

The effect of different processing of corn grain on gas production kinetics and *in vitro* digestibility in Taleshi COWS

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Abstract

In this experiment, the gas test technique was used to investigate the effect of various processing of corn grain on the amount of gas production and its components, digestibility of organic matter, and metabolic energy and net energy for lactation. A completely randomised design with 5 treatments and 3 replications were used. The treatments consisted of control treatments (untreated corn grain), steam flaked corn grain, extruded corn grain, roasted and rolled corn grain, and rolled only corn grain. Data were analysed using SPSS software and ANOVA. To compare the different treatments, Duncan's multiple range tests were used for mean comparison at $P \leq 0.05$. The difference in gas production among treatments during the different hours of incubation was significant ($P < 0.01$). The gas produced during the first hours of incubation (up to 8 hours) was significantly higher in treatments treated with heat and steam (treatment 2 and 3) and the means were different compare to other treatments ($P < 0.01$). Treatments 5 (rolled corn grain) produced significantly less gas than other treatments ($P < 0.01$). The effect of experimental treatments on all parameters of gas production was significant ($P < 0.01$). The least amount of potential of gas production (76.77 ml) and gas production from insoluble fraction (68.14%) were for treatment 3, which had a significant difference with other treatments ($P < 0.01$). The gas production rates in treatments 2 and 3 (respectively, 0.082 and 0.079 ml/ h) was not significantly different but was significantly higher than other treatments ($P < 0.01$). The effect of experimental treatments on organic matter digestibility, metabolizable energy and net energy for lactation was significant ($P < 0.01$). According to the results of this study, it seems that processing of corn could effectively alter the fermentation pattern and digestibility of corn grain in the rumen.

Keywords: Processed corn; Gas test; Digestibility; Energy

Introduction

Corn is the main feedstuff in dairy cattle nutrition which can supply energy and some proteins. A large proportion of starch in rolled corn escapes from ruminal fermentation (DePeters et al., 2003); therefore, different methods of corn processing were developed over the years. Grain tissue plays a major role in the rate and location of starch digestion in ruminants (Philippeau et al., 1999). The physical form of starch and its relationship with protein and cellular cohesion of starch units in seed affects the availability of microbes to nutrients inside the seeds. The chemical structure of starch granules and their interactions with the protein section in the endosperm fraction also affects the speed and amount of rumen fermentation (Ramirez et al., 1985).

The use of processed feedstuffs results in faster rumen development and more accessible carbohydrates (Streeter et al., 1993). The amount of starch availability in the rumen could influence some variables such as milk fat percentage, rumen pH and volatile fatty acid profiles. Cereal processing can increase its digestible compounds through the transformation of starch. The processing type will have lots of effects on the digestibility of dry matter, starch and protein (Hale, 1980). Simply performing mechanical processes significantly increases the digestibility of corn. On the other hand, by rolling or grinding of some corn grains the digestibility of whole corn is increased by approximately 25%. About 75% of the corn starch is disappeared in the rumen, while steam and flaking the corn, could increase this to about 95%. When starch is cooked during processing, it can easily be fermented in the rumen. The most digestible performance could be achieved when grains (corn, sorghum, wheat and barley) are processed in by steam flaking. The optimal use of starch is important for improving the production efficiency. Feeding with cereal grains whose starch is too gelatinized can reduce the animal performance, or because of acidosis, reduced feed intake, lameness and reduced digestibility of nutrients. The use of processed grains also increases the digestible energy (Zinn, 1990). Flaked corn will increase the proportion of propionate to acetate ratio in the rumen and reduce the loss of methane considerably (Palizdar et al., 2014a). The purpose of this study was to investigate the effect of corn processing on the characteristics of rumen fermentation, gas production and organic matter digestibility.

Materials and Methods

To perform this experiment, a completely randomized design with 5 treatments and 3 replications was used. The treatments consisted of control treatments (untreated corn grain), steam flaked corn grain, extruded corn grain, roasted and rolled corn grain, and rolled only corn grain. All treatments were obtained from industrial group of Ariadan Company- Iran.

Three fistulated Taleshi cows were used for collecting rumen liquor and they fed with a maintenance diet which contained experimental corns for one week. The gas test technique was done as previous experiments (Palizdar et al., 2014b) in Animal Science Research Institute. *In vitro* incubation was performed using 30 ml of buffered rumen fluid according to the method of Menke and Steingass (1988). A 200 mg of sample was weighed and placed in 100 ml graduated glass syringes. Buffer mineral solution was prepared according to Menke and Steingass (1988), and placed in a water bath at 39°C under continuous flushing with CO₂. Rumen fluid was collected after the morning feeding from two ruminally fistulated, Iranian Taleshi steers 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 steers was mixed and filtered through four layers of cheesecloth and flushed with CO₂. Rumen fluid was added to the buffered mineral solution (1:2 v/v), which was maintained in a water bath at 39°C. Rumen fluid mixed with buffered mineral solution with constant stirring, while maintained in a water bath at 39°C. 30 ml of buffered rumen fluid was dispensed into syringes containing the samples. All handling was under continuous flushing with CO₂. 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 after which, the initial volume recorded. The syringes were then 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. Cumulative GP data were fitted to the exponential equation:

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

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), "c" the GP rate constant for "b" and "t" is the time of incubation (h). Data on *in vitro* gas

production were subjected to analysis of variance in a completely randomized design using the SPSS program. 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

The amount of gas produced in different treatments during different hours of incubation showed a significant difference ($P < 0.01$; Table 1). The amount of gas produced after two and four hours of incubation was higher in treatments two (steamed flaked corn) and three (extruded corn) and showed a significant difference with other treatments ($P < 0.01$). Treatments five (rolled only corn) produced significantly less gas than other treatments. The highest and the lowest amount of gas after 6 and 8 hours of incubation were in treatments two and five, respectively, each separately showing a significant difference with other treatments ($P < 0.01$). The amount of produced gas in the control treatment was significantly higher than the other treatments ($P < 0.01$) after 24 hours of incubation. This increase was observed in the amount of gas produced in treatments four and five after 48 hours, which continued until the end of the incubation period ($P < 0.01$).

Table 1: Effect of experimental treatments on gas production during incubation times (ml/200 mg DM)

Treatment	Incubation time (h)								
	2	4	6	8	12	24	48	72	96
1	7.33 ^{b*}	12.33 ^b	16.83 ^c	23.82 ^c	44.82 ^b	73.65 ^a	87.15 ^a	89.82 ^b	91.31 ^b
2	8.63 ^a	18.37 ^a	29.20 ^a	39.87 ^a	52.12 ^a	66.09 ^c	76.61 ^b	79.89 ^c	80.84 ^c
3	8.34 ^a	17.63 ^a	26.14 ^b	32.91 ^b	42.04 ^c	55.90 ^d	65.03 ^c	68.02 ^d	68.96 ^d
4	5.99 ^c	10.83 ^c	16.33 ^c	28.49 ^c	52.49 ^a	72.32 ^{ab}	89.65 ^a	93.32 ^a	96.82 ^a
5	5.16 ^d	7.49 ^d	10.49 ^d	16.66 ^d	39.32 ^d	55.90 ^{bc}	88.65 ^a	92.65 ^{ab}	94.33 ^{ab}
SEM	0.36	1.10	1.84	2.11	1.47	1.73	2.56	2.70	2.77
P- value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

1) Control treatment (untreated corn grain), 2) steam flaked corn grain 3) extruded corn grain 4) roasted and rolled corn grain, and 5) rolled only corn grain. *Means within a column with different superscripts differ significantly ($P < 0.05$). SEM = standard error of mean.

The effect of experimental treatments on all parameters of gas production was significant ($P < 0.01$; Table 2). The lowest potential of gas production (a + b) (76.77 ml) and gas production from the insoluble fraction (68.14) were seen in treatment 3 (extruded corn grain), which had a significant difference with other treatments ($P < 0.01$). The control treatments and treatments four and five did not differ significantly in potential of gas production and gas production from the insoluble fraction (b), but had significant differences with treatments two ($P < 0.01$). The relative gas production rate in treatments two and three (respectively, 0.082 and 0.079 ml/h) was not significantly different ($P < 0.05$) but was significantly higher than other treatments ($P < 0.01$). The lowest relative rate of gas production was seen in treatment 5 (0.044 ml/h), which had a significant difference with other treatments ($P < 0.01$).

Table 2: Effect of experimental treatments on gas production coefficients

Treatment	a+b*	c	b
	ml	ml/h	ml
1	93.33 ^a	0.054 ^b	98.17 ^a
2	79.60 ^b	0.082 ^a	81.57 ^b
3	67.76 ^c	0.079 ^a	68.14 ^c
4	94.50 ^a	0.056 ^b	99.95 ^a
5	93.09 ^a	0.044 ^c	99.95 ^a
SEM	2.82	0.003	3.43
P-Value	<0.01	<0.01	<0.01

1) Control treatment (untreated corn grain), 2) steam flaked corn grain 3) extruded corn grain 4) roasted and rolled corn grain, and 5) rolled only corn grain. * a+b : potential of gas production, c: rate of gas production and b: gas production from insoluble but fermentable fraction. SEM = standard error of mean.

The effect of treatments on organic matter digestibility, metabolizable energy and net energy for lactation was significant ($P < 0.01$; Table 3). Control treatment was not significantly differed with treatments two and four for OMD, metabolizable energy and net energy for lactation. Also there was not any significant difference between treatment 2, 4 and 5, although treatment 3 had the lowest OMD, ME and NEL compared to other treatments.

Table 3: Effect of treatment on organic matter digestibility, net energy for lactation and metabolizable energy.

Treatment	OMD %	NEL Mj. Kg ⁻¹ DM	ME Mj.Kg ⁻¹ DM
1	83.15 ^a	8.17 ^a	12.72 ^a
2	80.41 ^{ab}	7.28 ^c	12.10 ^b
3	71.35 ^c	6.11 ^d	10.71 ^c
4	81.82 ^{ab}	8.02 ^{ab}	12.51 ^{ab}
5	78.82 ^b	7.67 ^b	12.04 ^b
SEM	1.16	0.203	0.194
P-Value	<0. 01	<0. 01	<0. 01

1) Control treatment (untreated corn grain), 2) steam flaked corn grain 3) extruded corn grain 4) roasted and rolled corn grain, and 5) rolled only corn grain. OMD: organic matter digestibility, ME: metabolizable energy and NEL: net energy for lactation.

Discussion

Gas production at different incubation times

The amount of gas production at the initial incubation times (2, 4 and 6 h) was affected by the treatments, so that the processed corn had different gas values than the control. The results of this study is in agreement with DePeters et al (2003), which they showed that using steam-flaked corn increased the amount of gas produced during the initial hours of incubation. Also, in the early hours of fermentation, the amount of gas produced from extruded corn was higher than untreated corn treatment (control). Faster fermentation in the early hours of incubation indicates a faster digestion of starch and soluble carbohydrates (DePeters et al., 2003). Compared to studies which used forages treatments (require long time for fermentation of fibre), initial 6 to 8 hours of incubation demonstrated some differences in the rate of starch fermentation of cereals (DePeters et al., 2003). Due to using heat and moisture processing for corn grain, the starch will have gelatinized and more exposed to microorganisms that it causes starchier fermentation and thus lead to increased gas production. Steaming destroys the internal protein matrix that surrounds the starch granules. This will ultimately increase the availability of more microbial enzymes and enzymes in the mammalian body to digest more corn starch (Burkholder, 2004; Getachew et al., 2001). The thinner the flake, the greater the gelatinization or solubilization of starch and the rate of enzymatic hydrolysis (Moore et al., 1992; Plascencia and Zinn, 1996).

The quantities of gas produced after 24 hours of incubation are used to estimate the amount of energy and digestibility of organic matter (Menke and Steingass, 1988). Reducing the amount of gas produced by treatments (2 and 3) after 24 h of incubation compared with other treatments indicates that the amount of fermentation in these two treatments were lesser than others. A large proportion of starch in rolled or cracked corn escaped ruminal fermentation and therefore will not ferment in the rumen (DePeters et al., 2003). The difference in the amount of gas production between the extruded corn and the steam-flaked corn is different because of their physical characteristics, so that in the process of extruding compare to steam flaking, the moisture will suddenly escape the grains and the product obtained will have more dry matter (Moore et al., 1992).

Gas production coefficients

In this experiment, it was shown that potential of gas production for treatments 2 and 3 was lesser than other treatments. The reason for the lower potential of gas production in extruded corn treatment compared to the control treatment can be explained by the fact that extruding of corn with direct heat may lead to 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).

This effect can also be seen in the reduction of *b* fraction, which in the third treatment that contains extruded corn, the amount of gas produce from *b* fraction decreased, which indicates that the non-degradable fraction has increased in treatment 3.

Steaming causes abrupt changes in starch granules and produces fewer non distinct starchy missiles. The puffing process will also lead to individual development of starch granules into thin-layer interplanetary complex. Although proteins remain intact in these processes, but the surrounding protein that is present in individual starch granules is destroyed (Theurer, 1986).

A major effect of proper grain processing is to shift the site of starch digestion from the intestines to the rumen, with concomitant increases in percentage digested within both sites. It was observed that treatments 2 and 3 had the highest relative gas production rate. This suggests that processes using heat and steam are effective on the

structure of starch granules and corn grain pericarp, which provides more bacterial access to starch and increases the speed of digestion in the rumen. Crushing alone (as in treatments 1 and 5) did not alter the structure of starch granules in a protein matrix (Streeter et al., 1993). Pericarp (external shell) in complete corn grain prevents the bacteria from binding and thus prevents digestion (Bergman et al., 1965; Huntington, 1997). It was also shown that the last treatment which contained rolled dry corn, had the least amount of relative gas production rate, which indicated that rolling cannot increase the availability of microbes in rumen fluid to corn starch lonely and lots of resistant starch to will be produced in this treatment. Philippeau (1999) suggested that grain tissue plays a major role in ruminal starch digestibility. It was reported that the difference in starch granular structure may justify the rate of gas production in cereal grains (Philippeau et al., 1999).

Digestibility of organic matter and metabolisable energy and net energy for lactation

The results of this study showed that the digestibility of organic matter in the treatment two (steam flaked corn) was higher than other treatments, which was in agreement with the results of Plascencia and Zinn (1996) (Plascencia and Zinn., 1996), which showed that organic matter digestibility of steam flaked corn was 15% more than rolled corn grain. Processed grains also have higher metabolisable energy than unprocessed grains, which can be attributed to the reduction in methane from processed cereal grains and, in addition, processed cereals reduced the fermentation in the large intestine. In the present study, it was shown that the amount of metabolisable energy in extruded corn was the least that can be due to the Maillard reactions. These reactions reduce fermentation of starch in rumen and decrease its rumen digestibility, which is evident in treatment 3 in the amount of digestibility of its organic matter (71.35%) compared with other treatments.

Regarding the higher digestibility of organic matter in treatment 2 (80.4%) and treatment 4 (81.8%) compared to other treatments, the increase in net energy of lactation was also predictable. Steaming increased digestible energy, metabolisable energy and net energy for lactation by up to 29, 36 and 33 percent, respectively. Our results are in agreement with several studies. They have been reported the starch digestibility for complete maize 59%, rolled corn grain 78% and steam flaked corn 83% (Owens et al, 1997; Owens, 2005a, 2005b; Owens and Zinn, 2005; Palizdar et al, 2012).

Conclusion

According to the results of this study, it seems that the processing operations are effective on the fermentation and digestibility of corn grain. All types of hot - dry, cold - dry, as well as hot - wet processing have been effective in changing the parameters of *in vitro* fermentation. Among these processing methods, extruding had the greatest detrimental effect on the digestibility of organic matter. Moreover, extruding and steam flaking had the highest impact on the rate of gas production which increased this parameter relative to the control treatment. Consequently, it seems that for increasing the rate of fermentation of corn in the rumen the steam flaking is the best technique.

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