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Studies on the effects of different chemical additives on the nutritive value of ensiled barley distillers' grain (BDG) using in vitro techniques

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Research Paper

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An experiment was carried out to evaluate four different acids as additives (Lactic: $C_3H_6O_3$, Acetic: CH_3COOH, Hydrochloric: HCl and Sulfuric:H_2SO_4 acid) and a control group (without any additive) for their effects on chemical composition, *in vitro* gas production and *in situ* dry matter disappearance of ensiled barley distillers grain (BDG). Dry matter (DM) content of ensiled BDG ranged from 22.12 to 23.85 %, Crude protein (CP), Ash content, Neutral detergent fiber (NDF) and Acid detergent fiber (ADF) ranged from 22.70 to 23.55 % DM, 3.62 to 5.47% DM, 47.30 to 58.40 % DM and 37.45 to 39.45% DM respectively. BDG treatment with sulfuric acid, significantly influenced the NDF and ADF contents than other treatments ($p<0.05$), so this parameters decreased than those of control group. Treatment of BDG with 0.5% sulfuric acid had the lowest NH3-N and pH than the corresponding control group ($P<0.05$). There was a higher content of effective degradability (ED) and dry matter disappearance (24hours) for this treatment (0.5% Sulfuric acid). All gas production parameters such as cumulative gas production (after 24, 48 and 96 h) and constant rate of gas production (C_{gas}) were highest for ensiled BDG with 0.5% sulfuric acid compared to those of control group ($p<0.05$). It appears that a low level of chemical additives (especially sulfuric acid) may be effective to improve nutritive value of ensiled BDG.

Key words: Chemical additive, barley distillers' grain, ensiled, *in situ*, *in vitro*

INTRODUCTION

Barley distillers' grain (BDG) is a valuable by-product of distillery industry, especially when cereal grains such as barley is used to produce ethanol. These by-products are rich in crude protein (CP), crude fiber (CF), ether extract (EE), vitamins and minerals. The nutrient contents of BDG except of starch, are proportionally higher than those of other by-products. It contains high protein and crude fiber (Dhiman et al., 2003). Therefore, it is mostly used as a protein supplement for cattle. When BDG fed as wet, care needs to be taken to assure that it does not deteriorate before being fed. Since the wet BDG are an excellent media for microbial growth and has been shown to support the growth of yeast and mold, it is better to feed the material as soon as possible after having received it from the brewery (Wyss, 1997; Wadhwa et al., 1995; Aning et al., 1994). It is best not to store the material much longer than a week to 10 days especially important for hot or warm areas (Aning et al., 1994). Kim et al. (1996) reported that wet BDG could be stored for 10 days in spring, 5 days in summer and 30 days during

winter. Feed mixtures containing BDG will spoil quickly, so any excess feed that animals have not consumed should be removed and discarded.

The palatability of wet BDG will decline with increasing storage time. Also it seems that the high moisture content of BDG gives problems associated with their transportation and preservation; hence two possible alternatives for conservation are ensiling and drying (Polan et al., 1985). Drying by means of heat, costs energy and it might be decrease the nutritive value of them, but the ensiling has the advantage to reduce these problems and to increase the reservation time. Indeed, ensiling is a method of forage preservation through stabilizing fermentation process by decreasing the pH within minimum fermentation period. Rasco et al. (1989) found that protein and NDF contents were the most affected by drying method. In silage, lack of oxygen and the accumulation of lactic acid inhibit its microbial metabolism and preserves nutrients (Ranjit and Kung, 2000).

Successful silage fermentation depends on achieving both anaerobic conditions and a low pH. The low pH is usually accomplished through the fermentation of sugars in the crop to lactic acid by lactic acid bacteria, which decreases plant enzyme activity and prevents the proliferation of detrimental anaerobic microorganisms, especially clostridia and enterobacteria (Yang et al., 2004). Many by-products (especially BDG) have been used as animal feeds (kazemi et al., 2009; McKendrick et al., 2003) and ensiling is sometimes used to prevent moist disorders of by-products after production. However, a normal fermentation is often difficult to achieve when by-products are ensiled alone because of high-moisture contents and lack of fermentable carbohydrate. Moreover, acetic and butyric acid production may increase especially after long time of storage (Imai, 2001), and spoilage might occur when by-products are exposed to air after silo opening (Schneider et al., 1995; Nishino et al., 2003).

Chemical additives are one of the most efficient feed additives for mould prevention. For example, sulfuric acid, a biostatic agent used successfully for preservation of high moisture grain (Eslamian et al., 2013) and it has decreased the disorders of spoiling after ensiling. Application of formic acid into the silage, has anti-bacterial effect on many bacteria species (especially lactic acid bacteria), also limited fermentation and reduction in organic acid content of silage is reported by many researchers (Polan et al., 1985; Filya et al., 2004). *In vitro* gas production procedure and *in situ* dry matter disappearance has done by many researchers (Kazemi et al., 2013; Menke and Steingass, 1988; Gencoglu et al., 2011).

The various chemical additives are substances which have the function of controlling chemical and biological reactions in the ensilage, however the lack of technical knowledge concerning the additive, dosage and methods of application of them, may generate negative consequences to the development of microorganisms benefic to the fermentation. Distillery residues from BDG contaminate the environment, but there is little information available about the effect of chemical additives and ensiling on the chemical composition and ruminal digestion kinetics and gas production by BDG. Thus, the aim of this study was to study the ensiling process, chemical composition, rumen *in situ* degradation and *in vitro* gas production of BDG treated with different chemical additive during the ensiling process.

MATERIALS AND METHODS

Preparation of treated silages

Wet barley distillers' grain (BDG) was obtained from company in iran (Niro malt company, Mashhad, Iran). Wet BDG (DM of 25%) were treated by sulfuric acid (H_2SO_4; pure of 98%), Acetic acid (CH_3COOH; pure of 96%), Hydrochloric acid (HCl; pure of 37%) and Lactic acid ($C_3H_6O_3$; pure of 90%) (apiece=0.5% of DM). A control group (without any additive) was prepared. Chemical additives supplied from a trade mark (Merck Company, Germany). The chemically treated samples were filled into nylon bags (Ankom Technology, America) with 0.2 mm thickness, then tied up and kept at room temperature on cemented floor. After having stored for 60, silages were opened, the dry matter (DM) was determined by oven drying at 60 ^{o}C (Memmert 854) for 48 h. After drying, the samples were ground through a 1 mm screen for gas test and 2 mm screen for *in situ* dry matter disappearance (Cyclotec 1883; Sample Mill, Memmert GmbH Co, Germany).

Chemical analysis

Samples of ensiled BDG were analyzed for Kjeldahl N and crude protein was calculated as Kjeldahl N × 6.25 (AOAC 1990). Organic matter (OM) and ASH content were determined according to (AOAC 1990). Neutral detergent fiber (NDF) and Acid detergent fiber (ADF) were determined according to methods described by Van-Soest et al (1991). The ammonia nitrogen (NH3-N) content of silages was determined according to Anonymous (1986). The pH value of the ensiled BDG was determined by a glass electrode (Metrohm, Model 691, Metrohm AG Ltd. Switzerland) immediately after opening according to the British standard method (Anonymous, 1986).

In situ trial

In situ dry matter disappearance of ensiled BDG was studied following the nylon bag technique described by

Table 1. Chemical composition of ensiled barley distillers' grain.

Treatments	Composition (%)				
	DM	CP	Ash	NDF	ADF
Control	23.20[ab]	23.55[a]	5.47[a]	58.40[a]	39.45[a]
0.5%Lactic acid	22.86[b]	23.00[ab]	3.90[b]	58.36[a]	39.34[a]
0.5%Acetic acid	22.12[c]	22.90[ab]	3.80[b]	58.15[a]	39.40[a]
0.5%Hydrochloric acid	23.57[a]	22.70[b]	3.62[b]	58.35[a]	39.34[b]
0.5%Sulfuric acid	23.85[a]	23.30[ab]	3.70[b]	47.30[b]	37.45[b]
SEM	0.21	0.26	0.12	0.25	0.29

[a, b] means in the same column with different superscript differ significantly (P<0.05); DM = Dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; Control=ensiled brewers' grain without any additive; SEM= Standard error of mean.

Mehrez and Ørskov (1977). Four sheep (44.6±2.0 Kg) fitted with ruminal fistulae were applied for this experiment. The animals were fed with 0.8 Kg DM alfalfa hay and 0.4 Kg DM concentrates (165 g CP/Kg of DM/ head/day, at 08.00 and 17:00 hours. Dried samples (5 g) were weighed into 9 ×17 cm polyester bags (52 µm pore size),and 5 bags were prepared for each sample and each incubation time. Ruminal incubation times were 2, 4, 8, 16, 24, 48, 72 and 96 hours. All bags were inserted at the same time, just before the morning feeding (i.e., 08:00 hours). The bags were soaked in distilled water before being incubated (39 ◦C for 15 min). At the end of each incubation time, bags were rinsed with cold mineral free distilled water until the rinse water was clear. Zero time disappearance was obtained by washing un-incubated bags in a similar way. All washed bags were dried by using oven dryer (60 ℃ for 48 hours). Disappearance of DM at each incubation time was calculated from the proportion remaining after being incubated in the rumen.

In vitro gas test trial

In vitro gas test was carried out as described by Menke and Steingass (1988). Prior to the morning feeding, rumen contents were obtained from four adult sheep via their rumen fistula. The donor animals were fed with 0.8 Kg DM alfalfa hay and 0.4 Kg DM concentrate (165 g CP/Kg DM/head/day, at 07.00 and 17.00 hours (at maintenance). Rumen fluid was pooled into a pre-warmed thermos flask, capped and immediately transported to laboratory in ice. Rumen content was then strained through 4 layers of cheesecloth. The laboratory handling of rumen fluid was carried out under a continuous flow of CO_2. Approximately, 200 mg DM from each sample was measured into each syringe. Then, it was filled with 30 ml of medium consisting of 10 ml of rumen fluid and 20 ml of buffer solution as described by Menke and Steingass (1988). Four syringes (without any sample) as blank were also measured and then were placed in a water bath at 39°C. Gas production was recorded directly after 2, 4, 8, 12, 16, 24, 36, 48, 72 and 96 hours post incubation.

Calculation and statistical analysis

The data about dry matter disappearance calculated using the technique of Ørskov and McDonald (1979), while effective DM degradability (EDMD) was calculated according to Ørskov and McDonald equation (1979). Cumulative gas production data was estimated following Osuji et al. (1993). Data on *in situ* DM degradation, chemical composition, fermentation factors (NH3-N and pH) and *in vitro* gas production were subjected to analysis of variance (ANOVA) in a completely randomized design, using the Statistical Analysis System (SAS) program General Linear Model procedure (SAS, 9.1). Significant means were compared by means of the Duncan's multiple range tests. Mean differences were calculated at $P < 0.05$. Standard errors of means were calculated from the residual mean square in the analysis of variance.

RESULTS AND DISCUSSION

Chemical composition of ensiled BDG is presented in Table 1. The chemical analysis revealed that generally BDG had a good nutrient profile (Table 1) due to ensiling (60 days). There was significant differences for chemical composition between treatments than those of control group (P<0.05). The ensiling process with different chemical additives, apparently altered carbohydrate composition of ensiled BDG. The content of NDF and ADF in sulfuric acid treatment was numerically less than control group (Table 1). Dry matter (DM) content of acetic acid and lactic acid treatments was lower than the control group (P<0.05). It is highly likely that water is added to the silage environment because of the nature of acetic acid and lactic acid in the reaction environment. Acetic acid has been considered to be held responsible for the increased aerobic stability, and acts as an inhibitory effect on the organisms cause spoilage. Therefore, stability increases exponentially with acetic acid

Table 2.The effect of chemical additives on silage pH and ammonia-N.

Treatments	Parameters	
	$NH3\text{-}N_{(mg/dl)}$	pH
Control	8.20[a]	3.87[a]
0.5%Lactic acid	7.60[b]	3.60[b]
0.5%Acetic acid	7.30[b]	3.78[ab]
0.5%Hydrochloric acid	8.10[a]	3.65[b]
0.5%Sulfuric acid	7.20[b]	2.70[c]
SEM	0.16	0.06

[a, b, c, d, e] means in the same column with different superscript differ significantly (P<0.05); Control=ensiled brewers' grain without any additive; SEM= Standard error mean.

concentration. When microorganisms use organic matter (especially readily fermentable carbohydrates) for their life activity during fermentation (McDonald et al., 1991), the content of DM content in silage might be reduced. In this trial, 0.5% Sulfuric acid had the highest DM than control group and other treatment, so it seems there was a little dry matter fermentation after ensiling according to McDonald et al.,(1991) report. Crude protein (CP) content was highest for control group, followed by Sulfuric acid treatment and lowest for hydrochloric acid treatment (Table 1). Silages with a high CP content and high solubility, such as BDG, can increase ammonia concentration of rumen (Charmley and Veira, 1990). Distillers dried grains contain a significant amounts of both rumen degradable (RDP) and rumen un-degradable protein (RUP), and post-ruminal digestibility of the RUP which was found to be high (Ingalls, 1994; Stern et al., 1995; O'Mara et al., 1997). The present study has revealed that as compared to those of control, silages treated with chemical additives had lower ash (P<0.05). Ash in by-products comes from two sources i) internal, e.g. minerals like calcium, magnesium, potassium and phosphorus, and ii) external, e.g. dirt, bedding, sand, etc. The less level of ash observed in the ensiled BDG treated with chemical additives may have been due to the additive than modified the mineral content in BDG. The ensiling process with different chemical additives, apparently altered carbohydrate composition of ensiled BDG. NDF of ensiled BDG with 0.5% Sulfuric acid (47.30%) was significantly (P < 0.05) lower than other treatments. The highest NDF content (58.40%) was observed when BDG was ensiled without any additive followed by 58.36% and 58.35% for BDG ensiled with 0.5% Lactic acid and 0.5% Hydrochloric acid at the same period. Also ADF content of ensiled BDG with 0.5% Sulfuric acid (37.45%) was significantly (P < 0.05) lower than other treatments. ADF content was the highest for BDG ensiled without additive. So, the use of sulfuric acid was effective in reducing the concentration of cell wall components of the silages when compared with the control treatment. The effect of chemical additives on silage pH and ammonia-N is presented in Table 2. The pH and NH3-N of BDG silage was affected by the additive type. Maximum (3.87) and minimum (2.70) pH was recorded for control group and 0.5% Sulfuric acid treatment respectively. The maximum and minimum of NH3-N obtained for control group and 0.5% Sulfuric acid respectively. Activity by environment protease enzymes is responsible for the conversion of true protein into smaller peptides, NH3-N and individual amino acids. This process is distinct from the catabolism of amino acids by certain micro flora in silage resulting in the production of ammonia, amides and amines (McDonald et al., 1991). The extent of proteolysis is dependent upon the crop species (Papadopoulos and McKersie, 1983) and the rate and extent of both drying (Muck, 1987) and pH decline (Charmley et al. 1991). So, NH3-N concentration in silages shows the degree of protein degradation. It seems treatment of BDG with 0.5% Sulfuric acid have had a less CP degradation in the silage media. The studies by Winters et al. (2002) and Rajcakova *et al*. (2005) have shown that proteolysis decreases in silages treated with a biological additive (*L. plantarum*). It is well known that cell breakdown and the resultant release of plant juices are prerequisites for the decreasing of significant of pH value during ensiling, and the complete exclusion of fresh air from the silage mass is usually expected to result in cell breakdown within the initial hours of ensiling (Greenhill, 1964). Often the high pH value was the main factor to result in the *Clostridium Spp*. activity occurring in the silage. The pH value of well-fermented high-quality silage with DM 200 g/Kg should be 4.2 or lower (Weissbach, 2003). Also According to McDonald et al. (2002) silage with a pH range of 3.8 to 4.2 is considered well preserved. Effect of different chemical additive on dry matter disappearance parameters of BDG during ensiling is presented in Table 3. Application of 0.5 % Sulfuric acid increased the degradation of dry matter (24 hours post incubation) and potentially degradable fraction (b) (P<0.05) than control group by 8 % and 8.15% respectively (p<0.05). Mean of effective degradability for dry matter was 48.60 to 57.10% for treatments (Table 3).The "a" fraction (Rapidly soluble fraction) was the highest for control group (P<0.05). Effective dry matter degradability of ensiled BDG with 0.5% Sulfuric acid, was significantly higher than the corresponding other treatments (P<0.05). Reported effective degradability of dry matter by Mustafa et al.,

Table 3. Effect of different chemical additive on dry matter disappearance parameters of barley distillers' grain during ensiling.

Treatments	Parameters (%)				
	a	b	c	ED	D_{24h}
Control	18.50^{a}	63.55^{c}	0.030^{c}	50.90^{b}	54.12^{b}
0.5%Lactic acid	10.80^{d}	71.34^{a}	0.035^{b}	48.60^{d}	46.95^{d}
0.5%Acetic acid	16.75^{b}	65.50^{b}	0.037^{b}	49.10^{cd}	53.16^{b}
0.5%Hydrochloric acid	16.50^{b}	60.65^{d}	0.037^{b}	49.90^{bc}	50.60^{c}
0.5%Sulfuric acid	12.75^{c}	71.70^{a}	0.047^{a}	57.10^{a}	62.12^{a}
SEM	0.21	0.37	0.004	0.40	0.43

$^{a, b, c, d, e}$ means in the same column with different superscript differ significantly (P<0.05); a=Rapidly soluble fraction (%); b= potentially degradable fraction (%); c=Constant rate of degradable fraction of b (%/h^{-1}); ED=Effective degradability of dry matter (%); Control=ensiled brewers' grain without any additive; D_{24h}=Dry matter disappearance after 24 h incubation; SEM= Standard error of mean.

Table 4. Effect of different chemical additive on estimated parameters of barley distillers' grain from *in vitro* gas production during ensiling.

Treatments	Parameters			
	$B_{gas(ml)}$	$C_{gas(ml/h)}$	$24h_{(ml)}$	$48h_{(ml)}$
Control	206.52^{d}	0.043^{b}	122.95^{c}	159.95^{c}
0.5%Lactic acid	190.73^{e}	0.036^{c}	110.70^{d}	145.70^{d}
0.5%Acetic acid	210.03^{c}	0.041^{b}	128.22^{b}	164.22^{b}
0.5%Hydrochloric acid	220.62^{b}	0.033^{c}	129.81^{b}	165.00^{b}
0.5%Sulfuric acid	227.10^{a}	0.056^{a}	162.50^{a}	203.70^{a}
SEM	1.02	0.001	0.61	0.52

$^{a, b, c, d, e}$ means in the same column with different superscript differ significantly (P<0.05); B_{gas}= cumulative gas production after 96h incubation (ml/1g of DM); C_{gas}= rate of gas production (ml/h/1g of DM); 24h=Cumulative gas production after 24h incubation; 48= Cumulative gas production after 48h incubation; Control=ensiled brewers' grain without any additive; SEM: Standard error of mean.

(2000) was lower ($p < 0.05$) for barley-based distillers' grains (43.9 %) rather than this experiment. Effect of different chemical additive on estimated parameters of BDG from *in vitro* gas production during ensiling is presented in Table 4. The highest cumulative gas production (96 hours) was observed when BDG was ensiled with 0.5% Sulfuric acid. On the other hand, lowest value was recorded from the use of 0.5% Lactic acid. Proportions of fermentation products depend on feed composition, especially CP and carbohydrates (Kamalak et al., 2005). However, most of CP and carbohydrate are degraded by microorganisms and creates more gas in the culture media. In contrast Kamalak et al. (2005) reported that there was a negative relationship between NDF and ADF contents with cumulative gas production after 96 hours post incubation. So it is possible that the result of high cumulative gas production from 0.5% Sulfuric acid may have been due to less ADF and NDF contents. Proportions of fermentation products depend on feed composition, especially CP and carbohydrates. However, most CP is degraded by microorganisms and creates less gas (Getachew et al., 2000). Therefore, more work towards evaluating the pathology of the rumeno-reticular cell kinetics using the above mentioned diet and their effects on other visceral organs especially liver and kidneys including clinical pathology of blood and urine are warranted.

CONCLUSION

The present work has shown that the application of chemical additives generally had a positive effect on BDG silage characteristics in terms of lower pH and less ammonia nitrogen concentration. Evaluation of silages can be carried out using both *in situ* dry matter disappearance and *in vitro* gas production. Based on the results of the present study, the addition of chemical additives to BDG before ensiling may be recommended. Generally, application of 0.5% Sulfuric acid showed better fermentation characteristics than other additives investigated.

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