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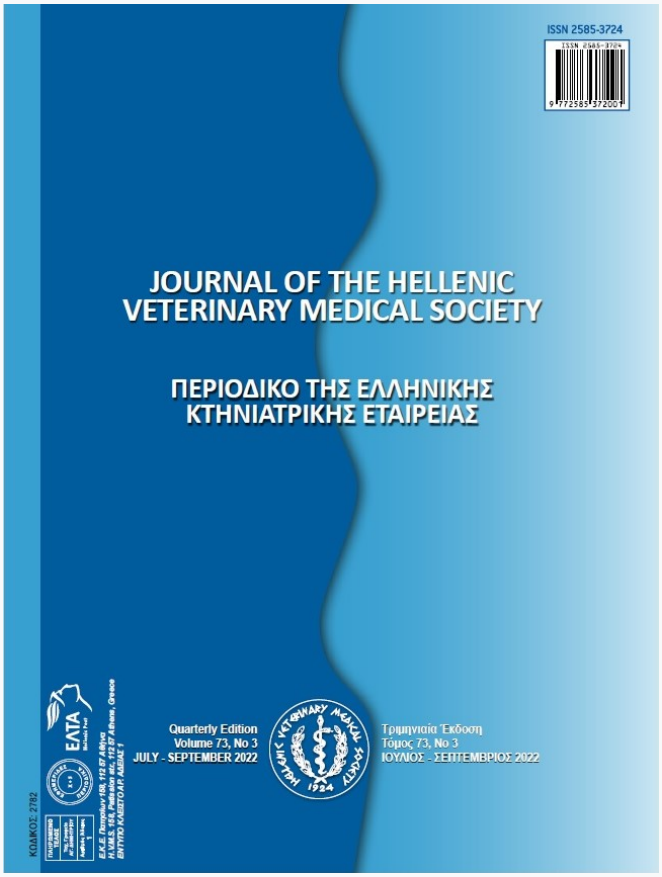

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Effects of different heat processing methods of rapeseed on ruminal and post-ruminal nutrient disappearance

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ABSTRACT: Due to improving the nutritive value of oilseeds and changing their digestion site in ruminants, processing, including heat treatment, seems necessary. The present research was conducted to evaluate the effect of applying heat processing such as roasting, microwaving and autoclaving on nutritive values; the amount and rate of degradability in rumen, and the disappearance of rapeseed nutrients in rumen, post-rumen and total tract. This evaluation was performed using mobile nylon bags techniques; three-step method of digestion and cornell net carbohydrate protein system (CNCPS) fraction. A completely randomized design was used to investigate the effect of applying heat processing, and SAS software was used to analyze the data. The field emission scanning electron microscope (FESEM) was used to monitored the effect of heat treatment on surface of rapeseed. The application of heat processing in this research (roasting, microwaving and autoclaving) had no significant effect on the chemical composition of rapeseed ($P>0.05$). The results obtained from mobile nylon bags method and three-step digestion method showed that raw rapeseed has the highest disappearance of DM and CP in rumen and therefore has a significant difference with processed seeds ($P<0.05$). Also, the disappearance of Dry matter (DM) and Crude protein (CP) of processed rapeseed in intestines was significantly higher than raw seed ($P<0.05$), and this was higher than other processing for autoclaved rapeseed. According to the results obtained from CNCPS protein fractionation, applying heat processing altering protein fractionation ($P<0.05$). Applying microwave processing has created cracks in the surface of the rapeseed wall, and this condition was not observed in the wall surface of other heated seeds. In general, it can be said that in addition to increasing the digestibility of rapeseed in the entire gastrointestinal tract, applying heat processing reduces its degradability in the rumen and has increased the disappearance of nutrients in the intestine, that it can be stated the digestion site is altered from rumen to intestine, which can prevent the loss of protein sources in ruminant feed.

Keywords: CNCPS, heat processing, mobile nylon bag, rapeseed

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INTRODUCTION

Whole rapeseed contains approximately 21% CP and 43% crude fat (CF) and have a proper amino acid composition for ruminants (Munoz et al., 2019). Due to the low water requirement of rapeseed plant and its high adaptation to the climatic conditions of countries with arid and semi-arid climates such as Iran, and considering the leading drought conditions, it can be said that rapeseed can be another substitute for other oilseeds in ruminant nutrition and on the other hand according to the high fat amount of seed and also the high quality of the oil resulting from it, the cultivation of this seed has become important.

Whole rapeseed has a hard shell that contains about 16% of the DM of the seed and acts as a barrier against microbial digestion in the rumen, so rapeseed is digested to a small extent in ruminants unless the seed cover is broken (Nega and Woldes 2018). On the other hand, as a result of breaking the seed shell, the seed protein is rapidly degraded in the rumen. Therefore, processing methods that break down the seed shell but reduce the degradability of protein in the rumen, and increase its nutritional value for ruminants, become important.

Physical and chemical processing, including heat processing can reduce protein degradability in rumen (Palangi and Majit 2019) and the bio-hydrogenation of fatty acids in the rumen by changing the protein matrix of oilseeds (Lashkari et al., 2015). Heat applying, such as microwave irradiation, creates transverse bridges within the peptide chains and between the chains with carbohydrates, and reduces the solubility of the protein (Paya et al., 2016) and the rumen degradability of the protein. Heat processing can alter the digestion site of nutrients, especially protein, and change digestion site from rumen to the post ruminal (Paya and Taghizadeh 2020). Hence, heat processing is widely used in ruminant nutrition. Heat processing makes the release of N-NH_3 in the rumen slower by making the protein more resistant to degradation. It has also been reported that applying heat to oilseeds protects unsaturated fatty acids from complete ruminal bio-hydrogenation and thus can improve and increase the concentration of unsaturated fatty acids in ruminants products (Paya and taghizadeh 2020).

Due to the lack of comprehensive information regarding the effect of various heat processing such as roasting, heat application along with humidity and microwaving on ruminal and post ruminal degradability and rapeseed protein fractionation, the present research was conducted to investigate various heating methods on the ruminal and post ruminal degradability using mobile nylon bags methods, three-step laboratory method of digestion and protein fractionation for rapeseed.

MATERIAL AND METHOD

Rapeseed Preparation and Heat Processing

Rapeseed used in this research was prepared from the livestock feed stores (VW29+V76, Mamaqan) East Azerbaijan Province, Iran. Three types of heat processing were used to process rapeseed. Heating rapeseeds were performed in two ways: dry heating using an oven at 120° C for 30 minutes and heating along with pressure and humidity that was performed by autoclave at 120° C for 30 minutes. Also, for processing rapeseed with microwave, 100 gr of rapeseeds were placed in a Pyrex pan (30×30×10 cm) with the height of 1-2 cm and were subjected to microwave irradiation at a power of 800 W for 4 minutes (Paya and taghizadeh 2020).

Chemical Compounds

To obtain information about rapeseed nutrients composition, DM, CP, crude fat, organic matter, Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) were measured according to the proposed AOAC (2005) and methods.

The method that described by Licitra et al., (1996) was used to determine various fractions of the protein. According to the report of Sniffen et al. (1992), feed protein has been composed of 5 fractions: non-protein nitrogen (A), rapid (B1), intermediate (B2) and slow (B3) rate degradability true protein, and inaccessible nitrogen (c). Fraction A (non-protein nitrogen) was estimated based on subtracting the total nitrogen from the true protein. Fraction C was also obtained by measuring the ADF nitrogen of the feed. To determine the ratio of fractions B1, B2 and B3, true protein measurement, soluble nitrogen in buffer solvent, ADF and NDF according to the method of Licitra et al. (1996) were used.

The true protein was determined using nitrogen deposited on Whatman 541 paper after applying TCA on the feed and using a Kjeldahl apparatus. Fraction B1 was obtained by subtracting the amount of soluble protein in the buffer solvent from the true protein. Fraction B2 was calculated by subtracting insoluble nitrogen in neutral detergent from soluble nitrogen in the buffer solvent. Fraction B3 was calculated by

subtracting insoluble nitrogen in acid detergent from insoluble nitrogen in neutral detergent.

Modified Three-Step Digestion Method

An modified method of Calsamiglia and Stern (1995) was used to perform this experiment. In this method, the raw and processed rapeseeds were incubated in the rumen for 12 hours and then the nitrogen amount of residual was measured and the residues from incubation were transferred to 50 ml vials. At this stage, three empty vials were also considered as Blank (to neutralize the nitrogen amount of the enzymes used). Then, 10 ml of pepsin-hydrochloric acid solution (2 g of pepsin in 1 liter of 0.1 N hydrochloric acid at 37° C) was added to each vial and incubated at 38° C for 1 hour. Then 0.5 ml of 1 N sodium hydroxide solution and 13.5 ml of pancreatin phosphate buffer (68 g KH_2PO_4 in 1 liter of distilled water at 37° C with a pH of exactly 7.8 and 6 g of pancreatin that was added to it) was added to each vial. The samples were incubated again for 24 hours in a 38° C incubator and then 3 ml of TCA solution (100 g of trichloroacetic acid in 100 ml of distilled water) was added to each vials. The vials were kept at room temperature for 15 minutes then centrifuged by 10000 g and 5 ml of the upper solution of each vial was taken, and its nitrogen was measured. Finally, using the Calsamiglia and Stern (1995) equations the digestibility of seeds was calculated.

Mobile Nylon Bags Method

The method recommended by Taghizadeh et al. (2005), which was a modified method of DeBoer et al. (1987), was used to determine the disappearance of DM and CP of the tested foodstuffs in intestines. A Holstein steers (age: 10 month, weight: 370 kg) with a T-shaped intestinal cannula was used for this purpose. Steer was fed with mixed ratio (ME: 2.14 Mcla /kg DM and CP: 17%) containing 70% forage (dry alfalfa) and 30% concentrate feeds (30% barley grain, 30% corn grain, 18% soybean meal, 15% wheat bran, 5% cotton seed meal, 1.5% vitamin supplement and Minerals, and 0.5% salt) prepared according to the recommendation of the National Research Association (NRC 2001). The animal was given ratio twice a day (9 in the morning and 4 in the afternoon). Water was available to the animal during the experiment.

Initially, 5 g of each tested feedstuff (raw and processed rapeseed) was ground with a 2 mm sieve and was poured inside nylon bags made of synthetic polyester fiber with the dimensions of 6×12 cm and the pore diameter of 45 micrometers and incubated in the rumen for 12 hours. After the mentioned time, the bags were removed from the rumen and washed with cold water until clear water came out from bags. The bags were then dried at 65° C temperature and the rumen disappearance of DM and CP of the samples were determined. Then the remaining samples were poured into polyester bags with the dimensions of 3×2 cm with a pore size of 45 micrometers. Six bags were considered for each sample. The bags were released by intestinal T-shaped canola at the beginning of the steer's small intestine (one bag every half hour) and collected from the feces after 24 to 48 hours. After collecting the bags from feces, all the bags were completely washed and dried with cold water (65° C for 48 hours) and the amount of DM and CP were determined. Finally, the DM and protein disappeared in the intestine was calculated based on the equations reported by DeBoer et al. (1987).

Field Emission Scanning Electron Microscope (FESEM) Procedure

To stabilize the sample, 4% glutaraldehyde solution was used for 12 hours at 4° C temperature and then detergent solution (4% Glutaraldehyde in 2M Sodium Cacodylate) was used for 15 minutes. For dehydration of the samples, the Parakhia (2017) method was used in which a series of dilutions of 30, 50, 70, 80 and 100 acetones were used that the samples were placed in each one of the solutions for 15 minutes. Finally, after drying the samples at 38° C for 30 minutes, gold plating was applied on the samples and photography was performed by FESEM (MIRA3 TESCAN) device.

Statistical Model Used

The data (disappearance of rumen, intestines and the total gastrointestinal tract) were statistically analyzed in a completely random design with the statistical model of $Y_{ij}=\mu+T_i+e_{ij}$ and using SAS statistical software version 9.2 (2012), and the mean were compared by Duncan method with 95% probability level.

RESULTS

Chemical composition

The chemical composition of raw and processed rapeseed have been reported in Table 1. As it is clear from the results obtained from the research, the application of heat processing in this research (roasting, microwaving and autoclaving) have not had a significant effect on the chemical composition of rapeseed.

However, the application of heat processing altered the chemical composition numerically, and this point is more evident in NDF and ADF, and their amounts are lower in heated seeds. According to the results of the present research, a slight decrease in the NDF and ADF due to microwave heating was observed.

Table 1. Chemical composition of raw and heat treated rapeseed (% of DM; n=4)

	Nutrients				
	CP	OM	NDF	ADF	EE
Unheated	20.9	94.2	20.1	17.3	39.4
Roasted	20.7	95.3	18.5	15.1	41.0
Microwave	20.4	94.1	19.3	17.0	40.4
Autoclaved	21.3	94.2	19.6	15.5	40.0
SEM	0.38	0.43	0.81	0.78	0.98
P value	0.39	0.19	0.60	0.16	0.68

CP: Crude protein; OM: Organic matter; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; EE: Ether extract; SEM: Standard error of the mean

Ruminal and Post Ruminal Degradability

To determine the ruminal and post-ruminal disappearance of raw and processed rapeseed, the method of mobile nylon bags and three-step laboratory methods Calsamiglia and Stern (1995) were used.

The DM and CP disappearance of raw and processed rapeseeds with three processes of roasting, microwaving and autoclaving have been reported in Tables 2 and 3. The obtained results showed that the heat processing used in this research could reduce the ruminal degradability of DM and CP of rapeseed, and this possibly lead to the supply of more nutrients and feed protein to the small intestine. The results obtained from mobile nylon bags method showed that raw rapeseed have the highest disappearance of DM and rumen CP and therefore have a significant difference with processed seeds (P<0.05). Also, the intestines disappearance of DM and CP of roasted, processed with microwave and autoclaved rapeseed was significantly higher than raw seed (P<0.05), and it was higher for autoclaved rapeseed than other processes.

The results showed that the DM and CP disappearance in the total gastrointestinal tract for processed rapeseed has significant difference from heat (roasting, microwaving and autoclaving) and this means that the application of heat processing in this research in addition to altering the digestion site of DM from the rumen to the intestine, has also increased digestibility and has compensated for the reduction of ruminal disappearance in processed seeds, that this trend has also been observed regarding the disappearance raw and processed rapeseeds CP.

Table 2. Ruminal, post-ruminal and total tract nutrient disappearance of raw and heat treated rapeseed by mobile nylone bag technique (%)

	Ruminal	Post-ruminal	Total tract
DM			
Unheated	18.2^{ab}	51.7^{c}	69.9^{c}
Roasted	19.1^{a}	56.4^{b}	75.5^{ab}
Microwave	17.9^{ab}	59.2^{a}	77.1^{a}
Autoclaved	16.9^{b}	56.8^{b}	73.7^{b}
SEM	0.52	0.56	0.76
P value	0.04	<0.0001	0.0002
CP			
Unheated	18.5^{a}	49.2^{b}	67.7^{b}
Roasted	15.6^{b}	60.1^{a}	75.7^{a}
Microwave	15.2^{b}	59.9^{a}	75.1^{a}
Autoclaved	14.7^{b}	59.8^{a}	74.5^{a}
SEM	0.72	0.66	0.66
P value	<0.0001	<0.0001	<0.0001

SEM: Standard error of the mean
Means in the same columns not sharing the same superscript are different ($p < 0.05$).

Table 3. Ruminal, post-ruminal and total tract nutrient disappearance of raw and heat treated rapeseed by modified three-step digestion technique (%)

	Ruminal	Post-ruminal	Total tract
DM			
Unheated	18.2^{ab}	48.7^{c}	66.9^{b}
Roasted	19.1^{a}	52.3^{b}	71.4^{a}
Microwave	17.9^{ab}	54.6^{a}	72.5^{a}
Autoclaved	16.9^{b}	52.9^{ab}	69.8^{a}
SEM	0.52	0.60	0.84
P value	0.073	0.0001	0.003
CP			
Unheated	18.5^{a}	46.4^{c}	64.9^{b}
Roasted	15.6^{b}	55.5^{b}	71.1^{a}
Microwave	15.2^{b}	57.1^{ab}	72.3^{a}
Autoclaved	14.7^{b}	57.9^{a}	72.6^{a}
SEM	0.72	0.64	0.64
P value	<0.0001	<0.0001	<0.0001

SEM: Standard error of the mean
Means in the same columns not sharing the same superscript are different ($p < 0.05$).

CNCPS Protein fractions

The percentage of CNCPS protein fractions of raw and heated treated rapeseed has been reported in Table 4. This fraction includes non-protein nitrogen (Fraction A), rapid degradable true Protein (Fraction B1), intermediate degradable true protein (Fraction B2), slow degradable true protein (Fraction B3), and indigestible protein (Fraction C). The purpose of this research in this section is to study the effect of some heat processing (roasting, microwaving and autoclaving) on various fractions of the rapeseed protein (CNCPS). In general, a significant difference was observed between various fractions of the protein ($p<0.05$). Fraction A decreased in all three types of processing, that this reduction was significant due to heat application ($p<0.05$).By applying heat processing, fraction B1 of rapeseed increased significantly ($p<0.05$) and the highest increase was in autoclaving process. For fraction B2, only the application of roasting process caused a significant increase ($p<0.05$).

The amount of fraction B3 increased significantly due to the application of roasting processing ($p<0.05$). Fraction C is non-degradable and has a direct relationship with thermal damage.

Table 4. The percentage of CNCPS protein fractions of raw and heated treated rapeseed

	Protein fractions (% CP)				
	A	B1	B2	B3	C
Unheated	9.7^{a}	43.2^{c}	33.5^{b}	9.3^{a}	4.0^{b}
Roasted	7.4^{b}	48.2^{a}	36.2^{a}	3.8^{b}	4.1^{ab}
Microwave	7.2^{b}	48.0^{a}	34.7^{ab}	4.8^{b}	5.1^{a}
Autoclaved	8.3^{b}	46.3^{b}	35.8^{ab}	4.4^{b}	5.0^{ab}
SEM	0.35	0.38	0.78	0.33	0.33
P value	0.0009	<0.0001	0. 047	<0.0001	0.058

SEM: Standard error of the mean
Means in the same columns not sharing the same superscript are different ($p< 0.05$).

Field Emission Scanning Electron Microscope (FESEM) Procedure

In this research, using the field emission scanning electron microscope, the rapeseed surface was photographed after applying heat processing (Fig. 1, 2 and 3). As it is specified in the images, applying microwave processing has created cracks in the surface of the rapeseed wall, and this condition was not observed in the wall surface of other heated seeds. In Fig. 4 the crack resulting from microwave application is also observed.

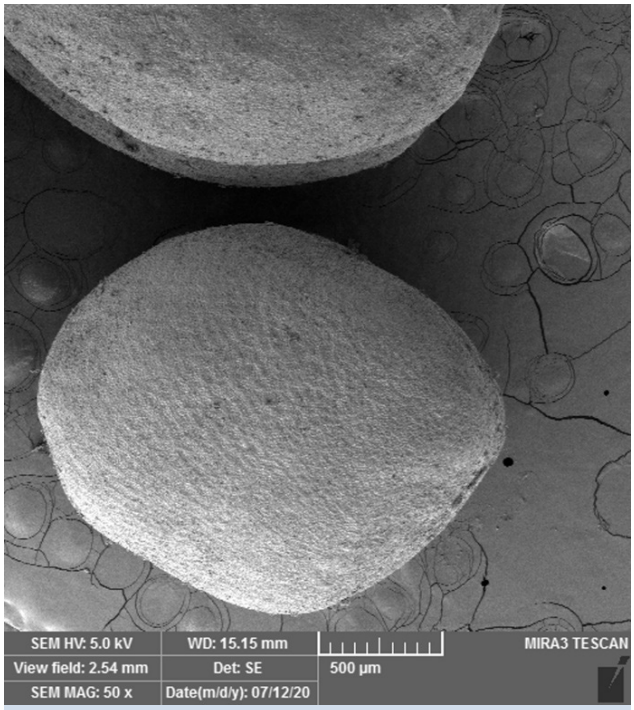

Fig. 1 FESEM (MIRA3TESCAN) image for roasted rapeseed.

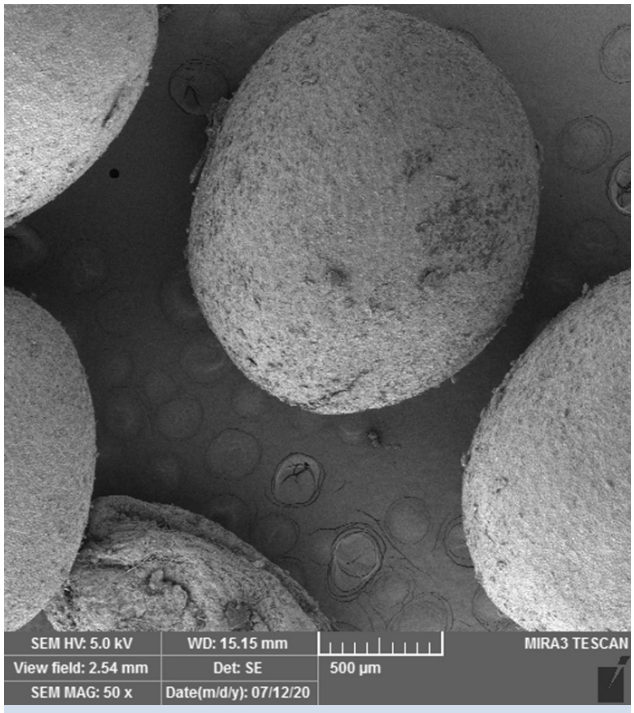

Fig. 2 FESEM (MIRA3TESCAN) image for autoclaved rapeseed

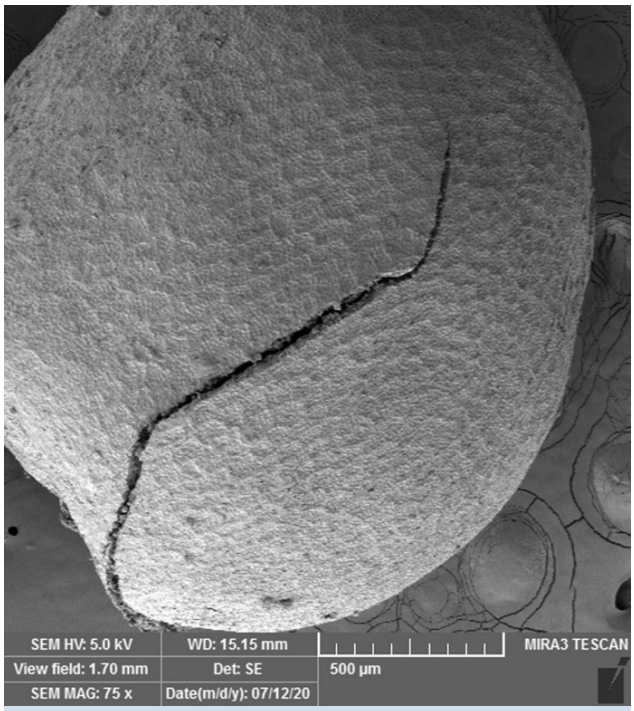

Fig. 3 FESEM (MIRA3TESCAN) image for microwave irradiated rapeseed

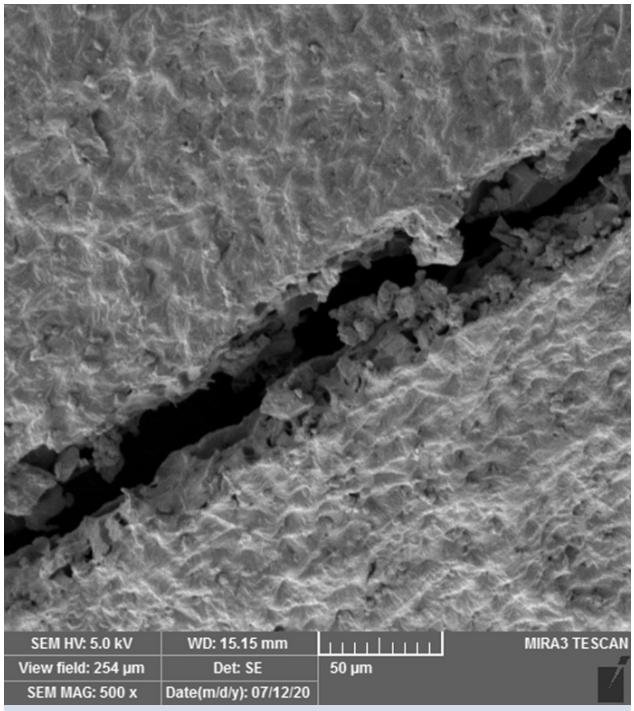

Fig. 4 FESEM (MIRA3TESCAN) image for microwave irradiated rapeseed

DISCUSSION

Chemical Compounds

The application of heat processing in this research (roasting, microwaving and autoclaving) have not had a significant effect on the chemical composition of rapeseed and considering the point that rapeseeds used for all treatments are from one plant variety and are purchased from one place, the lack of difference in chemical composition between all treatments can be justified. It seems that the reduction of some seed wall compounds during the heat application, especially in roasting and autoclaving, has reduced the NDF and ADF amounts in the treated seeds, that such a reduction in the cell wall has been reported due to heat application in other fat seeds such as soybeans (Fathi-nasiri et al., 2008) and flaxseed (Lashkari et al., 2015). One of the reasons that can cause a significant change in the amount of feedstuff cell wall is a factor that can eliminate the ester bond between phenolic acids and lignin-bonded polysaccharides and heat processing change the amount of these compounds less. According to the conducted studies regarding microwave radiation and other ionizing radiations such as gamma and electron, most researchers (Sadeghi and Shawrang 2008; Shawrang et al., 2011) agree on this idea that radiation has no significant effect on chemical compounds such as cell wall, but no enough reason was found in references indicating that microwave radiation does not affect chemical compounds.

Ruminal and Post Ruminal Degradability

The obtained results showed that the heat processing used in this research could reduce the ruminal degradability of DM and CP of rapeseed, and this possibly lead to the supply of more nutrients and feed protein to the small intestine. But these results will be acceptable when this processing does not have a negative effect on the disappearance of DM and CP of rapeseed in the small intestine, which was also examined in this experiment.

In ruminant determination of nutritional value of feeds and the effect of various processes including heat treatment is important, also it is important to study the amount of disappearance in the rumen, intestine and the entire gastrointestinal tract, which can be examined using methods such as nylon bags, gas production technique and three-step laboratory method (Ayasan et al., 2021).

The results obtained from mobile nylon bags method showed that raw rapeseed have the highest disappearance of DM and rumen CP. Such a decrease in ruminal disappearance for oil seeds due to heating has been reported for soybean seed (Fathi-Nasri et al., 2008) and flaxseed (Lashkari et al., 2015). Reduction

of ruminal degradability of rapeseed due to heat application (all three types of processing) can be due to change in the internal composition of rapeseeds, which increases their resistance against ruminal degradability.

Palangi and Majit (2019) reported that the application of heat could significantly reduce the degradability of DM and CP in the rumen, citing changes in the physical structure of chemical compounds such as feed protein and carbohydrates.

The higher post ruminal disappearance of the protein for heat treated rapeseeds confirms this hypothesis that due to the heat applied in this experiment and for a certain period of time, only the primary Millard products are formed and the production of products with higher degrees of Millard that do not have the digestibility in post rumenal has been prevented.

Considering the point that a significant difference between the degradability of DM and the CP of heat treated rapeseed (all three methods) indicate that breaking the rapeseed cover by microwaving may not be sufficient for effective degradation by rumen microorganisms, that such a case has also been reported by Wang et al. (1997) and Shirmohammadi et al. (2021).

CNCPS Protein fractions

This fraction includes non-protein nitrogen (Fraction A), rapid degradable true Protein (Fraction B1), intermediate degradable true protein (Fraction B2), slow degradable true protein (Fraction B3), and indigestible protein (Fraction C). Fraction A is the same as non-protein nitrogen, and consuming nitrogen more than necessary and ingesting large amounts of non-protein nitrogen into the rumen may cause its loss through ammonium conversion and eventually excretion in the form of urea in the feces and urine (NRC 2001). Fraction B1, or true protein, has a high rate of degradation and is degraded almost completely in the rumen (Chrenkova et al., 2014). Fraction B2, or true protein with the intermediate degradation rate, makes up the highest amount of CP. Heating and generally over-processing of feedstuffs destroy B2 fraction of proteins and make them insoluble, and in this case fractions B3 and C increase (Mirzaii-Alamoti et al., 2005; Chrenkova et al., 2014). In fact, B2 is a fraction of the true protein that has degradability both in the rumen and post rumen. The difference between this fraction and B3 is in the solubility of fraction B3 in acid detergent and insolubility in neutral detergent, while B2 has this solubility in both solutions. Since fraction B2 is calculated by the difference method, all errors due to measurement are entered in this fraction, which is probably one of the reasons for the difference between the values reported in the present experiment and others.

Fraction B3 is not dissolved in neutral detergent but is dissolved in acid detergent and its amount is calculated by subtracting the amount of insoluble protein in acidic detergent from the insoluble protein in neutral detergent. Heating foodstuffs increases the B3 fraction of them (Espinos et al., 2020; Hangshu et al., 2021).

Fraction C contains an inaccessible and insoluble protein in an acid detergent. This fraction contains proteins bound to lignin and tannins and products obtained from the millard reaction that have high resistance to degradation by rumen bacteria and digestive enzymes, so its amino acids are not absorbed in the post rumen (NRC 2001).

Field Emission Scanning Electron Microscope (FESEM) Procedure

There is little information and images regarding the surface of oilseeds after applying heat processing. As it is specified in the images, applying microwave processing has created cracks in the surface of the rapeseed wall, and this condition was not observed in the wall surface of other heated seeds. In conventional processing methods, heat is transferred from an external heat source to the feeddstuff, but in the microwave method, heat is generated from within the feedstuff. In microwave processing, rapid heat generation begins inside the seed and the production of water vapor puts pressure on the outer layer, which causes the seed shell to rupture, which has not been reported in other heat processing.

CONCLUSIONS

The results showed that the application of heat treatment reduced the disappearance of DM and CP in the rumen. This is important in the diet of high-yield ruminants, as it reduces the loss of valuable protein sources in the form of ammonia. Therefore, it can be said that autoclaving and microwave irradiation is an effective method to increase the efficiency of rapeseed CP. The intestinal disappearance of undegraded DM and CP in the rumen indicates that the heat application to rapeseed has increased the disappearance of DM

and CP in the intestine. Heat prossecing also increases the digestibility of DM and CP in the whole gastrointestinal tract, which means that heat application, especially autoclaving and microwave of rapeseed, were effective methods for changing the site of digestion of DM and CP from rumen to small intestine.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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