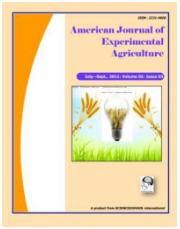

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Fermentation Characteristics, In Situ Rumen Degradation and Aerobic Stability of Whole Crop Barley Ensiled with Urea or Aqueous Ammonia

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Research Article

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ABSTRACT

Various chemical compounds might be added to forage to maintain or improve the quality value of a crop ensiled. The aim of the present experiment was to evaluate the fermentation characteristics, in situ rumen degradation and Aerobic Stability of whole crop barley ensiled with Urea or aqueous Ammonia. In the first experiment, Whole crop barley was harvested (35% DM), chopped, and then ensiled using laboratory silos (n= 4) as untreated (UT) or treated with urea (10, 20, 30 and 40 g kg^{-1} DM; U1, U2, U3 and U4, respectively) or aqueousammonia (10 and 20 g kg^{-1} DM; A1 and A2, respectively) for 30 days. Standard procedures were used to determine the chemical composition of the samples. The pH of the aqueous silage extract was determined using a pH meter. Ammonia-N concentration was determined in acidified silage extract (5 ml of the extract + 5 ml of 0.2 M HCl) using a distillation method. Four sheep (live weight: 44±3 kg) fitted with rumen fistulae were used. Approximately 5 g DM of each sample was placed in a polyester nylon cloth bag (10 × 12 cm, pore size of 52 µm, n=4), then incubated in the rumen for 0.0, 2, 4, 8, 16, 24, 48, 72 and 96 h. Rumen removal bags were washed in cold running water and dried in oven (60 ℃, 48 h), then weighted to determine DM disappearance. The equation of $P= a+b (1-e^{-ct})$ was applied to determine the coefficients (a= quickly degradable fraction, b= slowly degradable fraction, c= fractional degradation rate constant). Both urea and anhydrous ammonia caused a significant ($P <0.05$) increase in silage pH and NH_3-N, and CP concentrations. The slowly degradable fraction (b) of the silage treated with urea was significantly ($p <0.05$) higher than those of the untreated sample. Potential degradability of U4 was higher than other treatments. In a second experiment, whole crop

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barley was harvested (32.5% DM), chopped, and then ensiled (n= 4) for 35 days as untreated (UT) or treated with urea (23.4 g Kg^{-1}DM) or aqueous ammonium (13.1 g Kg^{-1}DM), to obtain a final application rate of about 0.35% N of fresh forage weight. The population of yeasts and molds (colony-forming unit= CFU) was determined by spread plating of filtered extract silages on malt extract agar. Aerobic stability was defined as the time it took for the temperature in the silage masses to rise 2 ℃ above ambient temperature. Whileboth of urea and ammonia had no effect on the initial number of mold in the silages, these treatments significantly lowered CFU of yeast in fresh silages. In this experiment both Ammonia and urea had a significant effect on inhibition of rising temperature and yeast in aerated silages. Urea and ammonia treatments significantly enhanced aerobic stability of silages.

Keywords: Whole crop barley silage; urea, ammonia; in situ degradability; aerobic stability.

1. INTRODUCTION

Whole crop cereal silages are the most important fodder crops for feeding dairy cows in Iran. Various microorganisms, enzymes and chemical compounds have been added to forage to maintain or improve the nutritive value of a crop ensiled (Arbabi et al., 2008; Kung et al., 2003; Vatandoost et al., 2010). As is the nature of most biological systems, there is a considerable variation in the outcome of using these additives. Some additives, which have proven to be effective in this respect, include chemicals based on ammonia-N additives (Alliet al., 1983; Hill and Leaver, 1999; Kung et al., 2000). It has been reported that ammonia and urea are practical and relatively inexpensive sources of non-protein nitrogen which can be used to increase the protein concentration of low protein forages such as cereal silage (Carr et al., 1984). McDonald et al. (1991) reported that ammonia used as an additive in silage to improve the nitrogen content of the product. On the other hand, ammonia-N inhibit yeasts and molds growth and can markedly improve the stability of cereal silages and lowered the peak temperatures of aerated silage due to its fungicidal properties, and thus increased bunk life (Hassoun et al., 1990; Kung et al., 2000). Hence the aim of the present study was to evaluate the fermentation characteristics, in situ rumen degradation and microbial characteristics of whole crop barley ensiled with various amounts of urea and aqueous ammonia in laboratory scale silos.

2. MATERIALS AND METHODS

2.1 Ensiling Procedures

In experiment 1, whole crop barley was harvested (35% DM), chopped, and then ensiled (n= 4) for 40 days. Approximately 3.25 kg of the forage from each treatment was packed into a laboratory scale polyethylene tube to achieve a packing density of about 220 kg of DM per m^3. The silos were immediately sealed by Polypropylene Screw Cap on top with a rubber seal and stored at ambient in varying temperature. The forage was ensiled as untreated (UT) or treated with the following additives; urea (10, 20, 30 and 40 g kg^{-1} DM; U1, U2, U3 and U4, respectively), aqueous ammonia (10 and 20 g kg^{-1} DM; A1 and A2, respectively), for each replicates per each treatment. These samples were evaluated to determine chemical composition and in situ degradation parameters of dry matter. In experiment 2, whole crop

barley was harvested (32.5% DM), chopped, and then ensiled (n= 4) for 35 days. Approximately 3.35 kg of the forage from each treatment was packed into a laboratory scale polyethylene tube to achieve a packing density of about 225 kg of DM per m^3. The silos were immediately sealed by Polypropylene Screw Cap on top with a rubber seal and stored at ambient in varying temperature. The forage was ensiled as untreated (UT) or treated with urea (23.4 g Kg^{-1} DM) or aqueous ammonium (13.1 g Kg^{-1} DM), to obtain a final application rate of about 0.35% N of fresh forage weight. These samples were evaluated to determine Microbial Analysis and Aerobic stability.

2.2 Chemical Analysis

Representative samples of fresh chopped whole crop barley and the silages were collected, oven dried to a constant weight at 60° C, and ground to pass through a 2 mm-screen for later analysis. Standard procedures were used to determine the chemical composition of the samples. Crude protein (CP) was determined according to the Kjeldahl procedure (AOAC, 2004) on the Tecator Auto-analyzer (1030). Determination of neutral detergent fiber (NDF) was made using the method of Van Soest et al. (1991). Samples of fresh silage (approximately 50 g) were mixed with 450 ml distilled water, and the silage extraction was made. Then, silage pH was determined using a portable pH meter (Metrohm 691, Swiss). Five ml of the silage extract was mixed with 5 ml of 0.2 N HCl. Ammonia-N concentration of the acidified silage extract was determined using distillation method (Kjeltec 2300 Autoanalyzer, FossTecator AB, Hoganas, Sweden). Ensiling DM recovery determined as the weight of difference between silo DM content at sealing and opening.

2.3 In Situ Technique

The ruminal degradable parameters of dry matter (DM) of the silages were determined using in situ procedure (Fathi Nasri et al., 2006). Four sheep (45 Kg, body weight) fitted with rumen fistulae were used in the present study. The bags (10×12 cm) were made of polyester nylon cloth with a pore size of 48 µm. Approximately, 5 g DM of each sample was placed in each bag, and four bags per each treatment were incubated for each time (2, 4, 8, 16, 24, 48, 72, 96 h). After removal the bags from the rumen, they were washed in cold running water and dried in anair-forced oven (60° C, 48 h). Zero time disappearance was obtained by washing rumen-unincubated bags in a similar way. After that, the bags were weighed and analyzed to determine the ruminal degradable parameters.

2.4 Microbial Analysis

After 35 days of ensiling, and after 6 days of aerobic exposure, approximately, 25 g sample of each silage sample was blended in 225 ml of sterile 25% strength Ringer's solution (Oxoid BR0052G) and homogenized for 1 min, and then was filtered through a double layer of cheesecloth into sterile tubes for microbial analysis. The filtered extract was used for the population of yeasts and molds by spread plating on malt extract agar (Oxoid CM0059) which had been acidified with 85% lactic acid (0.5% vol/vol) after autoclaving. Subsequent serial 10-fold dilutions were made (with the same diluter), to obtain 30 to 300 colonies per dish. The plates were incubated at 32^o C for 48–72 h and those containing a minimum of 30 and a maximum of 300 colony-forming units were enumerated.

2.5 Aerobic Stability

Three kilograms of well mixed silage from the bottom portion of each silo (free of visible spoilage) were placed back (without packing) into clean silos and a thermocouple wire was inserted into the center of each silage mass and recorded the temperature every 30 min. Aerobic stability was defined as the time it took for the temperature in the silage masses to rise 2^{o} C above ambient temperature.

2.6 Calculating and Statistical Analysis

The equation of $P= a+b\ (1-e^{-ct})$ was applied to determine the coefficients of a= quickly degradable, b= slowly degradable and c= constant rate of degradation of the incubated samples at t= time (Ørskov and McDonald, 1979). Effective Degradability (ED) of DM was then calculated according to the equation of Ørskov and McDonald. (1979), where ED= a+ ((b×c)/ (k+c)), where k is the rumen outflow rate assumed to be 2, 4 or 6% $h^{-1}$and a, b and c are as described before and the potential of degradability (PD) was measured by a+b. The data on microbial populations were transformed to log10 CFU $g^{-1}$of DM of forage or silage prior to statistical analysis. Chemical data are presented on a DM basis. Data of silage of pH and chemical components (NDF, NH3-N and CP), microbial and aerobic stability were statistically analyzed using complete randomized design. The statistical model was $Y_{ij}= \mu+ T_i+ \varepsilon_{ij}$, where Y_{ij}= dependent variable, μ= dependent valuable mean, T_i= effect of treatment, εij= residual error term. Data were analyzed using the GLM procedure of SAS. The Duncan procedure was used to test the mean significant difference at $P <0.05$.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition

Chemical composition of the untreated and treated whole crop barley silage (WCBS) is shown in Table 1. In the present study, the pH, NDF, CP and NH3-N was significantly affected ($P <0.05$) when urea or ammonia were applied. The pH of silages was dependent on the level of urea and ammonia application. Urea treated silages at rate of 30 or 40 g kg^{-1} DM increased pH significantly and in ammonia treated silages, application of 20 g kg^{-1} DM of ammonia increased pH ($P <0.0001$). These finding agree with those of Sarwatt et al. (1995) and Kung et al. (2000) who stated that the addition of urea and ammonia in cereal silages causes an increase in pH. As expected, in this study urea and ammonia enhanced the ammonia-N concentration in silages which also increased pH in silages this experiment. The data of CP content of the silages showed that urea caused an increase in the percentage of CP by about 3.25 units and ammonia caused an increase of about 1.8 units ($P <0.01$). This agrees with previous published data that reported the addition of urea to cereal silages significantly increased CP content (Buchanan-Smith, 1982). It has been previously reported that ammonia reduces plant proteolysis during ensiling of forages (Buchanan-Smith, 1982); in addition, forages treated with ammonia have been shown to be higher in insoluble N (Huber et al., 1979) and true protein (Buchanan-Smith, 1982).

Losses of NDF in the silages varied between 2 to 45 g kg^{-1} DM depending on the level of urea applied to the silo. Similarly, losses of NDF for ammonia treated silage varied between 12 and 22 g kg^{-1} DM and High level of urea and ammonia lowered significantly NDF content of silages ($P <0.01$). This support previous data who reported the presence of urea decreased structural carbohydrate content in whole crop cereal silages (Adesogan et al.,

1998; Deschard et al., 1988; Guney et al., 2007; Tetlow, 1992). Adogla-Bessaa et al. (1999) reported that Urea had a potential role in the increasing degradation of NDF during conservation, both by upgrading of nutritive value and through forage preservation.

3.2 In Situ Ruminal DM Degradation

Data of ruminal in situ degradation parameters of DM are shown in Table 2. Results indicated that the slowly degradable fraction (b) of DM of the silage treated with urea (U4) was significantly higher than those of the untreated samples ($p < 0.05$). Previous study reported that both urea and ammonia increase the digestibility of DM, OM and cell wall components of silages (Bolsen et al., 1996; Budag et al., 2009). Ammonia as a result of hydrolysis of urea, may cause to change in chemical composition of the cell wall, that usually render it more available to ruminal bacteria (Dawson and Steen, 2000; Mason et al., 1989). Davis (1980) reported that the addition of ammonia to forages increases the digestibility due to the solubilization of hemicellulose and delignification. Ammonia combines with the residual moisture in hay forming ammonium hydroxide that breaks the lignin-cellulose bonds in the cell walls of the forage. It also solubilizes some of the complex carbohydrates in the plant and swells plant fiber, thereby allowing for greater rumen microbial breakdown of the forage that caused to improvement in digestibility of forages (Kuhl, 1982). In this study, the slowly degradable parameter of DM was high in treatment U4 and A2 while coefficient c was lower which may provide a lower digestion in the rumen. In this case, feeds remain into the rumen for a longer time and rate of pass decrease. These factors caused to decline in the utilization of nutrients or potential of degradability (a+b) of dry matter in U4 treated silage.

3.3 Microbial Analysis and Aerobic Stability

Data of pH, DM, DM recovery, mold and yeast of fresh silages and pH, Mold and yeast of aerated silages after 6 days are shown in Table 3. Generally both Ammonia and urea treatments caused an increase in ammonia-N concentration in silages and had a significant effect on inhibition of rising temperature and yeast in silages after opening and exposure to the air. Microbiological data suggested that the addition of both of urea and ammonia had no effect on the initial number of mold in the silages, but after exposure, growth was delayed. In the case of yeast, this condition observed only for urea treated silage. As a whole, when exposed to air, silages can spoil rapidly due to the growth of yeasts (Henderson, 1979; Woolford et al., 1982). In the present study a rapid rise in temperature during aerobic exposure was observed in untreated silage. These findings are similar to those of Woolford et al. (1982), who reported that yeasts are essentially responsible for the aerobic instability in corn silage.

Urea impaired the DM recovery of whole crop barley silage by more than 2.8% of dry matter. It is in contrast to the findings of Huber et al. (1979, 1980), but in agreement with Bolsen et al. (1992). The reasons for these findings are unclear but varying recoveries of ammonia and types of silos used in different studies (bunks versus lab silos) could be factors affecting DM recoveries.

Data of aerobic stability are shown in fig. 1. The data showed that both urea and ammonia increased significantly aerobic stability ($p < 0.05$). Aerobic deterioration of conserved feeds is a complex process combining physical, chemical and microbiological factors.

Table 1: Chemical composition of whole crop barley silage as an untreated or treated with urea or aqueous ammonia

Item	Fresh forage	Treatments							S.E.M	P
		UT	U1	U2	U3	U4	A1	A2		
pH	6.78	4.07^{c}	4.35^{c}	4.75b^{c}	7.10^{a}	6.90^{a}	4.32^{c}	5.65^{b}	0.411	*
NH3-N (ml/dl)	--	9.1^{f}	26.8^{d}	46.7^{c}	80.2^{b}	94.7^{a}	17.6^{e}	31.7^{d}	3.59	*
CP (g kg^{-1} DM)	75.4	79.8^{f}	84.1^{e}	90.2^{d}	103^{b}	113^{a}	90.3^{d}	98.2^{c}	1.14	*
NDF (g kg^{-1} DM)	640	552^{a}	550^{a}	537^{ab}	527^{b}	507^{c}	540^{ab}	530^{b}	7.63	**

^{a,b,c,d,e,f}Means with different letters in the same row w differed significantly at P <0.05;
UT= untreated; U1= 10 g kg^{-1} DM; U2= 20g kg^{-1} DM; U3= 30 g kg^{-1} DM; U4= 40 g kg^{-1} DM; A1= 10 g kg^{-1} DM; A2= 20 g kg^{-1} DM;
**: (P <0.0001); **: (P <0.01).*

Table 2: In situ dry matter degradable coefficients of whole crop barley silage as an untreated or treated with urea or aqueous ammonia

Coefficients	Treatments[1]						
	UT	U1	U2	U3	U4	A1	A2
a	0.31± 0.02	0.31± 0.01	0.31± 0.02	0.30± 0.01	0.32± 0.01	0.28± 0.02	0.28± 0.01
b	0.52± 0.03^{b}	0.52± 0.02^{b}	0.54± 0.02^{ab}	0.56± 0.03^{ab}	0.59± 0.03^{a}	0.56± 0.03^{ab}	0.58± 0.03^{ab}
c	0.043± 0.007^{a}	0.037± 0.005^{ab}	0.035± 0.006^{ab}	0.029± 0.004^{b}	0.025± 0.004^{b}	0.035± 0.005^{ab}	0.025± 0.004^{b}
ED (0.02)	0.66	0.65	0.65	0.64	0.64	0.64	0.60
ED (0.04)	0.58	0.56	0.56	0.54	0.54	0.54	0.50
ED (0.06)	0.52	0.51	0.50	0.49	0.49	0.49	0.45
PD	0.84	0.84	0.85	0.86	0.91	0.84	0.86

^{a,b}Means in each row with unlike superscript letters differ Significance at P <0.05.
a= rapidly degradable; b= slowly degradable; c= fractional degradation rate constant.
ED (effective degradability) = a+((b×c)/ (k+c)) where k is the rumen outflow rate assumed to be 0.02, 0.04 or 0.06 h^{-1}.
PD (potential degradability) = a+b; UT= untreated; U1= 10 g kg^{-1} DM; U2= 20 g kg^{-1} DM; U3= 30 g kg^{-1} DM; U4= 40 g kg^{-1} DM; A1= 10 g kg^{-1} DM; A2= 20 g kg^{-1} DM.

Table 3: Chemical and microbial composition of whole crop barley silage as an untreated or treated with urea or aqueous ammonia after 35 d of fermentation.

Item	Treatment			S.E.M	P
	Untreated	Urea	Ammonia		
Fresh silages					
PH	4.10[b]	4.52[a]	4.45[a]	0.05	0.0007
DM	32.1[b]	31.8[b]	32.8[a]	0.11	0.0005
DM Recovery	93.8[a]	90.0[b]	91.8[ab]	0.68	0.0121
Mold, log cfu g^{-1}	3.43	3.49	3.24	0.09	0.1702
Yeast, log cfu g^{-1}	4.28[a]	3.97[b]	3.84[b]	0.07	0.0042
After 6 days aerated					
PH	7.42	5.61	5.35	0.06	<.0001
Mold, log cfu g^{-1}	4.79[a]	4.68[ab]	4.04[b]	0.22	0.0776
Yeast, log cfu g^{-1}	5.97[a]	4.35[b]	4.04[b]	0.22	0.0004

[a,b,c,d]*Means in columns with unlike superscripts differ (P <0.05).*
Urea: Urea applied at rate of 23.4 g Kg^{-1}DM to obtain a final application rate of about 0.35% N of fresh forage weight (assuming 32.5% DM).
Ammonia: Aqueous ammonia applied at rate of 13.1 g Kg^{-1}DM to obtain a final application rate of about 0.35% N of fresh forage weight (assuming 32.5% DM).

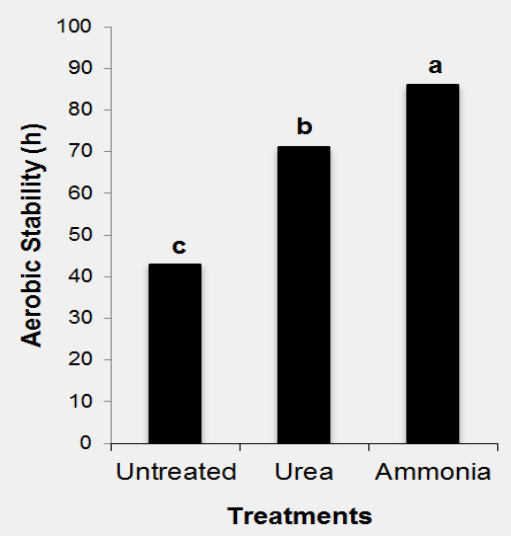

Fig. 1. Temperature changes as an indication of aerobic deterioration in untreated, urea treated or ammonia treated whole crop barley silage.
[a,b,c]*Bars with different letters differ (P <0.05).*
Urea: Urea applied at rate of 23.4 g Kg^{-1}DM to obtain a final application rate of about 0.35% N of fresh forage weight (assuming 32.5% DM).Ammonia: Aqueous ammonia applied at rate of 13.1 g Kg^{-1}DM to obtain a final application rate of about 0.35% N of fresh forage weight (assuming 32.5% DM).

Oxidation of substrates such as lactic acid, acetic acid, water soluble carbohydrate and starch is a important process in generation of heat during aerobic deterioration of conserved feeds (Hill and Leaver, 2002). The rise of temperature in silos that were not treated with urea or ammonia at ensiling was rapid during the first days of exposure, possibly reflecting

development of populations of spoilage micro-organisms in the silo (Muck and Bolsen, 1991). Urea additive in silage hydrolysis is in the presence of bacterial ureases (Dixon et al., 1976), the temperature of the forage mass and the presence of water in the forage conservation system that led to rapid production of ammonia during the early stages of ensiling (Hill and Leaver, 1999). The presence of ammonia-N from urea or ammonia treatments in high concentrations in conserved feeds inhibits microbial activity during ensiling and subsequent aerobic deterioration. In this case, previous study reported that Ammonia toxicity is related to a high pH that maintained the undissociated form of NH3 which is toxic to fungal cells (DePasquale and Montville, 1990; McDonald et al., 1991). Alli et al. (1983) found that urea additive breaks down to ammonia in the silo, and has a toxic effect on yeasts and molds. In present experiment, urea and ammonia caused to significantly increase pH and ammonia-N concentration that the effect of this condition on microbial communities was observed by a reduction in mold and yeast proliferation. This condition reduced the rate of change of temperature in the silage mass in the first days of exposure to air. This is agreed with Hassoun et al. (1990) who reported that ammonia-N applied in the cereal silage might alter the relative efficiency of chemical upgrading as well as the degree of microbial suppression during aerobic deterioration.

4. CONCLUSION

Data of the present study indicate that the use of urea or ammonia in WCBS cause to increase the pH and NH3-N concentration. This findings support previous results (Hill and Leaver, 2002; Kung et al., 2000). Applying this kind of additives in WCBS had a benefit when in situ DM degradation was considered. Present data demonstrate that the DM degradable coefficient of WCBS for fraction of b was improved when treated with urea or ammonia. This effect might be due to the microbial attachment and cell wall degradation of whole crop cereal silage as reported by Hill and Leaver (2002).

The level of urea and ammonia applied at ensiling affected the rate of deterioration of silages after exposure to air. Microbial activities during the exposure of silage lead to a rapid increase in silo temperature (Henderson, 1979; Woolford et al., 1982). In this study the addition of urea and ammonia at ensiling markedly reduced initial populations of yeasts and after exposure to air, prevented proliferation of yeasts and mold that caused to delayed the onset of heating rather than those of the untreated silages. Generally both Ammonia and urea caused to increase ammonia-N in the treated silages and had a significant effect on inhibition of rising temperature, mold and yeast of the silages aerobically.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Alli, I., Fairbairn, R., Baker, B. E., Phillip, L. E., Garino, H. (1983). Effects of Anhydrous Ammonia on Fermentation of Chopped, High-Moisture Ear Corn.

Alli, I., Fairbairn, R., Baker, B.E., Phillip, L.E., Garino, H. (1983). Effects of Anhydrous Ammonia on Fermentation of Chopped, High-Moisture Ear Corn. J. Dairy Sci., 66(11), 2343-2348.

Bolsen, K.K., Ashbell, G., Weinberg, Z.G. (1996). Silage fermentation and silage additives: review. Asian-Austr. J. Anim. Sci., 9, 483–493.

Bolsen, K.K., Sonon, R.N., Dalke, B., Pope, R., Riley, J.G., Laytimi, A. (1992). Evaluation of inoculant and NPN silage additives: a summary of 26 trials and 65 farm-scale silages. Kansas Agric. Exp. Sta. Rpt. Prog., 651, 101–102.

Buchanan-Smith, J.G. (1982). Preservation and feeding value for yearling steers of whole plant corn ensiled at 28 and 42% dry matter with and without cold flow ammonia treatment. Can. J. Anim. Sci., 62, 173-180.

Budag, C., Demirel, M., Celik, S., Bakici, Y. (2009). The effects of adding urea and molasses to corn harvested at dough stage on silage fermentation quality, In vitro organic matter digestibility and metabolic energy contents. J. Anim. Vet. Adv., 8, 1921-1924.

Carr, S.B., Hammes, R.C., Moe, Jr. A.J., McGilliard, M.L. (1984). Corn silage preservation with anhydrous ammonia, live culture microbial, or organic acid-based additives. J. Dairy Sci., 67, 1474-1481.

Davis, G.V. (1980). Ammoniation of roughages and grain.formula feed conference. Garden city branch of kansas state agricultural experimental stations. January., 21-22, 1980.

DePasquale, D.A., Montville, T.J. (1990). Mechanism by which ammonium bicarbonate and ammonium sulfate inhibit mycotoxigenic fungi. Appl. Environ. Microbiol., 56, 3711–3717.

Fathi Nasri, M.H., Danesh Mesgaran, M., France, J., Cant, J.P., Kebreab, E. (2006).Evaluation of models to describe ruminal degradation kinetics from in situ ruminal incubation of whole soybeans. J. Dairy. Sci., 89, 3087-3095.

Guney, M., Demirel, M., Celik, S., Bakici, Y., Levendoglu, T. (2007). Effects of urea, molasses and urea plus molasses supplementation to sorghum silage on the silage quality, in vitro organic matter digestibility and metabolic energy contents. J. Boil. Sci., 7, 401-404.

Henderson, A.R. (1979). Studies on the aerobic stability of commercial silages. J. Sci. Food Agric., 30, 223–228.

Hill, J., Leaver, J.D. (2002). Changes in chemical composition and nutritive value of urea treated whole crop wheat during exposure to air. Anim. Feed Sci. Technol., 102, 181–195.

Huber, J.T., Foldager, J., Smith, N.E. (1979). Nitrogen distribution in corn silage treated with varying levels of ammonia. J. Anim. Sci., 48, 1509.

Huber, J.T., Smith, N.E., Stiles, J. (1980). Influence of time after ensiling on distribution of nitrogen in corn silage treated with ammonia. J. Anim. Sci., 51, 1387–1392.

Kuhl, G. (1982). Ammonia boosts forage quality. Dairy Herd Management. August, 1982. 16.

Kung, L. Jr., Robinson, J.R., Ranjit, N.K., Chen, J.H., Golt C.M., Pesek, J.D. (2000). Microbial Populations, Fermentation End-Products, and Aerobic Stability of Corn Silage Treated with Ammonia or a Propionic Acid-Based Preservative. J. Dairy Sci., 83, 1479–1486.

McDonald, P., Henderson, A.R., Herson, S.J.E. (1991). The Biochemistry of Silage, 2nd edition. Chalcombe Publication, Marlow, UK.

Nocekو J.E. Grant, A.L. (1987). Characterization of in situ nitrogen and fiber digestion and bacterial nitrogen 897-903. I. contamination of hay crop forages preserved at different dry matt& percentages. J. Anim. Sci., 64, 552-564.

Ørskov, E.R., McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rates of passage. J. Agric. Sci., 92, 499–503.

Sarwatt, S.V., Urio, N.A., Ekem, A. (1995). Evaluation of some tropical forages as silage. Nutr.Abstr. Rev. Series B., 65, 4836.

Song, M.K., Kennelly, J.J. (1989). Effect of ammoniated barley silage in ruminal fermentation, nitrogen supply to the small intestine, ruminal and whole tract digestion, and milk production of Holstein cows. J. Dairy Sci., 72, 2981-2990.

Van Soest, P.J., Robertson, J.B., Lewis. B.A. (1991).Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74, 3583-3597.

Woolford, M.K., Bolsen, K.K., Peart, L.A. (1982). Studies on the aerobic deterioration of whole crop cereal silages. J. Agric. Sci. Camb., 98, 529–535.