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Effects of Urea, Molasses and Fibrolytic Enzymes on Nutritional Value of Date Palm (*Phoenix dactylifera*) Leaves Silage

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Authors' contributions

This work was carried out in collaboration between all authors. Author HK designed the study, authors KS and MYEI managed the analyses of the study and performed the statistical analysis. Author MS managed the literature searches, wrote the protocol, and the draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: This research was conducted to study changes of chemical composition and digestibility parameters of date palm leaves silage by adding urea, molasses and Fibrolytic Enzymes.
Study Design: The experimental treatments were: date palm leaves silage without additive (T1), date palm leaves silage with 4% of urea (T2), date palm leaves silage with 10% of molasses (T3), date palm leaves silage with 3 g of enzyme mix (T4), date palm leaves silage with 4% of urea and 10% of molasses (T5), date palm leaves silage with 4% of urea and 3 g of enzyme mix (T6), date palm leaves silage with 10% of molasses and 3 g of enzyme mix (T7), date palm leaves silage with 4% of urea, 10% of molasses and 3g of enzyme mix (T8). Results of chemical compounds were analyzed using complete randomized design (CRD) the data were analyzed using the GLM procedure SAS, (2000). Duncan's multiple range tests (p = 0.05) were used to determine statistical difference between treatment means.

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Place and Duration of Study: Sample: Department of Animal Science, Zabol university, between October 2009 and July 2010.
Methodology: In order to study changes of chemical composition, first, 50 kg of date palm leaves was collected and then crushed into 1-3 cm pieces for silage. Then, it was stored in plastic buckets by 4% of urea, 3 g of enzyme mixture and 10% of molasses.
Results: The results showed that treatment 2 had the highest amount of crude protein (CP) and treatment 1 had the least amount of CP with a significant difference ($p<0.05$). Treatment 5 had the least amount of Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) and treatment 1 had the highest ADF and NDF ($p <0.05$). Also, the studied treatments showed significant difference in degradability at different incubation times ($p<0.05$) so that treatments 5 and 8 demonstrated the highest degradability and treatment 1 showed the lowest degradability.
Conclusion: In general, considering information and results obtained from chemical composition, degradability and digestibility of the studied treatments, it could be concluded that silage with treatments 8 and 5 had better results compared with other treatments.

Keywords: Date Palm; digestibility parameters; fibrolytic enzymes; Molasses; Urea.

1. INTRODUCTION

Iran is one of the countries with the major parts located in arid and semiarid regions and its annual rainfall rate is low [1]. Water shortage and hot and arid climatic conditions cause woody property and low rate of crude protein along with decrease in digestibility of the plants [1]. High level of Iran's farmlands and many tropical countries in the world has been covered with date palms. In developing countries, shortage of food resources is considered important for feeding the livestock so that the ruminants in these countries feed on residues of crops (such as straw and corncob) [2]. Date palm with scientific name of *Phoenix dactylifera* is one of the important products in Iran. Each of different parts of palm tree has a good potential for use as animal feed. These materials can be divided into two classes of chlorophylls and residues of palm fruit. One of the most important parts of palm tree is its chlorophylls material. These materials which include leave and empty bunches of fruit can be used in two fresh and silage hay forms as animal feed and major residues of palm trees which include unripe fruits, very low fruits, date pit etc. are applied as animal feed. Based on the conducted researches, foliar parts of the palm tree (leaflets and the entire leaf of the date) can be a good substitute for straw as the ration of goats.

Production of milk and its compounds in Holstein dairy herbs which receive date palm leaf instead of barley straw is not so different from those which received barley straw [3]. Therefore, in the regions where these wastes are abundantly found, they can be replaced with barley straw in animals' ration [3]. Due to abundance of cellulose materials and low digestibility in date palm leaves and low crude protein rates, they should be processed before being used in animals' ration. Therefore the aim of this study was the effects of proceeding date palm leaves with Urea, Molasses and Fibrolytic Enzymes on nutritional value and degradability parameters of Date Palm leaves silage.

2. MATERIALS AND METHODS

2.1 Sample Preparation and Analytical Technique

First, date palm leaves were collected from Sistan Palm Gardens and then the collected samples were transferred to animal feed laboratory of University of Zabol. First, date palm leaves were cut with sizes of 3 to 4 cm, poured into plastic buckets with capacity of 5 kg and then additives including molasses; urea and enzyme were added to all treatments but the control one which only included date palm leaves at different levels. Then, they were compressed completely so that its air was discharged to some extent. The remaining oxygen was discharged from the buckets by injecting CO_2 for 2 min. At the end, lid of buckets was completely closed and they were kept at room temperature for 50 days. At the end of ensiling period bags were opened and the representative samples taken for subsequent analyses. The experimental treatments were: date palm leaves silage without additive (T1), date palm leaves silage with 4% of urea (T2), date palm leaves silage with 10% of molasses (T3), date palm leaves silage with 3 g of enzyme mix (T4), date palm leaves silage with 4% of urea and 10% of molasses (T5), date palm leaves silage with 4% of urea and 3 g of enzyme mix (T6), date palm leaves silage with 10% of molasses and 3 g of enzyme mix (T7), date palm leaves silage with 4% of urea, 10% of molasses and 3g of enzyme mix (T8). Dry Matter (DM) content was determined by drying the samples in an oven at 60ºC for 48 h. The dried samples were ground to pass a 2 mm sieve and their CP content was measured by kjeltec auto (1030 Analyzer Tecator), ADF and NDF by Van Soest et al. [4] method.

Data of chemical compounds were subjected to analysis as a completely randomized design, the data were analyzed using the GLM procedure SAS, (2000). Duncan's multiple range tests ($p = 0.05$) were used to determine statistical difference between treatment means.

2.2 In sacco Measurements

The nylon-bag technique was used to measure the rumen degradability of the sun-dried. Four Holestin steers with the average initial live weight of 450±10 kg fitted with the permanent ruminal fistula in individual pens were used. The steers were offered a diet containing 50:50 forage to concentrate in 2 equal portions daily (0700 and 19.00). Samples of about 3 g were weighed into polyester nylon bags with pore size of 44 µm and dimensions of 17x10 cm and incubated in the rumen for periods of 0, 2, 4, 8, 16, 24, 48, 72 and 96 h. After removal the bags were thoroughly washed under tap water and were dried to constant weight at 60ºC. The rapidly soluble materials were estimated by washing the bags containing samples after soaking in water without incubation in the rumen. The DM and CP degradation data were fitted to the following exponential equation Orskov et al. [5].

$$P = a + b\ (1 – e\text{-}ct)$$

Where:

P = Potential degradability or degradation of DM and CP at time t
a = The rapidly soluble fraction
b = The potentially degradable DM or CP
c = The constant rate of degradation of b parameter (percentage h^{-1})

The obtained data from degradability measurements were analyzed by the Fig. P software (Biosoft Corporation Durham, NC USA).

3. RESULTS AND DISCUSSION

Results of Chemical Compounds of Experimental Treatments of Date Palm Leaves Silage.

The results obtain of chemical composition shown in Table 1.

Mean percent of dry matter of date palm leaves silage varied between 93.90 and 96.27% among all treatments. The average of the experimental treatments showed significant ($p<0.05$) difference in mean of the dry matter. The highest percent of dried matter was observed in treatment 3 (96.27) and the above results were confirmed with results by Balakhial et al [6] who reported that adding molasses caused increase of dried matter of silage. The lowest rate of dried matter was related to treatment 6 (93.90%). It seems that adding enzyme and urea supplement had no considerable effect on dried matter and the obtained results were congruent with the results of researchers in 2004 which were conducted on Sorghum silage and concluded that adding molasses and enzyme caused increase of dried matter of Sorghum silage but enzyme and urea supplement had no effect on the dried matter [7].

Table 1. The means percent of chemical composition of treatments

Treatments	WSC	ADF	NDF	EE	CP	OM	ASH	DM
1	0/93^{f}	81/51^{a}	83/61^{a}	1/66d	5/89^{f}	93/41^{b}	6/58^{d}	95/50^{c}
2	1/24^{e}	80/75^{b}	83/55^{a}	1/66d	13/46^{a}	94/37^{a}	5/62^{e}	94/33^{g}
3	2/14^{b}	63/48^{f}	71/69^{f}	1/74cd	8/95^{d}	92/53^{bc}	7/46^{ab}	96/27^{a}
4	1/17^{e}	77/49^{c}	83/52^{a}	1/81c	7/96^{e}	93/24^{b}	6/75^{d}	95/21^{d}
5	1/57^{c}	60/55^{h}	75/41^{d}	2/82^{a}	11/09^{b}	93/00^{bc}	6/99^{cd}	94/77^{f}
6	1/40^{d}	71/82^{d}	81/64^{c}	1/85^{c}	10/20^{c}	93/23^{b}	7/01^{cd}	93/90^{h}
7	2/68^{a}	66/75^{e}	82/40^{b}	1/77^{cd}	10/12^{c}	92/19^{c}	7/80^{a}	95/97^{b}
8	2/76^{a}	62/59^{g}	74/07^{e}	2/18^{b}	10/01^{c}	92/15^{c}	7/20^{bc}	95/03^{e}
SEM	0/0073	1/07	0/0015	0/006	0/013	0/21	0/054	1/06

Columns means followed by the same letter are not significantly different at 0.05 probability level
WSC: water soluble carbohydrate, ADF: acid detergent fiber , NDF: neutral detergent fiber ,EE : ether extract , CP: crude protein, OM: organic matter, DM: dry matter

Rate of organic matter varied between 92.15 and 94.37% among all the treatments (Table 1). The lowest rate of organic matter was related to treatment 8 (92.15%) and the highest rate of organic matter was related to treatment 2 (94.37%). There was significant difference between treatments 2 and 3 at 5% level of significance. There was organic matter in treatment 2 (94.37%) and treatment 3 (92.53%), which could be due to the presence of urea in treatment 2 and molasses in treatment 3. In another report, researchers have declared that rate of the organic matter of date palm leaves is 90.2% without additive [8]. Ziaei [9] also declared that rate of organic matter in date palm leaves with urea silage was higher than that of date palm leaves with molasses silage.

Rate of ash in treatments varied between 5.62 and 7.80%. Rate of ash available in treatments had a reverse relationship with organic matter that was the highest rate related to treatments 3, 7 and 8 and the lowest rate related to treatment 2. There was no significant difference between treatments 1 and 4. Researchers who added molasses to wet weight of

red clover concluded that adding molasses increased silage ash and decreased organic matter [10].

Researchers also declared that adding molasses to sorghum silage caused increase of ash and decrease of organic matter [11] and the obtained results were congruent with the above results. In another report, researchers declared that increase of urea to silage of date to branches enhanced organic matter and decreased ash of the date palm leaves [12]. Researchers also declared that rate of ash of date palm was 5.67% without additive [13].

The highest rate of crude protein was in treatment 2 (13.46%) and the lowest crude protein was related to treatment 1(5.89%). The obtained results were congruent with the results of Sadeghi [12] in which adding levels of 0, 0.5 and 1% of urea to silage of cotton leaves caused increasing in crude protein.

The results were congruent with results of Lattemae [10] who added 0, 40 and 100 l/ton of molasses to the red clover based on wet weight for silage and the results showed that molasses decreased crude protein to some extent. There was no significant difference between treatments 6, 7 and 8 at ($p<0.05$).

Ziaei [9] reported that rate of crude protein of date palm leaves silage 5% of urea was 7.80% and also declared that rate increased by 9% by adding beet pulp (5%) and urea (5%) concurrently to date palm leaves and rate of crude protein increased by 9%. Researchers mentioned that crude protein rate of date palm leaf without additive was 5.1% [13]. In another report, researchers declared that crude protein rate of date leaf was 4.61% without additive [8].

Average ADF percent of different treatments studied in terms of dry matter is shown in Table 1. There was significant difference between all treatments in terms of ADF. The highest ADF was related to treatment 1 (81.51%) and the lowest ADF was related to treatment 5 (60.55%). Researchers declared that adding molasses to sorghum silage caused decrease of ADF and NDF for two reasons. The first reason was increase in activity of lactobacilli and bacteria in combination with silage followed by increase in NDF and ADF microbial degrading activity of silage content and the second reason was related to molasses itself which lacked NDF and ADF [11].

Researchers concluded that adding urea and molasses to silage of date palm leaves caused ADF decrease of leaves [15]. Researchers concluded in a study that supplementing urea with molasses caused ADF decrease of sorghum silage [7].

Considering Table 1, it was found that there was significant difference between treatments 2 and 4 at $p <0.05$. Effect of fiber degrading enzymes was higher than that of urea. It was found that there was significant difference between treatment 6 including date palm leaves silage with 4% of urea and 3 g of enzyme mix and treatment 7 including date palm leaves silage with 10% of molasses and 3 g of enzyme mix at $p<0.05$. It seems that effect of molasses with enzyme was higher than urea with enzyme.

Kafilzadeh et al. [8] reported that ADF of date palm leaves was 47.9% without additive (it was mentioned in another report that ADF of date palm leaves was 58.9% without additive [13]. Ziaei [9] reported that ADF of date palm leaves treated with 5% of urea was 37.3% and their ADF treated with 5% of urea and 10% of beet pulp was 42.2%. He declared no significant difference between them. In another report, he also declared that ADF of the date

palm leaves which was treated with 5% of urea and 10% of molasses was 42% and ADF of date palm leaves which was treated with 5% of urea and 10% of citrus pulps was 30.5% and mentioned that there was no significant difference between them [9].

Average NDF percent of different treatments studied in terms of dry matter is shown in Table 1. The highest percent of NDF was related to treatment 1 (83.16%) and the lowest rate was related to treatment 8 (74.07%). Researchers found that adding molasses to silage of sugar cane leaves caused decrease of NDF in silage [14]. Sadeghi et al. [12] reported decrease of NDF in leaves by adding molasses to the date palm leaves and the obtained results were congruent with the above results.

There was no significant difference between treatments 1, 2 and 4 at 5%. It is probable that urea and enzyme did not have considerable effect on NDF. There was significant difference between treatment 5 and treatment 6 at 0.05%. This issue was due to the presence of molasses in treatment 5. Researchers reported that NDF rate of date palm leaves was 75.8% without additives [13]. In another report, NDF of date palm leaves was reported as 61.5% without additives [8]. Ziaei [9] reported that NDF was 79.6% by adding 5% of urea to date palm leaves and NDF was 85.3% by adding 5% of urea with 5% of citrus pulps to date palm leaves. He also declared in another report that NDF of date palm leaves treated with 5% of urea and 10% of citrus pulps was 77.4%.

Marinas et al. [15] reported that, when ratio of leave to plant stem decreased in maturity stage, ratio of Neutral Detergent Fiber to lignin stage of plant increased.

As observed in Table 1, fat rate of the studied treatments varied from 1.66 to 2.82%. The highest crude fat was related to treatment 5 (2.82%) and the lowest crude fat to treatment 1 (1.66%). Considering Table 1, it was observed that there was significant difference between treatment 5 which included date palm leaves silage with 4% of urea and 10% of molasses and treatment 8 which included date palm leaves silage with 4% of urea, 10% of molasses and 3 g of enzyme mix at $p<0.05$. It was likely that rate of free fatty acids would increase by adding fiber degrading enzymes to silage and its effect on improvement of fermentation but there was no significant change and it is probable that there was no suitable condition for enzyme activity. In another report, researchers declared that fat rate of the date seed powder was 6.9% [7]. In Table 1 mean percent of carbohydrates of different studied treatments is mentioned in terms of dry matter.

The highest rate of Water Soluble Carbohydrate (WSC) was related to treatment 8 and the lowest rate to treatment 1. Increasing carbohydrates in treatments 7 and 8 were due to the presence of molasses in these treatments because molasses is a suitable carbohydrate source. Researchers added 0, 40 and 100 l/ton of molasses to the red clover based on wet weight for silage and results showed that rate of water soluble carbohydrates increased by adding molasses to silage [10]. Considering Table 1, there was no significant difference between treatments 7, 8 and treatments 2.4 at ($p<0.05$).

Considering Table 1, there was significant difference between treatments 1 and 4 at $p<0.05$ probably due to the presence of enzyme in treatment 4 compared with treatment 1. Cellulose and hemicelluloses enzymes released some soluble carbohydrates by degrading some NDF and ADF during this process [14].

Cold water soluble carbohydrates and their set are called WSC [16]. About 650 g was combined with molasses dry matter to form soluble carbohydrates [16]. The highest rate of

these carbohydrates was sucrose [17]. Therefore, WSC silage increased by adding molasses. In a study which was conducted on hay, adding 40 g of molasses to hay increased rate of water soluble carbohydrate from 70 g per kg to 190 g |per kg n terms of dry matter [13].

3.1 Results of Parameters of Dry Matter Degradation of the Studied Treatments

The highest rate of degradability in Section (a) was related to treatments 5, 3 and 8 and the lowest rate was related to treatments 2 and 4 and it was also observed that there was no significant difference between treatments 1, 2, 4 and 3, 5 at $p<0.05$. Researchers showed advantages of adding carbohydrates to hays and mentioned that fermentation improved in this case because molasses containing soluble carbohydrates was digestible and was fermented and degraded rapidly in rumen which caused degradability of dry matter [18]. Although fiber degrading enzymes increase rate of the hay soluble carbohydrates, generally, they have a lower effect on their digestibility [17]. The highest rate of degradability of insoluble but degradable part (b) was related to treatments 5 and 8 (38.03 and 37.95), respectively, and the lowest rate was related to treatments 1, 2, 3, 4, 6, 7 and there was no significant difference between them at ($p>0.05$).

C value (degradation rate constant) indicated instantaneous degradation speed of Section b in hour/percent and there was no significant difference between experimental treatments ($p>0.05$) (Table 2). a+b degradability coefficient (potential degradability) varied between 29.29 and 53.6% in the studied treatments. The highest degradability coefficient was related to treatment 5 and the lowest rate was related to treatment 4 and, considering Table 2, it was found that there was no significant difference between treatments 3, 5, 8 and treatments 7, 1, 2, 4, 6 at ($p>0.05$).

Table 2. Mean percent dry matter degradability of different treatments at different times of incubation

Times	1	2	3	4	5	6	7	8	SEM
3	8/58^{c}	7/64^{d}	14/64^{a}	9/31^{c}	15/22^{a}	12/18^{b}	12/32^{b}	14/51^{a}	0/259
6	12/65^{d}	11/25^{e}	18/90^{a}	12/09d^{e}	17/89^{a}	15/45^{bc}	15/21^{c}	16/47^{b}	0/462
12	15/63^{d}	13/78^{e}	21/32^{a}	14/53^{e}	20/81^{a}	19/69^{b}	17/31^{c}	21/48^{a}	0/265
24	18/19^{cd}	15/95^{e}	24/90^{a}	17/88^{d}	23/80^{ab}	24/27^{ab}	19/24^{c}	23/38^{b}	0/386
48	22/05^{c}	19/26^{e}	27/11^{b}	20/80^{d}	30/95^{a}	26/46^{b}	21/05^{d}	31/18^{a}	0/277
72	26/86^{c}	22/02^{e}	30/83^{b}	24/16^{d}	34/95^{a}	30/09^{b}	24/47^{d}	35/01^{a}	0/619
96	30/23^{c}	26/81^{d}	33/54^{b}	26/96^{d}	40/46^{a}	32/16^{b}	27/22^{d}	41/07^{a}	0/817
A	9/32^{d}	8/66^{d}	14/89^{a}	9/03^{d}	15/03^{a}	10/61^{c}	13/09^{b}	14/20^{ab}	0/460
B	25/82^{b}	23/92^{b}	19/22^{b}	20/25^{b}	38/03^{a}	20/88^{b}	25/51^{b}	37/95^{a}	3/789
a+b	35/14^{b}	32/59^{b}	34/10^{a}	29/29^{b}	53/06^{a}	31/49^{b}	61/38^{b}	52/14^{a}	2/80
C	0/054^{a}	0/014^{a}	0/028^{a}	0/023^{a}	0/011^{a}	0/041^{a}	0/014^{a}	0/012^{a}	0/0005
ED									
0/02	20/93^{d}	18/20^{f}	25/93^{b}	19/43^{e}	28/60^{a}	24/60^{c}	20/83^{d}	28/53^{a}	0/092
0/05	15/73^{d}	13/70^{e}	21/70^{a}	15/20^{d}	21/96^{a}	20/00^{b}	17/23^{c}	21/60^{c}	0/108
0/08	13/73^{d}	12/13^{e}	19/80^{a}	13/40^{d}	19/66^{a}	17/70^{b}	15/96^{a}	19/23^{a}	0/110

Mean percent of effective degradability of dry matter per hour did not show significant differences between all the studied treatments (for 0.02, 0.05 and 0.08) ($p<0.05$). The

highest degradability percent of dry matter was related to treatments 5 , 8 for rate of passage of 0.02 and to treatments 8, 5 and 3 for rate of passage of 0.05 and 0.08 and the lowest rate of passage at all three levels was related to treatment 2 ($p<0.05$) (Table 2). Considering Table 2, there was no significant difference between treatments 5, 8 and treatments 1, 7 at 0.05% for rate of passage of 0.02 in terms of dry matter degradability. There was no significant difference between treatments 5, 3, 8 and 1, 4 at 0.05% for rate of passage of 0.05 in terms of dry matter degradability.

Researchers reported that the highest degradability percent of dry matter for date palm leaves and mushroom and crushed date leaves is 9.63 and 9.85% for rate of passage of 0.02 % and 4.08% and 3.50% for rate of passage of 0.08%, respectively [8].

4. CONCLUSION

Considering information and results obtained from chemical composition, degradability and digestibility of the studied treatments, it could be concluded that silage with treatments 8 and 5 had better results compared with other treatments.

ETHICAL APPROVAL

Principles of animal care were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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