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Degradation Characteristics of Infrared Processed Barley Grain and Its Feeding Effects on Ruminal pH of Sheep

Research Article

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ABSTRACT

This study was conducted to investigate the effects of infrared processing of barley for 60, 90, 120 and 150 seconds (s) on protein hydrophobicity, *in vitro* protein digestibility, degradation characteristics of protein and starch and its feeding effect on ruminal pH of sheep. The surface hydrophobicity of protein increased ($P<0.04$) as processing time increased. The degradation rate and effective rumen degradability of protein and starch decreased up to 90 s and remains constant at higher processing times ($P<0.05$). *In vitro* protein digestibility of barley increased at processing time of 60 and 90 s, remain constant at 120 s, then decreased at 150 s ($P<0.05$). Electrophoretic analysis showed aggregation of barley proteins that remain undegradable for longer time in the rumen. Ruminal pH of sheep fed processed barley was higher ($P<0.05$) than those fed untreated sample. There were no significant differences in ruminal pH of sheep fed barley processed at times over 90 s. It was concluded that infrared processing for 90 s, as shortest time, could decrease rate and extent of protein and starch degradation of barley in the rumen.

KEY WORDS barley grain, infrared, *in sacco*, ruminal degradation, starch.

INTRODUCTION

Barley is the primary source of energy in concentrates in many parts of the world. Protein content of barley grain is little than oilseed meals; however, as this grain constitutes most part of concentrated in ruminant nutrition, main part of diet protein comes from it. Protein degradability of barley grain in the rumen is high and a little of it escape to small intestine (Ortega-Cerrilla *et al.* 1999; Prestl Økken, 1999; Sadeghi and Shawrang, 2008). Another limitation in barley feeding is occurrence of acidosis, because of high starch degradability in the rumen. Some studies conducted to resolve these problems with introducing different processing methods (Mc Niven *et al.* 1994; Mc Niven *et al.* 1995; Ortega-Cerrilla *et al.* 1999; Tothi *et al.* 2003; Sadeghi and Shawrang, 2008; Solanas *et al.* 2008). However, long processing time and low energy efficiency of heat treatments and environmental pollution of chemical treatments are limiting factors in their applications. Afzal *et al.* (1999) reported that the use of infrared radiation resulted in much faster barley drying when it was compared to conventional methods. It employs short time and high temperature to treat cereals or legumes before their final applications in food and feed (Pan and Atungulu, 2010). It has been reported that infrared processing could increase dry matter and nutrient digestibility in swine and poultry (Igbasan and Guenter, 1997; Zhang *et al.* 2003). In ruminant experiments, infrared processing has been used on oilseeds (Wang

et al. 1997; Wang *et al.* 1999; Mustafa *et al.* 2002). Authors found that infrared processing reduced ruminal degradability and increased intestinal digestibility of total and essential amino acids of canola seed. Recently, Mc Allister and Sultana (2011) reported that infrared treatment for 60 seconds could decrease ruminal crude protein degradability of cereal grains. We could not find investigation around the proper processing time of infrared on barley grain. Our hypothesis was that infrared processing of barley grain can decrease ruminal protein and starch degradation with increasing *in vitro* protein digestibility in a dose manner. Therefore, the purposes of this study were to evaluate the effects of infrared processing at different times on protein hydrophobicity, electrophoretic pattern, ruminal crude protein and starch degradation characteristics of barley grain and its feeding effect on ruminal pH of sheep.

MATERIALS AND METHODS

Sample preparation and treatments

Barley grain samples (variety Fajr) were obtained from three batches at field of Agriculture Research Institute (Karaj, Iran). Samples (three samples from each batch) were infrared processed for 60, 90, 120 and 150 s using a micronizer equipped with infrared irradiator (Helen Infrared, Philips, UK) with a temperature of approximately 220 ˚C. Surface temperature of the grains during irradiation was monitored using thermocouples attached to data logging system. Final exit temperature on leaving the micronizer ranged between 110 and 120 ˚C. Treated samples were spread on a tabletop and cooled to room temperature for 1 h before being packed in zipper bags and kept at 4 ˚C. For chemical analysis, ten grams of these samples were ground to pass a 1-mm screen, then were packed in low-density polyethylene air locked pouches and stored at -18 ˚C. Remainder of each sample was ground to pass a 3 mm screen for *in sacco* study and reserved as mentioned above. Chemical analysis was carried out on three samples from each batch in duplicate, but for *in sacco* trial, samples of each batched pooled.

In sacco experiment

Three 60 kg wethers (age 15 months) with rumen fistulas were used to determine disappearance extent and rate of starch and crude protein under confined feeding and housing conditions. Wethers were offered a diet of alfalfa hay and a barley-based supplement (800 g kg^{-1} of ground barley, 60 g kg^{-1} of molasse, 100 g kg^{-1} of soybean meal, 40 g kg^{-1} of trace mineral salt and 2 g kg^{-1} of vitamin A, D and E premix). On a dry matter basis, the basal diet contained 873 g kg^{-1} of alfalfa hay and 127 g kg^{-1} of supplement and it was offered at 0.02 of body weight daily in two equal portions at morning and afternoon. Water and a trace mineral block were provided for each wether *ad libitum*. Wethers were adapted to the basal diet for 10 d before initiating the trial. Procedure of ruminal incubation followed the method of Ørskov and Mc Donald (1979). Five grams of untreated and treated samples were weighed in duplicate into nylon bags (9×12 cm; 45 µm pore size) for each incubation period and each sheep. Each series with 30 samples (two replicates×five incubation periods×three animals for each treatment) for each batch were incubated in the ventral sac of rumen for 4, 8, 12, 24 and 48 hours. Immediately after removal from the rumen, samples were put in ice water to stop the microbial fermentation. The bags were washed three times for 5 min in a turbine washing machine (Jata, model 582, Spain). Bags were then dried to a constant weight at 60 ˚C for 48 h and weighed. The dried samples were stored at -18 ˚C for analysis of nitrogen and starch. Three bags were soaked in water bath for 30 min at 20 ˚C to estimate readily available crude protein and starch fractions of barley grain. After soaking, bags were machine washed and oven-dried.

Ruminal pH measurement

In a completely randomized design with five treatments and three replicates, fifteen Shal wethers (average body weight 65 kg) were fed diets (2.1 kg per day at 8:00 and 15:30) containing 40% alfalfa hay and 60% untreated or infrared processed barley-based supplements with mentioned components in previous section for one week. At day 7 of trial, rumen liquors were obtained before and 3 hour after feeding from each wether and pH were measured using portable digital pH meter (Hanna instrument, CA, USA). Before initiating the trial, wethers were adapted to experimental diets for 10 d.

Monitoring protein subunits

Protein subunits were fractionated by a SDS-PAGE discontinuous system according to Laemmli (1970). The ruminal undegradable fractions from each incubation period were ground (0.25 mm particle size) and replicate samples pooled. Twenty milligrams of dried untreated or irradiated barley grain was placed into 750 µL SDS-PAGE sample buffer. After 30 min of throughout mixing (vortex and inverse), samples were immersed at 90 ˚C for 3 min, centrifuged at 10000 × *g* for 1min and 25 µL of each sample was then loaded into the sample cell of gel. Electrophoresis of proteins used a 0.125 g mL^{-1} acrylamide resolving gel (1×110×140 mm) with 0.0375 g mL^{-1} acrylamide stacking gel. Gels were kept at a constant current of 30 mA until the bromophenol blue marker dye reached the bottom of the gel. Protein fixation and staining were completed simultaneously using a solution of Coomassie brilliant blue. Gel

destaining used 300 mL per litre methanol and 70 mL per litre acetic acid solution.

In vitro crude protein digestibility

Digestibility of rumen undegraded crude protein was estimated using the three-step *in vitro* procedure of Calsamiglia and Stern (2005). Samples containing 15 mg nitrogen were incubated for 1 h in 10 mL solution (0.1 N HCl) containing 1 g per litre of pepsin (Sigma P-7012, Sigma). Following incubation, 13.5 mL of phosphate buffer (pH 7.8) containing 37.5 mg of pancreatin (Sigma P-7545, Sigma) was added and samples incubated at 38 ˚C for 24 h. Undigested protein was precipitated using concentrated tri-chloric acid (3 mL). Supernatant was analyzed for soluble crude protein using a Kjeldahl method (AOAC, 1995).

Chemical analysis

Barley samples were analyzed for dry matter by oven-drying 1 g sample in triplicate as AOAC (1995). Nitrogen was determined according to AOAC (Method 984.13) and crude protein calculated as nitrogen × 6.25. The method of Mc Cleary *et al*. (1994) was used for determination of starch. No correction for sugar was carried out. Thus, the determined content of starch represents starch plus sugar. Barley protein was isolated from samples according to the method of Wu (1986), and then surface hydrophobicity of the protein-rich fraction of samples was determined according to the method of Hayakawa and Nakai (1985). Chemical analysis was carried out in duplicate.

Data fitting and statistical analysis

Digestion kinetics of crude protein and starch were determined according to the equation of Ørskov and Mc Donald (1979). The various degradability parameters for the nylon bags data were analyzed as a randomized complete block design, using wethers as blocks. Data of protein hydrophobicity, *in vitro* digestibility and ruminal pH were analysed at a completely randomized design. Analyses were carried out using the general linear model procedure of SAS for Windows version 9.1 (SAS Institute Inc., Cary, NC). When a significant difference was found, means were separated using Duncan's multiple range tests. Differences were considered to be significant if P≤0.05.

RESULTS AND DISCUSSION

Effects on hydrophobicity

Surface hydrophobicity of proteins related to untreated and processed barley is shown in Figure 1. Infrared processing for 60, 90, 120 and 150 s increased (P<0.05) the surface hydrophobicity of proteins by 5, 24, 46 and 48%, respectively, compare to untreated samples. There were no significant differences between untreated and 60 s or between 120 and 150 s processed samples.

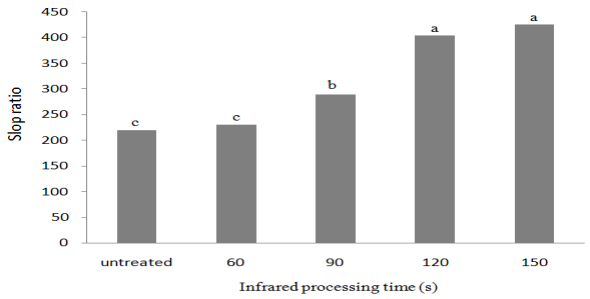

Figure 1 The surface hydrophobicity of untreated and infrared processed barley protein-rich fraction (slop ratio)

Effect on electrophoretic patterns

The sensitivity of proteins to untreated barley grain under proteolytic degradation is depicted in Figure 2. Most of the protein subunits of untreated barley grain disappeared after 6 h of ruminal incubation and of processed barley remained until 12 h of incubation.

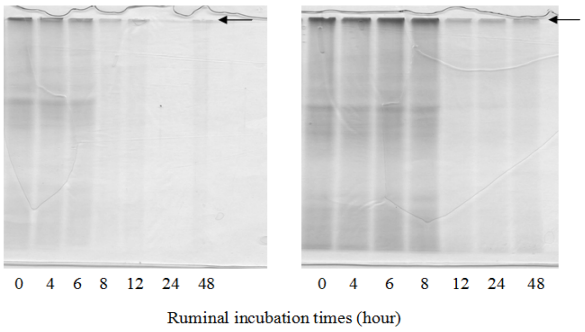

Figure 2 Electrophoretic profile of undegraded protein subunits for untreated (left) and 90 s infrared processed (right) barley grains incubated in the rumen. Arrow shows frontline of running gel, in which large molecules could not penetrate

Effects on ruminal protein degradability

There were differences (P<0.05) among degradation parameters and effective crude protein degradability of untreated and infrared processed barley grain (Table 1). Infrared processing for 60, 90, 120 and 150 s decreased the washout fraction "*a*" of protein by 22, 36, 55 and 54%, respectively, when it was compared to untreated samples. Processing up to 120 s, significantly increased the potentially degradable fraction "*b*" of crude protein, extending time to 150 s decreased it numerically.

The lowest "*a*" fraction and the highest "*b*" fraction were related to barley irradiated for 120 s and for untreated sample *vice versa*. Duncan test showed that infrared processing for 60 s decreased (P<0.05) constant degradation rate of protein in the rumen by 70% when it was compared to untreated sample.

However, extending processing time had no significant effect on it. As a consequence, the effective crude protein degradability of 60, 90, 120 and 150 s processed barley grain at outflow rate of 0.05 h^{-1} decreased (P<0.05) by 16, 18, 24 and 25%, when it was compared to untreated sample, respectively.

Effects on ruminal starch degradability

Washout fraction "*a*", degradable fraction "*b*", degradation rate "*c*" and effective degradability of starch related to untreated and processed barley grain has been shown in Table 2.

Washout fraction of 60 s processed sample was decreased (P<0.05) by 27%, when it was compared to untreated one, but extending processing time had no effect on it. Degradable fraction of starch increased as processed times increased; but over 120 s differences disappeared. Like to parameters of protein, the lowest "*a*" fraction and the highest '*b*" fraction related to processed sample was for 120 s. Processing for 60 and 90 s decreased (P<0.05) degradation rate of starch by 24 and 55%, respectively, when it was compared to untreated sample; and over 90 s, no significant effect was observed.

There was no significant difference among samples irradiated for 90 s and above for effective rumen degradability of starch.

Effective rumen degradability of starch decreased (P<0.05) up to 90 s, but increasing processing time had no further effect (P>0.05).

Effects on ruminal pH

Ruminal pH of sheep fed rations containing untreated and processed barley grain is shown in Figure 3.

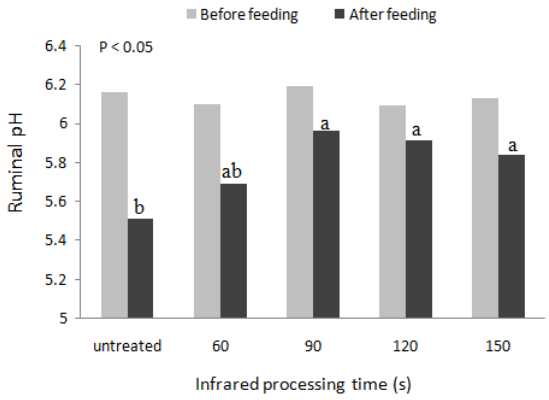

Figure 3 Ruminal pH of wethers fed diet supplemented with untreated or infrared processed barley grain

Infrared processing increased (P<0.05) ruminal pH of sheep compared to untreated sample. There were no significant differences among ruminal pH of sheep fed barley processed at times over 90 s.

Effect on *in vitro* protein digestibility

As shown in Figure 4, infrared processing for 60 and 90 s increased (P<0.05) *in vitro* protein digestibility of barley by 12 and 22%, respectively, when it was compared to untreated sample.

Table 1 Crude protein degradation characteristics of untreated and infrared processed barley grain

Treatments*	Degradation parameters**				Degradability at different ruminal passage rate (per hour)		
	a	*b*	*a* + *b*	*c*	0.02	0.05	0.08
Untreated	0.367^{a}	0.569^{e}	0.936^{b}	0.298^{a}	0.899^{a}	0.852^{a}	0.812^{a}
60	0.286^{b}	0.668^{d}	0.966^{a}	0.089^{b}	0.831^{b}	0.714^{b}	0.638^{b}
90	0.234^{c}	0.729^{c}	0.963^{a}	0.085^{b}	0.824^{b}	0.693^{c}	0.610^{c}
120	0.157^{d}	0.780^{a}	0.937^{b}	0.083^{b}	0.788^{c}	0.648^{d}	0.559^{d}
150	0.169^{d}	0.752^{b}	0.921^{b}	0.083^{b}	0.775^{c}	0.638^{d}	0.552^{d}
SEM	0.0147	0.0213	0.018	0.0061	0.0240	0.0253	0.0274

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
* Treatments: untreated and infrared processed barley for 60, 90, 120 and 150 s.
** *a*: the washout fraction; *b*: the potentially degradable fraction and *c*: the rate of degradation.

Table 2 Starch degradation characteristics of untreated and infrared processed barley grain

Treatments*	Degradation parameters**				Degradability at different ruminal passage rate (per hour)		
	a	*b*	*a* + *b*	*c*	0.02	0.05	0.08
Untreated	0.336^{a}	0.601^{d}	0.937^{c}	0.241^{a}	0.891^{a}	0.834^{a}	0.787^{a}
60	0.244^{b}	0.634^{c}	0.878^{b}	0.182^{b}	0.815^{b}	0.741^{b}	0.684^{b}
90	0.228^{b}	0.684^{b}	0.912^{a}	0.108^{c}	0.805^{b}	0.696^{c}	0.621^{c}
120	0.212^{c}	0.709^{a}	0.921^{a}	0.109^{c}	0.811^{b}	0.698^{c}	0.621^{c}
150	0.214^{c}	0.672^{b}	0.899^{a}	0.112^{c}	0.784^{c}	0.679^{c}	0.606^{c}
SEM	0.0185	0.0205	0.0260	0.0055	0.0231	0.0242	0.0261

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
* Treatments: untreated and infrared processed barley for 60, 90, 120 and 150 s.
** *a*: the washout fraction; *b*: the potentially degradable fraction and *c*: the rate of degradation.

There was no significant difference between protein digestibility of processed samples for 90 and 120 s. Increasing time to 150 s decreased protein digestibility compared to 120 s, even reach to lower than untreated barley grain by 2%.

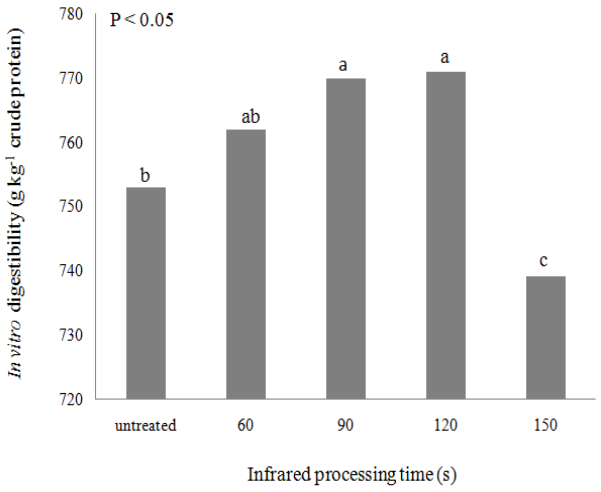

Figure 4 The *in vitro* crude protein digestibility of untreated and infrared processed barley grain

Ruminal protein degradation

The main purpose of the present study was to examine the effect of infrared processing on degradation characteristics of protein and starch in the rumen. Various processing times were also evaluated for introducing proper one. For interpreting the results, the surface hydrophobicity of protein was measured and electrophoretic patterns of protein subunits were monitored. Based on other heat treatments, we hypothesized that infrared processing in a dose manner result in decreasing protein and starch degradation in the rumen. In the literature, there was no study about the effects of infrared processing on ruminal degradation of cereals. A reduction in protein solubility in infrared processed barley samples was found by Fasina *et al.* (1999). Similar reductions in protein solubility have been reported in infrared toasted peas, cowpeas, beans and lentils (Arntfield *et al.* 1997; Mwangwela *et al.* 2007). Zheng *et al.* (1998) attributed the reduction in protein solubility of infrared toasted legumes to hydrophobic interactions, which render the protein less soluble in water. In the present study, the effective crude protein degradability of infrared processed barley grains decreased, but not in a dose manner. Decreasing in effective degradability of protein in barley by dry heat processing and microwave irradiation reported previously (Prestl Økken, 1999; Sadeghi and Shawrang, 2008). The optimum processing time of barley grain by dry heat was reported to be 30 min and with microwave 4 min (Prestl Økken, 1999; Sadeghi and Shawrang, 2008).

The modes of heat production in various sources differ, which affect optimum processing time. In dry heat processing, heat penetrate from the surface to inside, which result in non homogenous cooking. The surface overheated and burn and inside remain raw. But infrared penetrate to feed material and processed it homogenously. Infrared waves strike the material, a part of the energy which is absorbed making the constituent molecules to vibrate. During the vibration, inter-molecular friction among the molecules occurs and results in heat generation. The mechanisms behind the protection of protein against ruminal degradation in heat-treated feedstuffs are chemical reactions. As shown in Figure 1, an increase in the surface hydrophobicity of the protein occurred, possibly by changing the barley protein conformation to expose more hydrophobic sites (Prestl Økken, 1999). Heat treatment of protein will result in denaturation of protein and thermodynamically transform the proteins to a more resistant structure. In addition, heat processing can result in formation of cross-linkages between amino acids and reducing sugars (*i.e.,* Maillard reaction), or between proteins (*i.e.,* iso-peptide bonds). In Figure 2, cross-linked products of protein molecules were observed in top of gel that could not penetrate the running gel. This electrophoresis pattern regarding cross-linking of proteins was also observed in the microwave processing of barley grain (Sadeghi and Shawrang, 2008). As it is shown in Figure 4, infrared irradiation up to 120 s increased *in vitro* protein digestibility compared to untreated barley grain. In native structure of globular proteins (barley Hordeins), many hydrophobic amino acids are buried inside the molecule. Heat induces the unfolding of the protein and denaturation, thus exposing non-polar groups that were previously blocked (Prestl Økken, 1999). Therefore, this processing exposed hydrophobic amino acids (especially aromatics) that are position groups for active site of pepsin and trypsin enzymes (Murray *et al.* 2003). Reducing protein digestibility of samples processed at 150 s may be related to producing the Maillard products.

Ruminal starch degradation

Infrared processing alters starch degradation parameters of barley grain in the rumen, with decreasing in the washout fraction, increasing in the degradable fraction and decreasing in degradation rate. As a consequence, effective degradability of starch decreased as processing time up to 90 s increased. Similar to our results, in heat treated barley grain, Tothi *et al.* (2003) found a decrease in washout fraction, an increase in degradable fraction and a decrease in degradation rate of starch. In our study, effective degradability of starch decreased with processing, but in the study of Tothi *et al.* (2003) any change occurred in effective degradability of starch. In our study degradability of starch

remains constant at processing time over 90 s. This observation is in contrast to the study of Sadeghi and Shawrang (2008) in which starch degradability of microwave toasted barley grain at short period of irradiation increased and at longer periods decreased compared to control. It is likely that mechanism actions of various heat treatments differ and type of chemical reactions occur maybe differ with duration of processing and source. Differences in processing condition (source of heat and water contents) and *in sacco* experiments may be the reason of contradiction. In the present study, barley samples were processed with moisture content of 9%, but in the study of Sadeghi and Shawrang (2008), moisture of barley samples increased to 25%, which could affect on heat behaviour on starch. In the study of Mc Niven *et al*. (1994), a potential to increase the starch fraction escaping rumen degradation by heat treatment of barley was found. Increasing in starch escaping from the ruminal degradation related to protection of granules from microbial enzymes by denaturated proteins around them. Starch granules are embedded in a protein matrix, which protects the starch and must be degraded to some degree to allow amylolysis and digestion of the starch granules (Emami *et al.* 2010; Ren *et al.* 2010). A strong positive correlation between rumen degradation of starch and protein in a study (Goelema *et al.* 1999) with heat processing of feeds was found. Similar effects obtained with formaldehyde and sodium hydroxide treatment, indicated that treatment may create a protein matrix more resistant to proteolysis, thereby reducing rumen degradation of both protein and starch (Mc Niven *et al.* 1995; Sadeghi and Shawrang, 2008). As stated by Goelema *et al*. (1999), heat treatment could affect the extent of starch degradation by making the protein matrix more resistant to proteolysis. Other reactions that proceed at higher processing time, such as isopeptide crosslinks involving amino acids, such as lysine and serine, asparagine and glutamine and methionine and tryptophan may explain the additional effects of processing time on degradability of starch. Chemical reactions between starch molecules within the granules, thereby increasing the degree of crystallinity upon infrared irradiation may be another explanation for the decreased degradability of starch after heat treatment (Ren *et al.* 2010). Infrared irradiation like to dry heat treatment may also reduce the wash-out fraction with the formation of retrograded starch as reported by Goelema, *et al.* (1999). Retrogradation occurs when the gelatinised starch is cooled and leads to the formation of secondary resistant starch. Very little research has been carried out for studying the effects of infrared processing on chemical modification of starches.

Increasing of ruminal pH in wethers fed ration containing processed barley grain related to decrease in starch degradation rate as reported in Table 2. Barley starch is readily fermentable carbohydrate by ruminal microbes which produce volatile fatty acids and lactic acid. Decreasing in availability of starch or postponing its fermentation resulted in an increase of ruminal pH (Pauly *et al.* 1992). Extending processing time over 90 s had no significant effect on ruminal pH, because they had no further effect on starch degradation rate.

CONCLUSION

The results of this study indicated that infrared processing for 90 s, as shortest time, could decrease degradability of protein and starch, a characteristic that related to escape of these constituent of feed from the rumen to small intestine. Escaping of starch from the rumen was obtained by a decrease in solubility and degradation rate of starch. This change in degradation parameters of starch is useful in ruminant nutrition, because it could reduce acidosis when ration containing high amount of barley grain was offered to ruminants. In future studies, addition of infrared processed barley grain to diet of ruminants and determining volatile fatty acid profile and performance are recommended. Comparison of this method concerning processing cost with others can be useful for bring it to industrial scale application.

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REFERENCES

Afzal T.M., Abe T. and Hikida Y. (1999). Energy and quality aspects during combined FIR-convection drying of barley. *J. Food Eng.* **42,** 177-182.

AOAC. (1995). Official Methods of Analysis. 16th Ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Arntfield S.D., Scanlon M.G., Malcolmson L.J., Watts B., Ryland D. and Savoie V. (1997). Effect of tempering and end moisture content on the quality of micronized lentils. *Food Res. Int.* **30,** 371-380.

Calsamiglia S. and Stern M.D. (1995). A three-step *in vitro* procedure for estimating intestinal digestion of protein in ruminants. *J. Anim. Sci.* **73,** 1459-1465.

Emami S., Meda V., Pickard M.D. and Tyler R.T. (2010). Impact of micronization on rapidly digestible, slowly digestible, and resistant starch concentrations in normal, high-amylose, and waxy barley. *J. Agric. Food Chem.* **58,** 9793-9799.

Fasina O.O., Tyler R.T., Pickard M.D. and Zheng G.H. (1999). Infrared heating of hulless and pearled barley. *J. Food Process. Preserv.* **23,** 135-151.

Goelema J.O., Smits A., Vaessen L.M. and Wemmers A. (1999). Effect of pressure toasting, expander treatment and pelleting on *in vitro* and *in situ* parameters of protein and starch in a mixture of broken peas, lupins and faba beans. *Anim. Feed Sci. Technol.* **78,** 109-126.

Hayakawa S. and Nakai S. (1985). Relationship of hydrophobicity and net charge to the solubility of milk and soy proteins. *J. Food Sci.* **50,** 486-491.

Igbasan F.A. and Guenter W. (1997). The influence of micronization, dehulling, and enzyme supplementation on the nutritive value of peas for laying hens. *Poult. Sci.* **76,** 331-337.

Laemmli U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. **227,** 680-684.

Mc Allister T.A. and Sultana H. (2011). Effects of micronization on *in situ* and *in vitro* digestion of cereal grains. *Asian-australas. J. Anim. Sci.* **24,** 929-938.

Mc Cleary B.V., Solah V. and Gibson T.S. (1994). Quantitative measurement of total starch in cereal flours and products. *J. Cereal. Sci.* **20,** 51-58.

Mc Niven M.A., Hamilton R.M.G., Robinson P.H. and De Leeuiwe J.W. (1994). Effect of flame roasting on the nutritional quality of common cereal grains for ruminants and non-ruminants. *Anim. Feed Sci. Technol.* **47,** 31-40.

Mc Niven M.A., Weisbjerg M.R. and Hvelplund T. (1995). Influence of roasting or sodium hydroxide treatment of barley on digestion in lactating cows. *J. Dairy Sci.* **78,** 1106-1115.

Murray R.K., Granner D.K., Mayes P.A. and Rodwell V.W. (2003). Harper's Biochemistry. 26th Ed. Mc Graw-Hill, New York, NY, USA.

Mustafa A.F., McKinnon J.J., Christensen D.A. and He T. (2002). Effects of micronization of flaxseed on nutrient disappearance in the gastrointestinal tract of steers. *Anim. Feed Sci. Technol.* **95,** 123-132.

Mwangwela A.M., Waniska R.D., Mc Donough C. and Minnaar A. (2007). Cowpeas cooking characteristics as affected by micronisation temperature: a study of the physicochemical and functional properties of starch. *J. Sci. Food Agric.* **87,** 399-410.

Ortega-Cerrilla M.E., Finlayson H.J. and Armstrong D.G. (1999). Protection of starch in barley against rumen degradation by glutaraldehyde and formaldehyde as assessed by the dacron bag technique. *Anim. Feed Sci. Technol.* **77,** 83-90.

Ørskov E.R. and Mc Donald I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci. Camb.* **92,** 499-503.

Pan Z. and Atungulu G.G. (2010). Infrared Heating For Food And Agricultural Processing. 1th Ed. CRC Press, FL, USA.

Pauly T., Spörndly R. and Udén P. (1992). Rumen degradability in *sacco* of physically and chemically treated oat and barley grain. *J. Sci. Food Agric.* **58,** 465-473.

Prestl Økken E. (1999). *In situ* ruminal degradation and intestinal digestibility of dry matter and protein in expanded feedstuffs. *Anim. Feed Sci. Technol.* **77,** 1-23.

Ren G., Li D., Wang L., Özkan N. and Mao Z. (2010). Morphological properties and thermoanalysis of micronized cassava starch. *Carbohyd. Polym.* **79,** 101-105.

Sadeghi A.A. and Shawrang P. (2008). Effects of microwave irradiation on ruminal dry matter, protein and starch degradation characteristics of barley grain. *Anim. Feed Sci. Technol.* **141,** 184-194.

Solanas E.M., Castrillo C., Jover M. and De Vega A. (2008). Effect of extrusion on *in situ* ruminal protein degradability and *in vitro* digestibility of undegraded protein from different feedstuffs. *J. Sci. Food Agric.* **88,** 2589-2597.

Tothi R., Lund P., Weisbjerg M.R. and Hvelplund T. (2003). Effect of expander processing on fractional rate of maize and barley starch degradation in the rumen of dairy cows estimated using rumen evacuation and *in situ* techniques. *Anim. Feed Sci. Technol.* **104,** 71-94.

Wang Y., Mc Allister T.A., Zo Bell D.R., Pickard M.D.M., Rode L. and Cheng K.J. (1997). The effect of micronization of full fat canola seed on digestion in the rumen and total tract of dairy cows. *Can. J. Anim. Sci.* **77,** 431-439.

Wang Y., Mc Allister T.A., Pickard M.D., Xu Z., Rode L.M. and Cheng K.J. (1999). Effect of micronizing full fat canola seed on amino acid disappearance in the gastrointestinal tract of dairy cows. *J. Dairy Sci.* **82,** 537-544.

Wu Y.V. (1986). Fractionation and characteristics of protein rich material from barley after alcohol distillation. *Cereal. Chem.* **63,** 142-145.

Zhang Z., Nyachoti C.M., Arntfield S., Guenter W. and Cenkowski S. (2003). Effect of micronization of peas and enzyme supplementation on nutrient excretion and manure volume in growing pigs. *Can. J. Anim. Sci.* **83,** 749-754.

Zheng G.H., Fasina O., Sosulski F.W. and Tyler R.T. (1998). Nitrogen solubility of cereals and legumes subjected to micronisation. *J. Agric. Food Chem.* **46,** 4150-4157.