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RESEARCH ARTICLE

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Use of *in vitro* gas production technique to evaluate the effects of microwave irradiation on sorghum (*Sorghum bicolor*) and wheat (*Triticum sp.*) nutritive values and fermentation characteristics

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ABSTRACT

Effects of microwave irradiation (900 W) for 3, 5 and 7 min on the nutritive value of sorghum and wheat grains were evaluated by *in vitro* gas production technique. Gas volume was recorded at 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h of incubation and kinetics of gas production were estimated using model: $GP = A \exp\{- \exp[1 + (be/A)(LAG - t)]\}$. Cumulative gas production at 24 h was used for estimation of metabolizable energy, net energy for lactation, short chain fatty acids, digestible organic matter and microbial protein. For sorghum grain, microwave irradiation increased cumulative gas production for most times of incubation linearly. Microwave treatments for 5 and 7 min increased the *A* fraction linearly in both cereal grain, whereas the maximum rate of gas production (*b*) decreased linearly only in wheat grain. Microwave treatments for 3, 5 and 7 min increased ($P<0.05$) metabolizable energy, net energy for lactation and short chain fatty acids content of sorghum grain, but not of wheat grain. It was concluded that microwave irradiation changed the gas production parameters resulting changed ruminal fermentation characteristics that can be considered in ration formulation.

Key words: sorghum grain, wheat grain, microwave irradiation, *in vitro* gas production

Introduction

Cereal grains are the primary source of energy in highly productive ruminant diets to maintain their high production. Wheat (*Triticum sp.*) and sorghum (*Sorghum bicolor*) are traditionally used for animal feeds and have different susceptibility to fermentation in the rumen. Grain fermentability largely determines the feed value of grain for ruminants. It affects the site of starch digestion and microbial protein supply (Herrera-Saldana et al., 1990) and has an important effect on the rumen environment since it is related to changes in ruminal pH, volatile fatty acid (VFA) production, and cellulolytic activity (Lanzas et al., 2007), and also ultimately affects dry matter intake and milk yield (Herrera-Saldana et al., 1990).

There has always been a keen interest in the industrial application of microwaves to improve conventional processes, with the intent of taking advantage of its shorter startup time, faster heating, energy efficiency, space savings, precise process control, selective heating and final products with improved nutritive quality (Sumnu, 2001). Cereal grain treatments such as dry–heating, heat–moisture, physical and chemical processing and microwave treatment can alter their rumen availability and fermentation. Heat–moisture treatment typically employs prolonged heating of starch at a temperature range of 90-100°C at limited quantities of moisture insufficient to gelatinize the starch (Sair, 1967). Theurer et al. (1999) reported that heat–moisture increased starch availability in rumen for microorganisms and it can be caused by disruption of the protein matrix surrounding the starch granules in the grain endosperm and disorganization of the starch granules.

Microwave irradiation seems applicable to grain processing, so far it has not been used on a commercial scale.

Microwave energy penetrates a food or feed material and produces a volumetrically distributed heat source, due to molecular friction resulting from dipolar rotation of polar solvents and from the conductive migration of dissolved ions. The dipolar rotation is caused by variations of the electrical and magnetic fields in the product (Alton, 1998). Water, the major constituent of most food or feed products, is the main source for microwave interactions due to its dipolar nature (Fakhouri & Ramaswamy, 1993).

Sadeghi & Shawrang (2006) used an *in situ* technique to evaluate effects of microwave irradiation on ruminal dry matter, crude protein and starch degradation of cereal grain, but we could not find data on effects of microwave irradiation on *in vitro* gas production.

The objective of the present study was to determine effect of microwave irradiation on sorghum and wheat grain (different in rumen degradation) *in vitro* fermentation patern and their nutritive values such as ME (metabolizable energy), NEl (net energy for lactation), DOM (digestible organic matter), MP (microbial protein) and SCFA (short chain fatty acid) by gas production technique.

Materials and Methods

Sample preparation and microwave irradiation

The samples of wheat and sorghum grain were collected from dairy farm in northwest of Iran. Grains were adjusted to 30% moisture by addition of water to samples of known moisture content. The moisture content of samples was determined by oven–drying method according to AOAC (2004). Three 500 g samples of moistened grain were placed in a Pyrex pan (28×28×6 cm) with 1–2 cm height and were subjected to microwave irradiation (Butane microwave oven BC380W, Iran; emitting a 2450 MHz microwave frequency) at a power of 900 w (1.8 w/g microwave energy) for 3, 5 and 7 min. During the microwave irradiation samples were rotated to decrease in temperature gradients and to result in more uniform temperature distributions.

Chemical analysis

Dry matter, ash, crude protein and ether extract of sorghum and wheat grain were determined according to procedures of AOAC (2004). NDF and ADF of sorghum and wheat grain were determined using the methods of Van Soest et al. (1991). NDF was determined with using sodium sulfite, without washing with acetone, without α-amylase and expressed without residual ash.

In vitro gas production

Samples (300 mg) were weighed into 100 ml serum vial. McDougall (1948) buffer solution was prepared and placed in a water bath at 39°C. Rumen liquor samples were obtained from the two weathers that were fed on a diet comprising (DM basis), 550 $g.kg^{-1}$ alfalfa hay, 400 $g.kg^{-1}$ barley grain, 48 $g.kg^{-1}$ wheat bran and 2 $g.kg^{-1}$ lime stone at maintenance level (NRC, 1985). Rumen fluid was collected after the morning feeding. Rumen fluid was pumped with a manually operated vacuum pump and transferred into pre-warmed thermos flask, combined, filtered through four layers of cheesecloth and flushed with CO_2. Each feed sample was incubated in triplicate with 20 ml of rumen liquor and buffer solution (1:2). Three vials containing only the rumen fluid/buffer solution and no feed sample was included with each test and the mean gas production value of these vials was termed the blank value. The vials were sealed immediately after loading and were affixed to a rotary shaker platform (lab-line instruments Inc Melors dark, USA) and housed in an incubator. Gas production was measured in each vial after 2, 6, 12, 24, 48 and 72 h of incubation using a water displacement apparatus (Fedorak & Hrudey, 1983).

Calculations and statistical analysis

To describe the dynamics of gas production over time the following Gompertz function (Schofield et al., 1994) was chosen:

$$GP = A \exp \{-\exp [1 + (be/A)(LAG - t)]\}$$

where *GP* is cumulative gas production (ml), *A* – the theoretical maximum of gas production (ml), *b* – the maximum rate of gas production (ml/h) that occurs at the point of inflection of the curve, *LAG* – the lag time (h), which is defined as the time-axis intercept of a tangent line at the point of inflection, *t* is time (h) and *e* is the Euler constant. The parameters *A*, *b*, and *LAG* were estimated using Marquardt method with NLIN procedure of SAS 9.1. The effects of microwave irradiation times on cumulative gas production and its parameters were subjected to the GLM procedure (SAS Inst. Inc., Cary, NC) as a 2 (grain) × 4 (Microwave irradiation time) factorial experiment. Orthogonal polynomial contrast was used to examine their responses (linear and quadratic) to increasing the level of microwave irradiation individually.

The metabolizable energy and net energy for lactation content of feeds and short chain fatty acid and digestible organic matter were calculated using equations of Menke & Steingass (1988) and Getachew et al. (2002) as:

- ME (MJ/kg DM) = 1.06 + 0.1570GP + 0.0084CP + 0.022CF − 0.0081CA (n = 200, r^2 = 0.94)
- NEl (MJ/kg DM) = –0.36 + 0.1149GP + 0.0054CP + 0.0139CF − 0.0054CA (n = 200, r^2 = 0.93)
- DOM (% DM) = 9.00 + 0.9991GP + 0.0595CP + 0.0181CA (n = 200, r^2 = 0.92)
- SCFA (m mol / 200 mgDM) = 0.0222GP – 0.00425

Where *GP* is 24 h net gas production (ml / 200 mg DM); *CP*, *CF* and *CA* are crude protein, crude fat and crude ash (% DM), respectively.

Microbial protein was calculated as 19.3 g microbial nitrogen per kg DOM according to Czerkawski (1985).

Results and Discussion

Chemical composition of feedstuffs

The chemical composition of sorghum and wheat grain is shown in Table 1. Crude protein contents of sorghum and wheat grain were 10.8 and 14.9% DM, respectively. The NDF contents of sorghum and wheat grain were 22.4 and 15.7% DM, respectively. There were some differences and variations between chemical compositions in current study comparing with some other researches (Nikkhah et al., 2004; Lanzas et al., 2007). These variations in chemical composition of grains can be due to different original materials and growing conditions such as geographic, seasonal variations, climatic conditions and soil characteristics. In fact, this difference led to different nutritive values, because chemical composition is the initial and important index of the nutritive value of feeds (Maheri-Sis et al., 2011).

In vitro gas production

The mean values of *in vitro* cumulative gas production “corrected for blank” and estimated kinetic parameters by Gompertz model (*A* and *b*) for untreated and microwave treated for both grains are shown in Table 2 and Table 3, respectively. For sorghum grain, cumulative gas production was linearly increased by altering period of microwave irradiation. There was quadratic effect observed after 12 h of incubation with increase in time of microwave irradiation. This treatments increased also linearly (P<0.01) *A* fraction, whereas a quadratic effect of irradiation was also detected (Table 2). Although microwave treatment increased (P<0.01, linear effect) the theoretical maximum of gas production by 10%, 13% and 8% for 3, 5 and 7 min irradiation times of microwave, there were no significant effect for the maximum gas production rate and the lag time (LAG) among untreated and microwave treated sorghum grain. Cumulative gas production, and its *A* and *b* fractions were also affected (P<0.05) by microwave treatment for wheat grain. Microwave treatments increased (P<0.05) the *A* fraction and also decreased (P<0.05) the rate of gas production (*c*) of wheat grain linearly. When sorghum and wheat were treated with microwave irradiation, the cumulative gas production increased. Similar results have been observed by other steam processing (Azarfar et al., 2009; Parnian & Taghizadeh, 2009).

Incubation of feedstuff with buffered rumen fluid *in vitro*, the carbohydrates are fermented to short chain fatty acids (SCFA), gases, mainly CO_2 and CH_4, and microbial cells. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate (Wolin, 1960; Sallam et al., 2007) and substantial changes in carbohydrate fractions were reflected by total gas produced (Deaville & Givens, 2001). Gas production from protein fermentation is relatively small as compared to carbohydrate fermentation, while contribution of fat to gas production is negligible (Wolin, 1960). The linear increase in cumulative gas production of grain with increasing irradiation time suggests that microwave treatment increases the availability of carbohydrate for rumen microorganisms.

Table 1. *Chemical composition of the cereal grains (%DM).* [†]

Variable	Cereal grains	
	Wheat grain	Sorghum grain
DM	90.6	89.7
CP	14.9	10.8
EE	2.1	2.8
NDF	15.7	22.4
ADF	3.4	13.9
Hemicellulose[††]	12.3	8.5
Ash	2.9	3.8

Legend: [†] Three samples analyzed for each feed.
[††] Hemicellulose = NDF – ADF

Processing methods employ proper combinations of moisture and heat, in order to improve the rates of *in vitro* amylolytic attack of starch in cereal grains by both ruminal microbial and pancreatic enzyme sources (Theurer, 1986). Bilbao-Sáinz et al. (2007) demonstrated that microwave irradiation improved gelatinization of starch in wheat grain that led to chemical and physical changes in the starch granule. The extent of starch gelatinization is dependent on the amount of water present, processing time and temperature (Williams & Bowler, 1982). The little and more slowly the starch swells, the less starch gelatinization occurs (Davis, 1994).

It is well-documented that sorghum must be more vigorously processed than wheat, barley or corn to achieve optimum digestibility of the grain (Hale, 1973). The digestibility of sorghum protein is consistently lower than corn, wheat and barley proteins, a fact that affects ruminal starch digestibility. Unlike wheat grain, the sorghum proteins are more difficult to extract using classical solvent extraction techniques (Pomeranz, 1978). These observations indicate the important role of the protein digestibility in sorghum feeding value and especially starch availability. Although sorghum is fed primarily for its energy value in starch, the endosperm protein matrix must be disrupted if sorghum feeding potential has to be realized (Rooney & Pflugfelder, 1986). Disruption of linkages between protein matrix and starch granule (Theurer et al., 1999), hydrogen bonds and water absorption (Sadeghi & Shawrang, 2006) facilitated microbial or enzyme degradation of the starch granule. Unlike sorghum, wheat grain is considered as one of the most rapidly digested species among the cereal grain (Herrera-Saldava et al., 1990; Owens et al., 1997), which is associated with increased risks of health problems in cattle (Pozdíšek & Vaculová, 2008). Nutritionists have identified that ruminants require wheat, but it has low rumen degradability and high whole tract digestibility (Pozdíšek & Vaculová, 2008). Sadeghi & Shawrang (2008) reported that microwave irradiation decreased ($P<0.05$) ruminal starch degradation rate of barley grain. However, contradictory findings have also been reported (Sadeghi & Shawrang, 2006).

Table 2 *Effects of microwave irradiation on cumulative gas production and its parameters of wheat and sorghum grain.*

Microwave (min)[a]	Wheat				Sorghum				SEM	Contrast *P–value*[*]			
	0	3	5	7	0	3	5	7		WL	WQ	SL	SQ
Cumulative gas production													
2 h	11.8	10.9	16.9	15.6	14.9	15.4	15.8	18.0	1.40	*	NS	NS	NS
4 h	60.1	59.9	58.6	59.5	37.9	49.5	48.6	53.3	4.21	NS	NS	NS	NS
6 h	105.1	97.0	97.4	96.2	60.1	78.1	77.9	81.7	3.81	NS	NS	*	NS
8 h	165.4	156.0	151.3	146.2	87.5	107.3	151.3	146.2	4.50	**	NS	*	NS
12 h	224.2	218.8	206.4	204.3	117.3	139.1	144.9	141.4	5.32	**	NS	*	NS
16 h	254.4	250.2	244.3	243.8	146.4	170.4	178.6	171.2	5.64	NS	NS	*	*
24 h	272.9	264.4	275.3	277.3	190.2	219.1	229.3	215.1	5.83	NS	NS	*	*
36 h	288.6	278.3	301.3	306.5	242.6	275.3	286.6	267.9	5.89	**	NS	*	**
48 h	298.5	289.5	315.8	325.7	272.2	306.5	313.8	298.5	4.97	***	NS	**	**
72 h	307.3	297.2	327.7	338.7	293.0	327.5	335.5	320.2	4.83	***	*	**	**
96 h	311.7	302.7	335.5	346.5	304.2	336.2	344.8	327.7	4.93	***	NS	**	**
Gas parameters													
A	296.1	287.3	316.7	327.11	295.7	327.9	334.9	319.5	4.47	***	NS	**	***
b	24.2	23.7	18.7	17.8	8.3	9.5	10.3	9.2	0.63	***	NS	NS	*
LAG	1.62	1.73	0.82	0.65	–0.96	–1.55	–1.03	–2.23	0.319	***	NS	NS	NS

Legend: [a] 0: untreated sorghum grain, 3: microwave irradiated sorghum grain for 3 min, 5: microwave irradiated sorghum grain for 5 min, 7: microwave irradiated sorghum grain for 7 min. *A* : theoretical maximum of gas production (ml); *b* : maximum rate of gas production (ml/h); *LAG* : lag time (h); for all models the R^2 was > 0.99. NS: non–significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Values bearing different superscript in a row differ significantly ($P < 0.05$). * Polynomial contrasts, W - Wheat grain, S - sorghum grain, L - linear effect and Q - quadratic effect.

Table 3. *Effect of microwave irradiation time on estimated nutritive values of wheat and sorghum grain*.

Microwave (min)[a]	Wheat				Sorghum				SEM	Contrast *P–value*			
	0	3	5	7	0	3	5	7		WL	WQ	SL	SQ
ME	9.77	9.51	9.85	9.91	7.15	8.06	8.38	7.93	0.183	NS	NS	*	*
NEL	6.00	5.81	6.06	6.10	4.08	4.75	4.98	4.66	0.134	NS	NS	*	*
SCFA	1.20	1.17	1.21	1.22	0.84	0.96	1.01	0.95	0.025	NS	NS	*	*
DOM	64.47	62.78	64.96	65.36	47.73	53.50	55.54	52.70	1.166	NS	NS	*	*
MP	77.77	75.73	78.36	78.84	57.57	64.53	67.00	63.57	1.407	NS	NS	*	*

Legend: [a] 0: untreated sorghum grain, 3: microwave irradiated sorghum grain for 3 min, 5: microwave irradiated sorghum grain for 5 min, 7: microwave irradiated sorghum grain for 7 min. ME: Metabolizable Energy (MJ/kg DM); NEL: Net Energy for Lactation (MJ/kg DM); SCFA: Short Chain Fatty Acids (m.mol/200 mg DM); DOM: Digestible Organic Matter (% DM); MP: Microbial Protein (g).

Decreased *A* fraction for 7 min microwave treated sorghum grain compared to treated sorghum for 3 and 5 min seems that this is related to overheating effects that lead to form the intermolecular disulfide-cross linkages between sulfur-containing amino acids of its kaffirins because of long treatment time. Ezeogu et al. (2008) suggested that disulfide bonding and an increase in β–sheet structure of protein occurred with heating, disulfide bonding increased and was the greatest in the vitreous endosperm. Because an increased disulfide-bonded protein matrix limits the expansion of the starch granules, amylase access is limited, too. Sadeghi & Shawrang (2008) reported that over increasing the microwave irradiation time decreased solubility of DM in feedstuffs. The authors supposed that chemical reactions (such as Millard reaction) occurring during heat processing are responsible for the reduction in ruminal degradation.

Estimated energy values, short chain fatty acids, organic matter digestibility and microbial protein

The predicted metabolizable energy (ME, MJ/kg DM), net energy for lactation (NEl, MJ/kg DM), short chain fatty acids (SCFA, mM), digestible organic matter (DOM) and microbial protein (MP) from gas production for sorghum and wheat grain are presented in Table 3. For untreated sorghum grain, DOM was in agreement with other *in vitro* study (Pedersen et al., 2000). The ME, NEl, SCFA, DOM and MP were affected linearly and quadratically ($P < 0.05$) by microwave treatment. There were no differences for estimated ME, NEl, SCFA, DOM and MP among untreated and microwave treated wheat grain. *In vitro* rumen fermentation methods offer accurate and rapid estimates of differences in potential feed digestibility. The higher SCFA predicted from gas production in microwave treatment sorghum was due to a high absolute gas production, which most evident during the first 24 h of incubation. It seems that the gas production from cereal grains incubated *in vitro* in buffered rumen fluid was closely related to the production of SCFA which was based on carbohydrate fermentation (Opatpatanakit et al., 1994). Getachew et al. (2002) reported the close association between SCFA and gas production *in vitro,* using this relationship to estimate the SCFA production from gas values, which is an indicator of energy availability to the animal.

Conclusion

It was concluded that microwave irradiation changed the gas production parameters resulting changed ruminal fermentation characteristics that can be considered in ration formulation. Further study is suggested to determine effects of different microwave powers and times on fermentation kinetics of sorghum and wheat grain using *in vitro* techniques.

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