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

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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 cellulosytic activity of ruminal 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 cellulosates. 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.
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 experiment. Four beef cattle (Japanese Shorthorn, 3 heifers and 1 steer; average body weight 467 ± 39 kg) were used in the 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 O₂-free CO₂ gas. After sampling, the bottle was immediately and anaerobically warmed in a portable incubator (39 ± 1°C). Ruminal fluid was anaerobically incubated at 39°C for 24 h using an air-circulating incubator. The samples were shaken during incubation at a rate of 1.15 ml/min. The temperature of the injector and detector was set at 250°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, Tokyo, 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 NH₃-N in the clear sample of centrifuged ruminal fluid was determined by the catalysed indophenol colorimetric reaction [16,17].

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, NH₃-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 at 10 min for 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 umol 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-20, 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 NH₃-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 NH₃-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 NH₃-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 caproate, 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<sup>a</sup>            | 6.15<sup>b</sup>| 6.12<sup>b</sup>| 6.09<sup>b</sup>| 0.084| < 0.001           |
|           | mmol/l                       |                 |       |       |      |                   |
| Total volatile fatty acids | 38.3<sup>a</sup> | 51.6<sup>a</sup>| 52.6<sup>a</sup>| 52.2<sup>a</sup>| 2.07 | 0.0178            |
| Acetate   | 27.0<sup>a</sup>            | 36.3<sup>a</sup>| 36.7<sup>a</sup>| 36.5<sup>a</sup>| 1.47 | 0.0281            |
| Propionate| 6.15<sup>a</sup>            | 7.27<sup>a</sup>| 7.63<sup>a</sup>| 7.66<sup>a</sup>| 0.218| 0.0254            |
| Butyrate  | 4.13<sup>a</sup>            | 6.62<sup>a</sup>| 6.77<sup>a</sup>| 6.67<sup>a</sup>| 0.382| 0.0170            |
| Isobutyrate| 0.19<sup>a</sup>           | 0.27<sup>a</sup>| 0.29<sup>a</sup>| 0.29<sup>a</sup>| 0.012| < 0.001           |
| Valerate  | 0.38<sup>a</sup>            | 0.45<sup>a</sup>| 0.47<sup>a</sup>| 0.46<sup>a</sup>| 0.012| 0.0056            |
| Isovalerate| 0.29<sup>a</sup>          | 0.43<sup>a</sup>| 0.46<sup>a</sup>| 0.43<sup>a</sup>| 0.020| 0.0017            |
| Caproate  | 0.19                        | 0.26            | 0.30  | 0.26  | 0.021| 0.3252            |
| Acetate/Propionate ratio | 4.38<sup>a</sup> | 4.97<sup>a</sup>| 4.79<sup>a</sup>| 4.75<sup>a</sup>| 0.078| 0.0362            |

Values represent means with standard error of means (n=4).

*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<sup>a</sup>            | 6.25<sup>a</sup>| 6.19<sup>a</sup>| 0.115| < 0.001           |
|           | mmol/l                       |                 |       |      |                   |
| Total volatile fatty acids | 33.6<sup>a</sup> | 43.3<sup>a</sup>| 44.1<sup>a</sup>| 1.63 | < 0.001           |
| Acetate   | 23.3<sup>a</sup>            | 30.7<sup>a</sup>| 31.3<sup>a</sup>| 1.13 | < 0.001           |
| Propionate| 5.37<sup>a</sup>            | 6.61<sup>a</sup>| 6.83<sup>a</sup>| 0.23 | 0.0034            |
| Butyrate  | 3.47<sup>a</sup>            | 4.94<sup>a</sup>| 5.06<sup>a</sup>| 0.239| < 0.001           |
| Isobutyrate| 0.21<sup>a</sup>          | 0.26<sup>a</sup>| 0.25<sup>a</sup>| 0.009| 0.0202            |
| Valerate  | 0.32                        | 0.36            | 0.35  | 0.014| 0.3875            |
| Isovalerate| 0.26<sup>a</sup>          | 0.31            | 0.28  | 0.018| 0.5524            |
| Caproate  | 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).

*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 (mmol/l) | 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 |
|                      | 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      | 69.5 ± 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 | 14.0 ± 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 |        |        |        |
|                      | Valerate | 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 |        |        |        |
|                      | Isovalerate | 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 |        |        |        |
|                      | NH₃-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 |
|                      | NH₃-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 NH₃-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 NH₃-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 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 be 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 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 as 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 rumen 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.

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