ISSN: 2008-8140
E-ISSN: 2322-3618

Urmia University

VETERINARY RESEARCH FORUM

Volume 11, Number 1
March 2020

An International Quarterly Journal Published by Faculty of Veterinary Medicine, Urmia University, Urmia, I.R. Iran

ORIGINAL ARTICLE

Veterinary Research Forum. 2020; 11 (1) 59 – 65

doi: 10.30466vrf.2017.84395.2022

Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Different processing effects on nutritive value of barely grains by gas production method

Abbas Ali Ahmadi Naghadeh[1], Younes Ali Alijoo[2]*, Abbas Ali Naserian[3]

[1] PhD Candidate, Department of Animal Sciences, Faculty of Agriculture, Urmia University, Urmia, Iran ,[2] Department of Animal Sciences, Faculty of Agriculture, Urmia University, Urmia, Iran, [3]Department of Animal Sciences, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.

Article Info

Article history:

Received: 11 December 2017
Accepted: 06 March 2018
Available online: 15 March 2020

Keywords:

Extruding
Gas production technique
Incubation
Processing

Abstract

The purpose of this study was to investigate the effects of warm and cold physical as well as chemical processing methods on the nutritive value of barley grains by gas production technique. The processing methods included milling, steam flaking, extruding and soaking up the grain in water containing 1.00% citric acid, 1.00% propionic acid and 0.01 M sulfuric acid. Two-hundred mg of milling samples were incubated in special 100 mL glasses and the amount of gas produced at different hr was measured. The data were analyzed in a completely randomized design. The results showed that different treatments did not have a significant effect on chemical composition of barley seeds. Other methods of processing compared to the grinding method, significantly reduced the total amount of produced gas. In the 72 hr incubation period, the lowest amount of gas production was in the extruded (245.6 mL per g dry matter) treatment. However, there was no significant difference between the two methods of extruding and flaking. The highest percentage of digestible organic matter was associated with propionic acid (64.90%), while the steam cracking method (58.74%) was the least. Among the processing methods, the highest amounts of methane production, total protozoa population and volatile fatty acid concentration were related to the grinding method and the least amount of extrusion treatment was observed during 24 hr of incubation. Different experimental treatments had a significant effect on ammonia nitrogen condensation and the highest level was observed in milling. According to our results, processing methods such as extrusion and flaking may improve the grain nutritive value.

Introduction

Barley grain is one of the most common feed grains used in diets for dairy and beef cattle. Because the endosperm of the barley kernel is surrounded by pericarp, which is extremely resistant to microbial degradation in the rumen.[1] In addition, the barley seeds are surrounded by fiber with low digestibility. Unlike the corn grain that is well chewed, the barley grain is resistant to chewing and if the whole grain is fed to the animal, a significant amount of it will enter the stool.[2] Therefore; the dry barley grain needs to be processed to improve its use by dairy and beef cattle.

The processing increases the germs access to starch and the rate and degree of starch degradation in the rumen. Although processing is necessary to maximize the use of barley grain by cattle, processing too much grain increases the starch degradation in the rumen, which often leads to feeding intake amount reduction.[3] Also, the increase in the speed and severity of starch degradation in rumen causes an elevation in concerns about bloating, acidosis, lameness, liver abscesses, and food intake problems associated with gastrointestinal abnormalities.[4] A desirable change in starch digestion site requires processing methods or conditions increasing starch flow to the duodenum without digestion reduction throughout the gastrointestinal tract. Additionally, glucose uptake in the duodenum may reduce gluconeogenesis and increase ruminate productivity.[5] Each of the processing methods has advantages and disadvantages and the choice of appropriate method among available methods requires comparing these treatments under the same conditions.

***Correspondence:**

Younes Ali Alijoo. PhD
Department of Animal Sciences, Faculty of Agriculture, Urmia University, Urmia Iran
E-mail: y.alijoo@urmia.ac.ir

Cold and hot mechanical methods of barley processing include pelleting, roasting and steam lamination and chemical methods comprise sodium hydroxide, form-aldehyde, ammonia and urea processing that the use of these chemicals in human and animal nutrition was considered toxic and has been banned in many countries.[1]

Organic acids such as propionic acid and citric acid are poor in acids and commonly found in food. Unlike the chemicals listed above, these acids are not toxic to humans and animals and they are used extensively in food industries. Organic acids including citric acid increase starch tolerance to barley digestibility.[6] From the proposed ways to increase the share of digestible starch by barley seeds, processing with organic acids can help to form a hydrogen bond in the structure of starch granules, to open the amylopectin strands and to form a link between carbohydrate and protein (Millard's reaction).[7] Also, the processing of barley seed with organic acids reduces the accessibility of amylase enzymes of rumen bacteria to starch granules.[8]

It is not suggested to evaluate high feed intake and digestibility through the use of an alive animal due to reasons such as being time-consuming and expensive. The close relationship between ruminate fermentation results and gas production method has been reported previously.[9] Such gas production method is also advantageous since it enables the examination of the feeds fermentation process and a large number of samples in the short time. Another advantage of this method is determining the quantitative and qualitative rates and digestibility of an oral substance.[10] Several studies have addressed the use of barley processing methods;[1,11-13] however, scant attention has been given to the comparison of different methods of cold, hot and chemical processing of barley grain simultaneously. Thus, the present study plans to investigate various methods of barley processing including grinding, steaming, extruding and processing with citric acid, sulfuric acid and propionic acid on fermentation parameters using gas production technique.

Materials and Methods

The implementation of different stages of experiments was carried out at the Laboratory of Karaj Agricultural Research Center (Karaj, Iran). Three 15-kg batches of dry hulless whole kernel barley (Sahara variety) were provided from the market.

Processes. Six types of processing were included barley grain milling using a mill and a 2.00 mm screen, flaking with steam where the seeds were exposed to steam for 40 min and immediately from the rollers of an industrial machine that each of them rotate at the same speed,[11] the barley was extruded (the extrusion temperature was 130 ˚C) before being extruded, barley seeds contained approximately 20.00 to 22.00% moisture.

The extruder was a single screw extruder and the die diameter was 5.80 or 1.59 cm. Residence time in the barrel was approximately 25 sec, with the barrel temperature reaching 128 to 133 ˚C. The extrudate had a moisture level of about 5.00 to 6.00%.[14]

Barley seed processing with acids. Barley seeds were soaked for 24 hr in water containing the specified amount of each acid (soaking up the grain in water containing 1.00% citric acid, 1.00% propionic acid and 0.01 M sulfuric acid) and then used for gas testing.

Chemical analysis. Approximate analyses of food content including dry matter (DM), crude protein (CP; Kjeldahl), crude fat (Soxhlet) and crude ash were achieved according to methods of Association of Official Analytical Chemists[15] and neutral detergent fibers (NDF) and acid detergent fibers (ADF) were determined using van Soest *et al*. method.[16]

***In vitro* gas production test.** Samples, one from each batch, were simultaneously incubated in diluted rumen fluid to measure rumen fermentability as gas production according to Menke and Steingass.[17] Three runs were carried out. Briefly, about 200 mg of each sample was weighed into graduated 100-mL glass syringes, then 50.00 mL of diluted rumen fluid (buffer to rumen ratio of 2:1, v/v) collected from the rumen of three fistulated lactating dairy cows was added. Rumen liquor was maintained in a warm insulated flask, filtered through two layers of cheesecloth and used within 20 min from the collection. Before injection into syringes, the medium was saturated with CO_2 and the pH was corrected to 6.50 to 6.60. Donor cows were fed a total mixed ration (16.20% CP, 28.50% starch,0 and 35.00% NDF on a DM basis) formulated according to the Nutrient Requirement of Dairy Cattle[18] for an average body weight of 600 kg, 140 DIM and 35 kg milk yield (3.75% fat and 3.35% protein). The bulk of the diet, on a DM basis, was corn silage (31.20%), dehydrated alfalfa hay (16.70%), grass hay (4.10%) and energy-protein supplement (48.00%). Syringes were then placed vertically in a water bath at 39.00 ˚C. Blanks samples (diluted rumen fluid only) and an internal standard (Gelose 80 maize starch; Penford Food Ingredients Co., Centennial, USA) were also incubated. Samples from each batch were considered as experimental replicates. Gas production was measured at 2, 4, 6, 8, 10, 12, 24, 48, 72, 96 and 120 hr of incubation and again a separate incubation system for 24 hr was performed, in order to determine the effect of experimental treatments on the methane production and fermentation parameters such as short chain fatty acids, pH, ammonia nitrogen and protozoa population with three replications.

Cumulative gas production values were fitted to the potential equation according to the model of Ørskov and McDonald as follows:[19]

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

where, a = gas production from the immediately soluble fraction, b = gas production from the insoluble fraction, $a + b$ = potential degradability, c = gas production rate constant for insoluble fraction b and t = incubation time.

For protozoa enumeration, 50.00% formalin solution was mixed and maintained at room temperature 50:50 with 5.00 mL of the solution in the glass and counted with an electron microscope with a magnification of 40 times. The pH of each glass was measured immediately after opening and recorded using a digital pH meter (TitroLine easy titrator; Schott Instruments GmbH, Mainz, Germany).

According to Getachew *et al*., the metabolizing energy (ME; MJ per kg DM) of each feed can be obtained using the amount of gas produced in the laboratory and each chemical composition as follws:[9]

$$ME = 1.06 + (0.157 \times GP) + (0.084 \times CP) + (0.22 \times CF) - 0.081 \times CA$$

Also, the amount of digestible organic material (DOM) through the proposed formulas of Menke and Steingass is:[20]

$$DOM\ (\%) = 0.9991 \times GP + 0.0595 \times CP + 0.0181 \times CA + 9$$

In these equations, GP (mL) is gas produced from 200 mg of DM in 24 hr and CP, CF and CA are crude protein, crude fat and crude ash (% dry materials), respectively.

The amounts of each acetate ester, propionate and butyrate fatty acids were determined via a gas chromatography machine (PU4410, Glass column 1.65 × 4.6 mm; Philips, Cambridge, UK) using the internal standard (2-ethylbutyric acid). The ratio of acetate to propionate was also calculated.[21]

Ammonia nitrogen concentration was determined by the phenol-hypochlorite method using a spectrophotometer (M501; Spectronic CamSpec Ltd., Leeds, UK) at 630 nm.[22]

Experimental Design. The data were statistically analyzed in a complete randomized design using the MIXED procedure of SAS (version 9.1; SAS Institute, Cary, USA) with the following model:[23]

$$Yi = \mu + T_i + e_i$$

where, Yi is the observation of i, μ is the average of total observations, T_i is the effect of treatment and e_i is the error.

Results

The chemical composition of processed barley grains is presented in Table 1. The results showed that different treatments did not have a significant effect on the chemical composition of barley seeds.

The results of the gas production test using rumen fluid are shown in Table 2. The quantities of produced gas and the b and c coefficients in 120 hr indicated that different methods of barley processing have a significant effect on the amount of produced gas ($p < 0.05$). It should be noted that the process of gas production after 48 hr had a low velocity, which after 72 hr the numbers did not change compared to the previous hr.

Table 1. The analysis of barley processed nutrients composition (% of dry matter).

Processing*	DM	CF	CA	CP	NDF	ADF
Milled	92.61	1.90	2.66	12.50	24.21	6.70
Steam flaking	87.80	2.13	2.56	12.90	22.20	6.60
Extruded	93.48	1.85	2.76	12.44	23.70	7.60
Sulfuric acid	46.85	2.20	2.40	12.77	25.25	7.45
Citric acid	47.51	1.85	2.48	12.64	25.34	7.14
Propionic acid	47.77	2.15	2.22	12.71	24.89	8.08
SEM	0.87	0.05	0.03	0.31	0.45	0.54

DM: Dry matter; CF: Crude fat; CA: Crude ash; CP: Crude protein; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; and SEM: Standard error of means.

* Processing included: Milled barley; Steam flaking barley; Extruded barley; Barley grain wetting in water containing 1.00% citric acid, 1.00% propionic acid and 0.01 M sulfuric acid.

The lowest amount of gas production is among the extruded processing treatments. However, there was no significant difference between extruding and steam flaking methods. Also, processing with different acids in 8 hr of incubation significantly reduced the amount of gas.

Metabolizable energy (ME) and the percentage of DOM are shown in Table 3. The results showed that different processing methods did not affect the estimated parameters significantly.

The effects of different methods of barley processing on pH, methane production, volatile fatty acid concentration, ammonia nitrogen, and protozoans are shown in Table 4. The minimum pH after 24 hr of incubation was related to milling, but this difference was not significant. Among treatments, milling and extruding methods respectively showed the highest and lowest amount of methane and total protozoa production in 24 hr of incubation ($p < 0.05$). The results of volatile fatty acid concentration in Table 4 depict that the lowest concentration of total volatile fatty acids (TVFAs) was observed in warm processes.

The treatments showed significant differences in ammonia nitrogen concentration after 24 hr of incubation, with the highest concentration of ammonia nitrogen in the grinding method ($p < 0.05$).

Discussion

In this study, the range of processed CP samples was between 12.50% and 12.90%. Due to the use of the same source of seeds for different processing methods, the same chemical composition is not unexpected. The results of nutrients analysis in this study are inconsistent with the results of studies on Iranian barley varieties.[11,24,25]

During the various processes, chemical changes such as gelatinization and then cooling in the structure of grains are created that change the chemical bonds and affect the digestibility of the material. From the viewpoint of gas production reduction in the steam flaking and extruding, it can be associated with the dextran formation, cooling of gelatinized starches and a digestive secondary structure

Table 2. The production of gas at incubation times (mL per g DM) using rumen fluid.

Time (hr)	Processing*						SEM
	Milled	Steam flaking	Extruded	Citric acid	Sulfuric acid	Propionic acid	
2	15. 33	12.12	11.13	13.00	12.75	12.88	2.65
4	30.16 a	22.20 b	21 .60 b	24.24 b	24.40b	23.10 b	2.15
6	39.08 a	25.11b	24.40b	26.30 b	28.20 b	27.40 b	5.44
8	48.10 a	31.10 b	29.33 b	36.40 b	38.00 b	37.60 b	8.41
10	69.60 a	44.50 b	37.80 b	58.78 a	56.80 a	57.10 a	10.48
12	84.90 a	56.58 a b	44.10 b	75.30 a	78.70 a	76.30 a	12.71
24	191.40 a	160.40 b	138.70 c	183.40 a	179.17 a	180.18 a	13.55
48	270.50 a	234.80 b	208.70 c	267.80 a	270.00 a	272.40 a	12.56
72	281.28 a	248.24 b	245.60 b	274.01 a	278.24 a	275.62a	14.01
96	282.10 a	250.90 b	247.20 b	275.13 a	279.81 a	276.14 a	14.63
120	282.28 a	251.21 b	247.60 b	278.00 a	279.92 a	276.36 a	14.48
Coefficient†	290.88 a	270.58 c	267.27 d	286.58 b	287.41 b	287.64 b	4.45
Coefficient‡	0.064 d	0.082 b	0.087 a	0.074 c	0.075 c	0.072 c	0.0015

* Processing included: Milled barley; Steam flaking barley; Extruded barley; Barley grain wetting in water containing 1.00% citric acid, 1.00% propionic acid and 0.01 M sulfuric acid.

† Potential gas production (mL per 200 mg of DM); ‡ Rate fermentation section b (per hr); and SEM: Standard error of means.

abcd Different letters in each row indicate significant differences at the 0.05 level.

Table 3. Estimated parameters for processing seeds.

Estimated parameters	Processing*						SEM
	Milled	Extruded	Steam flaking	Citric acid	Sulfuric acid	Propionic acid	
ME (MJ Kg DM^{-1})	11.00	10.28	10.52	10.26	9.87	10.78	0.11
DOM (%)	64.60	59.12	58.74	64.40	64.50	64.90	0.74

* Processing included: Milled barley; Steam flaking barley; Extruded barley; Barley grain wetting in water containing 1.00% citric acid, 1.00% propionic acid and 0.01 M sulfuric acid.

ME: Metabolized energy; DOM: Digestible organic matter; and SEM: Standard error of means.

Table 4. Effects of various methods of barley treatment on pH, methane and protozoa (per mL rumen fluid at 24 hr of incubation).

Parameters	Processing*						SEM
	Milled	Steam flaking	Extruded	Citric acid	Sulfuric acid	Propionic acid	
pH	6.15	6.80	6.90	6.40	6.38	6.45	0.049
Methane (mm200mg DM^{-1})	45.60 a	39.20 c	38.60 c	42.24 b	41.40 bc	42.00 b	0.87
Protozoa (10^5 mL^{-1})	2.94 a	1.85 b	1.80 b	2.01 b	1.98 b	2.00 b	0.15
TVFAs (mm per 200 mg DM)	54.00 a	44.00 b	43.60 b	53.60 a	46.60 b	50.64 ab	4.00
Acetic acid (mol per 100 mol)	57.60 a	44.30 b c	42.30 c	46.90 b	48.10 b	44.45 bc	2.07
Propionic acid (mol per 100 mol)	23.30 c	36.90 a	37.50 a	32.00 b	31.10 b	33.31 b	1.40
Butyric acid (mol per 100 mol)	20.30	18.70	19.50	21.10	20.80	22.30	2.28
Acetic acid to Propionic acid	2.45a	1.22cd	1.12d	1.47b	1.55b	1.33bc	0.17
Ammonia (mg L^{-1})	20.10 a	13.40 c	12.40 c	15.60 b	14.30 b	15.30 b	0.75

* Processing included: Milled barley; Steam flaking barley; Extruded barley; Barley grain wetting in water containing 1.00% citric acid, 1.00% propionic acid and 0.01 M sulfuric acid.

TVFAs: Total volatile fatty acids; SEM: Standard error of means.

abcd Different letters in each row indicate significant differences at the 0.05 level.

formation. In addition to the long-term exposure of the steam to the potential shape, it can strengthen the protein, fat and starch binds in the endosperm barley increasing the resistance of starch to microbial fermentation.[11]

In a report by Svihus *et al*., studying the properties of cereal starch, it was concluded that applying gentle heat to moisture and in a short run can cause puffing of starch granules and starch gelatinization in the grain increasing its fermentation capacity in the rumen.[25] Steam flaking treatment reduces the production of very fine particulate material during processing,[1] which can produce less gas. In a study investigating various methods of processing such as microwaving, flaked, roasted and milled treatments, it has been shown that the lowest amount of gas was due to the flaked method.[11] Engstrom *et al*., have considered that the decrease in barley grain digestibility can be related to the process of barley grain processing with steam due to the increase of insoluble nitrogen in acid detergent by the Millard reactions.[26] Reportedly, a decrease in the degradability of starch by the steam flaking process *in vitro*, which is consistent with the results of this study.[27,28] Since the highest amount of produced gas during the first 24 hr is due to soluble carbohydrates,[29] it is likely that the milled barley grain is among the other treatments having the highest soluble carbohydrate content, which is the highest in produced gas.

Among the treatments, the highest percentage of DOM was related to propionic acid treatment. The lowest amount of ME was observed from treatment with sulfuric acid. These values are considered as a primary factor, but regarding the different physical properties of foods in the rumen and digestive disparities in the lower parts of the digestive tract, the values calculated by regression equations are not necessarily the best result, regardless of the specific circumstances of each feed at any time. Also, the error in the estimation of the approximate feed decomposition is due to the fact that the numbers generated by the formula are skewed. The results of the estimated parameters in this study are consistent with the results of other studies on different processing methods.[11,30]

The results of the study on Holstein cows using citric acid and lactic acid in barley seed treatment have indicated an increase in rumen pH six hr after ingestion, which has been related to increased stomach resistance to rumen digestion.[23] The results of the pH in this study are consistent with the results of studies on the treatment of cereal grains with organic acids reporting that they modulate the rumen resistance of nutritious grain seeds and reduce digestive problems,[8,31] resulting in improved performance and health. Inclusion of citric acid will maintain desire pH for stability of the rumen.[6]

One of the goals of livestock nutrition researchers is to change the rumen microbial ecosystem to improve livestock feed conversion.[32] Along with the production of rumen fatty acids during digestion, hydrogen is produced as an intermediate product, partly in methane production reactions[33] and the process of propionate production.[34] Ruminal protozoa provide hydrogen to produce methane gas by methanogen bacteria.[33] Therefore, compounds or processes that reduce the protozoa population can reduce methane production.

Reductions in gas production represent a decline in the production of volatile fatty acids. Regarding the relationship between methane production with two fatty acids and propionic acid, it is evident that lower methanogen reaction results in lower acetic acid and propionic acid production.[35] On the other hand, the major acetate is the end product of substances metabolism in protozoa[36] and thus protozoa population reduction will reduce acetic acid.[20] The combined reductions of methane and protozoa population have been observed in various studies along with acetic acid concentration decrease.[20,36]
It should be noted that the high amount of produced gas indicates the high metabolic energy as well as fermentable nitrogen and other nutrients necessary for the activity of the micro-organisms. Some hot treatments lead to physical and chemical changes in starch granules through breaking the hydrodynamic bands and absorbing water making them gelatinized resulting in their increased accessibility to fungal degradation by microorganisms. On the other hand, the formation of cross-links between amino acids and reducing sugars (Millard's reaction) or between proteins (iso-peptide bands)[37] and the discovery of protein strands, could be responsible for the reduction of rumen protein degradation during the heat treatment.[38] The effect of heat on the level of CP digestion depends on the amount of moisture content, temperature and processing time.[39] Alteration of the proteins three-dimensional structure in the barley grain by heat and these proteins digestibility reduction can explain the lower amount of gas production in the thermal processing of seeds. Thermal treatments reduce rumen degradation through a photolytic-resistant protein matrix formation. Thermal processes are effective in reducing the solubility of perineum and starch in the rumen and increasing the amount of protein and starch entering the small intestine for effective digestion and absorption.[40-42] Therefore, the lowest amount of ammonia nitrogen in steam flaking and extruded heat treatment is not unpredictable.

Considering that the purpose of cereal processing is to change the seed digestibility and to increase the amount of resistant starch, the results showed that the different methods of hot processing in this study compared to the method of grinding had reduced effect on fermentation parameters and the rate of gas production. Therefore, steam flaking, extruded and acid treatment methods without negative effect on the chemical composition of the seeds can be used to prevent the rapid fermentation of starch in the rumen and occurrence of digestive problems such as acidosis.

Acknowledgments

Authors would like to thank Urmia University for funding, laboratory and technical supports.

Conflict of interest

The authors have no conflicts of interest.

References

1. Dehghan-Banadaky M, Corbett R, Oba M. Effects of barley grain processing on productivity of cattle. Anim Feed Sci Technol 2007; 137(1-2): 1-24.
2. Beauchemin KA, McAllister TA, Dong Y, et al. Effects of mastication on digestion of whole cereal grains by cattle. J Anim Sci 1994; 72(1): 236-246.
3. Allen MS. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J Dairy Sci 2000; 83(7): 1598-1624.
4. Yang WZ, Beauchemin KA, Rode LM. Effects of barley grain processing on extent of digestion and milk production of lactating cows. J Dairy Sci 2000; 83(3): 554-568.

5. Kassem MM, Thomas PC, Chamberlain DG, et al. Silage intake and milk production in cows given barley supplements of reduced ruminal degradability. Grass Forage Sci 1987; 42(2): 175-183.
6. Harder H, Khol-Parisini A, Zebeli Q. Treatments with organic acids and pullulanase differently affect resistant starch and fiber composition in flour of various barley genotypes (*Hordeum vulgare* L.). Starke 2015; 67: 512-520.
7. Deckardt K, Khol-Parisiniand A, Zebli Q. Peculiarities of enhancing resistant starch in ruminants using chemical methods: Opportunities and challenges. Nutrients 2013; 5(6): 1970-1988.
8. Iqbal S, Zebeli Q, Mazzolari A, et al. Feeding barley grain steeped in lactic acid modulates rumen fermentation patterns and increases milk fat content in dairy cows. J Dairy Sci 2009; 92(12): 6023-6032.
9. Getachew G, Makkar HPS, Becker K. Tropical browses: Content of phenolic compounds, *in vitro* gas production and stoichiometric relationship between short chain fatty acids and *in vitro* gas production. J Agric Sci 2002; 139(3): 341-352.
10. Taghizadeh A. The estimation of feed value of energy supplement using *in vitro* gas production technique. In proceedings: Canadian Society of Animal Science; CSAS Annual Meeting. Guelph, Canada: 2004: 134.
11. Parand A, Taghizadeh A. Investigation of the digestibility of processed barley corn with different methods using the production method and the microbial enzyme source [Persian]. J Anim Sci Res (Agricultural Science) 2010; 20(2): 1-13.
12. Parnian Khaje Dizaj F, Taghizadeh A, Moghadam GH, et al. Use of *in vitro* gas production technique for evaluation of nutritive parameters of barley and corn grain treated by different microwave irradiation times [Persian]. J Anim Sci Res (Agricultural Science) 2010; 21(1): 15-27.
13. Tabatabaei M, Najafnejad B, Zamani P, et al. Study of the nutritional value of barley seeds and seeds of some raw and rough beans by *in vivo* and *in vitro* methods [Persian]. J Anim Sci Res (Agricultural Science) 2011; 21(2):11 -21.
14. Hudyma WT. Extruded barley fed to early weaned calve. MSc thesis. Montana State University. Bozeman, USA: 1989.
15. Official methods of analysis of the AOAC. 15th ed. Arlington, USA: Association of Official Analytical Chemists 1990; 323.
16. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991; 74(10): 3588-3597.
17. Menke K, Steingass H. Estimation of the energetic feed value from chemical analysis and *in vitro* gas production using rumen fluid. Anim Res Dev 1988; 28: 7-55.
18. National Research Council. Nutrient requirements of dairy cattle. 7th ed. Washington DC, USA: National Academy Press 2001.
19. Ørskov ER, McDonald I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J Agric Sci 1979; 92(2): 499-503
20. Menke KH, Steingass H. Estimation of the energetic feed value from the gas formation determined *in-vitro* with rumen juice and the chemical analysis. II. Regression equation [German]. Animal nutrition overviews 1987;15: 59-94.
21. Patra AK, Kamra DN, Agarwal N. Effect of plant extracts on *in vitro* methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. Anim Feed Sci Technol 2006; 128: 276-291.
22. Broderick GA, Kang JH. Automated simultaneous determinations of ammonia and total amino acids in ruminal fluid and *in vitro* media. J Dairy Sci; 1980(63): 64-75.
23. Laki A, Dehghan Banadaky M, Zali A, et al. Investigation of treated barley grain with lactic and citric acid on performance and feeding behavior of Holstein cows. J Anim Produc 2016; 18 (2): 249-260.
24. Ghorbani GR, Hadj-Hussaini A. *In situ* degradability of Iranian barley grain cultivars. Small Rumi Res 2002; 44(3): 207-212.
25. Svihus B, Uhlen AK, Harstad OM. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. Anim Feed Sci Technol 2005; 122(3-4): 303-320
26. Engstrom DF, Mathison GW, Goonewardene LA. Effect of β-glucan, starch, and fiber content and steam vs. dry rolling of barley grain on its degradability and utilization by steers. Anim Feed Sci Technol 1992; 37: 33-46.
27. Osman HF, Theurer B, Hale WH, et al. Influence of grain processing on *in vitro* enzymatic starch digestion of barley and sorghum grain. J Nutr 1970; 100: 1133-1139.
28. Sveinbjornsson J, Murphy M, Uden P. *In vitro* evaluation of starch degradation from feeds with or without various heat treatments. Anim Feed Sci Technol 2007; 132: 171-185.
29. Taghizadeh A, Janmohamadi H, Moghadam GA, et al. *In vitro* gas production profiles in some concentrate ingredients. J Anim Vet Adv 2008; 7 (2): 137-139.
30. Hosseini Z. Common methods of food decomposition [Persian]. Shiraz, Iran: Shiraz University Press 2010; 37-55.
31. Kazemi-Bonchenari M, Salem AZM, López S. Influence of barley grain particle size and treatment with citric acid on digestibility, ruminal fermentation and microbial protein synthesis in Holstein calves. Animal 2017; 11(8): 1295-1302.

32. Busquet M, Calsamiglia S, Ferret A, et al. Effects of cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continuous culture. J Dairy Sci 2005; 88: 2508-2516.
33. Morgavi DP, Forano E, Martin C, et al. Microbial ecosystem and methanogenesis in ruminants. Animal 2010; 4: 1024-1036.
34. Alemua AW, Dijkstra J, Bannink A, et al. Rumen stoichiometric models and their contribution and challenges in predicting enteric methane production. Anim Feed Sci Technol 2011; 166-167: 761-778.
35. García-González R, Lopez S, Fernandez M, et al. Dose response effects of *Rheum officinale* root and *Frangula alnus* bark on ruminal methane production *in vitro*. Anim Feed Sci Technol 2008; 145(1-4): 319-334.
36. Hess HD, Kreuzer M, Diaz TE, et al. Saponin rich tropical fruits affect fermentation and methanogenesis in faunted and defaunated rumen fluid. Anim Feed Sci Technol 2003; 109: 79-94.
37. Hayase F, Kato H, Fujimaki M. Racemization of amino acid residues in proteins and poly (L-amino acids) during roasting. J Agri Food Chem 1975; 23(3): 491-494.
38. Finley JW. Effects of processing on proteins: An overview. In: Phillips RD, Finley JW (Eds). Protein quality and the effects of processing. New York, USA: CRC Press 1988; 1-7.
39. Broderick GA, Wallace RJ, Orskov ER. Control of rate and extent of protein degradation. In: Physiological aspects of digestion and metabolism in ruminants. New York, USA: Academic Press 1991; 541-591.
40. Yu P, Sprague M, Egan AR, et al. Comparison of raw and roasted narbon beans (*Vicia narbonensis*) on performance and meat sensory attributes of lambs fed a roughage-based diet. Anim Feed Sci Technol 2001; 92(1-2): 1-16.
41. Mustafa AF, Seguin P. Effects of stage of maturity on ensiling characteristics and ruminal nutrient degradability of oat silage. Arch Anim Nutr 2003; 57(5): 347-357.
42. Murphy SR, Mc Niven MA. Raw or roasted lupin supplementation of grass silage for beef steers. Anim Feed Sci Technol 1994; 46(1-2): 23-35.