



## Effect of Treated Barley Grain with Sodium Hydroxide, Urea and Formaldehyde on Degradability of Crude Protein Using in situ

Ahmad Ahmadi<sup>1,\*</sup>, Mehdi Moghaddam<sup>2</sup>, Akbar Taghizadeh<sup>3</sup> and Alireza Safamehr<sup>4</sup>

<sup>1\*</sup>Department of Animal Sciences, Faculty of Agriculture, Maragheh Branch, Islamic Azad university, Maragheh, Iran

<sup>2</sup>Department of Animal Science, Faculty of Agriculture, Science and Research Branch, Islamic Azad university, Tehran, Iran

<sup>3</sup>Department of Animal Sciences, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

<sup>4</sup>Department of Animal Sciences, Faculty of Agriculture, Maragheh Branch, Islamic Azad university, Maragheh, Iran

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#### Corresponding Author:

Ahmad Ahmadi  
vahmadi2010@yahoo.com

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### ABSTRACT

The present study was carried out to determine the influence of treated barley grain with sodium hydroxide, urea and formaldehyde on degradability of crud protein and dry matter using in situ technique in Gize sheep. Two fistulated sheep with average BW  $45 \pm 2.5$  kg were used in a complete randomized design. The ruminal dry matter (DM) and crud protein (CP) disappearance was measured at 2, 4, 6, 8, 12, 16, 24, 36 and 48 h. The experimental treatments were A: barley grain treated with 3.5% sodium hydroxide and 1.5% urea, B: barley grain treated with 3.5% sodium hydroxide and 0.4% formaldehyde, C: barley grain treated with 3.5% urea and 0.4% formaldehyde. Parameters of crud protein (CP) for soluble fractions were (a) 41.063, 9.58 and 45.9% and fermentable fractions were (b) 51.16, 85.13 and 51.77% for treatments A, B and C, respectively. According to the survey results, it is clear that grain treated with chemical digestion has high feed potential and if further investigation in ruminant diets can be used as an alternative feed.

**Key words:** Barley grain, Formaldehyde, In-situ, Sodium hydroxide, Urea.

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## INTRODUCTION

Barley is a cheap cereal which is used in many countries, especially in European countries, to reduce production costs (Sharifi *et al.*, 2005). Barley grain is a widely used source of energy and easily digestible carbohydrates in ruminants particularly cattle diets (Yang *et al.*, 2000). The average crude protein content of barley grain is about 12% dry matter which is of low quality and lacks the lysine (Soufisiavash and Janmohammadi, 2004). Despite the low percentage of protein, due to the large amount of this substance in the diet, it provides a substantial portion in diet. Due to the high rate of solubility and the protein composition of barley grain, its degradability is so

high that increases the rate and extent of rumen fermentation of carbohydrates, bloating, acidosis, lameness, and hepatic disorders (Yang *et al.*, 2000). The process of grain can be effective in reducing the rate and extent of ruminal degradation of dry matter and protein, and decreasing metabolic disorders (Dehghan Banadaky *et al.*, 2007b). Various techniques have been developed for processing grains and feed ingredients to increase the efficiency of high cereal diets to improve palatability. Therefore, to improve animal performance, processed cereal grains can affect digestibility, rate, and site of digestion (McNiven *et al.*, 1995). Several processing methods are known to increase the efficiency of digestion and nutritional value of grains for animals (Rowe *et al.*, 1999). However, due to the high cost of physical and mechanical processing, more attention is needed to review the use of chemical methods in improving the nutritional quality of cereals in animal food (Dehghan Banadaky *et al.*, 2007b). Chemical treatments including hydroxides are used to reduce the resistance of the seed coat and formaldehyde is used to reduce the microbial digestion of protein. Formaldehyde is a chemical treatment of barley that reduced ruminal degradability of crude protein and starch grains (Ahmadi, 2011). To obtain the best performance of the seeds, their digestibility should be determined (Rowe *et al.*, 1999). Fermentation pattern and digestion sites can have a significant effect on the nature of available nutrients in animals (Dehghan Banadaky *et al.*, 2007a). Also, due to the limited sources of energy and fuel, high cost of physical and mechanical processing cereal grain, it is necessary to review the chemical methods to improve the nutritional quality of cereals in ruminants (Dehghan Banadaky *et al.*, 2007a). Therefore, the purpose of this study was to investigate the effect of processing grain with urea, sodium hydroxide, and formaldehyde on degradability of protein and optimum use of processing chemicals using nylon bags technique.

## MATERIALS AND METHODS

### Barley grain collection

Barley grain was obtained from *Ajhdar* local variety of department of agriculture of Malekan, Iran. The experimental treatments were A: barley grain treated with 3.5% sodium hydroxide and 1.5% urea, B: barley grain treated with 3.5% sodium hydroxide and 0.4% formaldehyde, C: barley grain treated with 3.5% urea and 0.4% formaldehyde that were prepared in the laboratory. Besides, 3 parts of solution and 1 part of barley were mixed in plastic containers and were kept in room temperature and away from sunlight for 60 days; samples were taken out of the containers and dried in the sunlight and milled in a 2 mm size to be used in other phases of the experiment. Animals used in this experiment were fed at maintenance level. The animals were fed with a mixture of 60% forage and 40% concentrate diet (Ørskov and McDonald, 1979).

### Chemical composition

Feedstuffs dry matter (DM, method ID 934.01), ash (method ID 942.05), ether extract (EE, method ID 920.30), and crude protein (CP, method ID 984.13) were determined by procedures of AOAC (1999). The neutral detergent insoluble fiber (NDF) and acid detergent fiber (ADF) concentrations were determined using the methods of Van Soest *et al.* (1991), without sodium sulphite. Neutral detergent insoluble fiber was analyzed without amylase with ash included.

### Measured in situ

To estimate the degradability of the nylon bag technique, the food samples were milled with a special mill and 2-mm sieve (Moghaddam *et al.*, 2012). 5 grams of each nutrient were poured into bags made of synthetic polyester fiber as  $6 \times 12$  cm and pore diameter of 50 mm. Two fistulated sheep with average BW  $45 \pm 2.5$  kg were used in a complete randomized design To

determine the degradation at time zero, sample bags were washed under tap water for 15 minutes. Incubation times were 0, 2, 4, 6, 8, 12, 16, 24, 36 and 48 h. After each incubation time, the bags were removed and rinsed with cold water until the water is completely cleared out. After washing, bags were incubated for 24 h at a temperature of 65 ° C to evaporate and for 24 h at 105 °C in oven (Moghaddam *et al.*, 2012). Degradation parameters (soluble, insoluble, and fixed rate of degradation) were calculated with Naway. For matched degradation data used from  $P=a+b(1-e^{-ct})$  that a= The degradation of soluble fraction (%), b= The degradation rate of insoluble fraction (%), c= The constant degradation rate (%/h), t=The incubation time (h), e= The constant factor (2.718) and P= The degradation rate at the time t. Effective degradability was calculated at  $ED=a+(b \times c)/(c+k)$  that k is passage rate which were considered in this study 0.02.

#### Statistical analysis

The obtained data from in situ study was analyzed according to a completely randomized design with 4 replicates by the GLM procedure (SAS, 2002). The treatment means were compared by the Duncan test.

## RESULT AND DISCUSSION

The chemical composition of treatments is presented in Table 1. The data show that treatment A had the most (95.5%) and treatment B had the least (94.5%) amount of dry matter which were in agreement with the findings of Parand and Taghizadeh (2009), for barley grain (93.8%) and Nikkhah *et al.*, (2007), for Barley grain processed with 3.5% hydroxide sodium (88.6%) ( $P<0.05$ ). Regarding the percentage of crude protein treatment C (16.31%) and treatment B (9.66%) had the highest and the lowest amount of crude protein. The findings of this study in this regard are in line with the findings of Nikkhah *et al.*, (2007), for barley treated with 3.5% urea (15.01%) and barley treated with 3.5% hydroxide sodium (10.51%). However, the findings of the present study differed from the data reported by Taghizadeh and Nemati (2008), (11.56%), Taghizadeh *et al.*, (2003), (10.5%) and Yang *et al.*, (2000), (13.5%) for barley grain. This difference can be attributed to processing barley with urea, NaOH, and formaldehyde in this study. Significant differences were observed among treatments of crude protein in this study ( $P<0.05$ ). These differences are pertained to the used urea for per 1.5% used urea solution about 3% is added to the crude protein in grain. According to table 1, there were significant differences in dry matter, crude protein, acid detergent fiber, and neutral detergent fiber in tested food ( $P<0.05$ ). There were also differences between the amounts of acid detergent fiber, neutral detergent fiber, crude protein, and neutral detergent fiber obtained in this study and the NRC (2001). These differences can be attributed to the effects of the treatments.

**Table 1. The chemical composition of feeds (% DM)\***

Treatments	DM	CP	NDF	ADF	ADIN
A	95.5 <sup>a</sup>	12.9 <sup>b</sup>	36.6 <sup>a</sup>	8.1 <sup>b</sup>	1 <sup>b</sup>
B	94.5 <sup>c</sup>	9.66 <sup>c</sup>	35.5 <sup>b</sup>	10.1 <sup>a</sup>	1.2 <sup>a</sup>
C	95.1 <sup>b</sup>	16.31 <sup>a</sup>	35.9 <sup>b</sup>	6 <sup>c</sup>	0.75 <sup>c</sup>
SEM	0.1105	0.04055	0.1972	0.1914	0.03785

\*DM=dry matter, CP=crude protein, NDF=neutral detergent fibre, ADF=acid detergent fibre, ADIN=acid detergent insoluble nitrogen.

a,b,c Within a column, means without a common superscript letter differ ( $P<0.05$ ).

\*\*Standard error means of the difference amount three treatments means.

According to the results reported in tables 2 and 3 at different times of incubation, treatments A and C are the highest and lowest DM disappearance values, respectively. Also, according to

the results obtained at 0 h of incubation, treatment C (15.41%) had the lowest and treatment A (23.52%) had the highest rate of dry matter disappearance that there were significant differences in all treatments ( $P < 0.05$ ). Taghizadeh *et al.*, (2001), reported (10.6%) of barley grain ruminal DM disappearance at 0 h. Taghizadeh and Nemati (2008), reported the rate of DM disappearance in unprocessed barley (18.145%).

**Table 2. Means of dry matter degradation of feeds by incubation at different times in the in situ method (% DM)**

Treatment	Incubation times (h)								
	0	2	4	6	8	16	24	36	48
A	23.52 <sup>a</sup>	32.62 <sup>a</sup>	45.79 <sup>a</sup>	54.66 <sup>a</sup>	69.93 <sup>a</sup>	83.97 <sup>a</sup>	94.55 <sup>a</sup>	95.89 <sup>a</sup>	96.64 <sup>a</sup>
B	20.24 <sup>b</sup>	32.23 <sup>a</sup>	36.96 <sup>b</sup>	47.2 <sup>a</sup>	69.54 <sup>a</sup>	83.94 <sup>a</sup>	86.66 <sup>b</sup>	95.56 <sup>a</sup>	95.65 <sup>a</sup>
C	15.41 <sup>c</sup>	24.35 <sup>b</sup>	36.6 <sup>b</sup>	41.93 <sup>a</sup>	69.01 <sup>a</sup>	80.64 <sup>b</sup>	83.33 <sup>c</sup>	88.8 <sup>b</sup>	90 <sup>b</sup>
SEM**	0.5458	0.6283	0.5777	0.4138	1.0255	1.0177	0.6059	0.2186	0.2421

<sup>a,b,c</sup> Within a column, means without a common superscript letter differ ( $P < 0.05$ ).  
 \*\*Standard error means of the difference amount three treatments means.

Also, showed significant differences in the incubation time 0 h among unprocessed barley grains, processed by autoclaving at 120 °C for 5 min and 20 min, and treated at 100 °C for 5 min and 20 min for the disappearance of DM, respectively. The differences between the results of the experiments can be attributed to the plant species, climatic conditions of the region, conditions of grain growth, and other environmental conditions. Since the DM is a mixture of crude protein, fat, carbohydrates, and vitamins and the tested foods treated with formaldehyde were different with regard to these nutrients, therefore the reduced ruminal degradation of grain starch and crude protein is not due to the toxicity effects of formaldehyde on microorganisms in the rumen, but rather it is due to the methylene crosslinking proteins in the barley field that reduced the sensitivity of microbial degradation of barley protein and microorganisms access to starch. Consequently, it increases the delay phase in rumen degradation of protein and starch (Dehghan Benadaki *et al.*, 2007a).

**Table 3. The parameters estimated from the dry matter degradability coefficients of feeds**

Treatments	Degradation coefficients			ED	RSD
	a	b	c		
A	20.94 <sup>a</sup>	77.62 <sup>b</sup>	0.099 <sup>a</sup>	84.13 <sup>a</sup>	4.99 <sup>b</sup>
B	10.54 <sup>c</sup>	89.28 <sup>a</sup>	0.098 <sup>a</sup>	84.67 <sup>a</sup>	6.99 <sup>a</sup>
C	17.97 <sup>b</sup>	73.23 <sup>c</sup>	0.102 <sup>a</sup>	79.23 <sup>b</sup>	6.55 <sup>a</sup>
SEM**	0.6924	0.7944	0.0213	0.179	0.1926

a=Dry matter solution at zero time (%), b=Fermentable material (%), c=Constant degradability coefficients at time t (%/h), ED=Effective degradation (The passage of time  $t=0.02$ ), RSD= Residual standard deviation.

<sup>a,b,c</sup> Within a column, means without a common superscript letter differ ( $P < 0.05$ ).

\*\*Standard error means of the difference amount three treatments means.

The findings of the present study indicated that treatment A had the highest DM degradation in all hours of incubation which can be caused by urea and sodium hydroxide effects. In addition, a large amount of DM degradation can be as a result of high levels of NDF and ADF of this grain. Treatments a (20.94%) and B (10.54%) had the highest and lowest a coefficient value for DM, respectively, that due to the high solubility of urea, these results are predictable and justifiable. Taghizadeh and Nemati (2008), reported DM solution at zero time (coefficient a)

value of (19.5%) for unprocessed barley grain that regardless of the treatment effect is consistent with the results of the present experiments. Also, Taghizadeh *et al.*, (2001), reported a coefficient value for barley (14%). Treatments B (89.28%) and C (73.23%), had the highest and lowest fermentable material (coefficient b), respectively. Taghizadeh and Nemati (2008), reported coefficient b value for barley (78%) that are similar to the values obtained for treatment A in this study. The results reported in this study revealed that the coefficients a and b indicated significant differences among treatments which were due to the treatment effects ( $P < 0.05$ ). Barley treated with NaOH improves fiber digestibility and reduces fluctuations in rumen pH and rumen degradability of starch and nitrogen (McNiven *et al.*, 1995).

Means of the data presented in tables 4 and 5 show that in zero-hour of incubation, treatments A (49.98%) and C (10.01%) had the highest and lowest rumen CP disappearance ( $P < 0.05$ ). This difference can be due to processing barley with urea that is highly soluble and hydroxide sodium which decreases ruminal pH fluctuations, and therefore in increases proteolytic activity of the rumen microorganisms that it has caused higher CP degradation. Considering the high solubility of urea and the effect of formaldehyde fixation properties, these findings are reasonable. Taghizadeh *et al.*, (2001), reported untreated barley grain CP degradation of (5.85%) in 0 h incubation. A part from the effects of animals, plant species, and environmental conditions, and because of processing the grain with the chemicals, these differences in CP disappearing are justifiable. The data obtained from this study showed that treatments A (96.2%) highest and C (97.87%) lowest CP disappearance in 48 h incubation. Taghizade *et al.*, (1380), and Taghizadeh and Nemati (2007), found unprocessed barley grain CP degradation in 48 h incubation about (68%) and (59.59%), respectively.

**Table 4. Means of crude protein degradation of feeds by incubation at different times in the in situ method (% DM)**

Treatment	Incubation times (h)								
	0	2	4	6	8	16	24	36	48
A	49.98 <sup>a</sup>	52.94 <sup>a</sup>	65.21 <sup>a</sup>	71.84 <sup>a</sup>	79.67 <sup>a</sup>	83.19 <sup>a</sup>	90.15 <sup>a</sup>	94.10 <sup>a</sup>	96.2 <sup>a</sup>
B	40.93 <sup>b</sup>	51.36 <sup>a</sup>	54.55 <sup>b</sup>	55.13 <sup>b</sup>	77.05 <sup>ab</sup>	82.85 <sup>a</sup>	86.97 <sup>b</sup>	92.95 <sup>b</sup>	95.2 <sup>b</sup>
C	10.01 <sup>c</sup>	27.79 <sup>b</sup>	41.91 <sup>c</sup>	44.66 <sup>c</sup>	74.53 <sup>b</sup>	77.35 <sup>b</sup>	86.10 <sup>b</sup>	92.77 <sup>b</sup>	94.87 <sup>b</sup>
SEM**	0.4948	0.7451	0.3757	4.7580	0.7856	0.6135	0.7427	0.3255	0.3289

<sup>a,b,c</sup> Within a column, means without a common superscript letter differ ( $P < 0.05$ ).

\*\*Standard error means of the difference amount three treatments means.

These differences can be attributed to plant species, animal effect, conditions for plant growth, environmental conditions and treatments effects. Since the proportion of rumen ammonia nitrogen and urea nitrogen transfer rates correlate negatively with the rumen, therefore the change in the proportion of dietary nitrogen digested in the rumen can alter the rumen and urea nitrogen recycle and this effect can be extensively increased by processing barley. It can also increase ruminal starch digestion and subsequent nitrogen recycle and urea nitrogen microbial degradation to rumen (Kiran and Mutsvangwa, 2007). In cereal starch granules are protected by a combination of protein which can combine with aldehydes, form covalent bonds through the amino group of amino acids and this protective protein keeps starch granules from being degraded by microorganisms. Therefore, treating barley with formaldehyde can reduce rumen degradation of starch. As a result of this, reproduction and growth rate of microorganisms is delayed and consequently proteolytic activity gets limited and the rate of protein degradation does not increase (Row *et al.*, 1999).

Crud protein degradability coefficients of the treatments presented in table 5 show that coefficient a had the highest and lowest values for treatments C (45.9%) and B (9.58%), respectively ( $P < 0.05$ ). These results were predictable due to the high solubility of urea. Treatments B (85.13%) and A (51.16%) had the highest and lowest coefficient b that were not significantly different, but the high difference between treatment B and other treatments can be due to the hydroxide OH factor that causes the destruction of protective starch protein granules and the penetration of microorganisms into the feed particles and facilitates digestion (Row *et al.*, 1999). With urea, microorganisms can readily get the needed nitrogen from urea and the feed protein is preserved, but due to increased late stage of protein and starch breakdown in the rumen with the presence of formaldehyde, causes the protein fermentation during over time in the rumen (Navidshad and Jafari Sayadi, 2000). It has been reported that the low nutritional value of this product is due to the presence of trypsin, beta-glucan and phytase as anti-nutritional factors.. These data are less than results of the study done by Taghizadeh and Nemati (2008), for the coefficient b (65%) for unprocessed grain. Chemical treatments included hydroxides to reduce the resistance of the seed coat and formaldehyde consumption to reduce microbial digestion of proteins. Also formaldehyde can be bonded with a protein and Inhibit invasion microorganisms to protein degradation. Endosperm contains starch granules which is surrounded with a combination of protein and Non-starch polysaccharides, using the solubility properties of proteins can be attached to the endosperm in such glutelins which are soluble in alkanes and alcohols, with the addition of chemical compounds eliminated the protective protein (Row *et al.*, 1999).

**Table 5. The parameters estimated from the crude protein degradability coefficients of feeds**

Treatments	Degradation coefficients			ED	RSD
	a	b	c		
A	41.063 <sup>b</sup>	51.16 <sup>b</sup>	0.1497 <sup>a</sup>	86.167 <sup>a</sup>	3.12 <sup>a</sup>
B	9.58 <sup>c</sup>	85.13 <sup>a</sup>	0.12 <sup>b</sup>	82.633 <sup>b</sup>	6.56 <sup>b</sup>
C	45.9 <sup>a</sup>	51.77 <sup>b</sup>	0.07 <sup>c</sup>	86.167 <sup>a</sup>	5.77 <sup>b</sup>
SEM**	0.795	1.153	0.00415	0.4	0.3436

a=Crude protein solution at zero time (%), b=Fermentable material (%), c=Constant degradability coefficients at time t (%/h), ED=Effective degradation (The passage of time  $r=0.02$ ), RSD= Residual standard deviation.

a,b,c

Within a column, means without a common superscript letter differ ( $P < 0.05$ ).

\*\*Standard error means of the difference amount three treatments means.

Urea entered into the rumen by bacterial urease is rapidly hydrolyzed to ammonia and so, ruminal ammonia density can rise and the efficient conversion of ammonia into microbial protein requires fulfilling two conditions. First, the ammonia density must be lower than the desired conditions and second, the microorganisms must have a readily available source of energy for protein synthesis. Feeding operations to achieve these requirements is the urea mixing with other feeds (For a long period of use and the amino). Such feeds should have a small amount of rumen degradable protein and greater amount of easily fermentable carbohydrates (Navidshad and Jafari Sayadi, 2000). In some studies, barley treated with urea has been a significant increase in milk production and reduced the rate of DM degradation (Robinson and Kennelly, 1988). Reduced protein and starch degradation in the rumen without negative effects on digestibility of rumen has been observed due to the effects of ammonia on barley grain. Increased ruminal propionate and decreased acetate and reduction in rumen pH with consumption ammonia grain in lactating cows, can be marked degradation and fiber fermentable and reduction in starch degradation (Campling,

1991). Many studies have been done on the processing of barley grain treated with sodium hydroxide. In some of these studies, increase in milk production, and food consumption and changes in milk composition have been reported (Bull, 1995). McNiven *et al.*, (1995), reported processed barley with NaOH improve fiber digestibility and reduces fluctuations in rumen pH and starch and nitrogen rumen degradability. Also, increasing acetate and reduced ruminal is a sign of improvement in fiber digestion in the rumen due to better pH stability and decrease in its acidity (Dehghan Benadaki *et al.*, 2007a).

To overcome the problems of easily digestible protein system, metabolizable protein system was offered (Taghizadeh and Farhomand, 2007). To this end, the protein degraded by rumen microorganisms including the protein with rapid degradability of and the protein with slow degradability and is provided in the form of effective degradable protein and was computed that it will enter the intestine in the form of microbial protein. Also, the digestible undegradable which is part of food protein, that is not undegraded in rumen but is digested in small intestine. The data in Table 6, among the treatments in this experiment, treatments C (95.16%) and B (61.64%) had the highest and lowest metabolizable protein that have a significant difference ( $P < 0.05$ ).

**Table 6. The parameters estimated from the metabolizable protein of feeds**

Treatments	ERDP	DUP	MP
A	108.542 <sup>b</sup>	9.212 <sup>a</sup>	78.677 <sup>b</sup>
B	88.295 <sup>c</sup>	5.135 <sup>b</sup>	61.64 <sup>c</sup>
C	141.991 <sup>a</sup>	4.287 <sup>b</sup>	95.16 <sup>a</sup>
SEM**	0.64	0.7	0.4496

ERDP=Effective ruminal degradable protein, DUP=Digestible undegradable protein, MP=Metabolizable protein.

The differences in the amounts of metabolizable protein in treatments A, B and C could be related to the differences in crude protein and degradation properties of these materials that has led to a decrease in MP in treatment B. The existing difference in the MP of the studied samples can be due to differences in chemical composition, climate variability, variety of different protein, especially insoluble protein in buffer and insoluble protein in acid, and also is related to differences in the cell wall and especially to the protein trapped in the wall (ADIN).

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