

## Article

# Assessment of the Nutrient Value and In Vitro Rumen Fermentation Characteristics of Garlic Peel, Sweet Potato Vine, and Cotton Straw

Huiru Chen <sup>1,†</sup>, Qianqian Sun <sup>1,†</sup>, Changxin Tian <sup>2</sup>, Xiangfang Tang <sup>3</sup>, Ying Ren <sup>1,\*</sup> and Wenxun Chen <sup>1,\*</sup><sup>1</sup> Hubei Key Laboratory of Animal Nutrition and Feed Science, Wuhan Polytechnic University, Wuhan 430023, China<sup>2</sup> Guangdong Haida Group Co., Ltd., Guangzhou 511400, China<sup>3</sup> State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing 100193, China

\* Correspondence: ranee1974@163.com (Y.R.); cwx17@whpu.edu.cn (W.C.)

† These authors contributed equally to this work.

**Abstract:** This experiment was conducted to determine the nutrient composition of three agricultural by-products, namely garlic peel, sweet potato vine, and cotton straw, calculate their relative feeding value, effective energy value, and other indexes, and comprehensively evaluate their nutrient value by combining with rumen in vitro fermentation technology, with the aim of providing data references for the development and utilization of non-conventional feed resources for ruminants. The results showed that: 1) the dry matter (DM), ash, ether extract (EE), and crude protein (CP) contents of cotton straw were significantly higher than the other two feeds ( $p < 0.05$ ), while the acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents of garlic peel were highly significantly higher than the others ( $p < 0.05$ ); 2) the relative feed value (DMI, DDM, TDN, RFV, and RFQ) and effective energy value (GE, DE, ME, NE<sub>m</sub>, NE<sub>g</sub>, and NE<sub>L</sub>) indexes of cotton straw were significantly higher than garlic peel and sweet potato vine ( $p < 0.01$ ); 3) after 48 h of in vitro fermentation, the dry matter degradation rate (IVDMD) of sweet potato vine was significantly higher than the other two feeds ( $p < 0.01$ ), and the cumulative gas productions (mL) and estimated gas parameters (a, b, a + b, and c) of sweet potato vine were significantly ( $p < 0.01$ ) higher than those of garlic peel and cotton straw; 4) the sweet potato vine had lower pH but higher NH<sub>3</sub>-N compared to garlic peel and cotton straw ( $p < 0.05$ ). The sweet potato vine had higher propionate, iso-butyrate, butyrate, iso-valerate, and total VFA than the other two roughages, which also had the lowest acetate-to-propionate ratio. Garlic peel produced the lowest acetate, while it produced the highest valerate ( $p < 0.05$ ). These findings demonstrate that all three by-products have high potential as livestock feed based on their nutritive value parameters. Comparatively, sweet potato vines exhibit higher feeding value due to their relatively moderate NDF content and superior rumen fermentation performance.

**Keywords:** agricultural by-products; nutritive value; rumen fermentation; gas production**Citation:** Chen, H.; Sun, Q.; Tian, C.; Tang, X.; Ren, Y.; Chen, W.

Assessment of the Nutrient Value and In Vitro Rumen Fermentation Characteristics of Garlic Peel, Sweet Potato Vine, and Cotton Straw.

*Fermentation* **2024**, *10*, 464. <https://doi.org/10.3390/fermentation10090464>

Academic Editor: Alessio Siciliano

Received: 16 August 2024

Revised: 4 September 2024

Accepted: 5 September 2024

Published: 7 September 2024



**Copyright:** © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

In the current structure of the global food system, up to 40% of all arable land and over 30% of cereal crop production are allocated for animal feed [1]. Feed ingredient demand worldwide has rapidly increased due to the further increase in consumption of animal products. The scarcity of high-quality forage feed has emerged as a significant constraint for animal husbandry in China. Meeting the rapidly increasing demand for animal products with additional feed derived from food grains will further aggravate food insecurity in China. To address the forage feed shortage in the livestock industry, the trend in feed development is increasingly focusing on identifying and utilizing alternative raw materials as efficient feed resources [2]. A growing body of literature indicates that repurposing crop

byproducts for use in livestock feeds enhances sustainability and mitigates the environmental impact of livestock production [3–5]. The crop farming industry of China generates a significant quantity of byproducts, primarily consisting of crop straw, chaff, residues, and leaves [6,7]. Crop byproducts are typically rich in digestible fiber and suitable as feeds for livestock but undesired for human consumption. They are considered a viable alternative as livestock feed. Incorporating these byproducts into dairy cow diets as a substitute for traditional forages or concentrates can offer multiple advantages, such as extending limited forage supplies, reducing feed costs, improving milk performance, and lowering the incidence of ruminal acidosis [8]. Therefore, the full utilization of crop byproduct as ruminant feed will contribute to the reduction in feed costs and the sustainable development of the livestock industry.

Garlic (*Allium sativum* L.), originating from Central and South Asia, is an annual bulbous herb belonging to the Alliaceae family [9]. The average annual production of garlic in China now exceeds 9 million tons, accounting for more than 70% of the global total production [10]. In recent years, there has been a significant increase in demand for garlic on the international market, both as a spice and in traditional medicine. This surge has resulted in a substantial quantity of garlic byproducts, such as garlic peel, leaf, and straw [11]. Garlic byproducts are abundant in protein, total carbohydrates, and ash, making them potential ingredients for ruminant feed. Garlic and its derivatives are rich in bioactive organosulfur compounds, including allicin, allixin, and allylsulfides, which endow garlic products with antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, and other physiological properties [12]. Garlic and its by-products emit a unique aroma that stimulates appetite, enhancing feed intake, and promoting digestion by increasing gastric juice secretion and gastrointestinal motility [13]. Zhu et al. [14] reported that the average daily gain (ADG) and feed conversion ratio (FCR) of Hu lambs improved with the supplementation of garlic peel at 80 g/kg (DM basis) over a 56 day period. Moreover, garlic powder and its extracts have been incorporated into ruminant diets to manage parasite infections, leveraging the antiparasitic properties of garlic, with studies revealing significant positive outcomes [15,16]. Despite these promising findings, there is still a paucity of literature on the effects of garlic byproducts on the nutrient intake and digestibility of ruminants. Consequently, more research is needed to fully exploit the potential of garlic byproducts in ruminant feeding.

Sweet potato (*Ipomoea batatas* L.) ranks among the top five essential food crops globally [17]. In 2022, China's sweet potato planting area was  $2.2 \times 10^6$  hm<sup>2</sup>, with a total yield of  $4.7 \times 10^7$  tons, accounting for 29.8% and 54.2% of the global sweet potato planting area and total production, respectively [18]. Virtually every part of the plant, including the stem, leaf, and root, is edible. However, these components differ in their nutrient, bioactive compound, non-nutrient, and antinutrient compositions. China is the largest producer of sweet potatoes globally, which represents more than 60% of the total worldwide production [19,20]. Sweet potato is mainly grown for roots, but a large volume of sweet potato vines (stems and leaves) was left after harvesting. These vines are a potential feedstuff for livestock, with moderate crude protein content (11%–17%), high digestibility (> 62%), and high moisture content [21], but it was reported that sweet potato vines have up to 67.7 g/kg DM-soluble condensed tannins. There is limited information regarding the impact of tannins on the degradability and utilization of sweet potatoes. Consequently, the optimal use of sweet potato vines as feed is hindered by the lack of comprehensive data on their nutritional value.

China is the world's largest producer, consumer, and exporter of cotton, accounting for 23.19% of global cotton production, while also generating over 30 million tons of straw [22]. As an agricultural byproduct, cotton straw is nutrient-rich, featuring a high content of crude fiber (60.63% to 70.10%) and acid detergent lignin (ADL) (10.29% to 18.55%), but a lower CP content (5.67% to 7.45%) [23]. The high lignin, cellulose, and hemicellulose content, along with free gossypol in cotton straw, results in poor palatability and low intake and digestibility by cattle and sheep, but some of these components still can be absorbed

and utilized by cattle and sheep. In fact, the nutrient content varied significantly among different parts of cotton straw, including the main stem, lateral branches, and leaves [24]. Cotton leaves had the highest crude protein content (12.03%–18.15%), lower cellulose and lignin content, but higher hemicellulose content (11.62%), making them more digestible. The main stem of cotton straw contained the highest levels of cellulose (46.16%–47.92%) and lignin (16.16%–20.18%), rendering it the least digestible part. Factors such as cultivation area, variety, planting and harvesting timing, cultivation practices, post-harvest storage conditions, storage duration, and other variables influence the nutrient content of different parts of cotton stalks. Therefore, to enhance the utilization rate of cotton straw, it is essential to minimize the use of coarse cotton stems and maximize the utilization of fine stems and leaves of cotton straw. Therefore, increasing the utilization of cotton straw as a feed resource holds significant practical importance for alleviating the forage shortage for ruminants in major cotton-producing areas.

Utilizing crop byproducts for animal feeds is crucial for the effective management of organic resources in China. This practice enhances the nation's food self-sufficiency and reduces dependence on imported feeds. Therefore, the objective of this study was to determine the potential nutritive value of garlic peel, sweet potato vines, and cotton straw and provide data for their utilization in ruminant feed formulations. This was performed through an analysis of chemical composition, estimation of relative feeding values, and in vitro ruminal fermentation for the utilization of these three non-conventional feed resources in ruminant nutrition.

## 2. Materials and Methods

### 2.1. Experimental Design and Treatments

The experiment was conducted at Evergreen Garden in Wuhan City, Hubei Province. Three roughages were evaluated as substrates: garlic peel, sweet potato vine, cotton straw. Details regarding the roughages and their sources are provided in Table 1. All samples were dried in an electric constant temperature drying oven (DHG-9140A, Shanghai, China) at 65 °C and ground to pass through a 1 mm screen (Henglian Co., Dalian, China). In the experimental analysis of chemical composition and in vitro rumen incubation, material from 3 biological replicates was used.

**Table 1.** The ingredient name and source of the three unconventional roughages.

Ingredient Name	Production Place	Harvest Season	Harvest Manner
Garlic peel	Linyi, Shandong, China	May, 2023	Mechanical
Sweet potato vine	Lianyungang, Jiangsu, China	August, 2023	Mechanical
Cotton straw	Alaer, Xinjiang, China	September, 2023	Manual

### 2.2. Chemical Composition Analysis

Following the methods outlined by the Association of Official Analytical Chemists [25], various parameters were measured: dry matter (DM) was determined via the drying method, crude protein (CP) content was assessed using an automatic Kjeldahl nitrogen tester (NKY6160, Wanghai Environmental Technology Co., Ltd., Shanghai, China), and crude ash (ash) content was measured with a muffle furnace (MF-N, Dutt Scientific Instruments Ltd., Shanghai, China). The ether extract (EE) content was evaluated using a Soxhlet extractor (CY-SXT-02, Chuanyi Experimental Instrument Co., Ltd., Shanghai, China). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using a fiber analyzer (TY-SF22, Tianyan Instrument Co., Ltd., Weifang, China) [26].

### 2.3. Relative Feeding Value and Effective Energy Analysis

Relative Feeding Value (RFV), dry matter intake (DMI), and digestible dry matter (DDM) were assessed according to the following formula [27]. Total digestible nutrient

(TDN) was calculated using the method of Lithourgidis et al. [28]. Relative forage quality (RFQ) was calculated using the method of Moore et al. [29]. The specific formula is as follows:

$$\text{DMI (\% BM)} = 120/\text{NDF}$$

$$\text{DDM (\% DM)} = 88.9 - 0.779 \times \text{ADF}$$

$$\text{TDN (\% DM)} = -1.291 \times \text{ADF} + 101.35$$

$$\text{RFV} = \text{DMI} \times \text{DDM}/1.29$$

$$\text{RFQ} = \text{TDN} \times \text{DMI}/1.23$$

Gross energy (GE) content was measured with an adiabatic bomb calorimeter (XRY-1A+, Changji Geological Instrument; Shanghai, China). The method of Lithourgidis et al. [28] was used to calculate net energy for milk production ( $\text{NE}_\text{L}$ ). Based on the TDN, the formulas provided by the NCR (2001) [30] were employed to calculate digestible energy (DE), metabolizable energy (ME), net energy for maintenance ( $\text{NE}_\text{m}$ ), and net energy for gain ( $\text{NE}_\text{g}$ ). The specific formula are as follows:

$$\text{DE (MJ/kg DM)} = 0.04409 \times \text{TDN} \times 4.184$$

$$\text{ME (MJ/kg DM)} = 0.82 \times \text{DE}$$

$$\text{NE}_\text{m} \text{ (MJ/kg DM)} = 1.37 \times \text{ME} - 0.138 \times \text{ME}^2 + 0.0105 \times \text{ME}^3 - 1.12$$

$$\text{NE}_\text{g} \text{ (MJ/kg DM)} = 1.42 \times \text{ME} - 0.174 \times \text{ME}^2 + 0.0122 \times \text{ME}^3 - 1.65$$

$$\text{NE}_\text{L} \text{ (MJ/kg DM)} = [1.044 - (0.0119 \times \text{ADF})] \times 2.205$$

#### 2.4. In Vitro Rumen Incubation and Gas Determination

Garlic peel, sweet potato vine, and cotton straw were used as the substrates for the fermented samples, and 1 g of each was weighed and put it in 150 mL culture bottles. The three substrates were used as 3 treatments and run in triplicate for in vitro rumen incubation. Ruminal fluid samples were collected from three ruminally fistulated, nonlactating, nonpregnant 4 year-old Holstein cows ( $650 \pm 32$  kg) cared for in accordance with the Wuhan Polytechnic University Animal Care and Use (Hubei, China; approval number: 2010-0029) guidelines. The cannulated animal handling and management procedures were reviewed and approved by the Ethics and Research Committee of Wuhan Polytechnic University (Hubei, China; approval number: 2010-0029). The donor cow was housed and maintained on a diet of silage, hay, and grain (75:25 forage-to-concentrate ratio, ad libitum) in a feed bunk for a total of 13.8 kg of DM of available feed per cow per day at the Wuhan Polytechnic University Dairy Research Farm. The cows were provided water ad libitum in the morning and evening. Rumen contents were extracted from each cow using a vacuum pump (V-i120SV, VALUE; Wenling, China), combined in equal volumes, and filtered through four layers of gauze. The filtrate was then immediately added to a glass jar containing buffer (prepared according to Menke et al. [31]), forming an in vitro culture solution with a rumen-fluid-to-buffer ratio of 1:2. This solution was maintained anaerobic by flushing with  $\text{CO}_2$  and kept ready for use. The incubation was carried out using a thermostatic water bath shaker (ZHSY-50, Zhichu Instrument; Shanghai, China).  $\text{CO}_2$  was introduced into the bottle, after which 60 mL of in vitro culture solution was quickly added. The bottle was then sealed with a rubber cap and incubated at  $39^\circ\text{C}$  constant-temperature shaking bath for 48 h.

Gas production constants were applied based on the model by [32] and fitted to the gas production kinetics curve of [33]:

$$y = a + b(1 - e^{-ct}) \quad (1)$$

where  $y$  represents the gas production at time  $t$ ;  $a$  is the gas production from rapidly degradable fraction (mL);  $b$  is the gas production from slowly degradable fraction (mL);  $c$  is gas production rate (%/h); and  $t$  is the incubation time (in hours).

At the conclusion of the experiment, the filter bags were removed, quickly rinsed with cold water, and then dried at  $65^\circ\text{C}$  to a constant weight. The dry matter (DM) residues were subsequently removed to calculate in vitro digestibility. Rumen fluid samples were

centrifuged at  $15\,000\times g$ , and the volatile fatty acids (VFAs) in the supernatants were analyzed using gas–liquid chromatography (Agilent 7890, Palo Alto, CA, USA) following the procedure described by Wang et al. [34]. The ruminal ammonia concentration was measured according to the method of Shahinian and Reinhold [35]. The Coomassie Brilliant Blue G-250 assay was employed to determine the microbial protein (MCP) levels in the rumen [36].

### 2.5. Statistical Analysis

Data were initially collated using Microsoft Excel 2019, and subsequent analysis was conducted via one-way ANOVA using SPSS 26.0. The fermentation substrate was considered fixed effects, while fermentor was considered random effect. The following model was used for these variables:

$$Y_{ij} = \mu + \alpha_i + \beta_j + u_{ij}$$

where  $\mu$  = population mean,  $\alpha_i$  = mean effects of the  $i$ th substrate,  $\beta_j$  = mean effects of the  $j$ th fermentor,  $u_{ij}$  = experimental error. Differences between groups were assessed with Duncan's multiple comparisons, with  $p < 0.05$  considered statistically significant.

## 3. Results

### 3.1. Chemical Composition of the Agricultural By-Products

The chemical composition of the raw materials is shown in Table 2. The DM content of the three ingredients ranged from 90.63% to 95.71%, with cotton straw having the highest content of 95.71%. Cotton straw displayed the highest ash content of 21.32%. The CP contents of garlic peel, sweet potato vine, and cotton straw were higher than 10%, with the highest value 18.85% found in cotton straw. The CF content of garlic peel was significantly higher than that of sweet potato vine and cotton straw, with garlic peel also having the highest NDF (56.55%) and ADF (44.84%) contents.

**Table 2.** The chemical compositions of the three agricultural by-products (DM basis, %).

Items	Garlic Peel	Sweet Potato Vine	Cotton Straw
DM	90.63	90.68	95.71
Ash	12.28	12.01	21.32
EE	0.64	1.68	6.23
CP	17.66	12.43	18.85
CF	33.28	20.71	11.41
ADF	42.87	39.46	26.59
NDF	56.55	44.84	29.48

### 3.2. Energy and Relative Feeding Value

As shown in Table 3, DMI, DDM, TDN, RFV, and RFQ of cotton straw were significantly higher than those of garlic peel and sweet potato vine ( $p < 0.05$ ), while garlic peel had the lowest relative feeding value in all of them. In terms of effective energy values (Table 4), GE, DE, ME,  $NE_m$ ,  $NE_g$ , and  $NE_L$  were also the highest for cotton straw while the lowest for garlic peel.

**Table 3.** Relative feeding value of the three agricultural by-products.

Items	Garlic Peel	Sweet Potato Vine	Cotton Straw	SEM	$p$ Value
DMI(% BM)	2.12 <sup>C</sup>	2.68 <sup>B</sup>	4.07 <sup>A</sup>	0.36	<0.001
DDM(% DM)	48.97 <sup>C</sup>	57.23 <sup>B</sup>	66.92 <sup>A</sup>	2.00	<0.001
TDN(% DM)	51.19 <sup>C</sup>	55.15 <sup>B</sup>	68.49 <sup>A</sup>	3.32	<0.001
RFV	80.56 <sup>C</sup>	118.72 <sup>B</sup>	211.24 <sup>A</sup>	22.91	<0.001
RFQ	88.31 <sup>C</sup>	120 <sup>B</sup>	226.69 <sup>A</sup>	26.49	<0.001

Note: Different uppercase letters in the same row indicate significant differences ( $p < 0.01$ ), and the same letters or no letters indicate no significant differences ( $p > 0.05$ ).



**Table 4.** Effective energy value of the three agricultural by-products (MJ/kg DM).

Items	Garlic Peel	Sweet Potato Vine	Cotton Straw	SEM	p Value
GE	15.31 <sup>C</sup>	15.59 <sup>B</sup>	16.20 <sup>A</sup>	0.17	<0.001
DE	9.44 <sup>C</sup>	10.17 <sup>B</sup>	12.63 <sup>A</sup>	0.61	<0.001
ME	7.74 <sup>C</sup>	8.34 <sup>B</sup>	10.36 <sup>A</sup>	0.50	<0.001
NE <sub>m</sub>	6.09 <sup>C</sup>	6.8 <sup>B</sup>	9.94 <sup>A</sup>	0.62	<0.001
NE <sub>g</sub>	4.58 <sup>C</sup>	5.17 <sup>B</sup>	7.95 <sup>A</sup>	0.63	<0.001
NE <sub>L</sub>	0.06 <sup>B</sup>	0.06 <sup>B</sup>	0.08 <sup>A</sup>	0.04	<0.001

Note: Different uppercase letters in the same row indicate significant differences ( $p < 0.01$ ), and the same letters or no letters indicate no significant differences ( $p > 0.05$ ).

### 3.3. Gas Production and Gas Parameters of Three By-Products

The IVDMD, IVNDFD, and cumulative volume of gas production of the three unconventional roughages are presented in Table 5. The IVDMD of roughage feeds ranged from 72.09 to 78.74%, being the highest for sweet potato vine, while the lowest for garlic peel ( $p < 0.05$ ). The highest IVNDFD was also found in sweet potato vine (72.56%) than in garlic peel (58.28%) and cotton straw (59.07%). The cumulative gas production volume increased with longer incubation times. After 48 h of incubation, gas production ranged between 89.90 and 147.05 mL per 1.00 g of substrate. At all incubation periods, the cumulative gas production (mL) from sweet potato vine was significantly ( $p < 0.001$ ) higher than that from garlic peel and cotton straw. Furthermore, the estimated parameters (a, b, a + b, and c) for sweet potato vine were significantly higher than those for the other substrates.

**Table 5.** In vitro rumen degradation rate, gas production, and gas parameters of the three by-products.

Items	Garlic Peel	Sweet Potato Vine	Cotton Straw	SEM	p Value
Rumen degradation rate					
IVDMD, %	72.09 <sup>B</sup>	78.74 <sup>A</sup>	75.82 <sup>B</sup>	1.41	0.026
IVNDFD, %	58.28 <sup>B</sup>	72.56 <sup>A</sup>	59.07 <sup>B</sup>	2.88	0.003
Gas production, mL/g					
4 h	4.83 ± 0.09 <sup>B</sup>	10.55 ± 0.44 <sup>A</sup>	2.48 ± 0.11 <sup>C</sup>	1.03	<0.001
8 h	24.08 ± 2.06 <sup>B</sup>	38.65 ± 1.76 <sup>A</sup>	19.00 ± 2.38 <sup>B</sup>	2.74	<0.001
12 h	51.45 ± 2.70 <sup>B</sup>	72.00 ± 3.36 <sup>A</sup>	38.55 ± 1.68 <sup>C</sup>	4.38	<0.001
24 h	85.20 ± 2.41 <sup>B</sup>	113.75 ± 4.21 <sup>A</sup>	67.13 ± 1.78 <sup>C</sup>	5.99	<0.001
36 h	102.28 ± 2.08 <sup>B</sup>	136.08 ± 3.63 <sup>A</sup>	81.90 ± 1.85 <sup>C</sup>	6.87	<0.001
48 h	112.23 ± 2.21 <sup>B</sup>	147.05 ± 3.05 <sup>A</sup>	89.90 ± 2.14 <sup>C</sup>	7.21	<0.001
Gas parameters					
a, mL	41.83 ± 4.30 <sup>A</sup>	44.64 ± 5.95 <sup>A</sup>	13.68 ± 2.75 <sup>B</sup>	4.83	0.002
b, mL	95.08 ± 5.13 <sup>B</sup>	127.90 ± 7.73 <sup>A</sup>	100.17 ± 2.98 <sup>B</sup>	5.25	0.005
a + b, mL	136.91 ± 1.24 <sup>B</sup>	172.54 ± 2.37 <sup>A</sup>	113.85 ± 1.93 <sup>C</sup>	7.35	<0.001
c, %/h	3.82 ± 0.18 <sup>AB</sup>	4.39 ± 0.22 <sup>A</sup>	3.60 ± 0.16 <sup>B</sup>	0.01	0.041

Note: IVDMD, in vitro dry matter digestibility; IVNDFD, in vitro NDF digestibility; a, gas production from rapidly degradable fraction; b, gas production from slowly degradable fraction; a + b, potential gas production; c, gas production rate. Different uppercase letters in the same row indicate significant differences ( $p < 0.01$ ), and the same letters or no letters indicate no significant differences ( $p > 0.05$ ).

### 3.4. Rumen Fermentation Products of The Three By-Products

Data on in vitro fermentation products of different by-products are presented in Table 6. For the three by-products, there was a significant difference in pH<sub>48</sub>, NH<sub>3</sub>-N, MCP, and total VFAs. The sweet potato vine had lower pH and MCP but higher NH<sub>3</sub>-N compared to garlic peel and cotton straw ( $p < 0.05$ ). Furthermore, the content of total VFA and acetate-to-propionate ratio (A/P) differed ( $p < 0.05$ ) among the three by-products. The sweet potato vine had higher propionate, iso-butyrate, butyrate, iso-valerate, and total VFA than the other two by-products, which also had the lowest acetate-to-propionate ratio. Garlic peel produced the lowest acetate, while producing the highest valerate.

**Table 6.** The rumen fermentation characteristics of the three roughages in vitro.

Items	GarlicPeel	Sweet Potato Vine	Cotton Straw	SEM	p Value
pH <sub>48</sub>	6.60 <sup>A</sup>	6.56 <sup>B</sup>	6.67 <sup>A</sup>	0.02	< 0.001
NH <sub>3</sub> -N, mg/dL	4.01 <sup>B</sup>	4.57 <sup>A</sup>	4.47 <sup>A</sup>	0.10	0.031
MCP, mg/mL	132.96 <sup>A</sup>	120.05 <sup>B</sup>	135.00 <sup>A</sup>	2.68	0.044
VFA content					
Acetate, mmol/L	29.19 <sup>B</sup>	33.18 <sup>A</sup>	34.17 <sup>A</sup>	0.72	< 0.001
Propionate, mmol/L	11.10 <sup>C</sup>	13.14 <sup>A</sup>	12.24 <sup>B</sup>	0.30	0.001
Iso-butyrate, mmol/L	0.18 <sup>B</sup>	0.40 <sup>A</sup>	0.19 <sup>B</sup>	0.03	< 0.001
Butyrate, mmol/L	1.86 <sup>B</sup>	2.24 <sup>A</sup>	2.17 <sup>AB</sup>	0.14	0.030
Iso-valerate, mmol/L	0.23 <sup>B</sup>	0.63 <sup>A</sup>	0.23 <sup>B</sup>	0.06	< 0.001
Valerate, mmol/L	0.49 <sup>A</sup>	0.45 <sup>AB</sup>	0.40 <sup>B</sup>	0.02	0.023
Acetate to propionate ratio	2.64 <sup>AB</sup>	2.49 <sup>B</sup>	2.73 <sup>A</sup>	0.10	0.022
Total VFA, mmol/L	43.09 <sup>B</sup>	50.14 <sup>A</sup>	48.52 <sup>A</sup>	1.10	< 0.001

Note: Different uppercase letters in the same row indicate significant differences ( $p < 0.01$ ), and the same letters or no letters indicate no significant differences ( $p > 0.05$ ).

#### 4. Discussion

##### 4.1. The Chemical Composition of the Three By-Products

Garlic peel is an inedible byproduct of garlic. In this study, the CP, EE, and NDF contents of garlic peel were 17.66%, 0.64%, and 56.55%, respectively. The results were aligned with previously published data [37,38], while the CP content determined in this study was higher, which may be related to the different growing environment and harvesting period of garlic. The sweet potato vines comprise both leaves and stems. Studies have shown that the crude protein content of the sweet potato stem is between 10 and 14% [39], and it contains high levels of water-soluble carbohydrates [40]. Baba et al. [41] evaluated the nutritional content of 12 varieties of sweet potato vines, revealing that their crude protein levels varied from 10.82% to 20.58% and crude fiber from 21.14% to 35.37%. In the present study, the CP and CF content of the sweet potato vine were 12.43% and 20.71%, which were close to the results of the above studies. Additionally, the CP content of garlic peel and sweet potato vines is similar to that of *Leymus chinensis*, while their EE content is lower than that of *Leymus chinensis* and alfalfa, as well as other high-quality roughages [42].

Cotton straw, a byproduct of cotton, and some of its parts can be absorbed and utilized by cattle and sheep. The nutrient content of different parts of the cotton straw varied widely, with the highest crude protein content in the leaves (12.03%–18.15%), the second highest in the lateral branches (6.22%–6.82%), and the lowest in the main stem (3.97%–5.22%) [43]. The main stems of cotton stalks contain high levels of cellulose and lignin, making them less digestible. In contrast, cotton leaves have lower levels of cellulose and lignin but higher levels of hemicellulose, which are more easily utilized [24]. The protein content of cotton straw measured in this study was 18.85% and crude fiber was 11.41%. This is mainly related to our sampling method. The samples collected were mainly cotton leaves and contained a small amount of cotton fiber, which resulted in high protein content and low fiber content of the samples. In the Xinjiang region of China, cotton straw is primarily utilized for grazing [44], and sheep predominantly feed on the cotton leaves and lateral branches of the straw. In addition, the CP content of cotton straw is close to that of alfalfa, while their ADF and NDF contents are lower than those of alfalfa, oat grass, and ryegrass [42,45]. The lower fiber content facilitates the digestion and absorption of cotton straw by ruminants and can increase their feed intake. In conclusion, the above three ingredients have good nutritional composition and can be used as a potential source of roughage for ruminants.

##### 4.2. Relative Feeding Value and Effective Energy Analysis

Forage quality encompasses both nutritive value and intake potential. The quality of roughages is primarily determined by the amount and composition of their structural carbohydrate contents. Specifically, the neutral detergent fiber (NDF) and acid detergent fiber (ADF) fractions, which constitute the plant cell walls, are key parameters influencing intake and digestibility. The relative feed value (RFV) and relative forage quality (RFQ)

are widely used indexes reflecting the quality of roughages based on their NDF and ADF content [46]. Relative feed value (RFV) is a quality-related parameter based on ADF and NDF concentrations of alfalfa (RFV = 100) [47]. In this study, the RFV and RFQ of sweet potato vines and cotton straw both exceeded 100, indicating that these two roughages have relatively high overall feed value. RFV is a calculated value based on the ADF and NDF contents of the feed, and its predictive accuracy depends on the precision of ADF and NDF in estimating DDM and DMI values, which introduces certain limitations. Therefore, although the RFV of sweet potato vines and cotton stalks is higher than that of alfalfa at full bloom, their actual feed value requires further comprehensive evaluation.

Energy is the key to sustaining life activities of livestock, and the effective energy value of feed is related to its nutrient composition. In this study, the GE range of the three roughages was between 15.31 and 16.20 MJ/kg, slightly lower than that of oat grass (17.71 MJ/kg) and alfalfa (17.1 MJ/kg) [48,49], but they can meet the energy requirements of ruminants in their diet. The DE values were estimated from the TDN values, while the ME values were calculated by multiplying the DE values by the coefficient 0.82; the  $NE_m$  and  $NE_g$  values were estimated from the ME values [30]. Therefore, the results of their significance analyses were all in agreement with TDN, and thus the energy value of cotton straw was significantly higher than the other two roughages.

#### 4.3. Ruminal DM Degradation and Gas Production Analysis

Rumen DM degradation in roughage is influenced by the cellulose content and the degree of lignification, which reflect the difficulty of degradation in ruminants [50]. The composition of neutral detergent fiber (NDF) and acid detergent fiber (ADF) in roughage affects the degradation rate in the rumen, with higher contents negatively correlated with the rate of degradation [51]. This study revealed significant differences in DM degradation among garlic peel, sweet potato vine, and cotton straw. In fact, there were variations in the NDF and ADF contents among the three forages in this study, which could be a contributing factor to the differences in DM and NDF degradation among them.

Degradation of the organic fraction of feed by rumen microorganisms is the main source of gas produced by feed in the rumen. In vitro gas production is an important indicator of the digestibility of feeds [52], and the level of in vitro gas production can directly reflect the nutritional quality of feed samples and indicate the metabolism of rumen microorganisms. Lei et al. [53] showed that there is a positive correlation between the magnitude of gas production and digestibility. The three roughages in this experiment had sufficient substrate fermentation in the early stage; the gas production increased with time and the GP rate was faster; and then the substrate decreased in the late stage and the GP production gradually leveled off. The degree of feed degradation by rumen microorganisms in the pre-fermentation period increased with time, but the degradable material gradually decreased in the later period, so the GP also increased slowly. In addition, the content of VFA and  $NH_3$ -N accumulated in the artificial rumen culture solution gradually increased with the extension of in vitro fermentation time [54], resulting in a change in the fermentation environment of the artificial rumen culture solution, leading to a certain degree of inhibition of rumen microbial activity. In addition, other substances, such as insoluble carbohydrate components and phytochemicals, can also affect gas production. In this study, the sweet potato vine exhibited the highest 48 h cumulative gas production, suggesting that a significant portion of fermentable components in the sweet potato vine were utilized.

#### 4.4. In Vitro Fermentation Fluid Parameters

The pH of rumen fluid is a crucial indicator that visually reflects the state of rumen fermentation [55]. If the pH is too high or too low, it will affect the activity of rumen microorganisms, leading to fermentation abnormalities. The pH of the three types of roughage in this study ranged from 6.56 to 6.67, falling within the optimal pH range (5.5–7.5) for the rumen. This range helps to maintain a stable fermentation environment



in vitro and is conducive to the growth of rumen microorganisms.  $\text{NH}_3\text{-N}$  is generated through the breakdown of nitrogen-containing compounds in the feed, which can reflect the degradation of protein in the diet and its reuse by microorganisms to synthesize bacterial proteins, serving as a crucial marker for rumen metabolism. An optimal  $\text{NH}_3\text{-N}$  level can support the proliferation of microorganisms and the synthesis of bacterial proteins. Hoover et al. [56] demonstrated that the optimal  $\text{NH}_3\text{-N}$  concentration for rumen microbial growth ranges from 3.3 to 8.0 mg/dL, and in fact, the  $\text{NH}_3\text{-N}$  concentration varied from 1 to 76 mg/dL. In this experiment, the  $\text{NH}_3\text{-N}$  concentrations of the three roughages were 4.01–4.57 mg/dL, which fell within the normal concentration range.

Volatile fatty acids (VFAs) are key indicators of the fermentation ability of feed in the rumen. Acetate, propionate, and butyrate constitute 95% of the total VFAs produced in the rumen and provide up to 75% of the metabolizable energy (ME) for ruminants [57,58]. Acetate is the main precursor for the synthesis of lactic fat, and propionate is the precursor for the synthesis of glucose, which can supply most of the energy required by the organism. Studies have shown that roughage contains a large amount of fiber-like substances, which can be fermented in the rumen to produce more acetic acid [59]. In this experiment, all three roughages had the highest content of acetate (acetate > propionate > butyrate) after 48 h of fermentation, which is related to the fiber content of roughage, and their fermentation in the rumen would produce a relatively high proportion of acetate and a lower proportion of propionate. Notably, the fermentation of sweet potato vine produced the highest content of acetic acid, propionic acid, and total VFA, and the lowest acetate-to-propionate ratio. Therefore, compared with other roughage, sweet potato vine has better rumen fermentation performance and is more favorable for digestion and absorption by ruminants.

## 5. Conclusions

Garlic peel, sweet potato vine, and cotton straw all demonstrated high potential as livestock feed based on their nutritive value parameters. However, sweet potato vine appears to have a higher feeding value due to its moderate NDF content and higher feed intake, as well as a superior relative feed value. Further verification of the actual feeding effects of these three ingredients in animals is needed, as sweet potato vine appears to show higher gas production compared to the other two by-products.

**Author Contributions:** H.C.: project administration, methodology, formal analysis, investigation and writing—original draft. Q.S.: methodology, formal analysis and investigation. C.T.: methodology, formal analysis. X.T. and Y.R.: resources, review and editing. W.C.: administration, formal analysis, investigation and writing—original draft. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by The National Key Research and Development Program of China (NO. 2022YFD1301101).

**Institutional Review Board Statement:** The animal study protocol was approved by the Institutional Review Board of Animal Welfare of Ethics and Research Committee of Wuhan Polytechnic University 2010-0029.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data are available at a reasonable request to the corresponding authors.

**Acknowledgments:** We wish to acknowledge the support of Ying Ren and the technical staff of the Animal Care and Use Committee of Wuhan Polytechnic University in daily management.

**Conflicts of Interest:** Author Changxin Tian was employed by the company Guangdong Haida Group Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## References

1. Sandström, V.; Chrysafi, A.; Lamminen, M.; Troell, M.; Jalava, M.; Piipponen, J.; Siebert, S.; van Hal, O.; Virkki, V.; Kumm, M. Food system by-products upcycled in livestock and aquaculture feeds can increase global food supply. *Nat. Food* **2022**, *3*, 729–740. [\[CrossRef\]](#)
2. Makkar, H.P.S. Review: Feed demand landscape and implications of food-not feed strategy for food security and climate change. *Animal* **2018**, *12*, 1744–1754. [\[CrossRef\]](#)
3. van Hal, O.; de Boer, I.J.M.; Muller, A.; de Vries, S.; Erb, K.H.; Schader, C.; Gerrits, W.J.J.; van Zanten, H.H.E. Upcycling food leftovers and grass resources through livestock: Impact of livestock system and productivity. *J. Clean. Prod.* **2019**, *219*, 485–496. [\[CrossRef\]](#)
4. Wang, L.; Setoguchi, A.; Oishi, K.; Sonoda, Y.; Kumagai, H.; Irbis, C.; Inamura, T.; Hirooka, H. Life cycle assessment of 36 dairy farms with by-product feeding in Southwestern China. *Sci. Total Environ.* **2019**, *696*, 133985. [\[CrossRef\]](#)
5. Pinotti, L.; Luciano, A.; Ottoboni, M.; Manoni, M.; Ferrari, L.; Marchis, D.; Tretola, M. Recycling food leftovers in feed as opportunity to increase the sustainability of livestock production. *J. Clean. Prod.* **2021**, *294*, 126290. [\[CrossRef\]](#)
6. Shi, W.; Fang, Y.R.; Chang, Y.; Xie, G.H. Toward sustainable utilization of crop straw: Greenhouse gas emissions and their reduction potential from 1950 to 2021 in China. *Resour. Conserv. Recy.* **2023**, *190*, 106824. [\[CrossRef\]](#)
7. Zhao, X.; Li, R.-C.; Liu, W.-X.; Liu, W.-S.; Xue, Y.-H.; Sun, R.-H.; Wei, Y.-X.; Chen, Z.; Lal, R.; Dang, Y.P.; et al. Estimation of crop residue production and its contribution to carbon neutrality in China. *Resour. Conserv. Recy.* **2024**, *203*, 107450. [\[CrossRef\]](#)
8. Bradford, B.J.; Mullins, C.R. Invited review: Strategies for promoting productivity and health of dairy cattle by feeding nonforage fiber sources. *J. Dairy Sci.* **2012**, *95*, 4735–4746. [\[CrossRef\]](#)
9. Rouf, R.; Uddin, S.J.; Sarker, D.K.; Islam, M.T.; Ali, E.S.; Shilpi, J.A.; Nahar, L.; Tiralongo, E.; Sarker, S.D. Antiviral potential of garlic (*Allium sativum*) and its organosulfur compounds: A systematic update of pre-clinical and clinical data. *Trends Food Sci. Technol.* **2020**, *104*, 219–234. [\[CrossRef\]](#)
10. Hou, J.; Liu, C. Research progress on deep processing and industrialization of garlic resources in China. *Biot. Resour.* **2020**, *42*, 36–42. [\[CrossRef\]](#)
11. Chen, K.; Nakasone, Y.; Xie, K.; Sakao, K.; Hou, D.-X. Modulation of Allicin-Free Garlic on Gut Microbiome. *Molecules* **2020**, *25*, 682. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Qin, W.; Huber, K.; Popp, M.; Bauer, P.; Buettner, A.; Sharapa, C.; Scheffler, L.; Loos, H.M. Quantification of Allyl Methyl Sulfide, Allyl Methyl Sulfoxide, and Allyl Methyl Sulfone in Human Milk and Urine After Ingestion of Cooked and Roasted Garlic. *Front. Nutr.* **2020**, *7*, 565496. [\[CrossRef\]](#)
13. Savairam, V.D.; Patil, N.A.; Borate, S.R.; Ghaisas, M.M.; Shete, R.V. Allicin: A review of its important pharmacological activities. *Pharmacol Res.* **2023**, *8*, 100283. [\[CrossRef\]](#)
14. Zhu, W.; Su, Z.; Xu, W.; Sun, H.X.; Gao, J.F.; Tu, D.F.; Ren, C.H.; Zhang, Z.J.; Cao, H.G. Garlic skin induces shifts in the rumen microbiome and metabolome of fattening lambs. *Animal* **2021**, *15*, 100216. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Krstin, S.; Sobeh, M.; Braun, M.S.; Wink, M. Tulbaghia violacea and Allium ursinum Extracts Exhibit Anti-Parasitic and Antimicrobial Activities. *Molecules* **2018**, *23*, 313. [\[CrossRef\]](#)
16. Strickland, V.; Krebs, G.; Potts, W. Pumpkin kernel and garlic as alternative treatments for the control of Haemonchus contortus in sheep. *Anim. Prod. Sci.* **2009**, *49*, 139–144. [\[CrossRef\]](#)
17. FAOSTAT. Statistics Division of Food and Agriculture Organization of the United Nations. 2020. Available online: <http://www.fao.org/faostat/en/#data> (accessed on 23 December 2023).
18. Ji, H.; Zhao, H.; Zeng, Y.; Cheng, R.; Wang, S.; Wang, Y.; Zhao, H. Meta-analysis on the effect and influence factors of nitrogen application on tuber yield of sweet potato in China. *J. Plant Nutr. Fertil.* **2024**, *10*, 1–14. [\[CrossRef\]](#)
19. Mu, T.-H.; Li, P.-G. Chapter 2—Sweet potato: Origin and production. In *Sweet Potato*; Mu, T.-H., Singh, J., Eds.; Academic Press: Beijing, China, 2019; pp. 5–25.
20. Scott, G.J. A review of root, tuber and banana crops in developing countries: Past, present and future. *Int. J. Food Sci. Technol.* **2021**, *56*, 1093–1114. [\[CrossRef\]](#)
21. Ffoulkes, D.; Deb Hovell, F.; Preston, T. Sweet potato forage as cattle feed: Voluntary intake and digestibility of mixtures of sweet potato forage and sugarcane. *Trop. Anim. Prod.* **1978**, *3*, 140–144.
22. Zuo, X.; Bi, Y.; Wang, H.; Gao, C.; Wang, L.; Wang, Y. Multi-Suitability Comprehensive Evaluation of Crop Straw Resource Utilization in China. *Res. Environ. Sci.* **2015**, *25*, 159–166. [\[CrossRef\]](#)
23. Aireti, M.; Junyu, Z. Research Progress on Feed Application of Cotton Straw in Ruminant Production. *Chinese J. Anim. Nutr.* **2024**, *36*, 2761–2772. [\[CrossRef\]](#)
24. Ling, W.; Li, Y.; Sun, X.; Hou, Y. Exploration of cotton straw feeding in Xinjiang reclamation area. *Anim. Breed. Feed* **2021**, *20*, 58–60. [\[CrossRef\]](#)
25. AOAC. *Official Methods of Analysis*, 18th ed.; Association of Analytical Chemists: Gaithersburg, MD, USA, 2005.
26. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597. [\[CrossRef\]](#)
27. Rohweder, D.A.; Barnes, R.F.; Jorgensen, N. Proposed Hay Grading Standards Based on Laboratory Analyses for Evaluating Quality. *J. Anim. Sci.* **1978**, *47*, 747. [\[CrossRef\]](#)

28. Lithourgidis, A.; Vasilakoglou, I.; Dhima, K.; Dordas, C.; Yiakoulaki, M.J.F.C.R. Forage yield and quality of common vetch mixtures with oat and triticale in two seeding ratios. *Field Crops Res.* **2006**, *99*, 106–113. [\[CrossRef\]](#)
29. Moore, J.E.; Undersander, D.J. Relative forage quality: An alternative to relative feed value and quality index. In Proceedings of the 13th annual Florida ruminant nutrition symposium, Gainesville, FL, USA, 1 January 2002; pp. 16–29.
30. NRC. Nutrient requirements of dairy cattle. In *Energy*, 7th ed; National Academies Press: Washington, DC, USA, 2001; pp. 13–14.
31. Menke, K.; Raab, L.; Salewski, A.; Steingass, H.; Fritz, D.; Schneider, W. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. *J. Agric. Sci.* **1979**, *93*, 217–222. [\[CrossRef\]](#)
32. Webster, J. Experimental Agriculture. In *The Biochemistry of Silage*; McDonald, P., Ed.; Chalcombe Publications: Marlow, Bucks, UK, 1981; p. 200.
33. Ørskov, E.-R.; McDonald, I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci.* **1979**, *92*, 499–503. [\[CrossRef\]](#)
34. Wang, M.; Wang, R.; Janssen, P.; Zhang, X.; Sun, X.; Pacheco, D.; Tan, Z.L. Sampling procedure for the measurement of dissolved hydrogen and volatile fatty acids in the rumen of dairy cows. *J. Anim. Sci.* **2016**, *94*, 1159–1169. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Shahinian, A.H.; Reinhold, J.G. Application of the phenol–Hypochlorite reaction to measurement of ammonia concentrations in Kjeldahl digests of serum and various tissues. *Clin. Chem.* **1971**, *17*, 1077–1080. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [\[CrossRef\]](#)
37. Dhillon, G.S.; Kaur, S.; Brar, S.K. Perspective of apple processing wastes as low-cost substrates for bioproduction of high value products: A review. *Renew. Sust. Energy Rev.* **2013**, *27*, 789–805. [\[CrossRef\]](#)
38. Ogbuewu, I.P.; Okoro, V.M.; Mbajorgu, E.F.; Mbajorgu, C.A. Beneficial Effects of Garlic in Livestock and Poultry Nutrition: A Review. *Agric. Res.* **2019**, *8*, 411–426. [\[CrossRef\]](#)
39. Ishida, H.; Suzuno, H.; Sugiyama, N.; Inami, S.; Tadokoro, T.; Maekawa, A. Nutritive evaluation on chemical components of leaves, stalks and stems of sweet potatoes (*Ipomoea batatas* pair). *Food Chem.* **2000**, *68*, 359–367. [\[CrossRef\]](#)
40. Ali, A.I.M.; Wassie, S.E.; Korir, D.; Merbold, L.; Goopy, J.P.; Butterbach-Bahl, K.; Dickhoefer, U.; Schlecht, E. Supplementing Tropical Cattle for Improved Nutrient Utilization and Reduced Enteric Methane Emissions. *Animals* **2019**, *9*, 210. [\[CrossRef\]](#) [\[PubMed\]](#)
41. Baba, M.; Nasiru, A.; Kark, I.; Muh, I.; Rano, N. Nutritional Evaluation of Sweet Potato Vines from Twelve Cultivars as Feed for Ruminant Animals. *Asian J. Anim. Vet. Adv.* **2017**, *13*, 25–29. [\[CrossRef\]](#)
42. Liu, G.; Qi, D.; Dong, X.; Liu, H.; Liu, S. Basic Knowledge of Sheepgrass (*Leymus chinensis*). In *Sheepgrass (Leymus chinensis): An Environmentally Friendly Native Grass for Animals*; Liu, G., Li, X., Zhang, Q., Eds.; Springer: Singapore, 2019; pp. 1–51.
43. Lei, F.; Qing, J. Evaluation of feeding value of different parts of cotton straw. *Contemp. Anim. Husb.* **2009**, *1*, 25–27. (In Chinese)
44. Zhang, J.; Sun, L. Analysis of cotton straw feeding mode and current situation in Xinjiang. *Xinjiang Livest. Husb.* **2019**, *34*, 38–40. (In Chinese) [\[CrossRef\]](#)
45. Wan, W.F.; Li, Y.J.; Li, H.G. Yield and quality of alfalfa (*Medicago sativa* L.) in response to fertilizer application in China: A meta-analysis. *Front. Plant Sci.* **2022**, *13*, 1051725. [\[CrossRef\]](#)
46. Jeranyama, P.; Garcia, A. Understanding Relative Feed Value (RFV) and Relative Forage Quality (RFQ). In Proceedings of the Extension Extra, Brookings, SD, USA, 1 August 2004; p. 352.
47. Lin, S.; Norberg, S.; Combs, D. Genomics of Forage Quality in Alfalfa. In *The Alfalfa Genome*; Yu, L.-X., Kole, C., Eds.; Springer International Publishing: Cham, Switzerland, 2021; pp. 129–143.
48. Kobayashi, N.; Hou, F.; Tsunekawa, A.; Chen, X.; Yan, T.; Ichinohe, T. Appropriate level of alfalfa hay in diets for rearing Simmental crossbred calves in dryland China. *Asian Austral J. Anim.* **2018**, *31*, 1881–1889. [\[CrossRef\]](#)
49. Abdelraheem, N.; Li, F.; Guo, P.; Sun, Y.; Liu, Y.; Cheng, Y.; Hou, F. Oat hay as winter feed improves digestibility, nitrogen balance and energy utilization of Tibetan sheep (*Ovis aries*) in the Qinghai Tibetan Plateau. *Liv. Sci.* **2019**, *230*, 103854. [\[CrossRef\]](#)
50. Gutierrez, D.; Elias, A.; López, R.; Herrera, F.; Jordán, H.; García, L. Influence of a microbial additive on the voluntary intake of dry matter, neutral detergent fiber and indicators of the ruminal fermentation of goats fed *Brachiaria brizantha* hay. *Cuban. J. Agric. Sci.* **2012**, *46*, 211–216.
51. Du, S.; Xu, M.; Yao, J. Relationship between fibre degradation kinetics and chemical composition of forages and by-products in ruminants. *J. Appl. Anim. Res.* **2016**, *44*, 189–193. [\[CrossRef\]](#)
52. Dijkstra, J.; Kebreab, E.; Bannink, A.; France, J.; López, S. Application of the gas production technique to feed evaluation systems for ruminants. *Anim. Feed Sci. Technol.* **2005**, *123*, 561–578. [\[CrossRef\]](#)
53. Lei, Y.; Li, X.Y.; Wang, Y.; Li, Z.; Chen, Y.; Yang, Y.X. Determination of ruminal dry matter and crude protein degradability and degradation kinetics of several concentrate feed ingredients in cashmere goat. *J. Appl. Anim. Res.* **2018**, *46*, 134–140. [\[CrossRef\]](#)
54. McDonald, P.; Edwards, R.A. The influence of conservation methods on digestion and utilization of forages by ruminants. *Proc. Nutr. Soc.* **1976**, *35*, 201–211. [\[CrossRef\]](#)
55. Gunun, P.; Wanapat, M.; Anantasook, N. Effects of physical form and urea treatment of rice straw on rumen fermentation, microbial protein synthesis and nutrient digestibility in dairy steers. *Asian-Australas J. Anim. Sci.* **2013**, *26*, 1689–1697. [\[CrossRef\]](#)
56. Hoover, W.H.; Stokes, S.R. Balancing carbohydrates and proteins for optimum rumen microbial yield. *J. Dairy Sci.* **1991**, *74*, 3630–3644. [\[CrossRef\]](#)

57. Bergman, E.N. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol. Rev.* **1990**, *70*, 567–590. [[CrossRef](#)]
58. France, J.; Dijkstra, J. Volatile fatty acid production. In *Quantitative Aspects of Ruminant Digestion and Metabolism*; Nolan, J., Dobos, R., Eds.; CABI Publishing: Wallingford, Oxfordshire, UK, 2005; pp. 157–175.
59. Iwamoto, M.; Asanuma, N.; Hino, T.J.N.C.G. Effects of pH and Electron Donors on Nitrate and Nitrite Reduction in Ruminal Microbiota. *Nihon Chikusan Gakkaiho.* **2001**, *72*, 117–125. [[CrossRef](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.