

Rosa roxburghii Tratt Residue Can be Used as a Novel Feed Resource for Cattle

Xinyu Song

Guizhou University

Yi Yang

Guizhou University

Chong Wang

Wen Zhu

Wenxuan Wu

wxwu@gzu.edu.cn

Guizhou University

Research Article

Keywords: Rosa roxburghii tratt residue, growth performance, blood metabolites, rumen fermentation, cattle

Posted Date: April 9th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4211620/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Rosa roxburghii tratt residue (RRTR) is a regional and uncommon byproduct in Guizhou. Little information is available on whether RRTR can be used as feed for ruminants. In this study, the feasibility of using RRTR as a new feed resource for cattle was investigated by chemical composition analysis, *in vitro* gas production (Trial 1) and animal feeding experiments (Trial 2). In trial 1, compared to the commonly used fruit residues, RRTR had a medium proximate nutrient level; the *in vitro* gas production at 24 h (166.67 mL) and dynamics fell within the normal range. In trial 2, 16 cattle were allocated to the control and treatment groups, with 8 replicates of 1 cattle each. Cattle in the control group were fed a basal diet without RRTR, while those in the treatment group were fed a diet containing 30% RRTR. Feeding RRTR had little effect on the growth performance of the control and treatment cattle ($P > 0.05$). The feed-to-gain ratio was greater in the treatment group than in the control group throughout the trial period ($P < 0.05$). Compared with those in the control group, the plasma urea levels in the treatment group were lower ($P < 0.05$), and the levels of other plasma biochemical metabolites were not different between the two groups of cattle ($P > 0.05$). The *in vivo* rumen fermentation parameters did not differ between the control and treatment groups ($P > 0.05$). Our findings indicate that RRTR has a nutritional profile similar to that of commonly used fruit residues; improves plasma protein utilization efficiency; and has little negative impact on growth performance, blood metabolites, and rumen fermentation parameters in cattle. Accordingly, RRTR can serve as a novel feed resource for cattle.

Introduction

In recent years, animal husbandry has developed rapidly in China. This leads to an increase in feed consumption for animals, and the lack of forage resources has severely restricted the expansion of the ruminant husbandry industry. Therefore, the use of unconventional feed resources based on local conditions has become an important strategy for maintaining the supply of animal husbandry. Many researchers have attempted to use fruit residue and found it effective at replacing part of the feed. Gowda et al. (2015) reported that the inclusion of pineapple residue in silage was a valuable feed replacement of traditional corn silage and that the ensiling of pineapple residue could improve the economic benefits for sheep and cows. Yi et al. (2023) reported that adding 2.5% citrus pulp to the broiler diet was more effective at utilizing carbohydrates through glycolytic/gluconeogenesis metabolism to affect the microbial composition and has important implications for improving gut health and economic benefits. In a review on the effect of apple residue on cattle and cows by Shalini et al. (2010), the authors reported that apple residue increased the average daily gain and metabolism of cattle and reduced feed costs for cows, suggesting that apple residue can safely be included in animal rations. These results indicate that fruit residue can increase growth performance and improve economic benefits and thus may be good alternatives to conventional feed.

Rosa roxburghii tratt (RRT), known as CiLi in Chinese, is a heliophilous, deciduous shrub species that primarily grows at altitudes ranging from 1,000 to 1,600 m in the hilly mountain areas of Guizhou Province, Southwest China, and mainly grows from mid-August to late September. RRT juice was reported

to have strong antioxidant activity due to its substantially greater amount of polysaccharides, vitamin C, superoxide dismutase (SOD), and phenolics than most other fruits. Moreover, Hu et al. (2023) reported that RRT supplementation in fermented broth alleviated the symptoms of type 2 diabetes in mice by modulating the gut microbiota and short-chain fatty acid production. RRT also contains catechins, which can scavenge free radicals, protect DNA from mutation, and reduce cholesterol levels.

RRT residue (RRTR) is the byproduct of RRT after the juicing process, often accounting for about 50% of the fresh weight of RRT. To date, most studies on RRTR have focused mainly on its small molecules. Zhou et al. (2019) reported that the insoluble dietary fibers extracted from RRTR could be directly applied to food raw materials. Li et al. (2023) suggested that RRTR might have potential as a functional food and a promising natural source of dietary fiber. By 2023, the yield of RRTR was as high as 150,000 tons in Guizhou Province. Currently, most RRTRs are discarded without further utilization (Wang et al. 2020), resulting in severe waste of resources and environmental pollution (Su et al. 2022). Thus, full use of RRTR is urgently needed.

Ruminants possess a unique ability to convert a variety of feedstuffs into high-quality protein for human consumption. Feeding byproducts from the processing of fruits have received increasing attention because of environmental consciousness, waste disposal regulations, and the economic competitiveness of livestock farming enterprises. Unlike other industrial waste, most residues from the processing of fruits have good and consistent qualities and nutritionally beneficial organic matter content that enables their use in feedstuffs for ruminant feeding. Generally, the most effective strategy for the long-term preservation of fruit residues is silage treatment, which can prevent fruit residues from spoiling and has received increasing attention. Unfortunately, to date, there has been no information on the utilization of RRTR as ruminant feed. Hence, this preliminary study was conducted to evaluate the feasibility of RRTR serving as a novel feed resource for cattle. By conducting *in vitro* gas production (Trial 1) and animal feeding experiments (Trial 2), we hypothesized that RRTR, as a fruit residue, also has the potential to be fed to ruminants.

Materials and methods

Animal care

The present study was reviewed and approved by the Animal Ethics Committee of Guizhou University (EAE-GZU-2022-E040).

Trial 1: Chemical composition of RRTR and *in vitro* gas production rumen fermentation parameters

Proximate nutrient analysis of RRTR

The RRTR was obtained from one RRT juice process factory located in Longli County, Guizhou Province. The fresh RRTR sample was prepared by drying at 65°C and grinding through a 40-mesh sieve. The DM content was assessed by the oven drying method, and the samples were dried to constant weight at

105°C (101A-3E, Shanghai Experimental Instrument Factory Co., Ltd.); the GE content was determined by the combustion method and isothermal automatic oxygen bomb CALORIMETER (Parr 6400 CALORIMETER); the CP content was determined by an automatic Kjeldahl nitrogen determination instrument (K1100, Haineng Future Technology Group Co., Ltd.) (AOAC, 1990); the OM content was calcined at high temperature and ashed to constant weight in a 550°C furnace (SX-4-10, Tianjin Test Instrument Co., Ltd.); and the contents of NDF and ADF were determined according to the methods of Van Soest et al. (1991) via an automatic fiber analyzer (Fibretherm FT 12, Gerhardt, Germany). The crude fiber (CF) content was calculated according to the formulation ($CF = -2.512 + 0.838 \times ADF$, $R^2 = 0.949$) proposed by Ma et al. (2018).

In vitro gas production and rumen fermentation

Animals and experimental design

Three 24-month-old Wuchuan black cattle (a local cattle breed) with a body weight of 270.67 ± 17.79 kg were selected as rumen fluid donors. They were placed and fed in a single pen with free access to water and exercise. The diet was formulated according to the Nutrient Requirement of Beef Cattle (2016) in the form of TMR with a forage-to-concentrate ratio of 30:70. Cattle were fed twice daily at 09:00 and 17:00.

Fermentation preparation

Ruminal fluid was collected from fasted cattle before the morning meal through an esophageal cannula via a vacuum pump (VP30, Labtech Instrument Co. Ltd., Beijing, China), which was inserted into the rumen after the 3 cattle were fed for 12 days. The ruminal fluid was filtered immediately through 4 layers of sterile gauze, which was added to a constant 39°C bottle. The culture time was 48 h. Before the culture, 4.0000 g of RRTR was weighed and placed in 250 mL of ANKOM fermentation bottles containing 50 mL of ruminal fluid (pH 5.86) and 40 mL of artificial saliva. The bottles were flushed with CO₂ gas before being capped with an ANKOM gas production module. An ANKOM RFS gas production measurement system was used to conduct the *in vitro* gas production ruminal fermentation experiment. RRTR was used as a fermentation substrate in 15 fermentation bottles, and 10 fermentation bottles were used as blanks for determining gas production. Bottles containing only ruminal fluid and artificial saliva served as blanks.

In vitro gas production and dynamics

According to the cumulative gas production (GP) pressure recorded by ANKOM software at all times, the *in vitro* GP was calculated by the following formula.

$$GP = V_j \times P_{\text{psi}} \times 0.068004084$$

where GP (mL) is the volume of gas produced at 39°C, V_j (mL) is the upper part of the liquid surface in the module bottle intervolum, and P_{psi} is the cumulative pressure recorded by the GMS software.

The NLIN process in SAS 9.4 statistical software was used to conduct nonlinear fitting calculations (Ørskov and McDonald, 1979) for gas production:

$$GP_t = a + b \times (1 - e^{-ct})$$

where GP_t (mL) is the gas production of the substrate sample at time t , a (mL) is the rapid fermentation part, b (mL) is the slow fermentation part, $a + b$ (mL) is the potential gas production, c (%/h) is the gas production rate, and t (h) is the fermentation time.

In vitro rumen fermentation parameters

Fermentation was stopped after 48 h of culture. The fermentation solution pH was measured by a pH detector (Seven2Go™, Mettler-Toledo International Inc.) and then centrifuged at 10 000 r/min at -20°C for 10 min. The supernatant was used to measure the concentration of ammonia nitrogen (NH_3-N) by colorimetry (Synergy™ H4, Berten Instruments, Inc.). The activities of carboxymethyl cellulase, cellobiase, and xylanase were measured according to the 3,5-dinitrosalicylic acid (DNS) colorimetric method. The VFA levels were measured by a GC-2010 Plus gas chromatograph (Shimadzu Corporation, Japan). The chromatographic conditions were as follows: column, SH-Rtx-WAX (30.00 m×0.25 mm×0.25 μm); carrier gas, high-purity nitrogen with a split ratio of 50:1; column temperature, 100°C for 2 min; flame hydrogen ion detector (FID) temperature, increased to 240°C at 5°C/min and held at 150°C for 2 min; gasification chamber temperature, 220°C; and sample volume, 1 μL. Crotonic acid was used as the internal standard.

The CH_4 production was calculated according to the following formula (Moss and Jouany, 2000):

$$CH_4\text{production}(\text{mmol/L}) = 0.45 \times \text{acetate} - 0.275 \times \text{propionate} + 0.4 \times \text{butyrate}$$

Trial 2: Animal feeding experiment

Animals and treatments

Sixteen cattle (17 ± 2.3 months; 241.22 ± 17.8 kg BW) were used as the experimental animals and were randomly allocated to a control group or a treatment group, with 8 replicates of 1 cattle. Cattle in the control group were fed a basal diet without RRTR, while those in the treatment group were fed a diet containing 30% (DM basis) RRTR to replace whole corn silage. The RRTR was silaged for 120 days before the current experiment. The experiment lasted 85 days and included a 15-day adaptation period and a 70-day trial period, which were divided into two 35-day stages. The level of RRTR gradually increased until its consumed amount stabilized as the final amount in the ration. The established RRTR level was 30%. The ratio of concentrate to roughage in the diet was 30:70. The ingredients and chemical composition of the diet fed to the cattle are shown in Table 1.

Table 1
The ingredients and nutrient levels of diets fed to cattle
in the animal feeding experiment

	Control	Treatment
<i>Ingredients</i> (% DM)		
Concentrate supplements ^{a)}	30	30
Whole corn silage	70	40
RRTR	0	30
Total	100	100
<i>Nutrient levels</i> ^{b)} (% DM)		
DM	96.05	96.06
NE _{mf} (MJ/kg)	4.23	4.54
CP	10.07	11.41
NDF	60.81	58.35
ADF	22.02	29.43
OM	95.10	94.63

^{a)} Composition (%): corn (29), wheat (30), soybean meal (11), distillers' dried grains with solubles (8.51), citric acid residue (8), wheat bran (10), limestone (1.2), CaHPO₄ (0.5), NaCl (1), NaHCO₃ (0.5), betaine (0.05), and premix (0.24).

^{b)} NE_{mf} was calculated according to the NRC (2016) and diet gross energy, while the others were actually measured.

^{c)} RRTR: *Rosa roxburghii* tratt residue; DM, dry matter; NE_{mf}, comprehensive net energy; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; OM, organic matter.

Measurement and analysis

Growth performance

During the experiment, all the cattle were weighed on an empty stomach before the morning feeding, and the initial body weight (IBW) was recorded. The provided feed and the remaining feed were accurately recorded. The average net gain (ANG), average daily gain (ADG), and ratio of dry matter intake (DMI) to ADG (F/G) were also calculated.

Plasma biochemical metabolites

Blood was collected from the jugular vein of all the cattle into disposable heparin sodium-containing vacuum tubes on days 35 and 70 before the morning meal. The blood samples were subsequently centrifuged at 4,000 rpm for 15 min, and the supernatants (plasma) were analyzed by an automatic biochemical analyzer (BS-360S, Shenzhen Mindray Bio-Medical Electronics Co., Ltd.). The plasma biochemical indicators included glucose (Glu), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total protein (TP), albumin (ALB), urea, aspartate transaminase (AST), and alanine transaminase (ALT).

In vivo ruminal fermentation parameters

On day 70 of the trial period, ruminal fluid was collected using a gastric vacuum extraction pump (VP 30, Beijing Laboratory Instrument Co., Ltd.) that was inserted into the rumen through the mouth of each cow. The pH of 50 mL of ruminal fluid was immediately measured using a Mettler-Toledo pH meter (Seven2Go, Mettler-Toledo International Inc.). The remaining ruminal fluid was then harvested by filtration through 4 layers of sterile gauze and used to measure the ruminal fermentation parameters, including $\text{NH}_3\text{-N}$ and the activities of carboxymethyl cellulase, cellobiase, and xylanase. The volatile fatty acid (VFA) concentration and CH_4 production calculations were the same as those in Trial 1.

Economic benefits

The economic benefits were calculated using the following formula based on the price of live cattle and feed cost during the experiment according to Sharif M et al. (2017).

Total feed cost (CNY) = total feed intake (kg) $\times$ feed price (CNY/kg)

Total weight gain income (CNY) = (final weight - initial weight) $\times$ live cattle price (CNY/kg)

Gross profit (CNY/cattle) = net weight gain income - total feed cost

Statistical analysis

All the data were collated by Microsoft Excel 2019, and statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The data on the chemical composition of the RRTR and *in vitro* gas production rumen fermentation parameters were subjected to descriptive statistical analysis and are presented as the means $\pm$ SDs. Statistical analyses of the animal feeding data were performed with one-way analysis of variance (ANOVA), followed by the SNK method for pairwise comparisons of the means between groups. All data from the animal feeding experiment are presented as the means, SEM represents the difference between the control group and treatment group, and the significance level was set as $P < 0.05$.

Results

Trial 1: Chemical composition and *in vitro* gas production rumen fermentation parameters of RRTR

Chemical composition of the RRTR

The chemical composition of RRTR is shown in Table 2. The DM content was 31.50%, the GE was 18.19 MJ/kg, the CP content was 11.99%, the NDF content was 53.71%, the ADF content was 47.22%, and the OM content was 97.03%. The CF content was calculated to be 37.06%.

Table 2
Chemical composition of the
RRTR

Item	Nutrients level
DM	31.50
GE (MJ/kg)	18.19
CP (% DM)	11.99
NDF (% DM)	53.71
ADF (% DM)	47.22
CF (% DM)	37.06
OM (% DM)	97.03

DM, dry matter; GE, gross energy; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; OM, organic matter.

***In vitro* gas production and dynamics of RRTR**

The *in vitro* gas production increased rapidly during the 24 h fermentation and gradually stabilized after 36 h, with 166.67 mL and 219.75 mL observed at 24 h and 48 h (Fig. 1), respectively.

The *in vitro* gas production dynamics of RRTR are listed in Table 3. The volume of the rapid fermentation part (a) was 11.66 mL, the volume of the slow fermentation part (b) was 252.01 mL, the potential gas production (a + b) was 263.67 mL, and the gas production rate (c) was 0.0395%/h. Accordingly, the DOM content and ME were calculated to be 416.75 g/kg and 3.51 MJ/kg, respectively.

Table 3
In vitro rumen fermentation gas production and dynamics of RRTR as a substrate

Item	Level
Accumulation of GP at 24 h (mL)	166.67 ± 25.24
Accumulation of GP at 48 h (mL)	219.75 ± 40.27
Rapidly degraded GP (a) (mL)	11.66 ± 3.65
Slowly degraded GP (b) (mL)	252.01 ± 80.95
Potential GP (a + b) (mL)	263.67 ± 80.82
Gas production rate (c) (%/h)	0.0395 ± 0.01

In vitro rumen fermentation parameters

As shown in Table 4, the *in vitro* rumen fermentation pH was 5.86. The NH₃-N concentration was 9.25 mg/dL. The enzyme activities of carboxymethyl cellulase, xylanase, and cellobioase were 0.34, 0.57, and 0.29 U/mL, respectively. The concentrations of TVFA, acetic acid, propionic acid, butyric acid, valeric acid, isobutyric acid, and isovaleric acid were 113.38, 75.59, 30.91, and 6.88, respectively. The proportions of acetic acid, propionic acid, and butyric acid in the TVFAs were 66.67%, 27.26%, and 6.07%, respectively. The ratio of acetic acid to propionic acid was 2.45, and the methane yield was 28.26 mmol/L.

Table 4
In vitro rumen fermentation parameters of RRTR

Item	Level
pH	5.86 ± 0.13
NH ₃ -N concentration (mg/dL)	9.25 ± 0.60
Carboxymethyl cellulose (U/mL)	0.34 ± 0.04
Xylanase (U/mL)	0.57 ± 0.05
Cellobiase (U/mL)	0.29 ± 0.03
TVFA (mmol/L)	113.38 ± 10.69
Acetate (%)	66.67 ± 0.67
Propionate (%)	27.26 ± 0.19
Butyrate (%)	6.07 ± 0.14
Acetate/propionate	2.45 ± 0.29
CH ₄ production (mmol/L)	28.26 ± 3.87

TVFA is the sum of acetate, propionate, and butyrate.

CH_4 (mmol/L) = $0.45 \times \text{acetate} - 0.275 \times \text{propionate} + 0.4 \times \text{butyrate}$.

Trial 2: Animal feeding experiment

Growth performance

The effect of RRTR on the growth performance of cattle is detailed in Table 5. RRTR treatment did not affect the DMI, FBW, ANG, or ADG throughout the entire trial period or the F/G ratio in stages 1 and 2 ($P > 0.05$). The F/G ratio of the cattle in the treatment group was greater than that of the control group for the whole trial period ($P < 0.05$).

Table 5
Effect of RRTR on the growth performance of cattle

	Control	Treatment	SEM	<i>P</i> value
Day 1 ~ 35				
IBW (kg)	237.13	236.25	44.29	0.99
DMI (kg/day)	6.92	7.09	0.92	0.90
FBW (kg)	276.22	271.22	48.47	0.94
ANG (kg)	39.09	34.97	5.42	0.60
ADG (kg/day)	1.12	1.00	0.15	0.60
F/G	6.47	7.19	0.51	0.33
Days 36 ~ 70				
Day 35 BW (kg)	276.22	271.22	5.42	0.60
DMI (kg/day)	7.60	7.91	1.46	0.88
Day 70 BW (kg)	299.75	305.25	61.78	0.95
ANG (kg)	44.43	42.43	9.17	0.95
ADG (kg/day)	1.27	1.21	0.26	0.88
F/G	6.23	6.73	0.31	0.28
Days 1 ~ 70				
IBW (kg)	237.13	236.25	44.29	0.99
DMI (kg/day)	7.06	7.55	1.24	0.78
Day 70 BW (kg)	299.75	305.25	61.78	0.95
ANG (kg)	83.04	78.11	14.43	0.81
ADG (kg/day)	1.19	1.12	0.21	0.81
F/G	6.09 ^b	6.78 ^a	0.20	0.03

IBW, initial body weight; DMI, dry matter intake; FBW, final body weight; ANG, average net gain; ADG, average daily gain; F/G, feed-to-gain ratio; day 35 BW, body weight on day 35; day 70 BW, body weight on day 70.

Plasma metabolites

Compared with the control group, the treatment group had reduced plasma urea levels of 71.1% and 53.8% in the first (days 1–35) and second (days 36–70) stages, respectively ($P < 0.05$; Table 6). No other

differences were detected in plasma Glu, TG, TC, LDL-C, HDL-C, TP, ALB, AST, or ALT concentrations or in the AST/ALT ratio between the 2 groups of cattle ($P > 0.05$).

Table 6
Effects of RRTR on plasma metabolites in cattle

	Control	Treatment	SEM	<i>P</i> value
Days 1 ~ 35				
Glu (mmol/L)	4.63	4.26	0.39	0.81
TG (mmol/L)	0.24	0.24	0.03	0.90
TC (mmol/L)	2.06	2.22	0.34	0.74
LDL-C (mmol/L)	0.43	0.39	0.11	0.81
HDL-C (mmol/L)	1.63	1.85	0.20	0.46
TP (g/L)	63.48	65.61	1.88	0.44
ALB (g/L)	31.50	32.44	1.50	0.66
Urea (mmol/L)	3.45 ^a	2.01 ^b	0.31	0.01
AST (U/L)	44.54	44.97	8.36	0.97
ALT (U/L)	41.18	46.61	7.60	0.62
AST/ALT	2.94	2.43	0.20	0.09
Days 35 ~ 70				
Glu (mmol/L)	4.02	4.04	0.22	0.96
TG (mmol/L)	0.21	0.21	0.03	1.00
TC (mmol/L)	2.65	2.48	0.41	0.78
LDL-C (mmol/L)	0.60	0.47	0.17	0.59
HDL-C (mmol/L)	2.03	1.95	0.21	0.80
TP (g/L)	62.90	62.34	2.74	0.89
ALB (g/L)	31.36	30.65	1.32	0.71
Urea (mmol/L)	3.08 ^a	2.00 ^b	0.26	0.01
AST (U/L)	42.81	41.49	6.72	0.89
ALT (U/L)	40.25	44.64	8.99	0.74
AST/ALT	2.79	2.37	0.19	0.15

Glu, glucose; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TP, total protein; ALB, albumin; AST, aspartate transaminase; ALT, alanine transaminase.

In vivo rumen fermentation parameters

The effects of RRTR on *in vivo* ruminal fermentation are shown in Table 7. There was no difference in the pH between the 2 groups of cattle ($P > 0.05$). Cattle fed the control diet had greater $\text{NH}_3\text{-N}$ concentrations than did those fed the control diet ($P < 0.05$). Xylanase activity was 17.4% lower in the treatment group than in the control group ($P < 0.05$), and carboxymethyl cellulose and cellobiase activity were unaffected ($P > 0.05$). The acetate/propionate ratio was 19.3% greater in the treatment group than in the control group ($P < 0.05$). There was no difference in the levels of TVFAs, acetate, propionate, or butyrate or in CH_4 production between the 2 groups of cattle ($P > 0.05$).

Table 7
The effect of RRTR on *in vivo* rumen fermentation parameters of cattle

Item	Control	Treatment	SEM	<i>P</i> value
pH	7.26	7.30	0.07	0.69
$\text{NH}_3\text{-N}$ concentration (mg/dL)	14.06 ^a	11.98 ^b	0.60	0.03
Carboxymethyl cellulose (U/mL)	0.32	0.32	0.02	1.00
Xylanase (U/mL)	1.49 ^a	1.22 ^b	0.07	0.02
Cellobiase (U/mL)	0.39	0.32	0.02	0.06
TVFA (mmol/L)	54.43	57.31	3.79	0.66
Acetate (%)	71.49	73.29	2.46	0.39
Propionate (%)	20.83	17.27	1.00	0.32
Butyrate (%)	7.68	9.44	0.49	0.09
Acetate/propionate	3.58 ^b	4.27 ^a	0.19	0.02
CH_4 production (mmol/L)	16.06	18.34	1.04	0.14

$\text{NH}_3\text{-N}$, ammonium nitrogen; TVFA, total volatile fatty acid; CH_4 , methane.

Economic benefits

There were no differences in feed cost or gross profit between the 2 groups of cattle for the 1st and 2nd stages or for the whole period ($P > 0.05$; Table 8).

Table 8
The effects of RRTR on economic benefits of cattle.

	Control	Treatment	SEM	<i>P</i> value
Days 1–35				
Feed price (CNY/kg)	2.3	1.87	-	-
Total DMI (kg/day)	242.32	248.23	32.29	0.90
Feed cost (CNY/head)	557.79	464.20	68.22	0.35
Net weight gain (kg)	39.09	34.97	5.42	0.60
Live cattle price (CNY/kg)	36	36	-	-
Total weight gain income (CNY/cattle)	1407.38	1258.88	8.60	0.60
Gross profit (CNY/cattle)	849.59	794.67	147.06	0.80
Days 36–70				
Feed price (CNY/kg)	2.3	1.87	-	-
Total DMI (kg/day)	266.00	276.77	47.85	0.88
Total feed cost (CNY/cattle)	612.29	517.58	99.02	0.54
Net weight gain (kg)	44.43	42.43	8.58	0.88
Live cattle price (CNY/kg)	36	36	-	-
Total weight gain income (CNY/cattle)	1599.43	1527.43	308.88	0.88
Gross profit (CNY/cattle)	987.13	1009.85	212.17	0.94
Days 1–70				
Feed price (CNY/kg)	2.3	1.87	-	-
Total dry matter intake (kg/day)	494.37	528.79	86.96	0.78
Total feed cost (CNY/cattle)	1137.95	988.88	180.61	0.57
Net weight gain (kg)	83.04	78.11	14.43	0.81
Live cattle price (CNY/kg)	36	36	-	-
Total weight gain income (CNY/cattle)	2989.29	2811.86	519.52	0.81
Gross profit (CNY/cattle)	1851.34	1822.98	344.69	0.95

CNY, Chinese yuan.

Discussion

Chemical composition of RRTR

Our current study is the first report on the nutrient level of RRTR mainly because it is a regional and almost unused fruit residue. Analyzing the chemical composition is the basis for further exploitation and utilization of any variety of feed. Compared to other commonly used fruit residues in previous studies, the CP level of RRTR (11.99%) was greater than that of apple residue (5.47–9.02%) (Kosmala et al., 2011; Afshar et al., 2011), pineapple residue (5.56–8.10%) (Gowda et al., 2015; Hu et al., 2023; Dieu et al., 2023), and citrus residue (6.17–7.3%) (Tayengwa and Mapiye, 2018). The NDF level of RRTR (53.71%) was lower than that of apple residue (61.2%) (Afshar et al., 2011) and higher than that of pineapple residue (19.58%) (Dieu et al., 2023) and citrus residue (24.2%, NRC, 2001). The ADF level of RRTR (47.22%) was greater than that of pineapple residue (9.40%) (Dieu et al., 2023) and citrus residue (22.2%, NRC, 2001) and was similar to that of apple residue (46.70%) (Afshar et al., 2011). Based on the calculated CF level (37.06%) through the model ($CF = -2.512 + 0.838 \times ADF$) (Zhou et al., 2019), we speculated that RRTR is classified as forage according to the international feed classification. From the chemical composition comparison between RRTR and other reported fruit residues, it seems that RRTR has a medium proximate nutrient level. To date, fruit residues have been widely used as forage for cattle (Gowda et al., 2015); thus, we argue that RRTR can also serve as a forage alternative for cattle. This speculation is verified by the following *in vitro* rumen fermentation and animal feeding experiments with cattle.

In vitro gas production and dynamics of RRTR

The *in vitro* gas production method is known for its ability to precisely evaluate the nutritional value of feed by calculating gas production through simulating rumen peristalsis *in vitro* to reflect the digestion of feed in the rumen. Gas production is a key index for measuring the extent of fermentation of substrates. In our experiment, the gas production of RRTR rapidly increased with time within 24 h and gradually tended to plateau. This profile is associated with the amount of substrate degradation during fermentation. GP dynamics are the result of regression analysis of gas production based on a classical mathematical model. This model is used to reflect the potential and utilization degree of a substrate. Our results showed that the rapidly degraded section (a), slowly degraded section (b), potential gas production section (a + b), and gas production rate (c) were 11.66 mL, 252.01 mL, 263.67 mL, and 0.0395%/h, respectively. Afshar (2011) also conducted an *in vitro* rumen fermentation experiment using 3 cattle as rumen fluid donors and apple residue as fermentation substrate, and their (a + b) values were lower than ours (263.67 vs. 77.82 mL), indicating that RRTR may be utilized more than apple residue for digestion. Margarette et al. (2023). reported that supplementation with proteolytic enzymes increased the *in vitro* nutrient degradation and fermentation characteristics of pineapple waste silage and that the (c) value was 0.45%/h without the addition of enzymes, which is higher than that in our study. Morteza et al. (2012). used citrus pulp silage as an *in vitro* gas fermentation substrate and reported that (c) was 0.05%/h, which was slightly greater than that in our study. Our findings showed that when RRTR was

utilized as a fermentation substrate, aberrant data for *in vitro* gas production dynamics parameters were not observed, whereas RRTR had no negative effect on the utilization of substrate.

In vitro rumen fermentation parameters of RRTR

Studies have shown that a pH range of 6.2 ~ 6.6 is conducive for rumen microbes to produce VFAs as an energy supply, and a pH range of 5.7 ~ 6.2 is conducive to internal metabolism via the utilization of $\text{NH}_3\text{-N}$ (Schmitz et al., 2018). The normal ruminal $\text{NH}_3\text{-N}$ concentration ranges from 5.0 to 30.0 mg/dL (Thao et al., 2014). If the $\text{NH}_3\text{-N}$ concentration is lower than 5.0 mg/dL, rumen microorganisms would have a reduced ability to synthesize protein due to insufficient N, which would result in rumen ammonia poisoning when the $\text{NH}_3\text{-N}$ concentration is greater than 30.0 mg/dL. In this experiment, the pH and $\text{NH}_3\text{-N}$ concentration of the RRTR were 5.86 and 9.25 mg/dL, respectively, which fall within the normal range. Our results indicate that the RRTR did not negatively affect rumen fermentation or nitrogen metabolism.

Cellulase activity is an important indicator reflecting the ability of rumen microorganisms to degrade cellulose in feed. The main cellulase enzymes in the rumen are carboxymethyl cellulase, xylanase and cellulobiase, which work together to degrade cellulose, hemicellulose, lignin and other substances that are difficult to absorb in feed. To the authors' knowledge, data on the cellulase activity of the RRTR have been insufficient until now. Therefore, our RRTR results are also the first to be reported. However, further studies are needed to compare cellulase activity among fruit residues.

VFAs are the products of carbohydrate degradation by rumen microorganisms and can provide 75% of the energy required by ruminants, and acetate, propionate, and butyrate account for 95% of the total VFAs produced in the rumen. The acetic acid in the rumen can be converted into lactate, and high lactate concentrations decrease the abundance of some microbes, including protozoa, methanogens, and cellulolytic bacteria, in the rumen; propionate can inhibit the growth of lactic acid bacteria in the rumen, reduce the production of lactic acid, increase the rumen pH, facilitate the activity of digestive enzymes, and promote the digestion and absorption of food (Kajikawa et al., 1990). The VFA content has been shown to be positively correlated with gas production (Fitri et al., 2021). Dieu et al. evaluated the *in vitro* fermentation (with 3 adult ewes as rumen donors) of 2 different varieties of pineapple residue (Smooth Cayenne and Sugarloaf) and reported that the TVFA level was 90.28 and 71.70 mmol/L (Dieu et al., 2023), which is lower than that in our study (114.78 mmol/L). This result may indicate that the RRTR has a greater ability to provide energy than pineapple residue for ruminants. The value of A/P is a key index for determining the rumen fermentation pattern. When the A/P ratio was greater than 2.5, acetic acid-type fermentation was dominant in the rumen, which was conducive to body fat synthesis. When the A/P ratio is less than 2.5, propionic acid-type fermentation is dominant, and more propionic acid is converted to glucose to provide energy. In this study, the *in vitro* rumen fermentation A/P ratio of RRTR was 2.45, which was slightly lower than the 2.5 observed for the propionic acid fermentation pattern.

Overall, the authors suggest that RRTR has the potential to serve as forage for cattle, and the following animal experiments were subsequently carried out to evaluate the feeding effect.

Trial 2: Effects of RRTR on growth performance, blood metabolites, and in vivo rumen fermentation parameters in cattle

Growth performance

Growth performance can reflect the growth state and metabolic health of animals and is commonly assessed based on the DMI, ADG, and F/G. The DMI can directly indicate feed palatability, the ADG can be used to evaluate feed conversion efficiency, and the F/G ratio can indicate the feed utilization efficiency. Oduguwa et al. (2020) reported that pineapple residue (supplemented with cassava peel) improved feed intake and growth performance in West African dwarf sheep and that sheep fed the same diet had greater ADG than animals in the other treatment groups. These authors concluded that feed containing ensiled pineapple fruit waste and cassava peel could be recommended to sheep farmers to improve feed intake and performance, especially during periods of austerity. In a study on pigs, Hsu et al. (2004) reported that the dietary inclusion of 15% citrus residue did not affect the ADG and that whole citrus residue silage and pressed citrus residue silage had greater ($P = 0.04$) feed conversion efficiency than did dried citrus residue. The results of the current study showed that neither the DMI nor the ADG differed significantly between the two groups of cattle, suggesting that RRTR has palatability and nutritional value similar to those of whole corn silage. There are 2 possible reasons for this phenomenon. First, RRTR is rich in flavor compounds and saccharine, which makes it highly palatable. Second, RRTR contains a variety of organic acids, which can promote gastrointestinal peristalsis, improve digestive capacity, and maintain acid–base balance, resulting in improved appetite. Unexpectedly, cattle in the treatment group had a significantly greater F/G ratio than did cattle in the control group. We are unsure why RRTR supplementation reduces the feed conversion ratio because the DMI and ADG are unaffected. A possible explanation might be attributed to the limited number of cattle in each group ($n = 8$). Accordingly, a large-scale trial involving more cattle is needed to evaluate the precise effect of RRTR on feed conversion efficiency.

Plasma metabolites

Plasma metabolites are important parameters for assessing metabolic status and body health. Plasma Glu is the primary indicator of the relative homeostasis of glucose catabolism and anabolism in the body. Plasma TG and TC are indicators of lipid catabolism and nutrient absorption in animals. Plasma LDL-C and HDL-C are the 2 forms of lipoprotein-incorporated cholesterol and function as cholesterol carriers and transporters, respectively; accordingly, they reflect cholesterol transport and lipid metabolism in the body. Plasma TP and ALB reflect the absorption and transformation of dietary protein in animals. Plasma ALT is mainly distributed in hepatocyte plasma, while AST is present in hepatocyte plasma and the mitochondria of hepatocytes; both indices are key indicators of amino acid metabolism and hepatocyte damage (Biasi et al., 1991; Parola et al., 1992) and play a critical role in the evaluation of animal health, growth, and development. Gowda et al. compared 2 TMRs comprising either 62% pineapple residue silage or maize green fodder silage with 38% compound concentrate feed in a 75-day feeding trial with sheep (n

= 10 per group) and found no difference in the serum total protein, urea, creatinine, glutamate oxaloacetate transaminase (SGOT), or glutamate pyruvate transaminase (SGPT) activities between the two groups (Gowda et al. 2015).

In our study, there were no differences in plasma Glu, TG, TC, LDL-C, HDL-C, TP, ALB, AST, or ALT concentrations or in the AST/ALT ratio between the 2 groups of cattle, but the plasma urea level was 71.1% and 53.8% lower in the treatment group than in the control group for the first (day 35) and second (day 70) blood sampling time points, respectively. As a product of protein catabolism, plasma urea can reflect dietary protein utilization efficiency. A reasonable plasma urea concentration range is generally suggested to be 2.0 ~ 7.1 mmol/L (Coma et al., 1995). If dietary protein is fully utilized by the body, it will be excessively catabolized into urea and excreted by the kidney, resulting in nutritional losses. The utilization of nitrogen is negatively correlated with the plasma urea level. In the present study, the plasma urea concentration in the treatment group was significantly lower than that in the control group in both stages, indicating that the inclusion of RRTR in the diet can enhance the plasma nitrogen utilization efficiency of cattle. This result may be associated with the compound. However, further investigations are needed to determine the exact underlying mechanism involved.

In vivo rumen fermentation parameters

Ruminal fermentation characteristics are largely affected by the composition of the diet, and it is reasonable to evaluate the feasibility of the RRTR diet based on rumen fermentation characteristics (Ma et al., 2024). Rumen pH is an important measurement for ruminants because it is associated with acid–base balance, especially for the survival of ruminal microbes. The normal rumen pH is reported to be in the range of 5.5 to 7.5. Sparkes et al. (2009) reported that replacing 30% of lucerne hay with fresh citrus pulp resulted in a significantly lower rumen pH (5.90 vs. 6.53) for ewes, indicating that citrus pulp affected rumen fermentation. In our experiment, there was only a slight difference in rumen pH (7.26 vs. 7.30), indicating that the inclusion of RRTR had no impact on the ruminal acid–base balance. Our results were supported by those of Fang J et al. (2019) and Soares et al. (2014), who reported that feeding apple pomace and citrus pulp had no significant effect on rumen pH in sheep and cows, respectively.

NH₃-N is an important source of nitrogen for rumen microbe growth and bacterial protein synthesis in ruminants. Devant et al. (2000) reported that after supplementation with a low-degrading protein and/or when the CP concentration was low, the ruminal NH₃-N concentration decreased to less than 5 mg/100 mL. In the review of Tayengwa and Mapiye (2018), the authors found that citrus residue (30% for ewes and 26% for lambs) resulted in varying ruminal VFA (154 mmol/L for ewes, 72.8 mmol/L for lambs) and NH₃-N (40 mg/dL for ewes, 6.09 mg/dL for lambs) concentrations, illustrating that the inclusion of citrus residue in ruminant diets improves the individual and total VFA profile by providing more lipogenic metabolizable nutrients. In our study, the NH₃-N concentration in the treatment group was significantly lower than that in the control group, indicating that RRTR can decrease the degradation of protein by ruminal microorganisms and increase the efficiency of protein utilization.

The ruminant diet contains a substantial amount of fibrous substances that rely on cellulase (carboxymethyl cellulase, fibrinosaccharase, and xylanase) secreted by microorganisms in the rumen for degradation and utilization. In a previous study, we found that the activities of carboxymethyl cellulase and cellobiase were unaffected, but the xylanase activity was 17.4% lower in the treatment group than in the control group throughout the trial period, possibly because xylanase activity was decreased in fermented RRTR.

VFAs are the main energy source for ruminants and an important carbon source for ruminal microbes, and their concentration and composition are important indicators of ruminal digestion and metabolism. Khaled et al. (2023) reported that increased VFA concentrations were associated with increased fermentable energy content and improved DMI and DM and OM digestibility. Ahn et al. (2022) reported that when 30% apple residue was supplemented in the diet of goats, the TVFA concentration was 144.8 mmol/L, which was significantly greater than that in a control group (80.6 mmol/L) without apple residue. In our study, there was no difference in TVFA, acetate, propionate, or butyrate levels between the control and treatment groups. This result indicated that there was no significant difference in overall digestibility between the 2 groups of cattle. Moreover, cattle in the control and treatment groups exhibited A/P values greater than 2.5, indicating an acetic acid-type rumen fermentation pattern.

Economic benefit

The economic benefit index can reflect whether RRTR is cost-effective when it is used as roughage and can, to some extent, provide an understanding of the feasibility of large-scale or long-term RRTR utilization in practice. Currently, RRTR is less expensive than conventional roughage because it has similar nutrients and can, in theory, replace conventional roughage in terms of feed cost. In this study, there were no differences in feed cost, net weight gain, or gross profit between the two groups of cattle. This result indicates that replacing whole corn silage with RRTR does not negatively affect feed cost or economic benefit and provides a good feed alternative for cattle.

Conclusions

Overall, RRTR has a medium level of proximate nutrients and normal *in vitro* rumen fermentation parameters and enhances plasma nitrogen utilization efficiency in cattle. RRTR feed supplementation has no negative impact on growth performance, blood metabolites, or *in vivo* rumen fermentation in cattle. These findings suggest that RRTR can be used as a novel feed alternative for cattle.

Declarations

Author Contributions

All authors contributed to the study conception and design. Conceptualization, visualization, writing the original draft was written by Xinyu Song. Sampling, formal analysis, data collection, experimental design, and data statistics were performed by Yi Yang. Methodology, investigation, project administration were

performed by Chong Wang. Fixing the original draft, project administration were performed by Wen Zhu. Methodology, fixing the original draft, resources, funding acquisition, and project administration was written by Wenxuan Wu. And all authors commented on previous versions of the manuscript, All authors read and approved the final manuscript.

Funding

The authors are grateful for the support by National Natural Science Foundation of China (31960674; U20A2057) and the Agricultural Plan Project of Science and Technology of Guizhou Province ([2020]1Y081, [2022]127).

References

1. Afshar Mirzaei-Aghsaghali, Naser Maheri-Sis, Mansouri H, et al (2011) Evaluating nutritional value of apple pomace for ruminants using *in vitro* gas production technique. *Annals of Biological Research* 2:100–106.
2. Ahn JH, Jo IH, Lee JS (2002) The Use of Apple Pomace in Rice Straw Based Diets of Korean Native Goats (*Capra hircus*). *Asian-Australasian Journal of Animal Sciences* 15:1599–1605. <https://doi.org/10.5713/ajas.2002.1599>.
3. Alexander G, Singh B, Sahoo A, Bhat TK (2008) *In vitro* screening of plant extracts to enhance the efficiency of utilization of energy and nitrogen in ruminant diets. *Animal Feed Science and Technology* 145:229–244. <https://doi.org/10.1016/j.anifeedsci.2007.05.036>.
4. Association Of Official Analytical Chemists (1990) Official methods of analysis : Supplements. Association Of Official Analytical Chemists, Arlington.
5. Bakshi MPS (2016) Waste to worth: vegetable wastes as animal feed. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 11:1–26. <https://doi.org/10.1079/pavsnr201611012>.
6. Beauchemin K (2009) Dietary mitigation of enteric methane from cattle. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 4:1–18. <https://doi.org/10.1079/pavsnr20094035>.
7. Beeson WT, Vu V, Span EA, et al (2015) Cellulose Degradation by Polysaccharide Monooxygenases. *Annual Review of Biochemistry* 84:923–946. <https://doi.org/10.1146/annurev-biochem-060614-034439>.
8. Bergman EN (1990) Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews* 70:567–590. <https://doi.org/10.1152/physrev.1990.70.2.567>.
9. Biasi F, Albano E, Chiarpotto E, et al (1991) *In vivo* and *in vitro* evidence concerning the role of lipid peroxidation in the mechanism of hepatocyte death due to carbon tetrachloride. *Cell Biochemistry and Function* 9:111–118. <https://doi.org/10.1002/cbf.290090208>.

10. Chashnidel Y, Bahari M, Teimouri Yansari A, Kazemifard M (2020) The Effects of Dietary Supplementation of Prebiotic and Peptide on Growth Performance and Blood Parameters in Suckling Zell Lambs. *Small Ruminant Research* 188:106121. <https://doi.org/10.1016/j.smallrumres.2020.106121>.
11. Chen G, Kan J (2018) Characterization of a novel polysaccharide isolated from *Rosa roxburghii* Tratt fruit and assessment of its antioxidant *in vitro* and *in vivo*. *International Journal of Biological Macromolecules* 107:166–174. <https://doi.org/10.1016/j.ijbiomac.2017.08.160>.
12. Chen H, Liu L, Zhu J, et al (2010) Effect of soybean oligosaccharides on blood lipid, glucose levels and antioxidant enzymes activity in high fat rats. *Food Chemistry* 119:1633–1636. <https://doi.org/10.1016/j.foodchem.2009.09.056>.
13. Cláudson Oliveira Brito, Ribeiro V, Paula A, et al (2020) Metabolizable energy and nutrient digestibility of shrimp waste meal obtained from extractive fishing for broilers. *Animal Feed Science and Technology* 263:114467. <https://doi.org/10.1016/j.anifeedsci.2020.114467>.
14. Coma J, Carrion D, Zimmerman DR (1995) Use of plasma urea nitrogen as a rapid response criterion to determine the lysine requirement of pigs². *Journal of Animal Science* 73:472–481. <https://doi.org/10.2527/1995.732472x>.
15. Cone JW, Becker P (2012) Fermentation kinetics and production of volatile fatty acids and microbial protein by starchy feedstuffs. *Animal feed science and technology* 172:34–41. <https://doi.org/10.1016/j.anifeedsci.2011.12.006>.
16. Crosswhite JD, Myers NB, Adesogan AT, et al (2013) The effect of dietary citrus pulp on the growth, feed efficiency, carcass merit, and lean quality of finishing pigs. *The Professional Animal Scientist* 29:345–358. [https://doi.org/10.15232/s1080-7446\(15\)30247-3](https://doi.org/10.15232/s1080-7446(15)30247-3).
17. De Boever JL, Cottyn BG, De Brabander DL, et al (1997) Prediction of the feeding value of maize silages by chemical parameters, *in vitro* digestibility and NIRS. *Animal Feed Science and Technology* 66:211–222. [https://doi.org/10.1016/s0377-8401\(96\)01101-7](https://doi.org/10.1016/s0377-8401(96)01101-7).
18. Devant M, Ferret A, Gasa J, et al (2000) Effects of protein concentration and degradability on performance, ruminal fermentation, and nitrogen metabolism in rapidly growing heifers fed high-concentrate diets from 100 to 230 kg body weight. *Journal of Animal Science* 78:1667–1676. <https://doi.org/10.2527/2000.7861667x>.
19. Dieu donné Kiatti, Alessandro Vastolo, Bossima Ivan Koura, et al (2023) The Chemical Characteristics and *In Vitro* Degradability of Pineapple By-Products as Potential Feed for Ruminants. *Animals* 13:3238. <https://doi.org/10.3390/ani13203238>.
20. Fang JC, Cao Y, Matsuzaki M, et al (2016) Effects of apple pomace-mixed silage on growth performance and meat quality in finishing pigs. *Animal Science Journal* 87:1516–1521. <https://doi.org/10.1111/asj.12601>.
21. Fang JC, Xia GJ, Cao Y (2019) Effects of replacing commercial material with apple pomace on the fermentation quality of total mixed ration silage and its digestibility, nitrogen balance and rumen fermentation in wethers. *Grassland Science* 66:124–131. <https://doi.org/10.1111/grs.12258>.

22. Fitri A, Obitsu T, Sugino T (2021) Effect of ensiling persimmon peel and grape pomace as tannin-rich byproduct feeds on their chemical composition and *in vitro* rumen fermentation. *Animal Science Journal* 92:e13524. <https://doi.org/10.1111/asj.13524>.
23. Gowda NKS, Vallesha NC, Awachat VB, et al (2015) Study on evaluation of silage from pineapple (*Ananas comosus*) fruit residue as livestock feed. *Tropical Animal Health and Production* 47:557–561. <https://doi.org/10.1007/s11250-015-0762-2>.
24. Hsu C K, Liao J W, Chung Y C, et al (2004) Xylooligosaccharides and Fructooligosaccharides Affect the Intestinal Microbiota and Precancerous Colonic Lesion Development in Rats. *The Journal of Nutrition* 134:1523–1528. <https://doi.org/10.1093/jn/134.6.1523>.
25. Hu X, Hu J, Leng T, et al (2023) *Rosa roxburghii*-edible fungi fermentation broth attenuates hyperglycemia, hyperlipidemia and affects gut microbiota in mice with type 2 diabetes. *Food Bioscience* 52:102432. <https://doi.org/10.1016/j.fbio.2023.102432>.
26. Hu Y, Shi T, Zhao W, et al (2023) Effects of Dried Blueberry Pomace and Pineapple Pomace on Growth Performance and Meat Quality of Broiler Chickens. *Animals* 13:2198–2198. <https://doi.org/10.3390/ani13132198>.
27. Huang DS, Li C, Qing C, et al (2022) Identification of polyphenols from *Rosa roxburghii* Tratt pomace and evaluation of *in vitro* and *in vivo* antioxidant activity. *Food Chemistry* 377:131922. <https://doi.org/10.1016/j.foodchem.2021.131922>.
28. Huang M, Xu Q, Deng XX (2014) L-Ascorbic acid metabolism during fruit development in an ascorbate-rich fruit crop chestnut rose (*Rosa roxburghii* Tratt). *Journal of Plant Physiology* 171:1205–1216. <https://doi.org/10.1016/j.jplph.2014.03.010>.
29. Kajikawa H, Yuji Nagasaki, Abe A (1990) Comparison between Colony Counting Method and Most Probable Number Method in Enumeration of Rumen Bacteria Fermenting Specific Substrates. *Nihon Chikusan Gakkaiho* 61:810–814. <https://doi.org/10.2508/chikusan.61.810>.
30. Khaled Gadulrab, Pola Sidoruk, Martyna Kozłowska, et al (2023) Effect of Feeding Dried Apple Pomace on Ruminal Fermentation, Methane Emission, and Biohydrogenation of Unsaturated Fatty Acids in Dairy Cows. *Agriculture* 13:2032. <https://doi.org/10.3390/agriculture13102032>.
31. Kosmala M, Krzysztof Kołodziejczyk, Zenon Zduńczyk, et al (2011) Chemical Composition of Natural and Polyphenol-free Apple Pomace and the Effect of This Dietary Ingredient on Intestinal Fermentation and Serum Lipid Parameters in Rats. *Journal of Agricultural and Food Chemistry* 59:9177–9185. <https://doi.org/10.1021/jf201950y>.
32. Li PD, Li C, Fu X, et al (2023) Physicochemical, functional and biological properties of soluble dietary fibers obtained from *Rosa roxburghii* Tratt pomace using different extraction methods. *Process Biochemistry* 128:40–48. <https://doi.org/10.1016/j.procbio.2023.02.021>.
33. Liu HW, Li K, Zhao JS, Wen D (2018) Effects of polyphenolic extract from *Eucommia ulmoides* Oliver leaf on growth performance, digestibility, rumen fermentation and antioxidant status of fattening lambs. *Animal Science Journal* 89:888–894. <https://doi.org/10.1111/asj.12998>.

34. Ma J, Fan X, Sun GJ, et al (2024) Replacing alfalfa hay with amaranth hay: effects on production performance, rumen fermentation, nutrient digestibility and antioxidant ability in dairy cows. *Animal Bioscience* 37:218–227. <https://doi.org/10.5713/ab.23.0232>.
35. Ma S N, Xu G S, Cui K, Ma T, Diao Q Y. Correlation analysis of CF, NDF and ADF in common feed of ruminants. *Feed Industry* 2018;39:52-59. (In Chinese)
36. Margarette K, Jae Ik Lee, Yu J, et al (2023) Supplementing Proteolytic Enzymes Increased the *In Vitro* Nutrient Effective Degradability and Fermentation Characteristics of Pineapple Waste Silage. *Fermentation* 9:218. <https://doi.org/10.3390/fermentation9030218>.
37. Menke KH (1988) Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research and Development* 28:7–55.
38. Morteza Kordi (2012) Influence of wheat bran as a silage additive on chemical composition, *in situ* degradability and *in vitro* gas production of citrus pulp silage. *AFRICAN JOURNAL OF BIOTECHNOLOGY* 11:12669–12674. <https://doi.org/10.5897/ajb12.767>.
39. Moss AR, Jouany J-P, Newbold J (2000) Methane production by ruminants: its contribution to global warming. *Annales de Zootechnie* 49:231–253. <https://doi.org/10.1051/animres:2000119>.
40. National Academies Of Sciences, Engineering, And Medicine (U.S.). Committee On Nutrient Requirements Of Beef Cattle (2016) Nutrient requirements of beef cattle. National Academies Press, Washington, Dc.
41. Nsahlai IV, Siaw D, Osuji PO (1994) The relationships between gas production and chemical composition of 23 browses of the genus *Sesbania*. *Journal of the Science of Food and Agriculture* 65:13–20. <https://doi.org/10.1002/jsfa.2740650104>.
42. Oduguwa BO, Sanusi GO, Fasae OA, et al (2020) Nutritive value, growth performance and haematological parameters of West African dwarf sheep fed preserved pineapple fruit waste and cassava by-products. *Nigerian Journal of Animal Production* 40:123–132. <https://doi.org/10.51791/njap.v40i1.671>.
43. Oluwafemi Babatunde Oduntan, Siyanbola Adewunmi Omitoyin, Sule Okanlawon Shakiru (2022) Pineapple Pomace as Replacement for Wheat Bran in a Practical Extruded Diet for Catfish (*Clarias gariepinus*). *Aceh journal of animal science* 7:41–46. <https://doi.org/10.13170/ajas.7.2.24150>.
44. Ørskov ER, McDonald I (1979) The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *The Journal of Agricultural Science* 92:499–503. <https://doi.org/10.1017/s0021859600063048>.
45. Parola M, Leonarduzzi G, Biasi F, et al (1992) Vitamin E dietary supplementation protects against carbon tetrachloride–induced chronic liver damage and cirrhosis. *Hepatology* 16:1014–1021. <https://doi.org/10.1002/hep.1840160426>.
46. Reed JD (1995) Nutritional toxicology of tannins and related polyphenols in forage legumes. *Journal of Animal Science* 73:1516–1528. <https://doi.org/10.2527/1995.7351516x>.
47. Rensburg van, Erasmus E, Du Toit Loots, et al (2005) *Rosa roxburghii* supplementation in a controlled feeding study increases plasma antioxidant capacity and glutathione redox state.

- European Journal of Nutrition 44:452–457. <https://doi.org/10.1007/s00394-005-0555-x>.
48. Schmitz R, Schnabel K, von Soosten D, et al (2018) Changes of ruminal pH, rumination activity and feeding behaviour during early lactation as affected by different energy and fibre concentrations of roughage in pluriparous dairy cows. *Archives of Animal Nutrition* 72:458–477. <https://doi.org/10.1080/1745039X.2018.1526535>.
49. Shalini R, Gupta DK (2010) Utilization of pomace from apple processing industries: a review. *Journal of Food Science and Technology* 47:365–371. <https://doi.org/10.1007/s13197-010-0061-x>.
50. Sharif M, Ashraf M, Mushtaq N, et al (2017) Influence of varying levels of dried citrus pulp on nutrient intake, growth performance and economic efficiency in lambs. *Journal of Applied Animal Research* 46:264–268. <https://doi.org/10.1080/09712119.2017.1294540>.
51. Soares L, Tadeu G, A.B. Schogor, et al (2014) Effect of abomasal or ruminal supplementation of citrus pulp and soybean oil on nutrient digestibility and ruminal fermentation of dairy cows. *Animal Feed Science and Technology* 189:123–129. <https://doi.org/10.1016/j.anifeedsci.2013.12.008>.
52. Sparkes JL, Chaves AV, Fung E, et al (2009) Effects of Replacing Lucerne (*Medicago sativa* L.) Hay with Fresh Citrus Pulp on Ruminal Fermentation and Ewe Performance. *Asian-australasian Journal of Animal Sciences* 23:197–204. <https://doi.org/10.5713/ajas.2010.90314>.
53. Su J, Fu X, Huang Q, et al (2022) Phytochemical profile, bioactivity and prebiotic potential of bound polyphenols released from *Rosa Roxburghii* fruit pomace dietary fiber during *in vitro* digestion and fermentation. *Food & Function* 13:8880–8891. <https://doi.org/10.1039/d2fo00823h>.
54. Subcommittee On Dairy Cattle Nutrition, Committee On Animal Nutrition, National Research Council (2001) Nutrient requirements of dairy cattle. Washington National Academy Press.
55. Suzika Pagán Riestra, Abner, Chin V, Rendel PF (2014) Pineapple and citrus silage as potential feed for small ruminant diets: fermentation characteristics, intake, nutrient digestibility, and aerobic stability. *DOAJ (DOAJ: Directory of Open Access Journals)* 27:37–46.
56. Tayengwa T, Mapiye C (2018) Citrus and Winery Wastes: Promising Dietary Supplements for Sustainable Ruminant Animal Nutrition, Health, Production, and Meat Quality. *Sustainability* 10:3718. <https://doi.org/10.3390/su10103718>.
57. Thao NT, Wanapat M, Cherdthong A, Kang S (2014) Effects of Eucalyptus Crude Oils Supplementation on Rumen Fermentation, Microorganism and Nutrient Digestibility in Swamp Buffaloes. *Asian-Australasian Journal of Animal Sciences* 27:46–54. <https://doi.org/10.5713/ajas.2013.13301>.
58. Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *Journal of Dairy Science* 74:3583–3597. [https://doi.org/10.3168/jds.s0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.s0022-0302(91)78551-2).
59. Wang C, Gao G, Huang J, et al (2017) Nutritive value of dry citrus pulp and its effect on performance in geese from 35 to 70 days of age. *Journal of Applied Poultry Research* 26:253–259. <https://doi.org/10.3382/japr/pfw069>.

60. Wang L, Shen C, Li C, Chen J (2020) Physicochemical, functional, and antioxidant properties of dietary fiber from *Rosa roxburghii* Tratt fruit modified by physical, chemical, and biological enzyme treatments. *Journal of Food Processing and Preservation* 44:e14858. <https://doi.org/10.1111/jfpp.14858>.
61. Wang SK, Xia J, Paepe KD, et al (2021) Ultra-high Pressure Treatment Controls *In Vitro* Fecal Fermentation Rate of Insoluble Dietary Fiber from *Rosa Roxburghii* Tratt Pomace and Induces Butyrogenic Shifts in Microbiota Composition. *Journal of Agricultural and Food Chemistry* 69:10638–10647. <https://doi.org/10.1021/acs.jafc.1c03453>.
62. Wu HY, Li MM, Yang XR, et al (2020) Extraction optimization, physicochemical properties and antioxidant and hypoglycemic activities of polysaccharides from roxburgh rose (*Rosa roxburghii* Tratt.) leaves. *International Journal of Biological Macromolecules* 165:517–529. <https://doi.org/10.1016/j.ijbiomac.2020.09.198>.
63. Yamane T, Nakatani H, Kikuoka N, et al (1996) Inhibitory effects and toxicity of green tea polyphenols for gastrointestinal carcinogenesis. *Cancer* 77:1662–1667. [https://doi.org/10.1002/\(sici\)1097-0142\(19960415\)77:8%3C1662::aid-cncr36%3E3.0.co;2-w](https://doi.org/10.1002/(sici)1097-0142(19960415)77:8%3C1662::aid-cncr36%3E3.0.co;2-w).
64. Yi LL, Zhang ZN, Li ZP, et al (2023) Effects of citrus pulp on the composition and diversity of broiler cecal microbes. *Poultry Science* 102:102454. <https://doi.org/10.1016/j.psj.2022.102454>.
65. Zhai XY, Ao HP, Liu WH, et al (2021) Physicochemical and structural properties of dietary fiber from *Rosa roxburghii* pomace by steam explosion. *Journal of Food Science and Technology* 59:2381–2391. <https://doi.org/10.1007/s13197-021-05254-7>.
66. Zhang L, Lu J, Okada H, et al (2017) Low-Power Highly Sensitive pH Sensor With μ dots Protective Structures for Monitoring Rumen in Cows in Real-Time. *IEEE Sensors Journal* 17:7281–7289. <https://doi.org/10.1109/jsen.2017.2757701>.
67. Zhou H, Wang CZ, Ye JZ, et al (2015) Effects of dietary supplementation of fermented *Ginkgo biloba* L. residues on growth performance, nutrient digestibility, serum biochemical parameters and immune function in weaned piglets. *Animal Science Journal* 86:790–799. <https://doi.org/10.1111/asj.12361>.
68. Zhou XL, Zhu GT, Lu Y, et al (2019) Optimization of insoluble dietary fiber preparation technology from *Rosa roxburghii* Tratt pomace by enzyme method. *IOP conference series* 330:042052. <https://doi.org/10.1088/1755-1315/330/4/042052>.

Figures

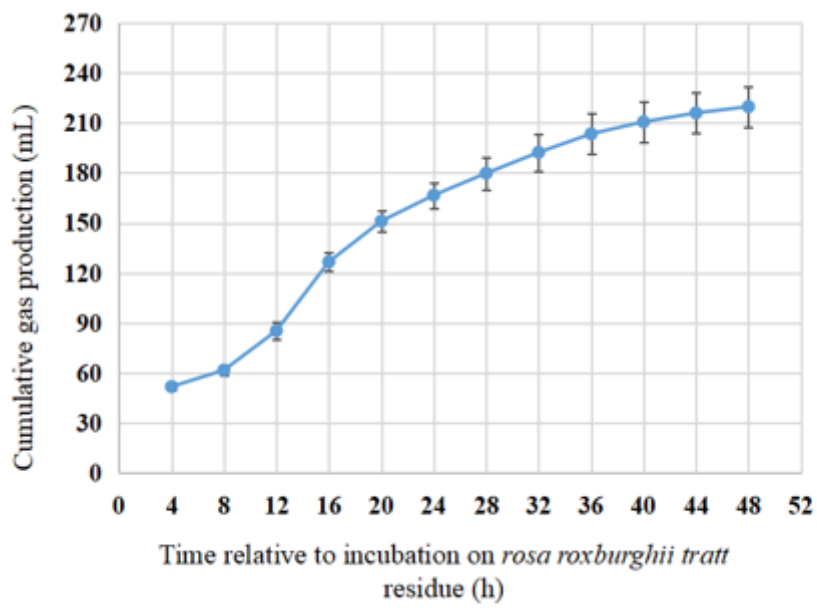


Figure 1

The 48-h *in vitro* rumen fermentation gas production of *Rosa roxburghii tratt* residue (RRTR) as a substrate