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Research Paper

Nutritional evaluation of ensiled brewers' grain by the *in vitro* and *in situ* techniques

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Abbreviations: BG, Brewers' grain; **NDF,** neutral detergent fiber; **ADF,** acid detergent fiber; **CP,** crude protein; **ED,** effective degradability; **DM,** dry matter; **EDMD,** effective dry matter degradability; **SAS,** statistical analysis system; **ANOVA,** analysis of variance; **LW,** light weight; **OM,** organic matter; **WSC,** water soluble carbohydrate; **ml,** milliliter.

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Abstract. This paper deals with the effects of ensiling on chemical composition, *in situ* degradability of dry matter and *in vitro* gas production parameters of wet brewers' grain (BG) with or without different additives. The BG ensiled at completely anaerobic conditions in double tubes. After 60 days of ensiling, the silages were opened for analysis. Although ensiled BG with 2% sulfuric acid had the least NDF, ADF, CP and pH than other treatments ($p < 0.05$), but it had the highest effective degradability (ED) and cumulative gas production (24 and 48 h after incubation) ($p < 0.05$). Adding of some additive (such as molasses and sulfuric acid) increased the cumulative gas production after 24, 48 and 96 h incubation than control group (without any additive). The results from gas production (cumulative gas production after 24, 48 and 96 incubation) and dry matter degradability parameters (such as rapidly soluble fraction (a) and ED) indicated that sulfuric acid (especially 2%) had a high antibacterial activity in BG silages and improved the nutritive value of BG after ensiling.

Keywords: Brewers' grain, ensiling, rumen degradability, *in vitro* gas production.

INTRODUCTION

The use of by-products as feedstuff for ruminant animals is becoming important in many parts of the world. Brewers' grains (BG) are by-products of the brewing industry that is prepared mainly from fermented barley to produce beer. BG has 230 to 290 g/kg CP (dry matter basis) and is high in digestible fiber (Pereira et al., 1998; Dhiman et al., 2003). BG is suitable for ruminants, particularly in dairy cows, to balance intake of large amounts of high starch diets, due to their fibrous nature and low energy content (Chiou et al., 1998; Dhiman et al., 2003). Many by-products from the food and beverage industries have been used as animal feeds, and ensiling is sometimes used to preserve moist by-products for subsequent feeding. However, acceptable fermentation is often difficult to achieve when by-products are ensiled alone due to high-moisture contents and lack of sugar substrates. Some chemical additives are added to silages, for example, organic acids are one of the most efficient feed additives for mould prevention. Also propionic acid, a biostatic agent also successfully used for preservation of high moisture grain (Filya et al., 2004; Samli et al., 2008), as well as has potential in suppressing microbial activity during short storage interval. Formic acid, as silage additive, has anti-bacterial effect on many bacterial species specially lactic acid bacteria; thus, addition of formic acid into silage results in limited fermentation and reduction in organic acid content of silage (Rooke et al., 1988; Filya et al., 2004). Sulfuric acid and molasses are one of the most efficient feed additives for mould prevention and promoters when BG ensiled. The rate and extent of fermentation of dry matter (DM) in the rumen are very important parameters for the nutrients absorbed in the gastrointestinal tract of ruminants. The nylon bag technique has been used for many years to provide an estimate of both rate and extent of DM degradation of by-product (*in situ* procedure) (Mehrez and Ørskov, 1977; Kazemi et al., 2012). Also, the *in vitro* gas production technique has widely been used during the last decades by ruminant nutritionists to study feed degradation during the last decades (Menke et al., 1979; Rymer et al., 2005). So the objective of this study was to investigate the effects of some additives (such as molasses and acid sulfuric) on silage fermentation, *in situ* dry matter degradability, *in vitro* gas production and chemical composition of ensiled BG.

MATERIALS AND METHODS

Ensiling of wet brewer's grain

BG (residues of barley after malting and extracting) was prepared from Niro Malt Company by Mr. Azghadi in Razavi Khorasan distinct (Mashhad, Iran). Four samples (3.5 kg) of wet BG was treated without any additive (as control group), with molasses (1 and 2% DM), sulfuric acid (1 and 2% of DM) separately and then compacted with vacuum in double-lined plastic tubes. Plastic tubes were sealed and stored in ambient temperature at about 25°C, up to 60 days. Mini-silos were opened after 60 days and samples were taken from each replication for analysis (four replication for each treatment). The pH value of the BG was determined by a glass electrode (Metrohm, Model 691) immediately after opening the silage tubes according to the British standard method (Anonymous, 1986). The ammonia nitrogen (NH3-N) content of silages was determined according to Anonymus (1986). Samples were dried at 60°C in oven dryer for 48 h and then milled with 2 mm mesh screen for chemical analysis. The ash content was determined at 525°C after 8 h oxidation. Crude protein (by Kjeltec 2300 Auotoanalyzer, Foss Tecator AB, Hoganas, Sweden) was analyzed by a standard Kjeldahl method (Beauchemin et al., 1994). Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) contents was determined according to Van Soest et al. (1991).

Ruminal degradability

The *in situ* DM degradability was carried out according to Mehrez and Ørskov (1977) procedure. Four Balochi male sheep (40 ± 5.7 kg LW) fitted with ruminal fistulae from the flock of the research farm of the Agricultural College at Ferdowsi University, Iran, were used in this study. Sheep fed with 1.5 kg DM alfalfa and 0.4kg DM concentrate (165 g CP/kg DM) per head and per day, at 8.00 a.m. and 6.00 p.m. Silage samples (2 g DM with 2 mm screen) were weighed into nylon bags (6.5 × 14 cm) with a 52 µm pore size. Samples were incubated in the rumen of each sheep after feeding at 08:00 a.m. for 0 (bags were washed with cold tap water without incubation), 2, 4, 8, 16, 24, 48, 72 and 96 h. After each incubation time, bags were removed from the rumen and rinsed with cold tap water, until the water remained clear. The bags were dried at 60°C for 48 h in an oven, and then weighed to determine DM disappearance. Ruminal disappearance at each incubation time was calculated as the difference between the residues and original samples. In each incubation time, six replications were used for each silage sample.

In vitro gas production

Procedure of *in vitro* gas production was performed according to Menke and Steingass (1988). Rumen fluid was obtained from four fistulated Balochi male sheep (40 ± 5.7 kg LW) before morning feed, normally fed with 1.5 kg DM alfalfa and 0.4 kg DM concentrate (165 g CP/kg DM) per head and per day, at 8.00 a.m. and 6.00 p.m. Ruminal content was immediately strained through four layers of cheesecloth to eliminate large feed particles and transferred to the laboratory in a prewarmed thermos. In an anaerobic condition, 50 ml of buffered rumen fluid [ratio of buffer to rumen fluid was 2:1, buffer were prepared as proposed by Menke and Steingass (1988)] was dispensed with pipette pump into a 120 ml serum bottle containing 1 g DM of the ensiled BG. Experiment repeated in two consecutive days (two runs, 5 replicates for each treatment). Then, each bottle was sealed with rubber stopper and aluminum cap and placed in a shaking water bath for 24 h at 38.6°C. Total gas values were corrected for blank incubation. To prevent accumulation of gas produced, head space gas pressure of each bottle was recorded using a pressure transducer (Theodorou et al., 1994) at 2, 4, 8, 16, 24, 48, 72 and 96 h of the incubation and then gas released.

Calculation and statistical analysis

The DM degradation data were fitted to the exponential equation $p = a + b (1 - e^{-ct})$ (Ørskov and McDonald, 1979) where p = DM disappearance in rumen at time t, a = the rapidly soluble fraction, b = the insoluble but fermentable fraction, c = the degradation constant rate of b (in $\%h^{-1}$). Effective DM degradability (EDMD) was calculated applying the equation of Ørskov and McDonald (1979):

Table 1. The NH_3-N and pH values of ensiled brewers' grain.

Treatments	Parameters	
	NH3-N	pH
Control	8.03^{b}	3.91^{a}
1% Molasses	8.84^{a}	3.70^{c}
2% Molasses	7.00^{c}	3.80^{b}
1% acid sulfuric	7.28^{c}	2.74^{d}
2% acid sulfuric	7.20^{c}	2.44^{e}
SEM	0.16	0.01

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

ED = a + [bc / (c+k)], where k = the rumen outflow rate (at level of 3 % per h). Cumulative gas production data were fitted to the exponential equation $y = b\ (1-e^{-ct})$ (Osuji et al., 1993) where b = the gas production from the readily soluble fraction and the insoluble fraction (ml), c = the gas production rate constant (ml/h), t = incubation time (h) and y = gas production at time of t (in ml). Gas pressure was converted into volume using an experimentally calibrated curve. Data on *in situ* DM degradation, chemical composition, fermentation factors (NH_3-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; using the Duncan's multiple range tests. Mean differences were considered significant at P < 0.05. Standard errors of means were calculated from the residual mean square in the analysis of variance.

RESULTS AND DISCUSSION

The NH_3-N and pH values of ensiled brewers' grain are presented in Table 1. Adding of additive (except 1% molasses) to the silage, significantly caused decrease of NH_3-N when compared to the control group ($p < 0.05$).Treatment by 1% molasses had the highest NH_3-N than other treatments ($p < 0.05$). By adding the additive to the silages, pH significantly decreased in comparison to the control group ($p < 0.05$). Treatment with 2% sulfuric acid significantly gives the smallest pH than the other treatments ($p < 0.05$). Ensiling is based on fermentation, which involves microbiological and enzymatic activity. Therefore, it is expected to be strongly influenced by characteristics of additives (molasses and sulfuric acid). The rate at which the pH drops is a function of the level of water soluble carbohydrate (WSC) and the epiphytic bacteria present on the crop prior to ensiling (Meeske et al., 2000). If insufficient lactic acid bacteria are present on the crop and the readily available sugar concentration is low at ensiling, this result in a slow drops in pH. So the results indicated that two additive (molasses and sulfuric) had a decreasing effect on pH. The NH_3-N concentration in silages shows the degree of protein degradation. Treatment of 1% molasses gave the highest NH_3-N value than the other treatments ($p < 0.05$) and it seems that degradability of CP in this treatment was increased (Table 1). The combined effects of plant and microbial enzymes can lead to extensive changes to the nitrogenous fractions during ensiling. Relatively high CP and pH of ensiled BG may have lead to extensive proteolysis, and hence increased the NH_3-N concentration (McDonald, 1991).

Chemical composition of ensiled brewers' grain is presented in Table 2. The treatments of sulfuric acid (1 and 2%) had the highest influence to the dry matter content than other treatments ($p < 0.05$). CP content of 2% sulfuric acid treated BG is lowest than other treatments ($p < 0.05$), as shown in Table 2 ($p < 0.05$). Ash content of control group and 2% molasses (5.40 and 5.08 respectively) were highest than other treatments. The contents of NDF and ADF were lowest for 1 and 2% sulfuric acid treated BG ($p < 0.05$). Generally the results indicated that: ensiling was effective on BG chemical composition. The results indicated that sulfuric acid more reacted with H_2O in the silage than molasses and as a result, the dry matter of silages has increased. Measurement of CP concentration in silages shows the degree of protein degradation after ensiling. So 2% sulfuric acid gave the lowest CP content than the control group and the other treatments ($p < 0.05$). Level of NDF digestibility is reported to be important criteria for feed quality (Bal et al., 1997). Valdez et al. (1988a, b) stated that reduction of DM and organic matter (OM) digestibility was caused by an increase in ADF and NDF contents which means decrease in ADF and NDF digestibility of silage. It showed that NDF and ADF contents of 1 and 2% sulfuric acid treated BG decreased after ensiling.

Estimated parameters of ensiled brewers' grain by the *in situ* procedure are presented in Table 3. The content of "a" parameters (Rapidly soluble fraction) was highest for 2% acid sulfuric than other treatments and so it seems that sulfuric acid has had a degradability function on physical and chemical structure of ensiled BG. The fraction of "b" was highest for 1% molasses than other treatments ($p < 0.05$). The fraction of "c" was highest for 1% sulfuric acid than other treatments ($p < 0.05$). Also effective degradability (ED) of dry matter was highest for 1 and 2 % sulfuric acid. Starch and cell wall contents of feedstuffs have a great impact on digestibility (Meeske et al., 2000). With the increase of starch content and the decrease of cell wall content of feedstuffs, feed value increases (Hart, 1990). Kamalak et al. (2005) reported that *in situ* DM disappearance after 48 and 96 h was negatively correlated with NDF. As mentioned above, 1 and 2% sulfuric acid treated BG, had lowest NDF and

Table 2. Chemical composition of ensiled brewers' grain.

Treatments	Composition				
	DM	CP	Ash	NDF	ADF
Control	22.90^{c}	23.77^{a}	5.40^{a}	58.44^{a}	39.00^{a}
1% Molasses	22.47^{c}	23.18^{a}	4.00^{c}	58.05^{a}	39.25^{a}
2% Molasses	22.69^{c}	23.89^{a}	5.08^{a}	57.93^{a}	39.27^{a}
1% Acid sulfuric	23.40^{b}	23.48^{a}	3.60^{d}	47.39^{b}	37.33^{b}
2% Acid sulfuric	24.44^{b}	21.93^{b}	4.40^{b}	44.43^{c}	34.62^{c}
SEM	0.17	0.23	0.12	0.18	0.28

$^{a, b, c, d}$ 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 mean.

Table 3. Estimated parameters of ensiled brewers' grain by the *in situ* procedure.

Treatments	Parameters			
	a	b	c	ED
Control	19.30^{b}	63.51^{e}	0.030^{d}	51.98^{c}
1% Molasses	17.16^{c}	71.94^{a}	0.038^{b}	48.08^{d}
2% Molasses	13.21^{d}	67.26^{c}	0.034^{c}	49.01^{d}
1% Acid sulfuric	12.55^{d}	70.38^{b}	0.046^{a}	55.72^{b}
2% Acid sulfuric	21.44^{a}	65.48^{d}	0.035^{c}	57.12^{a}
SEM	0.31	0.30	0.001	0.44

$^{a, b, c, d, e}$ 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; SEM = Standard error mean.

Table 4. *In vitro* gas production (ml) and estimated parameters of ensiled brewers' grain.

Treatments	Parameters			
	B_{gas}	C_{gas}	24 h	48 h
Control	194.83^{d}	0.030^{d}	109.85^{e}	144.81^{d}
1% Molasses	224.58^{a}	0.036^{e}	115.65^{d}	159.01^{c}
2% Molasses	205.80^{c}	0.043^{c}	122.04^{c}	160.13^{c}
1% Acid sulfuric	211.20^{b}	0.078^{a}	134.82^{b}	164.81^{b}
2% Acid sulfuric	227.15^{a}	0.054^{b}	161.49^{a}	203.15^{a}
SEM	1.13	0.001	0.67	1.02

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

ADF contents and as a result of this, ED of these treatments has increased.

In vitro gas production (ml) and estimated parameters of ensiled brewers' grain are presented in Table 4. With adding of additive material (molasses and sulfuric acid) to the silage, rate of gas production (c) and cumulative gas production at 24, 48 and 96 h after incubation significantly increased than control group (p < 0.05). The highest cumulative gas production (after 24, 48 and 96h incubation) was observed for 2% sulfuric acid (p < 0.05). It seems that, different chemical composition (such as NDF and ADF) of ensiled BG affected the rate and extent of gas production. Kazemi et al. (2012) reported that there was a negative correlation between NDF and ADF with gas production parameters. Also Kamalak et al. (2005) reported this negative correlation. The increase in gas production parameters for 1 and 2% sulfuric acid are possibly associated with decreased NDF and ADF contents. It is well known that gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate (Gatachew et al., 1998); whereas, protein fermentation does not lead to much gas production (Khazaal et al., 1995). The gas produced is directly proportional to the rate at which substrate degraded (Dhanoa et al., 2000). Additionally, kinetics of gas production is dependent on the relative proportions of soluble, insoluble but degraded, and undegradable particles of the feed (Getachew et al., 1998).

Conclusion

Quality of silages can be defined in many ways. *In vitro* gas production and *in situ* techniques can be considered as useful indicators, and low cost for the preliminary evaluation of the likely nutritive value of ensiled by-products. This study showed that applying of sulfuric acid (especially 2%) gave the highest *in vitro* cumulative gas production parameters and *in situ* dry matter degradability in comparison to the all evaluated treatments. Generally, the ensiling of BG with molasses and sulfuric acid caused to improving the nutritive value of them. Also, the result of this study showed that, supplementation of BG silages with different additives such as sulfuric acid and molasses can be effective in prevent of mould growth when it is compared with the control group (mould pollution of molasses and sulfuric acid treatments when opening, was less than the control group). Ensiled BG showed a great variation in chemical composition. Nonetheless, more research is needed to investigate effects of different additives on nutritive value of ensiled BG by *in vivo* and other *in vitro* methods.

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