Shiraz University

IJVR

ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol.25 No.4 Ser. No.89 2024

IRANIAN JOURNAL OF VETERINARY RESEARCH

Original Article

Nutrient content, *in-vitro* digestibility, and starch and protein molecular appearance of intact and ammoniated steamed-flaked and/or steamed-infrared heated-flaked barley grain

Honarmand, A.[1]; Vakili, A. R.[2]; Danesh Mesgaran, M.[2*] and Tahmasebi, A. M.[2]**

[1]Ph.D. Student in Animal Nutrition, Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran; [2]Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

[*]***Correspondence:*** *M. Danesh Mesgaran, Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran. E-mail: danesh@um.ac.ir*

[**]***Co-correspondence:*** *A. R. Vakili, Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran. E-mail: savakili@um.ac.ir*

10.22099/ijvr.2024.49168.7210

(Received 1 Jan 2024; revised version 14 Oct 2024; accepted 19 Nov 2024)

Abstract

Background: The impact of different physical and/or chemical treatments in cereal grains on starch morphology and ruminal digestibility has been evaluated. **Aims:** The effect of chemical and/or physical treatments on starch and protein molecular appearance and the *ex-vivo* digestibility of barley grain was studied. **Methods:** Treatments were: steam-flaked barley grain (SFB), SFB treated with ammonium bicarbonate (A), urea (U), and malic acid (M) (SFB_{AUM}), SFB treated with A, U, and lactic acid (L) (SFB_{AUL}), steam-infrared heated-flaked barley grain (SIFB), SIFB treated with A, U, and M ($SIFB_{AUM}$), and SIFB treated with A, U, and L ($SIFB_{AUL}$). Chemicals including A, U, M, and L were used as 56, 8, 10, and 10 g/kg dry matter (DM), respectively. Chemical composition and molecular morphology were determined using scanning electron microscopy and Fourier-transform infrared spectroscopy (FTIR). In situ mobile bag technique and *in vitro* batch culture procedure were used to estimate ruminal and post-ruminal digestibility. **Results:** Crude protein (CP) and starch concentrations in SFB_{AUL} were higher than the others ($P<0.05$). Starch granule morphology and protein structure were altered in the chemically treated samples. The potentially digestible fraction of DM was the highest in the SFB_{AUM} ($P<0.05$). Ruminal disappearance of DM, CP, and starch was improved in SFB_{AUL} and $SIFB_{AUL}$ compared with other groups ($P<0.05$). The highest post-ruminal digestibility of starch and CP was observed in $SIFB_{AUL}$ and SIFB ($P<0.05$). **Conclusion:** Present results indicate that chemical processing with L and applied steam-infrared heated-flaked in barley grain may improve *in vitro* digestibility of starch and CP and increase granule sizes.

Key words: Barley, Digestibility, Protein, Starch, Steam flake

Introduction

The efficiency of rumen fermentation and degradation of feed components is a key factor in improving the efficiency of feed utilization and profitability in modern dairy herds (Eastridge, 2006). The primary source of energy in high-yield dairy cows comes from starch, which also provides the host with glucose for ruminal microbial protein synthesis (Offner *et al*., 2003). Cereals are essential components of ruminant diets, but their rapid degradation severely impairs rumen fermentation and the health of their hosts. Barley is frequently fed to animals as a source of energy. However, when administered to cattle, barley grain must be processed to maximize digestion because its protective hull limits the extent of ruminal digestion (Holopainen-Mantila, 2015). Volatile fatty acids (VFA) are produced quickly from barley starch degradation, which might lead to an acidotic rumen pH (Emmanuel *et al*., 2008). Researchers have investigated chemical treatments using formaldehyde or NaOH to enhance the feed efficiency of cattle by modifying the nature and quantity of starch available to the rumen microbiota, thereby transferring a portion of starch digestion to the hindgut (Noziére *et al*., 2005). Previous studies have indicated that treating grains with acids might affect the rheological properties of starch content (Shen *et al*., 2019). Its reasons can be several physicochemical changes, such as the release of amylose and amylopectin from the starch granules and the construction of a molecular form that envelops the broken starch granule remnants (Rahmadani *et al*., 2023). Treating cereal

grains with mild acids such as malic acid (M) and lactic acid (L) is a new method that improves the nutrient composition and is safe for the animals that consume them (Humer and Zebeli, 2017). Treatment of barley grain with L and tannic acid with an additional heat treatment modulated ruminal fermentation profile, digestion, and increased fiber degradation (Deckardt *et al*., 2016). Iqbal *et al*. (2009) introduced a novel barley grain processing technology that entails steeping barley grain in L, a moderate organic acid, to modulate rumen fermentation patterns and maintain the metabolic health and high productivity of dairy cows. Steeping barley grain in L decreased the degradation rate of barley starch, increased the content of resistant starch, and consequently decreased rumen VFA concentrations (Iqbal *et al*., 2010). Protein sources, such as soybean meal, are expensive with limited accessibility in developing countries. Consequently, processing cereal grains with compounds containing ammonia and non-protein nitrogen (NPN) raises their crude protein (CP) content and also lowers feeding costs, which is economically and nutritionally important. The rate of rumen nutrient degradation of grains, including the degradation of starch and protein, has also been reduced using ammonia treatment. Additionally, this treatment has favorable impacts on the treated grain's CP content for ruminants, particularly in the case of maize. Also, some studies have reported the positive effects of ammonia treatment on animal performance, such as increasing milk production, milk protein, and lactose yield (Robinson and Kennelly, 1989). Since additional ammonia is bound to cereals and volatilization is constrained, the addition of urea to grains increases their CP level. In general, it has been argued that, when coupled with mechanical processing, the ammonization of whole grains might be a suitable processing method (Bradshaw *et al*., 1996). Cereal grains that are treated with alkalization could help with both spoilage and acidity. Because ammonia makes the rumen more alkaline, it is also predicted to slow the rate at which protons build up in the rumen, lowering the risk of reticuloruminal acidosis (Humer and Zebeli, 2017). Physical processing techniques such as pelleting, steam rolling, steam flaking, and toasting have been used to gelatinize starch granules using moisture, heat, and pressure (Svihus *et al*., 2005). The use of different combinations of heat, moisture, time, and mechanical action might affect the quality of processed barley grain (Kokić *et al*., 2013). Micronization is also a rapid (30-90 s) thermal treatment using infrared radiation (Aboud *et al*., 2019) and has great application potential in the feed industry due to the ease of construction and operation (Fasina *et al*., 1999). Awareness of the intrinsic molecular structure is essential to obtain the full knowledge of the feed. Spectral features of nutrients are associated with nutritional values and degradability characteristics. This relevance can be affected by nutrient use and animal performance (Xu *et al*., 2018). We hypothesized that ammoniated-acidification flaked barley grain may meliorate *in situ* degradation and *in vitro* first-order ruminal disappearance kinetics of its nutrients. Additionally, the chemical and physical treatment can alter the molecular morphology and structure, thereby influencing the chemical composition and digestibility of grains. The present study primarily aimed to evaluate the impact of various chemical processing methods on ruminal degradation of dry matter (DM), CP, and starch, as well as protein structures and starch granule morphology. The second objective of this study was to compare the effect of two different physical treatments (steam-flaked and/or steam-infrared heated-flaked) on barley grain molecular structure alternation and the extent and site of digestion.

Materials and Methods

Ethics statement

The animal study was evaluated and accepted by the Institutional Animal Care Committee, Ferdowsi University of Mashhad (Mashhad, Iran; protocol No. 101984).

Barley grain processing

For each chemical and physical processing run (n=4, including 3 replicates per each run), approximately 2 kg of DM of barley grain were used. Experimental treatments were: steam-flaked barley grain (SFB), SFB treated with ammonium bicarbonate (A), urea (U), and malic acid (M) (SFB_{AUM}), SFB treated with A, U, and lactic acid (L) (SFB_{AUL}), steam-infrared heated-flaked barley grain (SIFB), SIFB treated with A, U, and M ($SIFB_{AUM}$), and SIFB treated with A, U, and L ($SIFB_{AUL}$). Chemical additives including A, U, M, and L were applied during the steaming (35 min at 96°C) as 56, 8, 10, and 10 g/kg dry matter (DM), respectively. Then, steam cooked grains were divided into 2 groups; a portion of the steam-cooked grain was exposed to an infrared-heated for 55 s to reach internal kernel temperatures of 100°C, while grain surfaces were heated uniformly. All steamed grains were then passed through the roller mill in a flaker machine and were then flaked.

Nutrient composition and physical assessment

Nutrient composition

The samples were dried using an air-forced oven (60°C for 48 h) to determine DM, then grounded to pass through a 2 mm screen to be used for next analysis (Eyni *et al*., 2017). The concentration of ether extract (EE) and ash was measured using methods 945.38 and 945.18, respectively (AOAC, 2012). The determination of neutral detergent fiber (NDF) and acid detergent fiber (ADF) was carried out using Van Soest *et al*. (1991), while sodium sulfite and heat-stable alpha-amylase were not used and were expressed without residual ash. Nitrogen content was determined by the Kjeldahl method (Kjeltec 2300 Autoanalyser, Foss Tecator AB, Hoganas, Sweden), and CP was calculated as N × 6.25. The starch content was determined by the anthrone and sulfuric acid (Rose *et al*., 1991). The water-soluble carbohydrate

(WSC) level was measured using phenol/sulphuric acid described by Hall (2014). Determination of amylose and amylopectin content of the samples was carried out according to Hu *et al*. (2010). Data of nutrient composition with 4 replications were obtained.

Scanning electron microscopy procedure

Samples were cut using liquid N to arrive at excellent scanning quality for determining the particle shape and surface morphology of starch granules using a Hummer sputter coater for gold-platinum coating (process current 10 Å), then scanning electron microscopy (LEO 1450 VP, USA) photos were taken. All samples were examined at 25 kV accelerating voltage and ×2500 magnification.

FTIR of sample

The infrared (IR) absorbance band of the samples was performed using FTIR spectroscopy. Spectral data from all samples were prepared using a Thermo-Nicolet AVATAR 370 FT-IR (Avatar 370, Thermo Nicolet Corporation, America). The FTIR spectra were incorporated in the range of 400-4000 cm^{-1}. Then, regions in relevant to protein molecular structures were identified. The primary protein structures, amide I (1,720 to 1,577 cm^{-1}), and amide II (1,577 to 1,486 cm^{-1}) were quantified. The comparative contribution of α-helix and β-sheet protein secondary structure to the amide I [absorption band was assessed using the second derivative spectrum to find the α-helix (ca. 1,650 cm^{-1}) and β-sheet (1,638 cm^{-1}) component peaks (Yu, 2010)].

X-ray diffraction (XRD)

The XRD analysis of the samples was carried out on an X-ray diffractometer (XMP300, Unisantis, Georgsmarienhutte, Germany) at a Cu-K_α radiation wavelength of 1.54 Å, a target voltage of 45 kV, a current of 44 mA, and a diffraction angular range of 5-40° (2θ) with a 0.02° step size. Data were analyzed using Match software version 3.15.

In vitro batch rumen culture

In vitro ruminal disappearance of the nutrients from the samples was determined using a batch culture technique (Arroquy *et al*., 2005). The fermentation medium consisted of 400 ml cell-free ruminal fluid, cellobiose (0.05 g), resazurin (0.01 g), sodium carbonate ($NaHCO_3$) (4 g), cysteine-HCl (0.5 g), 150 ml mineral mixture 1 (composed of 3 g K_2HPO_4 in 1000 ml distilled water), 150 ml mineral mixture 2 (composed of KH_2PO_4 3 g, NaCl 6 g, $(NH_4)_2SO_4$ 6 g, $MgSO_4 \cdot 7H_2O$ 0.6 g, and $CaCl_2$ 0.6 g in 1000 ml distilled water), and 300 ml distilled water. Rumen fluid was obtained from 3 ruminal fistulated Holstein cows (625 ± 12 kg body weight, 150 ± 19 days in milk) that were fed a diet with a forage: concentrate ratio of 45:55; whit forages as 9 kg DM (30% alfalfa hay and 70% corn silage) and concentrate as 11 kg DM (25% barley grain, 25% corn grain, 20% soybean meal, 12% cottonseed meal, 10% wheat bran, 3% fish meal, 2% fat powder, 1% sodium carbonate, 0.5% DM calcium carbonate, 1% mineral and vitamin premix, and 0.5% salt). The daily ration was fed in three equal portions at 07:00 a.m., 5:00 p.m. and 7:00 p.m. Animals had access to water as *ad libitum*. The diet provides energy and nutrients required for 25 kg/day milk production (National Research Council, 2001). Rumen fluid was sampled prior to the morning feeding and instantly strained through four layers of cheesecloth to remove larger feed bits, and then moved to the laboratory. Subsequently, samples were centrifuged at 3000 g for 5 min. The supernatant was then centrifuged at 10,000 g for 20 min. Forty-five ml of the medium was transferred into a 100 ml bottle containing the tentative sample (500 mg of unprocessed or processed barley grain) and autoclaved at 120°C for 20 min. Then, each bottle was inoculated with 5 ml of the rumen liquid passed through a strainer (the bacteria-rich fluid), and anaerobic conditions were maintained through a CO_2 stream, following that, sealed with a rubber stopper and aluminum cap and, incubation was performed in an incubator at a temperature of 38.6°C for 2, 4, 8, 12, 16, 24, 36, and 48 h. At each sampling time, the bottles for that time were egressed from the incubator. Then, DM, CP, NDF, and starch in the remaindering samples were measured, and the disappearance was calculated (Naseroleslami *et al*., 2018). *In vitro* batch rumen culture was performed (three separate runs with three replicates in each run).

In Situ mobile bag technique

Ruminal, post-ruminal, and total tract DM, CP, and starch disappearances were assessed using the *in situ* mobile nylon bag technique (Kheirandish *et al*., 2022). To do so, three lactating Holstein cows equipped with a rumen fistula and an intestinal cannula (645 ± 17 kg body weight, 170 ± 11 days in milk) were used. Animals were fed a diet as described above. Approximately 6 g of any grounded sample was placed inside of a polyester bag (12 × 17 cm, 50 μm pore size, n=4) and placed into rumen concurrently just before the morning feeding. Bags were picked up after 12 h, then laundered using a washing machine. Bags were dried at 60°C for 48 h, using an air-forced oven dryer, then weighed and used to assess DM, CP, and starch contents. Approximately, 1 g of residual of each rumen incubated bag was placed in a mobile nylon bag (3 × 6 cm; 52 μm pore size; 6 bags per sample). The bags were placed in duodenum through the T-shaped cannulas at the rate of one bag every 30 min. Bags were removed from the emptied feces and washed in the washing machine until the water remained clear, then dried (60°C, 48 h). Finally, bags were weighed to determine DM and analyzed for protein and starch concentration. Rumen, intestine, and total tract digestibility were determined using three lactating Holstein cows, where four replicates from each experimental treatment were applied in each animal (n=12).

Calculation and statistical analysis

In vitro first-order kinetic parameters of DM, CP, and

starch disappearances of samples were assessed using a non-linear model. The exponential model was:

$D_{(t)} = D_{(i)}. \exp^{(-kd. time)} + I$

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 situ ruminal disappearance of DM, CP, and starch samples was estimated using the difference between the primary sample and the part residual after incubation in the rumen. Disappearance in the intestinal tract was computed by the difference between the rumen remnant after 12 h of incubation and the part remaining in samples recovered from feces. Multivariate analysis of the amide spectral region ($1,480^{-1}$, 720 cm^{-1}) was accomplished to illustrate the molecular structure difference among treatments using software R version 3, 3, 1. Data on the nutrient composition of the treatments with 4 replications were determined. *In vitro* first-order ruminal disappearance kinetics of nutrients were conducted during three runs. *In situ* ruminal, post-ruminal, and total tract digestibility were obtained using three lactating Holstein cows, where four replications of every treatment were applied. Consequently, all data were statistically analyzed as a completely randomized design using the generalized linear model (GLM) procedure of SAS version 9.4 (SAS, 2004). The statistical model was as follows:

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

Where,
Y: The dependent variable
μ: The overall mean
T_i: The effect of the processing
e_{ij}: Residual error

The Tukey's multiple comparison test was used to calculate and compare treatment means. Significance was expressed at P<0.05. Besides, orthogonal contrasts were constructed to evaluate 1) SFB and SIFB versus SFB_{AUM}, SFB_{AUL}, $SIFB_{AUM}$, and $SIFB_{AUL}$, 2) SFB, SFB_{AUM} and SFB_{AUL} versus SIFB, $SIFB_{AUM}$, and $SIFB_{AUL}$, and 3) SFB_{AUM} and $SIFB_{AUM}$ versus SFB_{AUL} and $SIFB_{AUL}$.

Results

Nutrient composition

The nutrient composition of the samples is presented in Table 1. The concentration of CP, starch, NDF, ADF, ash, amylose, and amylopectin was significantly changed when different processing methods were applied in the grain (P<0.05). The CP concentration was higher in SFB_{AUL} than the other treatments (P<0.05). The concentration of CP enhanced by 55% during chemical treatment compared with non-chemical treatment. According to the orthogonal contrasts, SFB_{AUL} and $SIFB_{AUL}$ had a higher mean of CP concentration compared with SFB_{AUM} and $SIFB_{AUM}$ (182.3 vs. 170.4 g/kg, P<0.05). The starch content of SFB_{AUL} was greater (562.5 g/kg) compared with those of other treatments. Treatment of the barley grain with L (SFB_{AUL} and $SIFB_{AUL}$) resulted in the higher mean starch content than that of M (P<0.05). The NDF concentration of the samples was significantly (P<0.05) altered among the treatments. Both SFB_{AUM} and $SIFB_{AUM}$ had higher NDF content compared with other samples. M caused a significant increase (22.26%) in the NDF content of the grain. A significant effect of treatments on ADF concentration was also found (P<0.05). The highest level of ADF was observed in SFB_{AUM}, and the lowest level was seen in SFB_{AUL}. The contrasts made between the acids used in the present experiment showed a significant difference between the SFB_{AUL} and $SIFB_{AUL}$ and SFB_{AUM} and $SIFB_{AUM}$ so that treated with M showed a higher ADF concentration than that of the flaked grains treated with L (P<0.05). The ash content of SFB was significantly higher than that of the others. The highest amylose concentration was observed in SFB. A

Table 1: Nutrient composition (g/kg DM) of physically and/or chemically treated barley grains

Parameters	Treatments						SEM	P-value			
								Treatment	Contrasts		
	SFB	SFB_{AUM}	SFB_{AUL}	SIFB	$SIFB_{AUM}$	$SIFB_{AUL}$			1	2	3
Crude protein	113.2^{e}	168.1^{d}	186.8^{a}	114.4^{e}	172.8^{c}	177.9^{b}	1.27	<0.001	<0.001	0.348	<0.001
Starch	560.3^{b}	558.3^{c}	562.5^{a}	561^{b}	556.8^{c}	560.2^{b}	1.03	0.026	0.203	0.247	0.003
Amylose	175.4^{a}	160.7^{b}	161.7^{b}	175.6^{a}	158.2^{b}	158^{b}	1.78	<0.001	0.376	0.250	0.705
Amylopectin	384.9^{b}	397.6^{a}	400.7^{a}	385.4^{b}	398.5^{a}	402.2^{a}	1.82	<0.001	0.923	0.250	0.705
Neutral detergent fiber	204.4^{b}	219.5^{a}	172.4^{d}	203.7^{b}	217.7^{a}	185.1^{c}	6.8	0.002	0.790	0.549	<0.001
Acid detergent fiber	74.7^{a}	76.9^{a}	62.4^{b}	69.8^{ab}	76.4^{a}	74.3^{a}	2.43	0.009	0.002	0.295	0.005
Ether extract	22.7	23.1	23.3	24.2	23.2	23.2	1.01	0.947	0.355	0.590	0.887
Ash	29.5^{a}	24.7^{b}	25.7^{ab}	25.7^{ab}	23.3^{b}	24.1^{b}	0.92	0.007	<0.001	0.010	0.331
Water soluble carbohydrate	36	35.2	34	36.8	35.3	33.5	2.30	0.913	<0.001	0.951	0.529

SFB: Steam-flaked barley grain, SFB_{AUM}: Steam-flaked barley grain treated with ammonium bicarbonate, urea and malic acid, SFB_{AUL}: Steam-flaked barley grain treated with ammonium bicarbonate, urea and lactic acid, SIFB: Steam-infrared heated-flaked barley grain, $SIFB_{AUM}$: Steam-infrared heated-flaked barley grain treated with ammonium bicarbonate, urea and malic acid, and $SIFB_{AUL}$: Steam-infrared heated-flaked barley grain treated with ammonium bicarbonate, urea and lactic acid. $^{a, b, c, d, e}$ Means with different superscript letters in each row indicate significant difference (P<0.05). SEM: Standard error of the means. Contrasts: 1) SFB and SIFB versus SFB_{AUM}, SFB_{AUL}, $SIFB_{AUM}$ and $SIFB_{AUL}$, 2) SFB, SFB_{AUM} and SFB_{AUL} versus SIFB, $SIFB_{AUM}$ and $SIFB_{AUL}$, and 3) SFB_{AUM} and $SIFB_{AUM}$ versus SFB_{AUL} and $SIFB_{AUL}$

Table 2: *In vitro* first-order ruminal disappearance kinetics of dry matter, crude protein, starch, and neutral detergent fiber (NDF) of physically and/or chemically treated barley grains

Parameters	Treatments						SEM	P-value			
								Treatment	Contrasts		
	SFB	SFB_{AUM}	SFB_{AUL}	SIFB	$SIFB_{AUM}$	$SIFB_{AUL}$			1	2	3
Dry matter											
D	0.64[d]	0.86[a]	0.76[c]	0.81[b]	0.77[bc]	0.77[bc]	0.02	0.001	0.002	0.137	0.027
I	0.35[a]	0.13[d]	0.23[b]	0.18[c]	0.23[b]	0.22[b]	0.01	<0.001	0.001	0.035	0.003
K_d	0.1[b]	0.13[a]	0.14[a]	0.11[b]	0.14[a]	0.13[a]	0.01	0.025	0.001	0.942	0.865
Crude protein											
D	0.66[c]	0.77[b]	0.78[b]	0.67[c]	0.79[b]	0.84[a]	0.02	0.003	<0.001	0.142	0.144
I	0.32[a]	0.22[b]	0.21[b]	0.32[a]	0.2[b]	0.15[c]	0.02	<0.001	<0.001	0.077	0.090
K_d	0.07	0.08	0.09	0.07	0.08	0.09	0.01	0.310	0.027	0.998	0.621
Starch											
D	0.72[b]	0.80[a]	0.83[a]	0.73[b]	0.81[a]	0.84[a]	0.02	0.001	<0.001	0.575	0.058
I	0.28[a]	0.19[b]	0.17[b]	0.27[a]	0.19[b]	0.16[c]	0.01	<0.001	<0.001	0.401	0.007
K_d	0.13	0.13	0.14	0.13	0.14	0.14	0.01	0.875	0.315	0.525	0.919
NDF											
D	0.51[b]	0.58[a]	0.61[a]	0.51[b]	0.58[a]	0.62[a]	0.03	0.054	0.003	0.807	0.225
I	0.47[a]	0.41[b]	0.37[b]	0.47[a]	0.40[b]	0.36[b]	0.02	0.019	0.001	0.733	0.089
K_d	0.07	0.09	0.07	0.07	0.08	0.07	0.01	0.499	0.202	0.661	0.139

SFB: Steam-flaked barley grain, SFB_{AUM}: Steam-flaked barley grain treated with ammonium bicarbonate, urea and malic acid, SFB_{AUL}: Steam-flaked barley grain treated with ammonium bicarbonate, urea and lactic acid, SIFB: Steam-infrared heated-flaked barley grain, $SIFB_{AUM}$: Steam-infrared heated-flaked barley grain treated with ammonium bicarbonate, urea and malic acid, and $SIFB_{AUL}$: Steam-infrared heated-flaked barley grain treated with ammonium bicarbonate, urea and lactic acid. [a, b, c] Means with different superscript letters in each row indicate significant difference (P<0.05). SEM: Standard error of the means. Contrasts: 1) SFB and SIFB versus SFB_{AUM}, SFB_{AUL}, $SIFB_{AUM}$ and $SIFB_{AUL}$, 2) SFB, SFB_{AUM} and SFB_{AUL} versus SIFB, $SIFB_{AUM}$ and $SIFB_{AUL}$, and 3) SFB_{AUM} and $SIFB_{AUM}$ versus SFB_{AUL} and $SIFB_{AUL}$. D: Potentially digestible fraction, I: Indigestible fraction, and K_d: Fractional rate constant of digestion (/h)

significant contrast was found between non-chemical (SFB and SIFB) and chemical processing, so that chemical additives reduced the concentration of amylose (P<0.05).

In vitro batch rumen culture

The parameters related to the first-order kinetics of digestibility of DM, CP, starch, and NDF of the physically and/or chemically processed barley grains are shown in Table 2. Potentially digestible fraction of DM was the highest for SFB_{AUM}, intermediate for SIFB, $SIFB_{AUM}$, $SIFB_{AUL}$, and SFB_{AUL}, and the lowest for SFB (P<0.05). The highest amount of the indigestible fractions of DM was observed in SFB. The result of contrasts between the types of acids and also the types of physical processing methods showed that using M as well as infrared heated-flaked processing caused a decrease in the indigestible fraction of DM (P<0.05). The lowest fractional constant rate of DM was assigned for SFB and SIFB. Potentially digestible fraction of CP varied from 0.66 to 0.84 with a mean of 0.75; SIF_{AUL} had the highest and SFB had the lowest CP first order digestible fraction (P<0.05). The highest (P<0.05) amount of indigestible fraction of CP was observed in SFB and SIFB. The potential digestible fraction of CP increased by 19.40% following chemical treatment (P<0.05). The highest potential digestible fraction of starch was seen in $SIFB_{AUL}$. In chemical treatment, the potential digestible fraction of starch was increased (P<0.05) compared with non-chemically treated grain (0.72 *vs.* 0.83). Using L (SFB_{AUL} and $SIFB_{AUL}$) leads to a significant reduction of the indigestible fraction of starch compared with those treated with M (SFB_{AUM} and $SIFB_{AUM}$, P<0.05). A significant difference in the potentially digestible fractions of NDF was seen between the treatments (P<0.05). Both SFB and SIFB had the highest indigestible fraction of NDF, and $SIFB_{AUL}$ had the lowest chemical treatment caused to increase the potentially digestible fractions of NDF, while the indigestible fraction of NDF was decreased.

Ruminal, post-ruminal and total tract nutrient disappearances

Disappearance of ruminal, post-ruminal, and total tract of DM, CP, and starch are illustrated in Table 3. A significant difference (P<0.05) was observed in the ruminal disappearance of DM, CP, and starch between the samples. The highest ruminal CP digestibility was seen in SFB_{AUL}, and the lowest was noticed in SIFB. Ruminal starch disappearance was also affected by the treatments. Both SFB_{AUL} and $SIFB_{AUL}$ had higher ruminal starch disappearance than those of other treatments. The result of contrasts demonstrated that chemical treatment through steamed flaking processing, increased the ruminal disappearance of DM, CP, and starch (P<0.05). The post ruminal digestibility of DM was the highest for SIFB (0.57) compared with other treatments. The post-ruminal digestibility of CP was the highest for SIFB, while SFB_{AUL} had the lowest. Also, the highest post-ruminal digestibility of starch among the treatments belonged to $SIFB_{AUL}$, and the lowest value of this parameter was related to SFB. The use of M for chemical treatment of grains (SFB_{AUM} and $SIFB_{AUM}$) increased post-ruminal digestibility of CP and starch compared with chemical treatment by L (SFB_{AUL} and $SIFB_{AUL}$, P<0.05). The post-ruminal digestibility of CP and starch in steam flaked barley grain was greater than barley grain processed with the steam-infrared heated-

flaked method, as shown by the contrasts (P<0.05). The total tract DM, CP, and starch digestibility of samples was significantly affected by the treatments (P<0.05). Total tract DM digestibility in both SFB_{AUL} and $SIFB_{AUL}$ was higher (P<0.05) than that of the other groups. The highest value of the total tract CP digestibility was observed in SFB_{AUL} and $SIFB_{AUL}$. It was also perceived that total tract CP digestibility was lower for SIFB than those of the others. Total tract starch digestibility in both SFB_{AUL} and $SIFB_{AUL}$ was higher than those of the other samples (P<0.05). Chemical treatment increased (P<0.05) total tract DM, CP, and starch digestibility compared with non-chemical treatment. The results indicated a significant difference between acids, so that using L increased total tract DM, CP, and starch digestibility compared with M (P<0.05).

Scanning electron microscopy

Figure 1 shows scanning electron microscopy images of protein matrix and starch granules of steamed flaked and steamed-infrared heated-flaked barley grains. The size of starch granules and the percentage type of granules are shown in Table 4. It was understood that protein matrix and starch granules were influenced in all chemically treated samples. The starch granules, as well as their arrangement and size, were deformed in the flaked barley grain. Due to high temperature, moisture, and pressure during steamed flaking or steam-infrared heated-flaking, starch granules were swelled, and the median particle sizes of starch granules increased dramatically.

FTIR protein molecular structures

FTIR spectrum of each treatment is shown in Fig. 2. An FTIR spectrum in a general comparison of all samples is demonstrated in Fig. 3. The findings revealed that physical and/or chemical treatment had an effect on barley grain protein molecular spectrum range, as shown by the spectrum results. The sensitive region related to amide I raised due to C=O stretching of the peptide group is 1700-1600 cm^{-1} and is associated with the sum of the overlapped structures such as β-sheet and α-helix. Both SFB_{AUL} and $SIFB_{AUL}$ had the greatest wavenumber peak in amide I, amide II, α-helix, and β-sheet compared

Table 3: Ruminal, post ruminal and total tract disappearances of dry matter, crude protein and starch of physically and/or chemically treated barley grains using *in situ* mobile nylon bag technique

Parameters	Treatments						SEM	P-value			
								Treatment	Contrasts		
	SFB	SFB_{AUM}	SFB_{AUL}	SIFB	$SIFB_{AUM}$	$SIFB_{AUL}$			1	2	3
Ruminal											
DM	0.63^{c}	0.66^{b}	0.71^{a}	0.59^{d}	0.63^{c}	0.70^{a}	0.01	<0.001	<0.001	0.006	<0.001
CP	0.32^{d}	0.62^{b}	0.72^{a}	0.24^{e}	0.58^{c}	0.70^{a}	0.01	<0.001	<0.001	<0.001	<0.001
Starch	0.89^{b}	0.89^{b}	0.91^{a}	0.88^{c}	0.89^{b}	0.91^{a}	0.005	<0.001	<0.001	0.001	<0. 001
Post-ruminal											
DM	0.56^{a}	0.52^{b}	0.52^{b}	0.57^{a}	0.54^{b}	0.53^{b}	0.01	0.003	0.002	0.207	0.381
CP	0.88^{a}	0.82^{cd}	0.81^{d}	0.89^{a}	0.84^{b}	0.83^{bc}	0.005	<0.001	<0.001	<0.001	0.024
Starch	0.71^{d}	0.97^{b}	0.98^{ab}	0.73^{c}	0.97^{b}	0.99^{a}	0.002	<0.001	<0.001	<0.001	0. 002
Total tract											
DM	0.84^{b}	0.83^{bc}	0.86^{a}	0.82^{c}	0.83^{bc}	0.86^{a}	0.005	<0.001	0.003	0.098	<0.001
CP	0.92^{c}	0.93^{b}	0.95^{a}	0.91^{d}	0.93^{b}	0.95^{a}	0.002	<0.001	<0.001	0.382	<0.001
Starch	0.96^{bc}	0.97^{ab}	0.98^{a}	0.96^{bc}	0.97^{ab}	0.98^{a}	0.007	<0.001	<0.001	0.482	<0.001

SFB: Steam-flaked barley grain, SFB_{AUM}: Steam-flaked barley grain treated with ammonium bicarbonate, urea and malic acid, SFB_{AUL}: Steam-flaked barley grain treated with ammonium bicarbonate, urea and lactic acid, SIFB: Steam-infrared heated-flaked barley grain, $SIFB_{AUM}$: Steam-infrared heated-flaked barley grain treated with ammonium bicarbonate, urea and malic acid, and $SIFB_{AUL}$: Steam-infrared heated-flaked barley grain treated with ammonium bicarbonate, urea and lactic acid. $^{a, b, c, d}$ Means with different superscript letters in each row indicate significant difference (P<0.05). SEM: Standard error of the means. Contrasts: 1) SFB and SIFB versus SFB_{AUM}, SFB_{AUL}, $SIFB_{AUM}$ and $SIFB_{AUL}$, 2) SFB, SFB_{AUM} and SFB_{AUL} versus SIFB, $SIFB_{AUM}$ and $SIFB_{AUL}$, and 3) SFB_{AUM} and $SIFB_{AUM}$ versus SFB_{AUL} and $SIFB_{AUL}$

Table 4: The distribution the size of starch granules, type of granules and relative crystallinity of physically and/or chemically treated barley grains

Treatments	Average diameter of starch granules (µm)	Percentage of type of starch granule			Relative crystallinity (%)
		A-granules (>15 µm)	B-granules (5-15 µm)	C-granules (<5 µm)	
SFB	15.76	8.6	91	0.4	21.22
SFB_{AUM}	14.96	8.3	91.2	0.5	19.83
SFB_{AUL}	17.29	9.4	90.1	0.5	19.76
SIFB	18.33	10.6	88.8	0.6	21.31
$SIFB_{AUM}$	16.22	9.7	89.9	0.4	20.15
$SIFB_{AUL}$	17.76	9.4	90.2	0.4	19.62

SFB: Steam-flaked barley grain, SFB_{AUM}: Steam-flaked barley grain treated with ammonium bicarbonate, urea and malic acid, SFB_{AUL}: Steam-flaked barley grain treated with ammonium bicarbonate, urea and lactic acid, SIFB: Steam-infrared heated-flaked barley grain, $SIFB_{AUM}$: Steam-infrared heated-flaked barley grain treated with ammonium bicarbonate, urea and malic acid, and $SIFB_{AUL}$: Steam-infrared heated-flaked barley grain treated with ammonium bicarbonate, urea and lactic acid

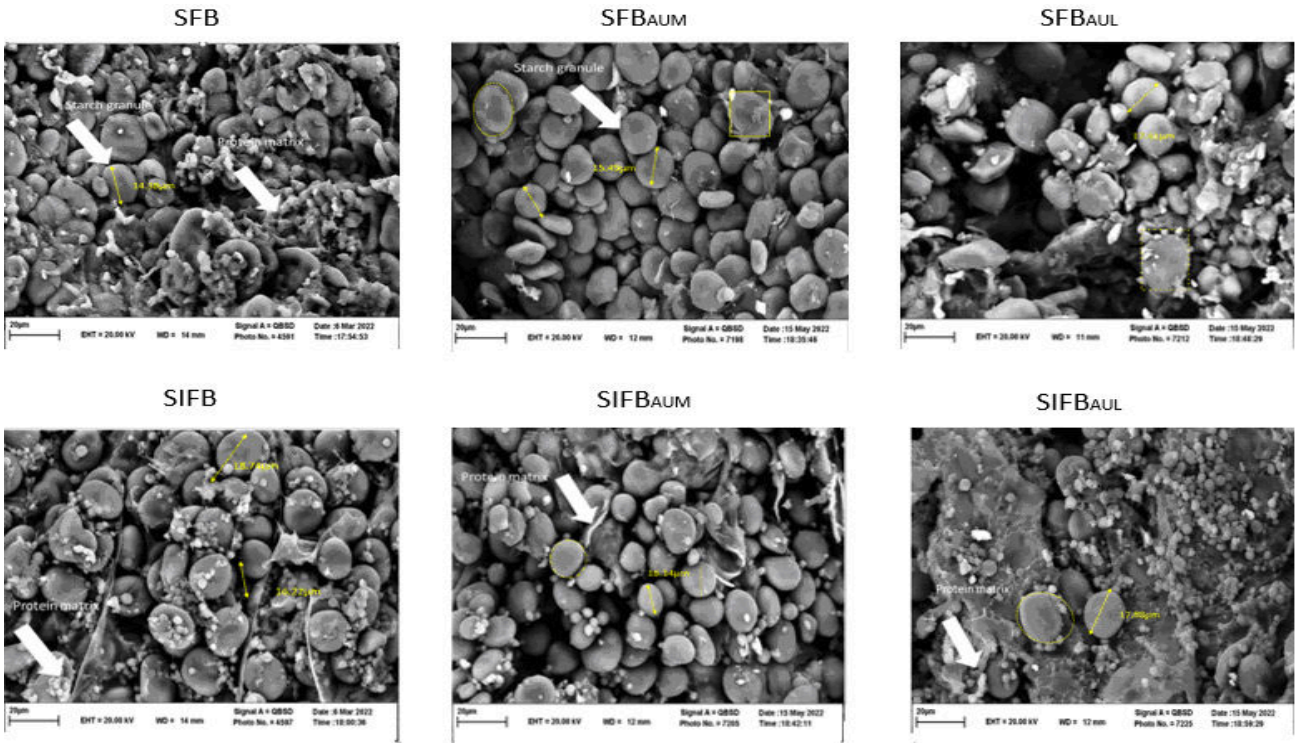

Fig. 1: Scanning electron microscopy morphology of starch granules of physically and/or chemically treated barley grains. **SFB:** Steam-flaked barley grain, **SFB$_{AUM}$:** Steam-flaked barley grain treated with ammonium bicarbonate, urea and malic acid, **SFB$_{AUL}$:** Steam-flaked barley grain treated with ammonium bicarbonate, urea and lactic acid, **SIFB:** Steam-infrared heated-flaked barley grain, **SIFB$_{AUM}$:** Steam-infrared heated-flaked barley grain treated with ammonium bicarbonate, urea and malic acid, and **SIFB$_{AUL}$:** Steam-infrared heated-flaked barley grain treated with ammonium bicarbonate, urea and lactic acid

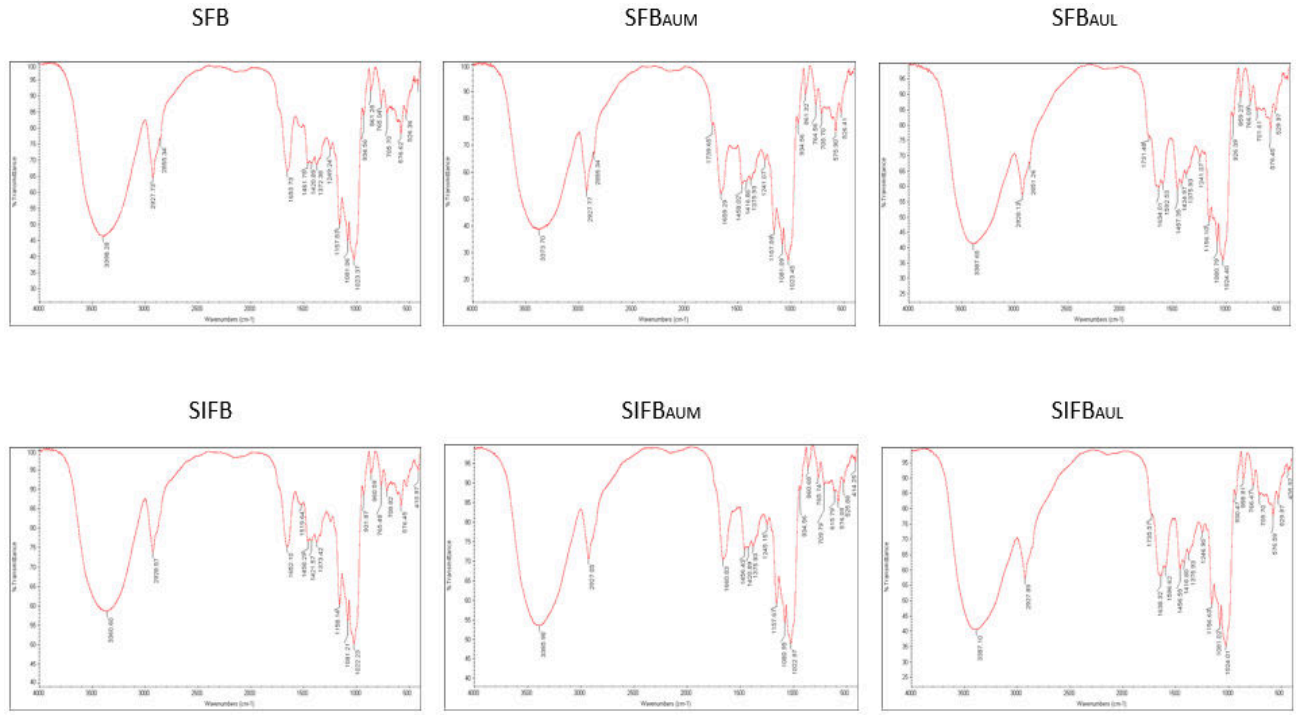

Fig. 2: Typical Fourier transform infrared spectroscopy (FTIR) of full molecular spectrum of physically and/or chemically treated barley grains. **SFB:** Steam-flaked barley grain, **SFB$_{AUM}$:** Steam-flaked barley grain treated with ammonium bicarbonate, urea and malic acid, **SFB$_{AUL}$:** Steam-flaked barley grain treated with ammonium bicarbonate, urea and lactic acid, **SIFB:** Steam-infrared heated-flaked barley grain, **SIFB$_{AUM}$:** Steam-infrared heated-flaked barley grain treated with ammonium bicarbonate, urea and malic acid, and **SIFB$_{AUL}$:** Steam-infrared heated-flaked barley grain treated with ammonium bicarbonate, urea and lactic acid

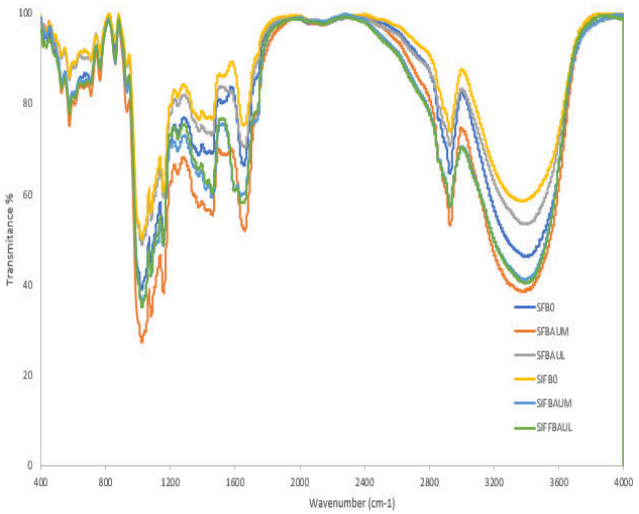

Fig. 3: Fourier transform infrared spectroscopy (FTIR) of physically and/or chemically treated barley grains. SFB: Steam-flaked barley grain, SFB_{AUM}: Steam-flaked barley grain treated with ammonium bicarbonate, urea and malic acid, SFB_{AUL}: Steam-flaked barley grain treated with ammonium bicarbonate, urea and lactic acid, SIFB: Steam-infrared heated-flaked barley grain, $SIFB_{AUM}$: Steam-infrared heated-flaked barley grain treated with ammonium bicarbonate, urea and malic acid, and $SIFB_{AUL}$: Steam-infrared heated-flaked barley grain treated with ammonium bicarbonate, urea and lactic acid

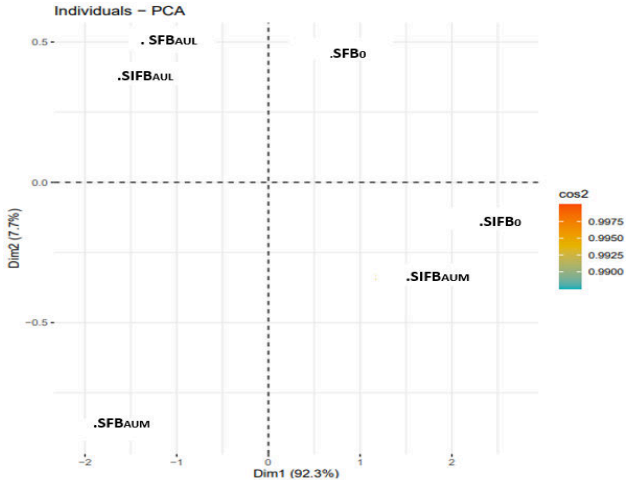

Fig. 4: Principle component analysis (PCA) whole spectra of protein molecular structures physically and/or chemically treated barley grains. SFB: Steam-flaked barley grain, SFB_{AUM}: Steam-flaked barley grain treated with ammonium bicarbonate, urea and malic acid, SFB_{AUL}: Steam-flaked barley grain treated with ammonium bicarbonate, urea and lactic acid, SIFB: Steam-infrared heated-flaked barley grain, $SIFB_{AUM}$: Steam-infrared heated-flaked barley grain treated with ammonium bicarbonate, urea and malic acid, and $SIFB_{AUL}$: Steam-infrared heated-flaked barley grain treated with ammonium bicarbonate, urea and lactic acid

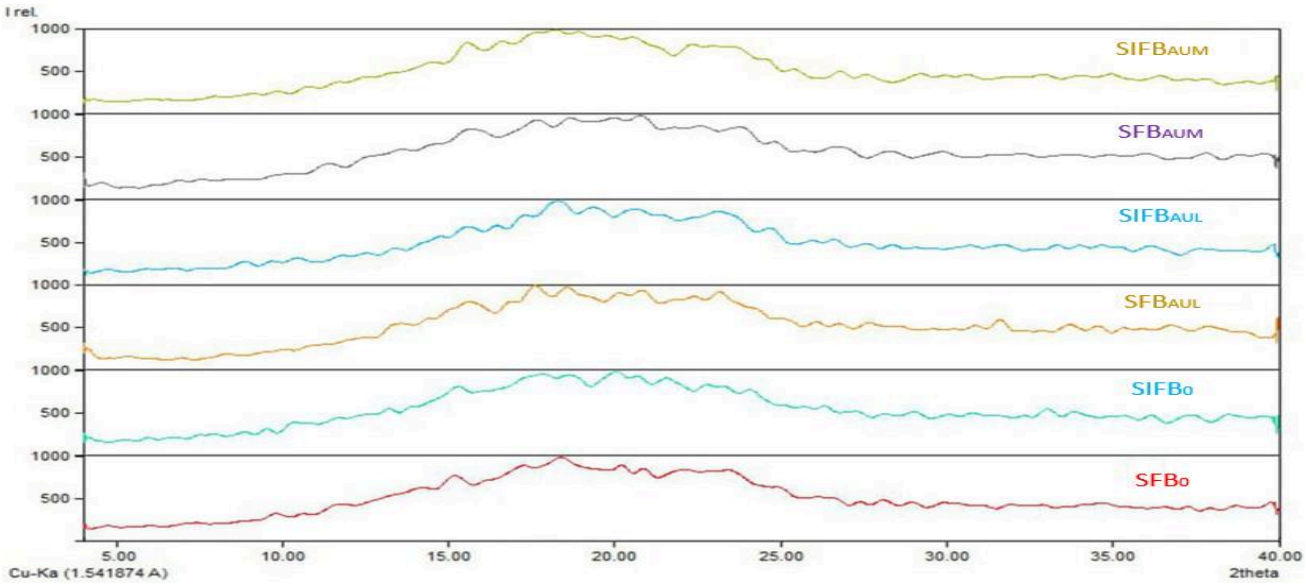

Fig. 5: The X-ray diffraction pattern of physically and/or chemically treated barley grains. SFB: Steam-flaked barley grain, SFB_{AUM}: Steam-flaked barley grain treated with ammonium bicarbonate, urea and malic acid, SFB_{AUL}: Steam-flaked barley grain treated with ammonium bicarbonate, urea and lactic acid, SIFB: Steam-infrared heated-flaked barley grain, $SIFB_{AUM}$: Steam-infrared heated-flaked barley grain treated with ammonium bicarbonate, urea and malic acid, and $SIFB_{AUL}$: Steam-infrared heated-flaked barley grain treated with ammonium bicarbonate, urea and lactic acid

with those of other samples. The PCA analysis indicated that the samples could be fully distinguished from each other because of the absence of heavy overlapping Fig. 4.

X-ray diffraction (XRD) spectra

The X-ray diffraction spectra of starch of physically and/or chemically treated barley are represented in Fig. 5. The relative crystallinity degree of treatments is supplied in Table 4. All samples showed very unique patterns when analyzed by XRD. The highest and lowest percentages of crystallinity were concerned with SIFB and $SIFB_{AUL}$. The striking point is that using acids had a higher effect on reducing the percentage of crystallinity.

Discussion

Nutrient composition

Our results illustrated that chemical and physical processing of barley grain ameliorated chemical compositions because the starch and CP contents were increased. One reason for the rise in protein content can be attributed to the addition of organic acids, urea, and ammonium bicarbonate to barley grains before physical processing. Present results confirmed the finding of Naseroleslami *et al*. (2018), who found that treating barley grain with alkaline compounds (ammonium, sodium hydroxide, and double sulfate of aluminum and potassium (alum)) significantly enhanced the CP content compared with untreated barley grain. Ammonization is an idea to increase dietary N content, promote increased ruminal pH, and regulate the rumen microflora, resulting in improved feed digestibility, performance, and wellbeing in beef and dairy cows (Belanche *et al*., 2021). The effect of organic acids on the CP concentration of processed grain has been considered in several studies. Harder *et al*. (2015) processed barley grains with 5% solutions (vol/vol) of citric acid or L for 24 h and heated at 22°C or oven-heating at 50°C. They reported that the concentration of CP in barley grain was significantly decreased, especially after chemical treatments with organic acids. They explained the decline in CP of processed barley could be related to washing out of soluble protein fractions mainly consisting of NPN or small peptides. In the present study, a reduction of NDF concentration in the L was seen. Present results confirmed the finding of Harder *et al*. (2015) who reported that, acids cause an increase in the hydrolysis of insoluble fiber fractions. It has been indicated that both heat and moisture during grain steam-flaking may alter starch content and reduce NDF and ADF (Rahimi *et al*., 2020). Another description may be due to the increase in CP, which resulted in a passive reduction (proportional reduction) in fiber. Chemical processing methods with mild acids can improve the nutrient value by modifying the starch content in treated grains (Rahmadani *et al*., 2023). The granules of starch enclosed in a protein matrix can react with organic acids (Harder *et al*., 2015). In our study, amylose was lower for chemically processed barley grains (Table 1, $P<0.05$). It is possible that the amylose was impressionable to hydrolysis by organic acid treatment (Harder *et al*., 2015). Chemical treatment reduced Ash content due to an increase in the solubility of minerals (Deckardt *et al*., 2014). Intensification hydrolysis of phytate in barley grain by processing with L could be rationale for the decrease of the Ash content (Metzler-Zebeli *et al*., 2014).

In vitro and *in situ* nutrient digestibility

In the present study, ruminal disappearance of DM, CP, and starch of flaked barley grain treated with L was increased. The combination of moisture, heat, and rolling causes the crystalline structure disruption, polysaccharide dissolution, and ruptured granule diffusion. This causes the starch granules to gelatinize (swell after absorbing water), altering the amount of starch fermented in the rumen and starch intestinal digestibility escaping the rumen degradation (Rahimi *et al*., 2020). In our study, amylose concentration in $SIFB_{AUL}$ was the lowest, indicating a more potentially digestible fraction of starch and ruminal and post-ruminal starch disappearance in this treatment. Chemical processing techniques have been found to be highly successful in reducing the proportion of rumen degradable starch and soluble fiber fraction, thereby enhancing the value of the slowly degradable fraction (Rahmadani *et al*., 2023). According to Naseroleslami *et al*. (2018), there was no significant difference in DM disappearances for barley grains during incubation in the rumen and post-rumen when treated with alkaline compounds (alum, liquid ammonia, and sodium hydroxide). However, when the *in situ* mobile nylon bag procedure was used, treated barley grain with alum had higher post-ruminal and total tract CP and starch disappearances compared with other treatments. They described the interaction between β-glucans and fiber fraction with viscosity, showing that raising the fiber fraction caused the feed ingredients to become more viscous, which slowed down the degradation of starch. The rate of rumen degradation can be slowed by using rolling, pelleting, and steam-flaking methods, allowing more CP and starch to bypass the rumen and enter the small intestine (Tosta *et al*., 2019a). Heat treatments have the potential to decrease rumen degradation and increase the bypass protein (Iommelli *et al*., 2022). In contrast with our results, steam flaking reduced rumen degradable protein for wheat, maize, and barley grains to varying degrees (Chrenkova *et al*., 2018). Present use of infrared radiation in chemically processed barley grain resulted in a significant decrease in the rapidly soluble portions of both protein and reservoir carbohydrates. However, it was observed that the treatment of radiation led to an increase in the slowly degradable fraction of starch when compared with the unprocessed grains (McAllister and Sultana, 2011). It is important to note that SIFB had a lower ruminal CP digestibility than SFB (Table 3). Taghavi *et al*. (2023), discovered that protein subfraction values are altered by microwave irradiation. The scenario of a decrease in the rate of ruminal CP degradation in the treated barley grains is represented by the transition from the ruminally degradable fraction to the partially ruminal degradable. The observed phenomenon can be ascribed to the denaturation of proteins under conditions of elevated temperature and moisture (Theodoridou and Yu, 2013). However, Peng *et al*. (2014) hypothesized that high temperatures and moisture could create cross-linkages between amino acids and sugars, increasing fiber bond CP.

Scanning electron microscopy

Present scanning electron microscopy images obtained from the barley grain, undergone chemical and physical processing, appeared to have potential for understanding any changes in the ruminal, post-ruminal, and total tract of DM and starch. Heat processing caused

granules to appear and enabled bacterial joining with higher colony quantities, causing high degradability of starch and DM in barley grain due to the high accessibility of fermentable carbohydrates (Rahmadani *et al*., 2023). Infrared radiation causes rapid internal heating, which evaporates the water within the grain, while high pressure ruptures the protein coat of the grain (Shirmohammadi *et al*., 2021). In this experiment, physical processing of barley grain showed some swelling and cracking in the starch granule, an increase in granules size due to gelatinization, and a loss of their crystalline structure, depending on how long the processing was done. Besides, the protein matrix is denaturized during the microwave heat treatment due to internal to external cracking (Taghavi *et al*., 2023). The protein matrix enveloping the starch granules has the potential to diminish the degree of starch digestion. The degree of starch gelatinization is influenced by the processing technique and various operational elements (Rahimi *et al*., 2020; Kokić *et al*., 2022).

FTIR protein molecular structures

The nutritional value, quality, and digestive fate of proteins are affected by secondary protein structures; the altered model-fitted α-helix to β-sheet ratio shows varied nutritive quality and protein availability (Yu, 2007). In this study, spectral bands linked to protein molecular structures were found between 1720 and 1450 cm^{-1}. These wavenumbers were similar to those found by Liu *et al*. (2013), Peng *et al*. (2014), and Gholizadeh *et al*. (2021). The most noticeable vibration bands of protein structure are connected to the amide I and II areas. The findings of the present study indicated that the different processing (chemical treatment and physical processing) of barley grains led to alterations in their inherent structures, which were largely supported by the previously published results of Prates *et al*. (2018), who examined the effect of three dissimilar heat methods to determine the relation between protein degradation and accessibility and protein molecular structure profiles in dairy cows. Yu (2011) suggested that heat processing may not have an impact on protein quality, which is not confirmed with our findings. Sun *et al*. (2018) showed that any changes in protein and carbohydrate spectral profiles caused by heating are connected to sources of protein and carbohydrate. PCA is a multivariate analysis technique that can be applied to IR spectrum analysis (Yu, 2005). This analysis can classify and differentiate inherent chemical structural differences and identify the primary sources of variation in protein fingerprint spectra (approximately 1720-1450 cm^{-1}). The generated PCA showed that the protein structures in processed barley grain can be put into separate groups. This means that the protein molecule structures in the samples are nearly different. It has been demonstrated that the ratio of α-helix to β-sheet correlates positively with effectively degraded CP and insoluble true protein but negatively with fiber-bound protein (Tosta *et al*., 2019b). There is a relationship between the composition of protein molecular structure and the changes observed in the digestibility of proteins in the rumen and intestines. The difference in digestibility between the treatments confirmed it.

X-ray diffraction (XRD) spectra

Based on the present results of starch X-ray diffraction patterns, the crystalline form of starch can be districted to A type, B type, C type, or V type (Van Hung *et al*., 2016). Examination of patterns showed some strong diffraction peaks when the diffraction angular 2θ are 15°, 18°, 19°, 20°, and 23°, which are typical A-type diffraction characteristics. Similar results have been observed by Chen *et al*. (2022), who reported that barley starch had an A crystalline packing arrangement with main diffraction peaks at around 15, 17, 18, and 23 (2θ). The position and intensity of the diffraction peaks are related to both chemical and physical processing methods. The striking point is that chemical processing had a higher effect on reducing the percentage of crystallinity in barley grain. A reduction in crystallinity was observed in soybean straw treated with alkaline compounds (sodium hydroxide and calcium oxide, Aslaniyan *et al*. (2023)). Our results confirm it because chemical treatment decreased crystallinity in barley starch. Decreasing the degree of polymerization, increases the solubility in water because the molecular weight is decreased, and this might increase the entropy driving force for dissolution due to the abating number of intermolecular interactions (Swantomo *et al*., 2017). The difference in *in vitro* and *in situ* nutrient digestibility among the samples may be due to this.

Present results indicate that treatment of barley grain with L during steaming improves the CP and starch content of flaked barley grain. In addition, the results show that treatment of barley grain with L or M enhance the ruminal potentially digestible fraction of DM, CP, starch, and NDF. While a reduction of the indigestible fraction of the nutrient is recorded. Overall, physical and/or chemical treatment of barley grain may introduce a new strategy to increase the nutrients entering post-rumen and intestinal digestibility. Both scanning electron microscopy and FTIR images demonstrate a high potential for physical and/or chemical treatment used in the present study for barley grain processing, as seen in the changing of starch morphology and protein structure.

Acknowledgements

The authors are grateful to Ferdowsi University of Mashhad, Iran, for their financial support (3/55045). We wish to thank Central Laboratory of Ferdowsi University of Mashhad for providing valuable help FTIR, SEM, and XRD analysis of the samples.

Conflict of interest

The authors confirm that the study was conducted in the absence of any commercial or financial relevant that could be interpreted as a potential conflict of interest.

References

Aboud, SA; Altemimi, AB; Al-HiIphy, A; Yi-Chen, L and Cacciola, F (2019). A comprehensive review on infrared heating applications in food processing. Molecules. 22: 4125-4145.

AOAC (2012). *Official methods of analysis*. (19th Edn.), Association of Official Analytical Chemists, Washington, D.C., PP: 121-130.

Arroquy, JI; Cochran, RC; Nagaraja, TG; Titgemeyer, EC and Johnson, DE (2005). Effect of types of non-fiber carbohydrate on *in vitro* forage fiber digestion of low-quality grass hay. Anim. Feed Sci. Technol., 120: 93-106.

Aslaniyan, A; Ghanbari, F; Kouhsar, JB and Shahraki, BK (2023). Comparing the effects of gamma ray and alkaline treatments of sodium hydroxide and calcium oxide on chemical composition, ruminal degradation kinetics and crystallinity degree of soybean straw. Appl. Radiat. Isot., 191, 110524.

Belanche, A; Martín-García, I; Jiménez, E; Jonsson, NN and Yañez-Ruiz, DR (2021). A novel ammoniation treatment of barley as a strategy to optimize rumen pH, feed degradability and microbial protein synthesis in sheep. J. Sci. Food Agric., 13: 5541-5549.

Benninghoff, J; Paschke-Beese, M and Südekum, KH (2015). *In situ* and *in vitro* ruminal degradation of maize grain and untreated or xylose-treated wheat, barley and rye grains. Anim. Feed Sci. Technol., 210: 86-93.

Bradshaw, WL; Hinman, DD; Bull, RC; Everson, DO and Sorensen, SJ (1996). Effects of barley variety and processing methods on feedlot steer performance and carcass characteristics. J. Anim. Sci., 74: 18-24.

Burakowska, K; Górka, P; Kent-Dennis, C; Kowalski, ZM; Laarveld, B and Penner, GB (2020). Effect of heat-treated canola meal and glycerol inclusion on performance and gastrointestinal development of Holstein calves. J. Dairy Sci., 103:7998-8019.

Chen, X; Ma, M; Liu, X; Zhang, C; Xu, Z; Li, H; Sui, Z and Corke, H (2022). Multi-scale structure of A- and B-type granules of normal and waxy hull-less barley starch. Int. J. Biol. Macromol., 200: 42-49.

Chrenkova, M; Formelova, Z; Ceresnakova, Z; Dragomir, C; Rajsky, M; Cismileanu, A and Weisbjerg, MR (2018). Rumen undegradable protein (RUP) and its intestinal digestibility after steam flaking of cereal grains. Czech J. Anim. Sci., 63: 160-166.

Deckardt, K; Khiaosa-ard, R; Grausgruber, H and Zebeli, Q (2014). Evaluation of various chemical and thermal feed processing methods for their potential to enhance resistant starch content in barley grain. Starch-Stärke. 66: 558-565.

Deckardt, K; Metzler-Zebeli, BU and Zebeli, Q (2016). Processing barley grain with lactic acid and tannic acid ameliorates rumen microbial fermentation and degradation of dietary fibre *in vitro*. J. Sci. Food Agric., 96: 223-231.

Eastridge, ML (2006). Major advances in applied dairy cattle nutrition. J. Dairy Sci., 89: 1311-1323.

Emmanuel, DGV; Dunn, SM and Ametaj, BN (2008). Feeding high proportions of barley grain stimulates an inflammatory response in dairy cows. J. Dairy Sci., 91: 606-614.

Eyni, B; Mesgaran, MD; Vakili, A and Valizadeh, R (2017). *In vitro* yield of microbial-n from fermentation of glucogenic and lipogenic diets provided by different sources of rumen degradable amino acids. J. Vet. Sci. Technol., 8: 4-10.

Fasina, OO; Tyler, RT; Pickard, MD and Zheng, GH (1999). Infrared heating of hulless and pearled barley. J. Food Process. Preserv., 23: 135-151.

Gholizadeh, H; Naserian, AA; Yari, M; Jonker, A and Yu, P (2021). Crude protein fractionation, in situ ruminal degradability and FTIR protein molecular structures of different cultivars within barley, corn and sorghum cereal grains. Anim. Feed Sci. Technol., 275: 114855.

Hall, MB (2014). Selection of an empirical detection method for determination of water-soluble carbohydrates in feedstuffs for application in ruminant nutrition. Anim. Feed Sci. Technol., 198: 28-37.

Harder, H; Khol-Parisini, A and Zebeli, Q (2015). Modulation of resistant starch and nutrient composition of barley grain using organic acids and thermal cycling treatments. Starch-Stärke. 67: 654-662.

Holopainen-Mantila, U (2015). Composition and structure of barley (*Hordeum vulgare* L.) grain in relation to end uses. Dissertation, University of Helsinki.

Hu, G; Burton, C and Yang, C (2010). Efficient measurement of amylose content in cereal grains. J. Cereal Sci., 51: 35-40.

Humer, E and Zebeli, Q (2017). Grains in ruminant feeding and potentials to enhance their nutritive and health value by chemical processing. Anim. Feed Sci. Technol., 226: 133-151.

Iommelli, P; Zicarelli, F; Musco, N; Sarubbi, F; Grossi, M; Lotito, D; Infascelli, F and Tudisco, R (2022). Effect of cereals and legumes processing on *in situ* rumen protein degradability: A review. Fermentation. 8: 363.

Iqbal, S; Zebeli, Q; Mazzolari, A; Bertoni, G; Dunn, SM; Yang, WZ and Ametaj, BN (2009). Feeding barley grain steeped in lactic acid modulates rumen fermentation patterns and increases milk fat content in dairy cows. J. Dairy Sci., 92: 6023-6032.

Iqbal, S; Zebeli, Q; Mazzolari, A; Dunn, SM and Ametaj, BN (2010). Feeding rolled barley grain steeped in lactic acid modulated energy status and innate immunity in dairy cows. J. Dairy Sci., 93: 5147-5156.

Kheirandish, P; Danesh Mesgaran, M; Javadmanesh, A; Mohri, M; Khafipour, E and Vakili, SA (2022). Effect of processed barley grain on *in vitro* rumen fermentation and fate of nitrogen metabolism. Iran. J. Appl. Anim. Sci., 12: 663-675.

Kokić, B; Dokić, L; Pezo, L; Jovanović, R; Spasevski, N; Kojić, J and Hadnađev, M (2022). Physicochemical changes of heat-treated corn grain used in ruminant nutrition. Animals. 12: 2234.

Kokić, B; Lević, J; Chrenková, M; Formelová, Z; Poláčiková, M; Rajský, M and Jovanović, R (2013). Influence of thermal treatments on starch gelatinization and *in vitro* organic matter digestibility of corn. Food Feed Res., 40: 93-99.

Liu, B; Thacker, P; McKinnon, J and Yu, P (2013). In-depth study of the protein molecular structures of different types of dried distiller's grains with solubles and their relationship to digestive characteristics. J. Sci. Food Agric., 93: 1438-1448.

McAllister, TA and Sultana, H (2011). Effects of micronization on the *in situ* and *in vitro* digestion of cereal grains. Asian-Australas. J. Anim. Sci., 24: 929-939.

Metzler-Zebeli, BU; Deckardt, K; Schollenberger, M; Rodehutscord, M and Zebeli, Q (2014). Lactic acid and thermal treatments trigger the hydrolysis of myo-inositol hexakisphosphate and modify the abundance of lower myo-inositol phosphates in barley (*Hordeum vulgare L.*). PLoS One. 9: 101-166.

Naseroleslami, R; Mesgaran, MD; Tahmasbi, A; Vakili, SA

and Ebrahimi, SH (2018). Influence of barley grain treated with alkaline compounds or organic extracts on *ex vivo* site and extent of digestion of starch. Asian-Australas. J. Anim. Sci., 31: 230-236.

National Research Council (2001). *Nutrient requirements of dairy cattle*. 7th Rev. Edn., Washington D.C., National Academy Press.

Noziére, P; Rémond, D; Lemosquet, S; Chauveau, B; Durand, D and Poncet, C (2005). Effect of site of starch digestion on portal nutrient net fluxes in steers Br. J. Nutr., 94: 182-191.

Offner, A; Bach, A and Sauvant, D (2003). Quantitative review of *in situ* starch degradation in the rumen. Anim. Feed Sci. Technol., 106: 81-93.

Peng, Q; Khan, NA; Wang, Z and Yu, P (2014). Moist and dry heating-induced changes in protein molecular structure, protein subfractions, and nutrient profiles in camelina seeds. J. Dairy Sci., 97: 446-457.

Prates, LL; Lei, Y; Refat, B; Zhang, W and Yu, P (2018). Effects of heat processing methods on protein subfractions and protein degradation kinetics in dairy cattle in relation to protein molecular structure of barley grain using advanced molecular spectroscopy. J. Cereal Sci., 80: 212-220.

Rahimi, A; Naserian, AA; Valizadeh, R; Tahmasebi, AM; Dehghani, H; Sung, KI and Nejad, JG (2020). Effect of different corn processing methods on starch gelatinization, granule structure alternation, rumen kinetic dynamics and starch digestion. Anim. Feed Sci. Technol., 268: 114572.

Rahmadani, M; Susanto, I; Prasetya, RD; Kondo, M; Nahrowi, N and Jayanegara, A (2023). Modification of dietary rumen degradable starch content by chemical processing of feed ingredients: A meta-analysis. Anim. Sci. J., 94: e13834.

Robinson, PH and Kennelly, JJ (1989). Influence of ammoniation of high-moisture barley on digestibility kinetics of rumen ingesta turnover, and milk production in dairy cows. Can. J. Anim. Sci., 69: 195-203.

Rose, R; Rose, CL; Omi, SK; Forry, KR; Durall, DM and Bigg, WL (1991). Starch determination by perchloric acid vs enzymes: evaluating the accuracy and precision of six colorimetric methods. J. Agric. Food Chem., 39: 2-11.

SAS Institute (2004). SAS®/STAT Software. Release 9.4, SAS Institute, Inc., Cary, NC, USA.

Shen, YZ; Ding, LY; Chen, LM; Xu, JH; Zhao, R; Yang, WZ; Wang, HR and Wang, MZ (2019). Feeding corn grain steeped in citric acid modulates rumen fermentation and inflammatory responses in dairy goats. Animal. 13: 301-308.

Shirmohammadi, S; Taghizadeh, A; Hosseinkhani, A; Moghaddam, GA; Salem, AZ and Pliego, AB (2021). Ruminal and post-ruminal barley grain digestion and starch granule morphology under three heat methods. Ann. Appl. Biol., 178: 508-518.

Sun, B; Rahman, MM; Tar'an, B and Yu, P (2018). Determine effect of pressure heating on carbohydrate related molecular structures in association with carbohydrate metabolic profiles of cool-climate chickpeas using Globar spectroscopy. Spectrochim. Acta, Part A. 201: 8-18.

Svihus, B; Uhlen, AK and Harstad, OM (2005). Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. Anim. Feed Sci. Technol., 122: 303-320.

Swantomo, D; Giyatmi, G; Adiguno, SH and Wongsawaeng, D (2017). Preparation of microcrystalline cellulose from waste cotton fabrics using gamma irradiation. Eng. J., 21: 173-182.

Taghavi, M; Taghizadeh, A; Mehmannavaz, Y; Hoseinkhani, A; Mohammadzadeh, H; Macit, M; Palangi, V and Lackner, M (2023). Degradability of *Vicia ervilia* grain using *in situ* and CNCPS methods, and model-based analysis of its ruminal degradation. Fermentation. 9: 419.

Theodoridou, K and Yu, P (2013). Application potential of ATR-FT/IR molecular spectroscopy in animal nutrition: revelation of protein molecular structures of canola meal and presscake, as affected by heat-processing methods, in relationship with their protein digestive behavior and utilization for dairy cattle. J. Agric. Food Chem., 61: 5449-5458.

Tosta, MR; Prates, LL; Christensen, DA and Yu, P (2019a). Effects of processing methods (rolling vs. pelleting vs. steam-flaking) of cool-season adapted oats on dairy cattle production performance and metabolic characteristics compared with barley. J. Dairy Sci., 102: 10916-10924.

Tosta, MR; Prates, LL; Christensen, DA and Yu, P (2019b). Biodegradation kinetics by microorganisms, enzymatic biodigestion, and fractionation of protein in seeds of cool-climate-adapted oats: Comparison among oat varieties, between milling-type and feed-type oats, and with barley grain. J. Cereal Sci., 89: 102814.

Van Hung, P; Vien, NL and Phi, NTL (2016). Resistant starch improvement of rice starches under a combination of acid and heat-moisture treatments. Food Chem., 191: 67-73.

Van Soest, PV; Robertson, JB and Lewis, BA (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.

Xu, N; Liu, J and Yu, P (2018). Alteration of biomacromolecule in corn by steam flaking in relation to biodegradation kinetics in ruminant, revealed with vibrational molecular spectroscopy. Spectrochim. Acta, Part A. 191: 491-497.

Yu, P (2005). Applications of hierarchical cluster analysis (CLA) and principal component analysis (PCA) in feed structure and feed molecular chemistry research, using synchrotron-based Fourier transform infrared (FTIR) micro spectroscopy. J. Agric. Food Chem., 53: 7115-7127.

Yu, P (2007). Protein molecular structures, protein subfractions, and protein availability affected by heat processing: a review. Am. J. Biochem. Biotechnol., 3: 66-86.

Yu, P (2010). Plant-based food and feed protein structure changes induced by gene-transformation, heating and bio-ethanol processing: A synchrotron-based molecular structure and nutrition research program. Mol. Nutr. Food Res., 54: 1535-1545.

Yu, P (2011). Dry and moist heating-induced changes in protein molecular structure, protein subfraction, and nutrient profiles in soybeans. J. Dairy Sci., 94: 6092-6102.

Zhao, Y; Yan, S; He, Z; Anele, UY; Swift, ML; McAllister, TA and Yang, W (2016). Effect of starch content and processing method on *in situ* ruminal and *in vitro* intestinal digestion of barley grain in beef heifers. Anim. Feed Sci. Technol., 216: 121-128.