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# Study of some additives effect on chemical composition and nutritive value of phragmites australis forage silage with *in* vitro gas production method

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**ABSTRACT:** This study was carried out to evaluate changes in chemical composition and nutritional value of common reed forage silage by*in vitro* gas production method. The experiment arranged in a factorial design 2×2×2 on based a completely randomized design. After determination of dry matter and chemical composition, gas production test and their parameters investigated in treatments.Results showed that the addition of mulberry leaves caused a significant increased in pH, CP, ASH content and reduction DM, EE, ADF and NDF content. Addition of barley flour caused a significant increased in EE, CP, ASH content and reduction pH, OM, ADF and NDF content. Addition of enzyme caused a significant increase CP and ASH content and reduction ADF and NDF content. Addition supplements mulberry leaves, barley flour and enzyme caused a significant different in CP, EE, ASH, OM, ADF and NDF content and there was no significant effect on the chemical composition. The findings obtained from *in vitro* gas production method revealed that the time incubation addition of mulberry leaves and barley flour caused increased, and enzyme except in times 2 and 4 caused increased gas productions value. In conclusion, considering the changes in cell wall contents, hemicelluloses contents and degradability values in the present study, it can be suggested that the use of additives mixed can be used to make good common reed forage silage.

***Keywords***: Study, Chemical, Phragmites Australis, Vitro.

## INTRODUCTION

Nowadays Attention to human nutrition considered one of the most complex contemporary problems in the developing world and especially in our country .Being located in a specific geographical conditions of water scarcity, loss, low rainfall and lack of optimal use of existing resources, all hands and cause feed followed by food shortages for the man created (Seyed momen, 2005).One of method that is somewhat less dependent on weather conditions and is used by dairy farmers for plants using natural fermentation (silage making) has the forage(Rowghani, 2008) .The common reed (CR) is one of the plants which grows in some area of Iran and other world countries especially in coastal wetlands. Feed resource restriction is the most problem in animal production in some area and native plants have a key role.*Phragmites australis* is a large perennial rhizomatous grass, or reed especially common in alkaline and brackish environments (Haslam, 1972). *Phragmites australis* used in the present experiment is a tall plant (2.0 - 4.0 m) found in freshwater wetlands, and it is interesting as a source of cellulose and energy.Ensiling Common reed forage while can be allowing long term storage too, it maintaind palatability and nutrient availability.Recently, in many tropical countries and regions, there has been a focus on indentifying and using locally available shrubs and tree leaves as a source of feeds or feed ingredients for ruminants because of their high nutritive value and positive effects on rumen function (Omar, 1999; Yao, 2000).Mulberry leaves are very palatable for sheep and goats and it has a high nutritional value and reported crude protein content of about 20% on dry matter (FAO, 1998). The mulberry leaves were high in nutritive value with protein content at higher than 21% in dry matter (Yao, 2000).Due to its high digestibility and excellent level of crude protein, mulberry foliage can be a comparable protein source to commercial concentrates for ruminal production (Singh and Makkar, 2002).Since mulberry leaves are rich in nitrogen as well as sulphur and minerals, supplementation into ruminant's diet could increase the efficiency of crop residues utilization in the feeding systems (Singh and Makkar, 2002).Traditionally, the milled cereals used at silage with the aim absorb water and prevent waste (McDonald ., 1990).Enzymes such as cellulose and xylanase most of all have been considered because cellulose and lignin consist the indigestible part plant cell walls and this enzyme are able broken link lignocellulosic and thus put material more susceptible to microbial digestion rumen (Tang ., 2008).no published data are available to show the effects of barley flour utilization in silage making on the fermentation parameters of common reed silage and its effects on animal productivity. Therefore in this research in order to improve the nutritional value common reed, deficiency of protein with the addition of mulberry leaves, deficiency of energy easily digestion in order to useful fermentation with the addition of barley flour and for break link lignocellulosic used of enzymes.

## MATERIALS AND METHODS

The experiment was conducted in animal Nutrition Laboratory of university of zabol. After common reed forage harvested on October 2012 and randomized collected and chopped to a length 2 cm. 5 kilograms of the harvested common reed were immediately placed in a plastic bag and then tightly silaged. The plastic bag was opened after 60 d of ensiling. After common reed forage collected, approximately 500 g of each treatment was oven-dried at 60°C and ground through a 1-mm screen for chemical analysis. All samples were analyzed for DM, OM, N, NDF, and ADF. The DM content was determined in triplicate by oven-drying at 105°C for 24 h. The OM was determined by Ashing at 550°C for 4 h. Total N content was determined according to the Kjeldahl method (AOAC, 1995; method 954.01). The contents of NDF and ADF were determined using the method of Van Soest. (1991), but NDF was assayed with the addition of a heat stable amylase (A3306, Sigma-Aldrich, St. Louis, MO) without sodium sulfite. Both NDF and ADF are expressed inclusiveof residual ash.

***Gas production test:***

Dried samples 200 mg of each treatment were incubated with rumen liquor and buffering solution (1:2) into calibrated glass syringes (100ml), in water bath, the temperature was 39 + 0.5ºC. The volumes of gas were measured and recorded at zero. 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours. Cumulative gas production data were fitted to the model of Orskov and McDonald.

$Y = b\ (1 - exp^{-ct})$

Where:

b = the potential gas production(ml), c = the gas production rate (ml/h), t = incubation time ( h), y = gas production at time (t)

The OMD of silage was calculated using equation of Menke.

OMD (%) = 14.88 + 0.889GP + 0.45CP + XA

Where:

GP = is 24 h net gas production (ml / 200 mg), CP = Crude protein (%), XA = Ash content (%)

ME (MJ/Kg DM) content of silage was calculated using equation of Menke as follows:

ME (MJ/kg DM) = $2.20 + 0.136GP + 0.057CP + 0.0029CP^2$

Where:

GP = is 24 h net gas production (ml/200mg), CP = crude protein

***Laboratory Analysis:***

pH was estimate by pH meter, Feed and feces samples were ground (1-mm screen) and analyzed for Ash (muffle furnace at 550°C for 4 h), crude protein (CP) (AOAC official method 954.01, AOAC, 1997, Kjeldahl N× 6.25), crude fiber (CF) (AOAC official method 978.10, AOAC, 1997), ether extract (AOAC official method 920.39, AOAC, 1997), ash free neutral detergent fiber (NDF) and acid detergent fiber (ADF) (VanSoest ., 1991).

***Statistical Analyses:***

All 2×2×2factorial design data from the experiment were analyzed by using the SAS (1998) GLM procedure according to the model:

$y = \mu + \alpha_i + \beta_j + \gamma_k + \alpha_i.\beta_j + \beta_j.\gamma_k + \alpha_i.\gamma_k + \alpha_i.\beta_j.\gamma_k + \varepsilon_{ijk}$where Yijk: observation from steer μ: the overall mean; αi: effect mulberry leaves (i = 1, 2), $\beta_j$: effect barley flour (j = 1, 2), $\gamma_k$: the effect enzyme(k = 1, 2), $\alpha_i.\beta_j$:effectinteraction mulberry leaves and barley flour, $\beta_j.\gamma_k$: effect interaction barley flour and enzyme, $\alpha_i.\gamma_k$: effect interaction mulberry leaves and enzyme, $\alpha_i.\beta_j.\gamma_k$:The effect of three additives and $\varepsilon_{ijk}$: residual effect. Results are presented as mean values with the standard error of the means.Differences among means with P<0.05 was accepted as representing statistical differences and tendencies of differences, respectively. The comparison among supplementation and trend of ratios responses were performed by orthogonal contrast using the GLM procedure.

## RESULTS AND DISCUSSION

***Results:***

***PH:***

Generally increases only with additives mulberry leaves and barley flour in the common reed forage silage, was significantly ( p< 0.05 ) lead to drop in the pH Table (1).

The chemical composition of silage: theresulted chemical composition is presented in Table 1. The crude protein content increased significantly (p<0.05) with additive each three additive.the high value 20/03 was shown intreatment 8that was containing all additives and the low value 9/62 recorded in the control (without additives). The Ashgenerally increased in treatments containing mulberry leaves.the high Ash content 15/76 was found in treatment containing 40% mulberry leaves (treatment 2) and low value 14/22 in control (without additives).Acid detergent fiber ADF and Neutral detergent fiber NDF were significantly (p<0.05) decreased with increase enzyme.

Table 1: Means chemical composition common reed forage silage with mulberry leaves, barley flour and enzyme

| Treatment | DM | EE | OM | ASH | ADF | NDF | CP | pH |
|---|---|---|---|---|---|---|---|---|
| control | 37/53 | 2/50$^{de}$ | 85/76$^{a}$ | 14/23$^{c}$ | 38/73$^{a}$ | 69/94 | 9/62$^{e}$ | 4/68 |
| Control+40% mulberry leaves | 29/81 | 1/66$^{e}$ | 84/23$^{c}$ | 15/76$^{a}$ | 37/08$^{b}$ | 65/89 | 14/65$^{b}$ | 5/68 |
| Control+10%barley flour | 37/11 | 2/69$^{ab}$ | 85/20$^{c}$ | 14/80$^{c}$ | 38/95$^{a}$ | 67/63 | 13/03$^{cd}$ | 4/19 |
| Control+enzyme | 36/66 | 2/23$^{d}$ | 85/49$^{b}$ | 14/50$^{b}$ | 38/19$^{ab}$ | 67/36 | 12/47$^{d}$ | 4/67 |
| Control+40%mulberry leaves+10%barley flour | 33/17 | 2/98$^{ab}$ | 85/16$^{c}$ | 14/84$^{c}$ | 30/44$^{d}$ | 57/47 | 14/52$^{b}$ | 4/96 |
| Control+40%mulberry leaves+enzyme | 33/13 | 2/90$^{ab}$ | 85/10$^{c}$ | 14/90$^{c}$ | 34/40$^{c}$ | 61/82 | 13/76$^{bc}$ | 5/51 |
| Control+enzyme +10%barley flour | 35/05 | 3/15$^{a}$ | 85/14$^{c}$ | 14/86$^{c}$ | 33/30$^{c}$ | 62/47 | 13/05$^{dc}$ | 4/08 |
| Control+40%mulberry leaves+10%barley flour+enzyme | 35/60 | 2/35$^{d}$ | 84/60$^{d}$ | 15/40$^{b}$ | 33/06$^{c}$ | 53/88 | 20/03$^{a}$ | 5 |
| M | ** | ** | ** | ** | ** | ** | ** | ** |
| B | ns | ** | ** | ** | ** | ** | ** | ** |
| A | ns | ** | ns | ns | ** | ** | ** | ns |
| MB | ** | ns | ns | ns | * | ** | * | ns |
| MA | ** | ns | ** | ** | ** | ns | ns | ns |
| AB | ns | ** | ** | ns | ns | ns | ** | ns |
| AMB | ns | ** | ** | ** | ** | ns | ** | ns |
| SEM | 0/478 | 0/184 | 0/0561 | 0/0561 | 0/808 | 1/339 | 0/6075 | 0/165 |

Columns having different superscript significantly (P<0.05) differ. CP: Crude protein, DM: Dry matter, EE: Ether extract, OM: Organic matter, ADF: Acid detergent fiber, NDF: Natural detergent fiber. M: effect mulberry leaves, B: effect barley flour, A: the effect enzyme, MB: effect interaction mulberry leaves and barley flour, BA: effect interaction barley flour and enzyme, MA: effect interaction mulberry leaves and enzyme, AMB: The effect of three additive SEM : stander error of means.

Gas Production Characteristics:The results of in vitro GP test for different treatment are summarized in Table 2.in the study with increasing incubation time resulted increasing of the gas production.effects all gas production parameters were significantly (p< 0.05). Fraction (b), gas produced from slowly degradable partandthe potential gas production (a+ b), were significantly (p<0.05) increased due to the edditive enzyme.Whereas in this study itwas found that the containing all additives treatmenthave significantly (p < 0.05) higher gas production rate fraction (c) than the control treatment.

Organic Matter Digestibility and Metabolizable Energy: The Organic matter digestibility andmetabolizable energy increased significantly (p<0.05)with addition barley flour and enzymeand higher was in treatment containing barley flour and enzyme (treatment 7).

Table 2: Means gas production volume (ml/200 mg DM) treatment containing common reed forage silage with mulberry leaves, barley flour and enzyme

| Treatment | 2 | 4 | 6 | 8 | 12 | 24 | 48 | 72 | 96 |
|---|---|---|---|---|---|---|---|---|---|
| **control** | 3/28 | 7/69$^{d}$ | 9/83$^{d}$ | 10/81$^{e}$ | 14/58$^{f}$ | 25/56$^{e}$ | 39/32$^{d}$ | 45/39$^{d}$ | 49/15$^{d}$ |
| **Control+40% mulberry leaves** | 3/57 | 9/34$^{c}$ | 12/30$^{C}$ | 13/95$^{d}$ | 18/86$^{d}$ | 29/20$^{d}$ | 41/84$^{c}$ | 46/71$^{dc}$ | 49/72$^{dc}$ |
| **Control+10%barley flour** | 4/60 | 9/53$^{c}$ | 12/17$^{c}$ | 13/32$^{d}$ | 17/60$^{e}$ | 29/44$^{d}$ | 42/77$^{c}$ | 47/71$^{c}$ | 50/50$^{dc}$ |
| **Control+enzyme** | 3/11 | 7/36$^{d}$ | 9/82$^{d}$ | 10/80$^{e}$ | 14/24$^{f}$ | 26/68$^{e}$ | 41/41$^{c}$ | 47/48$^{c}$ | 51/24$^{bc}$ |
| **Control+40%mulberry leaves+10%barley flour** | 4/82 | 11/29$^{b}$ | 15/62$^{b}$ | 17/78$^{b}$ | 23/43$^{b}$ | 35/24$^{a}$ | 48/04$^{a}$ | 53/19$^{a}$ | 55/52$^{a}$ |
| **Control+40%mulberry leaves+enzyme** | 5/58 | 12/31$^{a}$ | 17/05$^{a}$ | 19/19$^{a}$ | 24/93$^{a}$ | 34/44$^{a}$ | 45/27$^{b}$ | 50/36$^{b}$ | 52/65$^{b}$ |
| **Control+enzyme +10%barley flour** | 3/92 | 8/82$^{c}$ | 11/77$^{c}$ | 13/24$^{d}$ | 17/49$^{e}$ | 31/06$^{c}$ | 45/29$^{b}$ | 51/34$^{b}$ | 55/43$^{a}$ |
| **Control+40%mulberry leaves+10%barley flour+enzyme** | 4/59 | 10/82$^{b}$ | 14/93$^{b}$ | 16/57$^{c}$ | 21/66$^{c}$ | 33/15$^{b}$ | 46/93$^{a}$ | 53/09$^{a}$ | 55/96$^{a}$ |
| **M** | ** | ** | ** | ** | ** | ** | ** | ** | ** |
| **B** | * | ** | ** | ** | ** | ** | ** | ** | ** |
| **A** | ns | ns | ** | ** | ** | ** | ** | ** | ** |
| **MB** | * | ** | ** | ** | ** | ** | ns | ns | * |
| **MA** | ** | ** | ** | ** | ** | ns | ns | ns | * |
| **BA** | ** | ** | ** | ** | ** | ** | ** | ns | ns |
| **MBA** | ns | ** | ** | ** | ** | ** | ** | ** | ** |
| **SEM** | 0/5037 | 0/5852 | 0/5767 | 0/5829 | 0/6911 | 0/7178 | 0/8304 | 0/9035 | 0/9662 |

Columns having different superscript significantly (P<0.05) differ. M: effect mulberry leaves B: effect barley flour, A: the effect enzyme, MB: effect interaction mulberry leaves and barley flour, BA: effect interaction barley flour and enzyme, MA: effect interaction mulberry leaves and enzyme, AMB: The effect of three additive SEM : stander error of means.

Table 3: The parameters of the gas production, *in vitro* organic matter digestibility (IVOMD%) and metabolic energy (ME MJ/kg DM ).

| Treatment | b | C | OMD | DOMD | ME |
|---|---|---|---|---|---|
| **control** | 51/97$^{e}$ | 0/028$^{f}$ | 42/85$^{f}$ | 36/75$^{e}$ | 6/48$^{f}$ |
| **Control+40% mulberry leaves** | 49/86$^{d}$ | 0/039$^{dc}$ | 45/22$^{e}$ | 38/12$^{d}$ | 6/97$^{e}$ |
| **Control+10%barley flour** | 51/37$^{cd}$ | 0/037$^{d}$ | 48/60$^{d}$ | 41/37$^{c}$ | 7/64$^{dc}$ |
| **Control+enzyme** | 55/01$^{b}$ | 0/029$^{f}$ | 50/02$^{c}$ | 42/27$^{c}$ | 7/84$^{bc}$ |
| **Control+40%mulberry leaves+10%barley flour** | 55/01$^{b}$ | 0/046$^{b}$ | 47/92$^{d}$ | 41/26$^{c}$ | 7/48$^{d}$ |
| **Control+40%mulberry leaves+enzyme** | 50/82$^{cd}$ | 0/055$^{a}$ | 52/20$^{b}$ | 45/43$^{b}$ | 8/10$^{b}$ |
| **Control+enzyme +10%barley flour** | 57/25$^{a}$ | 0/032$^{e}$ | 56/01$^{a}$ | 48/99$^{a}$ | 9/27$^{a}$ |
| **Control+40%mulberry leaves+10%barley flour+enzyme** | 55/82$^{ab}$ | 0/041$^{c}$ | 50/93$^{bc}$ | 45/20$^{b}$ | 7/92$^{b}$ |
| **M** | ** | ** | ** | ** | ns |
| **B** | ** | * | ** | ** | ** |
| **A** | ** | ** | * | * | ** |
| **MB** | ** | ** | * | ns | ** |
| **MA** | ** | ** | ** | ns | ** |
| **BA** | ns | ** | ** | ** | * |
| **MBA** | * | ** | ** | ** | ** |
| **MSE** | 0/8533 | 0/00137 | 0/8057 | 0/6788 | 0/1211 |

Columns having different superscript significantly (P<0.05) differ. M: effect mulberry leaves B: effect barley flour, A: the effect enzyme, MB: effect interaction mulberry leaves and barley flour, BA: effect interaction barley flour and enzyme, MA: effect interaction mulberry leaves and enzyme, AMB: The effect of three additive SEM : stander error of means, a + b : potential gas production ( ml ), c : gas production rate during incubation ( ml/ h ), IVOMD : *In vitro* organic Matter Digestibility(%)., IVDOMD : *In vitro* organic Matter Digestibility in dry matter(%), ME = Metabolic energy (MJ/kg DM).

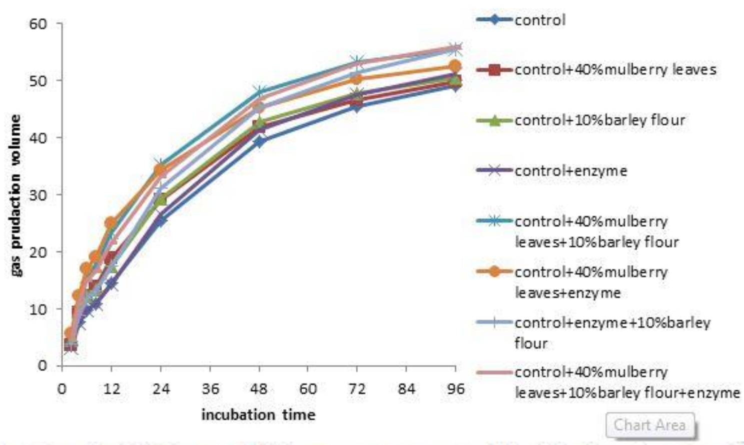
Discussion: In this study the pH lowest value 4/08 was in 10% barley flour + enzyme sellulas (treatment7) which is expected due to improve the activity of lactic acid bacteria to produce lactic acid and discourage the clostridia. khadem(2009) reported The concentration of lactic acid was higher inbarley flour mixed alfalfa silage(BAS) compared to that in ordinary alfalfa silage (AS) which could be due to the use of barley flour for fortifying alfalfa silage.result showed thataddition of barley flour to silage decreased the pH. ADF and NDF decreased with addtivies enzyme because enzyme are able broken link lignocellulosic these results were inconsistent with Zobelletal (2000). In contrast Ash was increased with additives mulberry leaves due to the high was mineral content.Verstegen (2011) reported that Ash content in mulberry leaves was (13.6%).This results revealedhighest value of CP wasat treatment8 .this might have occurred due to additive Together mulberry leaves and barley flour especially mulberry leaves. Mulberry leaf are of a high protein quality comparable to or better than soybean meal (Machii, 1989), and it could be an alternative supplemental feed, comparable and in some cases superior to alfalfa, to replace conventional feed resources for goat (Nguyen ., 2005) and sheep (Doran ., 2007).Verstegen (2011) reported that CP content of the mulberry leaves was the highest (22.3%)Among the four forages studied.the value reported in the previous and present studies indicates that mulberry leaves could be a high protein forage for farm animals.

the high value gas prouduction was shown in treatment5that was containing mulberry leaves and barley flour andthe low value recorded in the control treatment. The Negative relationship showed between Rate and volume of gas produced with ADF and NDF content, This finding was similar to that found by hadietal(2003).

In this study the Acid detergent fiber (ADF) and Neutral detergent fiber (NDF) had negative relationship with (b) and ( c ) decreased ADF and NDF content lead to high gas production.

Researchers with research on Parameters of nutritional barley grain and cornreported that isthe amount of barley starch 59/2%(Herrera-Saldana , 1990),The amount of corn starch, 71% (sadeghi and shorang., 2006), therefore can be said that Much of the gas production is from the fermentation carbohydrates, especially starch (parnyanekhajedizj., 2010), that duetotheincrease gas production could by additivebarley flour. The all estimated parameters of gas productionfraction (b), (a+b) and (c) were increased with the addition only of barley flour to common reed forage silage.The in vitro organic matter digestibility (IVOMD) , in vitro organic matter digestibility in dry matter (IVDOMD) and metabolic energy (ME) showed increasing containing additives treatment than to control treatment and was highest content in treatment 7.

Conclusion: This result concluded that the addition of three additives improved the quality of common reed forage silage, chemical composition, in vitro organic matter digestibility, metabolic energy and fermentation activities.




Gas prudaction (ml/200mg DM) treatments studied in incubation diffrent times

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