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Physico-Chemical Properties and Digestibility of Watermelon Vines Ensiled with Urea and Molasses

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Abstract

An experiment was undertaken to estimate some physico-chemical properties and nutrients digestibility of watermelon vines ensiled with 2% urea and four different levels of molasses (0%, 5%, 10% and 15%) for 30 days. A completely randomized design with 4X5 (4 levels and 5 incubation period) for factorial arrangements was adopted for executing the experiment. The ensiled materials before and after 8, 15, 22, 30 days of incubation were tested for chemical composition, lactic and acetic acids concentration. In-vitro dry matter digestibility (IVDMD), in-vitro crude protein digestibility (IVCPD) and in-vitro crude fiber digestibility (IVCFD) were measured at the last day of the fermentation period. Addition of molasses and fermentation period had significant ($P\leq0.05$) effects on physico-chemical properties of the silage of watermelon vines. The pH value of the silage with 0% molasses was relatively higher than in silage with 15% of molasses and decreased with increasing fermentation period. The dry matter, crude protein contents, lactic and acetic acids concentration were significantly ($P\leq0.05$) increased with increasing molasses levels and fermentation times while crude fiber content was decreased. The highest values of IVDMD, IVCPD and IVCFD were recorded by silage supplemented with 15%, 10% and 5% of molasses, whereas the lowest value was recorded by 0% of molasses. The increase of molasses levels resulted in significant ($P\leq0.05$) improvement of physical characteristics (color and smell) of watermelon vines silage. This study indicated that the addition of molasses enhanced the physico-chemical and nutritional properties of the silage of watermelon vines incubated with urea.

Key words: watermelon vines, silage, lactic, acetic acid, digestibility

المستخلص

أجريت التجربة لتقدير بعض الخصائص الفيزيوكيميائية وهضمية المغذيات لعرشة البطيخ المسولجة بإضافة 2% يوريا ومستويات مختلفة من المولاس (0% , 5% , 10% و 15) لمدة 30 يوما. نفذت التجربة بالتصميم كامل العشوائية بالتوزيع العاملي. فتحت المواد المسولجة أسبوعيا لمدة (أربعة اسابيع) لتحديد المتغيرات الفيزيوكيميائية و وتركيز حمضي اللاكتيك والأسيتك . تم قياس الهضمية لكل من المادة الجافة والبروتين الخام والألياف الخام عند أخر يوم لفترة التخمير. أشارت النتائج أن لإضافة المولاس وفترة التخمير تأثير معنوى ($P\leq0.05$) على الخصائص الفيزيوكيميائية . كانت أعلى قيمة للأس الهيدروجيني للسيلاج عند مستوى 0% مولاس مقارنة ب 15% مولاس وقلت هذه النسبة بزيادة مستوى المولاس وفترة التخمير . زاد محتوى المادة الجافة والبروتين الخام للسيلاج المعامل بالنسب 5% و 10% و 15% زيادة معنوية ($P\leq0.05$)

خلال فترة التخمير بالمقارنة مع 0% مولاس. علاوة على ذلك كان للمستوى العالى من المولاس (15%) تأثير معنوى ($P \leq 0.05$) فى تقليل محتوى الألياف الخام. وعلى صعيد أخر , ارتطبت الزيادة فى مستويات المولاس مع زيادة تركيز حمضي اللاكتيك والأسيتك خلال فترة التخمير . سجلت أعلى هضمية للمادة الجافة والبروتين الخام والالياف الخام عند مستويات 15% , 10% ثم 5% مولاس بينما سجلت أقل هضمية عند المستوى 0% مولاس. نتج عن زيادة مستوى المولاس تحسين معنوى ($P \leq 0.05$) فى الخصائص الفيزيائية للسيلاج (اللون والرائحة) بينما لم يكم هنالك أى تأثير معنوى ($P>0.05$) . على قوام السيلاج . أشارت هذه الدراسة إلي أن إضافة المولاس حسن من الخصائص الفيزيوكيميائية والتغذوية لسيلاج عرشة البطيخ المسولج مع اليوريا.

الكلمات المفتاحية : عرشة البطيخ، السيلاج، حمض اللاكتك و السترك، الهضمية

Introduction

Sudan is a country of diversified ecological conditions including climate, vegetation and soil, which result in an enormous wealth of diversified indigenous genetic resources of crops of which watermelon is an example. The Western part of Sudan is an important region for the diversity of watermelon where different cultivars and uses are known, especially in the Kordofan region. In 2012 the Arab Organization for Agricultural Development (AOAD) estimated that Sudan production of watermelon was 515.00 Million Tons.

In the dry season, pastures dry up and available herbage can hardly meet the maintenance requirement of the animals, particularly in late summer. In Sudan the crop residues have potential to be used as non-conventional roughages practically during dry season (Atta Elmnan *et al.*, 2007; 2009; 2011). However, the high fiber content, low percentage of soluble carbohydrate and relative low levels of fermentable and by-bass protein are responsible for the low nutritional value of these crop residues (Atta Elmnan *et al.*, 2007; 2009; 2011). The improvement of their nutritive value through physical, chemical or biological methods could reduce the bulkiness and enhancing their nutritive value (Alamin, 2008).

Interest in conserving by-products by ensiling is steadily increasing due to the increase in their use as animal feeds (Megias *et al.*, 1998; Kayouli and Lee 2000; Bakshi *et al.*, 2006; Kholif *et al.*, 2007). Properly ensiled silage from high moisture by-products can replace costly feeds such as maize silage in ruminant diets (Itavo *et al.*, 2000; Lallo *et al.*, 2003 and Pirmohammadi *et al.*, 2006).

There is a wide range of valuable by-products and residues resulting from food crops, cropping systems and food processing which are often inefficiently or completely unused or are largely wasted due to the inability of farmers to use them before they spoil, as a result of seasonal production peaks and troughs, consequently these by-products often become pollutants. Therefore, it can be preserved through seasonally abundant for later feeding during periods of feed shortage (Kayouli and Lee 2000). Therefore, the present study was designed to make silage from watermelon vines and determine the effect of different levels of molasses and fermentation period on nutritive value and quality of the watermelon vines silage incubated with 2% urea.

Martials and methods

Study area:

The experiment was conducted in the Laboratory of the Department of Animal Nutrition, Faculty of Animal production, University of Khartoum, Shambat.

Collection of the experimental materials

The watermelon vines were collected from Elseleet area. Commercial sugar cane molasses were purchased from local market.

Silage making

Green watermelon vines (stems and leaves) were chopped treated with 2% urea then the samples were divided into four groups according to the level (0, 5, 10 and 15%) of molasses. All samples were kept into airtight plastic bags and then the preserved samples were observed for physical observation, chemical analysis and in-vitro digestibility determination.

Processing and preservation of the materials

Green watermelon vines were chopped into 2-3cm length by knife and wilted for four days (to reduce water content to be 60%). Three thousand and two hundred grams of chopped watermelon vines were placed in a plastic bowl, mixed well with 2% urea then the quantity was divided into four groups regarding the level of molasses (0, 5, 10 and 15%). The properly mixed samples were poured into previously labeled glass jar, pressed, squeezed sufficiently to make airtight by hand pressure and tightly closed. Then the ensiled samples were kept at room temperature 25°C for 30 days. During incubation period the ensiled samples of each treatment were opened at each fermentation period (0, 8, 15, 22, 30 days). Each level had three replicates for any opening period.

Three silos of each treatment were opened at each fermentation period (30, 35 and 40 days

Observation and collection of samples

During the incubation period samples were taken from each treatment for chemical analysis. Samples were air dried and ground with the grinding machine for about 1mm in diameter for chemical analysis and *in- vitro digestibility* test.

pH value

The pH value was measured by pH meter, three samples were taken from each treatment weekly, these samples were put in a beaker (capacity 50 ml) and mixed with 20 ml of distilled water, and then pH in the tested solution was recorded for each bottle.

Chemical analysis

Treated watermelon vines samples were analyzed for dry matter (DM), crude protein (CP) and crude fiber (CF) according to (AOAC, 1990). Lactic acid and acetic acid concentration were determined using method described by (Dalaly and Alhakim 1987).

In-vitro digestibility

Dry matter samples from each sample were subjected to 48 hrs microbial digestion period with Mc Douglass buffer, rumen fluid mixture in sealed plastic bottles, followed by 48 hrs digestion with pepsin in week acid (Tilley and Terry 1963) at the last day of fermentation period.

Color, smell and texture

The physical changes (color, smell and texture) of all ensiled samples were observed and documented by questionnaire in the last day of the experiment.

Statistical analysis

Data obtained from the experiment were subjected to analysis of variance (ANOVA) according to completely randomize design with 4x5 factorial arrangement (Steel and Torrie 980), means between treatments were compared using the least significant difference (LSD). Data from questionnaire for silage quality was analyzed by (ANOVA) for completely randomize design and the means were separated by the (LSD).

Results and discussion

The effect of addition of different levels of molasses (0%, 5%, 10% and 15%) and incubation period on the nutrients content of the watermelon vines silage incubated with 2% urea was illustrated in Table 1 and Figure 1. The results indicated that the dry matter content was significantly ($P \leq 0.05$) increased with increasing molasses levels and fermentation period. When the ensiling process duration increased a constant increase in dry matter was noticed till the end of experiment. The lowest value was obtained by 0% level of molasses in day zero, whereas the highest value recorded by 15% level molasses in day 30 (Figure 1). The highest DM content recorded by 15% level of molasses in the last day of incubation period may be due to addition of molasses which can improve fermentable sugar contents of ensiling material and improve dry matter content. Man and Wiktorsson (2001) and McDonald *et al.*, (2010) stated that increase in dry matter in the silage correlated with addition of molasses, because the molasses contain high soluble carbohydrates for microbial fermentation, On other hand, Yunus *et al.*, (2000) and Man and Wiktorsson (2001) reported that use of molasses as silage additive led to reduce the loss of nutrient by early pH decline and stabilization of the medium. Similar results was reported by Khan *et al.*, (2006) who indicated that higher dry matter content in grasses ensiled with molasses may be due the reduction of plant and microbial enzymes because of early drop in pH and early constancy of the medium compared with grasses ensiled without molasses.

Table 1 Chemical composition (%) of watermelon vines silage supplemented with different levels of molasses

Level of molasses	DM	CP	CF
0%	44.4^{c}	32.0^{b}	22.2^{a}
5%	45.7^{c}	31.3^{b}	21.2^{ab}
10%	49.6^{b}	32.3^{b}	20.4^{ab}
15%	53.6^{a}	34.6^{a}	19.7^{b}
SEM$\pm$	1.05	0.60	1.21

DM= dry matter, CP= crude protein, CF= crude fiber, SEM= standard Error and a, b, c Mean value in the same column having different superscripts were significantly variable ($P \leq 0.05$).

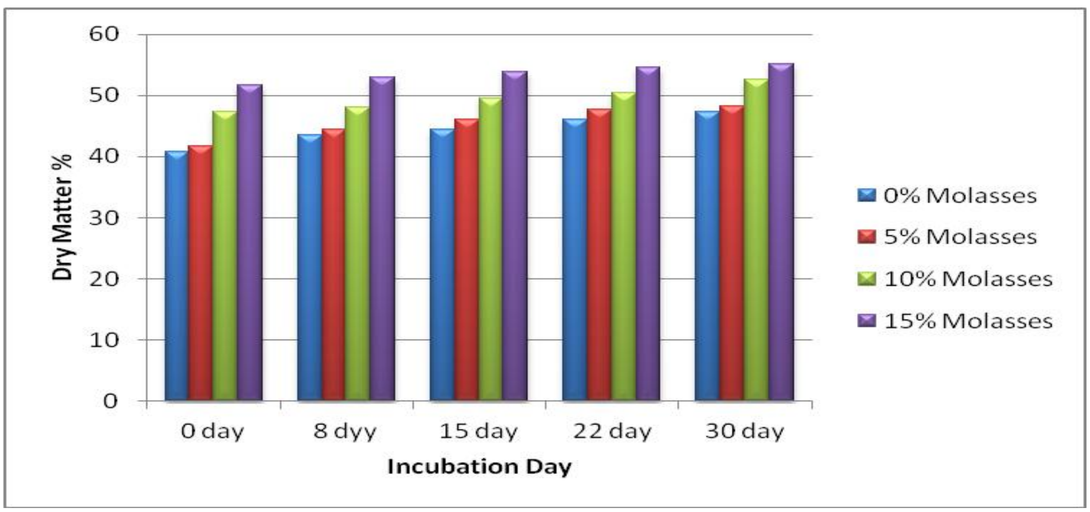

Figure 1 Dry matter content (%) of watermelon vines silage supplemented with different levels of molasses during fermentation period

The addition of different levels of molasses significantly ($P \leq 0.05$) increased crude protein content of watermelon vines silage at level 15% of molasses, whereas there was no significant differences ($P > 0.05$) between control (0%) and other two treatments 5% and 10% molasses Table 1. The lowest value of CP was obtained in 0% and 5% level of molasses in day zero, whereas the highest value recorded by 15% level molasses in day 30 (Figure 2). The increase of crude protein content which associated with the increasing the molasses level may be due to the readily available energy, which was used by the microorganism for the growth and increase microbial protein in silage. Tolera and Sundstol (2000) stated that the microbial nitrogen supply improved the increasing supply of nitrogen, fermentable carbohydrate, sulfur and probably the other essential nutrient. Similar results were observed by Man and Wiktorsson (2003) Sunijders and Wouters (2004) who stated that protein content increased with increasing incubation period and molasses level.

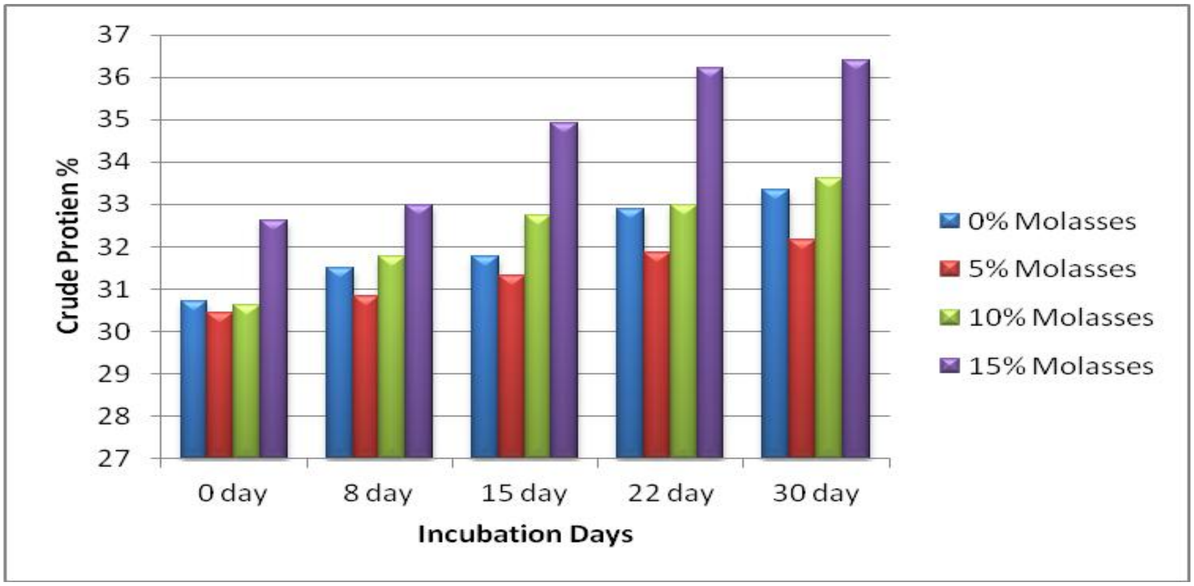

Figure 2 Crude protein content (%) of watermelon vines silage supplemented with different levels of molasses during fermentation period

In this study crude fiber was decreased significantly (P≤0.05) at high level (15%) of molasses Table 1, moreover, increasing incubation period length led to obvious reduction in crude fiber content (Figure 3). After 30 days of fermentation, CF content reduced by about 29.1%, 27.8, 27.1 and 25% for 15%, 10% and 5% level of molasses respectively. This decline in CF content among different treatments may be due to addition of 2% urea to watermelon vines silage. The chemical action of urea on silage disturbs the cell wall component resulting in increasing the soluble fraction (Atta Elmanan *et al.,* 2007). Moreover, may be due to the effect of fermentation occurred during the incubation period led to degradation of one or more of the cell wall components (Bostami *et al.,* 2008). Similar results were observed earlier by Fazaeli *et al.,* (2003) and Guney *et al.,* (2007) who reported that the fiber content reduced due to ensiling period.

The changes in pH value of watermelon vines silage due to molasses and fermentation periods are shown in Table 2 and Figure 4. Maximum pH was found in control (0%) level of molasses and it was decreased with increasing the incubation period, moreover, the association of progressive increase in molasses levels and fermentation period occasioned considerable reduction in pH value Figure 4. This result is powered by previous studies obtained by Becker *et al.*, (1970); Henderson (1993); Tosi *et al.*, (1995), Keady (1996), Liue and Guo (2002), and Mc Donald *et al.* , (2010), who found that the addition of molasses reduced pH value in treated silage.

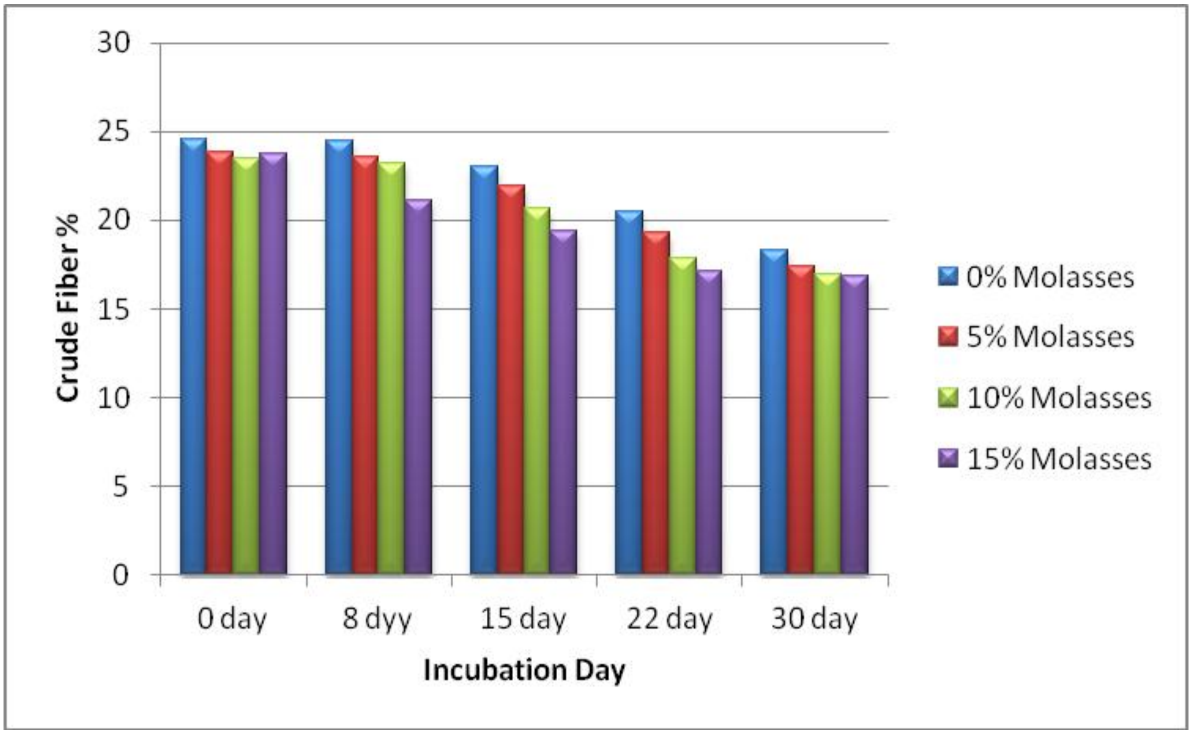

Figure 3. Crude fiber (%) content of watermelon vines silage supplemented with different levels of molasses during fermentation period

Yunus *et al.*, (2000) reported that addition of molasses lowered silage pH due to increase lactic acid production. Addition of molasses to ensiling material has improved the availability of fermentable sugars for anaerobic fermentation that lead to higher acid production and thus lower silage pH (Nisa *et al.*, 2005).

Table 2 pH value of watermelon vines silage supplemented with different levels of molasses

Level of molasses	pH
0%	7.41[a]
5%	6.79[ab]
10%	6.42[bc]
15%	6.00[c]
SEM$\pm$	0.36

SEM= standard Error of mean and a, b, c Mean value in the same column having different superscripts were significantly variable ($P \leq 0.05$).

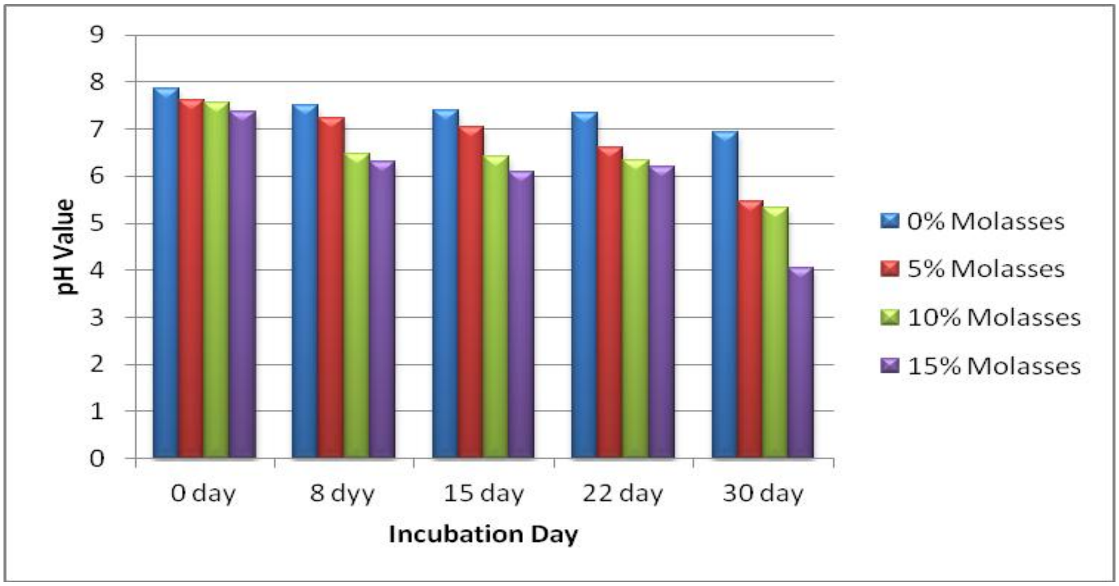

Figure 4 pH value of watermelon vines silage supplemented with different levels of molasses during fermentation period

The concentration of lactic acid and acetic acid content as affected by different levels of molasses and fermentation period were illustrated in Table 3, (Figure 5) and (Figure 6). Minimum lactic acid and acetic acid concentration was found at 0% levels of molasses at all fermentation period. However, lactic acid and acetic acid were steady increased significantly ($P \leq 0.05$) with increase the level of additive and fermentation period (Figures 5 and 6).

The High production of lactic acid content and corresponding lower pH of material ensiled with molasses were attributed to the availability of easily fermentable sugars for better growth of lactic acid bacteria (Bureenok *et al.*, 2005; Sarwar *et al.*, 2006 and Touqir *et al.*, 2007). The high production of lactic acid lowers the silage pH and terminates the microbial activity in ensiled material (McDonaled *et al.*1991 and Bolsen *et al.*, 1996). On other hand the high production of acetic acid may be due to some hydrolysis that occurs during ensilage resulting in liberating pentose's which may be fermented to acetic acid by most types of lactic acid bacteria as mentioned by Nisa *et al.*, (2005) and Wilkinson (2005).

Table 3 Lactic acid and acetic acid (%) concentration of watermelon vines silage supplemented with different levels of molasses

Level of molasses	Lactic acid	Acetic acid
0%	0.15^{b}	0.10^{b}
5%	0.22^{ab}	0.15^{ab}
10%	0.35^{a}	0.23^{a}
15%	0.40^{a}	0.27^{a}
SEM	0.09	0.06

SEM= standard Error of mean and a, b, c Mean value in the same column having different superscripts were significantly variable ($P \leq 0.05$).

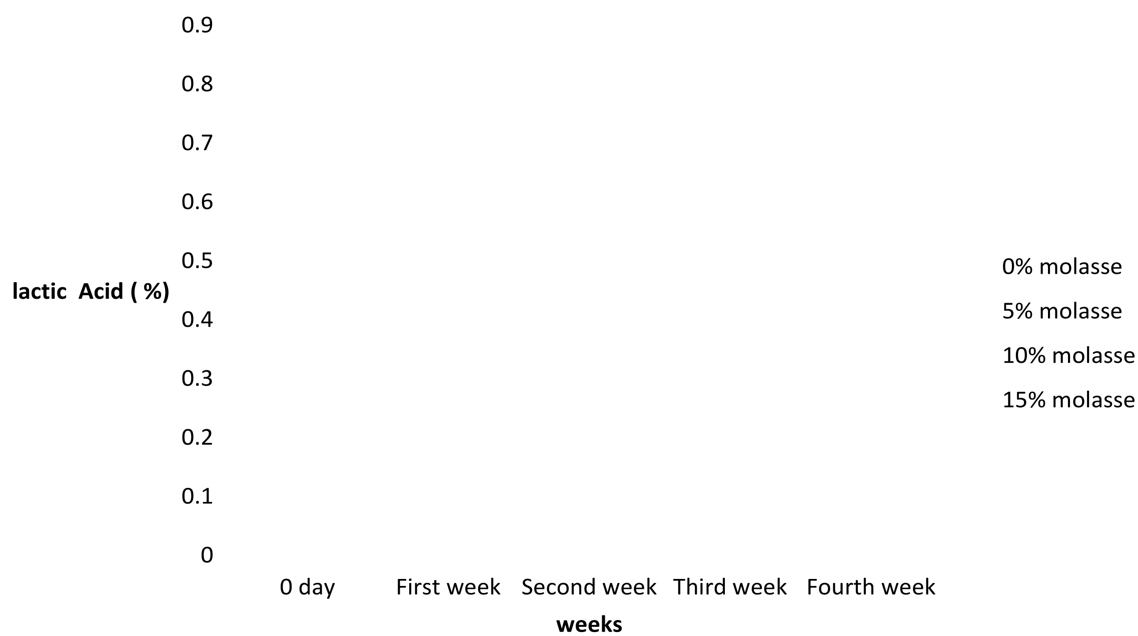

Figure 5 Lactic acid concentration (%) of watermelon vines silage supplemented with different levels of molasses during fermentation period

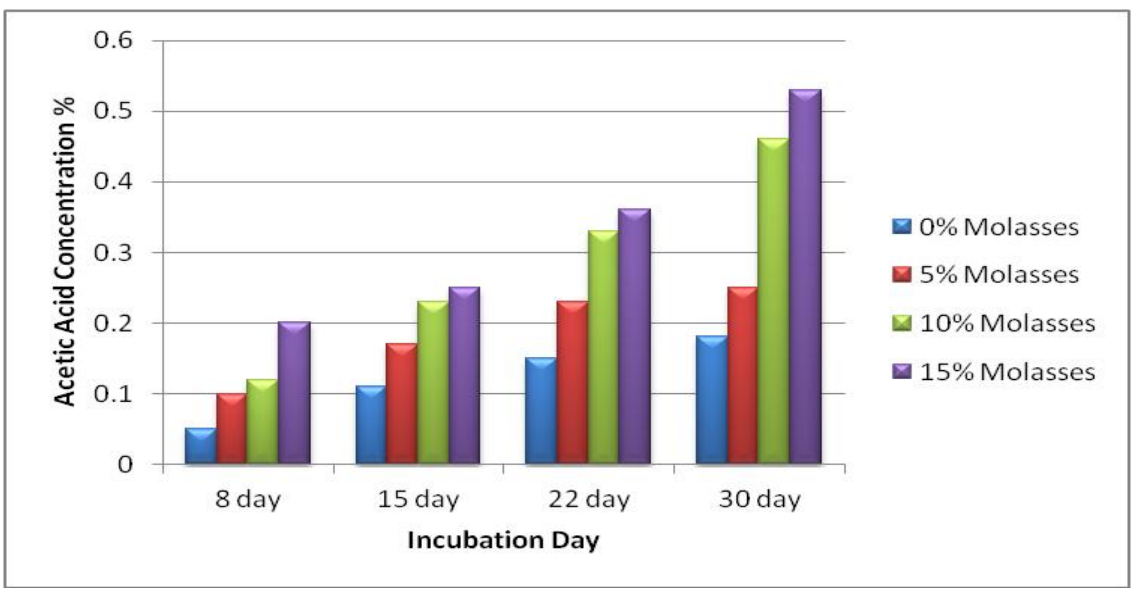

Figure 6 Acetic acid concentration (%) of watermelon vines silage supplemented with different levels of molasses during fermentation period

The physical properties of watermelon vines silage at different levels of molasses were shown in Table 4. Results indicated that the treatment without molasses had ammonia smell and pale color, whereas treatments with molasses had green brown color and typical silage smell. A liaison between incubation period and increases of molasses levels significantly ($P \leq 0.05$) enhanced the silage color and smell, whereas there was no significant ($p > 0.05$) effect on texture of silage. Fermentation with addition of molasses indicates silage quality as well as color and smell and no fungal growth (Snijders and Wouters, 2004). In the present study based on color and smell the silage considered to be acceptable with 15% molasses than those 5% and 10% molasses. Contrary results were obtained by Man and Wiktorson (2003) who reported that the low level of molasses was better than high level of molasses in terms of color also Bostami *et al.,* (2008) stated that the level of 5% molasses may be sufficient for producing good quality silage. The presence of fungal growth is undesirable because it uses silage nutrients and sometimes produced toxins substances (Man and Wiktorsson, 2003). The lack of agreement with above mentioned researchers; in high and low ratio of molasses may be due to the use of 2% urea in the present study, which is considered preservative agent (Kim and Koch , 2012). This assumption reinforced by not observing the growth of any fungi in the watermelon vines silage.

Table 4 Physical evaluation of watermelon vines silage supplemented with different levels of molasses

Level of molasses	Smell	Color	Texture
0%	2.80^{a}	3.30^{a}	1.00^{a}
5%	1.80^{b}	1.80^{b}	1.00^{a}
10%	1.30^{c}	1.50^{b}	1.00^{a}
15%	1.20^{c}	1.20^{b}	1.00^{a}
SEM	0.17	0.25	0.05

SEM= standard Error of mean and a, b, c Mean value in the same column having different superscripts were significantly variable ($P \leq 0.05$).

The *In-vitro* digestibility of dry matter digestibility (IVDMD), crude protein digestibility (IVCPD) and crude fiber digestibility (IVCFD) of watermelon vines silage were illustrated in Table 5. The maximum digestibility of DM, CP and CF were achieved by15% levels of molasses. IVDMD was increased significantly ($P \leq 0.05$) due to increase the molasses level, this agreed with findings reported by Shultz *et al.,* (1974) who mentioned that the ensiling grasses with 1% urea and 20% molasses for 40 days caused a significant increase in the in vitro dry matter digestibility (IVDMD).

The reason for this improvement in IVDMD may be due to ensiling which quick the action of urease enzyme to release ammonia which acts as an important role in breaking down bonds that linked lignin, cellulose and hemicellulose together (Klopfenstein 1978) and increased rate of cellulose and hemicellulose degradation (Tarkow and Feist 1969).

The enhanced of IVCPD may probably due to the chemical effect of ammonia produced from urea analysis during ensiling. Merchen and Satter (1983) related the improvement CPD to the increased amounts of nitrogen that available to microorganisms, due to the increased solubility of silage nitrogen and the ratio of the non protein nitrogen during ensiling, taking in account that ammonia is the essential source of nitrogen to many species of bacteria.

The improvement of CF digestibility may be due to digestion of cell wall and cellulose, Gupta and Pradhan (1977) reported that ensiling wheat straw with a mixture of urea and molasses increased the digestion of cell wall and cellulose. Shultz et *al.*, (1974) demonstrated that the microorganisms in silage degraded cellulose and hemicelluloses of ryegrass treated with urea and molasses during ensiling. Elpat Evskii and Zharkov (1961) noticed that the cellulose content of the roughages was markedly decreased during silage fermentation. The increase of IVDMD, IVCPD and IVCFD with addition of molasses was also demonstrated by (Hiep *et al.*, 2008).

Table 5 *In-Vitro* Digestibility of dry Matter, crude protein and crude fiber (%) of watermelon vines silage supplemented with different molasses level

Level of molasses	IVDMD %	IVCPD %	IVCFD %
0%	66.4^{d}	52.3^{c}	45.1^{c}
5%	72.8^{c}	57.9^{bc}	48.1^{b}
10%	75.7^{b}	60.9^{b}	49.1^{ab}
15%	78.1^{a}	68.8^{a}	49.7^{a}
SEM	0.10	2.52	0.53

IVDMD= In-vitro dry matter digestibility, IVCPD= in-vitro crude protein digestibility, IVCFD= in-vitro crude fiber digestibility, SEM= standard error of mean and a, b, c mean value in the same column having different superscripts were significantly variable ($P \leq 0.05$).

Conclusion

The Results obtained from the present study indicated that the addition of molasses improved physio-chemical properties and *in-vitro* digestibility of watermelon vines silage; therefore, it can be used effectively in animal feeding, especially during dry season.

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