ whole or cracked cottonseed). Diets contained (% DM): chopped rice straw (20.0%), cassava chip (40.0%), soybean meal (7.0%), cottonseed (10.0%), dried brewers grain (5.0%), tomato pomace (5.0%), molasses (8.0%), urea (1.5%), salt (0.5%), oyster shell (0.3%), di-calcium phosphate (0.2%), mineral-vitamin mix (0.3%), sulfur (0.2%), tallow (1.0%) and sodium bicarbonate (1.0%). Diet composition was approximately 63% DM, 16% CP and 69% TDN. Ruminal fermentation parameters (pH, NH_3 and VFA) and performance were similar among diets (15.5 kg/d DM intake, 18.9 kg/d FCM, 4.30% milk fat, 3.34% milk protein). However, TMR silage led to a lower proportion of oleic acid (C18:1) in milk fat (27.0 vs. 24.4% of total FA, for fresh

vs. ensiled TMR). This response was likely due to the partial biohydrogenation of unsaturated FA during silage fermentation.

Recently, Myyaji and Nonaka (2018) compared the digestion and performance of dairy cows fed fresh or ensiled TMR. The TMR contained rye silage, beet pulp, soybean meal, mineral-vitamin mix, and dry-rolled or steam-flaked hulled rice. Ensiling TMR improved starch (91.6 vs. 97.2%) and DM digestibility (72.3 vs. 77.6%), tended to increase DM intake (22.4 vs. 23.0 kg/d) and resulted in greater milk yield (36.4 vs. 37.6 kg/d, for fresh and ensiled TMR, respectively). The ruminal concentration of VFA (97.1 vs. 101.8 mM) was slightly higher in cows fed ensiled TMR. Despite the higher ruminal fermentability, TMR silages led to slightly greater concentrations of ruminal NH_3 (7.27 vs. 8.81 mg/dL) and higher urinary N excretion (124 vs. 152 g/d). In a companying paper, the authors reported higher ruminal degradability of CP for those TMR silages (Miyaji et al., 2017). Meanwhile, compared with the fresh TMR, daily secretion of milk protein was higher in cows fed TMR silage (1.24 vs. 1.31 kg/d, respectively) (Myyaji and Nonaka, 2018). A potential of TMR silages for high-producing cows was demonstrated in this study.

3.2. Ensiled TMR for growing ruminants

The TMR silages have also been used for feeding growing sheep and cattle. Cao et al. (2010) reported the effects of ensiling a TMR on N balance, ruminal fermentation and methane production in sheep. Compared with fresh TMR, ensiled TMR had higher contents of digestible energy (13.8 vs. 14.6 MJ/kg DM) and digestible CP (94 vs. 105 g/kg DM), which contributed to lower fecal N excretion (8.21 vs. 7.08 g/d). On the other hand, ensiling the TMR increased the urinary N excretion (9.76 vs. 11.81 g/d). Meanwhile, N retention was similar between treatments (5.2 g/d). The ruminal concentration of total VFA (2 and 4 h after feeding) was higher in sheep fed TMR silage (88.4 and 87.4 mM vs. 127 and 116 mM, for fresh and ensiled TMR, respectively). Compared to fresh TMR, the ensiled TMR reduced methane emissions by 10 L/d by 9.84 L per kg of DM intake, by 17.3 L per kg of digestible DM intake, by 0.54 L per kg of metabolic BW and by 20.9 J per kJ gross energy intake. The authors argued that lower methane production was due to the conversion of lactic acid (formed during silage fermentation) to propionic acid, an electron-consuming reaction in the rumen.

Meenongyai et al. (2017) examined the effect of ensiling on the nutritive value of a grass-based TMR for beef cattle. Thirty crossbred Zebu-Holstein steers were

blocked and received one of three experimental diets for 188 d. The treatments were 1) fresh TMR with fresh Napier grass, 2) fresh TMR with Napier silage, and 3) TMR silage (containing the Napier grass). For treatments 1 and 2, forage and concentrate were mixed before feeding the animals. Experimental diets contained (DM basis): Napier grass (41.15%), cassava pulp (6.00%), cassava chip (5.00%), corn (9.97%), rice bran (14.00), palm kernel meal (6.91%), soybean meal (5.00%), sugar (9.16%), salt (0.50%), urea (2.00%) and minerals (0.30%). On average, diets had 47% DM and 15% CP. The pH values of rations 1, 2 and 3 were 4.7, 4.0 and 3.5, respectively. Total-tract digestibility was lower for the fresh TMR containing grass silage (60.54%) compared with fresh TMR containing fresh Napier grass (71.13%) or TMR silage (65.51%). The DM intake, ADG and feed efficiency were similar across treatments.

Recently, our research group carried a trial to compare the nutritive value of TMR silages containing different protein sources for finishing beef cattle (Lazzari et al., unpublished). Thirty-two Nellore heifers were blocked by initial BW and received one of four dietary treatments. The TMR silages contained (DM basis): sugarcane bagasse (13%), rolled corn (59.4 to 68.4%), corn gluten feed (15%), limestone (0.6%), mineral mix (2%) and one of the following protein sources: urea (1%), soybean meal (ensiled with all ingredients) (7.1%), soybean meal (omitted at ensiling but supplied at TMR feeding = nonfermented) (7.1%) or rolled soybean grain (10%). All diets contained 13% CP and 10.5% roughage NDF (DM basis). The TMR silage formulated with soybean grain contained approximately 4.7% ether extract (EE), whereas the remaining diets contained approximately 3.0% EE. There was no difference in animal performance [DM intake, average daily gain (ADG), carcass gain, dressing and feed efficiency] among diets formulated with urea or soybean meal (ensiled or nonfermented). However, compared with other treatments, TMR silage containing soybean grain improved the ADG (1.22 vs. 1.49 kg/d) and carcass gain (0.890 vs. 1.010 kg/d), probably due to the trend of higher DM intake (8.02 vs. 9.17 kg/d), higher energy supply by EE and, perhaps, lower ruminal methane emission due to the unsaturated FA from the soybean grain. Hence, balancing protein in TMR silage with urea or soybean meal (ensiled or not) does not affect the performance of finishing cattle. On the other hand, the inclusion of soybean grain (and perhaps other oilseeds) is a feasible strategy to improve the nutritive value of TMR silages for beef cattle.

4. Final remarks

Marketing TMR silages represents a promising business opportunity for agroindustry, whereas feeding TMR silages is an alternative to simplify nutritional management.

Among nutritional changes during TMR storage, a decrease in true-protein content and an increase in starch digestibility (which often leads to higher feed efficiency) have been consistently reported. Further research is warranted to fine-tune the supply of metabolizable protein, vitamins and additives via TMR silages.

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