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Microwave Heat Treatment Induced Changes in Forage Hay Digestibility and Cell Microstructure

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Abstract: To investigate the effects of microwave (MW) treatment on hays nutritive value, five types of hay (50 g) were treated with MW for 0 (control), 20, 40, 60, and 80 s (0, 440, 880, 1320, and 1760 kJ kg^{-1} of MW energy, respectively) and analyzed for nutritive value prior to scanning by an electron microscope to observe microstructure changes. The dry matter (DM) % of hays were increased with increasing treatment time ($p < 0.001$). The improved DM and digestible organic matter in the DM were recorded from MW treated lucerne (60 s), wheat (40 s), and canola (20 s) ($p < 0.001$), which might have been due to the MW ruptured cell wall. The MW energy required for maximal DM digestibility improvement was linearly related to control hay crude protein content ($r^2 = 0.79$; $p < 0.001$). Overall, the study showed MW treatment can increase some hays' digestibility. In addition, the crude protein content from control hay may be a proxy to indicate DM digestibility improvement.

Keywords: thermal treatment; nutritional value; feed quality; SEM-IA; conserved forage

1. Introduction

Forage hay is a major conserved forage used worldwide for ruminant production. However, the high concentration of cell walls in hay products could limit ruminant intake and access to nutrients by rumen microbes [1–3]; therefore, it often results in suboptimal ruminant performance (Chaudry and Miller 1996).

A range of physical, chemical, and biological treatments have been previously explored to investigate the effect on hay digestibility [4]. Microwave (MW) treatment is a non-ionizing electromagnetic physical heating method which proved to be effective for the improvement of the nutritional value of food [5,6] due to its safe, quick, and energy-efficient properties [7]. In the human food industry, MW heating is widely used for thawing frozen food, heating up, pre-cooking, blanching, baking, pasteurizing, and sterilizing food [8]. The rapid increase of inner temperature in the MW treated material due to heating, generally known as "thermal run-way," is usually linked to the dielectric properties of the material that may cause plant cell wall destruction [9] and might increase the utilization of previously unavailable cell nutrients [10].

There are a few studies have explored concentrate feed (e.g., grain) nutritive value change in relation to MW heating [11,12]. These studies demonstrated that MW treatment reduced rumen dry matter (DM) degradability and increased availability of bypass protein in the small intestine, which may potentially lead to improved ruminant performance. Brodie, et al. [13] found that MW treatment increased in small scale pepsin-cellulase in vitro dry matter digestibility (15%) of lucerne hay compared to control, Dong, et al. [14] found similar improvement in MW treated wheat straw under in sacco organic matter degradability (20%) study in Yak compared to a control. Based on these studies, it can be hypothesized that MW treatment can enhance the nutritive value of animal feed (grain/roughage) independent of compositional and structural differences (e.g., high-quality concentrates vs low quality roughage, legume vs non legume, etc.) However, the information related to MW treatment effect on forage hay nutritive value and exploration on the potential mechanisms of the changes is limited. Therefore, this study aimed to explore microwave heat treatment effect on forage hay nutritive values and cell microstructure.

2. Materials and Methods

2.1. Experimental Design

A small-scale pepsin-cellulase in vitro experiment was conducted at the Dookie laboratory, the University of Melbourne, Dookie Campus, Australia. Five different forage hays commonly used for sheep production in southern Australia were selected. Each forage hay was subjected to five distinct level of MW energy within three replicates.

2.2. Sample Preparation and Microwave Treatment

The five forage treatments consisted of lucerne (Medicago sativa), canola (Brassica napus), pasture (perennial ryegrass; Lolium perenne. L + white clover; Trifolium repens), oat (Avena sativa), and wheat (Triticum aestivum) hay. Wheat and canola hay (loose, 18–20 cm) were collected from the Dookie Campus sheep farm at The University of Melbourne, northern Victoria, Australia. Lucerne and pasture hay bale (50 cm × 30 cm) were purchased from a farm supplies store in Shepperton, northern Victoria, Australia. Oat hay (loose, 25–50 cm) was collected from a sheep farm near Dookie Campus, northern Victoria, Australia. All the selected hay samples were previously sundried (DM ranges from 89%–91%); as a result, further drying was not required. Each of the hays was sub-sampled from the different point of the bag or bale of the hay to make a composite bulk sample for MW treatment and analysis.

Each treatment consisted of three replications (50 gm/replication) and was subjected to MW treatment. According to the designed MW treatments, hay samples were treated for 0 (control), 20, 40, 60, or 80 s (s), which is equivalent to 0, 440, 880, 1320, and 1760 kJ kg^{-1} of MW energy, respectively, using a bench-scale MW oven (EMS8586V, Sanyo, Tokyo, Japan). The MW oven had a cavity dimension of 370 mm × 380 mm × 210 mm, an operational frequency of 2.45 GHz, and a rated power level of 1.1 kW. Based on the pre-experimental test, the maximum MW treatment time (80 s) in this study was selected as it was the closest time to start burning the hay. In order to achieve a constant treatment pattern in all the treated samples, the turntable of the MW oven was marked with the container dimension before treatment to minimize the MW treatment inconsistency. All the treated samples were cooled at room temperature for 3 h (h) before grinding them through a 1 mm sieve in a benchtop ultra-centrifugal benchtop hammer mill (RETSCH, ZM 200, Germany).

2.3. Chemical Analyses

Samples of the MW treated hay were analyzed for DM g/kg (method 934.01) and ash content g/kg (method 942.05) according to [15]. Organic matter (OM) g/kg was calculated as 100 g/kg–ash g/kg. The crude protein (CP) g/kg was determined using the Kjeldahl method (method 954.01 [15]). The neutral detergent fiber (NDF) [16], and acid detergent fiber (ADF) ([15]; method 973.18) were analyzed used an ANKOM200 Fiber Analyzer Unit (F57 filter bag, ANKOM Technology, Fairport, NY, USA).

2.4. In Vitro Pepsin-Cellulase Digestibility

The in vitro pepsin-cellulase digestibility was determined as described by the Australian Fodder Industry Association Manual [17]. Per Method-1.7 R., pepsin enzyme from porcine gastric mucosa from Sigma Aldrich Australia (Lyophilized powder, ≥2500 units/ mg protein E1%/280) and crystalline cellulase from Trichoderma viride (Onozuka FA, Yakult Pharmaceutical Ltd., Tokyo, Japan) were used.

2.5. Scanning Electron Microscope Image Analysis (SEM-IA)

Microwave treated hay was imaged via a scanning electron microscope (SEM). For SEM analysis, hays with the highest digestibility improvement (lucerne (60 s), canola (20 s), and wheat hay (40 s)) were used to compare with their control treatment, respectively. However, canola hay was eliminated due to its high fragility and made it difficult to prepare a representative sample for analysis. Representative SEM images were captured for wheat and lucerne hay from their top surface and core center surface area. SEM imaging was then conducted on a ThermoFisher Teneo system in the Bio21 Microscopy Facility at The University of Melbourne, Australia. Further, the SEM images were analyzed to quantify each image's microstructural changes using image processing software Fiji [18].

2.6. Statistical Analysis

Data were analyzed using GenStat (Version 16, VSN International Ltd., Hemal Hempstead, 20 UK). A two-way analysis of variance (ANOVA) was conducted for chemical composition and digestibility parameters. The analysis included the MW treatment as factor 1 and forage type as factor 2, and "replications" as a block. A least significant difference (LSD) test (Fisher's unprotected) was used to distinguish among means at a 95% confidence level. From the ANOVA result, minimal MW energy required (MME) for different forages to achieve maximal DM digestibility (DMD) was identified. A linear regression analysis was then conducted to establish the relationships between baseline organic nutrients from control forage and MME.

3. Results

3.1. Chemical Composition of Microwave Treated Hays

The control forage nutritive value determined in this study was within the standard range for hays commercially evaluated and reported at the Feed Test division of the Australian Wool Testing Authority (AWTA Limited, Melbourne, VIC, Australia).

The DM content of hays without MW treatment in this study was between 890 and 930 g/kg (fresh weight basis). In general, the DM content of hays increased with increasing MW treatment time (Table 1). However, no difference was observed in DM content in MW treatment and forage type interaction ($p = 0.197$). Similarly, different hays showed no difference in OM, NDF, and ADF change due to MW treatment and forage type interaction (Table 1). However, the effect of MW treatment increased oat hay NDF content at 60 s of treatment ($p = 0.026$), and ADF content of canola, pasture and wheat hay increased ($p < 0.001$) at 80 s (Table 1).

Hays used in this study had CP ranging from 70 to181 g/kg without MW treatment (Table 1). There was a significant interaction ($p = 0.007$) observed in CP between the MW treatment and forage type (Table 1). The MW treatment in canola hay for 20 s and oat hay for 40 s had increased CP compared with the control, but the other hays had no difference in CP compared to their controls (Table 1).

3.2. In Vitro Pepsin-Cellulase Digestibility

Both the dry matter digestibility (DMD) and digestibility of organic matter in the DM (DOMD) contents of lucerne, canola, and wheat hays increased when treated with MW for 60, 20, and 40 s ($p < 0.001$), respectively, compared with their controls (Table 1). On the other hand, DMD of pasture and oat did not show any effect up to 60 s treatment. When MW treatment for was 80 s, both hay digestibility measures started to reduce dramatically.

Table 1. Chemical composition and digestibility (g/kg dry matter basis, unless stated otherwise) of hays treated by microwave (MW) treatment.

Parameters	MW Time (MT) (s)	Forage Type (FT)					MT		FT×MT	
		Lucerne	Canola	Pasture	Oat	Wheat	LSD	*p*-Value	LSD	*p*-Value
DM g/kg (fresh weight basis)	0	930.7 ^{aC}	902.2 ^{aD}	928.6 ^{aC}	904.5 ^{aA}	896.0 ^{aC}				
	20	935.9 ^{aBC}	911.9 ^{aCD}	931.2 ^{aBC}	922.1 ^{aA}	913.0 ^{aB}				
	40	939.7 ^{aB}	925.6 ^{aBC}	939.8 ^{aC}	912.3 ^{aA}	916.5 ^{aAB}	0.36	<0.001	0.81	0.197
	60	940.0 ^{aB}	942.6 ^{aAB}	941.6 ^{aAB}	923.3 ^{aA}	923.7 ^{aA}				
	80	947.5 ^{aA}	948.1 ^{aA}	943.2 ^{aA}	930.3 ^{aA}	921.3 ^{aA}				
OM (g/kg)	0	912.0 ^{aA}	924.2 ^{aA}	922.4 ^{aA}	926.4 ^{aA}	943.1 ^{aA}				
	20	910.6 ^{aA}	928.0 ^{aA}	923.0 ^{aA}	937.3 ^{aA}	944.8 ^{aA}				
	40	911.0 ^{aA}	929.3 ^{aA}	922.8 ^{aA}	934.5 ^{aA}	943.4 ^{aA}	0.32	0.137	0.71	0.336
	60	911.7 ^{aA}	926.9 ^{aA}	916.3 ^{aA}	931.3 ^{aA}	941.9 ^{aA}				
	80	909.4 ^{aA}	929.1 ^{aA}	914.5 ^{aA}	931.3 ^{aA}	945.5 ^{aA}				
CP (g/kg)	0	180.8 ^{aA}	115.4 ^{cdeB}	070.3 ^{gA}	90.9 ^{fA}	116.4 ^{cdA}				
	20	186.2 ^{aA}	144.9 ^{bA}	058.2 ^{gA}	100.7 ^{efA}	109.5 ^{cdefA}				
	40	188.6 ^{aA}	112.5 ^{cdefB}	064.2 ^{gA}	114.6 ^{cdeA}	116.3 ^{cdA}	0.67	0.716	1.50	0.007
	60	193.8 ^{aA}	110.8 ^{cdefB}	065.3 ^{gA}	107.0 ^{defA}	108.8 ^{cdefA}				
	80	190.5 ^{aA}	122.6 ^{cB}	069.6 ^{gA}	108.1 ^{cdefA}	110.9 ^{cdefA}				
NDF (g/kg)	0	315.4 ^{aA}	604.1 ^{aA}	660.3 ^{aA}	467.1 ^{aB}	562.2 ^{aA}				
	20	311.3 ^{aA}	605.8 ^{aA}	634.3 ^{aC}	486.5 ^{aAB}	555.1 ^{aA}				
	40	328.7 ^{aA}	642.3 ^{aA}	632.8 ^{aB}	493.9 ^{aAB}	549.3 ^{aA}	0.80	0.026	1.78	0.156
	60	335.0 ^{aA}	625.6 ^{aA}	608.2 ^{aC}	494.9 ^{aA}	544.9 ^{aA}				
	80	336.5 ^{aA}	651.6 ^{aA}	664.5 ^{aA}	484.4 ^{aAB}	577.3 ^{aA}				
ADF (g/kg)	0	241.2 ^{aA}	432.5 ^{aBC}	395.3 ^{aAB}	312.7 ^{aA}	307.7 ^{aB}				
	20	230.6 ^{aA}	420.8 ^{aC}	391.9 ^{aAB}	313.5 ^{aA}	301.0 ^{aB}				
	40	245.5 ^{aA}	452.0 ^{aAB}	399.2 ^{aAB}	311.4 ^{aA}	313.9 ^{aAB}	1.04	<0.001	2.32	0.358
	60	256.5 ^{aA}	448.5 ^{aAB}	375.0 ^{aB}	320.7 ^{aA}	308.4 ^{aB}				
	80	256.3 ^{aA}	466.8 ^{aA}	416.3 ^{aA}	326.1 ^{aA}	335.2 ^{aA}				
DMD (g/kg)	0	658.1 ^{eD}	484.2 ^{lB}	558.3 ^{hiAB}	719.0 ^{abA}	623.5 ^{fgBC}				
	20	679.3 ^{dCD}	532.7 ^{jA}	574.4 ^{hA}	733.5 ^{aA}	631.0 ^{fB}				
	40	701.0 ^{bcBC}	455.0 ^{mC}	575.9 ^{hA}	731.1 ^{aA}	655.9 ^{eA}	0.90	<0.001	2.01	<0.001
	60	732.6 ^{aA}	427.8 ^{nD}	553.3 ^{iB}	723.5 ^{aA}	657.5 ^{eA}				
	80	714.7 ^{abAB}	434.9 ^{nACD}	512.1 ^{kC}	685.8 ^{cdB}	608.8 ^{gC}				
DOMD (g/kg)	0	581.5 ^{eD}	396.9 ^{mB}	501.8 ^{jBC}	653.0 ^{abA}	550.1 ^{fgA}				
	20	609.8 ^{dC}	444.1 ^{lA}	515.9 ^{ijAB}	662.3 ^{aA}	569.8 ^{fA}				
	40	629.0 ^{cBC}	357.5 ^{nC}	536.0 ^{ghA}	658.6 ^{abA}	584.7 ^{eB}	0.83	<0.001	1.85	<0.001
	60	665.5 ^{aA}	335.3 ^{nD}	478.8 ^{kC}	651.9 ^{abA}	595.4 ^{deB}				
	80	641.0 ^{bcAB}	349.2 ^{noC}	451.1 ^{lD}	608.6 ^{dB}	529.1 ^{hiC}				

Means with different superscripts (a, b, c or d . . .) are significantly different from one another due to forage type and MW treatment interaction (FT×MT). Means with different superscripts (A, B, C, or D) are significantly different from one another within the forage due to MW treatment (MT). The LSD is the Least Significant Difference value, which indicates a statistically significant difference among means at a probability of 95%. Fisher's unprotected LSD has been applied in this study. Two-way ANOVA has been used to observe the significance due to microwave treatment and forage type interaction (FT×MT). One-way ANOVA has been used to observe the significance only due to microwave treatment (MT). DM = Dry matter, OM = Organic matter, CP = Crude protein, NDF = Neutral detergent fiber, ADF =Acid detergent fiber, DMD = Dry matter digestibility, DOMD = Digestibility of organic matter in dry matter.

3.3. Scanning Electron Microscope Image Analysis (SEM-IA)

Observation of the control sample showed intact cell structure and surfaces. In contrast, MW-treated wheat and lucerne samples taken from both the top and the central core showed severely disrupted structures and highly exposed areas (Figures 1 and 2). Furthermore, the pixel intensity of the MW treated lucerne hay central core sample showed the highest pixel intensity (35.742), followed by MW treated top sample (33.521) and control (22.415). In case of wheat hay, the pixel intensity for control, top and central core samples was 8.335, 23.579, and 37.345, respectively (Figure 2).

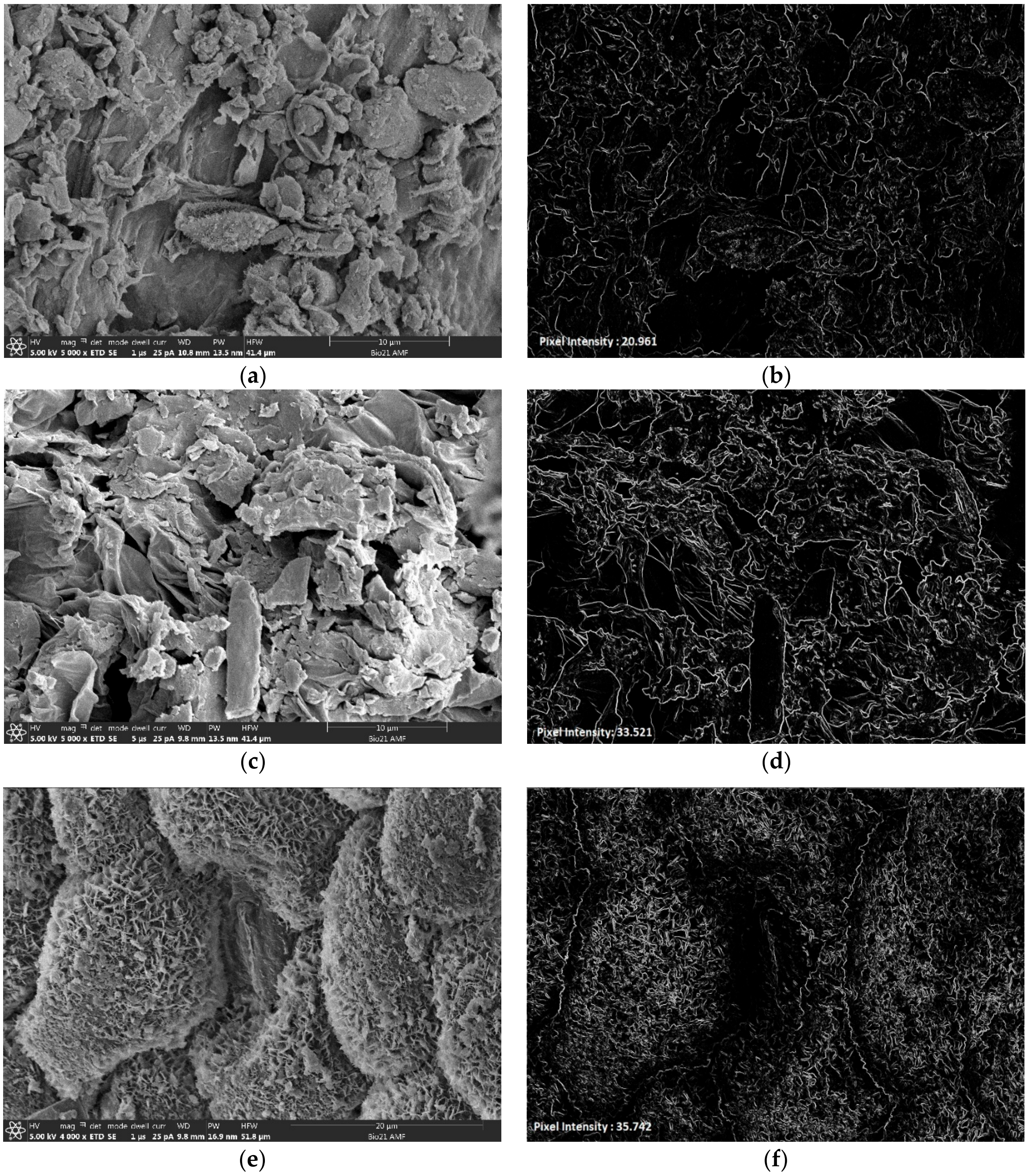

Figure 1. Scanning electron microscope images of lucerne hay, (**a**,**b**) SEM and Fiji analytical image of untreated hay, respectively, (**c**,**d**) SEM and intensity variance image MW treated (taken from the top of the sample), respectively, (**e**,**f**) MW treated (taken from the center core of the sample), respectively.

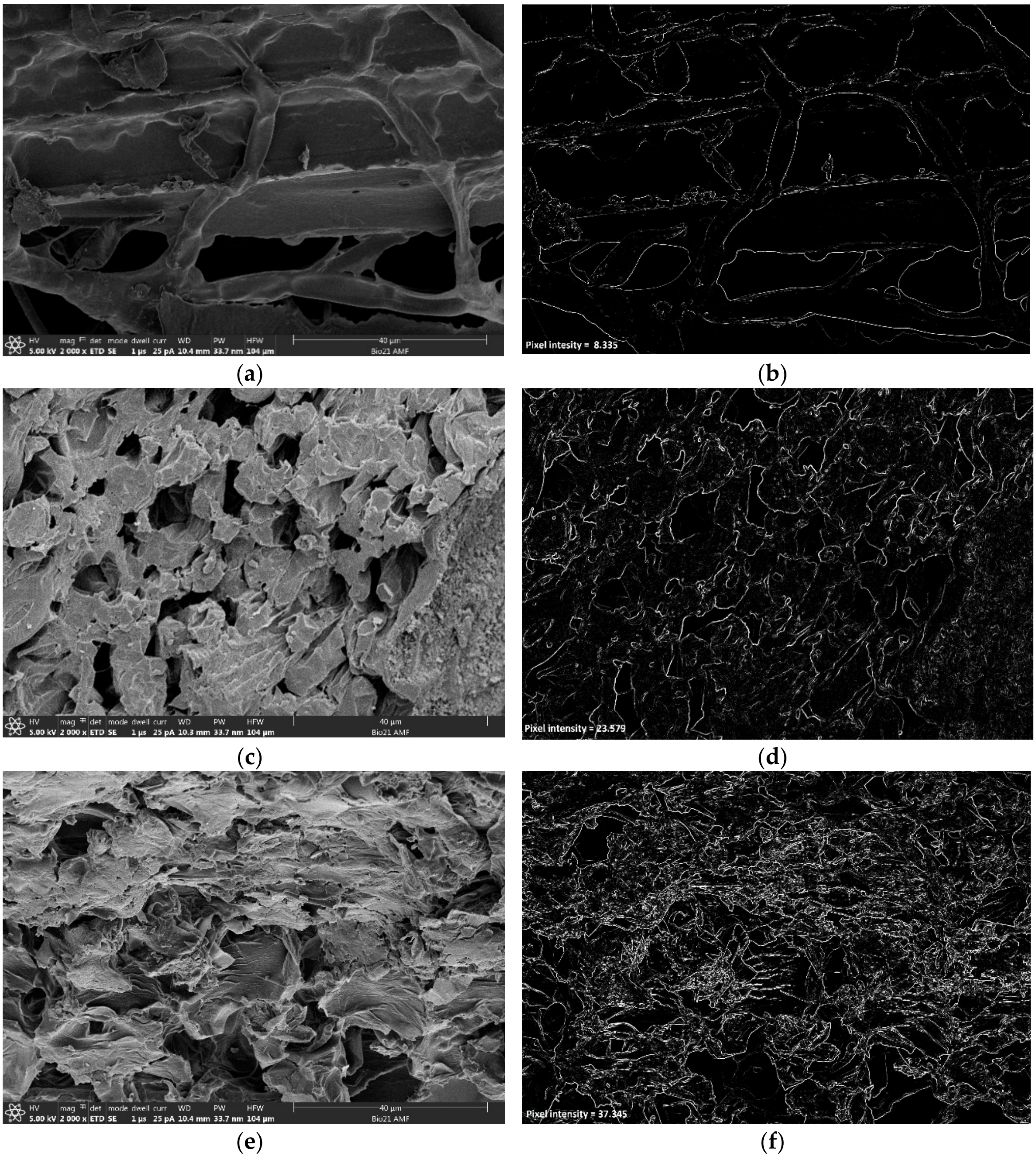

Figure 2. Scanning electron microscope images of wheat hay, (**a**,**b**) SEM and Fiji analytical image of untreated hay, respectively, (**c**,**d**) SEM and intensity variance image MW treated (taken from the top of the sample), respectively, (**e**,**f**) MW treated (taken from the center core of the sample), respectively.

3.4. Relationship between Baseline Organic Nutrients and Minimum MW Energy Required for Maximum DMD% Increase

A positive relationship was observed between the CP content of the control hays and the maximum DMD increase (e.g., lucerne hay baseline CP content 181 g/kg vs. DMD increased by 11%) due to MW treatment (Figure 3a). The minimum MW energy (MME) input (kJ kg^{-1}) for maximum DMD% increase from hays showed a strong positive relationship (Figure 3b).

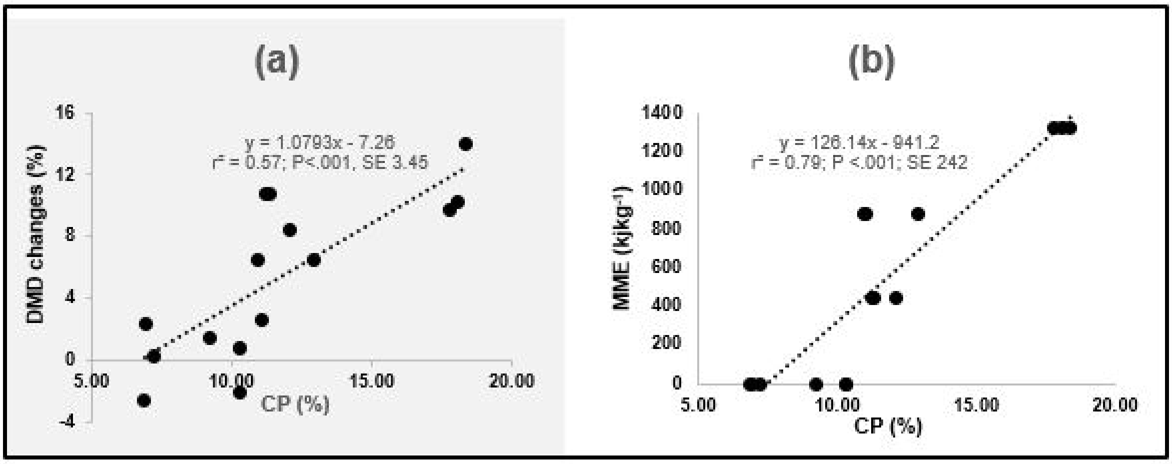

Figure 3. Relationship between crude protein (CP; g/kg) of control hays with DMD changes (%) due to MW treatment (**a**) and minimum MW energy input (MME; kJ kg^{-1}) to achieve maximal dry matter digestibility and CP g/kg (**b**).

The MME inputs for lucerne, canola, oat, pasture, and wheat hay were 1320, 440, 0, 0, and 880 kJ kg^{-1}, respectively, in this study. The relationships between the MME input and organic nutrients parameters from control forage are shown in Table 2.

Table 2. Relationships between the minimal MW energy (MME) input for five tested forage hays to achieve maximal dry matter digestibility and organic nutrients (crude protein and neutral detergent fiber) from control hays.

Prediction Equation #	Sample Size	r^2	SE	*p*-Value
$MME_1 = -941.2 + 126.1$ CP [1] g/kg	15	0.79	242	<0.001
$MME_2 = 1932 - 26.9$ NDF [2] g/kg	15	0.37	423	0.01

Equation has been predicted based on multiple linear regression model. MME_1 = CP: MW energy input for crude protein; MME_2 = NDF: MW energy input for neutral detergent fiber.

A moderate relationship was observed between NDF content of control forage and MME input (Table 2). A strong positive linear relationship was observed between control forage CP content and MME (Table 2). However, there was no significant ($p = 0.688$) relationship observed between MME and DMD.

4. Discussion

4.1. Chemical Composition

In general, the DM content increased gradually with increasing MW treatment time in this study, which agrees with previous studies [14,19]. The inside-out heating mechanism associated with MW treatment facilitated steam generation in the cell wall, which induced rapid heating, which caused, ultimately, the disruption of the cell wall leading to quick evaporation in the plant hay material [20].

In consideration of forage type and MW treatment interaction, it was observed that CP content of canola and oat hay after 20 s and 40 s MW treatment increased by 25% and 15% compared with the control, respectively. The causes of this improvement are unknown, while other hays did not show any changes in the CP content. One possible reason could be due to the increase of detection of unavailable cell wall-bound CP during the determination of CP [21], which might become available for detection due to cell wall destruction in this study (Figures 1 and 2). Further, other feed studies suggested that exogenous protein factors (structure, content of antinutritional factor, starch and non- starch polysaccharides) and endogenous factors (cross-linkage, molecular structure, hydrophobicity) [22] could be altered by heat treatment [23], which could in turn impact on protein availability and utilization in animals [13]. Future work is needed to better understand how and why CP content change in response to MW treatment.

Most of the previous research focused on MW treatment on concentrate feed (grain and meal) and hardly reported fiber fraction change of the feed. In the current study, the ADF and NDF content of different forage hays showed no difference when the interaction of MW treatment and forage type was considered. Further, the NDF and ADF content in wheat hay were not impacted by the MW treatment alone. This result agrees with [14], who found no difference in ADF content and NDF content of wheat straw treated with MW (750 W) for 240 s and 480 s.

4.2. Dry Matter Digestibility and Digestibility of Organic Matter in Dry Matter

The DMD and DOMD contents were affected by the MW treatment, although the effect was not constant across different hays in this study. Lucerne hay showed the highest increase of all hays in the present study, with increases in in DMD content (11%) and DOMD content (14%) at 60 s MW treatment compared with the control. The level of increase in the DMD content of lucerne hay was similar to the finding of Brodie et al. (2012) [13]. The study showed that DMD increased by 14.9% when treated for 80 s MW treatment (750 W) compared with the control. Brodie et al. (2012) [13] speculated that the increase of DMD content was possibly due to the destruction of the cell microstructure, which is similar to what was observed in the current study (Figures 1 and 2). The cell microstructure destruction from MW treatment might increase nutrient availability and access by enzymes (e.g., CP digestibility). Canola hay showed a 10% and 12% increase in DMD and DOMD content, respectively, at 20 s MW treatment compared with the control. Wheat hay showed a 5% and 8% increase in DMD and DOMD content at 40 s MW treatment compared with the control. The increasing trend of digestibility in this study was supported by Dong et al. (2005) [14], who found a 20% increase in ruminal OM degradability in a nylon bag degradability study of 240 s MW treated wheat straw. Furthermore, a number of researches on concentrate feed (e.g., grains, seed, and seed meal) showed that, MW treatment increase digestibility of concentrate feeds [12,24,25]. However, the improvement of digestibility level in forage hay is comparatively lower than concentrate feeds. This is may be due to the difference in nutrient content, density, and cellular structure [26].

4.3. Scanning Electron Microscope Image Analysis

Observation with SEM image confirmed that cell wall intactness was visible in orderly in the control sample irrespective of their high sun-drying texture during hay processing (Figures 1 and 2). An extensive cellular surface breakdown could be seen in the MW treated SEM images. The MW treatment might induce cell microstructures to disintegrate and initiate the loosening of intactness [10]. The central core sample showed more disruption than the top surface sample, which is likely due to the unevenness of MW heating [27]. The pixel intensity represents the primary pixel value, which helps to identify and classify differences in images based on texture, smoothness, and pattern [28]. In the present study, higher intensity variance in the MW treatment sample in comparison to the control indicates that there might be some potential destruction that occurred in the cell microstructure of the lucerne and wheat hay (Figures 1 and 2). Previous studies also supported this finding in the present study. For example, Choi et al. (2006) [10] found that due to MW treatment, destruction in the microstructure of cell surface occurred in soybean grain, which eventually increased the extraction of soluble soy protein. Another study with rapeseed also reported microstructure disruption due to MW treatment [29]. However, it is important to note that the level of microstructure destruction in grain and forage hay may not be the same, as they are different from each other based on morphological and chemical structure.

4.4. Relationships between the Minimal Microwave Energy Input for Different Hay to Achieve Maximal Dry Matter Digestibility Increase

The current study showed a strong positive relationship between MME and baseline CP content of control hays (Figure 3b), and to the best of our knowledge, this is the first time a study reported such relationship. Hays with higher CP content generally required more input energy to achieve maximum DMD content improvement and the optimum MW energy for lucerne hay (1320 kJ kg^{-1}) in present study is similar to the optimum energy calculated in the study conducted by Brodie et al. (2012) [13] (1200 kJ kg^{-1} calculated from 0.750 kW MW power used 80 s treatment time for 0.05 kg sample). In the present study, positive changes in DMD of hays were also found to be related to the baseline CP content of hays (r^2 = 0.57). A previous study also suggested that the improvement of digestibility (with vs without any processing) considerably depends on the control feed CP content and its digestibility [30]. However, changes with other baseline organic matter (e.g., ADF and NDF) were not as strong as CP content in this study. The reason is unknown for this relationship. Further research is required to understand and validate the relationship.

It is important to note that the influences of MW heating on the material are affected by dielectric properties [9], each biological material possess different degree of dielectric properties, which ultimately affect the temperature distribution in the target material. Responses in any heated material depend on the temperature distribution, retention, and equilibrium [31,32]. These mechanisms regulate the heat-induced changes in any materials. Therefore, the changes in the nutrient composition and their digestibility depends on the temperature distribution in the hays. Further research into the dielectric properties of the forage hays used in this study is required to understand the relationships between major nutritive value parameters (e.g., CP and DMD) and dielectric properties of the materials in order to better understand the MME required to achieve maximum change in nutritive characteristics.

5. Conclusions

The current study confirmed that MW treatment can improve the DM and OM digestibility of lucerne, canola, and wheat hays. Furthermore, it has also shown that the changes in digestibility could be related to the cell microstructure destruction due to MW treatment of hay. A major finding in this study is that the relationship between the baseline CP content of hay, MME, and DMD changes, indicating that baseline CP content of hay can be used to predict the minimal MW energy input is required for maximum DMD improvement. It is important to note that forage hay is typically used as a maintenance ration for ruminant production during periods of unavailability of fresh forage, but if the quality of hay can be improved, it can be used as a good source of nutrients for formulating ruminant diet. However, this is a preliminary study to understand the MW treatment on hay nutritive value, and with the present data it can be postulated that a consolidated research project would find an in-depth detailed mechanism associated to a higher digestibility of hays.

Author Contributions: M.S.R.S., G.B., B.C. and L.C. participated in the conceptualization and design of the study, M.S.R.S. and R.K. carried out the experimental procedure and analysis. L.C. supervised by the experimental work. M.S.R.S. and L.C. carried out the analysis of data. M.S.R.S., G.B., B.C., R.K., E.C. and L.C. writing, drafting and editing of the manuscript. M.S.R.S. and E.C. participated in the image analysis of the scanning electron microscopy images. and drafting the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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