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An Overview on the Factors Affecting Water-soluble Carbohydrates Concentration during Ensiling of Silage

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ABSTRACT

Water-soluble carbohydrates (WSC) concentration during the ensiling process is influenced by both controllable and uncontrollable factors such as temperature, moisture level, fertilizers, additives nutrients, and time of ensiling of silage. The WSC contents may vary among the different fodder and forage species. The ensiling temperature has a limiting impact on water-soluble carbohydrates and their concentration decrease with increasing temperature. Crops should be harvested at optimum moisture and dry matter level to reach the required concentration of water-soluble carbohydrates to produce organic acids. Water-soluble carbohydrate concentration decreased with the crop's maturity due to the accumulation of carbohydrates in the grains. The evening cut has more concentration of WSC than that of the morning cut due to the photosynthesis process. The contents of WSC can be increased by using different kinds of additives during the ensiling process. Ensiling time has not much influence on the water-soluble carbohydrates. To understand these factors, we have a detailed review of the factors affecting the WSC of silage.

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INTRODUCTION

With the increase in population, the utilization of natural resources also increases, which causes depletion of natural resources. Human beings are destroying their natural grasslands for their social benefit; as a result, the natural food chain is disturbed. The livestock industry is currently facing problems of food scarcity. It is a big challenge for scientists to meet the required nutrition of the animals. Researchers are trying to preserve the silage for a long period with good quality to meet with food demand of the livestock industry. The preservation of silage for a long period and as well as for good quality silage depends upon both controllable and uncontrollable factors. Carbohydrate in the forage crops is one the most important nutrient because it is a fermentable substrate for the ensiling process of the silage.

A carbohydrate is a bio-molecule composed of carbon (C), hydrogen (H), and oxygen (O) atoms, frequently with an H-O atom ratio of 2:1 and with empirical formula CH_2O. These are formed during photosynthesis in plants' leaves, and certain bacteria obtained energy from sunlight to assimilate CO_2. These organisms can form carbohydrates, proteins, and lipids in the presence of solar energy if CO_2, H_2O, and inorganic sources of nitrogen (N) are accessible easily. It shows that life on earth relies on this procedure of CO_2 assimilation, in which carbohydrates are the first intermediates. In the respiration process, the utilization of carbohydrates occurs by oxidation. Starch and sucrose are stored in plants in the form of

glucose.
Carbohydrates are distributed into four main groups; monosaccharides, disaccharides, polysaccharides, and oligosaccharides, and these comprise the non-structural carbohydrates (Quentin *et al.*, 2015; Stick and Williams, 2010). Monosaccharides are simple sugars, e.g., glucose, fructose, ribose. These are soluble in water. Disaccharides are the pair of monosaccharides and are also soluble in water, e.g., sucrose, maltose. At the same time, the polysaccharides are the large chains of the monosaccharides and insoluble in water e.g., starch, cellulose, glycogen. According to this classification of carbohydrates, we can classify the carbohydrates into two groups; water-soluble carbohydrates (WSC) and non-soluble carbohydrates (NSC). WSC plays an important role in producing organic acid like lactic acid, which is most needed for fermentation during the ensiling of silage. Silage making is an old agricultural practice that started more than 3,000 years ago. However, a rapid increase in the application of this technology occurred after the 1940s due to the mechanization of forage harvesting (Wilkinson *et al.*, 2003).
The prime motive for the writing of this article is to discuss the factors affecting the WSC's degradation during the ensiling process for the production of good quality and well-preserved silage.

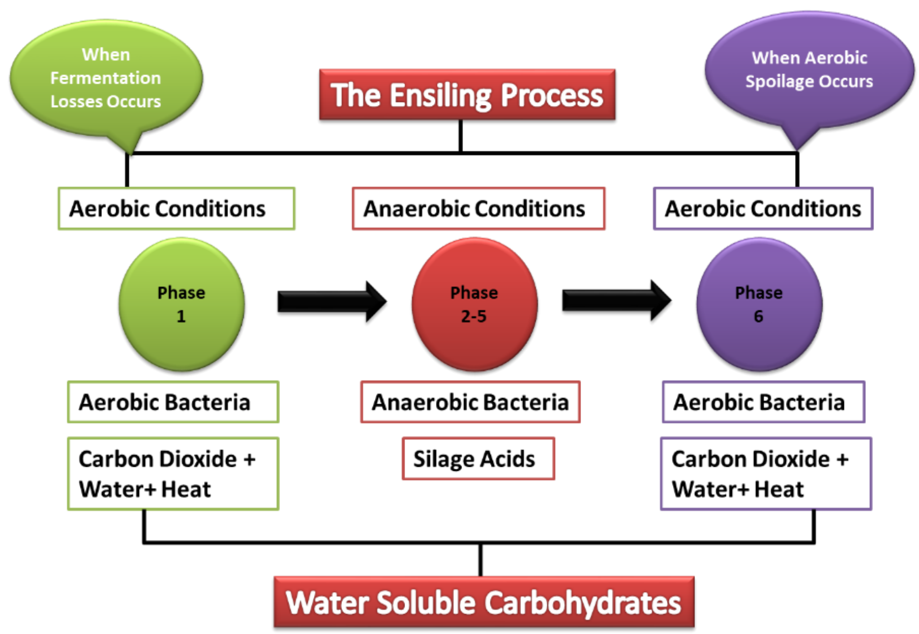

Figure 1. The Ensiling Process of Water Soluble Carbohydrates (WSC).

Impact of carbohydrates on plants and silage quality
Carbohydrates in forages are the fermentable source of energy in the rumen, and their improved congregations make better the nitrogen use efficiency (NUE) of dairy cows (Dickhoefer *et al.,* 2018). During the ensiling process of silages, carbohydrates are the key source of fermentable substrates (Wang *et al.,* 2019). Most forages are preserved during the ensiling process for use as animal feed. Under an anaerobic environment, WSC converted into organic acids and lactic acids with the help of lactic acid bacteria (LAB). Lactic acid is necessary for the fermentation of acids, and for competent preservation, it should consist of above 60 % of the total silage organic acids produced. As a result, pH is reduced, and the moist forages are preserved from spoilage microorganisms (Filya *et al.,* 2000). Carbohydrates are tremendously significant from a nutritional viewpoint and are the key fermentation substrate (Kung Jr *et al.,* 2018).
The nutritional level of the forage crop depends upon the following important three contents; carbohydrates, proteins, and lipids. These nutrients provide energy to animals and determine the crop's digestibility (Van Soest, 2018). Insoluble structural saccharides cellulose and hemicellulose or the storage forms such as starch and water-soluble polymers (fructans, glucose, etc.) are the

primary types of carbohydrates. WSC and protein are the supreme nutrients for livestock in the forage crop vegetative biomass, and they should maintain the quality of biomass during the postharvest. In forage crops, carbohydrates range from 50–75 % as dry matter (DM); if this amount is too low, it shows that the nutritional quality of the silage will be affected, and they can't preserve silage for a long period. Different types of enhancements of cereals are added to overcome the lower concentration of carbohydrates.

The concentration of carbohydrates in fodder and forage crop species may be affected by different kinds of factors such as climate, season, etc. Pelletier *et al.* 2010 reported that different forages have variable ranges of carbohydrate concentrations. The aggregated WSC concentration with starch for timothy (*Phleum pratense* L.), tall fescue, smooth bromegrass, reed canarygrass, alfalfa, orchard (*Dactylis glomerata* L.), and red clover fluctuate from 35 to 257 g kg^{-1} DM. Similar, the averaged concentrations of soluble carbohydrates without for white clover (*Trifolium repens* L.), meadow bromegrass (*B. biebersteinii* Roemer), and Kentucky bluegrass (*Poa pratensis* L.) (Marshall *et al.,* 2004; Baron *et al.,* 2005) range from 59 to 101 g kg^{-1} DM. Warm-season forage and fodder species have a higher concentration of WSC than that of the cool season. That's why corn and sorghum are most proffered to make good quality silage and for long-term preservation due to having a sufficient amount of WSC contents. Similarly, alfalfa has a lower concentration of the WSC, so different types of additives are used during ensiling to make the good quality silage of alfalfa (Pelletier *et al.,* 2010; Allen, 2013).

Intermediary metabolism, energy transfer and storage, and formation of plant structure are the key functions performed by carbohydrates in a plant. (Keller and Pharr, 2017). With the help of Calvin, light energy is fixed in carbohydrates, and these carbohydrates act as preliminary substrates for all intermediate pathways of plant functions (Adams *et al.,* 2018). In plants, energy is translocated in disaccharide sucrose (Quick and Schaffer, 2017) and stored in plant polymers in the form of starch and fructans (Pollock *et al.,* 2017). Carbohydrates perform important functions in the structural integrity of cells, tissues, and organs; and establish most plant cell walls (O'Neill and York, 2018). Carbohydrates are the storage products of energy. These are stored in the plants and animals in the form of starch and glycogen, respectively. These products are the polymers of glucose; when the surplus of glucose is available, they are deposited in cells in the form of granules. These polymers are broken down by enzymes when the body is exerting itself, and these become the fuel for the metabolic reaction. Plants store the starch in roots, tubers, and leafy parts during the photosynthetic activity, although some plants also store sucrose such as sugarcane and sugar beets. Human and animal diets mainly consist of sucrose and starch, and these must be broken down into their components before absorbing into the bloodstream.

Factors affecting the carbohydrates

Many controllable and uncontrollable factors like temperature, moisture content, dry matter (DM), time of harvesting, AM/PM cut, fertilizers, additives, ensiling or storage time etc. can affect the silage quality and influence the WSC concentration in fodder and forage species during the postharvest. It is very important to study these factors because the WSC is the primary substrate for the ensiling of silages.

Temperature

Climatic conditions like temperature may influence all phases of silage production and usage from the field to the feed bunk. (Ren *et al.,* 2019) reported the impact of ensiling temperature on the silage quality and found that for 90 days of silage ensiling, WSC contents were lower at 34°C as compared with -3 and 18 °C that represent inflated microbial propagation at 34 °C leading to rapid WSC utilization. This effect of temperature on WSC is represented in Figure 2. Furthermore, WSC contents are significantly affected by temperature, ensiling time and their interactions.

Lactic acid fermentation is also affected by high temperatures. During the process of ensiling, lactic acid bacteria (LAB) primarily convert WSC and a little lignocellulolytic material into organic acids (mostly lactic acid) under an oxygen-limited environment. Due to this beneficial process of acidification, the silages can be stored for a longer period with lesser chances of degeneration (Gallagher *et al.,* 2018; Ni *et al.,* 2017). As in Kim and Adesgon (2006) study, the higher ensiling temperature increased acid detergent insoluble crude protein (ADICP) concentration. Because this led to the formation of heat-damaged proteins through the Maillard reaction, this reaction involves in complexing of WSC or hemicellulose and amino acids under high temperatures. This happens because the Maillard reaction occurs in silages; the higher ensiling temperature decreases or falls within the same range from 35 to 40 °C (Muck *et al.,* 2003). As the high temperatures increased further, this led to an increase in the production of acetoin.

Acetoin can be oxidized or reduced into unwanted compounds like diacetyl and 2,3 butanediol, respectively. These products are categorically formed by *Streptococci*, *Pediococci*, and *Lactobacilli* species of bacteria when glucose is limiting, such as low-WSC corn forages, due to low fructose 1,6 biphosphatase for activating lactate dehydrogenase (Wagle *et al.,* 2021).

Kim and Adesogan (2006) reported that residual WSC concentration also decreased as ensiling progressed due to the formation of organic acids (lactic acid) by LAB, but the rate of WSC degradation was higher at cool temperature rather than the hot temperature. He also observed the lower production of acetate and lactate in the corn silage at higher temperatures. At high temperatures, lower grain yield also results in a decrease in starch concentration in corn silage due to the effect of temperature on starch synthase, the enzyme controlling the starch synthesis in the grain. Thalmann and Santelia (2017) reported that temperatures above 30°C irreversibly inactivate starch synthase, prohibiting starch accumulation in the kernel. However, a study conducted in Brazil found that corn silage production on intensive dairy farms in the south and southeast had adequate starch concentration (de Oliveira *et al.,* 2017). Few studies have compared the non-fiber carbohydrates (NFC) concentration among cool-season forage species. The limited available information reflects that red clover has a greater NFC concentration than alfalfa. Tall fescue has the highest NFC concentration among several cool-season kinds of grass (Pelletier *et al.,* 2010). In the tropics and subtropics regions, during the initial phases of fermentation, the temperature might be exceeded up to 40°C due to continuous respiration and aerobic microbial activity, when air is still present in the silo (Bernardes *et al.,* 2018). In tropical regions, the fermentation quality is improved when the grass is inoculated with the commercial lactic acid bacteria (LAB) due to high temperatures and low WSC contents (Gulfam *et al.,* 2017). Cai *et al.,* (1999) reported that *Pediococcus acidilactici* could be homolactic fermentation stable and decrease nutrient loss during the ensiling of low-WSC forage at 48 °C.

Dry matter and moisture content

Nowadays, ensiling is the easiest way for the supply of quality forage for ruminants. Silage quality is mostly exaggerated by raw material features such as moisture content (MC), WSC contents, and bacterial population. There are three crucial reasons to harvest the crops at the right moisture content: (1) for maximum yield, (2) preventing storage and field losses, and (3) to make sure the high palatability and maximum intake by the animals. In addition, the seepage is reduced when crops are harvested at the right moisture content.

High moisture (>70 %) has counteracted the rapid pH decrease, so it is not easy to produce quality silage due to higher concentrations of WSC and the population of LAB (Kung Jr *et al.,* 2010). Furthermore, increased moisture mostly tolerates higher chances of clostridial fermentation and effluvium loss, which leads to higher DM loss, increased butyric acid production, and extensive proteolysis, following which reducing digestibility of feed, feed intake, and NUE in that fermentable components are easily lost during the ensiling process. Non-protein nitrogen is ineffective in microbial nitrogen synthesis relative to true protein, and high butyric acid content might discount feed palatability (Borreani *et al.,* 2018).

A research was conducted by Yahaya *et al.* (2002) on the degradation of structural carbohydrates from orchard grass (*Dactylis glomerata* L.) ensiled at a different moisture level, i.e., low (LM, 40 %), medium (MM, 65 %), and high moisture (HM, 76 %). The fermentation quality of all silages was admissible. The loss of DM was very small (2–3 %) in all the silages. The water-soluble carbohydrates (WSC), gross energy, and hemicellulose losses increased with higher moisture levels during the ensiling. The hemicellulose losses in LM, MM, and HM silages were 7, 9 and 12 %, respectively. The losses of WSC were 23, 70, and 77 % in low moisture, medium moisture, and high moisture silages, respectively. These consequences show that ensiling the orchard grass at higher moisture levels increased the losses of hemicellulose and WSC, which would decrease the digestibility. The loss of WSC in orchard grass at various moisture levels is represented in Figure 3.

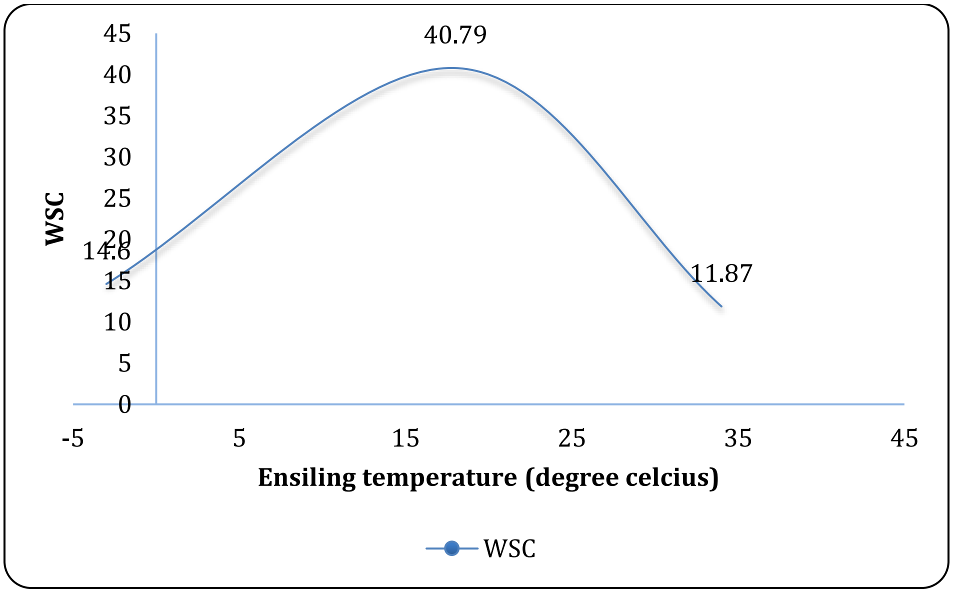

Figure 2. Effect of ensiling temperature on WSC contents.

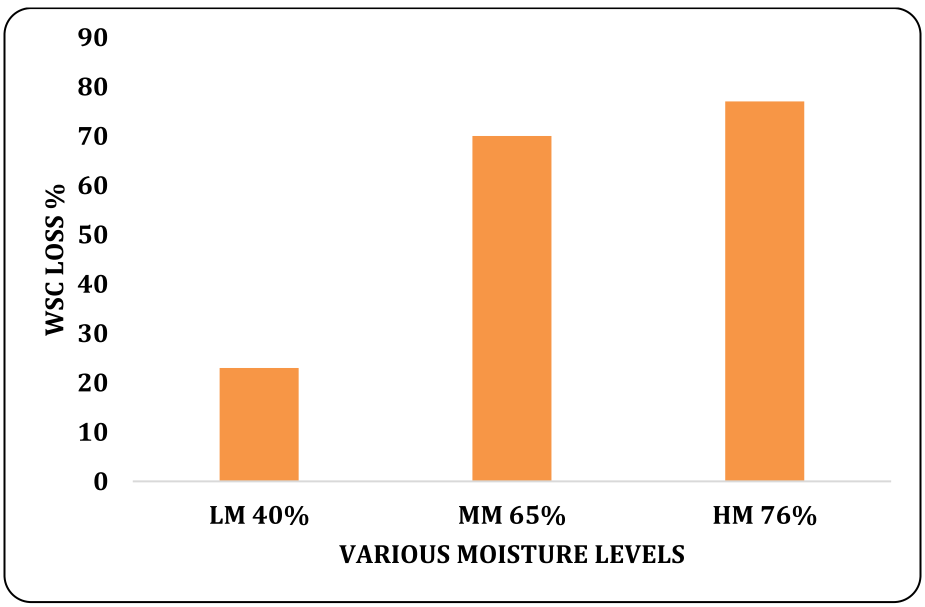

Figure 3. Relationship between WSC and moisture in orchard grass silage.

Plant sugar (carbohydrate) is required to produce organic acids (lactic acid) during the ensiling of fodder crops and species. Jones *et al.* (2004) stated that lactic acid bacteria should have carbohydrates available to produce organic acids, mainly lactic acid. If the carbohydrates are less available during the fermentation or ensiling, then the production of acids will stop. If the carbohydrates are less available, then the final pH of the silage will increase, and that will be too high to stop the growth of spoilage organisms. Two factors indicate the number of sugars or carbohydrates required for the maximum fermentation: crop species and water. In wet forage, to prevent the growth of undesired spoilage bacteria, a low pH is needed. That means that for the production of organic acids, more sugars or carbohydrates are required. Legumes need more organic acids to drop the pH than grasses or corn because these have a natural buffering capacity. Keeping this scenario in mind, the combination of high buffering capacity and low sugar or carbohydrate contents at harvest means alfalfa is especially susceptible to incomplete fermentation.

However, in the rainy season, alfalfa contains a lower concentration of WSC and DM and has a significantly higher buffering capacity that would cause lowering the DM and increasing the butyric acid (Ozturk *et al.,* 2006). Liu *et al.* (2018) reported that Gold Queen alfalfa is more capable of coping with drought stress due to having significantly more fractions of WSC contents. In this study, WSC shows a minor decrease in the spring season due to drought stress which means that WSC is less affected by the drought stress. Although, other fodder and forage species showed an increase in WSC due to drought stress (DaCosta and Huang, 2006; Nakayama *et al.,* 2007). The osmotic potential of the plants increased if the plant has a significant amount of water-soluble carbohydrates, which lead to uptake of the soil water and decrease the drought stress effects (Miranda-Apodaca *et al.,* 2018). Generally, DM content 30 %-35 % and WSC > 5 % DM are required to obtain good quality silage with high DM recovery (Guyader *et al.,* 2018).

Time of harvesting

Harvesting fodder and forage crops on the right stage and development are the most crucial factors to ensure the production of good quality silage. If the harvest has a high moisture level, then it will lead to poor fermentation and seep. On the other hand, if the harvested crop has a low moisture level, the kernel will become harder and less digestible, and chances of yeast and molds growth will increase as well as, the time of harvesting has a significant impact on the carbohydrates concentration in the crop plants. Xie *et al.* (2012) studied the nutritive composition of the wheat crop at three maturity stages (flowering, milk, and dough) and found that with increasing maturity, the WSC contents decreased quickly from the milk stage to the dough stage due to the accumulation of starch in the grain. Similar results were also reported by (Filya, 2003). However, lactic acid production was still sufficient at the dough stage to ensure the silage with good fermentation quality—WSC concentration at different maturity stages, as shown in Figure 4.

Cazzato *et al.* (2011) conducted a study on the production and quality of safflower forage at three different harvest stages (PB, STB, FS) and found that the WSC contents in forage before ensiling with not wilting were ranged from 128, 105, and 100 g/kg DM at PB, STB and FS stages, respectively. The water-soluble carbohydrate contents were almost similar at STB and FS stages, but WSC contents showed the highest value when the forage was harvested at the PB stage. Water-soluble carbohydrates contents and buffering capacity values found in their study were lower in late harvesting stages and higher in earlier harvesting stages. However, all three harvesting times were favorable to produce the lactic acid for good quality silage. The high WSC substrate allowed lactic acid fermentation and a good conservation quality in all the harvesting stages of the safflower. Li *et al.* (2016a) reported that the WSC contents of fresh forage of Siberian wild rye at different maturity stages (sprouting stage, flowering stage, and milky stage) was >6.0-7 % DM (Teller *et al.,* 2012). They also reported that the combined treatment of *Lactobacillus plantarum* and Lactobacillus buchneri increases WSC contents in silage at the milky and sprouting stage. This phenomenon happened due to the higher number of *Lactobacillus plantarum* in the ensiling materials. Thus, in the alpine region, the best-recommended harvesting time is the flowering stage of the Siberian wild rye having sufficient WSC contents for ensiling.

Leptocanna chinensis low WSC contents and LAB counts; that's why it is not easy to ensile (Zhang *et al.,* 2014). *L. chinensis* is often harvested at advanced maturity for yield. In terms of nutrition aspects of silage, the stage of maturity at harvest is an immensely crucial factor. Advanced maturity may adversely impact the ensiling characteristics of forages because of decreased WSC contents, increased cell-wall deposition, and difficulty of excluding air from the ensiled forage (McEniry *et al.,* 2014). It remains unclear that which is the best stage of maturity to harvest *L. chinensis* for ensiling. Zhang *et al.* (2016) reported in their study that WSC content was higher in the early heading stage than that of the late heading stage of *L. chinensis* before the ensiling. Also, forage digestibility and consumption by animals are influenced by the forage crop's maturity (Opsi *et al.,* 2013). So, harvesting the crops at the most suitable stage of plant growth would increase the feeding value of silage. Rabelo *et al*. (2015) studied the chemical composition of corn silages harvested at five maturity stages (early dent (ED), 1/3 of milk line (ML), 1/2 ML, 2/3 ML, and black layer (BL) and found that the NFC (non-fiber carbohydrates) was higher in 2/3 ML stage. The lowest concentration was seen in the ED stage. The total carbohydrates were higher in the 1/3 ML stage and lowest in the 2/3 ML stage.

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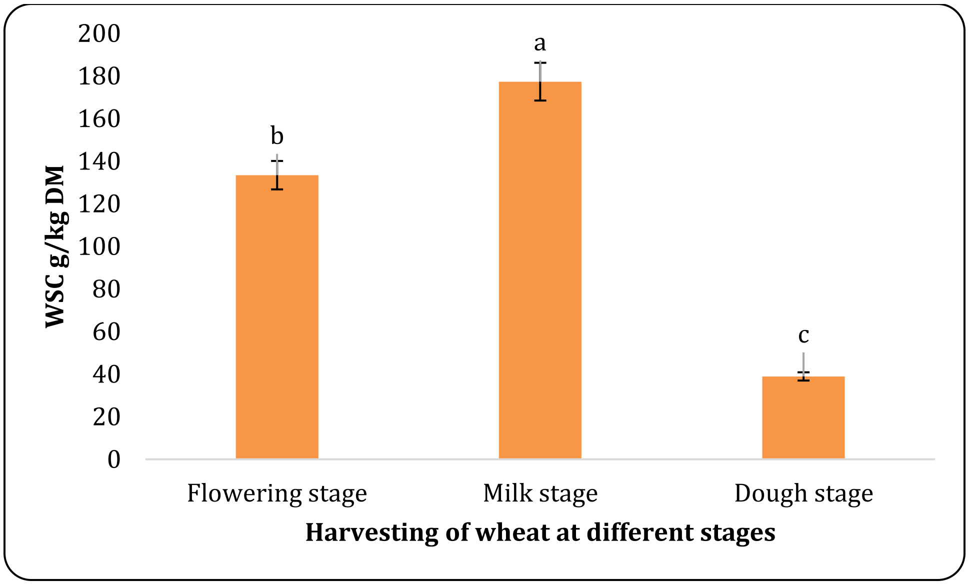

Figure 4. WSC in wheat at different harvesting stages.

Silage fermentation is a multiplex process affected by material characteristics like DM, buffering capacity, WSC, and epiphytic microflora. Therefore, the composition of alfalfa at harvest defines the ensiling process as well as the silage quality. Water-soluble carbohydrates contents and DM increase as alfalfa matures, positively impacting silage quality, but the buffering capacity decreases with maturity. Guo *et al.* (2019) compared the first cut alfalfa and second cut alfalfa harvested from the BS to the IFS stage. They concluded that the second cut of alfalfa had the lower DM and WSC contents, but the buffering capacity, epiphytic aerobic bacteria, and harmful microorganism counts were higher than the first cut. Although both cuts of alfalfa harvested at FFS had a similar DM, WSC contents and harmful microorganism counts with first cut alfalfa harvested at a similar stage. The higher buffering capacity of alfalfa could lead to a slow decline of silage pH and more protein hydrolysis during the initial fermentation (Buxton *et al.,* 2003).

The non-fiber carbohydrates (NFC) concentration may vary with stage of development, and due to the confounding effect of the climatic conditions on the time of harvest, the results are inconsistent (Wilman, 2004). In the most northern agricultural area of eastern Canada, a study has been conducted. They reported no consistent differences in forage NFC in tall fescue cut 2, 3, or 5 times per year (Drapeau *et al.,* 2005). The concentration of NFC did not influence by the shorter intervals between the harvests. However, as the ambient air temperatures decrease in the fall, the delaying harvest can result in a significant increase in NFC concentration. In eastern Canada's most northern agricultural area, the tall fescue NFC concentration increased from 60 g/kg of DM to 150 g/kg DM in early September to late October, respectively (Drapeau *et al.,* 2007). The temperature influences plant growth; if the temperature is not optimum for the growth, then carbohydrates accumulate as photosynthesis generates the NFC that is not immediately incorporated into plant tissues due to the decline in growth rates of the plant. However, photosynthesis continues at the same rate while growth slows down. No general conclusions could be drawn in a study, the difference in NFC concentrations between summer and spring with the forage species, conducted in eastern Canada (Pelletier *et al.,* 2010). However, alfalfa harvested in early October had a greater NFC concentration than that harvested in June or August (128 vs. 91 and 102 g/kg of DM, respectively) (Morin *et al.,* 2012a). Perennial forage crops harvested in late fall (e.g., October in eastern Canada) are likely to have much greater NFC concentrations than those harvested in early fall or summer.

Time of cutting (AM/PM)

Nonstructural carbohydrates are the vital source of fermentable substrates during the ensiling process and a key source for readily fermentable energy of the rumen microbes. During the day, plant carbohydrate concentration increased due to photosynthetic carbohydrate synthesis exceeds utilization. This activity in the plant leads to emphasize that cutting should be

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done during the day when the NFC concentration in the plants is highest. Many studies have been conducted among different forage and fodder species, particularly in the sunny days, and concluded that NFC concentration would be higher if the cutting of the crop should be done later in the day (Burns *et al.,* 2007; Burns *et al.,* 2005). In both timothy (Morin *et al.,* 2012a) and alfalfa (Morin *et al.,* 2011), the NFC concentration occurs after sunrise between 11 and 13 h, and this is the optimal period for harvesting the crops with higher NFC and this time is the same in spring, summer, and autumn for alfalfa (Morin *et al*., 2012b).

Plant cells remain viable after cutting and continue to use NFC for respiration until they die. Wilting conditions has also an impact on the NFC. Alfalfa NFC concentration decrease during the first day of wilting. This rate of decrease is not affected by the time of cutting. Consequently, at the end of the wilting period under optimal drying conditions, PM cut alfalfa has more NFC than AM cut. However, recent research has shown that nighttime NFC losses in late PM alfalfa are lower than AM cut. Alfalfa cut and laid directly on the ground reduces wilting time by up to 9 hour under poor drying conditions and exhibits increased NFC concentration compared with alfalfa that is swathed and dried in windows. When wilting conditions are conducive for rapid drying and with two consecutive sunny days, producers using this practice can produce wilted alfalfa forage with greater NFC concentrations in less than 24 h. Some NFC, such as fructans in timothy, are prone to degradation during the wilting period, partly due to higher activities of specific hydrolase enzymes (Ould-Ahmed *et al*., 2017). The fate of these carbohydrates during ensiling is also unclear; in one study, only 2 % of lactic acid bacteria (LAB) possessed the ability to degrade this polysaccharide (Winters *et al*., 1998).

Soluble carbohydrates such as glucose, fructose, and sucrose are quantitatively the most important substrates for silage fermentation. Consequently, concentrations of NFC decrease during fermentation, and the extent of this decrease in NFC concentration varies with silage DM concentration. This decrease in NFC concentration during the fermentation process can lead to lower silage quality, so the good nutritive quality of silage can only be achieved by harvesting the forages at the highest NFC concentration. As outlined above, the concentration of NFC in alfalfa increases if the alfalfa is cut in the afternoon and wilted in wide swaths. Tremblay *et al*. (2014) investigated the implication of this practice for silage fermentation. Differences in alfalfa NFC concentration at ensiling (10 to 36 g/kg of DM) due to PM cutting, with or without wide swaths, were reduced to between 0 and 15 g/kg of DM, mostly as a result of the utilization of NFC during fermentation. When NFC concentration differences at ensiling due to PM cutting were greater than 10 g/kg of DM, silage conservation attributes were improved with lower pH, greater concentrations of lactate (+8 to +20 g/kg of DM), and lower concentrations of volatile fatty acids (VFA) (−8 to −11 g/kg of DM) and ammonia nitrogen (−6 to −11 g/ kg of total N). Improved silage conservation attributes, along with a greater residual starch concentration from NFC enriched alfalfa, can increase the nutritive value of silage and improve milk production.

Fertilizer

Fertilizers are simply planting nutrients applied to the agricultural field to supply the required elements, which are deficient naturally in the soil. These nutrients have a great impact on the fertility of the soil and as well as on the growth and development of the plant. These nutrients are applied to the soil to increase the yield of the crop. So, in the fodder and forage crop species, these nutrients play an important role in the composition of plants and the quality of silage. The composition of forage is also influenced by applying fertilizer to the soil. Li *et al*. (2016b) reported that CP and WSC contents increased significantly as the nitrogen application rose from 0 to 225 kg/ha. They indicated that N should be applied to forage (whole crop) wheat at a similar rate according to the rate applied to grain wheat. Although more N application might increase the digestible DM yield, there is a risk of NO_3-N accumulation. In addition, LAB count increased significantly with the increased N application from 0 to 225 kg/ha. That might be attributed to increased WSC and CP contents at high N application, which are vital nutrients for the growth of LAB with strict nutrition requirements.

In contrast, (Asano *et al*., 2018) concluded that N fertilization did not harm the quality of reed silage. The fermented silage quality becomes poor when the WSC contents of the material fall below 10 % of DM (Borreani *et al*., 2018). The effect of N fertilization was only related to CP in a reed. Protein is synthesized from N absorbed through the roots and from carbohydrates, such as WSC; therefore, a large N supplement decreases the amount of

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WSC in plants due to a stimulation of carbohydrate consumption. The WSC content of reed in their study was only 5.3 % of DM. Therefore, the WSC contents were half the value required for producing good quality silage. The most effective approach would add both additives LAB and a substrate to get the high-quality silage of the reed.

Another study conducted by (Skonieski *et al*., 2017) to check the effect of different nitrogen (N) rates (0, 60, 120, 240, 480) kg/ha on the nutritional quality of maize and found that the NFC concentration went higher at first three N rates, but it decreased at last two N rates and, there was the same scenario with the total carbohydrates (TC). Hence, it showed that NFC and TC concentration in maize crops increases with increasing the N fertilization rates at a certain level; further increasing the N rates would decrease NFC and TC concentration. The conventional 100 % NPK and 50 % CM+50 % NPK fertilizers improve the plant growth, corn-soybean DM yield, CP, and quality of the corn-soybean silage. However, the effect of chemical fertilizers CM and BF and their combinations were not serious for WSC concentration. CM contains a high N concentration, and it has become the most crucial manure due to its lower price and environment-friendly characteristics. So, the smallholding farmer can easily afford it and increase the crops' DM yield (Baghdadi *et al*., 2018).

Lowering N fertilization has been shown to increase NFC and reduce CP concentrations of several cool-season grass species—for example, timothy, orchardgrass (*Dactylis glomerata* L.), and tall fescue (Pelletier *et al*., 2009)—but it may also reduce total yield. Timothy silage quality is reduced by increased application of N fertilizer, primarily at early developmental stages, and this can be attributed to a reduction in WSC concentration and an increase in the buffering capacity of the forage. Therefore, the ensiling properties of timothy are less favorable when high rates of N fertilizer are applied (Tremblay *et al*., 2005). Increasing N fertilizer application generally increases herbage crude protein concentration (Keady *et al*., 2000) and buffering capacity (Tišma *et al*., 2018) and reduces dry herbage matter (Whitehead, 1995) and WSC (Tremblay *et al*., 2005) concentrations. Similar, residual silage WSC concentration was higher for the zero N fertilizer than for the high N fertilizer treatment (King *et al*., 2013). It is common to raise the rate of N fertilizer application from 0 to 125 kg/ha, then buffering capacity of herbage increased and herbage DM and WSC concentrations decreased during pre-ensiling (King *et al*., 2012). It was reported that the addition of urea to pomegranate pulp lowers the acid detergent fiber (ADF), neutral detergent fiber (PDF), acid detergent lignin (ADL), and WSC content (Ozcan and Kilic, 2018).

Additives

Lactic acid bacteria (LAB) and water-soluble carbohydrates (WSC) are vital aspects for higher silage quality during the ensiling process (Ni *et al*., 2017). Out of the various additives, L inoculants and M (molasses) have been suggested as an effectual stimulant to improve silage quality through enhancing the L load and fermentable substrate (WSC), respectively (Li *et al*., 2014; Ni *et al*., 2015). Water-soluble carbohydrate (WSC) is a crucial aspect for silage fermentation, and the concentration of more than 5 % DM was shown to be important for guaranteeing acceptable fermentation quality. Although, soybean has a low level of WSC content of 1 %, which indicates that good preservation of soybean without any additives during ensiling is impossible. When soybean is treated with L and M inoculants, the pH decreased due to an increased level of WSC, leading to good quality silage (Ni *et al*., 2017).

Inoculation with LAB reduced silage pH and WSC concentration but enhanced the DM concentration and recovery. When LAB is inoculated in laboratory-scale silos, WSC concentration, NH_3-N concentration, and molds are reduced, and DM concentration increased. In contrast, no effect was observed on DM concentration; NH_3-N concentration was reduced while the WSC concentration increased when LAB was inoculated in farm-scale silos (Oliveira *et al*., 2017). The trends for farm-scale silos to have greater WSC concentration and fewer LAB maybe because the harvested forage for the former is left in the field for longer periods than mini-silos. The longer drying durations may have decreased epiphytic bacterial viability, increased moisture loss, and decreased plant respiration, and collectively, these factors likely increased the residual WSC in forages (Weinberg *et al*., 2010).

The lack of effects of LAB inoculation on the fermentation and DM recovery of corn and sorghum silages is notable because these forages are perhaps the most widely ensiled for dairy production in the United States. The lack of response to inoculation is probably because these forages contained sufficient WSC concentrations for the fermentation and high epiphytic bacterial populations, and low buffering capacities (Carvalho *et al*., 2014).

Unlike in corn and sorghum silages, LAB inoculation improved the fermentation of legume and grass silages probably because of their low epiphytic flora, low WSC concentrations, and high buffering capacities (particularly for the legumes) (Arriola *et al*., 2015; Ogunade *et al*., 2016; Silva *et al*., 2016). The causes of the reduction in DM recovery by LAB inoculation of sugarcane silages are not clear, but they are probably associated with excessive fermentation due to the high WSC concentration of sugarcane. König *et al*. (2017) reported that the WSC content is adequate to produce good quality silage when LAB is used as an additive. At the early stage of crop maturity, LAB treatment stimulated efficient lactic acid fermentation resulting in lower residual WSC content and pH than formic acid and hexamethylenetetramine treatments. The effect of hexamethylenetetramine as a silage additive did not depend on the herbage fermentation coefficient, WSC content, or silage pH. The LAB-based additive was less efficient than chemicals only when the WSC content of the crop was low in trials with the early stage of crop development. The average WSC content of the treated silages with different additives was higher compared with the control silage of the Lupin-wheat. NAHe (sodium nitrite) treated silages contained less residual WSC than FA (formic acid) treated silage (König *et al*., 2019). Similar results were also reported by (Li *et al*., 2019) that FA increased the WSC of ryegrass, bur clover, and mixed silages.

The residual WSC contents increased by using the ethanol alone or combined with the inoculants compared to the control or *Lactobacillus plantarum* silages because the ethanol inhibited the loss of WSC by microorganisms. Zhang *et al*. (2011) reported in their study that, fermentation quality of the Napier grass increased by using ethanol as a silage additive. This additive inhibits the undesirable bacteria from using the WSC and reduces the silage losses during the earlier phases of fermentation. The results in the production of more LAB and producing good quality silage. The ensiling quality structural and nonstructural carbohydrate and enzymatic digestibility (ED) value of mature Napier grass silage improved through additives (Desta *et al*., 2016). Ethanol could reduce the loss of protein and WSC by inhibiting undesirable aerobic bacteria growth during the early stage of ensiling. Similar results were also reported by (Yuan *et al*., 2012) that wet hulls-barley distillers grains (WHDG) had higher WSC when treated with ethanol. The WSC content in TMR was highest when silage is treated with the combined ethanol and molasses additives and was lowest when treated with the *Lactobacillus plantarum* additive only (Yuan *et al*., 2015).

The preservation of the alfalfa is limited due to its less WSC and high buffering capacity (BC). Different kinds of additives are used to increase the preservation of the alfalfa silages' residual WSC and CP. Residual WSC increased when alfalfa is treated with formic acid compared to other treatments shown in Figure 5. Alfalfa harvested before the killing frost had enough WSC to start the ensiling process for lactic acid production. Lactic acid, acetic acid, and ethanol contents decreased when alfalfa is treated with the organic acids, but the residual WSC and CP increased, which results in the higher IVDMD (In vitro dry matter digestibility) (Li *et al*., 2016c). Furthermore, carbonate is also effective in increasing residual WSC and lactic acid in silage (Santos *et al*., 2009).

The inoculation of *Hedychium* with LAB would result in silage having high crude protein contents. However, the WSC and cell wall fiber components of this silage have significantly lower values. The aerobic stability is enhanced by using the *Hedychium* silages with molasses, while the LAB inoculants impaired the stability. However, the application of urea in the deficiency of WSC resulted in very bad preserved silage and instability in the air (Moselhy *et al*., 2015). Enzymes are used as a direct means to enhance fiber degradation by promoting the availability of WSC as a substrate for LAB (Ebrahimi *et al*., 2014; Nadeau *et al*., 2000; Tian *et al*., 2014). The enzymes like cellulose and hemicellulose are used to increase the WSC to ensure their availability for LAB to produce lactic acid, presumably by the degradation of a NDF and ADF (Colombatto *et al*., 2003; Li *et al*., 2014). The combined additives of *Lactobacillus plantarum* and cellulase, and *Lb. casei* and cellulase decreased the NDF, ADF, and hemicellulose content and increased WSC contents (Zhang *et al*., 2016).

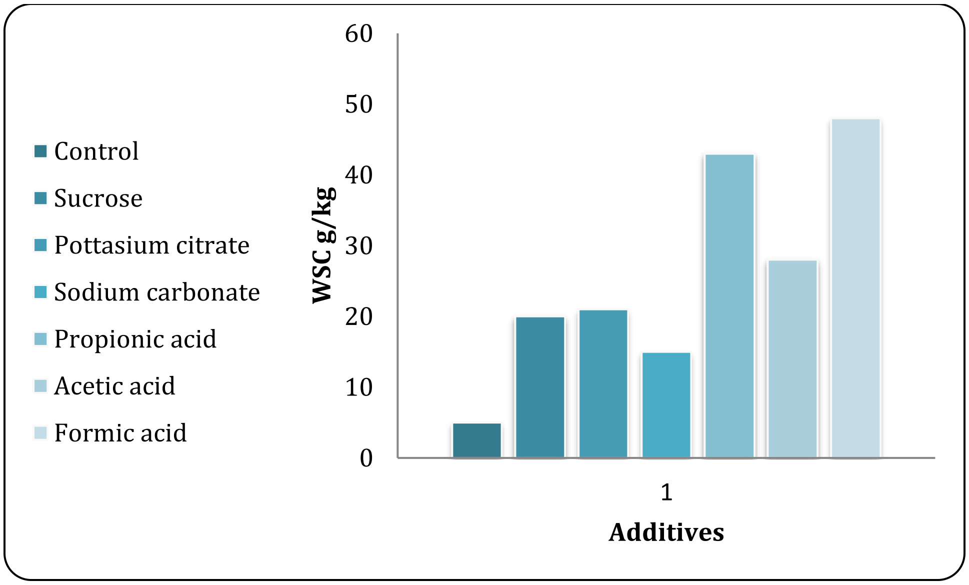

Figure 5. Effect of various additives on the WSC concentration during alfalfa ensiling.

Propionic acid is used to stop yeasts which digest the lactic acid and WSC when silage is exposed to the air. It is a worthwhile inhibitor of aerobic deterioration of silage (Woolford, 1975). A case has been studied by (Chen *et al*., 2016) and reported that the MP (molasses + propionic acid) silage has higher residual WSC contents than that of the control silage. Some scientists reported the effects of additives on the fermentation and nutritive quality of whole crop sorghum silage (Filya, 2003; Guan *et al*., 2002). A case has been studied by (Sifeeldein *et al*., 2019); they reported that the LAB treated sorghum silages suffered a faster aerobic deterioration than the control silage because these contained comparably high contents of residual WSC and lactic acid. Water-soluble carbohydrates (WSC) contents in all sweet sorghum silages decreased during the ensiling process. After five days of ensiling, silage inoculated with AZZ4 (*Lactobacillus plantarum* subsp. Plantarum) contained higher residual WSC contents than the other treatments. All the inoculants enhance the residual WSC contents relative to the control silage. It is due to the LAB inoculants that guarantee the fast and vigorous lactic acid fermentation and rapid reduction of the silage pH at early stages, which inhibit the loss of WSC fermented by spoilage bacteria.

Lynch *et al*. (2015) added cellulase and xylanase to corn forage before ensiling alone and combined with a ferulic acid esterase-producing (FAE) silage inoculant. They found that the combination resulted in a lower pH and higher WSC than the control silage after 70 days of ensiling. One would theorize that positive responses to the application of fibrolytic enzymes at ensiling would most likely be with low-WSC forages. This contention was recently supported, as ensiling of a low-WSC forage, *Leymus chinensis*, with inoculants and a cellulase improved the fermentation and the in vitro digestibility of this wild grass silage (Tian *et al*., 2014). Similarly, improvements were observed when fibrolytic enzymes were added to mixtures of low-WSC barley straw and corn silage at ensiling (Guo *et al*., 2014) and high-moisture low-WSC tropical forages (Khota *et al*., 2016). Enzymatic profiles and activity of mixed enzyme preparations are often unpredictable, further contributing to the variable responses observed with these additives.

Ensiling time or storage time

The main purpose of ensiling forages is to maintain the nutrients available for future feeding. There are four crucial phases when silage is ensiled aerobic, active fermentation, stable, and feed-out. The aerobic phase starts after harvesting and ensiling, and during this phase, plant and microorganism respiration occurs until oxygen is completely absent in the silo or the supply of substrate is used up. Once oxygen is absent, fermentation starts with bacterial production of fermentation end-products. This phase is characterized by the accumulation of lactic and acetic acids and a corresponding decline in pH. Proteolysis, the main mechanism responsible for disrupting the zein-proteins cross-linked to starch granules, occurs under acidic conditions suggesting that continuous alterations in fermentation profile as storage progressed may directly affect starch digestibility (Der Bedrosian *et al*., 2012). Similar results have been reported by (Arcari *et al*., 2016), as the ensiling time increased, the digestibility of starch increased.

Sariçiçek *et al*. (2016) reported that the WSC concentration of corn silage raised from 67 g/kg DM to 108 g/kg DM for the 90th and 202nd day, and the lowest concentration was found on the 118th day. Similar results were also reported by (Weinberg *et al*., 2011) that WSC concentration of corn silage in mini silos was 135.7 g/kg DM at the end of the five months.

CONCLUSION

The concentration of WSC is influenced by the different kinds of controllable and uncontrollable factors. The temperature has a limiting impact on the water-soluble carbohydrates. If the temperature is less during the ensiling, then the consumption of the WSC will be lower and vice versa. At the higher ensiling temperature, there are chances of production of heat-damaged proteins, which could lead to acetoin formation. This acetoin could be oxidized or reduced into undesired compounds that are not beneficial for silage quality. The losses of the water-soluble carbohydrates are higher at high moisture levels and lower at low moisture levels. Also, high moisture negatively impacts the rapid pH decrease, so it is not easy to produce good quality silage. So, the crop should be harvested when it has 60-70 % moisture content and 30-40 % DM. Harvesting the crop at the right stage and development is the most important factor in ensuring a sufficient amount of water-soluble carbohydrates for the ensiling process and preserving good quality silage. It has seemed that with increasing the crop's maturity, the carbohydrates concentration is decreased because carbohydrates start to accumulate in the grains. So, the crop should be harvested at the equitable stage to achieve good quality silage. The time of cutting of the forage crop also has an impact on the concentration of water-soluble carbohydrates. It is suggested that crops be harvested during the PM time rather than the AM time because PM cutting has higher water-soluble carbohydrates than AM cutting. The fertilizer also influences water-soluble carbohydrates (WSC) concentration. Their concentration is increased by increasing the application of nitrogen to the soil up to a specific level. Nowadays, CM has become the most important manure due to its reliability. The concentration of water-soluble carbohydrates can be increased or decreased by applying different additives like molasses, LAB, etc. Those crops with lower water-soluble carbohydrate contents are inoculated with additives to obtain good quality silage. Conclusively, the ensiling time does not influence the concentration of water-soluble carbohydrates during the ensiling process.

FUTURE OPPORTUNITIES

Water-soluble carbohydrates (WSC) are the main source of energy during the ensiling process. So, its availability during the ensiling process must be maintained to produce organic acids like acetic acid. Future studies must be focused on the water-soluble carbohydrate's degradation at different phases of fermentation to know this degradation mechanism very well for the production of high quality and well-preserved silage.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

All the authors have equal contributions.

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