applied sciences

MDPI

Article

Effects of Sprouted Barley with Different Cultivation Stages on Fermentation Characteristics and Degradation Kinetics in the Rumen

Ji-Yoon Kim [1,†], Dimas Hand Vidya Paradhipta [2,†], Young-Ho Joo [1], Myeong-Ji Seo [1], Chang-Hyun Baeg [1], Seung-Min Jeong [3] and Sam-Churl Kim [1,*]

[1] Division of Applied Life Science (BK21Four, Institute of Agriculture and Life Science), Gyeongsang National University, Jinju 52828, Republic of Korea; in42869@naver.com (J.-Y.K.); wn5886@gmail.com (Y.-H.J.); audwltj@naver.com (M.-J.S.); back5254@naver.com (C.-H.B.)
[2] Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia; dimashandvidyaparadhipta@ugm.ac.id
[3] Grassland & Forages Division, National Institute of Animal Science, RDA, Cheonam 31000, Republic of Korea; tmdals8077@naver.com
* Correspondence: kimsc@gnu.ac.kr; Tel.: +82-55-772-1947
† These authors contributed equally to this work.

Abstract: The present study investigated the effects of sprouted barley (SB) with different cultivation stages on fermentation characteristics and degradation kinetics in the rumen. The SB was cultivated in three different stages as follows: 0, 4, and 8 days. Dried samples from each cultivation stage of SB were incubated in the rumen buffer at 39 °C for 48 h in quadruplicate with three blanks. Dry matter (DM) and neutral detergent soluble carbohydrate concentrations of SB decreased linearly ($p = 0.001$) by increasing the cultivation stage, while crude protein, neutral detergent fiber (NDF), acid detergent fiber, and hemicellulose concentrations increased linearly ($p \leq 0.001$). Total volatile fatty acid and butyrate in the rumen decreased linearly ($p \leq 0.020$) by increasing the cultivation stage, while pH and propionate increased linearly ($p < 0.001$). The total degradation fraction of DM and NDF increased quadratically ($p \leq 0.003$). The fraction degradation rate of DM and NDF decreased linearly ($p \leq 0.001$) by increasing the cultivation stage, while the lag phase increased linearly ($p \leq 0.010$). The present study concluded that cultivated SB at 4 days was recommended for animal feed due to the highest nutrient degradation in the rumen without any adverse effects on fermentation characteristics.

Keywords: *in vitro*; rumen degradation kinetic; rumen fermentation; sprouted barley

Citation: Kim, J.-Y.; Paradhipta, D.H.V.; Joo, Y.-H.; Seo, M.-J.; Baeg, C.-H.; Jeong, S.-M.; Kim, S.-C. Effects of Sprouted Barley with Different Cultivation Stages on Fermentation Characteristics and Degradation Kinetics in the Rumen. *Appl. Sci.* **2024**, *14*, 364. https://doi.org/10.3390/app14010364

Academic Editors: Pedro A. R. Fernandes and Manuel A. Coimbra

Received: 23 November 2023
Revised: 28 December 2023
Accepted: 29 December 2023
Published: 30 December 2023

1. Introduction

Barley grain is used as a main ingredient for several traditional foods or beverages. The production of barley increases year by year to supply the demand market. During the grading process for food, defective barley grains are separated and become an agricultural by-product, mainly used as energy sources for animals. Meanwhile, hydroponic fodder production could be an alternative approach to improve its economic value [1–3]. In addition, some of the barley grain could be pre-sprouting, pre-germinating, gape, splitting, or skinning during harvesting, storage, or distribution due to environmental factors. Without any processing, those defective barley grains may have low economic value and could significantly reduce the farmer's income. The germination of grains changes the nutrient compositions of dry grain by converting starch into sugar, increasing protein content, or converting protein and fat into amino acids and fatty acids, respectively [1]. Sprouted barley (SB) is a dry barley grain that germinates and sprouts for 8 days [2–4]. It results in green shoots and roots that can provide green fodder as a fiber source for ruminant requirements. According to Dung et al. [3], dry matter (DM) concentration of barley grain decreased with sprouting activity, but crude protein (CP) and mineral concentrations

increased. Supplementation of SB in the steer diet could improve DM digestibility [4], rumen fermentation [5], and animal growth performance [6]. In sheep or goats, dietary SB increased total DM intake, feed efficiency, and body weight [7], and it also improved fermentation and enzyme activity in the rumen [5]. In addition, replacing wheat bran and wheat straw with SB had no adverse effect on milk yield and milk composition of lactating goats; still, it showed beneficial effects on health conditions, mortalities, conception rates, and abortion [8]. Moreover, supplementation of SB for non-ruminants such as chickens increased the egg-laying rate, fertility, and number of hatched chicks [9].

Nevertheless, the application of SB as a feed source did not always promise beneficial effects for the animal [10,11]. The cultivation stage of SB might be a significant influence on the success of SB application for ruminants. It was supported by many previous studies that reported no advantage of the SB over the barley grain because the digestibility of SB decreased along with an increase of cultivation stage [10,11]. The structural carbohydrates such as neutral detergent fiber (NDF) and acid detergent fiber (ADF) were reported to increase as the growths of root and green fodder [10,11]. In the appropriate cultivation stage, SB can present beneficial effects for ruminant diet. Therefore, the present study was conducted to investigate the effect of SB with different cultivation stage on fermentation characteristics and degradation kinetics of DM and NDF in the rumen.

2. Materials and Methods

2.1. Preparation of Sprouted Barley

The hulled barley grain (Youngyang hybrid) was germinated in Geochang Livestock Center, Geochang-gun, Gyeongnam Province, Republic of Korea (latitude 35°43′52.6″ N and longitude 127°55′40.4″ E). The hulled barley grain was washed and soaked with the tap water containing 0.1% hypochlorite to avoid fungal contamination. Then, these barley grains were transferred into hydroponic sprouting unit with the size 4.0 × 1.6 × 1.25 m as length × width × height, respectively. The hydroponic sprouting unit consisted of five shelves with the distance between shelves was 40 cm. Each shelf had two trays for germination with each tray had size 24 cm × 4 m as length × width. The soaked barley grains (6 kg) were spread in a tray with a thickness of 1.5–2.0 cm. Semi-automated irrigation sprayer was used without any additional supplement and the irrigation was occurred every four hours. The temperature of sprouting room was controlled at 21–22 °C with 75% of humidity. In the present study, barley grain was cultivated for 0 day, 4 days, and 8 days. The photograph of all cultivated of SBs for the present study was presented in the Figure 1. The height of cultivated SB at 4 days was approximately at 5.5–7 cm, while the height of cultivated SB at 8 days was approximately at 14.5–18 cm. Each cultivation stage of SB was sub-sampled homogeny (2 kg) for further analyses, such as chemical composition and in vitro rumen incubation.

2.2. Chemical Composition of Sprouted Barley

The sub-sampled SBs were dried at 65 °C for 48 h and ground to pass 1-mm screen using a cutting mill (Shinmyung Electric Co., Ltd., Gimpo, Republic of Korea) according to Lee et al. [12] for the measurement of chemical compositions. The dried sample would be also used for in vitro rumen incubation. The DM concentration was determined by drying sample (about 10 g) into the dry oven (OF-22GW, Jeio Tech, Seoul, Republic of Korea) at 105 °C for 24 h. The crude ash (CA) was determined with a muffle furnace at 550 °C for 5 h (AOAC [13]; method 942.05). The CP and ether extract (EE) were determined by the producers of Kjeldahl (AOAC [13]; method 984.13) using N analyzer (B-324, 412, 435 and 719 S Titrino, BUCHI, Flawil, Switzerland) and Soxhlet (AOAC [13]; method 920.39), respectively. The NDF and ADF were determined by using Ankom 200 fiber analyzer (Ankom Technology, Macedon, NY, USA) following the procedure of Van Soest et al. [14]. The hemicellulose (HEMI) was determined by calculating the differences between NDF and ADF. And then, the neutral detergent soluble carbohydrate (NDSC) was calculated as:

100 − (CP + EE + CA + NDF). The concentrations of CA, CP, EE, NDF, ADF, HEMI, and NDSC were expressed as % of DM.

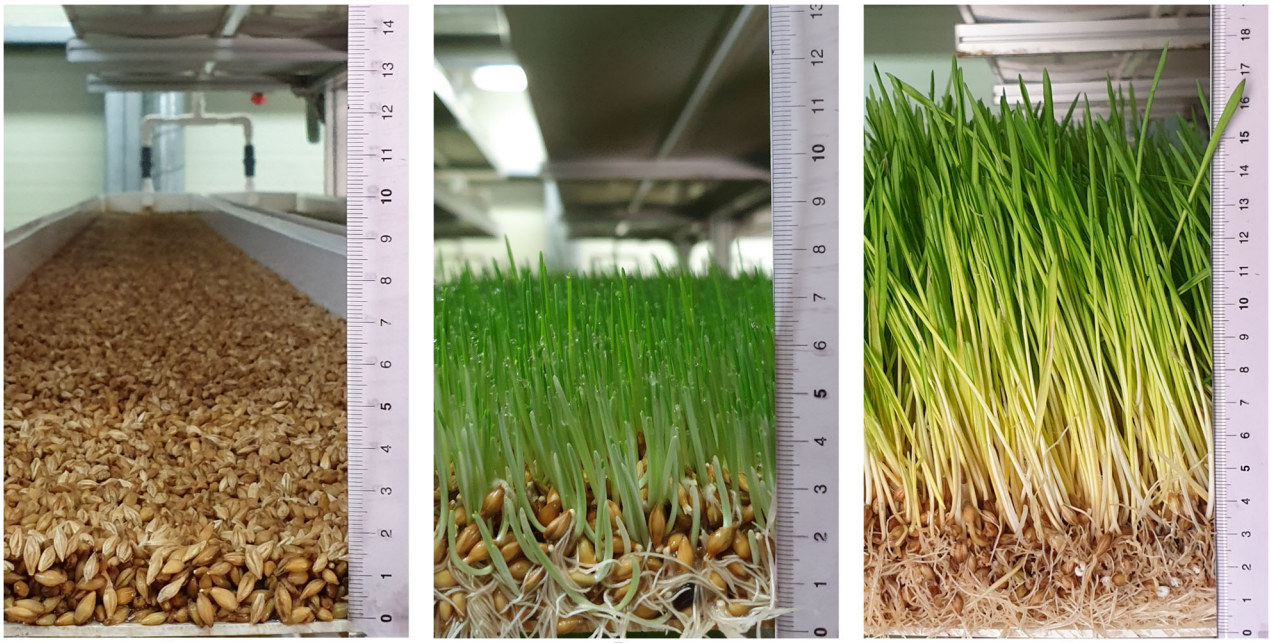

Figure 1. The photograph of cultivated sprouted barley at 0 (**A**), 4 (**B**), and 8 (**C**) days in the present study.

2.3. In Vitro Rumen Incubation

The procedure of animal care for cannulated Hanwoo heifers was approved by the animal ethical committee of Gyeongsang National University, Jinju, Republic of Korea (GNU-191011-E0050). Two non-pregnant cannulated Hanwoo heifers were maintained at the University farm as rumen fluid source. Daily, those heifers were fed rice straw and concentrate mix at a 4:1 ratio with the addition of vitamin-mineral premix. Rumen fluid was collected from cannulated heifers before morning feeding around 1 L for each cow. The rumen fluid was placed into 2 L-thermos during the transportation to the laboratory. The thermos was prepared in warm conditions (39 °C) before maintaining a similar temperature to the rumen. In the laboratory, the collected rumen fluid was composited and then filtered via two layers of cheesecloth. Rumen fluid was mixed with an anaerobic culture medium at a 1:2 ratio to prepare rumen buffer [15]. The ground SB (0.5 g) was weighted into an incubation bottle with rumen buffer (40 mL) [16]. Then, the incubation bottle was gassed with CO_2 and closed tightly to reach anaerobic conditions [16,17]. Four replications for each treatment were used along with three blanks. The bottles were placed into an incubator at 39 °C for 0, 1, 2, 4, 8, 24 and 48 h [17].

2.4. Digestibility and Fermentation Characteristics in the Rumen

After incubation, bottles were opened and transferred to 50 mL conical tube to separate orts and rumen buffer through centrifugation at 2568× *g* for 15 min (Supra 21k, Hanil Electric Corporation, Seoul, Republic of Korea, with rotor A50S-6C No.6). The difference of weight in DM basis between before incubation (dried sample) and after incubation (orts) was used to calculate in vitro DM digestibility (IVDMD). In addition, difference of NDF concentration between dried sample and orts was used to calculate in vitro NDF digestibility (IVNDFD). On the other side, supernatant was used to analyze rumen fermentation characteristics such as pH, ammonia-N, and volatile fatty acid (VFA) consisting of acetate, propionate, iso-butyrate, butyrate, iso-valerate, and valerate. The pH meter (SevenEasy, Mettler Toledo, Greifensee, Switzerland) was used to determine pH of rumen content.

The colorimetric method described by Chaney and Marbach [18] was applied to measure concentration of ammonia-N. In the assigned period of incubation, the rumen buffer was centrifuged at 5645× *g* for 15 min to separate the supernatant and the residue. Collected supernatant was used for VFA analysis [16,17]. The concentrations of each VFA profile were determined using HPLC (L-2200, Hitachi, Tokyo, Japan) fitted with a UV detector (L-2400; Hitachi, Tokyo, Japan) and a column (Metacarb 87H; Varian, Palo Alto, CA, USA) according to the method described by Muck and Dickerson [19].

2.5. Degradation Kinetics in the Rumen

Degradation kinetics of DM and NDF in rumen was determined by measuring IVDMD and IVNDFD, respectively, in each hour of incubation. These collected data were calculated into nonlinear regression procedure of Statistical Analysis Software version 9.3 (SAS, Cary, NC, USA) to fit with the model of McDonald [20] following:

$$Y = A + B\ (1 - e - c\ (t - L))\ \text{for}\ t > L$$

where A is the immediately degradable fraction; B is the potentially degradable fraction; A + B is total degradable fraction; C is the degradation rate of potentially degradable fraction; L is the lag phase; and t is time of incubation (h).

2.6. Statistical Analysis

All collected data in the present study were analyzed using polynomial contrast with PROC GLM of SAS. The orthogonal coefficients for linear and quadratic contrast were adjusted to account for the unequal spacing of cultivation stage (0 day, 4 days, and 8 days) with PROC IML of SAS before testing polynomial contrast [16]. The mean data of rumen pH, ammonia-N, and total VFA (including acetate, propionate, and butyrate) were also analyzed using polynomial contrast to clarify the pattern by incubation hour. Its orthogonal coefficients for linear, quadratic, and cubic were adjusted before to account for the unequal spacing of incubation hour (0, 1, 2, 4, 8, 24, and 48 h). Mean separation was performed by Tukey test and the significant differences were declared at $p \leq 0.05$.

3. Results

3.1. Chemical Composition of Sprouted Barley

The DM ($p = 0.001$) and NDSC ($p < 0.001$) concentrations linearly decreased by increasing the cultivation stage of SB (Table 1), while CP ($p = 0.001$), EE ($p = 0.001$), CA ($p = 0.020$), NDF ($p < 0.001$), ADF ($p < 0.001$), and HEMI ($p < 0.001$) linearly increased. The concentrations of DM (92.3 vs. 18.2%) and NDSC (65.4 vs. 33.4%) were higher ($p < 0.05$) at the cultivation stages of 0 day than 8 day. In contrast, the concentrations of CP (15.5 vs. 10.1%), EE (4.23 vs. 2.93%), CA (2.90 vs. 2.28%), NDF (44.0 vs. 19.3%), ADF (20.1 vs. 5.56%), and HEMI (23.9 vs. 13.7%) were higher ($p < 0.05$) at the cultivation stages of 8 day than 0 day.

3.2. Digestibility and Fermentation Characteristics of Sprouted Barley in the Rumen

Rumen pH decreased linearly ($p < 0.001$) in all cultivation stages of SBs over hour of incubation (Figure 2). On 0 h of rumen incubation, the cultivated SBs at 4 and 8 days had lower ($p < 0.01$) rumen pH than that of 0 day, while the cultivated SB at 4 days had lower ($p < 0.01$) rumen pH on 1 to 2 h than that at 0 and 8 days. From 4 to 24 h of rumen incubation, the cultivated SB at 4 days presented the lowest ($p < 0.01$) pH, while the cultivated SB at 8 days was the highest ($p < 0.01$). The ammonia-N concentration increased linearly ($p < 0.001$) in all cultivation stages of SBs over hour of rumen incubation (Figure 2). From 0 to 2 h of rumen incubation, the cultivated SB at 0 day had lower ($p < 0.05$) ammonia-N concentration than that at 4 and 8 days, whereas on 8 h of rumen incubation, the cultivated SB at 0 day had higher ($p < 0.05$) ammonia-N concentration than that at 4 and 8 days. Similar with ammonia-N pattern, total VFA concentration also increased linearly ($p < 0.001$) in all cultivation stages of SBs over hour of incubation (Figure 2). The cultivated SB at 0 day

had lower total VFA concentration than that at 4 and 8 days on 0 ($p < 0.01$), 1 ($p < 0.05$), 2 ($p < 0.01$), and 24 ($p < 0.01$) h of rumen incubation. On 8 h of rumen incubation, the cultivated SB at 0 day had higher ($p < 0.001$) total VFA than that at 8 days, while the cultivated SB at 4 days had no different compared to other cultivation stages of SBs.

Table 1. Effects of cultivation stages on chemical compositions of sprouted barley.

	Cultivation Stage, Days			SEM	Contrast	
	0	4	8		Linear	Quadratic
Dry matter (DM), %	92.3 ^{a}	30.4 ^{b}	18.2 ^{c}	0.482	0.001	0.254
Crude protein (CP), % DM	10.1 ^{c}	12.3 ^{b}	15.5 ^{a}	0.428	0.001	0.253
Ether extract (EE), % DM	2.93 ^{c}	3.65 ^{b}	4.23 ^{a}	0.112	0.001	0.253
Crude ash (CA), % DM	2.28 ^{b}	2.66 ^{ab}	2.90 ^{a}	0.165	0.020	0.994
Neutral detergent fiber (NDF), % DM	19.3 ^{c}	31.0 ^{b}	44.0 ^{a}	0.783	<0.001	0.927
Acid detergent fiber (ADF), % DM	5.56 ^{c}	12.5 ^{b}	20.1 ^{a}	0.360	<0.001	0.781
Hemicellulose (HEMI), % DM	13.7 ^{c}	18.4 ^{b}	23.9 ^{a}	0.577	<0.001	0.961
Neutral detergent soluble carbohydrate (NDSC), % DM	65.4 ^{a}	50.4 ^{b}	33.4 ^{c}	1.051	<0.001	0.288

$^{a\sim c}$ Means in the same row with different superscripts differ significantly ($p < 0.05$).

The concentration of acetate in the rumen decreased linearly ($p < 0.001$) in all cultivation stages of SBs over hour of incubation (Figure 3). However, the cultivation stage of SB did not affect the acetate concentration in all hours of incubation. On the other side, propionate showed the cubic pattern ($p < 0.001$) in all cultivation stages of SBs during in vitro rumen incubation, which increased during 8 h of incubation and then decreased after that (Figure 3). The cultivated SB at 0 day had lower ($p < 0.01$) propionate concentration than that at 4 and 8 days on 0 and 4 h of rumen incubation, but had higher ($p < 0.01$) on 2 h. On 24 h of rumen incubation, the cultivated SB at 8 days had highest ($p < 0.05$) propionate concentration than that at 0 and 4 days. The concentration of butyrate increased linearly ($p < 0.001$) in all cultivation stages of SBs over hour of incubation (Figure 3). On 2 h of rumen incubation, the cultivated SBs at 0 and 4 days presented lower ($p < 0.01$) butyrate concentration than that at 8 days. However, the cultivated SB at 0 day had higher ($p < 0.01$) butyrate concentration than that at 4 and 8 days on 4 and 24 h of rumen incubation.

The IVDMD ($p < 0.001$) and IVNDFD ($p = 0.005$) increased quadratically by increasing cultivation stage of SB (Table 2). The highest IVDMD of SB was presented by 4 days of cultivation, followed by 8 days of cultivation, and then by 0 day of cultivation ($p < 0.05$; 65.0 vs. 56.2 vs. 34.9%). The cultivated SB at 4 days had higher IVNDFD than that at 0 day ($p < 0.05$; 77.0 vs. 70.1%), while the cultivated SB at 8 days was not differ compared to other cultivated days. In the rumen fermentation characteristics, the pH increased linearly ($p < 0.001$) by increasing cultivation stage of SB. Otherwise, rumen concentrations of VFA ($p = 0.003$) and iso-valerate ($p = 0.036$) decreased linearly by cultivation stage of SB. The concentration of iso-butyrate increased quadratically ($p = 0.003$) by increasing cultivation stage of SB. The highest iso-butyrate was presented by cultivated SB at 8 days, then followed by cultivated SBs at 0 and 4 days ($p < 0.05$; 1.58 vs. 13.4 and 1.22%). Contrast with iso-butyrate, concentration of butyrate decreased linearly ($p = 0.020$) by increasing cultivation stage of SB. The cultivated SB at 0 day had higher butyrate concentration than cultivated SBs at 4 and 8 days ($p < 0.05$; 19.7 vs. 15.5 and 15.5%). The other individual VFAs were not affected by cultivation stage of SB. The acetate to propionate ratio decreased quadratically ($p < 0.001$) by increasing cultivation stage of SB. The cultivated SB at 0 day had higher ratio than that at 4 and 8 days ($p < 0.05$; 5.69 vs. 3.53 and 3.55).

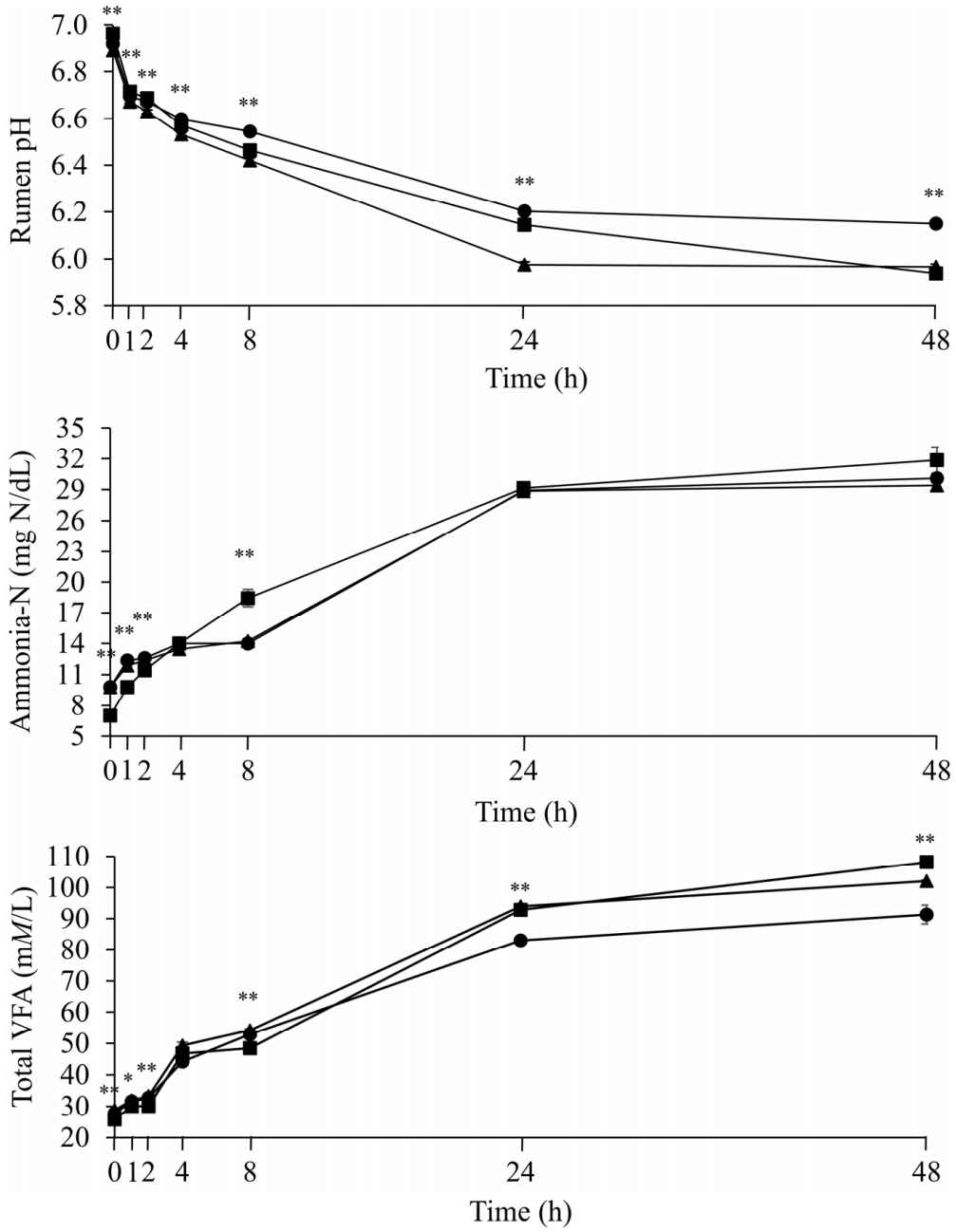

Figure 2. Effects of cultivation stages on rumen pH, and concentrations of ammonia-N and total VFA of sprouted barley incubated in rumen buffer for 48 h. Sprouted barley was cultivated at 0 (■), 4 (▲), and 8 (●) days, respectively. Based on polynomial contrast analysis, pH and concentrations of ammonia-N and total VFA had linear pattern ($p < 0.001$) by the incubation hour. Value differ between groups within same hour * $p < 0.05$ and ** $p < 0.001$.

3.3. Degradation Kinetics of Sprouted Barley in the Rumen

In DM degradation kinetics, the immediately degradable fraction ($p < 0.001$), the potentially degradable fraction ($p = 0.001$), and the total degradable fraction ($p < 0.001$) increased quadratically by increasing cultivation stage of SB (Table 3). The cultivated SBs at 4 days, 8 days, and then 0 day sequentially had the highest to lowest of the immediately degradable fraction ($p < 0.05$; 38.4 vs. 25.9 vs. 12.2 mL/g), the potentially degradable fraction ($p < 0.05$; 37.1 vs. 30.1 vs. 20.0 mL/g), and the total degradable fraction ($p < 0.001$; 75.5 vs. 56.0 vs. 32.2 mL/g). The fraction degradation rate decreased linearly ($p = 0.010$) by increasing cultivation stage of SB. On the other side, the lag phase increased linearly

($p < 0.001$) by increasing cultivation stage of SB. In NDF degradation kinetics, the immediately degradable fraction ($p < 0.001$) and the total degradable fraction ($p = 0.003$) had quadratic pattern by increasing cultivation stage of SB, where cultivated SB at 4 days had higher the immediately degradable fraction ($p < 0.05$; 43.4 vs. 23.6 and 23.0 mL/g) and the total degradable fraction ($p < 0.05$; 90.6 vs. 72.0 and 78.4 mL/g) than those at day 0 and 8 (Table 3). The potentially degradable fraction increased linearly ($p = 0.025$) by increasing cultivation stage of SB. The fraction degradation rate decreased linearly ($p = 0.007$) by increasing cultivation stage of SB, while the lag phase increased linearly ($p < 0.001$).

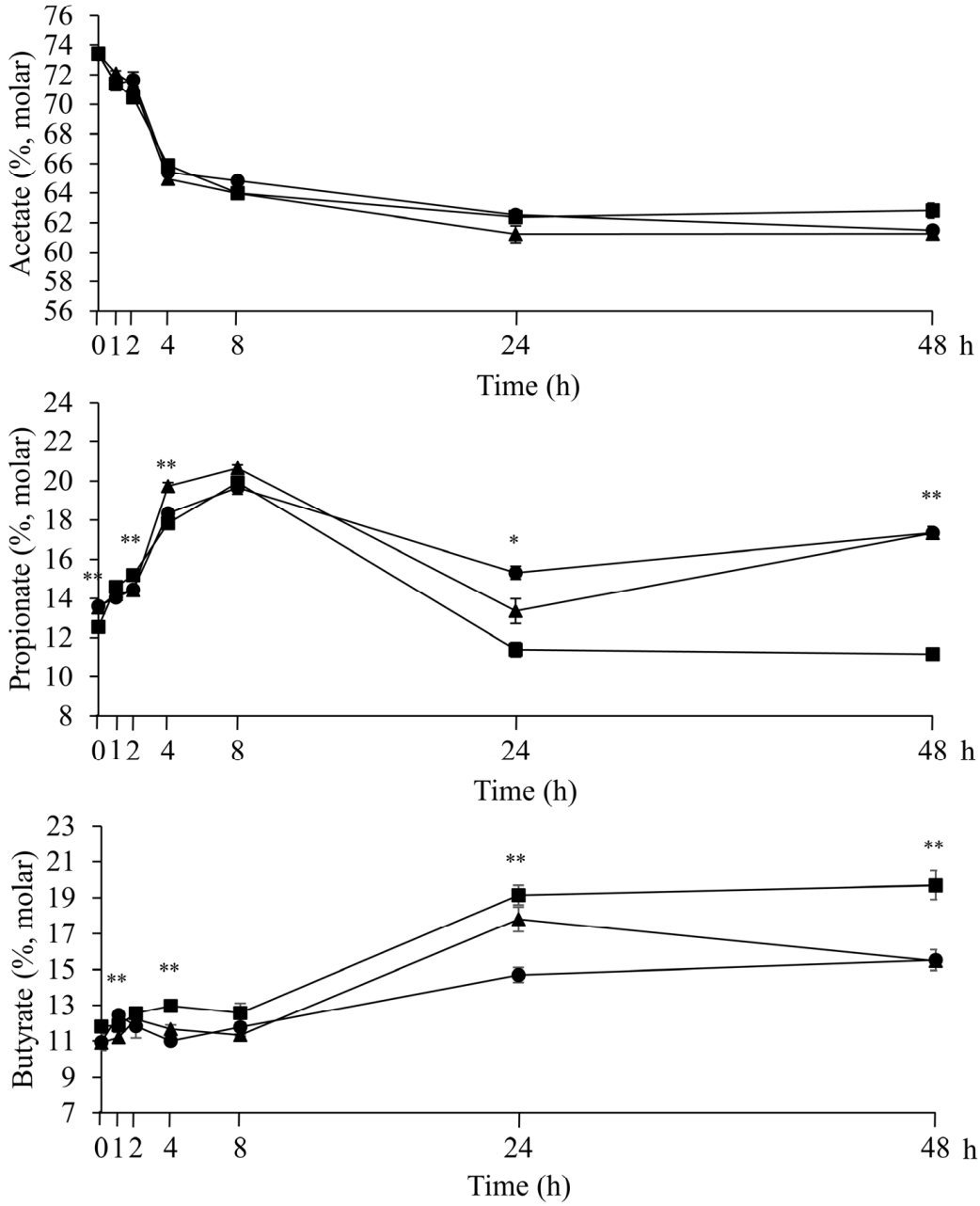

Figure 3. Effects of cultivation stages on concentrations of acetate, propionate, and butyrate of sprouted barley incubated in rumen buffer for 48 h. Sprouted barley was cultivated at 0 (■), 4 (▲), and 8 (●) days, respectively. Based on polynomial contrast analysis, concentrations of acetate and butyrate had linear pattern ($p < 0.001$) by the incubation hour, while the propionate concentration had cubic pattern ($p < 0.001$). Values differ between groups within same hour * $p < 0.05$ and ** $p < 0.001$.

Table 2. Effects of cultivation stages on in vitro rumen digestibility and fermentation characteristics of sprouted barley incubated for 48 h in rumen buffer.

	Cultivation Stage, Days			SEM	Contrast	
	0	4	8		Linear	Quadratic
In vitro digestibility, % DM						
DM	34.9 ^{c}	65.0 ^{a}	56.2 ^{b}	1.268	0.126	<0.001
NDF	70.1 ^{b}	77.0 ^{a}	74.2 ^{ab}	2.014	0.286	0.005
Fermentation characteristics						
pH	5.94 ^{b}	5.97 ^{b}	6.15 ^{a}	0.029	<0.001	0.097
Ammonia-N, mg N/dL	31.9	29.4	30.1	1.775	0.238	0.241
Total VFA, m*M*/L	108.2 ^{a}	102.1 ^{ab}	91.4 ^{b}	2.580	0.003	0.379
Acetate, % of molar	62.8	61.2	61.5	0.788	0.175	0.203
Propionate, % of molar	11.1 ^{b}	17.3 ^{a}	17.4 ^{a}	0.272	<0.001	0.145
Iso-butyrate, % of molar	1.34 ^{b}	1.22 ^{b}	1.58 ^{a}	0.047	0.300	0.003
Butyrate, % of molar	19.7 ^{a}	15.5 ^{b}	15.5 ^{b}	0.743	0.020	0.058
Iso-valerate, % of molar	3.29 ^{a}	3.13 ^{a}	2.39 ^{b}	0.287	0.036	0.249
Valerate, % of molar	1.85	1.59	1.66	0.115	0.175	0.059
Acetate:Propionate	5.69 ^{a}	3.53 ^{b}	3.55 ^{b}	0.087	0.048	<0.001

$^{a\sim c}$ Means in the same row with different superscripts differ significantly ($p < 0.05$).

Table 3. Effects of cultivation stages on dry matter and neutral detergent fiber degradation kinetics of sprouted barley incubated for 48 h in rumen buffer.

	Cultivation Stage, Days			SEM	Contrast	
	0	4	8		Linear	Quadratic
DM degradation kinetics						
A, mL/g of DM	12.2 ^{c}	38.4 ^{a}	25.9 ^{b}	1.228	0.793	<0.001
B, mL/g of DM	20.0 ^{c}	37.1 ^{a}	30.1 ^{b}	3.026	0.739	0.001
A + B, mL/g of DM	32.2 ^{c}	75.5 ^{a}	56.0 ^{b}	3.471	0.943	<0.001
C, %h	0.28 ^{a}	0.05 ^{b}	0.05 ^{b}	0.006	0.010	0.058
L, h	0.00 ^{b}	22.5 ^{a}	23.0 ^{a}	0.185	<0.001	0.358
NDF degradation kinetics						
A, mL/g of DM	23.6 ^{b}	43.4 ^{a}	23.0 ^{b}	1.992	0.761	<0.001
B, mL/g of DM	48.4 ^{ab}	47.2 ^{b}	55.4 ^{a}	1.676	0.025	0.046
A + B, mL/g of DM	72.0 ^{b}	90.6 ^{a}	78.4 ^{b}	1.937	0.226	0.003
C, %h	0.24 ^{a}	0.05 ^{b}	0.05 ^{b}	0.031	0.007	0.043
L, h	0.00 ^{c}	20.7 ^{b}	23.0 ^{a}	0.651	<0.001	0.057

A, the immediately degradable fraction; B, the potentially degradable fraction; A + B, the total degradable fraction; C, the fraction degradation rate; L, the lag phase. $^{a\sim c}$ Means in the same row with different superscripts differ significantly ($p < 0.05$).

4. Discussion

Similar to the previous studies [3,10,11], CP, EE, CA, NDF, ADF, and HEMI concentrations of SB increased by more extended cultivation stage, while DM concentration of SB was reported to decrease. As shown in Figure 1, the roots are more developed by the cultivation stages. Generally, these roots in the hydroponic cultivation system have a higher water-holding capacity than the stalk and leaves of SB, which could support the decreased DM concentration in the present study by cultivation stages. The NDSC concentrations of sprouted barley at 0 to 8 days were rapidly decreased by 30%, which might represent the use of energy sources during germination [1–3]. These explanations also can support the increased CP, EE, CA, NDF, ADF, and HEMI concentrations of sprouted barley by the cultivation stages. Meanwhile, an increase in the cultivation stage produced more green fodder carpets (roots) containing structural carbohydrates, such as NDF, ADF, and HEMI. The concentrations of NDF, ADF, and HEMI rapidly increased at 8 days of the cultivation

stage. From 0 to 8 days of cultivation stages, the increased concentrations of NDF, ADF, and HEMI were 24.7, 14.5, and 10.2%, respectively. The DM, CP, EE, NDF, and ADF concentrations of SB in the present study differed compared to previous studies [2,9–11]. These results might be due to the hybrid and cultivation stage differences.

The rumen pH decreased in all cultivation stages of SB over incubation hours, which was supported by an increase in total VFA concentration [21]. In general, cultivated SB at 4 days and 8 days presented high total VFA and ammonia-N concentrations in the early hour of incubation (4 h) than cultivated SB at 0 day. It could occur because the germination changed the starch and protein into free sugar and amino acid [1,2,22] that degraded faster into VFA and ammonia-N, respectively [21]. However, after 4 h of incubation, the barley grain presented the highest total VFA concentration in the rumen. Soluble carbohydrate is higher in the grain form than sprouted form [2,3,10], which was the main reason for this result. In general, rumen pH in SB cultivated at 8 day was lower than in SB cultivated at 4 day. Cultivated SB at 4 day had a lower structural carbohydrate than cultivated SB at 8 day (Table 1), which could decrease the digestibility (Table 3) and produce numerically lower VFA concentration, and then resulted in higher pH.

Acetate concentration of SB in the rumen decreased through the hour of incubation without any influence by the cultivation stage. This decrease in acetate concentration might be caused by decreasing rumen pH through the hour of incubation in the present study, which agreed with the previous studies [21,23]. The low rumen pH would inhibit the growth of fibrinolytic bacteria, where acetate was a result of structural carbohydrate degradation by these bacteria [21,24]. The propionate was higher in the early hour of incubation by cultivated SB at 0 day. However, cultivated SB at 0 day had the lowest propionate concentration from 4 to 48 h. The higher propionate concentration of cultivated SB at 4 and 8 days might be caused by the change of starch into free sugar by germination [1,2,22], where free sugar was degraded by rumen microbe into propionate [21,24]. The propionate showed a cubic pattern during incubation, which might be affected by the changing patterns of acetate and butyrate concentrations. It also could be partially supported by converting propionate into glucose [25] and lactate [26] through the gluconeogenesis of rumen microbes. Butyrate concentration increased slightly from 0 to 24 h of incubation. However, it was lower in SBs at 4 and 8 days compared to SB at 0 day on 48 h of incubation. In general, cultivated SB at 0 day showed a higher butyrate concentration with lower propionate concentration than cultivated SBs at 4 and 8 days during incubation. These results indicated that the germination could increase free sugar proportion and its degradation in the rumen.

The IVDMD and IVNDFD of SB after 48 h of incubation showed the quadratic effect by cultivation stage, where cultivated SB at 4 days had the highest. These results in the present study agree with the previous studies that the germination increased rumen digestibility because of a change of polymer nutrients into monomer or oligomer nutrients [1,2,22]. The increased nutrient digestibility in cultivated SBs at 4 and 8 days were also supported by the previous study, which were tested in the animal trial [4,5]. However, a more prolonged cultivation stage could decrease the digestibility of SB due to the increase of structural carbohydrates [1,10]. Therefore, in the present study, IVDMD and IVNDFD increased in cultivated SB at 4 days and then decreased in cultivated SB at 8 days. In general, the results of IVDMD and IVNDF have supported by the results of Peer and Leeson [2] that 1 to 4 days of SB cultivation were nutritionally superior for livestock diet including ruminant.

After 48 h of incubation, the result of rumen pH was supported by the result of total VFA in the present study. A high concentration of total VFA resulting from microbial degradation would decrease the rumen pH. In addition, the ammonia-N concentration also influenced the rumen pH [21], but its concentrations did not differ among the cultivation stages after 48 h of incubation. Interestingly, the total VFA concentration was a contrast to IVDMD results in the present study. Cultivated SB at 0 day had the highest total VFA concentration but the lowest IVDMD. Even though the grain barley highly contained soluble carbohydrates, but it had a lot of hull (Figure 1) that could not degrade in the

rumen. It was a reason for the low IVDMD of cultivated SB at 0 day in the present study. The barley grain was faster to degrade but had a lower total degradable fraction than it sprouting form (Table 3). In addition, the soluble carbohydrate of cultivated SB at 0 day could be another reason for high total VFA concentration that is also in agreement with by the results of Hobson and Stewart [21]. In the present study, cultivated SBs at 4 and 8 days had a higher propionate and a lower butyrate. Similar to our discussion above, these results may be caused by the changes in nutrient forms due to germination.

Supporting the IVDMD and IVNDFD results of the present study, the DM and NDF degradation kinetics results also indicated that 4 days was the best day for cultivating SB because it produced the highest total degradable fraction. The present study reported that the germination could increase the digestibility in the rumen, but the extended cultivation stage could be the opposite result. Cultivated SB at 8 days had lower degradable fraction than cultivated SB at 4 days due to higher structural carbohydrates, where hemicellulose and lignin might accrue and represented in the solid root of SB (Table 1; Figure 1). Increased structural carbohydrates (cellulose, hemicellulose, and lignin) of SB in the present study could decrease the degradation rate and increase the lag phase of DM and NDF degradation kinetics in the rumen, which agreed with a previous study [21].

5. Conclusions

The present study concluded that cultivation of SB at 4 days was recommended as a feed source for ruminants. It resulted in the highest nutrient degradability without adverse effects on fermentation characteristics in the rumen by in vitro study. Increasing cultivation stages could decrease the concentrations of DM and NDSC but increase the concentrations of CP, EE, CA, NDF, ADF, and HEMI. Cultivated SB at 8 days produced a strong solid root, which resulted in a low digestibility due to the high concentrations of structural carbohydrates compared to cultivated SB at 4 days. Generally, the defective barley grain is recommended to be processed as fodder for the ruminants, which could increase the economic value of by-products. In addition, using defective grains for sprouted fodder increases the nutritional values and helps to provide a good quality feed for sustainable farming.

Author Contributions: Conceptualization, J.-Y.K., D.H.V.P. and S.-C.K.; methodology, J.-Y.K., D.H.V.P. and Y.-H.J.; software, Y.-H.J., M.-J.S. and S.-M.J.; validation, J.-Y.K., Y.-H.J., M.-J.S. and C.-H.B.; formal analysis, J.-Y.K., D.H.V.P., M.-J.S. and C.-H.B.; investigation, D.H.V.P., M.-J.S. and S.-M.J.; resources, Y.-H.J. and S.-C.K.; data curation, J.-Y.K., D.H.V.P. and S.-C.K.; writing—original draft preparation, J.-Y.K. and D.H.V.P.; writing—review and editing, Y.-H.J. and S.-C.K.; visualization, J.-Y.K., D.H.V.P. and S.-C.K.; supervision, S.-C.K.; project administration, Y.-H.J. and S.-M.J.; funding acquisition, Y.-H.J. and S.-C.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded (Project No. 821069-03-3-HD020) by IPET (Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries), and Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.

Institutional Review Board Statement: The animal care and procedure to maintain cannulated heifers was approved by animal ethical committee of Gyeonsang National University (GNU-191011-E0050).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available on request from the corresponding author with justifiable reason.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Chavan, J.; Kadam, S.S. Nutritional improvement of cereals by sprouting. *Crit. Rev. Food Sci. Nutr.* **1989**, *28*, 401–437. [CrossRef] [PubMed]
2. Peer, D.J.; Leeson, S. Nutrient content of hydroponically sprouted barley. *Anim. Feed Sci. Technol.* **1985**, *13*, 191–202. [CrossRef]
3. Dung, D.D.; Godwin, I.R.; Nolan, J.V. Nutrient content and in sacco digestibility of barley grain and sprouted barley. *J. Anim. Vet. Adv.* **2010**, *9*, 2485–2492. [CrossRef]

4. Hafla, A.N.; Soder, K.J.; Brito, A.F.; Rubano, M.D.; Dell, C.J. Effect of sprouted barley grain supplementation of an herbage-based or haylage-based diet on ruminal fermentation and methane output in continuous culture. *J. Dairy Sci.* **2014**, *97*, 7856–7869. [CrossRef] [PubMed]
5. Farghaly, M.M.; Abdullah, M.A.M.; Youssef, I.M.I.; Abdel-Rahim, I.R.; Abouelezz, K. Effect of feeding hydroponic barley sprouts to sheep on feed intake, nutrient digestibility, nitrogen retention, rumen fermentation, and ruminal enzymes activity. *Livest. Sci.* **2019**, *228*, 31–37. [CrossRef]
6. Reed, J.J.; Bauer, M.L.; Loe, E.R.; Caton, J.S.; Lardy, G.P. Effects of processing on feeding value of sprouted barley and sprouted durum wheat in growing and finishing diets for beef cattle. *Prof. Anim. Sci.* **2005**, *21*, 7–12. [CrossRef]
7. Gebremedhin, W.K. Nutritional benefit and economic value of feeding hydroponically grown maize and barley fodder for Konkan Kanyal goats. *IOSR J. Agric. Vet. Sci.* **2015**, *8*, 24–30. [CrossRef]
8. Saidi, A.R.M.A.; Omar, J.A. The biological and economical feasibility of feeding barley green fodder to lactating Awassi ewes. *Open J. Anim. Sci.* **2015**, *5*, 99–105. [CrossRef]
9. Abouelezz, K.F.M.; Sayed, M.A.M.; Abdelnabi, M.A. Evaluation of hydroponic barley sprouts as a feed supplement for laying Japanese quail: Effects on egg production, egg quality, fertility, blood constituents, and internal organs. *Anim. Feed Sci. Technol.* **2019**, *252*, 129–135. [CrossRef]
10. Peer, D.J.; Leeson, S. Feeding value of hydroponically sprouted barley for poultry and pigs. *Anim. Feed Sci. Technol.* **1985**, *13*, 183–190. [CrossRef]
11. Fazaeli, H.; Golmohammadi, H.A.; Shoayee, A.A.; Montajebi, N.; Mosharrat, S. Performance of feedlot calves fed hydroponics fodder barley. *J. Agr. Sci. Technol.* **2011**, *13*, 367–375.
12. Lee, S.S.; Lee, H.J.; Paradhipta, D.H.V.; Joo, Y.H.; Kim, S.B.; Kim, D.H.; Kim, S.C. Temperature and microbial changes of corn silage during aerobic exposure. *Asian-Australas. J. Anim. Sci.* **2019**, *32*, 988–995. [CrossRef] [PubMed]
13. AOAC. *Official Method of Analysis*, 18th ed.; Association of Official Analytical Chemists: Washington, DC, USA, 2005.
14. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for dietary fiber, natural detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597. [CrossRef] [PubMed]
15. Adesogan, A.T.; Krueger, N.K.; Kim, S.C. A novel, wireless, automated system for measuring fermentation gas production kinetics of feeds and its application to feed characterization. *Anim. Feed Sci. Technol.* **2005**, *123*, 211–223. [CrossRef]
16. Paradhipta, D.H.V.; Joo, Y.H.; Lee, H.J.; Lee, S.S.; Noh, H.T.; Choi, J.S.; Kim, J.; Min, H.G.; Kim, S.C. Effects of inoculants producing antifungal and carboxylesterase activities on corn silage and its shelf life against mold contamination at feed-out phase. *Microorganisms* **2021**, *9*, 558. [CrossRef] [PubMed]
17. Lee, S.S.; Kim, D.H.; Paradhipta, D.H.V.; Lee, H.J.; Yoon, H.; Joo, Y.H.; Adesogan, A.T.; Kim, S.C. Effects of wormwood (*Artemisia montana*) essential oil on digestibility, fermentation indices, and microbial diversity in the rumen. *Microorganisms* **2020**, *8*, 1605. [CrossRef] [PubMed]
18. Chaney, A.L.; Marbach, E.P. Modified reagents for determination of urea and ammonia. *Clin. Chem.* **1962**, *8*, 130–132. [CrossRef]
19. Muck, R.E.; Dickerson, J.T. Storage temperature effects on proteolysis in alfalfa silage. *Trans ASAE.* **1988**, *31*, 1005–1009. [CrossRef]
20. McDonald, I. A revised model for the estimation of protein degradability in the rumen. *J. Agri. Sci.* **1981**, *96*, 251–252. [CrossRef]
21. Hobson, P.N.; Stewart, C.S. *The Rumen Microbial Ecosystem*, 2nd ed.; Blackie Academic and Professional: London, UK, 1997. [CrossRef]
22. Chung, T.Y.; Nwokolo, E.N.; Sim, J.S. Compositional and digestibility changes in sprouted barley and canola seeds. *Plant Food Hum. Nutr.* **1989**, *39*, 267–278. [CrossRef]
23. Russell, J.B. The importance of pH in the regulation of ruminal acetate to propionate ration and methane production in vitro. *J. Dairy Sci.* **1998**, *81*, 3222–3230. [CrossRef] [PubMed]
24. Sutton, J.D.; Dhanoa, M.S.; Morant, S.V.; France, J.; Napper, D.J.; Schuller, E. Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. *J. Dairy Sci.* **2003**, *86*, 3620–3633. [CrossRef] [PubMed]
25. Aiello, R.J.; Armentano, L.E.; Bertics, S.J.; Murphy, A.T. Volatile fatty acid uptake and propionate metabolism in ruminant hepatocytes. *J. Dairy Sci.* **1988**, *72*, 942–949. [CrossRef] [PubMed]
26. Weigand, E.; Young, J.W.; McGilliard, A.D. Volatile fatty acid metabolism by rumen mucosa from cattle fed hay or grain. *J. Dairy Sci.* **1974**, *58*, 1294–1300. [CrossRef]