Short communication

Chemical composition, *in vitro* dry matter digestibility, and triterpenoid contents in Reishi (*Ganoderma lingzhi*) mushroom beds

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Abstract This study considered the chemical composition, *in vitro* dry matter digestibility (IVDMD), and triterpenoid content in Reishi (*Ganoderma lingzhi*) mushroom beds (RMB). Crude protein, cellulose, and hemicellulose contents of RMB were 5.10, 44.97, and 3.76%, respectively. The IVDMD of RMB was 44.2%, which was a similar level to that of roughage such as rice straw. In addition, with antioxidant and anti-inflammatory activities, triterpenoids (ganoderic acid A, C2, C6, H, ganoderenic acid C, and D) remained in RMB. These results indicate that RMB can provide some nutrition and medicinal properties, and there is a possibility that it may be used as a feed resource.

Key words: Chemical composition, Reishi (*Ganoderma lingzhi*) mushroom bed, *in vitro* dry matter digestibility, Triterpenoids

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Introduction

Soaring feed prices gives rise to difficulties in the livestock environment. It can be expected that the utilization of unused resources which have been discarded until now can contribute to sustainable recycling-oriented livestock farming.

Reishi (*Ganoderma lingzhi*) is a traditional medicinal mushroom, and its extract contains many bioactive compounds. Triterpenoids are the primary bioactive components that contribute to medicinal properties of Reish mushroom. Reishi has been reported to have several functional benefits for humans, including hepatoprotective activity [5], antioxidant properties [8], antibacterial activity [9], and antivirus activity [20]. This has led to an increase in demand in Asia, due to increasing health consciousness and production is increasing [14]. The fruiting bodies are expensive, however, Reishi mushroom beds (RMB) are readily available as they are just discarded. Previously, we demonstrated that there was no problem with acceptability of RMB and total rumination time, when goats fed total mixed ration (RMB: concentrate=50:50) [11]. From the viewpoint of effective utilization of resources,

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it is necessary to examine the components of RMB. Furthermore, clarifying how many medicinal ingredients remain in the RMB, can contribute to the use of feed. This paper aims to reveal the feed components, *in vitro* digestibility, and triterpenoid content in RMB.

Materials and Methods

1. Samples

RMB (*Ganoderma lingzhi* strain BMC9049) was purchased from Bisouken Co. Ltd. (Fukuoka, Japan). The media was composed of beech sawdust: rice bran (4:1, v/v) in heat-sealed cultivation bags (3.6 L) with microfilter windows, resulting in final moisture of 55% in the mushroom bed. The environmental condition of the cultivation site was maintained at the temperature 23-25°C, humidity around 80%. The mushroom bed was cultivated until mature stage, spore not dispersed (30-35 age in weeks). After the fruit bodies were harvested, seven RMB were sufficiently air-dried for 90 days in the laboratory and crushed to less than 1 mm by a Willey-type mill for component analysis, *in vitro* dry matter digestion (IVDMD), and triterpenoid determination.

2. Chemical Composition

The proximate components of RMB were analyzed as described by AOAC [1]. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) in RMB were measured according to previous report [15]. NDF, ADF, and ADL values exclude residual ash. The difference between NDF and ADF, the difference between ADF and ADL, and ADL are reported as hemicellulose, cellulose, and lignin, respectively.

IVDMD was determined using the procedure reported by previous report [12] with minor modification. Two rumen fistulated ram fed with alfalfa hay *ad libitum* were used as rumen fluid donors. Rumen fluid obtained via rumen probe was used after filtered through two-layer gauze. An RMB sample (0.5 g) was placed in a centrifuge tube, and then 10 mL of filtered rumen fluid and 40 mL of McDougall's artificial saliva [7] were added. Tubes were incubated in a water bath at 39°C for 48 h under an anaerobic condition. During incubation, the tube was manually shaken at 0, 3, 6, 9, 24, 27, and 30 h after the start of the culture. The contents after incubation with the rumen fluid were filtered to nylon paper, and the residue was incubated in a 0.2% pepsin solution at 39°C for 48 h under an anaerobic condition. After incubation, IVDMD was measured.

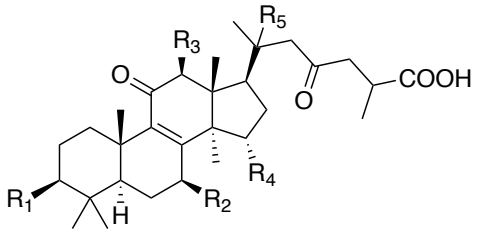
The study was conducted at The University of Shiga Prefecture. The procedures used in the experiments were approved by the Animal Care and Use Committee of the university (#2020-8).

3. Extraction

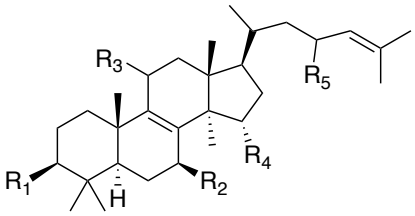
The samples (5 g) of RMB were extracted at room temperature for 24 h with 100 mL of methanol on a magnetic stirrer. After filtering (No.5A; ADVANTEC TOYO KAISHA, Ltd., Tokyo, Japan), the methanol extracts were rotary evaporated to dryness. Each extraction was conducted in triplicate.

4. Quantitative analysis of triterpenoids in RMB

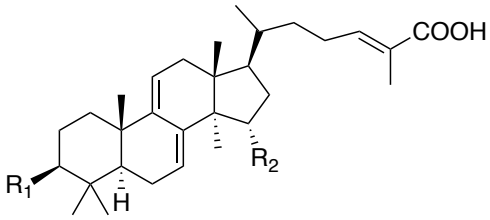
A total of 16 triterpenoids were quantified from methanol extracts of RMB. The 16 triterpenoids were as follows: Ganoderic acid A (**1a**), B (**1b**), C1 (**1c**), C2 (**1d**), C6 (**1e**), K (**1f**),



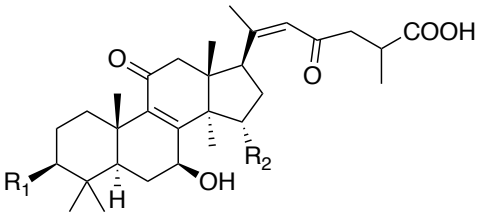
Ganoderic acid A (**1a**) R_1=O, R_2=R_4=OH, R_3=R_5=H
B (**1b**) R_1=R_2=OH, R_3=R_5=H, R_4=O
C1 (**1c**) R_1=R_4=O, R_2=OH, R_3=R_5=H
C2 (**1d**) R_1=R_2=R_4=OH, R_3=R_5=H
C6 (**1e**) R_1=R_3=OH, R_2=R_4=O, R_5=H
K (**1f**) R_1=R_2=OH, R_3=OAc, R_4=O, R_5=H
H (**1g**) R_1=OH, R_2=R_4=O, R_3=OAc, R_5=H
I (**1h**) R_1=R_2=R_5=OH, R_3=H, R_4=O



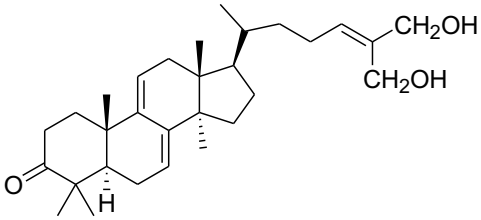
Ganoderic acid LM2 (**2a**) R_1=R_3=R_4=O, R_2=R_5=OH
DM (**2b**) R_1=R_2=O, R_3=R_4=R_5=H
Lucidal (**2c**) R_1=OH, R_2=O, R_3=R_4=R_5=H



Ganoderic acid TR (**3a**) R_1=O, R_2=OH
TN (**3b**) R_1=OH, R_2=OAc



Ganoderenic acid C (**4a**) R_1=R_2=OH
D (**4b**) R_1=R_2=O



Ganoderiol F (**5**)

Figure 1 Chemical structure of each triterpenoid quantified in methanol extract from Reishi

H (**1g**), I (**1h**); ganoderic acid LM2 (**2a**), DM (**2b**), lucidal (**2c**); ganoderic acid TR (**3a**), TN (**3b**); ganoderenic acid C (**4a**), D (**4b**); ganoderiol F (**5**) (Figure 1). Methanol extracts of RMB were dissolved in methanol and filtered through a 0.2 μm filter to provide samples for analysis. Triterpenoid content was determined by LC-MS/MS (model LC-MS/MS 8050; Shimadzu, Kyoto, Japan) in Multiple Reaction Monitoring mode [6].

Results and Discussion

1. The proximate components and IVDMD of RMB

The results for the chemical composition and IVDMD of RMB are summarized in Table 1. The DM% of RMB was 92.13%. Compared to forages such as barley and straw which have low CP levels from 6 to 10%, RMB had a similar or slightly lower level (5.16 %DM). From the results of detergent

Table 1 Chemical composition and *in vitro* dry matter digestibility of RMB

	%DM (mean ± SE, n=7)
Dry matter [a]	92.13 ± 0.15
Crude protein	5.16 ± 0.14
Ether extract	0.51 ± 0.04
Crude fiber	38.82 ± 0.42
Crude ash	4.40 ± 0.07
Nitrogen free extract	51.10 ± 0.32
Neutral detergent fiber	64.71 ± 0.74
Acid detergent fiber	60.89 ± 0.28
Acid detergent lignin	15.94 ± 0.41
in vitro dry matter digestibility	44.62 ± 0.41

[a]: based on air dry matter

fiber (NDF, ADF, and ADL) determination, hemicellulose was low level (3.82 %DM) and lignin was not high (15.94 %DM), but cellulose was high content (44.957 %DM). Reishi (*Ganoderma lingzhi*) is known as a white-rot fungus and decompose lignin, which is an indigestible fraction for ruminants, thus it can be expected to improve digestibility [17]. Previously reported that the fiber content of the oyster mushroom waste bed (mainly composed with wheat straw) was decreased, and the dry matter digestibility increased than that before mushroom growth [18]. In this study, the IVDMD of RMB was 44.62%, which was the similar to roughages such as rice straw (around 40%). From the above, RMB may be used as an alternative to low-quality roughage.

2. Triterpenoid contents in RMB methanol extracts and materials

Triterpenoid contents in RMB extract are shown in Figure 2. All triterpenoids were detected from RMB extracts except compounds **2c** and **5**. The compounds **1a** and **1d** were the major triterpenoids in strain BMC9049, containing about 60 mg/g extract of Reishi fruiting body [6]. In this study, the triterpenoids content of methanol extracts was relatively low level compared to the extracts of Reishi fruiting body, because RMB, which are mainly composed of beech sawdust, were used in this study. However, triterpenoids were detected in the waste mushroom beds because part of the fruiting body stalk and unharvested small fruiting bodies remain in the waste mushroom bed.

Previously reported that hippocampal neurons' superoxide dismutase (SOD) activity was significantly increased by compound **1a** [4]. It has also reviewed bioactive constituents about ganoderic acids, and mentioned compounds **1d** and **1e** showed antioxidant activity [16]. In terms of antioxidants, it has been reported that phenolic compounds from grapes contributed to improving total antioxidant capacity, catalase level, glutathione peroxidase level, and SOD activity in sheep [19]. As another example, feeding Holstein bulls with peppermint, cloves, and lemongrass, which are high in antioxidants, has been reported to improve blood antioxidant activity and reduce IgG levels [3]. Furthermore, identified compounds **1a** and **1g** were identified as contributing to the anti-inflammatory activity of Reishi (*Ganoderma lingzhi*) [5]. As the immune system of newborn calves is too weak to fight

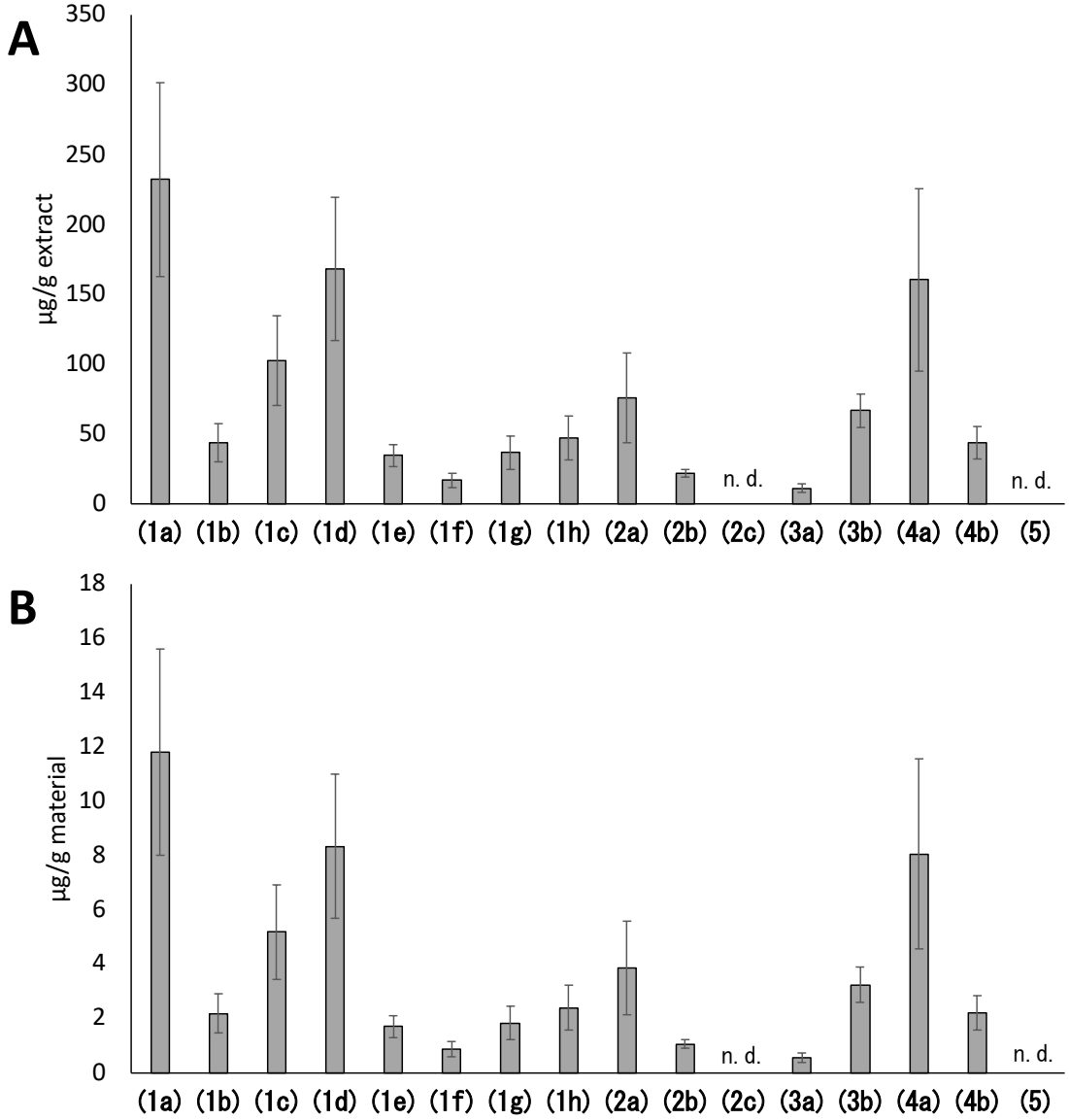


Figure 2 Content of each triterpenoid in methanol extract (μg/g extract) (**A**) and in materials (μg/g material) (**B**). Values are shown as the mean ± SE (n=7). The triterpenoid content (**B**) was calculated by multiplying the value of (**A**) by the extraction efficiency of each waste mushroom bed.

various infections, most diseases (for e.g., flu, diarrhea, and omphalitis) and death events affecting calves occur in the first few days after birth [10,13]. In addition, bovine mastitis is a common inflammatory disease in the udder of dairy cows that causes economic loss to dairy industries. The development of alternative strategies, especially the utilization of natural products has gained a lot of interests [2].

The above suggests that RMB feeding may positively affect the ruminant immune system. Our future research will be focused on relationship between degradation and metabolism of triterpenoids in rumen.

Conflict of interest

Authors declare no Conflict of Interests for this article.

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短　報

霊芝（*Ganoderma lingzhi*）廃菌床の化学成分、インビトロ乾物消化率および トリテルペノイド含量

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本研究では、薬用キノコである霊芝（*Ganoderma lingzhi*）廃菌床の飼料的利用を目的とし、その飼料成分と消化性および機能性成分であるトリテルペノイド類の残存量を調査した。その結果、霊芝廃菌床の粗タンパク質、セルロース、ヘミセルロース、*in vitro* 乾物消化率はそれぞれ、5.10、44.97、3.76、44.2%でありイナワラ等と同程度の値であった。また、霊芝の薬効成分であるトリテルペノイド類は ganoderic acid A、C2、C6、H、ganoderenic acid C、D が検出された。以上のことから、霊芝の廃菌床は一部代替粗飼料として利用できる可能性が示された。

キーワード: 化学成分、霊芝（*Ganoderma lingzhi*）廃菌床、インビトロ乾物消化率、トリテルペノイド

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