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Determination of the Best Method for Silage of Berseem Clover (*Trifolium alexandrinum*) in Humid Weather Conditions

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ABSTRACT

In humid conditions, we do not obtain dry forage. The best conditions for production of dry forage are silage. This experiment was carried out for evaluation and determination of suitable plant material for silage of berseem clover (*Trifolium alexandrinum* L.) in humid weather conditions. Berseem clover, grown in the north part of Iran with humid climate, was harvested at 40-45 cm height. Then single or mixed silage with different ratios of barley and maize milled grains (10 & 20%) and stubble of wheat and paddy (20 & 40%) were kept in the cement silages. The quality of silage materials were evaluated by measuring their pH, lactic acid and NH_4^+, 45 days after ensiling. Results showed that the best composition for silage of berseem clover is the combination of berseem clover with 20% of maize milled grains indices with pH 4.2, lactic acid 6.69 and NH_4^+ 1.48. © 2010 Friends Science Publishers

Key Words: *Trifolium alexandrinum*; Silage; Additive materials; Humid weather conditions

INTRODUCTION

A chief part of berseem clover (*Trifolium alexandrinum* L.) is ensiled to preserve its quality and nutritional value. It is possible through moisture and temperature reduction of silage and the use of additive materials such as chemicals and plant materials to silage contents. Among the plant materials, it can be referred to milled grains such as maize, oat and barley, also grain stubble and beet molasses (Fairchild, 1992; Kennedy & Mackie, 1995). Successful silage crop production is based on a number of key factors that include species and variety selection, soil type, soil pH, fertilizer regime, pest control, optimal harvest date and storage losses (O'Donvan *et al*., 2000; Ross *et al*., 2004a & b; King, 2007). Lactic acid, sulphuric acid, formic acid and propionic acid can also be mentioned as chemicals, which reduce the acidity of ensiled materials (Siadat, 1993). The quality of ensiled materials without the use of additive materials is variable. In general, cereals are ensiled better than other plant species. To add chemicals and plant materials to the silage contents will lead to enhanced quality of ensiled contents (Lattemae, 1997).

The quality of ensiled material depends on the composition and nutritional value of fodder, type of ensiled fodder, variety and operations of harvesting and silage preparation. Indices such as pH, lactic acid, ammonia, color, scent and taste of ensiled materials are used to determine the quality of silage (Edwards, 1997). Ensiling process is complete at a pH range of 4.2-4.6 (Khorasani *et al.*, 1993; Bolsen *et al.*, 1996). Optimal pH and total organic acids for wet ensiled materials should be between 3.8 and 4.2%, also 1.5 and 2.5%, respectively and the amount of lactic acid should be three times higher than acetic acid (Kniht, 1985). The presence of ammonia in the fodder silage is an index for decomposition of protein materials. The amount of ammonia used for this purpose is varied between 1 and 9.25% of silage protein. Graves and Williams (1999) showed that berseem clover can be ensiled by ratios of clovers, Poaceae (1:1) or clovers, barley (3:1). This increased the quality of silage by decreasing the moisture of silage contents. Almeida *et al.* (1989) through to expose of *Pennisetum purpureum* to sunlight for 4 and 8 h and to add 15 and 30% sugarcane to it, showed that the relative withering of fodder during 4 h and the following silage resulted in the improvement of dried material (28.6%), raw protein (9%), raw fiber (37.1%) and dried materials digestibility (53.4%). They also showed that devote of 15% of silage capacity to sugarcane, the amount of solved carbohydrates required for the fermentation of ensiled materials will be supplied. Cussen *et al.* (1995) demonstrated that using pure or mixed (*Lolium perenne* L.) silage with 30% and 60% of white clover, the amount of lactic acid is not related to the composition of plant materials in the silage, but increasing of cereals to the mixture leads to the extreme rise in lactic acid. Also the amount of ammonia was highly affected by the composition of ensiled plant materials.

The studies of Optiz and Jucken (1995) on the pure

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and mixed silage duration of grasses showed that the silage duration of fodder materials increased in line of increasing the share of grasses. The acidity of the ensiled materials showed that the stability of the silage contents was significantly influenced by its composition. In these conditions, the presence of grasses in the silage causes an undesirable effect on the pH of the silage. Feeding mixed ensiled fodder of berseem clover and barley stubble to cows and buffalos during hot months of the year increased their production to 5% (Alim, 1991). The studies showed that the silage of sorage maize, the composition of sorage maize along with sweet clover and sorage maize along with urea accompanied with the production of 74%, 67% and 74% nutrition unit; 9.7, 9.2 and 9.77 MJol energy and 90, 118, and 116 g protein per kg of dried material, respectively (Khitrinov & Khitrynau, 1994). The amount of digestible protein per each fodder unit in the mixed silage of maize along with sweet clover was more than that of maize along with urea sole silage of sorage maize (Khitrynau & Khamitsevich, 1994). The daily weight gain of the 8 months old calfs nourished from these materials for 152 days was 6.03, 6.54 and 6.44 g/kg, respectively (Khitrynau *et al*., 1994).

The objectives of this research were to obtain the kind and the best ratio of additive materials (10 & 20% of barley & maize milled grains+berseem clover, 20 & 40% of stubble of wheat and paddy+berseem clover, 20 & 40% of rice stubble+berseem clover, 20 & 40% of wheat stubble+berseem clover and sole berseem clover) (totally 9 treatments) in order to producing the silage of clover in humid environmental conditions (Guilan province) in the north part of Iran. Also to obtain an overall proportion of these materials, along with retaining of nutritional value of ensiled materials.

MATERIALS AND METHODS

This experiment was carried out in the research farm of Rasht Azad University, Iran, situated in latitude N 37.19 and E 46.36. The total rainfall of the region is 1420 mm, the average annual temperature is 16.5°C and the hottest and coldest months of the year are August with 31.9°C and February with 2.5°C.

The study was carried out in 9 treatments and 3 replications including 27 cement silages with the capacity of 1 m^3. Experimental treatments included the following silages: sole berseem clover (T_1), 90% berseem clover+10% milled barley (T_2), 80% berseem clover+20% milled barley (T_3), 90% berseem clover+10% milled maize (T_4), 80% berseem clover+20% milled maize (T_5), 80% berseem clover+20% rice stubble (T_6), 60% berseem clover+40% rice stubble (T_7), 80% berseem clover+20% wheat stubble (T_8) and 60% berseem clover+40% wheat stubble (T_9).

The results of the chemical analysis of the experimental materials are presented in Table I. Berseem clovers were harvested when they reached 40-45 cm (containing 15.7% dried materials) and were chopped off to segments 1-1.5 cm. The segments were stored in sterilized cement silages along with additive materials. The silages were covered with plastic after pressing. Silage temperature was measured and recorded twice a day. After 45 days the contents of the silage were taken out and selected 5 samples randomly from each silage and transmitted to the laboratory. After extraction the acidity of extracts were measured by pH-meter followed by filteration several times. Obtained solution was used for measuring the amounts of lactic acid, calcium, phosphorus, and ammonia using titration by sodium hydroxide 0.1 N, titration by EDTA, spectrophotometer and kjehldal methods, respectively. The solutions with pH 3.8 to 4.2 (90% berseem clover+10% milled barley, 80% berseem clover+20% milled barley, 90% berseem clover+10% milled maize, 80% berseem clover+20% milled maize) were selected for the rest of operations and the solutions with pH up to 5 were ignored.

Data were subjected to ANOVA (analysis of variance) and significant differences between treatments means were determined by MSTATC and STATISTICA softwares.

RESULTS AND DISCUSSION

Table II shows the summary of variance analysis of experimented characters. As shown in the table, the composition of ensiled materials had a significant effect at a probability of 1% on the pH reaction of silage environment and the amount of lactic acid and ammonia. Nonetheless the amount of ammonia and phosphorus of silage contents was not affected by the ensiled plant composition. Fig. 1 shows that to add rice stubble (80% berseem clover+20% rice stubble, 60% berseem clover+40% rice stubble) and wheat stubble (80% berseem clover+20% wheat stubble & 60% berseem clover+40% wheat stubble) had no result on reducing the acidity of silage environment and significant difference was not observed between the amount of pH in the silage environment and pH of the sole berseem clover silage.

The deficiency of sugar in the contents of stubble is one of the main reasons for inefficiency of rice and wheat stubble on the acidity of silage environment (Table I). Optiz and Jucken (1995) showed that the silage duration of fodder materials increase in line with increasing of the grasses share in the silage environment, but the presence of grasses in the silage has an undesirable effect on the pH of ensiled materials. Allocating 10% and 20% of the silage capacity to milled barley and maize grains decreased the silage acidity significantly, pH reached 5.0, 4.7, 4.4 and 4.2 in 90% berseem clover+10% milled barley, 80% berseem clover+20% milled barley, 90% berseem clover+10% milled maize and 80% berseem clover+20% milled maize treatments, respectively (Fig. 1). According to the literature the best single indicator of the effect of ensiling on the nutritive value of high moisture silage is pH. In general the lower the pH the better, since it indicates that a lactic

Table I: The results of chemical decomposition for experimental materials

Experimental materials	Chemical composition of experimental materials					
	Calcium	Phosphorus	Non-nitrogenous materials	Protein	Dry mater	Moisture
Berseem clover	1.54	0.25	34.3	18.7	15.4	84.3
Barley seed	0.15	0.22	38.69	10.5	86	14
Maize seed	0.18	0.3	68.18	8.6	85	15
Rice fodder	0.06	0.05	35	13	87	13
Wheat fodder	0.22	0.07	40	3.8	88	12

Table II: Condense of analysis variance of nett silo and compost silo (*Trifolium alexandrium*)

Source of variation	Degrees of freedom†		Mean squares				
			pH	Lactic acid (%)	NH_3(%)	Calcium (%)	Phosphorus (%)
Repetition	2	2	0.063	0.084	0.033	0.003	0.001
Treatment	8	3	1.104 **	2.448 **	2.66 **	0.035 NS	0.0003 NS
Experimental error	16	6	0.116	0.119	0.055	0.013	0.001
C.V%			6.55	15.6	9.01	8.65	13.87

† Column of right is for Freedoms Degree and column of left is pH
NS and **not significant and significant of probability for level 1% and 5%

acid type of fermentation has occurred. The most appropriate pH for wet ensiled materials is between 3.8 and 4.2 (Kniht, 1985; Iqbal *et al*., 2005). Iqbal *et al*. (2005) indicated that ensiling mott grass silage for 30 days had better pH (3.9 vs. 5.3 & 4.3) and lactic acid concentration (4.9 vs. 3.3 & 4.5%) compared to 10 and 20 days of ensiling periods. The low pH level and high lactic acid contents in mott grass silage ensiled was because of increased availability of fermentable carbohydrates (Iqbal *et al*., 2005).

The present experiments showed that the composition of 80% berseem clover+20% milled maize with creation of a proper pH, 4.2 (Fig. 1a) in the silage environment is a suitable composition and recommended for application in humid weather conditions. Graves and Williams (1999) demonstrated that berseem clover can be silaged by clover and graminae as well clover and barley grain with ratios of 2:1 and 3:1, respectively. Thus decrease in silage humidity causes increase in its quality.

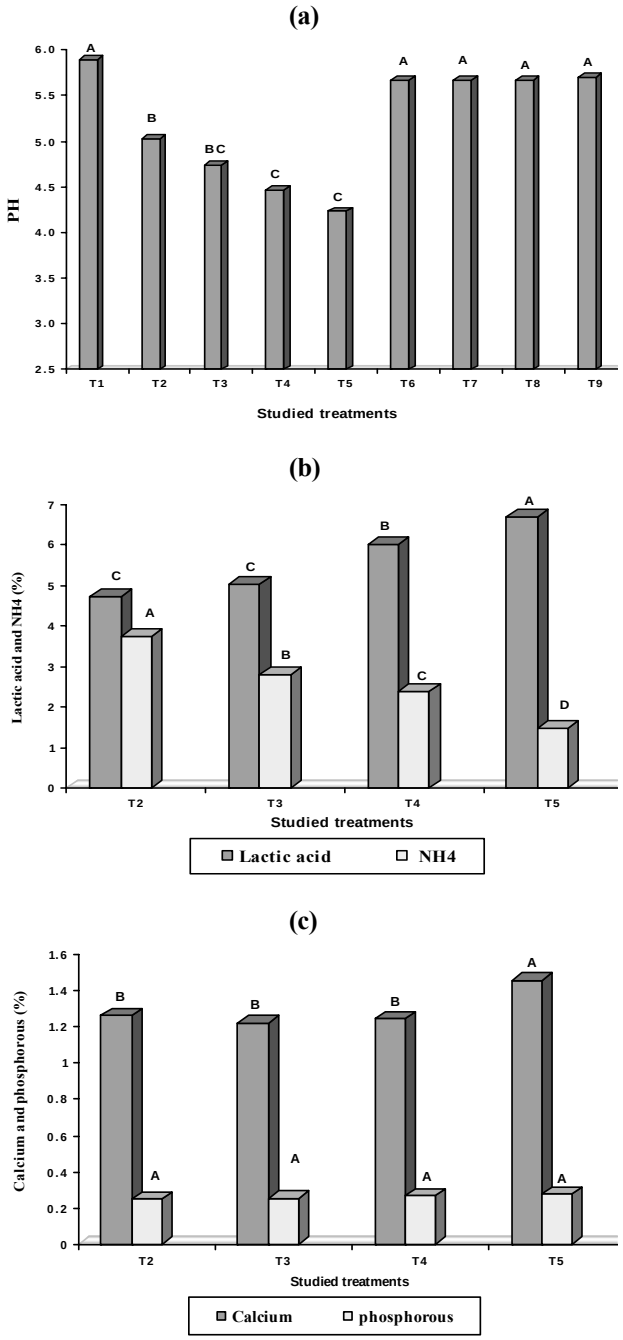
In current study, treatments with pH more than 5 were ignored and the qualitative evaluation of silage product was restricted to 90% berseem clover+10% milled barley, 80% berseem clover+20% milled barley, 90% berseem clover+10% milled maize and 80% berseem clover+20% milled maize treatments. Comparison of the means of lactic acid in the experimental treatments showed that the silage of 80% berseem clover+20% milled maize produced the highest amount of lactic acid (6.69%) (Fig. 1b). Reducing of the milled maize to 10% of silage's relative weight resulted in a significant decrease in the amount of lactic acid (6.02%) and increasing of the milled barley grains instead of maize grains led to a more decrease in the amount of lactic acid in the silage environment (in 90% berseem clover+10% milled barley & 80% berseem clover+20% milled barley equal to 4.73% & 5.03%, respectively) (Fig. 1b). Contrary to our results, Cussen *et al.* (1995) using the silage of (*Lolium perenne* L.) as both of sole and mixed with 30% and 60% of white clover showed that the amount of lactic acid in the ensiled contents had no relation to the composition of plant materials in the silage, while increasing in the mixture caused in an extreme rise in the lactic acid of the silage cereals environment. The presence of ammonia in the silage indicates an extreme decomposition in the protein materials and the amount is varied between 1% and 9.25% of protein materials (Holand & Brumer, 1999). Increasing milled maize to 10% and 20%, as compared to barley, resulted in a significant reduction in ammonia in the silage content. Thus the quality of silage was increased (Fig. 1b). As shown in Fig. 1b, the highest and the lowest amounts of ammonia in silage environment were produced by a composition of 90% of berseem clover+10% of milled barley (3.73%) and 80% of berseem clover+20% of milled maize grains (1.47%), respectively. The main reason for decreasing in the amount of ammonia and increasing of the share of maize is the large amount of sugar in maize grains compared to that of barley grains (Table I). Cussen *et al*. (1995) also showed that the amount of ammonium in the silage environment was highly affected by the composition of plant materials in the silage. Nutrient constituents can be an important parameter in determining the microbial pattern of ensiled plants such as maize (Bal & Bal, 2009). The fermentation process is highly influenced by availability of bacterial substrate (fermentable carbohydrates), crude protein content, moisture content and predominant bacteria during ensiling process (Khorasani *et al.*, 1993; Bolsen *et al.*, 1996).

As shown in Table II, no various compositions of the silage had significant effect on the amount of silage calcium and phosphorus, but the average amount of calcium in treatment of 80% of berseem clover+20% of milled maize grains (1.45%) was significantly more than other experimental levels. Therefore no the experimental plant materials had significant effect on the amount of silage calcium and phosphorus (Table I, II & Fig. 1c).

CONCLUSION

The best method for berseem clover silage in humid

Fig. 1: The effect of silage composition on acidity of silage medium (a), the amount of lactic acid and ammonia (b), the amount of calcium and phosphorus in materials containing silage (c)



weather conditions is the mixed silage of 80% berseem clover+20% of milled maize grains. Increasing the solved glusids in the silage environment resulted in a decreasing the acidity and ammonia and an increase in the amount of lactic acid in the ensiled materials. These conditions cause the silage stability also a tasty and high quality fodder for animal consumption.

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