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Replacement of corn grain with steam-flaked and extruded corn in total mixed ration on *in vitro* fermentation and volatile fatty acids concentration

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Abstract

The effect of using steam-flaked and extruded corn on *in vitro* fermentation of total mixed rations (TMR) was studied. Six dietary treatments were used: ration 1 (control) - corn grain at 20% in a TMR, ration 2 – stem-flaked corn at 20% in a TMR, ration 3 – extruded corn at 20% in a TMR, ration 4 – corn grain at 10% + steam-flaked corn at 10% in a TMR, ration 5 – corn grain at 10% + extruded corn at 10% in a TMR and ration 6 – steam flaked corn at 10% + extruded corn at 10% in a TMR. The TMR used in the *in vitro* evaluation was made up of the corn and or processed corn (20%) alfalfa hay (40%), beet sugar pulp (20%), soybean meal (10%) and barley grain (20%). A 200 mg sample of each ration was weighed and placed in 100 ml glass syringes. Each sample was then inoculated with artificial saliva and filtered rumen liquor (2:1 ratio), then incubated at 39°C in a ventilated oven. Gas production (GP) was recorded at 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours. At 96 hours, 10 ml of supernatant from each sample was taken for the determination of volatile fatty acids (VFA) concentration. The total *in vitro* gas and VFA produced were corrected for the blank. Production of acetate, propionate, valerate and isobutyrate was different ($P<0.05$) between treatments because the organic matter digestibility (OMD) was different as well and in treatment 2 the OMD was 80.41%. There was a significant difference between treatment 1 and other treatments including processed corn ($P<0.05$) for propionate concentration, while control treatment had the highest concentration of propionate (26.32 mol/100mol). Acetate to propionate ratio was significantly different between treatments ($P<0.05$). Moreover, treatment 1 had the lowest acetate to propionate ratio (1.71) in comparison to treatment 3, 5 and 6. Based on the results of this study; steam flaking and or extruding corn can potentially alter the VFA production in the rumen. Using processed corn in place of corn grain in TMR might alter rumen fermentation and probably increase the digestibility of feeds in dairy cows.

Keywords: steam-flaked corn; extruded corn; *in vitro*; total mixed ration; *in vitro* gas production; feed digestion

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Introduction

Different grain processing techniques have been used in an attempt to improve feed utilization by ruminants (Firkins et al., 2001). Corn, whose major nutrient is starch, is included in ruminant diets to boost the dietary energy content (Galyean, 1996). Moreover, processing of corn is used to increase extent of starch digestion in the rumen. Optimal starch utilization is fundamental to improve efficiency of animal productivity. For non-ruminants, starch from finely ground grain is fully digested, but for ruminants fed high concentrate diets, finely ground grain often causes metabolic diseases (Theurer et al., 1999). Therefore, rather than finely grinding corn, processes including steam-rolling, steam-flaking and fermentation (high moisture storage) are used to increase the extent of starch digestion from grains fed to ruminants (Nocek and Tamminga, 1991).

Nowadays, steam-flaking of corn grain is a classic method of processing in many countries (Soltani et al., 2009). Steam flaking of corn has been shown to

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increase starch digestibility in the rumen and using steam flaking of corn (SFC) the volatile fatty acids production in the rumen could be changed (Joy et al., 1997; Crocker et al., 1998). It is known that marked changes in the molar proportions of the concentrations of VFA in the rumen can be induced in response to a wide variety of dietary manipulations (Theurer et al., 1999; Owens et al., 1997). The consequences of these changes for metabolism in the body and ultimately for meat and milk production have been explored in numerous papers (Firkins et al., 2001; DePeters et al., 200;3 Soltani et al., 2009).

Steam flaking of cereals employs water and heat to cause swelling of starch granules, followed by rolling to further disrupt the swollen granules (Rooney and Pflugfelder, 1986). The original method of "steam processing and flaking" of corn and barley renders the starch fraction more readily available to rumen microorganisms and enzyme degradation than conventional methods of steam or dry rolling (Firkins et al., 2001). Firkins et al. (2001) reported that starch digestion improved as the steam-processed flake became thinner. Ruminal digestion of starch is important for bacterial growth, rumen health and the production of volatile fatty acids. Intestinal starch digestion provides free glucose, which can be used directly by the mammary tissue for milk production (Huntington, 1997; Knowlton et al., 1998; Huntington et al., 2006) and is estimated to yield 42% more energy than ruminally digested starch (Owens et al., 1986 & 1997; Owens and Zinn, 2005; Owens, 2005 a&b).

Measurements of actual rates of production of individual VFA would clearly be of greater value than simple rumen concentrations, but technical difficulties have demonstrated to be a serious problem. In a study by DePeters et al. (2003) an *in vitro* gas production technique was used to determine differences in starch availability that occurred with steam flaking of corn among eight feed mills in California. Total volatile fatty acids (VFA) production did not differ for mill or process at 8 or 72 hrs of incubation (DePeters et al., 2003). Propionate production was higher for steam flaked corn than whole corn at both 8 and 72 h (DePeters et al., 2003). Following their study, DePeters et al. (2003) concluded that the *in vitro* gas production technique could be used to determine the effect of grain processing on fermentation rate and volatile fatty acids production.

Determination of intake and digestibility of feedstuffs *in vivo* is time-consuming, laborious, and expensive and is unsuited for large-scale feed evaluation (Menke et al., 1979). The first report of Menke et al. (1979) demonstrated a high correlation between digestibility measured *in vivo* and predicted from an *in vitro* rumen gas production technique in combination with chemical composition. In addition this technique might be applied to study associative effects of various types of feeds, examine influences of feed additives on rumen fermentation, partitioning of fermented substrates into fermentation products, and assess the composition of gases from fermentation of various feeds. Unlike other *in vitro* techniques, such as Tilley and Terry (1963), Mehrez and Ørskov (1977) which is based on gravimetric measures that follow disappearance of substrate, gas test methods measure appearance of fermentation products.

Currently, there is a dearth of information on the effects of flaking and or extruding corn grain (used in TMR) digestion, as measured by gas production and VFA concentration and proportions. *In vitro* rumen gas production was used to compare sorghum grain hybrids that differed in endosperm colour (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). The objective of this research was to determine the effect of processing (flaking and extrusion) of corn grain used in TMR to estimate rumen fermentation and VFA production using the *in vitro* gas technique.

Materials and Methods

Dietary ingredients, processing and ration formulation

Unprocessed, extruded and SFC grain samples were obtained from Armaz Co (Tehran, Iran) The processed corn was acquired in two batches. Other ingredients used in ration formulation were also obtained from a commercial farm near Tehran.

Samples of steam flaked, extruded corn, unprocessed corn and all diet ingredients were ground in a Wiley mill (Arthur A. Thomas, Philadelphia, PA) to pass a 1mm sieve and stored at room temperature. Six dietary treatments were used: Treatment 1 (control) - corn grain at 20% in a TMR, Treatment 2 – stem-flaked corn at 20% in a TMR, Treatment 3 – extruded corn at 20% in a TMR, Treatment 4 – corn grain at 10% + steam-flaked corn at 10% in a TMR, Treatment 5 – corn grain at 10% + extruded corn at 10% in a TMR and Treatment 6 – steam flaked corn at 10% + extruded corn at 10% in a TMR. The TMR used in the in vitro evaluation was made up of the corn and or processed corn (20%), alfalfa hay (40%), beet sugar pulp (20%), soybean meal (10%) and barley grain (20%) which presented in Table 1.

Determination of gas production

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

Table 1: Ingredient composition (% of DM) of the treatments

	Treatment					
Ingredient	1	2	3	4	5	6
alfalfa hay	40	40	40	40	40	40
beet sugar pulp	10	10	10	10	10	10
soybean meal	10	10	10	10	10	10
barley grain	20	20	20	20	20	20
Corn grain	20	0	0	10	10	0
SFC	0	20	0	10	0	10
Extruded corn	0	0	20	0	10	10
total	100	100	100	100	100	100

mineral solution was prepared according to Menke and Steingass (1988), and placed in a water bath at 39°C under continuous flushing with CO_2. 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 CO_2. 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 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 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:

$Y = a + b\ (1 - \exp^{-ct})$ 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). At the end of the incubation 10 ml of supernatant from each sample was taken and used for the determination of volatile fatty acids (VFA) concentration. The VFA concentration was determined as described by DePeters et al. (2003). While the metabolizable energy (ME), net energy for lactation (NEL) contents and organic matter digestibility (OMD) were computed as described by Menke and Steingass (1988) in the equations below:

Equation 1: ME (MJ/kg DM) = 2.20 + 0.136 × Gp + 0.057 × CP+ 0.0029 × EE 2

Equation 2: NEL (MJ/kg DM) = 0.115 × GP + 0.0054 × CP + 0.014 × EE - 0.0054 × XA - 0.36

Equation 3: OMD (g/100 g DM) = 14.88 + 0.889 × Gp + 0.45 × CP + 0.0651 ×XA where,

CP is crude protein in g/100 g DM, XA ash in g/100 g DM, EE is ether extract in g/100 g DM and GP is the net gas production (ml) from 200 mg after 24 h of incubation.

Statistical analysis

Data is presented as means and standard errors of means. Data on *in vitro* gas production were subjected to analysis of variance using the SAS program's General Linear Model (GLM) procedure (SAS, 9.1, 2005) with mean comparison done using the least square means method. Mean differences were considered significant at $P<0.05$.

Results

Effect of experimental treatments on ME, NEL and OMD is presented (Table 2). There was a significant difference ($P<0.05$) in ME among treatments (Table 2). Treatment 2 (containing 20 percent SFC) had the highest ME content (12.10 MJ/kg DM) and was significantly different compared to other treatments. Treatment 1 had similar ME content compare to treatment 3, 4, 5 and 6 ($P>0.05$). NEL content of experimental treatments was significantly affected by processed corn in TMR ($P<0.05$). As well as results for ME, the NEL of Treatment 2 (containing 20 percent SFC) had the highest ME content (12.10 MJ/kg DM) and was significantly different, compared to other treatments. The OMD was significantly affected by treatments ($P<0.05$). Diet containing 20 percent SFC had the highest OMD value (80.4%) and was significantly different compared to other treatments ($P<0.05$). Treatment 2 had the lowest OMD value but had no significant difference with treatment 1, 4 and 5.

Effect of ration composition (experimental treatment) on VFA concentration in rumen liquor has been shown in Table 3. There was a significant difference ($P<0.05$) in acetate production among treatments (Table 3). Treatment 4 significantly produced the lowest acetate value compare to other treatments (treatment 3, 5 and 6, respectively). Propionate production was significantly affected by treatment ($P<0.05$) and the treatment 1 had the highest propionate value compare to other treatments (Table 3).

There was no significant difference ($P>0.05$) in butyrate production among the different rations. However, isobutyrate concentration was significantly different between treatments ($P<0.05$). Treatment 4 had the highest isobutyrate concentration (3.76 mol/100mol) compared to

Table 2: Effect of experimental treatments on ME (MJ/kg DM), NEL (MJ/kg DM) and OMD (g/100 g DM)

	Treatments*							Significant
Item	1	2	3	4	5	6	SEM	P-Value
ME	11.31^{bcd}	12.10^{a}	10.17^{d}	11.01 ^{dc}	11.24^{bc}	11.42^{b}	0.21	0.011
NEL	6.62^{bc}	7.28^{a}	6.11^{c}	6.36^{bc}	6.55^{bc}	6.71^{b}	0.17	0.011
OMD	75.26^{bc}	80.41^{a}	71.36^{c}	73.30^{bc}	74.76^{bc}	75.96^{b}	1.38	< 0.01

^{abcd} Within row means with different superscripts are significantly different at P ≤ 0.05. *Treatments explained in material and method section. SEM: standard error of mean.

Table 3: Effect of experimental treatments on VFA production (mol/100 mol) after 96 h incubation

	Treatments*							Significant
VFA†	1	2	3	4	5	6	SEM	P-Value
Acetate	45.02 ^{abc}	44.15 ^{bc}	45.99 ^{a}	43.36 ^{c}	45.68 ^{ab}	45.79 ^{ab}	0.58	0.045
Propionate	26.32 ^{a}	25.51 ^{b}	25.36 ^{b}	25.17 ^{b}	25.23 ^{b}	25.05 ^{b}	0.15	0.011
Butyrate	21.57	23.40	21.89	22.92	21.95	22.22	0.47	0.132
Isobutyrate	3.39^{b}	3.18 ^{bc}	3.03 ^{c}	3.76 ^{a}	3.33 ^{bc}	3.25 ^{bc}	0.099	<0.01
Valerate	3.68^{b}	3.74 ^{b}	3.70 ^{b}	4.77 ^{a}	3.79 ^{b}	3.66 ^{b}	0.14	<0.01
A: P $^{\spadesuit}$	1.71^{c}	1.73 ^{bc}	1.81 ^{a}	1.72 ^{c}	1.81 ^{ab}	1.82 ^{a}	0.027	0.026

^{abcd} Within row means with different superscripts are significantly different at P ≤ 0.05. *Treatments explained in material and method section. SEM: standard error of mean.

other treatments (P<0.01). The valerate concentration as well as isobutyrate was affected by treatments (P<0.01). Treatment 4 had the highest value for valerate production (4.77 mol/100mol) as well and was significantly different in comparison to other treatments. Acetate to propionate ratio was significantly affected by treatments (P<0.05). Treatment 1 and 4 had the lowest acetate to propionate ratio (1.71 and 1.72 respectively) compared to other treatments, and treatment 3, 5 and 6 had the highest acetate to propionate ratio.

Discussion

Using steam flaked corn in total mixed ration increased total ME, NEL and OMD of the diet when compared to the use of extruded or unprocessed corn (Table 2). The improvement in ME, NEL and OMD when steam-flaked corn is used in TMR as observed in this study is in agreement with observations of DePeters et al. (2003). Steam flaking of corn leads to greater gelatinization of starch which (gelatisation) facilitates the solubilization of starch (Plascencia and Zinn, 1996). The more soluble the starch is the more readily susceptible it becomes to enzymic hydrolysis in the rumen which translates to increased digestibility of corn starch and organic matter. Similar to our findings is the report by Firkins et al. (2001) who demonstrated that steam flaking of corn increased starch digestibility in both the rumen and total tract. Increase in digestibility and increased availability of ME in diets containing SFC could be the basis of their ability to provide increased energy to support milk production without a depression in dry matter intake (Joy et al., 1997). The change in VFA concentration in this study is not consistent to other study (Crocker et al., 1998). In that study, Crocker et al. (1998) used SFC which replaced dry rolled corn in the diet of lactating dairy cows. They reported that there was no effect on total VFA concentration in ruminal fluid and the concentration of acetate decreased while the concentration of propionate increased in a linear fashion with increasing SFC (Crocker et al., 1998). Other finding revealed no alteration in VFA production when steam rolled of corn was used in dairy ration (Reis and Combs, 2000). Although in the current study, using steam flake corn has changed VFA concentration. Total VFA concentration and VFA concentration relative to time of feeding were not different when SFC corn replaced dry rolled corn (Joy et al., 1997; Zinn et al., 2002). With steam-flaking, molar percentages of acetate and isovalerate were decreased, and propionate was increased (Joy et al., 1997; Zinn et al., 2002) but in the current study there was no difference in acetate concentration between SFC ration and control ration. In the current study, acetate concentration was not in the range of 60-65 mol/100 mol (Joy et al., 1997; Reis and Combs., 2000; Callison et al., 2001; Guyton et al., 2003). The discrepancy in research results may be explained by differences in the quantity of starch in the diet, the grain processing method and forage type (Knowlton et al., 1998). Most of the studies reviewed by Theurer et al. (1999a) included diets with alfalfa hay or corn silage as the principal forage. Simpson (1984) stated that increasing the amount of steam flaked corn in the diet could lead to a linear increase in ruminal starch digestion. Consistently post-ruminal starch digestion of corn would increase with flake density and steam used for corn processing. The volatile fatty acids in the rumen could be modified with the rate of starch digestibility. In contrast to the present study, other reports have also shown that there is a tendency towards increase concentration of propionic acid when the steam flaked of corn is used in the diet (Hale, 1973; Svihus et al., 2005; Soltani et al., 2009).

Conclusion

In conclusion, the results of the current study showed that the use of processed corn, especially SFC in a total mixed ration could improve the overall diet digestibility and may alter VFA production in the rumen. Using processed grain like SFC instead of corn grain in total mixed rations may alter rumen fermentation and probably increase the digestibility of ruminant feeds.

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