Original scientific paper

EFFECTS OF REPLACING LOW STARCH BY-PRODUCTS FOR BARLEY GRAIN ON *in situ* RUMEN DEGRADABILITY AND *in vitro* GAS PRODUCTION PROPERITES OF A TOTAL MIXED RATION ABSTRACT

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ABSTRACT
This study was conducted to study the effects of different sources of low starch by-products substituted for barley grain on *in situ* rumen degradability and *in vitro* gas production properties of a total mixed ration. The experimental treatments consisted of 370 g/kg roughages and 630 g/kg concentrates. The total mixed diets contained 100 g /kg dry matter (DM) barley grains (BG, control diet), sugar beet pulp (SBP, treatment 2), wheat bran (WB, treatments 3), or dried citrus pulp (DCP, treatment 4). In the first experiment, the experimental diets were evaluated for *in-situ* rumen degradability by the nylon bag technique. In the second experiment, treatments were evaluated for *in vitro* gas production parameters. Data were analyzed as a completely randomized design. The data on *in situ* experiment indicated that non-soluble degradation fraction *(b)*, the fractional degradation rate *(c)* and potential degradability *(a+b)* were not affected by the diets (P>0.05). However, soluble degradable fraction (*a*) was highest for a diet with DCP (P<0.05). The *in vitro* gas production data showed there were no significant differences among treatments for gas produced at different times of incubation (P>0.05). Besides, the potential gas production (*b*), and OMD did not differ among treatments (P>0.05). But, the fractional rate of gas production (*c*) was lowest for diet with DCP (P<0.05). Still, SCFA varied among various treatments (P<0.05), and it was higher for DCP treatment than other groups. It concluded that replacing low starch by-products, such as dried citrus pulp and sugar beet pulp, for up to 10% of diet DM instead of barley grain can improve the DM digestibility of a total mixed ration without any adverse effect on ruminal fermentation in ruminants.

Key words: Barley grain, dried citrus pulp, starch, sugar beet pulp, wheat bran.

INTRODUCTION
The grain is often substituted for forage in high producing dairy cow diets to increase intake and milk yield. Reducing dietary NDF concentration usually increases DMI, probably by lowering the filling effect of the diet (Allen, 2000). However, increasing dietary starch can also negatively affect feed intake and milk production. Feeding less forage NDF reduces chewing time, and fiber digestibility is reduced when dietary starch concentration is increased (Grant and Mertens, 1992). Also, the grains are costly, and their inclusion in the animal diet further increases animal production cost. In the past, efforts have been made to minimize the use of grains in the diet of animals by substituting various agricultural by-products. There are several alternative strategies for increasing dietary energy content with a lower risk of its adverse side effects. One approach is to utilize carbohydrate sources in ruminant's diets to ferment faster and more extensive than forage NDF, and that mimic some of its beneficial

effects, but do not have the same adverse impact as starch fermentation (Afghahi and Esteghamat, 2015).

Adding non-forage NDF to low-forage diets might reduce the adverse effects of increased starch fermentation without increasing the filling effect of the diet to the same extent as forage NDF (Allen, 2000). Beet pulp, dry citrus pulp, and wheat bran are by-product feeds that contain a high concentration of neutral detergent soluble fiber (NDSF), especially pectin substances. Dry sugar beet pulp contains 250 g pectin /kg DM (Thibault et al., 1991). In citrus pulp, pectin comprises approximately 450 g/kg of cell wall component (Sunvold et al., 1995), and the content of total pectin in wheat bran is about 0.35% on DM basis (Bailoni et al., 2003). Pectin, a valuable constituent of ruminant’s feed, is degraded rapidly and extensively in the rumen but, unlike starch, yields little lactate, causing less of a decline in rumen pH (Barrios-Urdaneta et al., 2003). Some of the results in related reports about replacing SBP for grain in the total mixed ration are variable. Dann et al., (2014), partially replacing corn grains with SBP or wheat middlings, did not find any significant effects on ruminal fermentation, chewing behavior, milk production, and composition.

In a recent study, Garcia-Rodriguez et al. (2020) investigated the influences of replacing extruded maize (20% of total diet) by dried citrus pulp (20%) in a mixed diet on ruminal fermentation and methane production in rusitec fermenters. They indicated some positive effects of citrus pulp on diet degradability and *in vitro* fermentation parameters and concluded that maize in dairy sheep diets can be totally replaced by dried citrus pulp without negatively affecting ruminal fermentation.

Ertl et al. (2016) evaluated the impact of a complete substitution of common cereal grains and pulses with a mixture of WB and SBP in a high forage diet on dairy cow performance. They showed that dietary treatment did not affect milk production, milk composition, feed intake, or total chewing activity.

During the processing of wheat, about 20% of the DM results in the by-products as wheat bran, which indicates their high availability (Ertl et al., 2016). The use of wheat bran in livestock diets is recommended due to higher energy contents than forages. In comparison with forages, wheat barns NDF is fermentable, and more than 62% of rumen microorganisms are accessible; thus wheat bran can effectively be included in the dairy and fattening sheep and cattle diets as a part of forage or grains (Afghahi and Esteghamat, 2015; Oba et al., 1999).

So, the objective of this study was to evaluate the effects of different sources of low starch by-products substituted with barley grain on *in situ* rumen digestibility and *in vitro* gas production properties a total mixed ration.

MATERIALS AND METHODS

Experimental treatments

The experimental treatments were diets with 370 g forage /kg DM (barley silage and alfalfa hay), and 630 g concentrate /kg DM, comprised of 1) 100 g barley grain (BG)/kg DM (control diet), 2) 100 g sugar beet pulp (SBP)/kg DM, 3) 100 g wheat bran (WB)/kg DM and 4) 100g dried citrus pulp /kg DM (DCP) and diets formulated by NRC (2001) software. The feed ingredients and chemical composition of diets are shown in Table 1.

In situ rumen degradability

Measurements of *in situ* DM degradability of treatments were performed in 3 rumen-fistulated steers (650±12 kg BW, three years old) using the nylon bag technique (Ørskov and McDonald, 1979). The nylon bags (5 × 10 cm^2, pore size 45 μm) were filled with 5 g of samples and put into the rumen. Feed ingredients of the diet of steers are shown in Table 2. Steers were fed at maintenance level. The bags were removed at 2, 4, 8, 12, 24, 48, and 72 h after the start of incubation, and each bag was washed immediately with tap water until color disappeared. For the t_0 incubation time, the bags were simply washed in the water. *In situ* disappearance of DM was measured relative to the initial sample. The rate and extent of DM degradation were estimated according to the equation: $p = a + b\ (1 - e^{-ct})$ where: p is the amount degraded at time t, a and b represent soluble and non-soluble degradable fractions, respectively; c is the constant rate of degradation of the b fraction for DM and e is the natural logarithm (Ørskov and McDonald 1979). Effective degradability (ED) was calculated using the outflow rates (*Kp*) of 0.02, 0.03, 0.04 and 0.05%/h according to Ørskov et al. (1980) model: ED = a + (b × c)/(c + k), where ED is effective degradability, and a, b and c are the constants as described earlier in the non-linear equation above and k the rumen fractional outflow rates.

Table 1. Ingredient and chemical composition of the experimental diets

	Treatment[1]			
	BG	SBP	WB	DCP
Ingredients, g/kg DM				
Alfalfa	200	200	200	200
Barley silage	170	170	170	170
Soybean meal	100	105	95	115
Cottonseed	70	70	70	70
Corn grain	210	210	210	210
Cottonseed meal	110	105	115	95
Barley grain	100	0	0	0
Sugar beet pulp	0	100	0	0
Wheat bran	0	0	100	0
Dried citrus pulp	0	0	0	100
Fat	20	20	20	20
Limestone	7	7	7	7
Vitamin-Mineral mix[2]	10	10	10	10
Salt	3	3	3	3
Chemical composition, (%DM)				
CP	18.0	17.09	18.01	17.8
NDF	30.43	32.61	32.71	30.38
Forage NDF	17.95	17.95	17.95	17.95
Soluble NDF	9.0	10.5	8.75	11.3
ADF	20.13	21.97	20.91	20.91
NFC[3]	37.6	35.4	34.5	37.5
Starch	22.3	17.5	19.2	17.6
Sugar	5.3	5.9	5.5	7.7
NDSF[4]	10	12.03	9.5	12.3
Ether extract	6.1	6.9	6.3	6.1
Ash	7.9	8.3	8.3	8.4
Ca	0.8	0.9	1.0	1.01
P	0.5	0.5	0.5	0.5
NEl[5], MJ/kg	6.73	6.69	6.73	6.71

[1]BG: barley grain, SBP: sugar beet pulp, WB: wheat bran, DCP: dried citrus pulp.
[2]Contained (/kg of premix; DM basis): 330,000 IU of vitamin A, 60,000 IU of vitamin D, 1,000 IU of vitamin E, 16% Ca, 8.5% P, 6.3% Na, 4.5% Mg, 2,100 mg Zn, 1,500 mg Mn, 535 mg Cu, 12 mg Se, 45 mg I.
[3]NFC: non-fiber carbohydrates.
[4]NDSF: Neutral detergent soluble fiber.
[5]NEl was calculated based on NRC (2001).

Table 2. Feed ingredients of the diets of fistulae steers and sheep

Ingredients, (g/kg) DM of Diet	
Alfalfa	300
Corn silage	200
Concentrate	500
Composition of concentrate, (g/kg DM)	
Barley grain	550
Canola meal	150
Wheat Bran	280
Vitamin- mineral mix	10
Calcium carbonate	5
Salt	5
Chemical composition(g/kg DM)	
CP	133
NDF	372
ADF	223
ME(Mcal/kg)	2.62
Ca	10
P	6

In vitro gas production

In vitro gas production was carried out using the method described by Menke and Steingass (1988). Samples (200 mg) were weighed into 100 ml calibrated glass syringes (3replicates per sample). The buffered mineral solution was prepared 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 adult ruminally fistulated Balouchi sheep (42 ± 2.5kg BW, two years old), strained through four layers of cheesecloth, and flushed with CO_2. Feed ingredients of the diet of sheep are shown in Table 2. Sheep were fed at the maintenance level. The syringe was then filled with 30 ml of medium consisting of 10 ml rumen fluid and 20 ml buffer solution. Three syringes with only buffered rumen fluid were incubated and considered as the blanks. All handling was under continuous flushing with CO_2. The syringes were placed in a water bath at 39 °C. The syringes were gently shaken every two h, and the incubation terminated after recording the 96 h gas volume. Gas production was measured at 2, 4, 6, 8, 12, 24, 36, 48, 72, and 96 h. Total gas values were corrected for the blank incubation, and reported gas values are expressed in ml per 200 mg of DM. Rate and extent of gas production was determined for each feed by fitting gas production data to the nonlinear equation $Y = b\ (1-e^{-ct})$ (Ørskov and McDonald, 1979), where Y is the volume of gas produced at time t (ml), b is the potential gas production (ml/g DM), and c the constant rate of gas production (ml/h). Parameters b and c were estimated by an iterative least-squares method using a non-linear regression procedure of the statistical analysis systems (SAS 2003). in vitro organic matter digestibility (OMD, g/kg OM) were estimated according to Menke et al (1979).

OMD = 148.8 + 8.89 GP + 4.5 CP (g/kg DM) + 0.651 ash (g/kg DM),

where GP is net GP in mL from 200 mg of dry sample after 24 h of incubation.

Short-chain fatty acids (SCFA) were predicted as (0.0239 GV- 0.00601) (Getachew et al., 2002), where GV is total gas volume.

STATISTICAL ANALYSIS

Data on degradation parameters, gas production, gas production parameters, OMD, and SCFA were analyzed as a completely randomized design and subjected to a one-way analysis of variance. Significant differences between individual means were identified using Duncan's multiple range test (Snedecor and Cochran, 1980).

The used model was:

$Y_{ij} = \mu + T_i + e_{ij}$

Where µ = the common mean, T_i = the effect of treatments and e_{ij} = the random error.

RESULTS AND DISCUSSION

In situ rumen degradability

Data on degradation parameters for a dry matter of different treatments are presented in Table 3. In addition, the pattern of DM degradability of feeds content different treatments is shown in Figure 1. Results showed that the values of soluble degradable fractions (*a*) were significantly different (P<0.05) among treatments and it was highest in DCP and SBP treatments. Still, there were no significant differences among diets for the mean values of the insoluble degradable fraction (*b*) (P>0.05). Similarly, data indicated that there were no significant differences among treatments for fractional degradation rate (*c*) and potential degradability (*a*+*b*) (P>0.05). But, effective degradability was significantly different among treatments for all outflow rates, and it was highest for DCP and SBP treatments (P<0.0.5).

Table 3. Degradation parameters of different treatments

	Treatments[1]					
	BG	SBP	WB	DCP	SEM	*P*-value
Degradation parameters[2]						
a (mg/g)	275^{b}	289^{ab}	267^{b}	306^{a}	0.06	0.043
b (mg/g)	458	511	462	482	0.22	0.420
c ($\% \ h^{-1}$)	0.089	0.09	0.087	0.072	0.013	0.760
Potential Degradability (*a*+*b*) (mg/g)	733.9	801.4	729.4	790	19.7	0.126
Effective Degradability (%)						
Kp =0.02	64.97^{bc}	70.29^{a}	64.34^{c}	68.17^{ab}	0.894	0.026
Kp =0.03	61.83^{b}	66.71^{a}	61.13^{b}	64.43^{ab}	0.929	0.040
Kp =0.04	59.18^{b}	63.72^{a}	58.43^{b}	61.39^{ab}	1.056	0.075
Kp =0.05	56.90^{ab}	61.17^{a}	56.13^{b}	58.85^{ab}	0.177	0.120

[1]BG= barley grain, SBP= sugar beet pulp, WB= wheat bran, DCP= dried citrus pulp

[2]*a* and *b* represent soluble and non-soluble degradable fractions, respectively; *c* is the fractional degradation rate of the *b* fraction for DM, *Kp* is the passage rate.

Means along the same rows bearing different superscripts are significantly different (P < 0.05).

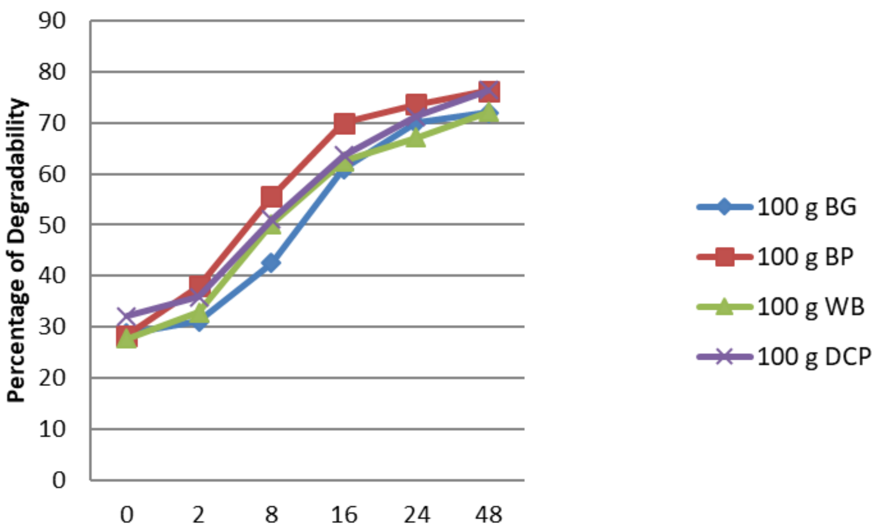

Figure 1. Pattern of DM degradability of feeds content different treatments (BG= barley grain, BP= sugar beet pulp, WB= wheat bran, DCP= dried citrus pulp).

As the data show (Table 3), the treatments containing DCP had the highest amount of *(a)* parameter, it possibly related to the higher NDSF content of this feedstuff as a soluble carbohydrate in compared with other diets, especially diet containing BG.

The DM and nutrients digestibility is related to the efficiency of feed use. The different published works showed that the citrus pulp offers high digestibility of DM and the main nutrients. Citrus pulp has a low neutral detergent fiber (NDF) content of 16%. In addition, its NDF includes a highly digestible compound, pectin, which, although a structural carbohydrate, is rapidly degradable in the rumen (Andrade et al., 2020).

Lashkari et al. (2017) investigated the digestibility of diets containing of 44% lucerne and 55.7% of the concentrate mixture composed citrus pulp (0, 223.7, 456.6 and 675.4 g/kg of DM) substituted for maize. They found that use of citrus pulp did not affect the digestibility of the DM, but citrus pulp inclusion levels promoted linear enhance in NDF digestibility, and had a quadratic effect for ADF. According to their study, the positive effect of substituting starch concentrates with feeds rich in easily degradable cell walls, such as citrus pulp, has generally been linked with a more appropriate ruminal environment for cellulolytic bacteria.

In another experiment with lambs, Tadayon et al. (2017) used citrus pulp to replace barley and maize grains, with inclusion levels in the diet at 0, 110 and 220 g/kg. They reported that the inclusion of dehydrated citrus pulp increased the digestibility of DM, OM, CP and NDF. The higher digestibility observed with the use of the citrus pulp may be associated to the greater digestibility of soluble carbohydrates of grains such as maize and barley (Miron et al. 2002). Moreover, pectin can promote a greater colonization of the particles, favoring its degradation in the rumen (Fondevila et al. 2002).

Voelker and Allen (2003) investigated the effect of substituting sugar beet pulp for high-moisture corn on the ruminal digestion of DM and OM. They reported that replacing highly digestible NDF for rapidly degraded starch in a high-concentrate diet did not reduce the amount of digested DM, but increased total diet digestibility by increasing NDF digestibility. Also, Sparkes et al. (2010) studied the effects of replacing Lucerne (*Medicago sativa* L.) hay with fresh citrus pulp on ruminal fermentation and ewe performance. They demonstrated that the replacement of 30% of a Lucerne diet with fresh citrus pulp improved *in vitro* dry matter digestibility.

In vitro gas production

Data on gas production in different times of incubation and gas production parameters, OMD, and SCFA are presented in Table 4. There were no significant differences among treatments for gas produced at different times of incubation (P>0.05). Potential gas production (*b*), and OMD did not differ among treatments (P>0.05). But, the fractional rate of gas production *(c)* was lowest for diet with DCP (P<0.05). Still, SCFA varied among various treatments (P<0.05), and it was higher for DCP treatment than others.

As the data show (Table 4), there was no dietary effect on the amount of gas produced at different times of incubation, OMD % and potential gas production fraction (*b*). The fractional rate of gas production *(c)* parameter of treatments was significantly different for a diet containing DCP compared with other treatments, and it was highest for diets containing BG and SBP. Perhaps this is due to the high level of pectin (NDSF) content in SBP and the high level of starch content in BG.

Garcia-Rodriguez et al. (2020) investigated the influences of replacing extruded maize (20% of total diet) by dried citrus pulp (20%) in a mixed diet on ruminal fermentation and methane production in rusitec fermenters. They showed some positive effects of citrus pulp on diet degradability and in vitro fermentation parameters. Garcia-Rodriguez et al. (2020) reported that fermenters receiving the DCP showed greater pH values and fiber disappearance and lower methane production than those fed extruded maize. Besides, in their study replacing extruded maize by DCP caused an increase in the proportions of propionate and butyrate and a decrease in acetate. Finally, they indicated that DCP can substitute extruded maize, promoting more efficient ruminal fermentation.

Table 4. Gas production parameters, estimated organic matter digestibility and short chain fatty acids

	Treatments[1]					
	BG	SBP	WB	DCP	SEM	*P*-value
Items[2]						
2h incubation	9.15	9.82	11.29	16.15	4.217	0.655
4h incubation	39.18	36.69	40.76	40.53	3.165	0.791
6h incubation	70.96	65.99	78.86	65.54	7.464	0.584
8h incubation	92.01	86.36	104.43	81.17	11.496	0.549
12h incubation	99.27	96.56	120.04	88.43	15.284	0.539
24h incubation	163.25	171.5	207.4	163.14	21.951	0.472
36h incubation	161.67	155.58	198.71	147.22	24.543	0.501
48h incubation	173.77	167.67	214.41	157.28	26.121	0.474
72h incubation	179.2	172.88	221.43	173.22	27.464	0.566
96h incubation	185.4	178.85	230.33	255.39	28.606	0.256
b	180.74	177.05	255.39	212.36	42.581	0.585
c	0.073[a]	0.075[a]	0.068[a]	0.043[b]	0.006	0.069
OMD (%)	52.78	53.85	52.44	51.89	0.718	0.383
SCFA (mmol/200mg DM)	0.82[b]	0.79[c]	0.76[d]	1.15[a]	0.006	<0.0001

[1]BG= barley grain, SBP= sugar beet pulp, WB= wheat bran, DCP= dried citrus pulp

[2] *b*: Potential gas production (ml/g DM); *c*: fractional rate of gas production (ml/h); OMD: Organic matter digestibility; SCFA: Short chain fatty acids

Sparkes et al. (2010) studied the effects of replacing Lucerne (*Medicago sativa* L.) hay with fresh citrus pulp on ruminal fermentation and ewe performance. They demonstrated that the replacement of 30% of a Lucerne diet with fresh citrus pulp increased total gas production.

In a study with growing lambs, Dhaked et al. (2002) reported that with replacement of 50% and 100% of maize grain with wheat bran in concentrate mixture, dry matter, digestible DM, organic matter, digestible OM, and total digestible nutrient was similar among treatments. Also, they found the digestibility (%) of all the nutrients and balances (g/day) of Ca, P and N were similar for all treatments. So, Dhaked et al. (2002) concluded that half of the maize grain can be safely and economically replaced with wheat bran from the concentrate mixture of growing lambs without any adverse effect on their performance and to support an 85–90g daily gain.

CONCLUSION

This study suggests that replacing low starch by-products, such as dried citrus pulp and sugar beet pulp, for up to 10% of diet DM instead of barley grain can improve the DM digestibility of a total mixed ration without any adverse effect on ruminal fermentation in ruminants. So, the use of these agricultural by-products would reduce the feed cost and the amount of human-edible ingredients used in the diet of ruminants.

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