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Influence of barley grain treated with alkaline compounds or organic extracts on *ex vivo* site and extent of digestion of starch

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Objective: Two e*x vivo* experiments were conducted to verify the effect of barley grain (*Nusrat cultivar*) treated with alkaline compounds (AC) including alum, ammonium, and sodium hydroxide or cation-exchanged organic extracts (OE) prepared from alfalfa hay, sugar beet pulp and *Ulva Fasciata*, on extent and digestion of starch.
Methods: In the first study, the *in vitro* first order disappearance kinetic parameters of dry matter (DM), crude protein (CP) and starch were estimated using a non-linear model ($D_{(t)} = D_{(i)} \cdot e^{(-k_d \cdot time)} + I$, where: $D_{(t)}$ = potentially digestible residues at any time, $D_{(i)}$ = potentially digestible fraction at any time, k_d = fractional rate constant of digestion (/h), I = indigestible fraction at any time). In the second experiment, the ruminal and post-ruminal disappearance of DM, CP, and starch were determined using *in situ* mobile nylon bag.
Results: Barley grains treated with alum and alfalfa extract had a higher constant rate of starch digestion (0.11 and 0.09/h) than others. Barley grain treated with OE had a higher constant rate of CP digestion and that of treated with AC had a higher constant rate of starch digestion (0.08 and 0.11/h) compared with those of the other treatments. The indigestible fraction of starch treated with alum and sugar beet pulp extract was higher than that of the control group (0.24 and 0.25 vs 0.21). Barley grain treated with AC and OE had significant CP disappearance in the rumen, post-rumen and total tract, and also starch disappearance for post-rumen and total tract compared with the untreated ($p<0.001$).
Conclusion: This study demonstrated that AC and OE might have positive effects on the starch degradation of the barley grain. In addition, treating barley grain with alum and sugar beet pulp extract could change the site and extend digestion of protein and starch.

Keywords: Alkaline Compounds; Barley Grain; First Order Kinetic; Nylon Bag; Organic Extract

INTRODUCTION

Cereal grains are the main ingredients of ruminant rations, but their rapid degradation seriously impairs rumen fermentation and the health of the host [1]. The most part of the digestible energy in cereal grains comes from starch. Starch digestion rate is momentous in attention to the venture of rumen acidosis, as a high rate of starch degradation in the rumen induces a severe fall in ruminal pH, which may diminish microbial protein synthesis, fibre digestion, and feed intake, and, in addition, negatively impact the metabolic condition of the animal [2]. Cereal starch can be an origin of glucose if a procedure of treating is applied, which decreases rumen degradation, thereby allowing it to be digested in the small intestine [3]. Therefore, the goals of grain processing include attempts to maximize total starch digestion in the animal, to optimize starch fermentation in the rumen to enhance volatile fatty acids yield while avoiding acidosis, and to advance starch availability in the small intestine [4,5]. Various processing methods have recently been applied to enhance the digestibility of barley, each of which requires special circumstances and different effects [6,1].

Because the starch digestion of the cereals in the rumen adversely affects the efficiency and health of the animal [7], so many methods including physical and chemical methods are applied to moderate its rumen disappearance rate [8,9]. Alkaline processing has been found to increase the digestibility of ruminant feeds [10]. This response to alkaline processing is due, in part, to solubilization of hemicellulose, as it is a major portion of the seed coat of most cereal grains [11]. However, information is still limited as to whether alkaline compounds (AC) can impact the rumen ecosystem when it is applied for barley grain processing. It has been proposed that alfalfa and sugar beet pulp are all nice sources of pectin having high cation exchange capacity (CEC). The CEC is a plant's ability to attract and bind hydrogen ions [12]. The CEC evaluates the ionized surface groups of fibre, which may affect the rate of adhesion of microorganisms to the fibre and, thus, the rate of digestion, and may also provide significant buffering capacity in the gastrointestinal tract [12]. When hydrogen ions are bound to a plant component rather than being free in the rumen, the rumen is less acidic [13]. High concentrations of free hydrogen ions in the rumen bring high rumen acidity. Hydrogen ions have a positive charge and plant cells usually have many negative charges on their surface and so, bind hydrogen ions. If the ions chemically adjoin the plant rather than being free, the rumen will become minor acidic and, so, feeding this group of grains (cereal) can prevent metabolical disorder in cows [14]. Thus, the microbes flourish faster and the rate of digestion of the plant cell is increased [14]. In ruminants, however, it is not obvious if this plant ability may apply to processing of cereal grain in view of starch digestion. In this study, assumed that organic treatment versus chemical manners of treating barley grain may alter the abundance or the metabolic activity of microbes in the rumen, most likely by changing the site and extension of the substrate availability. Therefore, the purpose of these experiments was to measure the effect of various chemical or organic treating on *in vitro* first order kinetic parameters of digestion and determining *in situ* mobile bag disappearance of the dry matter (DM), crude protein (CP), and starch of barley grain in the rumen, post-rumen, and total tract.

MATERIALS AND METHODS

Sample preparation and grain treatment

The barley grain used in this trial was *Nusrat* cultivar (temperate climate), obtained from the Seed and Plant Improvement Institute, Iran. The grain was treated with AC, including ammonium (liquid ammonia), sodium hydroxide (NaOH) and alum (double sulphate of aluminium and potassium) and organic extract (OE) with cation-exchanged capacity, obtained from sugar beet pulp (*Beta Vulgaris*), alfalfa hay (*Medicago Sativa*), and *Ulva Fasciata* (CEC = 565.5, 473.4, and 351 mmol hydrogen/kg neutral detergent fibre [NDF], respectively [15,12]). These plants were chosen due to high potential CEC in accordance with the study of McBurney [12]. Whole barley grain (DM = 890, starch = 620, and CP = 120 g/kg DM) was treated by soaking a known weight of grain in a specific volume of AC or OE solutions of appropriate concentration in airless bags for 30 days (sodium hydroxide and ammonium) or 48 h (alum and OE), following which the samples were dried at 65°C during 48 h using air-forced oven. In order to prepare OE, fifty grams of dried *Ulva Fasciata* or powdered sugar beet pulp soaked in 1 L of distilled water for 48 h at a speed of 120 RPM on a shaker. A sample (50 g) of powdered alfalfa hay was mixed with 1,000 mL of 70% ethanol and left for 10 minutes. The OE obtained by maceration method and filtered by Whatman filter paper and 25 mL of the extract solution sprayed on 100 g of barley grain and soaked in the laboratory flask (250 mL). In order to prepare AC, five grams of sodium hydroxide, 5 g pure alum and 5 mL ammonium dissolved in 100 mL distilled water for 100 g of whole barley grain and then stored in airless bags, and then opened and dried in the oven for 48 h at 65°C.

In vitro batch rumen culture

The anaerobic culture method that was utilized was the one illustrated by Dehority [16]. The fermentation medium was readied in accordance with Arroquy [17], including of 400 mL cell-free ruminal fluid, cellobiose (0.05 g), K_2HPO_4 (0.45 g), KH_2PO_4 (0.45 g), NaCl (0.90 g), $(NH_4)_2SO_4$ (0.90 g), $MgSO_4{\cdot}7H_2O$ (0.09 g), $CaCl_2$ (0.09 g), resazurin (0.01 g), $NaHCO_3$ (4 g), and cysteine-HCl (0.5 g) per litre of the medium. Rumen fluid was received from 3 Holstein lactating dairy cows fed a total mixed ration (including of 438 g/kg forage [DM basis]), contained 248 alfalfa hay and 190 corn silage (g/kg DM). The mixture of the concentrate (g/kg DM) was as follows: 233 barley grain, 138 wheat bran, 55 soybean meal, 87 cottonseed meal, 5 common salt, 7 sodium bicarbonate, 6 calcium carbonate and 9 mineral and vitamin supplement, strained through 4 layers of cheesecloth, and centrifuged at 3,000 RPM for 5 min. The supernatant was then centrifuged at 13,000 RPM for 15 min. Forty-five mL of the medium was transitioned into a 100 mL bottle, including the testable sample (450 mg of untreated or treated barley grain) and autoclaved in 120°C for 20 min. Then every bottle was inoculated with 5 mL of strained rumen liquid and finely bubbled with CO_2, sealed and incubated using a water bath (39°C). Prior to the inoculation, the rumen liquid was incubated for 1 h in an incubation chamber at 39°C to let large feed bits rise to the upside. In time, the inoculum, taking care neither to include the big particles that rose to the top nor that which sintered in the bottom, was introduced anaerobically into the fermentation flacon. The bottles were incubated for 4, 8, 12, 16, 24, and 48 h at 39°C, and then each bottle content was filtered through a 42 μm filter paper. The unfiltered content of each bottle was dried using the oven for 48 h at 60°C. Then DM, CP, and starch in the remaining ingredients were measured, and the disappearance was calculated.

In situ mobile nylon bag

Ruminal and post-ruminal digestion of DM, CP, and starch of

the samples were evaluated using the *in situ* mobile nylon bag techniques. Two Holstein steers (310 kg BW) with rumen fistula and T-shaped cannula in the proximal duodenum were used in the present study. Steers were fed 5.6 kg of DM of high-quality alfalfa hay, 1.3 kg of DM maize silage and 2.5 kg of DM concentrate (CP = 17 g/kg DM) per day. Bags were suspended in the rumen in a polyester mesh bag (9×17 cm; 50 μm pore size), which was filled with 6 g of dry ground samples and each feed sample was incubated in 6 replicates (3 replicates for each steer) in the rumen for 12 h. After that time, bags removed from the rumen and placed in a conventional washing machine; then the content of each bag was dried in an oven at 55°C until a constant weight was achieved before designation of DM disappearance [18]. After that, 1 g of each sample was placed in a nylon bag (3×6 cm; 52 μm pore size; 6 bags per sample). The bags were inserted for every 30 min into the duodenum through the T-shaped cannulas. Bags dropped within faeces were collected, washed until the water remained clear and then dried (60°C, 48 h). The content of each bag was analysed for DM, CP, and starch. The content of sample residuals, replicates within steers was pooled and DM, CP, and starch disappearance measured.

Chemical composition

Barley grains were milled to pass through a 1 mm Retsch Muhle mill (Retsch EPP 15×20, Germany), and dried using an air-forced oven at 60°C for 48 h. Nitrogen value was characterized using the Kjeldahl procedure (Kjeltec 2300 Autoanalyser, Foss Tecator AB, Hoganas, Sweden) and CP was estimated as N×6.25. Starch was determined by an anthrone/sulphuric acid method using glucose as standard and estimated as 0.9×glucose content [19]. The NDF (measured without alpha amylase and sodium sulphite) and acid detergent fibre (ADF; measured without alpha amylase and sodium sulphite). Samples were also analysed for ether extract and ash concentrations [20].

Statistical analysis

First order parameters of DM, CP, and starch disappearance of untreated or treated barley grain were estimated using an exponential model. The model was: $D_{(t)} = D_{(i)} \cdot e^{(-k_d \cdot time)} + I$, where: $D_{(t)}$ = potentially digestible residues at any time, $D_{(i)}$ = potentially digestible fraction at any time, k_d = fractional rate constant of digestion (/h), I = indigestible fraction at any time. The percentage disappearance of the DM, CP, and starch at 12 h incubation in the rumen was estimated as the difference between the primary sample and the part remaining after incubation in the rumen. Disappearance in the intestinal tract was calculated by the difference between the rumen residue after 12 h of incubation and the part remaining in samples recovered from faeces. Data were statistically analysed using a complete randomized design model as $Y_{ij} = \mu + T_i + \varepsilon_{ij}$, where Y is the analysed variable, μ is the overall mean, T_i is the effect of the barley grain processing (i = 1…7) and ε_{ij} is experimental error. Differences between samples in rumen, intestine and total tract disappearance of DM, CP, and starch were analysed using the MIXED procedure of SAS [21], with Duncan's multiple range test used for the comparison of means at $p<0.05$.

RESULTS AND DISCUSSION

Chemical composition

Chemical composition (g/kg DM) of the untreated and treated barley grain is presented in Table 1. Results showed that CP content was affected by treating with AC and OE and was lower for control group compared with those of the others ($p<0.05$). Starch content was higher for *Ulva Fasciata* rather than those of the other kinds of treatment ($p<0.05$). In barley grain, starch exists as granules encapsulated in a protein matrix, which can react with AC or OE. Thus, the differences in starch contents might also be caused by differences in effects of AC or OE on the protein matrix of the endosperm tissue. There was no difference among DM, NDF, and ADF.

First order parameters

The average fraction of DM disappearance is shown in Table 2. There was no significant difference between DM disappearances

Table 1. Chemical composition (g/kg dry matter) of untreated or treated barley grain with AC or OE

Parameters	CON[1)]	Treatments[2)]					
		AC			OE		
		AM	SH	AU	SBP ext.	AH ext.	ULF ext.
Dry matter	890	886.7	887.4	886.3	889	890.8	887.9
Crude protein	120[c]	124[ab]	127.3[a]	123.8[ab]	119.5[c]	127.2[a]	123.2[ab]
Starch	620.3[ab]	611.9[b]	619[ab]	610.7[b]	619.8[ab]	618[ab]	624.2[a]
Neutral detergent fiber	221.1	226.5	221.9	224	222	221.5	223.2
Acid detergent fiber	80	84.5	82.2	83.3	81.7	84.1	83.1
Ash	20.3	21.6	21	21.5	20.1	20.9	21.8
Ether extract	19	19.4	20.3	20	19.1	20.5	21

[1)] CON, control group.
[2)] AC, alkaline compounds; AM, ammonium; SH, sodium hydroxide; AU, alum; OE, organic extract; SBP, sugar beet pulp; AH, alfalfa hay; ULF, *Ulva Fasciata* extracts.
[a,b,c] Means within each row with differing superscripts are significantly different ($p<0.05$).

Table 2. *In vitro* first order ruminal disappearance kinetics of dry matter, crude protein and starch in untreated or treated barley grain with AC or OE

Parameters[1]	CON[2]	Treatments[3]						SEM	Contrast[4]	
		AC			OE					
		AU	SH	AM	AH ext.	SBP ext.	UlF ext.		AC vs OE	U vs T
Dry matter										
D	0.38	0.64	0.54	0.62	0.56	0.62	0.62	0.02	NS	NS
I	0.19	0.21	0.19	0.17	0.23	0.17	0.19	0.01	NS	NS
K_d	0.06	0.05	0.08	0.09	0.07	0.07	0.05	0.004	NS	NS
Crude protein										
D	0.61	0.60	0.66	0.62	0.70	0.69	0.03	0.31	NS	NS
I	0.22	0.32	0.19	0.22	0.17	0.16	0.02	0.69	NS	NS
K_d	0.06^{ab}	0.03^{c}	0.08^{a}	0.08^{a}	0.08^{a}	0.07^{ab}	0.07^{ab}	0.01	*	NS
Starch										
D	0.69	0. 70	0.67	0.68	0.67	0.70	0.66	0.008	NS	NS
I	0.21	0.22	0.18	0.24	0.25	0.20	0.22	0.09	NS	NS
K_d	0.07^{c}	0.06^{c}	0.08^{bc}	0.11^{a}	0.04^{d}	0.09^{bc}	0.07^{c}	0.006	*	NS

SEM, standard error of the means.
[1] D, potentially digestible fraction; K_d, fractional rate constant of digestion; I, indigestible fraction.
[2] CON, control group.
[3] AC, alkaline compounds; OE, organic extract; AU, alum; SH, sodium hydroxide; AM, ammonium; AH, alfalfa hay; SBP, sugar beet pulp; ULF, *Ulva Fasciata* extracts.
[4] U, untreated; T, treated; NS, non significant.

of treated barley grain with AC or OE in the comparisons made among the seven treatments (p>0.05) evaluated under the present study conditions. The fractional rate constant of DM digestion of the untreated or treated barley grain ranged from 0.05 to 0.09 with a mean of 0.07 (Table 2). As seen in Table 2, the fractional rate constant of CP disappearance demonstrated an increase (p< 0.05) among treatments (sodium hydroxide, alum, and sugar beet pulp extract). The *in vitro* first order parameters of the starch disappearance of treated barley grain with AC or OE are presented in Table 2. The fractional rate constant of starch digestion was affected by treating and it was higher (p<0.05) for alfalfa hay extract and alum than those of the other groups (0.09 and 0.11 vs 0.07/h). The fractional rate constant of starch digestion of treated barley grain increased (4%) by alum and (2%) by alfalfa hay extract treatment, than those of the other treatings. Indigestible fraction for starch was higher for barley grain treated with alum and sugar beet pulp extract (0.24 and 0.25/starch) than those of the others. Abdi [22] also reported that processing barley grain with the AC influenced the starch degradation. These results suggested that barley grain treated with alum and sugar beet pulp extracts could change the site and extent of starch digestion from the rumen to intestine. The reason why and how the processing with AC or OE affect starch degradation is complicated to illustrate, but several possibilities can be discussed. The change in proportion between soluble and insoluble β-glucans could be a description for the decrease in starch degradation with processing. It is proposed that the increase in soluble β-glucans practically leads to increased viscosity even by using *in vitro* method, thereby slowing down the starch degradation [23]. Another paraphrase could be related to the protein, as the content increases with these treatments. Different amounts of NDF in the barley grain treatments may cause higher digestion rates for alfalfa hay extract and alum than other treatments or control group. Stevnebo [3] also reported that the NDF fraction directly or indirectly has a clear impact on the ruminal digestion rate of starch barley grain. In summary, it appears that the fractional rate constant of digestion of treated barley grain is positively correlated with chemical treating. The differences in indigestible fraction among treatments may be associated with different structures of starch and protein matrix of the endosperm [24]. It is possible that the protein matrix encompassing starch granules is the main factor responsible for differences in the ruminal starch digestion of cereal grains and can be treated with the above features for breakdown protein matrix of starch granules in barley grain. Lanzas [4] showed that correlation between starch content and the fractional rate constant of digestion were poor (R^2 = 0.12). The findings of this experiment confirm the report of Stevnebo [3], who obtained similar results.

In situ mobile nylon bag

Parameters of ruminal, post-ruminal and total tract DM, CP, and starch disappearance of untreated or treated barley grain with AC or OE are shown in Table 3. During incubation in the rumen and post-rumen, there was no significant difference in DM degradabilities among the seven treatments. The results of this study support the findings of Dehghan Banadaky [25], who reported no difference in ruminal DM disappearances for barley grains when treated with AC, but observed conflict with post-ruminal DM disappearance. However, since this study was conducted only on alkaline processing, no conclusion can be drawn about the OE treating. There was a significant difference (p<0.05) in post-ruminal and total tract CP and starch disappearance of treated barley

Table 3. Disappearance of dry matter, crude protein and starch in untreated or treated barley grain using with *in situ* mobile nylon bag

Parameters	CON[1]	Treatments[2]						SEM	Contrast[3]	
		AC			OE					
		AU	SH	AM	AH ext.	SBP ext.	UlF ext.		AC vs OE	U vs T
Ruminal										
DM	0.83	0.83	0.82	0.84	0.83	0.85	0.84	0.05	NS	NS
CP	0.83^{bc}	0.82^{c}	0.81^{c}	0.87^{ab}	0.87^{a}	0.82^{bc}	0.89^{a}	0. 07	NS	NS
Starch	0.93	0.92	0.93	0.92	0.92	0.93	0.92	0.04	NS	NS
Post-ruminal										
DM	0.33	0.29	0.29	0.32	0.30	0.29	0.26	0.04	NS	NS
CP	0.38^{e}	0.76^{a}	0.55^{cd}	0.64^{bc}	0.53^{d}	0.68^{ab}	0.63^{bc}	0.09	NS	*
Starch	0.48^{c}	0.75^{a}	0.64^{c}	0.64^{b}	0.49^{c}	0.72^{a}	0.41^{c}	0.07	*	*
Total tract										
DM	0.89	0.88	0.88	0.89	0.88	0.89	0.88	0.01	NS	NS
CP	0.89^{c}	0.96^{a}	0.92^{bc}	0.95^{ab}	0.93^{bc}	0.97^{a}	0.96^{a}	0.02	NS	*
Starch	0.93^{b}	0.99^{a}	0.96^{ab}	0.95^{ab}	0.94^{b}	0.97^{a}	0.94^{b}	0.06	NS	*

SEM: standard error of the means; DM, dry matter; CP, crude protein.
[1] CON, control group.
[2] AC, alkaline compounds; OE, organic extract; AU, alum; SH, sodium hydroxide; AM, ammonium; AH, alfalfa hay; SBP, sugar beet pulp; ULF, *Ulva Fasciata* extracts.
[3] U, untreated; T, treated; NS, non significant; * significant at $p < 0.05$.
[a,b,c,d] Means within each row with differing superscripts are significantly different.

grain. Ruminal CP disappearance of treated barley grain with *Ulva Fasciata* extract was higher than those of the other groups. Treated barley grain with alum and sugar beet pulp extract had a higher post-ruminal and total tract CP and starch disappearance compared with other treatments when the *in situ* mobile nylon bag technique was used ($p<0.05$). Consequently, treating can be affected by protein disappearance, but is in conflict with the results of Dehghan Banadaky [25], who reported that barley grain control had a higher ruminal CP disappearance versus barley processing with AC. In that perusal, barley grain treated with sodium hydroxide had a higher value of post-ruminal CP disappearance. Treated barley grain with NaOH disrupts the seed coat by partial hydrolysis of hemicellulose and lignin. However, in the present experiment, alum, and sugar beet pulp extract has a higher post-ruminal CP disappearance than those of the other treatments. The highest post-ruminal CP (0.76, 0.68) and starch (0.75, 0.72) disappearance were observed in barley grain treated with alum and sugar beet pulp extract, respectively. The findings in this study support the results of Dehghan Banadaky [25], who noted intestinal and post abomasal starch disappearance were higher in chemically treated barley grain. This can be explained: in barley grain, a protein matrix surrounds the starch granules and ruminal pre-incubation increases intestinal digestibility of barley protein. Thus, ruminal pre-incubation promotes digestion of protein matrix and exposes the starch granules to enzymatic digestion in the small intestine [26]. This experiment supports Robinson [27], who reported increase in the flow of starch into the duodenum and reduction in its breakdown in the rumen as a result of chemical processing of barley grain. The total tract CP disappearance of treated barley grain with alum and sugar beet pulp extract were higher ($p<0.05$) than those of the other groups (0.96, 0.97), as shown in Table 3. It was also observed that the total tract CP disappearance was lower for the control group. The total tract starch disappearance of barley grain treated with alum and sugar beet pulp extract were higher than those of the other treatments ($p<0.001$). Digestibility of starch is generally inversely proportional to its amylose content because the amylose action is in the amorphous sections of the amylopectin [28]. The rumen degradation rate of starch is influenced by a number of interactions between kernel tissues and rumen microorganisms [24]. In barley grain, the protective effect of starch obtained with formaldehyde was related to the lower numbers of attachment sites for amylolytic microorganisms due to the strengthening of the protein matrix [25]. Perhaps supporting the view that chemical treating affects the extent of starch degradation by making the protein matrix more resistant to proteolysis [29], chemical reactions between starch molecules within the granules which is chemical treating may be another paraphrase for the decreased rumen degradation of starch (RDS) in barley grain. Comparisons between treated barley grain with AC and OE and other treatments that compared for the ruminal DM, CP, and starch disappearance was not a significant difference with the control group ($p>0.05$). A significant difference was found for the ruminal starch disappearance between AC and OE and chemical treating with other treatments and control group ($p<0.05$). Also, significant differences ($p<0.05$) were observed in the total tract CP and starch disappearance of the control group with various kinds of chemical treating. So we can expect that treating of barley grain with AC or OE can be prepared by the reduction of free hydrogen ions in the rumen that those bounded by the surface of feeds and prevent declining ruminal pH and, thereby reducing amylolytic bacteria [12]. This type of treating not only increases the flow of starch

into the duodenum but also prevent acidosis in dairy cows that consume more starch for supply energy. These findings suggested that chemical treating, especially when using alum and sugar beet pulp extract, influenced the amount of starch available in the intestinal tract. Moreover, our study supported the previous results by slowing down the ruminal degradation of starch from treated barley grain. Indeed, an increased starch disappearance in the small intestine may improve the efficiency of feed consumption by reducing waste of energy as methane [25].

CONCLUSION

By examining the results of this research, it may be concluded that chemical treating increases starch digestibility and may also alter the site and extent of starch digestion, but must not harmfully affect ruminal pH and cause digestive dysfunction.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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