The Effect of Adding Different Sources of Soluble Carbohydrates and the Sila Prime Bacterial Inoculum on the Fermentation of Egyptian Berseem Silage and its Nutritional Value

Ibrahim Hussein Abdal Sada Al-helali¹, Ali Ahmed Alaw Al-Qotbi¹ and Frokh Kafilzadeh²

¹College of Agriculture, Al-Qasim Green University, Iraq

²College of Agriculture, Razi University, Kermanshah

Abstract

The study was conducted in the Animal Production Department of the Faculty of Agriculture - Al-Qasim Green University for the period from 9/25/2019 to 9/18/2020, to investigate the effect of adding different sources of soluble carbohydrates (molasses 6% and dates syrup 6%) and the microbial inoculant Sila Prime S4X On the chemical composition and fermentation properties of Berseem silage. A factorial experiment was conducted with a factor of 32 X, the first factor, the source of soluble carbohydrates, with three sources (without adding soluble carbohydrates, adding 6% molasses, adding 6% dates syrup) and the second factor adding the microbial inoculant (without a inoculant or adding the microbial inoculant Sila Prime 10^6 for colonies units / g). The results of the chemical composition analysis showed that there was a significant increase (P < 0.01) in the dry matter when soluble carbohydrates were added to the Berseem silage, where the dates syrup addition treatment was excelled and gave 23.086 while there was no significant effect as a result of adding the microbial inoculant to the Berseem silage. As for the interaction effect, the results showed there was a significant increase (P <0.01) in the dry matter. The results of the study also showed that adding the microbial inoculant had a significant effect on the traits of the crude protein, as the treatment of adding the microbial inoculant was excelled 18.75.As for the effect of interaction, the results indicated that there was a significant increase (P < 0.01) in the treatment of adding dates syrup and without the microbial inoculant, which amounted to 20.07 on the remaining treatments, while the treatment of adding dates syrup without adding the microbial inoculant was the lowest value among the treatments, which amounted to 16.35 and for silage fermentation The results showed that the addition of soluble carbohydrate sources and microbial inoculant to Berseem silage led to a significant improvement (P < 0.01) for silage fermentation. The lowest pH was recorded at 4.076 and the highest concentration of lactic acid 364.386

also led to a significant increase (P < 0.01) in the residual level of soluble sugars.

Keywords: carbohydrates, sila prime bacterial inoculum, Egyptian Berseem silage

Introduction

All preserved food materials such as hay or silage play an important role in all countries where there is a restricted vegetation period such as winter or dry season. Preserved feeds worldwide are an essential component of ruminant diets during times when fresh crops are not available (Pahlow et al. 2003 and Jeroch, 2008). Therefore, ensiling, despite its widespread use as a means of preserving crops in many parts of the world to provide feed throughout the year or in periods of scarcity of pastures (Wilkinson 2012), can be used to the aforementioned aim. Silage in particular is considered as feed for productive livestock (Woolford, 1984). Egyptian Berseem (Trifolium alexandrinum L.) is a high-quality feed that is characterized by a high concentration of nutrients, mainly protein (15-25% dry matter), minerals (11-19%) and carotene (Feedipedia, 2013; Sharma et al., 1974). Berseem is special nutritional value because it is a legume feed(Hannaway et al., 2004). It is also very palatable and somewhat digestible. When fed alone, it can support a growth average of about 550 g / day, and milk production of about 10 kg / day (Chauhan et al., 1992). This was also confirmed by Das et al. (1999). Fresh green Berseem also has good nutritional value comparable to fresh alfalfa (Brink et al. 1988 and Fraser et al. 2004 and Yoelao et al. 1970). Egyptian berseem (Trifolium alexandrinum L.) is a high-quality feed that is characterized by a high concentration of nutrients, mainly protein (15-25% dry matter), minerals (11-19%) and carotene (Feedipedia, 2013 and Sharma et al., 1974). Berseem is special nutritional value because it is a legume feed(Hannaway et al., 2004). It is also very palatable and somewhat digestible. When fed alone, it can support a growth average of about 550 g / day, and milk production of about 10 kg / day (Chauhan et al., 1992). This was also confirmed by Das et al. (1999). Fresh green Berseem also has good nutritional value comparable to fresh alfalfa (Brink et al. 1988 and Fraser et al. 2004 and Yoelao et al. 1970). and Fulkerson et al. (2007) that the nutritional value of Berseem (protein and energy) can meet the requirements of relatively milk-producing cows (more than 30 L/day) provided that the cows consume a sufficient amount of dry matter to achieve this level of production. The addition of microbial inoculants and soluble carbohydrates is an important component during silage, where the addition of inoculants stimulates the fermentation of lactic acid as well as ensures a rapid decrease in the pH, thus improving silage preservation (Filya et al., 2000). Siddons et al. (2014)

indicated that the use of inoculants in silage is to stimulate the growth of the homogeneous fermentation lactic acid bacteria present on crops and plants naturally (epiphytic, hoLAB) and its dominance over fermentation and production of good quality silage and to avoid unwanted fermentation of soluble sugars and loss of dry matter that It occurs due to the presence of some harmful types of bacteria on crops and plants prepared for Ensiling. In addition, molasses contain large quantities of rapid degradation sugars 48-65%, which makes it a material of high nutritional value, and a source of energy in the nutrition of ruminants (Hashemi, 1991) The widespread use of molasses (75% dry matter) at an average of 10% or more to provide a source of carbohydrates, it accelerates the fermentation of Ensiling tropical weeds (Yang et al., 2006). The addition of molasses to 5% is effective enough to stimulate silage fermentation by producing lactic acid and lowering the pH of silage (Yunus et al., 2000). Moreover, adding molasses would provide sufficient levels of energy to revive the silage microorganism, leading to an increase in the rate of fermentation and the production of lactic acid (Balakhial et al., 2008). The present study aimed to investigate the possibility of Berseem Ensiling and study the effect of various sources of soluble Carbohydrate and the sila prime microbial inoculant on the chemical composition and some properties of silage fermentation.

Materials and methods

The study was conducted in the Animal Production Department of the Faculty of Agriculture - Al-Qasim Green University for the period from 9/25/2019 to 9/18/2020.to investigate the effect of the addition of different sources of soluble carbohydrates (6% molasses and 6% dates syrup) and Sila Prime S4X microbial inoculant on the chemical composition, fermentation properties and laboratory digestion of the RUSITEC method in Berseem silage. A factor experiment was conducted with two factors 3 X 2. The first factor was the source of soluble carbohydrates by three sources (without adding soluble carbohydrates, adding 6% molasses, adding 6% dates syrup) and the second factor was adding the microbial inoculant (without inoculant or adding the microbial inoculant Sila Prime 10 ⁶colonforming units / g and by four repetitions for each treatment. The third Berseem was harvested and then cut by hand into small parts and then wilted to obtain an appropriate level of dry matter for the purpose of Ensiling, with a process of constant stirring during wilting to prevent damage to the crop and allow for the greatest loss of moisture. 3% of barley powder was added to increase the content of soluble carbohydrates at a constant level for all sample, as well as adding minced wheat straw by 5% to increase the level of dry matter. The solutions prepared for the Ensiling were sprayed on the Berseem

according to the different treatments, and the mixture was mixed well to ensure that the added solution was distributed on all parts of Berseem, the Berseem filled in double nylon bags and pressed by hand to get the air out of them and the bags were sealed tightly.Suitable pits were prepared to store the prepared silage bags during the fermentation period that lasted 60 days after being filled with soil and compacted well.

Silage fermentation properties

To study the properties of silage Berseem fermentation by preparing the aqueous extract of silage according to the method of Levital et al. (2009) by mixing 50 g of silage with 500 mL of distilled water in a mixer device for 10 minutes and filtering through two layers of cheese cloth. It is then filtered by a filter paper. After that, the filtrate was transferred to 10 ml tubes and a few drops of a 50% solution of concentrated sulfuric acid were added and then distributed to a set of 2 ml Abendorf tubes. The tubes were preserved by freezing until further fermentation parameters were determined (Kazemi-Bonchenar et al., 2010). Silage fermentation properties included estimation of the pH, soluble sugars concentration, ammonia nitrogen, lactic acid, and TVFA total volatile fatty acids. The pH of the silage aqueous extract was estimated immediately prior to adding the acid and using the Mi 180 Bench Meter, which was previously set with buffer solutions 4, 7 and 10. The carbohydrates were first broken down into simple sugars using dilute hydrochloric acid, and the glucose was dehydrated in a hot acid medium to hydroxymethyl furfural. This compound with Anthrone determined a color compound product with a maximum absorption at the wavelength of 630 nm. The concentration of lactic acid was estimated according to the following equilibrium: Lactic acid concentration = (absorbance of the test sample / the absorbance of the standard solution) x 50 mL (Taylor, 1996). The ammonia nitrogen concentration was calculated according to the following equation:

N mg / 100 ml = [(ml volume of acid - ml planck) \times concentration \times 0.014 volume of solution] \times 100. Then the ammonia nitrogen concentration in the silage was recalculated as the percentage of total nitrogen.

Chemical Analysis

Chemical analyzes of silage sample were performed according to the method (AOAC, 2005) and (Levital et al. 2009). The different samples were dried in a drying oven at a temperature of 60 $^{\circ}$ C for a

period of 48 hours, and milled after being cooled and preserved until those analyzes were made. The dry matter content of the samples was estimated by drying at a temperature of 105 ° C for a period of 24 hours and the dry matter percentage was calculated. The organic matter content of the samples was estimated by burning the dried samples in an incineration oven at a temperature of 500 ° C for a period of 4 hours, and the percentage of organic matter was calculated. The content of the samples from the crude protein CP was estimated by estimating the nitrogen levels by the Kjeldal method.

Results and discussion

Table (1 and 2) represent the effect of adding different sources of soluble carbohydrates and the sila prime microbial inoculant on the chemical composition of Berseem silage and the effect of the interaction between them, which included dry matter, organic matter, crude protein and neutral fiber extract. The results of the current study showed that there were significant differences (P < 0.05) in the dry matter values of Berseem silage due to the addition of sources of soluble carbohydrates. Where the values of the inoculant addition treatments with and without the addition of the microbial inoculant and the molasses addition treatments with and without the addition of the microbial inoculant were excelled, where there were no significant differences between those treatments. The highest value among them was the treatment of adding dates syrup to the rest of the treatments amounted to 23,086, while the treatment without adding carbohydrates was the lowest value of the treatments amounted to 19,338, while there were no significant differences (P < 0.05) between the values of the treatments as a result of adding the microbial inoculant to Berseem silage. With regard to the effect of the interaction between the treatments of dry matter, where there were significant differences (P < 0.05) between the values of the treatments, where the values of the treatments of the adding dates syrup with and without the addition of the microbial inoculant and the addition of molasses with and without the addition of the microbial inoculant were excelled. There were no significant differences between those treatments, and the highest value among them was the value of the treatment of adding dates syrup with the microbial inoculant and amounted to 23,293 on my treatment without adding the soluble carbohydrates with the addition of the microbial inoculant and the treatment without adding the soluble carbohydrates and without the microbial inoculant, which was the lowest value among the treatments, reaching 19,323. This is due to the addition of mainly soluble carbohydrate sources and the microbial inoculant,

which led to a decrease in the dry matter loss during silage, which provided the energy needed for lactic acid bacteria, increased their numbers, increased lactic acid concentrations, and thus increased acidity and decreased pH as a result, which led to the inhibition of the activity of non-microorganisms. Desirable and reduced dry matter loss, Between Kim et al. (2021) that among the inoculants of silage, lactic acid bacteria play an important role in preserving and fermenting feed crops, where these bacteria work well to reduce the pH and inhibit unwanted microorganisms as well as control the loss of nutrients in the silage during the process. Silage, where these bacteria use plant compounds and simple carbohydrates to produce organic acids such as lactic acid and acetic acid. When silage is eaten by animals, these bacteria break down the secondary structures of the plant in the rumen. Rajabi et al. (2017) explained in a study they conducted on the effect of adding dates that are not suitable for human consumption on the chemical composition of Berseem silage and its digestibility in the rumen of the Kirmani sheep, where different proportions of dates were used (0, 5, 10, 15%) and the results showed improvement in the quality of silage and increase the dry matter content and the total energy content. Pasandi et al. (2012) obtained an increase in the dry matter of corn silage due to the addition of molasses, While Siddons et al. (2014) indicated through their study of the possibility of using molasses as effective additives in promoting lactic acid fermentation and hindering the fermentation of Clostridium bacteria and proteolysis as a result of lowering the pH. As well as reducing dry matter loss. The increase in the production of lactic acid may be due to the increase in the number of lactic acid bacteria as a result of adding the inoculant, which in turn consumes the available sugars, which leads to the preservation of dry matter and less loss in return (Babaeinasab et al., 2015). The addition of molasses leads to stimulating silage fermentation, which leads to changes in the chemical composition of the stored feed material, and the extent and nature of these ferments are affected by the type and level of soluble sugars present. This may result in a loss of dry matter at rates that increase with the progression of the ensiling stages (Can and Denek, 2007). As for the organic matter, the results of the current study showed that there are significant differences (P < 0.05) in the organic matter values of Berseem silage due to the addition of soluble carbohydrate sources, where the value of the dates syrup addition treatment excelled on the rest of the treatments amounting to 86.368, while the treatment without adding carbohydrates was the lowest for treatments, it amounted to 82,795. While there were no significant differences (P < 0.05) between the values of the treatments as a result of adding the microbial inoculant to Berseem silage. As for the effect of the interaction between the values of the organic matter in the different treatments, where there were significant differences (P < 0.05) between

the values of the treatments of the organic matter. Where the treatment of adding dates syrup with the microbial inoculant, which amounted to 87,110, and the treatment of adding dates syrup without adding the microbial inoculant excelled on the rest of the treatments. The treatment of adding molasses with and without adding the microbial inoculant excelled on the treatment without adding soluble carbohydrates with adding the inoculant and treatment without adding soluble carbohydrates and without the microbial inoculant, which was the lowest value among the treatments, reaching 82.623. This increase in the percentage of organic matter may be due to the addition of sources of soluble carbohydrates and microbial inoculant, as well as the decrease in the percentage of ash, as indicated in Table (4-2). Siddons et al. (2014) indicated the possibility of using molasses as effective additives in promoting lactic acid fermentation and impeding the fermentation of Clostridium bacteria and proteolysis due to the reduction of the pH, as well as reducing the loss of dry matter. Rajabi et al. (2017) stated that adding dates to Berseem silage led to a significant increase in dry matter, organic matter and total energy, of which dates contain higher levels of Berseem. The addition of molasses stimulated silage fermentation, which in turn led to changes in the chemical composition of the stored feed material. The extent and nature of this fermentation are affected by the type and level of soluble sugars present. This may result in a loss of dry matter at rates that increase with the progression of the ensiling stages (Can and Denek, 2007).

		studied traits				
Sources	Treatments	Dry matter	Organic matter	Organic matter	A neutral fiber extract	
	Without adding	19.338 b	82.795c	18.61 b	55.982 b	
ashahla	Sugars	±0.241	±0.446	±0.25	±0.601	
soluble	Adding molasses	22.903 a	84.887b	19.73 a	55.367 b	
carbohydrate	6%	±0.167	±0.457	±0.20	±1.994	
S	datas summer 0/6	23.086 a	86.368a	17.02 с	58.666 a	
	dates syrup%6	±0.346	±0.688	±0.26	±0.293	
lu	p val	0.0001	0.0004	0.0001	0.014	
5	Sig	**	**	**	*	
	Without adding	21.781 a	84.201a	18.16 b	58.033 a	
microbial	sila prime	±0.558	±0.507	±0.46	±0.715	
inoculant	adding sila prime	21.771 a ±0.562	84.681a ±0.659	18.75 a ±0.25	55.310 b ±1.184	

 Table (3) The effect of soluble carbohydrate source and microbial inoculant on the fermentation

 properties of Berseem silage (as shown in the table) ± standard error

0.006	0.002	0.325	0.975	p valu
**	**	NS	NS	Sig

CFU * colony-forming unit / g fresh alfalfa. The averages bearing different letters differ significantly at the level of * (P < 0.05) or ** (P < 0.01.(

Table (2) The effect of the interaction between the source of soluble carbohydrates and the microbial inoculant on the chemical composition of Berseem silage (% on the basis of dry matter) ± standard error

	studied t	microbial			
A neutral fiber extract	Organic matter	Organic matter	Dry matter	inoculant	Sources
55.032 ab	18.07 c	82.623b	19.323 b	Without adding	Berseem
0.995±	±0.12	±0.489	±0.313	sila prime	silage without
56.932 a	19.15 b	82.939b	19.353 b	adding cila primo	adding
0.309±	±0.29	±0.749	19.353 0	adding sila prime	carbohydrate
59.767 a	20.07 a	84.749ab	23.140 a	Without adding	Berseem
$0.232 \pm$	±0.27	±0.772	±0.266	sila prime	silage with
50.967 b	19.40 ab	84.865ab	22.666 a	Adding sile prime	the adding of
2.368±	±0.18	±0.616	±0.150	Adding sila prime	molasses 6%
59.300 a	16.35 d	85.626a	22.880 a	Without adding	Berseem
$0.212 \pm$	±0.12	±0.733	±0.485	sila prime	silage with
58.032 a	17.70 с	87.110a	23.293 a		adding of
0.300±	±0.10	± 1.144	23.293 a ±0.543	adding sila prime	dates syrup
0.300±	±0.10	±1,144	±0.545		6%
0.0002	0.0001	0.003	0.0001	p valu	
**	**	**	**	Sig	

CFU * blackboard unit / g fresh hay

Averages bearing different letters differ significantly at level * (P < 0.05).

As for the crude protein, the results of the current study showed that there were significant differences (P <0.05) in the crude protein values of Berseem silage as a result of adding sources of soluble carbohydrates, where the value of the addition of molasses excelled on the rest of the factors, amounting to 19.73. While the treatment of adding molasses was the lowest value of the transactions, reaching 17.02. While there were significant differences (P <0.05) between the protein values in the treatments as a result of adding the microbial inoculant to the silage, the highest value was 18.75 in the

treatment of adding the microbial inoculant, while the value of the treatment without adding the microbial inoculant was 18.16 the lowest value for the Berseem silage. As for the effect of the overlap between treatments for protein, where the results indicated that there were significant differences (P <0.05) between the values of the treatments, where the treatment of adding molasses without adding the microbial inoculant excelled on the rest of the treatments, reaching 20.07. While the treatment of adding molasses with the addition of the microbial inoculant excelled on the treatment without adding carbohydrates with the addition of the microbial inoculant, which excelled on the treatment of adding Berseem and without adding the microbial inoculant, which in turn excelled on the treatment without adding soluble carbohydrates and without adding the microbial inoculant and the treatment of adding dates syrup with the addition of the microbial inoculant, which was the lowest value among treatment was 16.35. The increase in the protein ratio can be explained by the added carbohydrate sources (dates syrup and molasses) and the microbial inoculant, while the slight decrease in the treatment of adding molasses without adding the inoculant may be due to the dates syrup containing soluble sugars suitable for proteolytic bacteria. Rasool (1999) indicated that using molasses may not only improve the energy content of silage, but also ensure a low pH and prevent proteolysis. Adesogan (2006) notes that the only effect of using molasses on fermentation may be a decrease in the concentration of ammonia nitrogen, indicating a lower rate of proteolysis compared to untreated silage. While Franco et al. (2019a) explained in a study they conducted on the effect of various additives (lactic acid bacteria, formic acid and formic acid salts) to improve the quality of fermentation and aerobic stability of red Berseem silage, the results indicated that there were no significant differences in protein content in the treatment of adding acid bacteria. Lactic as compared to control treatment. And in silage grass (Franco et al., 2019b.) Baytok et al. (2005) showed that adding molasses led to an increase in crude protein. Moreover, the use of lactic acid bacteria inoculants improved the quality of silage fermentation, which could lead to a decrease in the decomposition of crude protein in silage, and a decrease in the loss of nutrients, including protein, accordingly (Cao et al., 2013). It was observed in the study of Jatkauskas and Vrotniakiene (2006) in the silage of mixed grass and legumes as well as in the study of Winters et al. (2002) in the study of red Berseem silage .Siddons et al. (2014) demonstrated the possibility of using molasses as effective additives in promoting lactic acid fermentation and hindering the fermentation of Clostridium bacteria and proteolysis due to the reduction of the pH, as well as reducing the loss of dry matter. The results of Li et al. (2010) study showed that adding the plantarum L. inoculant increased the protein content by rates ranging between 0.14 and 0.62%. The results of the

current study also showed that there were significant differences (P < 0.05) in the values of the neutral fiber extract NDF of Berseem silage due to the addition of sources of soluble carbohydrates, where the value of the addition of molasses excelled on the rest of the workers and reached 58,666, while the treatment of adding molasses was the lowest value in the transactions, reaching 55,367. Whereas, significant differences (P < 0.05) were found between the values of neutralized fiber extract in the treatments as a result of adding microbial inoculant to Berseem silage, where treatment without adding microbial inoculant was superior to 58.033, while the value of adding microbial inoculant was 55,310, which is the lowest value for Berseem silage. As for the effect of the interaction between the neutral fiber extract treatments, where the results indicated the presence of significant differences (P < 0.05) between the values of the treatments. As the treatment of adding molasses and without adding the microbial inoculant to Berseem silage excelled on the rest of the treatments, it reached 59.767, and the treatment of adding molasses with and without adding the microbial inoculant excelled on the treatment without adding soluble carbohydrates with the addition of the microbial inoculant, which outperformed the treatment without adding the soluble carbohydrates and without the microbial inoculant and the treatment of adding molasses. With the addition of the microbial inoculant, which was the lowest value among the treatments, reaching 50.967. While Baytok et al. (2005), the enhancement of cell wall degradation due to the increased fermentation of silage due to addition of molasses is a possible cause of the decrease in silage content of neutral and acidic fiber extract. Abarghoei et al. (2011) showed that the decrease in neutralizing fiber extract content in silage to cell wall degradation by fiberolytic clostridia or acid hydrolysis (McDonald et al, 1991). Effect of addition of different sources of soluble carbohydrates and microbial inoculant on the fermentation properties of Berseem silage. Table (3 and 4) represent the effect of adding different sources of soluble carbohydrates and microbial inoculants on the properties of Berseem silage fermentation and the effect of interaction between them, which included pH, soluble sugars, lactic acid, total volatile fatty acids and individual volatile fatty acids, including stearic acid, propionic acid, Butyric acid, ammonia nitrogen. The results of the current study showed significant differences (P <0.05) in the pH values of Berseem silage due to adding sources of soluble carbohydrates, where the value of treatment without adding soluble carbohydrates excelled on the rest of the treatments and reached 5,301 while the treatment of adding dates syrup was the lowest value in the treatments, reaching 4,078, There were no significant differences between the treatment of adding molasses and the treatment of adding dates syrup. While there were no significant differences (P <0.05) between the values of the treatments due to the addition of the Sila Prime microbial inoculant to

the Berseem silage. As for the effect of the interaction between the values of the pH treatments in the current study, where there were significant differences (P < 0.05) between the values of the treatments, the treatment without adding excelled on the soluble carbohydrates without adding the microbial inoculant Sila Prime to the Berseem silage on the rest of the treatments. The highest value was 5,353 for treatment without adding soluble carbohydrates with the addition of the microbial inoculant, while there were no significant differences between the treatments for adding soluble carbohydrates with or without adding the microbial inoculant. While the treatment of adding dates syrup with adding the microbial inoculant was the lowest value among the treatments, reaching 4.076. The decrease in pH can be explained by the appropriate additions of dissolved carbohydrates, which provided the necessary energy for the lactic acid bacteria, and the increase in lactic acid concentrations as a result of this, the high acidity of the silage, and thus the decrease in the pH. Li et al. (2010) indicated that adding soluble sugars with the insecure rapid and effective fermentation that led to the rapid production and accumulation of lactic acid in early stages of ensiling, which led to a rapid decrease in the pH, and that adding molasses by 5% would be sufficiently effective. To stimulate silage fermentation by producing lactic acid and lowering the pH of silage (Yunus et al., 2000). As the lactic acid bacteria can, during the silage, represent the soluble carbohydrates with high efficiency to produce lactic acid in sufficient quantities to reduce the pH, inhibit the growth of harmful bacteria and improve the quality of silage (Cai et al., 2003).Li et al. (2010) indicated that adding soluble sugars with the insecure rapid and effective fermentation that led to the rapid production and accumulation of lactic acid in early stages of ensiling, which led to a rapid decrease in the pH, and that adding molasses by 5% would be sufficiently effective. To stimulate silage fermentation by producing lactic acid and lowering the pH of silage (Yunus et al., 2000). As the lactic acid bacteria can, during the silage, represent the soluble carbohydrates with high efficiency to produce lactic acid in sufficient quantities to reduce the pH, inhibit the growth of harmful bacteria and improve the quality of silage (Cai et al., 2003). Siddons et al. (2014) has shown to prove that the possibility of using molasses as effective additives in promoting lactic acid fermentation and hindering the fermentation of Clostridium bacteria and proteolysis due to the reduction of the pH, as well as reducing the loss of dry matter. Saeed (2012) stated that using molasses at a rate of 10% apparently secured the microorganisms of silage obtaining the necessary energy to ferment the soluble sugars lacking in straws and similar dry forages, thus increasing the activity of these organisms and producing large quantities of lactic acid, which enhances the decrease in the acidity. PH. As for the soluble sugars, the results of the current study showed significant differences (P <0.05) in the soluble sugars of Berseem silage as a result of adding sources of soluble carbohydrates, where the value of the addition of molasses and the addition of dates syrup, which amounted to 51,480 and 47,000 respectively, did not show significant differences. While the treatment without adding soluble carbohydrates was the lowest value in the treatments, reaching 20.728. Also, significant differences (P < 0.05) were found between the values of soluble sugars in the treatments as a result of adding the microbial inoculant Sila Prime to the Berseem silage, where the addition of the microbial inoculant excelled on the second treatment and reached 47,355, while the value of the treatment without adding the microbial inoculant was 32.118, which is the lowest value. As for the effect of the Interaction between the values of soluble sugars in the treatments, the results of the current study showed that there are significant differences (P <0.05) between the values of the treatments. As the treatment of adding molasses with the addition of the microbial inoculant to Berseem silage, which amounted to 69,096, excelled on the treatments of adding dates syrup without adding the microbial inoculant and the treatment of adding molasses and without the microbial inoculant, and these treatments did not show significant differences between them, which in turn excelled on the treatment without adding soluble carbohydrates with the addition of the inoculant. Microbiome, which was the lowest value among the treatments, reaching 19,514. The reason for the excelled of these treatments may be due to the carbohydrate additives, which led to an increase in the level of soluble sugars remaining in the Berseem silage. As for the low level of soluble sugars in treatment without adding soluble carbohydrates, it may be due to the lack of Berseem, which is considered one of the leguminous feeds, where Soluble sugars represent the main substance exposure to the activity of lactic acid bacteria (Rehman, 2011). Whereas the results of Li et al. (2010) showed that it is preferable to add the lactic acid bacteria inoculant with glucose in improving the quality of fermentation compared to adding the inoculant alone. In addition, the addition of the inoculant with glucose increased the dry matter and residual content of soluble sugars, as well as the crude protein(Aksu et al., 2006). Pereira et al. (2006) showed that the fermentation pattern, and hence the quality of silage, is influenced by many factors, including the dry matter content, the amount of soluble carbohydrates available, and colonies of basic lactic acid bacteria. In a study conducted by Franco et al. (2019a) on the effect of various additives (lactic acid bacteria, formic acid and formic acid salts) to improve the quality of fermentation and aerobic stability of red Berseem silage, the results indicated a lower level of soluble sugars in the treatment of adding lactic acid bacteria compared to the rest of the treatments.Franco et al. (2019) indicated a decrease in the soluble sugars in the treatment of adding lactic acid bacteria compared to the rest of the treatments in the grass silage. The sugar content, defined as the total amount soluble in fermentable water by fermented carbohydrate lactic acid bacteria, can be determined by the ability of plants to form silage (Steinhofel, 2008). The amount of fermentable base material required for the fermentation process varies from one crop to another and depends on various factors. The greater the buffer capacity and moisture content, the greater the amount of substrate required for fermentation (Muck, 1988). The results of the current study showed that there were significant differences (P < 0.05) in lactic acid concentrations of Berseem silage due to the addition of sources of soluble carbohydrates. Where the value of adding molasses and adding dates syrup, which reached 313,548 and 283,031 respectively, the treatment without adding excelled on soluble carbohydrates, which was the lowest in the treatments, reaching 124,548. The results also showed that there were significant differences (P <0.05) between the lactic acid values in the treatments as a result of adding the Sila Prime microbial inoculant to the Berseem silage, where the addition of the microbial inoculant excelled on the second treatment and reached 266.859, while the value of the treatment without adding the microbial inoculant was 213.00, which is the lowest values. As for the effect of the interaction between the lactic acid values in the treatments of Berseem silage, where the results of the current study showed that there are significant differences (P < 0.05) between the values of the treatments, where the treatment of adding molasses with the addition of the microbial inoculant to Berseem silage, which amounted to 364.386, excelled on the rest of the treatments. While the treatment of adding molasses without adding the microbial inoculant and the two treatments of adding dates syrup without adding the inoculant, which did not show significant differences between them, treatment without adding excelled on soluble carbohydrates with and without adding the microbial inoculant, which showed the lowest value among the treatments, reaching 122,515 and 126,580, respectively. This is due to the addition of soluble carbohydrates, which led to an increase in the numbers of lactic acid bacteria to provide the necessary energy for them, and thus the level of lactic acid concentrations increased as a result. Paya et al. (2015) concluded that the addition of inoculant to ensiling secured rapid and effective fermentation that led to rapid production and accumulation of lactic acid in the early stages of ensiling, which led to a rapid decrease in the pH. While Franco et al. (2019a) indicated in a study they conducted on the effect of various additives (lactic acid bacteria, formic acid and formic acid salts) to improve the quality of fermentation and aerobic stability of red clover silage, the results indicated a decrease in the concentration of lactic acid in the treatment of adding lactic acid bacteria compared to with a control treatment. Moreover, adding molasses would provide sufficient levels of energy to silage microorganisms, which leads to an increase in the rate of fermentation and production of lactic acid (Balakhial et al., 2008). The decrease in the pH level of silage may be associated with the addition of molasses alone or with the lactic acid bacteria inoculant to the increase in the production of lactic acid, which may be due to the increase in the number of microorganisms consuming the largest amount of available sugars (Kaiser et al., 2004). The lactic acid bacteria inoculant will not be able to produce sufficient quantities of lactic acid to reduce the pH to acceptable levels without adequate quantities of soluble sugars in the original feed crop (Seale, 1986. Therefore, sugar or other sugar-rich materials must be added to ensure good quality of fermentation in Silage sample (Li et al. 2010). As for the concentration of total volatile fatty acids, the results of the current study showed that there were significant differences (P <0.05) in the total volatile fatty acids values of Berseem silage due to the addition of sources of soluble carbohydrates. Where the value of treatment without adding soluble carbohydrates excelled on the rest of the workers, as it reached 1.383, while the treatment of adding dates syrup was the lowest value in the treatments, reaching 1.000. While there were no significant differences between the total volatile fatty acid values in the treatments as a result of adding the microbial inoculant to the silage. As for the effect of the interaction between the values of the total volatile fatty acid treatments, the results also did not show significant differences (P < 0.05) between the values of the treatments as a result of adding the sources of dissolved carbohydrates and the microbial inoculant to Berseem silage. The reason for the slight decrease in the total volatile fatty acids when adding soluble carbohydrates may be due to the reduction in the activity of some microorganisms due to the low pH. The addition of inoculants leads to an increase in the average of fermentation, resulting in a significant decrease (P < 0.05) in the pH, accompanied by a significant increase (P < 0.05) in the total concentration of fermentation acids (Acosta-Aragon et al., 2012). Arbabi and Ghoorchi (2008) indicated that the increased concentration of volatile fatty acids was the result of an increase in molasses level. This increase in the concentration of volatile fatty acids may be due to the activity of microorganisms, represented by lactic acid bacteria, which work to produce acids as a result of their representation of available sugars. Kaiser et al. (2004) indicated that the increase in fatty acid production may be due to the increase in the number of microorganisms naturally present on crops prepared for ensiling that consume the largest amount of available sugars. Schroeder et al. (2013) stated that volatile fatty acids represent the final product of the decomposition or assimilation of soluble sugars under anaerobic conditions.

 Table (3) The effect of soluble carbohydrate source and microbial inoculant on the fermentation

 properties of Berseem silage (as shown in the table) ± standard error

studied traits						
Ammonia nitrogen % from total nitrogen	Total volatile fatty acids mEq % dry matter	Lactic acid Mmol / L	soluble sugars Mg / dL	рН	Treatments	Sources
0.104 a ±0.009	1.383 a ±0.078	124.548 b ±10.94	20.728 b ±2.264	5.301 a ±0.035	Without adding Sugars	
0.111 a ±0.003	1.066 b ±0.101	313.548 a ±29.861	51.480 a ±8.453	4.181 b ±0.027	Adding molasses 6%	soluble carbohydrates
0.112 a ±0.001	1.000 b ±0.026	283.031 a ±15.81	47.000 a ±7.880	4.078 b ±0.039	dates %6 syrup	
0.588	0.0007	0.0001	0.003	0.0001	p valu	
NS	**	**	**	**	Sig	
0.114 a ±0.003	1.166 a ±0.082	213.892 b ±23.904	32.118 b ±4.634	4.488 a ±0.165	Without adding sila prime	microbial inoculant
0.104 a	1.133 a	266.859 a	47.355 a	4.552 a	adding sila	moculant
±0.005	±0.074	±32.925	±7.762	±0.172	prime	
0.140	0.724	0.015	0.037	0.119	p valu	
NS	NS	*	*	NS	Sig	

CFU * blackboard unit / g fresh hay

The averages bearing different letters differ significantly at the level of * (P <0.05) or ** (P <0.01).

 Table (4) The effect of the interaction between the source of soluble carbohydrates and the

 microbial inoculant on the properties of Berseem silage fermentation (according to the units

 shown against the fermentation criteria in the table) ± the standard error

		studied traits	5			
Ammonia nitrogen mg / 100 ml	Total volatile fatty acids mEq % dry matter	Lactic acid Mmol / L	soluble sugars Mg / dL	рН	Microbial inoculant	Treatments
0.114 a ±0.011	1.366 a ±0.084	126.580 c ±23.54	21.943 b ±4.567	5.250 a ±0.061	Without adding sila prime	Berseem silage without
0.093 a ±0.015	1.400 a ±0.147	122.515 c ±1.437	19.514 b ±1.444	5.353 a ±0.012	adding sila prime	adding carbohydra tes
0.116 a ±0.003	1.133 a ±0.209	262.709 ab ±40.80	33.864 ab ±8.514	4.136 b ±0.039	Without adding sila prime	Berseem silage with the
0.105 a ±0.004	1.000 a ±0.040	364.386 a ±27.804	69.096 a ±7.356	4.226 b ±0.024	Adding sila prime	addition of molasses 6%
0.111 a ±0.001	1.000 a ±0.040	252.386 b ±15.387	40.546 ab ±9.147	4.080 b ±0.071	Without adding sila prime	Berseem silage with the
0.112 a ±0.002	1.000 a ±0.040	313.677 ab ±17.434	53.454 ab ±13.354	4.076 b ±0.047	adding sila prime	addition of dates syrup 6%
0.416	0.05	0.0001	0.003	0.0001	p valu	
NS	*	**	**	**	Sig	5

CFU * blackboard unit / g fresh hay

Reference

- Abarghoei, M., Y. Rouzbehan and D. Alipour. (2011). Nutritive value and silage characteristics of whole and partly stoned olive cakes treated with molasses. J. Agric. Sci. Tech.13: 709-716.
- Acosta Aragon, Y., I. Rodrigues, U. Hofstetter and E. M. Binder. (2011). Mycotoxins in Silages: Occurrence and Prevention. Iranian J. Appl. Anim. Sci. 1 (1): 1-10
- 3. Adesogan, A. T. (2006). Factors Affecting Corn Silage Quality in Hot and Humid Climates.

In: Proc. 17th Florida Ruminant. Nutrition Conf. 108–127.

- 4. Aksu, T., E. Baytok, M. A. Karsli and H. Muruz (2006). Effects of formic acid, molasses and inoculant additives on corn silage composition, organic matter digestibility and microbial protein synthesis in sheep. Small Rumin. Res. 61:29-33.
- Arbabi, S., T. Ghoorchi and S. Hasani. (2010). The effect of delayed ensiling and application of propionic acid-based additives on the nutrition value of corn silage. Asian J. Anim. Sci. 4 (3): 132-140.
- Babaeinasab, Y., Y. Rouzbehan, H. Fazaeli and J. Rezaei (2015). Chemical composition, silage fermentation characteristics, and in vitro ruminal fermentation parameters of potatowheat straw silage treated with molasses and lactic acid bacteria and corn silage. J. Anim. Sci. 93:4377–4386.
- Balakhial, A., A. Naserian., A. H. Moussavi., F. E. Shahrodi., M. ValiZadeh. (2008). Changes in chemical composition and in vitro DM digestability of Urea and Molasses treated whole crop canola silage. J. Anim. Vet. Adv. 7(9): 1042-1044.
- Baytok, E., T. Aksu, M. A. Karsli, and H. Muruz (2005). The effects of formic acid, molasses and inoculant as silage additives on corn silage composition and ruminal fermentation in sheep. Turk. J. Vet. Anim. Sci. 29:469–474.
- 9. Brink, G. E. and T. E. Fairbrother (1988). Cool- and warm-season forage legume potential for the southeastern USA. Trop. Grassl., 22 (3): 116-125.
- Cai, Y., Y. Fujita, M. Murai, M. Ogawa, N. Yoshida, R. Kitamura and T. Miura (2003). Application of lactic acid bacteria (*Lactobacillus plantarum* Chikuso-1) for silage preparation of forage paddy rice. Japanese Journal of Grassland Science, 49 (5): 477–485.
- 11. Cao, Y., Y. Cai and T. Takahashi (2013). Ruminal digestibility and quality of silage conserved via fermentation by Lactobacilli. <u>http://dx.doi.org/10.5772/50816. -L13</u>.
- 12. Catchpoole, V. R and E. F. Henzell.(1971). Silage and silage- making from tropical herbage species. Herbage Abstr.41:213.
- 13. Chauhan, T. R.; Gupta, R.; Chopra, A. K., 1992. Comparative nutritive value of legume

hays fed to adult buffaloes. Buffalo J., 8 (3): 243-246.

- 14. Das, A. and G. P. Singh(1999) . Effect of different levels of berseem (*Trifolium alexandrinum*) supplementation of wheat straw on some physical factors regulating intake and digestion. Anim. Feed Sci. Technol., 81 (1-2): 133-149.
- 15. Denek, N. and A. Can (2007). Effect of wheat straw and different additives on silage quality and In vitro dry matter digestibility of wet orange pulp. J. Anim. Vet. Adv.6; 217.
- 16. Filya, I., G. Ashbellb, Y. Henb and Z. G. Weinberg (2000). The effect of bacterial inoculants on the fermentation and aerobic stability of whole crop wheat silage. Anim. Feed Sci. Technol., 88: 39-46.
- Franco, M., T. Stefansk, T. Jalava, A. Huuskonnen and M. Rinne (2019a) .Additive improve fermentation quality and aerobic stability of red clover silage under challenging condition. 18th International Symposium FORAGE CONSERVATION. Brno, Czech Republic, August 13-16, 2019.pp. 110-111.
- Franco, M., T. Stefansk, T. Jalava, A. Huuskonnen and M. Rinne (2019b). Effect of compaction, soil contamination and additive treatments on grass silage quality. 18th International Symposium FORAGE CONSERVATION. Brno, Czech Republic, August 13-16, 2019.pp. 108-109.
- Fraser, J., D. McCartney, H. Najda andZ. Mir (2004). Yield potential and forage quality of annual forage legumes in southern Alberta and northeast Saskatchewan. Can. J. Plant Sci., 84 (1): 143-155.
- 20. Fulkerson, W. J., J. S. Neal, C. F. Clark, A. Horadagoda, K. S. Nandra and I. Barchia (2007). Nutritive value of forage species grown in the warm temperate climate of Australia for dairy cows: Grasses and legumes. Livest. Sci., 107 (2-3):253-264.
- 21. Goering, H. K and P.J. Van Soest. (1970). Forage Fiber Analysis (apparatus, reagents, prosedures and some applications). USDA Agricultural Handbook No. 379.
- 22. Hannaway, D. B. and C. Larson (2004). Berseem Clover (Trifolium alexandrinum L.).

Oregon State University, Species Selection Information System.

- 23. **Hashemi, M. (1991).** Livestock, bird, and aquatic animal's nutrition (foods, feeding, and rations). Tehran: FarhangJame' Publications. First print. P930.
- 24. Jatkauskas, J. and V. Vrotniakienė (2006). Effects of silage fermentation quality on ruminal fluid parameters. Biologija. Nr. (4): 65–71.
- 25. Jeroch, D., W. Drochner and O. Simon (1999). Ernährung landwirtscha flichen Nutztiere. Berlin, Springer Verlage, 544 S.
- 26. Kaiser, A.G. (2004). Silage additives. In Successful silage, 2nd ed (Eds A.G. Kaiser, J.W. Piltz, H.M. Burns, & N.W. Griffiths), pp. 171–196. New South Wales, Australia: Dairy Australia and New South Wales Department of Primary Industries.
- Kazemi-Bonchenari, M., K. Rezayazdi., A. Nikkhah., H. Kohram and M. Dehghan-Banadaky. (2010). The effects of different levels of sodium caseinate on rumen fermentation pattern, digestibility and microbial protein synthesis of Holstein dairy cows. Afri. J. Biotech., 9: 1990-1998.
- Keady, T. W. J and J. J. Murphy (1997). The effects of treating low dry matter herbage with a bacterial inoculant or formic acid on the intake and performance of lactating dairy cattle. Anim. Sci. 64: 25–36.
- Kim, D., K. D. Lee and K. C. Choi (2021). Role of LAB in silage fermentation: Effect on nutritional quality and organic acid production—An overview. ALMS Agriculture and food, 6(1):216-234.
- 30. Levital, T., A.F. Mustafaa., P. Seguinb and G. Lefebvre (2009). Effects of a propionic acidbased additive on short-term ensiling characteristics of whole plant maize and on dairy cow performance. Anim. Feed Sci.Tech. 152: 21–32.
- 31. Li, J., Y. Shen and Y. Cai (2010). Improvement of fermentation quality of rice straw silage by application of a bacterial inoculant and glucose. Asian-Aust. J. Anim. Sci. 23(7): 901- 906.
- 32. McDonald, P., A. R. Henderson and S. J. E. Heron (1991). Microorganisms. In The

Biochemistry of Silage, Chapter 4, 2nd edn ed. McDonald, P., Henderson, A.R. and Heron, S.J.E. pp. 81–151. Abersytwyth, UK: Chalcombe Publications.

- 33. Muck, R. E. (1988). Factors influencing silage quality and their implications for management. Journal of Dairy Science. 71:2992-3002.
- 34. Pahlow, G., R. E. Muck., F. Driehuis., S. J. Oude Elferink and S. F. Spoelstra (2003). Microbiology of ensiling. Pages 31–93, in Silage Science and Technology. L Al-Amoodi, ed. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI. Pakistan. Int. J. Agri. Biol., 2002; 4: 186-192.
- 35. **Pasandi, m., R. Kamali and A. Kaviani (2012).** Use of sugar beet molasses to improve fermentation of sweet corn stem and leaf silage. Animal Science Journal 25:27-32.
- 36. Paya, H., A. Taghizadeh and S. Lashkari (2015). Effects of Lactobacillus plantarum and hydrolytic enzymes on fermentation and ruminal degradability of orange pulp silage. J. BioSci. Biotechnol. 4(3): 349-357.
- 37. Pereira, O. G., E. M. Santos, C. L. L. F. Ferreira, H. C Mantovani and D. C. S. Penteado (2006). Populações microbianas em silagem de capim-mombaça de diferentes idades de rebrotação. Anais da XLIII Reunião anual da sociedade brasileira de zootecnia. João PessoaPB, julho.
- Phillip, L. E. and V. Fellner (1992). Effects of bacterial inoculation of high moisture ear corn on its aerobic stability, digestion and utilization for growth by beef steers. J. Anim. Sci. 70, 3178–3187.
- 39. Rajabi, R, R. Tahmasbi, O. Dayani and A. Khezri (2016). Effect of addition of wasted date on chemical composition of ensiled alfalfa and rumen digestibility and rumen fermentation parameters in Kermani sheep .Animal Science Journal (Pajouhesh & Sazandegi) No 114 pp: 77-88.
- 40. Rasool, S., S.H. Raza and T.Ahmad (1999). Rumen metabolism of sheep fed silage containing poultry litter. FAO Electronic Conference on Tropical Silage.recent developments.

Grass and Forage Science. 68: 1-19.

- 41. **Rehman, A. U. (2011).** Chemical composition of oat silage and urea treated wheat straw as influenced by exogenous fibrolytic enzymes. MSc thesis, University of Agriculture, Faisalabad.
- 42. Saeed, A. A. (2012). Effect of addition of urea and ensiling period on the quality and chemical composition of wheat straw silages. Alqadisya J. Agric. Sci. 2 (2): 1-1.
- 43. Schroeder, J.W. (2013). Silage fermentation and preservation. NDSU Extension Service. AS1254.
- 44. Seale, D. R. (1986). Bacterial inoculants as silage additives. J. of Applied Bacteriology, vol. 61, no. 15, pp. 9–26,.
- 45. Sharma, V. V. and P. C. Murdia (1974). Utilization of berseem hay by ruminants. J. Agr. Sci., 83 (2): 289-293.
- 46. Siddons, R. C., C. Arricastres, D. L. Gale and D. E. Beever (2014). The effect of formaldehyde or glutaraldehyde application to lucerne before ensiling on silage fermentation and silage N digestion in sheep. British J. Nutr. 52, 391-401Silageprojekt. LFZ Raumberg-Gumpenstein, Institut Pflanzenbau undSilierung und Nacherwärmung von Silomais. Landtechnik. 59: 218-219.
- 47. Taylor, K.A.(1996). A simple colorimetric assay for muramic acid and lactic acid. Applied Biochemistry and Biotechnology. Jan 1;56(1):49-58.Technology. Agronomy Monograph no. 42. Madison, WI: American Society .
- 48. Wilkinson, J. M. and D. R. Davies. (2012). The aerobic stability of silage: key findings and recent developments. Grass and Forage Science. 68: 1-19.
- 49. Woolford, M. K. (1984). The silage fermentation. Microbiology Series vol. 14. New York.
- 50. Yang, H. Y., X. F. Wang, J. B. Liu, L. J. Gao, M. Ishii, Y. Igarashi and Z. J. Cui (2006). Effect of water soluble carbohydrate content on silage fermentation of wheat straw. J. Bioscience and Bioengineering. 101 (3): 232-237.

- 51. Yoelao, K., M. G. Jackson and I. Saran (1970). The effect of wilting berseem and lucerne herbage on voluntary drymatter intake by buffalo heifers. J. Agricultural Sci., 74 (01): 47-51.
- 52. Yunus, M., N. Ohba., M. Furuse and Y. Masuda (2000). Effects of adding urea and molasses on napier grass silage quality. Asian-Aus. J. Anim. Sci. 13:1542.