Animal Husbandry, Dairy and Veterinary Science

Research Article ISSN: 2513-9304

In vitro and *in vivo* ruminal fermentation of micronised wood powder for volatile fatty acid production in beef cattle

Yoshio Hamano[1]*, Takehiko Takahashi[2] and Hitosi Agematu[3]

[1]Field Education and Research Center, Faculty of Bioresource Sciences, Akita Prefectural University, Ohgata, Akita 010-045, Japan

[2]Department of Machine Intelligence and Systems Engineering, Faculty of Systems Science and Technology, Akita Prefectural University, Yurihonjo, Akita 015-0055, Japan

[3]Department of Applied Chemistry, Akita National College of Technology, Akita, Akita 011-8511, Japan

Abstract

The aim of the present study was to evaluate *in vitro* and *in vivo* ruminal fermentation of micronised wood powder in beef cattle. Micronised wood powder was prepared by pulverizing sapwood of Japanese cedar to a mean particle size of 52 μm. Ruminal fluid from four beef cows was anaerobically incubated with different amounts of micronised wood powder (50, 100, 200, or 400 mg per 30 ml rumen fluid) for 24 hours at 39°C. In the *in vivo* experiment, four cows were fed micronised wood powder for four weeks in a crossover design. During the initial feeding period of seven days, micronised wood powder was gradually increased to the equivalent to 0.3% of body weight, and then animals were fed micronised wood powder for three weeks at this level. In batch fermentation, total concentrations of volatile fatty acid were increased by micronised wood powder supplementation except at 50 mg. This response was mainly attributable to increased concentrations of the major volatile fatty acids of acetate, propionate, and butyrate. However, volatile fatty acid production from micronised wood powder by *in vitro* ruminal fermentation was low and limited, even when the supplemented levels were increased to 400 mg. The ruminal fermentability of micronised wood powder was shown by the lack of change in the volatile fatty acid profile during *in vivo* ruminal fermentation. Therefore, the present study found that ruminal microorganisms enable fermentation and digestion of lignocelluloses in micronised wood powder directly for volatile fatty acid production. However, further consideration of micronised wood powder, such as improvement in particle size or lignin, is needed to accelerate the fermentation kinetics of micronised wood powder in the rumen.

Introduction

Wood by-products of the lignocellulosic biomass have been tested to utilise its polysaccharides consisting of cellulose and hemicellulose as raw materials for bioethanol and biogas production [1]. Theoretically, the holocelluloses in woody materials could also be available as a desirable feedstuff for ruminants to obtain volatile fatty acids such as acetate, propionate, and butyrate. However, observations evaluating ruminal digestibility of woody lignocellulose have been limited. Sawdust is a simple form of a woody feedstuff that is able to play a role as a roughage substitute to maintain desirable rumen function and prevent abnormality of the gastrointestinal tract [2,3]. Although sawdust feeding has been reported to improve body weight gain in ruminants [4,5], this effect on performance does not contribute directly to volatile fatty acid production induced by ruminal microorganisms. El-Sabban *et al.*. [3] has reported that long-term feeding of oak sawdust did not affect the ruminal volatile fatty acid profile in steers.

Of the major components of the plant cell wall, cellulose is shielded by lignin and hemicellulose [6,7]. In addition, lignin covalently binds to hemicelluloses and gradually develops the strength and rigidity of cell walls [8]. Ruminal anaerobic digestion (fermentation) of holocelluloses is dependent on the cellulase activity caused by bacteria and fungi [6]. Hence, cross-linkages of lignin with holocelluloses or phenolic acids possibly reduce the cellulolytic activity of rumen microorganisms [9]. Wood by-product will also make it difficult to contribute to volatile fatty acid production in ruminants as mentioned above.

In recent studies, white-rot fungi inducing lignin degradation has been used to increase the digestibility of lignocellulose by ruminants [10-12]. It is possible, however, that ruminal digestion of woody lignocellulose is accelerated by exposing woody celluloses. Takahashi *et al.*. [13] developed the tandem-ring mill, a pulverising device capable of producing micronised wood powder. This device can pulverise wood chips of Japanese cedar to a maximum particle diameter of 28 μm. In addition, the physical form of micronised wood powder achieves an enzymatic saccharification rate of lignocelluloses that is higher than 80% [13]. Thus, the present study hypothesised that ruminal microorganisms can digest the lignocelluloses in micronised wood powder for volatile fatty acid production.

Therefore, the aim of the present study was to determine whether micronised wood powder directly contributes to volatile fatty acid production due to *in vitro* and *in vivo* rumen fermentation of beef cattle.

Correspondence to: Dr. Yoshio Hamano, Field Education and Research Center, Faculty of Bioresource Sciences, Akita Prefectural University, Ohgata, Akita 010-045, Japan, E-mail: yhamano@akita-pu.ac.jp

Key words: *beef cattle, micronised wood powder, biomass, lignocelluloses, ruminal fermentation, volatile fatty acids*

Received: March 15, 2017; **Accepted:** April 07, 2017; **Published:** April 10, 2017

Materials and methods

Animals and diets

The present study was approved by the Animal Care and Use Committee at Akita Prefectural University. Experiments consisted of two *in vitro* experiments and an *in vivo* examination. Four beef cattle (Japanese Shorthorn, 3 heifers and 1 steer; average body weight 467 ± 39 kg) were used in *in vitro* experiments (Experiments 1 and 2) and a crossover design for an *in vivo* experiment (Experiment 3). Animals were individually housed in a floor pen and were adapted by feeding a basal diet consisting of orchard grass hay (56.4% dry matter) and mixed feed (dry matter basis: 17.5% powdered alfalfa hay; 7.6% powdered soybean; 18.0% maize flakes; 0.5% powdered soy-sauce cake) for 3 weeks prior to experiments. In this study, soy-sauce cake, which has a pleasant odor to cattle, was used to mask the distinctive woody odor because micronised wood powder for cattle was observed to have low acceptability to cattle in a preliminary experiment. The basal diet equivalent to 0.9% of body weight was given twice daily at 0900 and 1600. Animals had free access to fresh water and a vitamin-mineral block. Micronised wood powder was mechanically prepared according to the method of Takahashi *et al.* [13] by pulverizing woody chips of sapwood of Japanese cedar (*Cryptomeria japonica* D. Don). Concentrations of cellulose, hemicellulose, and lignin in micronised wood powder (air-dried basis) were 41.2%, 16.1%, and 30.0%, respectively.

In vitro experiments

In these experiments, the digestibility of micronised wood powder was evaluated by volatile fatty acid production in batch fermentation with ruminal fluid. About 500 ml of ruminal fluid from four donor animals was individually obtained 5 h post-morning feeding using a stainless steel oral catheter for cattle (Sanshin Industrial Co. Ltd., Kanagawa, Japan) and a vacuum pump. Prior to sampling, the inside of the inner tube and a sampling bottle were filled with O_2-free CO_2 gas. After sampling, the bottle was immediately and anaerobically warmed in a portable incubator (39 ± 1°C). Ruminal fluid was transferred to the laboratory and then filtered through four layers of surgical gauze into a polypropylene beaker under flushing with O_2-free CO_2 gas. The ruminal fluid was mixed with a buffer (pH 6.8) as artificial saliva (292 mg K_2HPO_4, 240 mg KH_2PO_4, 480 mg $(NH_4)_2SO_4$, 480 mg NaCl, 100mg $MgSO_4 \cdot 7H_2O$, 64 mg $CaCl_2 \cdot 2H_2O$, 4,000 mg Na_2CO_3, and 600 mg cysteine hydrochloride) in accordance with the method of Lila *et al.* [14]. The ruminal fluid (10 ml) and buffer (20 ml) were immediately mixed in a 50 ml polypropylene test tube containing a substrate of micronised wood powder. The tubes were warmed in metal beads (Lab Armor Beads, Lab Armor LLC, OR, USA) set at 39°C beforehand. In Experiment 1, four supplementation levels of micronised wood powder substrate were prepared at 0 (control), 50, 100, and 200 mg per tube (n = 4). Furthermore, in Experiment 2, ruminal fermentation of micronised wood powder was evaluated at the levels of 0, 200, and 400 mg per tube (n = 4). As a control, the mixed ruminal fluid was incubated without micronised wood powder. Duplicate samples were screw-capped and further sealed with airtight tape after CO_2 flushing. The mixed ruminal fluid was anaerobically incubated at 39°C for 24 h using an air-circulating incubator. The samples were shaken during incubation using a twist-rotating shaker (about 40 rpm). After incubation, the pH was immediately measured, and a portion of the ruminal fluid was used for volatile fatty acid assay.

In vivo experiment

Four cattle fed the basal diet were used in a crossover design. In this experiment, small pellets of micronised wood powder were prepared using a pelletizer. The micronised wood powder pellets were crudely crushed using a grinder prior to feeding. Animals were fed micronised wood powder mixed with the basal diet twice daily. Control animals were fed the basal diet alone. In the initial 2 days period, micronised wood powder was given at a level of 0.1% of initial body weight. On Day 3, micronised wood powder supplementation was increased by 0.2% of initial body weight. Animals were then fed micronised wood powder at a maximal level of 0.3% (air-dried basis) of initial body weight per day. Ruminal fluid was withdrawn at 1400 on Days 2, 4, and 7 using an oral catheter. An initial sample (Day 0) was obtained on the day before micronised wood powder feeding. Micronised wood powder at the level of 0.3% of body weight per day was given continuously for 3 weeks, and body weight was measured again. Ruminal fluid for analysis of volatile fatty acids, NH_3-N, and pH was obtained at 1400 on Day 22. Thereafter, animals were fed the basal diet alone during an adaptation period for 3 weeks, and the same feeding trial was performed for 4 weeks in a crossover design.

Chemical analysis of rumen fluid

The ruminal fluid pH was measured with a pH meter (model 827 pH labs, Metrohm AG, Herisau, Switzerland). For volatile fatty acid determination, ruminal fluid was centrifuged for 10 min at 4°C (9,000 *g*), and 1.0 ml was mixed with 200 µl of a 25% metaphosphoric acid-formic acid mixture (3:1) [15]. In addition, 100 µl of an internal standard (20 µmol pivalic acid; Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) was added to the rumen fluid samples. The mixture was incubated for 30 min at 4°C and centrifuged (9,000 *g*) for 10 min at 4°C. The clear supernatant was transferred to a vial for gas chromatography. Volatile fatty acid values were determined using a gas chromatography system (model GC-2014, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector, an auto-injector (AOC-20i, Shimadzu), and a fused silica capillary column (Nukol, 30 m x 0.25 mm x 0.25 µm, Supelco, PA, USA). Helium gas was used as the carrier gas at a flow rate of 1.15 ml/min. The temperature of the injector and detector was set at 250°C. The temperature in the column oven was initially set at 90°C, then linearly increased to 185°C at a rate of 10°C/min, and held for 2.5 min. The sample volume injected was 1 µl, and the split ratio was 70:1. Short-chain fatty acids were identified and quantified by their retention times and peak area with standards (Tokyo Chemical Industry Co., Ltd.) using a computer program for data analysis (GC solution, Shimadzu). The concentration of NH_3-N in the clear sample of centrifuged ruminal fluid was determined by the catalysed indophenol colorimetric reaction [16,17].

Statistical analysis

Statistical analysis for analysis of variance (ANOVA) was performed using a computer program (StatView, Abacus Concepts Inc., Berkeley, CA). In Experiments 1 and 2, data on pH values and volatile fatty acid concentrations were analysed by one-way ANOVA. For the *in vivo* experiment (Experiment 3), alterations of pH value, volatile fatty acid concentrations, and NH_3-N concentration in response to gradually increased micronised wood powder feeding were statistically determined with two-way ANOVA as a 2 x 4 factorial arrangement (micronised wood powder treatments x sampling days). In Experiments 1 and 2, when a significant effect of micronised wood powder on the parameters was detected by ANOVA, Tukey's honestly significant difference test was used to determine significant responses

to micronised wood powder supplementation (KaleidaGraph, Synergy Software, PA, USA). In Experiment 3, final concentrations of volatile fatty acids and NH_3-N in *in vivo* ruminal fluid after micronised wood powder feeding for 4 weeks were compared with controls using Student's *t*-test (KaleidaGraph, Synergy Software).

Results

In vitro experiments

In Experiment 1, the responses of *in vitro* rumen fermentation to micronised wood powder supplementation at levels ranged from 50 to 200 mg, as shown in Table 1. Overall, supplemented levels of micronised wood powder reduced (P<0.05) pH values in incubated rumen fluid compared with the control. There was no significant difference among the levels of micronised wood powder in the extent of decreased pH. In volatile fatty acid production, concentrations of total volatile fatty acids increased (P<0.05) in micronised wood powder-supplemented groups at levels of 100 and 200 mg compared with the control. Concentrations of major volatile fatty acids of acetate and propionate were higher (P<0.05) in micronised wood powder-supplemented groups at the levels of 100 and 200 mg than in the control. Concentrations of butyrate increased (P<0.05) with micronised wood powder, regardless of its supplementation levels. The molar ratio of acetate to propionate was not altered at this micronised wood powder level, while micronised wood powder at 50 mg slightly increased (P<0.05) the molar ratio. Concentrations of other minor volatile fatty acids also increased (P<0.05) in the micronised wood powder-treated groups, except for capronate, but there was no alteration in the response to micronised wood powder-supplementation levels. Thus, these results indicate that micronised wood powder contributed to volatile fatty acid production in *in vitro* ruminal fermentation. However, the extent of the fermentation of micronised wood powder was not dependent on its supplementation level.

In Experiment 2, the results of *in vitro* ruminal fermentation of micronised wood powder at levels of 200 and 400 mg are shown in Table 2. In this experiment, a significant reduction in pH value due to micronised wood powder supplementation was also observed (P<0.05). Total volatile fatty acid concentrations were higher (P<0.05) in both micronised wood powder-supplemented groups than in the control, but the magnitude of the difference between the micronised wood powder levels was not significant. Similar to Experiment 1, concentrations of acetate, propionate, and butyrate increased (P<0.05) in both micronised wood powder-supplemented groups, compared with controls. However, there was no significant difference between the micronised wood powder groups. The molar ratio of acetate to propionate was not affected by micronised wood powder. In this experiment, micronised wood powder had no effect on concentrations of minor volatile fatty acids, while a slight increase in the isobutyrate concentration was observed in the micronised wood powder-supplemented group at 200 mg (P<0.05).

Table 1. Effects of micronised wood powder supplementation on *in vitro* ruminal fermentation for 24 hours (Experiment 1).

Treatment	Micronised wood powder (mg)					ANOVA (*P*-value)
	0	50	100	200	SEM	
pH	6.82^{a}	6.15^{b}	6.12^{b}	6.09^{b}	0.084	< 0.001
	mmol/l					
Total volatile fatty acids	38.3^{b}	51.6^{ab}	52.6^{a}	52.2^{a}	2.07	0.0178
Acetate	27.0^{b}	36.3^{ab}	36.7^{a}	36.5^{a}	1.47	0.0281
Propionate	6.15^{b}	7.27^{ab}	7.63^{a}	7.66^{a}	0.218	0.0254
Butyrate	4.13^{b}	6.62^{a}	6.77^{a}	6.67^{a}	0.382	0.0170
Isobutyrate	0.19^{b}	0.27^{a}	0.29^{a}	0.29^{a}	0.012	< 0.001
Valerate	0.38^{b}	0.45^{a}	0.47^{a}	0.46^{a}	0.012	0.0056
Isovalerate	0.29^{b}	0.43^{a}	0.46^{a}	0.43^{a}	0.020	0.0017
Capronate	0.19	0.26	0.30	0.26	0.021	0.3252
Acetate / Propionate ratio	4.38^{b}	4.97^{a}	4.79^{ab}	4.75^{ab}	0.078	0.0362

Values represent means with standard error of means (n=4).
^{a,b}Mean values within a row not sharing a common superscript letter were significantly different, P<0.05.

Table 2. Effects of micronised wood powder supplementation on *in vitro* ruminal fermentation for 24 hours (Experiment 2).

Treatment	Micronised wood powder (mg)				ANOVA (*P*-value)
	0	200	400	SEM	
pH	6.82^{a}	6.25^{b}	6.19^{b}	0.115	< 0.001
	mmol/l				
Total volatile fatty acids	33.6^{b}	43.3^{a}	44.1^{a}	1.63	< 0.001
Acetate	23.8^{b}	30.7^{a}	31.3^{a}	1.13	< 0.001
Propionate	5.37^{b}	6.61^{a}	6.83^{a}	0.23	0.0034
Butyrate	3.47^{b}	4.94^{a}	5.00^{a}	0.239	< 0.001
Isobutyrate	0.21^{b}	0.26^{a}	0.25^{ab}	0.009	0.0202
Valerate	0.32	0.36	0.35	0.014	0.3875
Isovalerate	0.26	0.31	0.28	0.018	0.5524
Capronate	0.08	0.12	0.11	0.010	0.3985
Acetate/Propionate ratio	4.44	4.64	4.58	0.037	0.0614

Values represent means with standard error of means (n=4).
^{a,b}Mean values within a row not sharing a common superscript letter were significantly different, P<0.05.

Table 3. Effects of micronised wood powder (MWP) on *in vivo* ruminal fermentation in beef cattle during initial feeding period (Experiment 3).

		Feeding period (day)					ANOVA (*P*-value)		
		0	2	4	7	Mean	Day	MWP	Interaction
pH	Control	6.60 ± 0.139	6.61 ± 0.098	6.73 ± 0.044	6.91 ± 0.027	6.71 ± 0.102	0.2941	0.8257	0.3306
	MWP	6.77 ± 0.103	6.72 ± 0.128	6.66 ± 0.110	6.76 ± 0.045	6.73 ± 0.094			
Total volatile fatty acids	Control	84.2 ± 3.3	79.4 ± 3.2	73.3 ± 4.7	61.7 ± 3.6	74.6 ± 2.75	0.1638	0.2315	0.0625
(mmol/l)	MWP	75.1 ± 4.2	77.3 ± 8.7	86.3 ± 7.0	77.5 ± 2.9	79.0 ± 2.96			
Volatile fatty acids composition (mol/100mol)									
Acetate (A)	Control	70.5 ± 0.22	71.1 ± 0.28	70.6 ± 0.51	70.9 ± 0.69	70.7 ± 0.21	0.4645	0.0356	0.9475
	MWP	69.4 ± 1.13	70.3 ± 0.42	69.5 ± 0.14	70.3 ± 0.49	69.8 ± 0.31			
Propionate (P)	Control	14.7 ± 0.67	14.2 ± 0.27	13.6 ± 0.22	13.5 ± 0.28	14.0 ± 0.67	0.3331	0.1849	0.5281
	MWP	16.4 ± 1.68	14.1 ± 0.25	14.6 ± 0.25	13.5 ± 0.18	14.6 ± 0.48			
Butyrate	Control	12.8 ± 0.47	12.6 ± 0.12	13.6 ± 0.38	13.2 ± 0.65	13.1 ± 0.22	0.0785	0.2143	0.4009
	MWP	12.4 ± 0.73	13.6 ± 0.59	13.8 ± 0.31	14.3 ± 0.27	13.5 ± 0.29			
Isobutyrate	Control	0.54 ± 0.076	0.59 ± 0.043	0.63 ± 0.026	0.66 ± 0.035	0.60 ± 0.025	0.3009	0.0502	0.5751
	MWP	0.53 ± 0.017	0.55 ± 0.020	0.57 ± 0.024	0.54 ± 0.039	0.54 ± 0.012			
Valeriate	Control	0.59 ± 0.021	0.77 ± 0.070	0.65 ± 0.029	0.83 ± 0.214	0.71 ± 0.056	0.2719	0.3155	0.5293
	MWP	0.54 ± 0.052	0.66 ± 0.074	0.73 ± 0.063	0.65 ± 0.018	0.64 ± 0.030			
Isovaleriate	Control	0.64 ± 0.095	0.58 ± 0.052	0.70 ± 0.046	0.73 ± 0.031	0.66 ± 0.031	0.3330	0.0863	0.5503
	MWP	0.60 ± 0.035	0.59 ± 0.028	0.62 ± 0.023	0.60 ± 0.041	0.60 ± 0.015			
Capronate	Control	0.19 ± 0.017	0.21 ± 0.037	0.20 ± 0.038	0.17 ± 0.038	0.19 ± 0.036	0.8672	0.6909	0.9791
	MWP	0.18 ± 0.015	0.20 ± 0.034	0.18 ± 0.009	0.18 ± 0.039	0.17 ± 0.039			
A / P ratio	Control	4.82 ± 0.222	5.03 ± 0.113	5.19 ± 0.103	5.26 ± 0.110	5.07 ± 0.079	0.0312	0.1084	0.5935
	MWP	4.37 ± 0.470	5.00 ± 0.074	4.76 ± 0.076	5.21 ± 0.096	4.83 ± 0.136			
NH_3-N (mg/l)	Control	92.6 ± 10.38	82.5 ± 6.12	90.4 ± 3.87	99.2 ± 2.57	92.6 ± 3.38	0.2362	0.5049	0.1396
	MWP	83.4 ± 5.85	89.3 ± 13.00	123.4 ± 20.77	94.3 ± 7.24	97.6 ± 7.07			
Values represent means with standard error (n=4).									

Table 4. Effects of micronised wood powder on *in vivo* ruminal fermentation in beef cattle after the constant feeding for 3 weeks (Experiment 3).

	Control	Micronised wood powder	SEM
Total volatile fatty acids (mmol/l)	96.9	99.2	3.80
Volatile fatty acids composition (mol/100 mol)			
Acetate	68.0	67.9	0.30
Propionate	14.3	13.7	0.49
Butyrate	15.4	16.2	0.37
Isobutyrate	0.65	0.63	0.026
Valerate	0.78	0.75	0.028
Isovalerate	0.68	0.65	0.038
Capronate	0.20	0.21	0.012
Acetate / Propionate ratio	4.79	5.00	0.185
NH_3-N (mg/l)	121	121	3.4
Values represent means with standard error of means (n=4).			

In vivo experiment

In Experiment 3, micronised wood powder feeding had no detrimental effect on feed intake and rumen fermentation in beef cattle throughout the *in vivo* experiment. All animals consumed the hay and feed mixed with micronised wood powder completely. Table 3 shows the results of pH value, total volatile fatty acid concentration and composition, and NH_3-N concentration in ruminal fluids taken from beef cattle fed micronised wood powder during the initial period of seven days. The gradual increase of micronised wood powder had no effect on pH values and total volatile fatty acid concentration in ruminal fluids over seven days. A significant difference between control and micronised wood powder feeding was shown only in the proportion of acetate (P=0.0356). Micronised wood powder feeding slightly lowered acetate, while there was no significant change in propionate or the molar ratio of acetate to propionate. In addition, the volatile fatty acid profile of rumen fluid taken from the cattle fed micronised wood powder for a further 21 days is shown in Table 4. Overall, the total concentration of volatile fatty acids was higher relative to that in the first period of seven days but not affected by the micronised wood powder feeding. In addition, the volatile fatty acid composition and NH_3-N concentration remained unchanged in the micronised wood powder-fed group.

Discussion

In the present study, increased volatile fatty acid production due to micronised wood powder supplementation was observed in *in vitro* batch incubation of ruminal fluid, although whether woody lignocelluloses fermented directly in the rumen contribute to volatile fatty acid production was untested. However, the extent of the *in vitro* fermentation of ruminal microorganisms was small, even if the micronised wood powder-supplemented levels rose, as shown in Experiments 1 and 2. A reduction in pH during ruminal fermentation occurs in response to enhanced concentrations of organic acids such as

volatile fatty acids or lactic acid [3]. The present study did not determine the lactic acid concentration in incubated ruminal fluid, but a slight reduction in pH would be attributable to the increased volatile fatty acid concentration. Thus, the *in vitro* digestibility of micronised wood powder was restricted in relation to the ability of rumen microorganisms to degrade lignocellulose degradation. In addition, the responsiveness of volatile fatty acid production to micronised wood powder was not consistent with the high saccharification rate (more than 80%) of micronised wood powder that was shown by an enzymatic assay. This inconsistency indicates that digestion of micronised wood powder, in fact, would be relatively slow during incubation of ruminal fluid. To attain a complete fermentation of the cellulose and hemicellulose in micronised wood powder for volatile fatty acid production, an incubation time longer than 24 hours might be required.

Although plant cell wall digestion is dependent on ruminal microorganisms consisting of bacteria, protozoa, and fungi, cellulolytic activity has been reported to vary with the interrelationship among different species of microorganisms [18]. Lee *et al.* [19] indicated that, in an *in vitro* monoculture derived and separated from rumen microorganisms, the ability of plant cell wall digestion is greater in the fungal fraction than in the bacterial fraction. However, in the present study, the lack of micronised wood powder fermentation in response to its supplemented levels would be associated with structure of lignocellulose rather than the population of rumen microorganisms or rumen flora (microbial ecosystem). Namely, the present study suggested that the lignin present in micronised wood powder avoided the cellulolytic activity of bacteria and fungi that will caused by contact with woody polysaccharides. Hence, the physical structure and shape of micronised wood powder might remain unsuitable for ruminal fermentation of microorganisms, even though a cellulolytic enzyme is substantially able to catalyse the degradation of cellulose, which must be exposed on the particle surface of micronised wood powder.

With regard to the volatile fatty acid profile, micronised wood powder-induced responses of major short-chain fatty acids were accurately sensitive under *in vitro* ruminal fermentation. In addition, the molar ratio of acetate to propionate was constant in the incubated ruminal fluid. In minor short-chain fatty acids, the presence of micronised wood powder during rumen fluid incubation was concomitantly accelerated, but these significant alterations were slight. These results suggested that the fermentation of micronised wood powder did not affect the proportion of predominant short-chain fatty acids with alteration of ruminal flora. However, the efficiency of conversion of glucose derived from woody cellulose and hemicellulose to volatile fatty acid production remains uncertain.

In the *in vivo* experiment, feeding of micronised wood powder did not contribute to stimulation of ruminal fermentation in beef cattle (Experiment 3), although the number of animals used for the trial was small. Among woody materials used for ruminant feed, sawdust has been examined previously. Feeding sawdust that has greater particle size relative to micronised wood powder will enable it to play a role as a roughage substitute responsible for desirable ruminal functions such as preventing abnormality of the gastrointestinal tract [3,5] rather than a role as an energy source producing volatile fatty acids. Anthony and Cunningham [4] reported that feeding hardwood sawdust produced the highest weight gain of steers and lambs during the fattening period. Improved body weight gain has also been observed in heifers ingesting pine sawdust [5]. However, El-Sabban *et al.*. [3] reported that oak sawdust did not contribute to volatile fatty acid production in the rumen fluid of steers *in vivo*. In the present study, the lack of micronised wood powder-dependent volatile fatty acid production would be attributable to the weak and insufficient digestibility of micronised wood powder in the rumen, as shown by batch incubation (Experiments 1 and 2). A previous report by El-Sabban *et al.*. [3] suggested that passage through the gastrointestinal tract would be fast when small particle size oak sawdust was fed to heifers. Micronised wood powder might rapidly pass through the rumen before fermentation of the micronised wood powder by ruminal microorganisms has progressed. Thus, it is desirable that the fermentation kinetics for micronised wood powder for 24 hours be rapidly enhanced to stimulate cellulolytic activity of rumen microorganisms. In addition, the acceptability of micronised wood powder produced from Japanese cedar to cattle should be considered because cattle dislike its distinctive odor in micronised wood powder. Chemical modification of micronised wood powder quality is also needed to extinguish the Japanese cedar odor undesirable to cattle.

In conclusion, the present study found that the lignocellulose of micronised wood powder actually contributed to volatile fatty acid production in *in vitro* ruminal fermentation. The micronised wood powder-induced volatile fatty acid production was attributed to elevated concentrations of the major short-chain fatty acids of acetate, propionate, and butyrate. However, the digestibility of micronised wood powder estimated from increased volatile fatty acid concentrations was relatively low and slow. Hence, in the *in vivo* experiment, micronised wood powder feeding induced no increment in volatile fatty acid production, while there was no detrimental effect of micronised wood powder on the properties of ruminal fermentation. Further modification of micronised wood powder preparation is needed to accelerate volatile fatty acid production from its cellulose and hemicellulose because lignin still present in micronised wood powder might inhibit the activity of rumen microorganisms.

Funding

This work was supported by a grant, Studies on Application and Practical Use of Research Seeds for Business, from Akita Prefectural University.

References

1. Das A, Mondal C, Roy S (2015) Pretreatment methods of ligno-cellulosic biomass: A review. *Journal of Engineering Science and Technology Review* 8: 141-165.

2. Dinius DA, Peterson AD, Long TA, Baumgardt BR (1970) Intake and digestibility by sheep of rations containing various roughage substitutes. *J Anim Sci* 30: 309-312.

3. el-Sabban FF, Long TA, Baumgardt BR (1971) Utilization of oak sawdust as a roughage substitute in beef cattle finishing rations. *J Anim Sci* 32: 749-755. [Crossref]

4. Anthony WB, Cunningham JP (1968) Hardwood sawdust in all concentrate rations for beef cattle. *J Anim Sci* 27: 1159-1160.

5. Cody Jr. RE, Morrill JL, Hibbs CM (1968) Evaluation of health and performance of bovines fed wood fiber as a roughage source or intake regulator. *J Dairy Sci* 51: 952 (Abstract).

6. Adney WS, Rivard CJ, Shiang M, Himmel ME (1991) Anaerobic digestion of lignocellulosic biomass and wastes - Cellulases and related enzymes. *Appl Biochem Biotechnol* 30: 165-183. [Crossref]

7. Boudet AM, Kajita S, Grima-Pettenati J, Goffner D (2003) Lignins and lignocellulosics: a better control of synthesis for new and improved uses. *Trends Plant Sci* 8: 576-581. [Crossref]

8. Vanholme R, Morreel K, Ralph J, Boerjan W (2008) Lignin engineering. *Curr Opin Plant Biol* 11: 278-285. [Crossref]

9. Adesogan AT, Ma ZX, Romero JJ, Arriola KG (2014) Ruminant Nutrition Symposium: Improving cell wall digestion and animal performance with fibrolytic enzymes. *J Anim Sci* 92: 1317-1330. [Crossref]

10. Okano K, Kitagawa M, Sasaki Y, Watanabe T (2005) Conversion of Japanese red cedar

(Cryptomeria japonica) into a feed for ruminants by white-rot basidiomycetes. *Anim Feed Sci Technol* 120: 235-243.

11. Okano K, Ohkoshi N, Nishiyama A, Usagawa T, Kitagawa M (2009) Improving the nutritive value of madake bamboo, Phyllostachys bambusoides, for ruminants by culturing with the white-rot fungus Ceriporiopsis subvermispora. *Anim Feed Sci Technol* 152: 278-285.

12. van Kuijk SJ, Sonnenberg AS, Baars JJ, Hendriks WH, Cone JW (2015) Fungal treated lignocellulosic biomass as ruminant feed ingredient: a review. *Biotechnol Adv* 33: 191-202. [Crossref]

13. Takahashi T, Sato Y, Ito K, Mori H (2014) Effect of agitation speed on enzymatic saccharification of dry-pulverized lignocellulosic biomass. *Renewable Energy* 62: 754-760.

14. Lila ZA, Mohammed N, Yasui T, Kurokawa Y, Kanda S, et al. (2004) Effects of a twin strain of Saccharomyces cerevisiae live cells on mixed ruminal microorganism fermentation in vitro. *J Anim Sci* 82: 1847–1854. [Crossref]

15. Filípek J, Dvorák R (2009) Determination of the volatile fatty acid content in the rumen liquid: comparison of gas chromatography and capillary isotachophoresis. *Acta Veterinaria Brno* 78: 627-633.

16. Chaney AL, Marbach EP (1962) Modified reagents for determination of urea and ammonia. *Clin Chem* 8: 130-132. [Crossref]

17. Souza NKP, Detmann E, Valadares Filho SC, Costa VaC, Pina DS, et al. (2013) Accuracy of the estimates of ammonia concentration in rumen fluid using different analytical methods. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 65: 1752-1758.

18. Morgavi DP, Sakurada M, Mizokami M, Tomita Y, Onodera R (1994) Effects of ruminal protozoa on cellulose degradation and the growth of an anaerobic ruminal fungus, Piromyces sp. strain OTS1, *in vitro*. *Appl Environ Microbiol* 60: 3718-3723. [Crossref]

19. Lee SS, Ha JK, Cheng K-J (2000) Relative contributions of bacteria, protozoa, and fungi to in vitro degradation of orchard grass cell walls and their interactions. *Appl Environ Microbiol* 66: 3807–3813. [Crossref]