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Effect of using steam flaked and extruded corn grain in total mixed ration on *in vitro* rumen fermentation kinetics and gas production

MH Palizdar*[1, 2], HR Mohammadian-Tabrizi[2], MR Pourelmi[2] and F Rafiee[2]

[1]Young Researchers Club of Islamic Azad University, Chalous Branch, Mazandaran, Iran
[2]Department of Animal Science, Islamic Azad University, Chalous Branch, Mazandaran, Iran

Abstract

This study was carried out to determine the effects of different processing methods on *in vitro* rumen fermentation kinetics and gas production (GP) of corn grain in total mixed ration. To evaluate different processed corn grain used in a total mixed ration, an *in vitro* rumen GP technique was utilized. Treatments included were 1) corn grain (20%) in a total mixed ration (TMR), 2) steam flaked corn (SFC) 20% in TMR, 3) extruded corn (20%) in TMR, 4) corn grain (10%) + SFC (10%) in TMR, 5) Corn grain (10%) + Extruded corn (10%) in TMR, 6) SFC (10%) + extruded corn (10%) in TMR. The TMR included alfalfa hay (40%), beet sugar pulp (10%), soybean meal (10%), barley grain (20%) and 20 percent of corn grain (CG) which was replaced by processed corn to prepare the experimental treatments. There were significant differences ($P<0.05$) in gas production among treatments throughout the incubation times. There was a significant difference between treatments at 12 h ($P<0.05$) for total GP and treatment 2 produced the highest volume of gas and the treatments 1, 3, 4 and 5 produced the lowest volume. After 24 h of incubation of samples, same trend was recorded and treatment 2 produced highest ($P<0.05$) GP compared to other treatments. Potential GP (a+b) was highest ($P<0.05$) for treatment 2 with no significant differences with treatments 1, 5 and 6. Consistent with a+b and b fractions, the rate of GP "c" was affected by treatments ($P<0.05$). As a result, steam flaking of corn seems to have potential to increase starch fermentation in the rumen. It is possible to alter the site and extent of digestion of cereal grains to increase the digestibility of concentrate feeds.

Keywords: steam flaked corn; extruded corn; *in vitro;* total mixed ration; digestion kinetics

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Introduction

Grains are fed to ruminants mostly to supply energy, and most of the digestible energy in cereal grains comes from starch. To maximize starch digestion by livestock, corn and sorghum grain must be processed. Corn is included in ruminant diets to increase the energy concentration of the diet (Galyean, 1996). Nutritionally, starch is the most important component of corn, and mechanical processing is used to increase extent of starch digestion in the rumen. For non-ruminants, starch from finely ground grain is fully digested, but for those ruminants fed high concentrate diets, finely ground grain often causes metabolic diseases (Theurer et al., 1999a). Therefore, rather than finely grinding corn, processes including steam rolling, steam flaking and fermentation (high moisture storage) are used to increase extent of starch digestion from grains fed to ruminants (Nocek and Tamminga, 1991). Livestock producers can increase the value of a feed through altering site and extent of digestion by means of grain processing. Preferably, optimal processing economically increases digestibility, processing also may alter the site of digestion but must not detrimentally affect ruminal pH and cause digestive dysfunction (Harmon and McLeod, 2001). Basically,

Corresponding author: MH Palizdar, Young Researchers Club of Islamic Azad University, Chalous Branch, Mazandaran, Iran.

grains are processed to improve their nutritional value. Processing methods and responses in site and extent of digestion have been reviewed extensively (Nocek and Tamminga, 1991; Huntington, 1997; Rowe et al., 1999; Theurer et al., 1999a; Firkins et al., 2001; Harmon and McLeod, 2001; Harmon and McLeod, 2005; Owens and Zinn, 2005; Owens 2005a&b; Huntington et al., 2006).

To form steam rolled or steam flaked grain, dry whole grain is moistened with steam and crushed between corrugated rolls. Owens (2005a) demonstrated that compared with steam flaked grain, steam rolled grain is steamed for a shorter time period, crushed flakes are thicker, and a smaller proportion of the starch will be gelatinized (fracturing of starch granules). Starch that is gelatinized is very rapidly and completely fermented within the rumen. However, starch in flaked grain can retrograde (harden to form digestion resistant starch) if the grain is cooled slowly (Ward and Galyean, 1999). As discussed by Zinn et al. (2002), effects of processing on the site and extent of starch digestion will vary with processing conditions like grain moisture, screen size or roll gap as well as fermentation moisture and steaming time. The primary factor limiting the extent of digestion either in the rumen or the intestines is the extent to which surface area is exposed for microbial or enzymatic attack (Owens 2005a&b). In addition, with more vitreous grain, encapsulation or imbedding of starch granules within a matrix of either protein or fibre delays or retards digestion.

Corn processing has been reported to increase starch digestibility (Galyean et al., 1979; Turgeon et al., 1983) and feedlot performance (Cole et al., 1976; Zinn et al., 2002) although results have not been consistent for all processing methods. In extensive reviews of published trials, dry corn processing like extruding of corn did not improve starch digestibility (Owens et al., 1986) or feedlot performance over whole shelled corn (Owens et al., 1997). New method of "steam processing and flaking" corn and barley renders the starch fraction more readily available to rumen microorganisms and enzyme degradation than conventional methods of steam or dry rolling. Starch digestion improved as the steam-processed flake became thinner (Firkins et al., 2001). Ruminal digestion of starch is important for bacterial growth, rumen health and the production of volatile fatty acids as well. Intestinal starch digestion provides free glucose, which can be used directly by the mammary tissue for milk production (Huntington, 1997; Knowlton et al., 1998), and is estimated to yield 42 percent more energy than ruminally digested starch (Owens et al., 1986).

Determination of intake and digestibility of feedstuffs *in vivo* is time-consuming, laborious, expensive, requires large quantities of feed and is unsuited for large-scale feed evaluation. Considerable effort has been directed towards development of *in vitro* techniques that accurately predict intake and digestibility *in vivo*. Since the first report of Menke et al. (1979), where a high correlation was reported between digestibility measured *in vivo* and predicted from an *in vitro* rumen gas production technique in combination with chemical composition, a considerable amount of researches have been done in *in vitro* rumen gas techniques to predict digestibility of feeds, effects of secondary compounds on rumen microbial activity, to assess kinetics of fermentation, associative effects of various types of feeds, examine influences of feed additives on rumen fermentation, partitioning of fermented substrates into fermentation products, and assessing the composition of gases from fermentation of various feeds. Currently, there is little field research available to compare the difference between flaked or extruded corn in TMR for dairy cattle by different feeds. *In vitro* rumen gas production was used to compare sorghum grain hybrids that differed in endosperm color (Streeter et al., 1993) and to evaluate effects of varieties, growing sites, and grain species (Opatpatanakit et al., 1994). Gas production based on yeast fermentation was used to evaluate processing method and grain sorghum type (Hinders and Freeman, 1969). *In vitro* gas production was used to compare forage quality (Siaw et al., 1993; Herrero et al., 1996; Wood and Manyuchi, 1997) and effects of various sources of fat on rumen fermentation (Getachew et al., 2001; Palizdar et al, 2012). We did not find any reports in the literature where *in vitro* rumen gas production was used to compare processed corn in different total mixed rations. The objective of this research was to use an *in vitro* gas fermentation system to determine the effects of corn grain processing in total mixed rations on fermentation kinetic and rumen gas production.

Materials and Methods

Extruded and steam-flaked corn (SFC) grain samples were obtained from a commercial processing industrial unit (Armaz. Co, Iran). Samples were obtained on two separate random dates. The whole corn (WC) represented unprocessed i.e., not steam treated and extruded corn. Samples were grounded in a Wiley mill (Arthur A. Thomas, Philadelphia, PA) to pass a 1 mm sieve and stored at room temperature. *In vitro* incubation was performed using 30 ml of buffered rumen fluid according to the method of Menke and Steingass (1988).

Treatments were 1) corn grain (20%) in a total mixed ration (TMR), 2) SFC (20%) in TMR, 3) extruded corn (20%) in TMR, 4) corn grain (10%) + SFC (10%) in TMR, 5) corn grain (10%) + extruded corn (10%) in TMR, 6) SFC (10%) + extruded corn (10%) in TMR. The TMR prepared according to Getachew et al. (2001) and included alfalfa hay (40%), beet sugar pulp (10%), soybean meal (10%), barley grain (20%) and finally 20% of WC which was replaced by processed corn to make the experimental treatments.

Approximately 200 mg of samples were weighed and placed in 100 ml graduated glass syringes. Buffer mineral solution was prepared and placed in a water bath at 39 degree Celsius under continuous flushing with CO_2. Rumen fluid was collected after the morning feeding from two ruminally fistulated, Iranian Taleshi steers (Weighing 350 kg, age 3 years old) and fed a total mixed ration consisting of approximately 40% chopped alfalfa hay and 60% concentrate ingredients. Rumen fluid was pumped with a manually operated vacuum pump from the rumen into pre-warmed thermos flasks. The rumen fluid from the two cows was mixed and filtered through four layers of cheesecloth and flushed with CO_2. The well mixed and CO_2 flushed rumen fluid was added to the buffered mineral solution (1:2 v/v), which was maintained in a water bath at 39°C and mixed. Rumen fluid was added to the buffered mineral solution with constant stirring, while maintained in a water bath at 39°C. About 30 ml of buffered rumen fluid was dispensed into syringes containing the samples. All handling was under continuous flushing with CO_2. After closing the clips on the silicon tube at the syringe tip, syringes were gently shaken and the clips were opened to remove gas by pushing the piston upwards to achieve complete gas removal. The clip was closed, the initial volume recorded and the syringes were affixed to a rotary shaker platform (Lab-line instruments Inc Melors dark, USA) set at 120 rpm housed in an incubator at 39°C. Incubation was completed in triplicate with readings of GP after incubation for 0, 2, 4, 6, 8, 12, 24, 48, 72 and 96 h for samples. Kinetics of total GP was calculated (Ørskov and McDonald, 1979) for each treatment. Differences in the composition and activity of rumen fluid inoculum were controlled by parallel measurements with incubation of buffered ruminal fluid without substrate (Blank test). Cumulative GP data were fitted to the exponential equation:

$$Y = a + b\,(1 - \exp^{-ct})$$

Where, Y is the gas produced at "t" time, "a" the GP from the immediately soluble fraction (ml), "b" the GP from the insoluble fraction (ml), "a + b" potential of GP (after 96 h) from fermentable fraction (ml/200 g DM), "c" the GP rate constant for "b" and "t" is the time of incubation (h).

Statistical analysis

Data on *in vitro* gas production were subjected to analysis of variance in a completely randomized design using the SAS program General Linear Model (GLM) procedure (SAS, 9.1, 2005). Significant means were compared using the least square means method. 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

Cumulative gas production (ml/200mg DM) at different incubation times is presented (Table 1).There was a significant difference ($P<0.05$) in gas production among treatments after 2h of incubation (Table 1). Corn grain (Treatment 1) produced the lowest volume of gas (7.4 ml/200mg DM) compared to other treatments after 2h of incubation ($P<0.05$). This trend was comparable for corn grain after 4h of incubation, although treatments containing steam flaked or extruded corn produced higher gas compare to treatment 1. Furthermore treatment 2, 3 and 6 produced the highest gas volume. The difference of gas production at 6 h after incubation was significantly different between treatments as well ($P<0.05$). The highest volume of gas production at 6 h after incubation was seen for treatment 2 and 6 which contained 20 and 10 percent of SFC. The lowest gas produced for treatment 1 at this time. After 8h of incubation the treatment 2 produced more gas ($P<0.05$) compare to other treatments, although there was no significant difference between treatments 3, 4 and 5 for gas production at this time.

There was a significant difference between treatments at 12 h ($P<0.05$) for total GP, and treatment 2 produced the highest volume of gas and the treatments 1, 3, 4 and 5 produced the lowest volume. After 24 h of incubation of samples, also same trend was recorded for treatment 1 that produced more gas compare to other treatments. The effect of processing on GP after 48h of

Table 1: *In vitro* gas production (ml/200mg DM) of treatments incubated in buffered rumen fluid at different incubation times

	Treatments*							Significant
Time (h)	1	2	3	4	5	6	SEM	P-Value
2	7.40^{c}	8.63^{a}	8.34^{ab}	7.87^{bc}	8.17^{ab}	8.58^{a}	0.15	<0.01
4	15.27^{d}	18.37^{a}	17.63^{ab}	16.53^{c}	16.98^{bc}	18.43^{a}	0.33	<0.01
6	22.04^{d}	29.20^{a}	26.14^{b}	24.40^{c}	24.84^{bc}	27.96^{a}	0.50	<0.01
8	29.44^{d}	39.87^{a}	32.91^{c}	31.80^{c}	32.07^{c}	36.40^{b}	0.71	<0.01
12	42.35^{c}	52.12^{a}	42.04^{c}	42.66^{c}	43.23^{c}	46.86^{b}	1.10	<0.01
24	60.30^{cb}	66.09^{a}	55.90^{c}	58.09^{bc}	59.74^{bc}	61.09^{b}	1.55	<0.01
48	70.54^{bc}	76.61^{a}	65.03^{c}	68.96^{bc}	70.90^{ab}	71.71^{ab}	0.15	0.011
72	73.21^{cd}	79.89^{a}	68.02^{d}	71.63^{cd}	73.57^{c}	74.21^{bc}	1.75	0.011
96	74.31^{bc}	80.84^{a}	68.96^{c}	72.89^{bc}	74.83^{b}	75.77^{ab}	1.74	<0.01

*1: Corn grain (20%) in TMR, 2: SFC (20%) in TMR, 3: Extruded corn (20%) in TMR, 4: Corn grain (10%) + SFC (10%) in TMR, 5: Corn grain (10%) + Extruded corn (10%) in TMR, 6: SFC (10%) + extruded corn (10%) in TMR; SEM: Standard Error of the Means.

Table 2: The gas production parameters of treatments calculated using F-curve software

Item	Treatments*						SEM	P-Value
	1	2	3	4	5	6		
a+b	77.85 ^{ab}	83.57 ^{a}	70.14 ^{c}	74.93 ^{bc}	76.93 ^{ab}	77.28 ^{ab}	2.25	0.028
b	75.85 ^{ab}	81.57 ^{a}	68.14 ^{c}	72.93 ^{cb}	74.93 ^{b}	75.28 ^{b}	1.96	0.011
c	0.067 ^{c}	0.082 ^{a}	0.079 ^{a}	0.071 ^{bc}	0.070 ^{c}	0.079 ^{a}	0.0014	0.010

*1: Corn grain (20%) in TMR, 2: SFC (20%) in TMR, 3: Extruded corn (20%) in TMR, 4: Corn grain (10%) + SFC (10%) in TMR, 5: Corn grain (10%) + Extruded corn (10%) in TMR, 6: SFC (10%) + extruded corn (10%) in TMR; a + b: potential GP (ml/200 mg DM); b: the GP from the insoluble fraction (ml); c: fractional rate of GP (ml/h); SEM: Standard Error of the Means.

incubation was also significant (P<0.05) in which at this time the lowest gas was recorded for treatment 3 (20 percent of extruded corn in TMR) and differed significantly with treatment 2, 5 and 6. This trend was comparable for 72 and 96h and the treatments differed significantly for total GP.

Effect of processing of corn on gas production parameters is shown in Table 2. There was a significant effect (P<0.05) for potential GP (a+b), and the treatment 2 had the highest (a+b) value. There was no significant difference between this treatment and treatments 1, 5 and 6 (P>0.05). Gas production from the insoluble fraction (b) was significantly affected by treatments (P<0.05), and the treatments 1 and 2 had the greatest "b" fraction with no significant difference between these two treatments. There was no significant difference between treatments 1, 4, 5 and 6 for parameter "b" but the lowest "b" fraction was recorded for treatment 3 which included 20 percent of extruded corn. In consistent with a+b and b, the parameter of fractional rate of gas production "c" was significantly affected and there was significant difference between treatments (P<0.05). Treatments 2, 3 and 6 had the highest and the treatments 1, 4 and 5 had the lowest "c" fraction.

Discussion

Steam flaking of corn increased *in vitro* total GP of TMR incubated in buffered rumen liquor. Processing of cereal grains, for example grinding and steam flaking of corn, increases digestibility of whole corn (DePeters et al., 2003). Consistent to our results, other findings confirmed that steam flaking of corn lead to produce more *in vitro* gas compared to unprocessed corn grain (DePeters et al., 2003). Gas production was higher at 8h for SFC than whole corn which is consistent to the study of DePeters et al. (2003). Inconsistent to our results, gas production did not differ between SFC and whole corn at 24 and 72h of incubation (DePeters et al., 2003), although we use the processed corn instead of whole corn in a totally mixed ration. (Plascencia and Zinn, 1996) reported that thin flaking of corn might lead to greater gelatinization of starch and facilitates higher solubilization. Soluble starch in the rumen is readily susceptible to enzymatic hydrolysis. Maximizing the rumen digestibility of corn starch may cause higher gas in the rumen. Steam flaking of corn could increase starch digestibility in both the rumen and total tract, so in diets containing SFC it can provide more energy to support milk production without a depression in dry matter intake (Firkins et al., 2001).

Hypothetically, increasing starch digestibility in the rumen should support more microbial protein synthesis. There should be an appropriate balance between starch digestion in the rumen and intestine to support microbial protein synthesis as well as a moderate escape of starch to duodenum (Oba and Allen, 2003). In the current experiment, rations containing SFC produced greater gas than other treatments confirmed earlier findings which concluded that gas production is related to starch reactivity and disappearance (DePeters et al., 2003). Diet contained 20 percent of extruded corn (treatment 3) had the lowest potential of gas production (a+b) that may nr due to dry heating of corn. Dry and hot processing of corn may possibly increase the maillard reaction and reduce starch solubility. Maillard or nonenzymatic browning reaction caused by heating and drying. An excess of heating of grains might increase Maillard reactions and the proportion of resistant starch, a fraction of starch that is less available to enzyme activity (Vicente et al., 2008).

The degree of damage of starch and extent of denaturation of protein in flaked grain varies with processing conditions. Moreover the 'b' fraction of treatment 3 was reduced compare to treatment 1 and 2, demonstrated that the site of digestion might shifted from the rumen to the intestines for extruded corn (Firkins et al., 2001).

Conclusion

Steam flaking or extruding of corn could modify the digestion kinetics of corn used in common rations of ruminants. Therefore, maximizing the rumen digestibility of starch while reducing the amount of corn grain using byproducts is a viable strategy when corn supplies are low and prices are high.

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