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Effects of *Lactobacillus plantarum* and Cellulase on Mixed Silages of *Amaranthus hypochondriacus* and Cornmeal: Fermentation Characteristics, Nutritional Value, and Aerobic Stability

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Abstract: In order to develop new feed resources, the aim of this study was to investigate the effects of moisture content, additives, and their interactions on the fermentation quality, aerobic stability, and in vitro digestibility of mixed silage of amaranth and cornmeal. The mass ratios of amaranth and cornmeal were 69:31, 76:24, and 84:16 for adjusting the moisture content of silage to 60% (W1), 65% (W2), and 70% (W3), respectively. The silage treatments included no additives (U), the addition of *Lactobacillus plantarum* (L), the addition of cellulase (E), and the addition of *Lactobacillus plantarum* + cellulase (M) mixed reagents. The results revealed that the pH and ammonia nitrogen (NH_3-N/TN) ratios were significantly lower in W1 than in W2 and W3 (3.66,19.3 g kg^{-1} TN vs. 3.70, 3.70, 20.0 kg^{-1} TN, 25.1 kg^{-1} TN, $p < 0.05$). Moreover, dry matter (DM), organic matter (OM), in vitro dry matter digestibility (*iv*DMD), in vitro organic matter digestibility (*iv*OMD), and in vitro crude protein digestibility (*iv*CPD) significantly increased ($p < 0.05$). Meanwhile, the aerobic stability of mixed silage containing amaranth and cornmeal decreased with increasing water content. The aerobic stability of the L, E, and M treatment groups was improved by 15, 105, and 111 h, respectively, compared with that of the control group at W1. The pH and NH_3-N/TN ratios were lower with the addition of E (E and M) than with the absence of E (U and L) (3.73, 20.1 g kg^{-1} DM vs. 3.64, 22.9 g kg^{-1} DM, $p < 0.05$). NDF and ADF were significantly lower with the addition of E than without the addition of E (598 g kg^{-1} DM, 145 g kg^{-1} DM vs. 632 g kg^{-1} DM, 160 g kg^{-1} DM, $p < 0.05$). However, CP, *iv*DMD, *iv*OMD, and *iv*CPD were significantly higher ($p < 0.05$). AA and NH_3-N/TN were significantly lower ($p < 0.05$) with the addition of L (L and M) than without the addition of L (U and E). In conclusion, the best fermentation quality, in vitro digestibility, and aerobic stability of amaranth and cornmeal mixed silage treated with *Lactobacillus plantarum* + cellulase (M) were achieved at 60% water content. The present study confirmed the potential of amaranth as silage and its potential application for improving feed quality and animal performance.

Keywords: amaranth; fermentation quality; nutritional value; in vitro digestibility; aerobic stability

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1. Introduction

With the development of livestock and poultry farming and the increased demand for feed resources, traditional silage cannot fully meet the needs of the farming industry. However, some of the other proteins required by ruminants, such as dairy cows, usually come from feed [1]. Therefore, the pursuit of reasonably priced, highly productive, and efficient novel protein feeds to completely or partially replace traditional protein feeds is highly important for improving the quality of animal products [2]. In this context, amaranth, as a plant-based protein feed rich in nutrients with unique properties, is considered a feed

resource with great potential. Amaranth can be grown in many areas of China, and the yield is generally 75,000–150,000 kg per hectare of fresh weight, with some varieties yielding as much as 225,000 kg, which is considered a promising feed resource [3].

Amaranth belongs to the genus Amaranthus of the family Amaranthaceae and is used as both human food and animal feed. It is rich in vitamins and minerals, with high protein content, high resistance, and high yield, making it a high-quality feed resource for ruminants [4,5]. In addition, the dry matter of amaranth has good degradability and fermentation properties, which can add value to ruminant feed. The addition of amaranth silage has been reported to increase body weight gain and reduce rumen methane emissions in male lambs [6–9]. However, amaranth cannot be preserved for a long period via conventional methods because of its high protein content, high water content, and thick stalks that cannot be easily dried into hay [4,5]. Therefore, silage is a good way to improve the utilization of amaranth, which can not only prolong the preservation time but also improve the palatability of the feed. Amaranth is characterized by high moisture and low soluble carbohydrate content. Thus, preserving amaranth directly via conventional silage methods is considered difficult. The soluble carbohydrate content of amaranth was found to be 50.46 g kg^{-1} dry matter (DM), meeting just the minimum threshold of 50 g kg^{-1} soluble carbohydrate content recommended for producing high-quality silage [10]. The addition of 10% cornmeal has been reported to improve the fermentation quality and apparent digestibility of silage [11]. Moreover, cornmeal, which is characterized by high contents of soluble carbohydrates (WSC) and dry matter, is a good mixed silage auxiliary that can directly increase the fermentation substrate, compensate for the lack of fermentable carbohydrates in amaranth, and reduce the water content to improve the fermentation success of silage. However, to our knowledge, few studies have investigated the effects of mixing amaranth silage with cornmeal during ensiling.

Silage success also depends on appropriate biological and chemical conditions that allow a rapid and sufficient decrease in the pH of the silage. Therefore, silage additives are recommended to manipulate fermentation and prolong aerobic stability [4]. Microbial additives such as lactic acid bacteria and cellulase can lead to a rapid drop in pH, facilitating the silage process and improving the fermentation quality [12].

The effects of water content, lactic acid bacteria, and cellulase on fermentation quality, in vitro digestibility, and aerobic stability of amaranth silage have been shown in previous studies, but their interactions have not been explored [13]. Therefore, the aim of this study was to explore the effects of moisture and additives on the fermentation quality, aerobic stability, and in vitro digestibility of mixed silage of amaranth and cornmeal by adding lactobacilli and cellulase individually or in a mixture. This study can provide more feed choices and ways to utilize the resources in the farming industry and promote the sustainable development of the livestock and poultry farming industry.

2. Materials and Methods

2.1. Experimental Materials and Design

The cultivation experiment was conducted in the experimental field of Jilin University (123.3° E, 44.1° N), China. Planting was in June 2020, and the plants were harvested in September. Amaranth was in full maturity at the time of harvesting.

The amaranth was chopped into lengths of approximately 1 to 2 cm before ensiling. The mixing ratios (*w*/*w*) of amaranth and cornmeal ingredients were 69:31, 76:24, and 84:16 in order to regulate the water content of the silage ingredients to 60%, 65%, and 70%, respectively. For each silage moisture level, the silage treatment was designed as follows: no additive (U), lactic acid bacteria inoculant (L), cellulase (E), and a mixed preparation of lactic acid bacteria and cellulase (M). For the lactic acid bacterial inoculant, Chikusou-1 (*Lactobacillus plantarum*) was obtained from Snow Brand Seed Co., Ltd. (Tokyo, Japan). Acremonium cellulase was obtained from Meiji Seika Pharma Co., Ltd., Tokyo, Japan (Lot No.: ACCF-6940). The lactic acid bacteria were dissolved in distilled water according to the manufacturer's instructions, after which the samples were sprayed evenly with a micro

sprayer and mixed thoroughly. The dosage of lactic acid bacteria was 4.7×10^6 colony-forming units (cfu) per gram of fresh weight (FW). Meanwhile, cellulase was applied at 50 mg kg^{-1} FW. It was dissolved in distilled water according to the manufacturer's instructions, after which the samples were sprayed evenly with a micro sprayer and mixed well. The actual enzyme activity was 4.2×10^3 U g^{-1} FW. The mixed silage was subsequently loaded into a 5-liter plastic silo. The silage density was 550.1 ± 20.0 kg m^{-3} FW, and the silo was kept at room temperature (21–25 °C) for anaerobic fermentation. After 60 days of fermentation, three replicate silos were opened for the determination of the chemical composition, fermentation quality, and in vitro digestibility of the silage. The remaining silage was repeatedly mixed for the aerobic stability tests.

2.2. Fermentation Quality Analysis

Once opened, silage samples were taken via the "tetrad" method. Subsequently, 20 g of silage was thoroughly mixed with 180 mL of distilled water and homogenized in polyethylene vacuum bags for 1 min. The sample was then extracted in a refrigerator at a constant temperature of 4 °C for 24 h and filtered through 4 layers of gauze and qualitative filter paper [14]. A portion of the resulting extract was used to measure the pH via a pH meter (PHSJ-4F, Yidian Scientific Instruments Co., Ltd., Shanghai, China). The other part was frozen and stored at −20 °C for the determination of organic acids and ammonia nitrogen (NH_3-N) contents. The NH_3-N content was determined by the Robinson method [15]. Lactic acid (LA), acetic acid (AA), propionic acid (PA), and butyric acid (BA) contents were determined by using high-performance liquid chromatography (column: ShodexRspak KC-811s-DVB gel column, Japan; detector: SPD-M10AVP; mobile phase: 3 mmol L^{-1} perchloric acid; flow rate: 1 mL min^{-1}; column temperature: 50 °C; detection wavelength: 210 nm; injection volume: 5 μL).

2.3. Chemical Composition Analysis, Energy, and In Vitro Degradability Analysis

The dry matter (DM) contents of fresh samples and silages were determined in a 65 °C oven for 48 h. The dried samples were ground and passed through a 1.0 mm sieve for chemical analysis. The contents of organic matter (OM) and crude protein (CP) were determined via the methods of the Official Association of Analytical Chemists [16]. The neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents were determined according to the methods reported by Van et al. [17]. The water-soluble carbohydrate (WSC) content was measured via the anthrone sulfate colorimetric method [18]. The buffering capacity (BC) was measured using the method of Playne and McDonald [19]. The gross energy (GE) content was determined by an oxygen cartridge calorimeter (SDAC1000, Sundy, Changsha, China).

In vitro degradability experiments were conducted according to the principles of the Laboratory Animal Guidelines for the Ethical Review of Animal Welfare. The study protocol was reviewed and approved by the Animal Ethics and Welfare Committee of Jilin University (Jilin, China; Approval Number: SY202009600). The dried silage sample (0.5 g) was placed in a filter bag (ANKOM F57; diameter of hole 25 μm; Ankang Technology; Macedon, NY, USA) and sealed with a hand pressure sealing machine (PFS-400; Zhejiang Dongfeng Packing Machine Co., Ltd. Wenzhou, China) for subsequent in vitro incubation. Before the samples were placed in them, the fiber bags were rinsed with acetone and then thoroughly air-dried for 5 h at 105 °C in a forced convection drying oven (VL 115, VWR, Shanghai, China). A total of 196 fiber bags were prepared (48 silage silos × 4 parallel samples + 4 blank controls). The filter bag was processed and then loaded into a 130 mL serum bottle. Four small-tailed billy goats were fed a mixture consisting of 40% corn silage, 20% alfalfa hay, and 40% concentrate (DM-based) twice daily. Rumen fluid was collected from these animals. The rumen fluid was maintained at a temperature of 39 °C under an atmosphere of carbon dioxide. The medium was filtered through four layers of coarse cotton cloth, and then the filtrate was mixed with McDougall artificial saliva at a 1:4 (v/v) ratio. Each serum vial was supplemented with a 60 mL mixture followed by incubation in

a CO_2 atmosphere at 39 °C. The incubation was performed in a water bath. After 72 h of incubation, the filter bag was removed from the serum vial and gently rinsed with cold distilled water until the water become clear. The fiber bags were gently squeezed to remove excess moisture and then dried in a forced convection oven at 100 °C for 24 h. The residue was then weighed and measured for in vitro dry matter digestibility (*iv*DMD) [20]. In vitro neutral deterrent fiber digestibility (*iv*NDFD) was determined by analyzing the residual NDF [21]. The formulas for *iv*DMD, *iv*NDFD, in vitro crude protein digestibility (*iv*CPD), and in vitro organic matter digestibility (*iv*OMD) are as follows: (respective weights of DM, NDF, CP, and OM before digestion-respective residual weights of DM, NDF, CP, and OM)/(respective weights of DM, NDF, CP, and OM before digestion).

2.4. Microbiological Analysis

Fresh samples (20 g) were mixed uniformly with 180 mL of sterile saline solution (0.85% NaCl) and shaken on a shaker for 30 min. Then, 1 mL of the homogenate was subjected to 10 × serial dilutions. Each gradient was prepared as 3 parallel replicates and poured into dishes. Finally, 100 μL of the dilutions at various concentrations were evenly applied to agar media as described below with coated rods. *Lactobacillus bacteria* were cultivated on De Man, Rogosa, and Sharp agar media (Budweiser Technology Co., Ltd., Shanghai, China) via incubation for 48 h under anaerobic conditions at 37 °C. Aerobic bacteria were cultivated on nutrient agar media via incubation at 37 °C (Hope Biotechnology Co., Ltd., Qingdao, China). Yeast and molds were grown on potato glucose agar media at 28 °C for 48 h (Budweiser Technology Co., Ltd., Shanghai, China). The numbers of microorganisms were counted on plates of 20–200 cfu. All microbial data were converted to log10 cfu g^{-1}. The results are reported on a fresh weight basis.

2.5. Aerobic Stability Analysis

Amaranth silage from each treatment was placed in a clean 1 L plastic bucket. A thermocouple wire was placed in the center of the amaranth silage, and the ambient temperature was recorded by the thermocouple line in the empty bucket using a data recorder (OHR-G100T; Hongrun Company, Ltd., Fuzhou, China). The silage temperature was recorded at 1 h intervals. The ambient temperature was also recorded every hour as a blank. Aerobic stability is the time taken for silage to reach a temperature 2 °C higher than the ambient temperature.

2.6. Statistical Analysis

The data were analyzed via the GLM program of SPSS statistical software (version 26; International Business Machine Corporation; Armonk, NY, USA) for each indicator according to the following model:

$$Y_{ijk} = a + W_i + E_j + L_k + (W \times E)_{ij} + (W \times L)_{ik} + (L \times E)_{jk} + (W \times E \times L)_{ijk} + b_{ijk}$$

In the above model, Y_{ijk} is the response variable, a is the overall mean, W_i is the fixed effect of the moisture content of silage material i (i = 1, 2, 3), E_j is the fixed effect of cellulase j (j = 1, 2), and L_k is the fixed effect of lactic acid bacteria k (k = 1, 2). $(W \times E)_{ij}$ is the interaction of silage feedstock moisture content i and cellulase j. $(W \times L)_{ik}$ is the interaction of silage feedstock moisture content i and lactic acid bacteria k. $(L \times E)_{jk}$ is the interaction of cellulase j and lactic acid bacteria k. $(W \times E \times L)_{ijk}$ is the interaction of moisture i, cellulase j, and lactic acid bacteria k. b_{ijk} is the residual error.

Multiple comparisons were made via Tukey's test on the basis of the results of significance tests for water content, enzyme treatment, bacterial treatment, and interaction [22].

3. Results

3.1. Chemical Composition and Microbial Counts of Fresh Materials

The chemical composition, gross energy, buffering capacity, and microbial counts of amaranth and cornmeal mixed silage are shown in Table 1. The DM, CP, and WSC contents

of amaranth were 185 g kg^{-1}, 124 g kg^{-1} DM, and 50.46 g kg^{-1} DM, respectively. The buffering capacity value of amaranth was 340 mEq kg^{-1} DM, which was 4.0 times higher than that of cornmeal (85.9 mEq kg^{-1} DM). The numbers of lactic acid bacteria, yeast, and mold adhering to the surface of amaranth were 2.42 log_{10} cfu^{-1}, 2.00 log_{10} cfu^{-1}, and 0.41 log_{10} cfu^{-1}, respectively.

Table 1. Characteristics of amaranth and cornmeal.

Item	Amaranth	Cornmeal
Chemical composition, energy, and buffering capacity		
Dry matter (g kg^{-1} FW)	185	873
Organic matter (g kg^{-1} DM)	876	981
Crude protein (g kg^{-1} DM)	124	91.4
Neutral detergent fiber (g kg^{-1} DM)	651	303
Acid detergent fiber (g kg^{-1} DM)	377	85.0
Acid detergent lignin (g kg^{-1} DM)	111	16.7
Water-soluble carbohydrate (g kg^{-1} DM)	50.46	103.32
Gross energy (MJ kg^{-1} DM)	18.0	19.5
Buffering capacity (mEq kg^{-1} DM)	340	85.9
Microbial counts		
Lactic acid bacteria (log_{10} cfu^{-1} FW)	2.42	ND
Yeast (log_{10} cfu^{-1} FW)	2.00	ND
Mold (log_{10} cfu^{-1} FW)	0.41	ND

DM, dry matter; FW, fresh weight; cfu, colony-forming units; ND, not detected.

3.2. Fermentation Quality of Amaranth and Cornmeal Mixed Silage

Table 2 showed the fermentation quality of the amaranth and cornmeal mixed silage. All the treatment groups had pH values less than 4.0 after 60 days of silage. The addition of L (L and M groups) or E (E and M groups) significantly ($p < 0.05$) decreased the pH of the silages. In addition, there was an interaction effect of W × E on pH ($p < 0.001$). Without the addition of E (U and L groups) significantly increased pH compared to with the addition of E (3.74, 3.72 vs. 3.64, 3.63, $p < 0.05$). However, this effect was greater in W2 than in W1 and W3. In contrast, the addition of L led to a decrease in pH (3.72, 3.63 vs. 3.74, 3.64), but L and W did not interact.

The water content significantly affected the LA and PA contents, with W2 having significantly higher LA content than W1 and W3 (20.3 g kg^{-1} DM vs. 16.9 g kg^{-1} DM, 14.6 g kg^{-1} DM, $p < 0.05$) and a significantly lower PA content (0.00 g kg^{-1} DM vs. 0.03 g kg^{-1} DM, 7.34 g kg^{-1} DM, $p < 0.05$). The AA content of amaranth and cornmeal mixed silage was significantly lower (16.3 g kg^{-1} DM, vs. 17.8 g kg^{-1} DM) in group L than in group U. The AA content of silage was significantly lower in group L than in group U. The AA content of silage was significantly lower in group L than in group U.

There was an interaction effect between W × E and W × L on the BA content ($p < 0.05$). The BA content resulting from the addition of E was significantly higher than that resulting from the addition of BA without E (1.26 g kg^{-1} DM, 1.81 g kg^{-1} DM vs. 0.649 g kg^{-1} DM, 0.246 g kg^{-1} DM). With the addition of E, W3 had the lowest BA content. However, without the addition of E, W1 had the lowest BA content. The BA content with the addition of L was significantly lower than that without the addition of L (0.246 g kg^{-1} DM, 1.26 g kg^{-1} DM vs. 0.649 g kg^{-1} DM, 1.81 g kg^{-1} DM). Without the addition of L, the BA content decreased with increasing water content. However, with the addition of L, the change in BA content with water content was not significant ($p > 0.05$).

Table 2. Fermentation quality of amaranth and cornmeal mixed silage prepared with lactic acid bacteria and cellulose.

Item ‡	Moisture	Average	Additives †				SEM	Significance of Main Effects and Interactions (*p*-Value)						
			U	E	L	M		W	E	L	W × E	W × L	L × E	W × L × E
pH value	W1	3.66	3.69 ^{Ab}	3.63 ^{a}	3.69 ^{Ab}	3.62 ^{a}	0.002	<0.001	<0.001	0.001	<0.001	0.179	0.217	0.093
	W2	3.70	3.77 ^{Bb}	3.64 ^{a}	3.73 ^{Bb}	3.64 ^{a}								
	W3	3.70	3.77 ^{Bc}	3.64 ^{a}	3.73 ^{Bb}	3.62 ^{a}								
	Average	3.68	3.74	3.64	3.72	3.63								
LA (g kg^{-1} DM)	W1	16.9	16.6 ^{Aab}	20.6 ^{b}	12.2 ^{a}	18.1 ^{b}	0.078	0.020	0.811	0.338	0.116	0.599	0.953	0.455
	W2	20.3	22.4 ^{B}	17.7	21.1	19.8								
	W3	14.6	14.3 ^{A}	16.3	15.7	12.0								
	Average	17.2	17.8	18.2	16.3	16.6								
AA (g kg^{-1} DM)	W1	17.1	13.7	18.3	9.10 ^{A}	10.1	0.090	0.041	0.941	0.047	0.090	0.199	0.514	0.126
	W2	18.3	22.0	13.7	20.4 ^{B}	17.2								
	W3	17.5	16.5	24.2	15.0 ^{AB}	14.1								
	Average	16.2	17.4	18.7	14.8	13.8								
PA (g kg^{-1} DM)	W1	0.03	0.11	ND	ND	ND ^{A}	0.131	0.002	0.082	0.681	0.053	0.836	0.535	0.671
	W2	ND	ND	ND	ND	ND ^{A}								
	W3	7.34	ND	19.3	8.30	1.76 ^{B}								
	Average	2.46	0.37	6.43	2.77	0.59								
BA (g kg^{-1} DM)	W1	1.67	0.902 ^{a}	3.85 ^{Cb}	0.152 ^{a}	1.78 ^{a}	0.009	<0.001	<0.001	0.006	<0.001	0.008	0.433	0.139
	W2	1.06	0.721	1.59 ^{B}	0.330	1.58								
	W3	0.198	0.323	ND ^{A}	0.255	0.017								
	Average	0.990	0.649	1.81	0.246	1.26								
NH_3-N (g kg^{-1} TN)	W1	19.3	21.0 ^{Ab}	19.9 ^{Aab}	18.1 ^{Aa}	18.3 ^{Aa}	0.016	<0.001	<0.001	<0.001	<0.001	0.036	<0.001	<0.001
	W2	20.0	21.4 ^{Ab}	19.2 ^{Aab}	21.1 ^{Bb}	18.4 ^{Aa}								
	W3	25.1	30.7 ^{Bc}	21.9 ^{Ba}	24.7 ^{Cb}	22.9 ^{Bab}								
	Average	21.5	24.4	20.3	21.3	19.9								

$^{A–C}$ Means of water contents within a column with different superscripts differ in the same additive treatment ($p < 0.05$); $^{a–c}$ Means of additives treatments within a row with different superscripts differ on the same water content ($p < 0.05$); SEM, standard error of the mean; W, moisture; U, no additive. † L, lactic acid bacteria; E, cellulase; M, the mixture of lactic acid bacteria and cellulase. ‡ DM, dry matter; LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; NH_3-N, ammonia nitrogen; TN, total nitrogen; ND, not detected.

There was an interaction effect between W × E, W × L, and L × E on NH_3-N/TN ($p < 0.05$). NH_3-N/TN decreased with decreasing water content (25.1 g kg^{-1} DM, 20.0 g kg^{-1} DM, and 19.3 g kg^{-1} DM, $p < 0.05$). However, without the addition of E, the mean NH_3-N/TN ratio was higher than that of the addition of the E group (22.9 g kg^{-1} DM vs. 20.1 g kg^{-1} DM, $p < 0.05$). Moreover, the NH_3-N/TN ratio with the addition of L was significantly lower than that without the addition of L (20.6 g kg^{-1} DM vs. 22.4 g kg^{-1} DM, $p < 0.05$). With the addition of L, W1 had the lowest NH_3-N/TN. Without the addition of L, W2 had the lowest NH_3-N/TN. The addition of L significantly reduced NH_3-N/TN, but with the addition of E, the mean NH_3-N/TN ratio was lower than that without the addition of E (19.9 g kg^{-1} DM vs. 21.3 g kg^{-1} DM, $p < 0.05$).

3.3. Chemical Composition of Amaranth and Cornmeal Mixed Silage

Table 3 showed the chemical composition of the amaranth and cornmeal mixed silage. The effects of water content and additives on the in vitro digestibility of silage are shown in Table 4. The DM content increased with decreasing water content (287 g kg^{-1}, 330 g kg^{-1}, 389 g kg^{-1}, $p < 0.05$). There was an interaction effect of L × E on the DM content ($p = 0.010$). The addition of L reduced the DM content of the silage. However, the effect of the E addition was greater than that without the addition of E (329 g kg^{-1} vs. 340 g kg^{-1}, $p < 0.05$). There was an interaction effect of W × E on the OM content ($p < 0.001$). With the addition of E, the OM content was significantly lower than that without the addition of E (940 g kg^{-1} DM, 941 g kg^{-1} DM vs. 942 g kg^{-1} DM, 942 g kg^{-1} DM, $p < 0.05$). However, this effect was lower for W2 than for W1 and W3.

There was an interaction effect between W × E and W × L on the CP content ($p < 0.05$). With the addition of E, the CP content was higher than that of the group without E (121 g kg^{-1} DM vs. 118 g kg^{-1} DM, $p < 0.05$). However, this effect is smaller for W1 compared to W2 and W3. With the addition of L, CP content increased ($p < 0.05$) with increasing water content. However, it remained unchanged without the addition of L.

There was an interaction effect of W × L on NDF content ($p = 0.024$). The NDF content in the addition of L treatment was significantly lower than that in the treatment without the L addition (631 gkg^{-1} DM, 588 g kg^{-1} DM vs. 632 g kg^{-1} DM, 607 g kg^{-1} DM, $p < 0.05$). With the addition of L, W1 had the highest NDF content, whereas without the addition of L, W3 had the highest NDF content.

There was an interaction effect of W × E on the ADF content ($p = 0.033$). The ADF content decreased with decreasing water content (190 g kg^{-1} DM, 153 g kg^{-1} DM, 115 g kg^{-1} DM, $p < 0.05$). Without the addition of E, the ADF content was significantly higher than that with the addition of E (160 g kg^{-1} DM, 160 g kg^{-1} DM vs. 146 g kg^{-1} DM, 143 g kg^{-1} DM, $p < 0.05$). However, this effect was smaller for W1 compared to W2 and W3.

There was an interaction effect between W × E and W × L on GE ($p < 0.05$). The addition of E increased the GE content under W2 (18.9 MJ kg^{-1} DM, 19.0 MJ kg^{-1} DM vs. 18.7 MJ kg^{-1} DM, 18.8 MJ kg^{-1} DM, $p < 0.05$), but there was no significant difference between W1 and W3 conditions with the addition of E. The effect of W × E on the GE content of silage with the addition of L was not significant ($p < 0.05$), and the effect of W × L on the GE content of silage with the addition of L was not significant ($p < 0.05$). In silage with the L addition, GE decreased ($p < 0.05$) with increasing water content but was unaffected ($p > 0.05$) in silage without the L addition.

Table 3. Chemical composition and energy of amaranth and cornmeal mixed silage prepared with lactic acid bacteria and cellulose.

Item ‡	Moisture	Average	Additives †				SEM	Significance of Main Effects and Interactions (*p*-Value)						
			U	E	L	M		W	E	L	W × E	W × L	L × E	W × L × E
DM (g kg^{-1})	W1	389	400 ^{Cb}	382 ^{Ca}	394 ^{Cb}	381 ^{Ca}	0.050	<0.001	<0.001	0.074	0.492	0.468	0.010	0.971
	W2	330	338 ^{Bb}	323 ^{Ba}	335 ^{Bb}	325 ^{Ba}								
	W3	287	296 ^{Ab}	280 ^{Aa}	292 ^{Ab}	280 ^{Aa}								
	Average	336	345	328	340	329								
OM (g kg^{-1} DM)	W1	952	954 ^{Cb}	951 ^{Ca}	953 ^{Cb}	951 ^{Ca}	0.015	<0.001	<0.001	0.832	<0.001	0.108	0.924	0.957
	W2	941	940 ^{B}	941 ^{B}	941 ^{B}	942 ^{B}								
	W3	931	933 ^{Ab}	929 ^{Aa}	933 ^{Ab}	929 ^{Aa}								
	Average	941	942	940	942	941								
CP (g kg^{-1} DM)	W1	118	119	118 ^{A}	116 ^{A}	118 ^{A}	0.022	<0.001	<0.001	0.766	0.002	0.017	0.773	0.035
	W2	120	117 ^{a}	122 ^{Bb}	118 ^{ABa}	121 ^{Bb}								
	W3	122	119 ^{a}	122 ^{Bb}	121 ^{Bab}	124 ^{Cb}								
	Average	120	118	121	118	121								
NDF (g kg^{-1} DM)	W1	627	643 ^{B}	608	644	613 ^{B}	0.368	0.004	<0.001	0.190	0.924	0.024	0.248	0.553
	W2	595	598 ^{A}	584	623	575 ^{A}								
	W3	621	654 ^{Bb}	628 ^{ab}	625 ^{ab}	576 ^{Aa}								
	Average	615	632	607	631	588								
ADF (g kg^{-1} DM)	W1	115	114 ^{A}	115 ^{A}	121 ^{A}	110 ^{A}	0.151	<0.001	<0.001	0.828	0.033	0.900	0.655	0.508
	W2	153	162 ^{B}	145 ^{B}	159 ^{B}	144 ^{B}								
	W3	190	204 ^{Cb}	177 ^{Ca}	201 ^{Cb}	176 ^{Ca}								
	Average	152	160	146	160	143								
ADL (g kg^{-1} DM)	W1	19.9	19.1 ^{A}	21.1 ^{A}	19.4 ^{A}	19.8 ^{A}	0.033	<0.001	0.478	0.713	0.393	0.854	0.193	0.110
	W2	25.5	25.5 ^{B}	25.9 ^{BC}	24.3 ^{B}	26.1 ^{B}								
	W3	34.0	35.7 ^{C}	32.1 ^{C}	33.2 ^{C}	35.2 ^{C}								
	Average	26.5	26.8	26.4	25.6	27.0								
GE (MJ kg $^{-1}$ DM)	W1	18.7	18.7 ^{B}	18.7 ^{B}	18.7 ^{B}	18.6 ^{B}	0.009	<0.001	0.274	0.467	<0.001	0.017	0.734	0.920
	W2	18.9	18.7 ^{B}	18.9 ^{C}	18.8 ^{B}	19.0 ^{C}								
	W3	17.9	18.0 ^{Ac}	17.9 ^{Aab}	18.0 ^{Abc}	17.8 ^{Aa}								
	SEM	18.5	18.5	18.5	18.5	18.5								

$^{A–C}$ Means of water contents within a column with different superscripts differ with the same additive treatment ($p < 0.05$); $^{a–c}$ Means of additives treatments within a row with different superscripts differ with the same water content ($p < 0.05$); SEM, standard error of the mean; W, moisture; U, no additive. † L, lactic acid bacteria; E, cellulase; M, the mixture of lactic acid bacteria and cellulase. ‡ DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; GE, gross energy.

Table 4. In vitro digestibility of amaranth and cornmeal mixed silage prepared with lactic acid bacteria and cellulase.

Item ‡	Moisture	Average	Additives †				SEM	Significance of Main Effects and Interactions (*p*-Value)						
			U	E	L	M		W	E	L	W × E	W × L	L × E	W × L × E
*iv*DMD (g kg^{-1})	W1	737	738 ^{C}	737 ^{C}	732 ^{C}	741 ^{C}	0.117	<0.001	<0.001	0.828	0.033	0.900	0.655	0.508
	W2	709	701 ^{B}	715 ^{B}	703 ^{B}	715 ^{B}								
	W3	680	669 ^{Aa}	690 ^{Ab}	671 ^{Aa}	690 ^{Ab}								
	Average	709	703	714	702	715								
*iv*OMD (g kg^{-1} DM)	W1	777	778 ^{C}	777 ^{C}	772 ^{C}	781 ^{C}	0.118	<0.001	<0.001	0.828	0.033	0.900	0.655	0.508
	W2	748	740 ^{B}	754 ^{B}	742 ^{B}	754 ^{B}								
	W3	719	708 ^{Aa}	729 ^{Ab}	710 ^{Aa}	729 ^{Ab}								
	Average	748	742	753	741	756								
*iv*CPD (g kg^{-1} DM)	W1	589	590 ^{Cb}	589 ^{Bab}	586 ^{Ca}	591 ^{Bb}	0.040	<0.001	<0.001	0.733	0.001	0.204	0.585	0.078
	W2	582	577 ^{Ba}	586 ^{Bb}	578 ^{Ba}	585 ^{ABb}								
	W3	574	568 ^{Aa}	578 ^{Ab}	571 ^{Aa}	580 ^{Ab}								
	Average	581	578	584	578	585								
*iv*NDFD (g kg^{-1} DM)	W1	579	594 ^{B}	560	595	565 ^{B}	0.355	0.004	<0.001	0.190	0.924	0.024	0.248	0.553
	W2	548	550 ^{A}	537	575	528 ^{A}								
	W3	572	605 ^{Bb}	579 ^{ab}	576 ^{ab}	529 ^{Aa}								
	Average	567	583	559	583	541								

$^{A–C}$ Means of water contents within a column with different superscripts differ with the same additive treatment ($p < 0.05$). $^{a–b}$ Means of additives treatments within a row with different superscripts differ with the same water content ($p < 0.05$). SEM, standard error of the mean; W, moisture; U, no, additive. † L, lactic acid bacteria; E, cellulase; M, the mixture of lactic acid bacteria and cellulase. ‡ *iv*DMD, in vitro dry matter digestibility; *iv*OMD, in vitro organic matter digestibility; *iv*CPD, in vitro crude protein digestibility; *iv*NDFD, in vitro neutral detergent fiber digestibility.

3.4. In Vitro Digestibility of Amaranth and Cornmeal Mixed Silage

There was an interaction effect of W × E on *iv*DMD, *iv*OMD, and *iv*CPD ($p < 0.05$). *iv*DMD, *iv*OMD, and *iv*CPD were significantly lower without the addition of E than with the addition of E (715 g kg^{-1} DM, 755 g kg^{-1} DM, 585 g kg^{-1} DM vs. 703 g kg^{-1} DM, 742 g kg^{-1} DM, 578 g kg^{-1} DM, $p < 0.05$). However, this effect was smaller in W1 compared to W2 and W3. There was an interaction effect of W × L on the *iv*NDFD ($p = 0.024$). The addition of L increased the *iv*NDFD content under W1 (595 g kg^{-1} DM, 565 g kg^{-1} DM vs. 594 g kg^{-1} DM, 560 g kg^{-1} DM, $p < 0.05$), but there was no significant difference between W2 and W3 conditions with the addition of E.

3.5. Aerobic Stability of Amaranth and Cornmeal Mixed Silage

The effects of moisture content and additives on temperature changes in mixed amaranth and cornmeal silage under aerobic conditions are shown in Figures 1–4. At 60% moisture content, the temperatures increased from 0 to 69 h in all the treatment groups, with greater increases in the U and L treatment groups. At 65% moisture content, the temperature of all the treatment groups increased from 0 to 78 h. At 70% moisture content, the temperature of all the treatment groups increased from 0 to 69 h, with greater increases in the U and L treatment groups. At 70% moisture content, the temperature increased significantly after 51 h in the L treatment group and after 69 h in the E treatment group. The aerobic stability decreased significantly ($p < 0.05$) with increasing water content. The aerobic stability of the E and M treatments was significantly higher than that of the U treatment at all water contents.

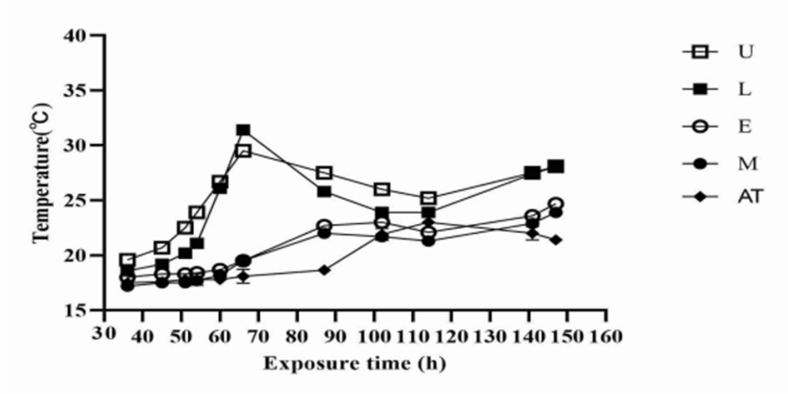

Figure 1. Effect of additive application on the temperature change in amaranth and corn powder mixed silage after exposure to aerobic conditions at 60% moisture content. U, control; L, lactic acid bacteria; E, cellulase; M, mixture of lactic acid bacteria and cellulase; AT, ambient temperature.

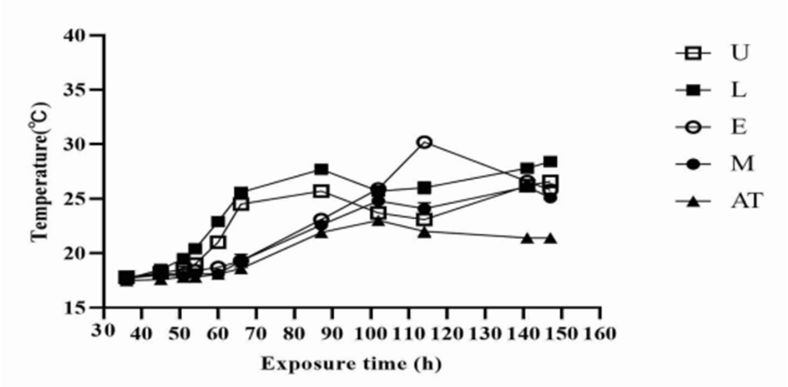

Figure 2. Effect of additive application on the temperature change in amaranth and corn powder mixed silage after exposure to aerobic conditions at 65% moisture content. U, control; L, lactic acid bacteria; E, cellulase; M, mixture of lactic acid bacteria and cellulase; AT, ambient temperature.

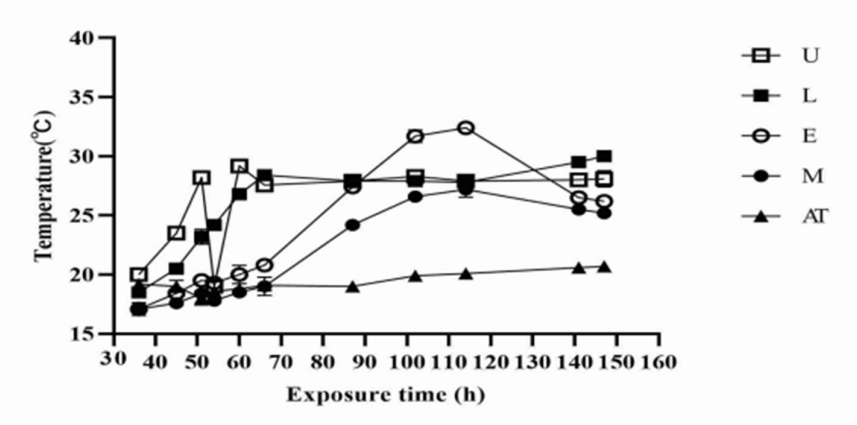

Figure 3. Effect of additive application on the temperature change in amaranth and corn powder mixed silage after exposure to aerobic conditions at 70% moisture content. U, control; L, lactic acid bacteria; E, cellulase; M, mixture of lactic acid bacteria and cellulase; AT, ambient temperature.

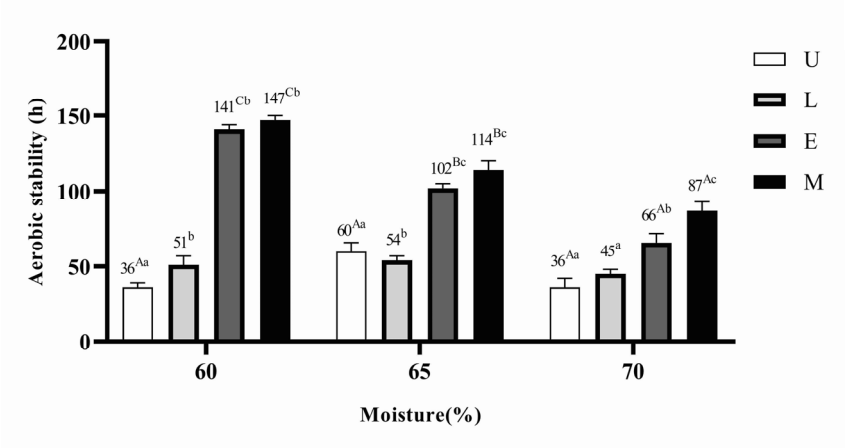

Figure 4. Time required for the temperature of amaranth and corn powder mixed silage to exceed room temperature by 2 °C after exposure to air. U, control; L, lactic acid bacteria; E, cellulase; M, mixture of lactic acid bacteria and cellulase. $^{A–C}$ Means of moisture contents with different superscripts differ under the same additive treatment ($p < 0.05$). $^{a–c}$ Means of additives treatments with different superscripts differ under the same moisture content.

4. Discussion

In our experimental hypotheses, we speculated that the addition of lactic acid bacteria and cellulase at W1 could induce earlier lactic acid fermentation in the mixed silage and improve fermentation quality, in vitro digestibility, and aerobic stability. As shown in Table 2, the M treatment had the best quality. According to Table 4, *iv*DMD and *iv*OMD were the highest in the M treatment at all water contents. As shown in Figure 1, the M treatment had the highest aerobic stability, which is consistent with our previous speculation.

4.1. Effects of Moisture and Additives on the Fermentation Quality of Amaranth and Cornmeal Mixed Silage

Water content is the main factor affecting silage quality. When the silage water content is too high, it can lead to a negative silage quality [23]. However, when the water content is too low, more pores are present in silage silos than in silages with higher water contents. Moreover, the low content of organic acids with antifungal activity (acetic acid) is not sufficient to inhibit the growth of yeasts, which can deteriorate quickly after opening [24]. Muck et al. reported that fermentation quality improved and nutrient losses decreased at a silage moisture of approximately 65% [25]. Therefore, three moisture levels of 60%, 65%, and 70% were used in the current study. The decrease in pH was more pronounced

at lower water contents. This may be due to the higher DM content at low water content, which provides more fermentation substrate. It enables lactic acid bacteria to produce large amounts of lactic acid while inhibiting the respiration of plant cells and reducing glycogen consumption and protein degradation. Yahaya et al. reported that high-moisture silage with a high pH value was not as effective in fermentation as low-moisture fermentation was, which is consistent with the results of the present study [26]. In the present study, W × E had an interactive effect, and the E addition treatment further reduced the pH of the silage. This is due to the addition of cellulase, which breaks down the plant cell wall during ensiling and provides soluble sugars to lactic acid bacteria. The increased sugar content during the early stages of ensiling promoted lactic acid bacteria colonization. This leads to a rapid increase in lactic acid and a decrease in pH, which in turn inhibits the protein hydrolyzing activity of harmful microorganisms and plant enzymes [27,28]. Generally, a low pH indicates a high lactic acid concentration, and the typical concentration of lactic acid in silage ranges from 2% to 4% DM. Interestingly, although the pH in this experiment was less than 4.0, the lactic acid content was not high. This may be because Enterobacteriaceae can convert nitrate to nitrite, which is then converted to NO and NO_3 in a 2:1 ratio under acidic conditions, resulting in a lower pH [24,29]. The BA content of the E-added or L-added treatments ranged from 0.00 to 1.78 g kg^{-1} DM. The low BA content indicated that lactic acid bacteria and cellulase preparations can reduce clostridial fermentation [30].

The NH_3-N/TN ratio is an indicator of protein hydrolysis activity, amino acid deamination, and decarboxylation. This is mainly because protein hydrolysis by *Clostridium perfringens* ferments amino acids through valine and leucine deamination and redox reactions between alanine and glycine. This usually indicates the degradation of nutrients in mixed silage [31]. The NH_3-N/TN ratios of all the silages in this study were within satisfactory limits (< 10% TN), indicating that extensive protein hydrolysis did not occur [2]. Li et al. reported that the addition of cellulase to cassava leaf silage significantly reduced NH_3-N, supporting the results of the present treatment. In addition, the addition of lactic acid bacteria can reduce the microbial diversity of clover, annual ryegrass, and their mixed silage and improve silage quality [32]. This may be due to the addition of exogenous lactic acid bacteria, shifting fermentation towards lactic acid with homofermentative lactic acid bacteria or towards acetic acid with fermentative lactic acid bacteria. It also reduces the growth of clostridia and molds in silage, which reduces the degradation of proteins via the silage process and results in the retention of more nutrients in the silage, which is consistent with the results of this experiment [33]. The combined action of lactic acid bacteria and cellulase improved fermentation quality, reduced the plant cell wall fraction and protein loss, provided more digestible substrates for rumen microbial fermentation, and promoted rumen digestion. The combined treatment of lactic acid bacteria and cellulase may have beneficial synergistic effects on the fermentation quality of amaranth and cornmeal mixed silage [28].

4.2. Effects of Moisture and Additives on the Chemical Composition and In Vitro Digestibility of Amaranth and Cornmeal Mixed Silage

The DM, GE, and in vitro digestibility of silage tended to decrease with increasing moisture. These results indicated that high-moisture mixed silage had high losses of WSC and hemicellulose and low digestibility. This is in line with the results of Yahaya et al.'s study on orchard grass [34]. At the same time, we found an interesting phenomenon: There was the highest DM in all treatment groups at W1, but the CP content was the lowest. This may be because the addition of cornmeal regulates moisture. However, the protein content of cornmeal was 32.6 g kg^{-1} DM lower than that of amaranth, thereby resulting in the lowest CP content in all the treatment groups at W1. This finding is similar to that of Mehrangiz et al., who reported that the addition of molasses could lead to amaranth fermentation, increasing DM concentration [35]. Mehrangiz et al. reported that the soluble and degradable CP fractions of amaranth, as well as effective CP degradability, were not

affected by wilting or the addition of any additives to silage [35]. However, compared with the no E treatment, the addition of the E treatment significantly increased the CP content in the mixed silage. This may be because cellulase disrupts the plant cell wall and releases more plant proteins. The plant proteins continue to synthesize new bacterial proteins that are more easily digested and absorbed by the animals, which in turn promotes digestion and degradation and improves the *iv*CPD. This was also indicated by the results of a previous study on the mixed silage of soybean residue and corn stover by Zhao et al. [36]. Compared with W2 and W3, W1 reduced the levels of ADF and ADL. This is due to the increase in raw material, which leads to a higher WSC content and lower NDF and ADF levels in silage [37]. High-moisture silage tends to have relatively high cellulose digestibility. Morrison reported a similar increase in cellulose digestibility due to the action of extracellular cellulase, which leads to the shortening of the cellulose chain length and makes it more susceptible to enzymatic attack [38]. Compared with the no E treatment, the addition of the E treatment significantly reduced the NDF and ADF contents, which is similar to the findings of Lynch et al. on corn silage [39]. This may be because the added fibrocystic enzymes increased the hydrolysis of cell wall carbohydrates, decreased their fiber content, and increased the WSC content. This result is in agreement with the findings of Foster et al., who reported that the addition of cellulase to warm-season legumes and Bahia grass silage increased the WSC content [37,40].

The *iv*DMD and *iv*OMD of the mixed silage in the M treatment were greater than those in the other groups, which may have been due to the reduction in DM loss from the silage with the addition of the L and E treatments. As a result, the levels of *iv*DMD and *iv*OMD in the rumen were elevated. A low *iv*NDFD was observed in mixed silage under the M treatment. This result may be due to two reasons. One is related to the hydrolysis of hemicellulose due to silage fermentation. Hemicellulose is acid-unstable under strongly acidic conditions, and silage fermentation leads to hydrolysis of the most readily available structural carbohydrates in feed [41]. Secondly, the addition of lactic acid bacteria enhanced NDF fermentation and increased hydrolysis. Moreover, cellulase treatment reduced the amount of available NDF degraded by rumen microorganisms in mixed silage [42]. At W3, in vitro digestibility was significantly increased in mixed silage under E and M treatments than under the U and L treatments. Therefore, we can infer that in vitro digestibility and NDF and ADF contents were negatively correlated, and our conclusions were the same as those of Bao et al. [37].

4.3. Effects of Moisture Content and Additives on the Aerobic Stability of Amaranth and Cornmeal Mixed Silage

Aerobic instability is the underlying cause of the loss of nutrients and DM, and mycotoxins produced from undesirable microorganisms also lead to health risks in human beings and animals. Therefore, aerobic stability is an important factor affecting the nutritional quality and subsequent feeding value of silage in ruminants [32]. Aerobic microorganisms metabolize and consume nutrients, and a change in silage temperature is usually used as an important parameter to evaluate the aerobic stability of silage [43].

AA is one of the most effective substances for inhibiting spoilage microorganisms to improve aerobic stability [44]. Interestingly, in this experiment, the aerobic stability of the mixed silage of amaranth and cornmeal decreased with increasing water content (Figure 4). However, the AA content did not decrease with increasing water content. This may be because a moist environment is more favorable for the growth of microorganisms such as yeasts and acetic acid bacteria, and acid-tolerant yeasts can survive in silage [45]. Increased yeast growth rate in high-moisture treatments was also demonstrated in a study of total mixed rations by Hao et al. [46].

The aerobic stability of the L, E, and M treatments improved at all water contents in this experiment. This occurred because the inoculated lactic acid bacteria have an anisolactic acid metabolic pathway that is capable of producing acetic acid during fermentation after the silos are opened. Thus, effectively controlling the yeast and filamentous fungi could

improve aerobic stability [47]. In addition, according to Kaewpila et al., the addition of cellulase can improve the aerobic stability of Napier Pakchong grass, which was consistent with our experimental results [20]. The exposure time of all the M treatment groups was longer than that of the other groups, which may be due to the synergistic effect of lactic acid bacteria and cellulase when used together. Many studies have shown that lactic acid bacteria or cellulase can improve the aerobic stability of mixed silage by lowering the pH and NH_3-N contents and reducing the abundance of yeasts and clostridia [27]. As a result, the M treatment group was more stable during aerobic exposure and presented reduced spoilage losses during silage fermentation.

4.4. Discussion of the Effects of Mixing Seed Amaranth and Maize Meal on Actual Production

The competence of veterinarians in the field of animal nutrition is essential for the promotion and maintenance of good health in livestock [48,49]. Moreover, the general public is becoming increasingly concerned with the process of food production and animal welfare [50]. However, in the veterinary training system, most graduating veterinarians lack knowledge in the field of animal nutrition [51]. This may be due to insufficient teaching time regarding animal nutrition in veterinary schools. Therefore, practitioners should increase their reading of the literature in the area of animal nutrition. This study may provide new guidance programs and solutions for veterinary feeding in terms of additive application and water content control to provide up-to-date best practice insights for preparing future professionals to meet the challenges discussed in this paper.

5. Conclusions

In summary, the silage water content, lactic acid bacteria, and cellulase affect the fermentation quality, nutrient content, in vitro digestibility and aerobic stability of mixed amaranth and cornmeal silage. In this study, the simultaneous addition of Lactobacillus and cellulase at 60% water content (the mass ratio of amaranth and cornmeal was 69:31) resulted in the lowest pH, PA, AA, and NH_3-N/TN and therefore the best fermentation quality. In addition, mixed silage under the above conditions presented the lowest content of ADF, the highest contents of *iv*DMD, *iv*OMD, and *iv*CPD, and higher contents of DM and OM, thus providing higher nutritional value and digestibility. However, further in suit experiments are needed in this experiment to evaluate the effects of amaranth and cornmeal silage mixtures on rumen growth performance.

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Data Availability Statement: The author is solely responsible for the completeness and accuracy of all data in the article. Further inquiries can be directed to the corresponding authors.

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

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