



**BIOACTIVE MYCO-NUTRIENTS OF ASEPTICALLY CULTURED FRUITING
BODIES OF *Coprinus comatus* (O.F. MÜLL.) PERS. ON RICE BRAN-ENRICHED
RUMINANTS' DUNG**

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ABSTRACT

This work established the growth and fruiting body production, and nutritive composition of *Coprinus comatus* aseptically cultivated on the formulated rice bran enriched dung of the different ruminants. Cow dung recorded the highest organic carbon ($35.40 \pm 0.1\%$) and nitrogen content ($1.20 \pm 0.0\%$). Horse dung significantly showed the shortest period of fruiting body development (8.67 ± 0.6 days) and produced the highest yield of 4.10 ± 0.4 g ($13.67 \pm 0.3\%$ bio-efficiency). Based on the composition analyses, fruiting bodies grown on cow dung recorded the highest amount of protein ($32.30 \pm 0.02\%$) and carbohydrates ($30.25 \pm 0.06\%$) while those harvested from the horse dung had the highest fiber ($11.31 \pm 0.04\%$) and ash ($13.16 \pm 0.01\%$) contents and the lowest amount of fat (2.54 ± 0.02). Out of six mycochemicals screened, four were found present in fruiting bodies grown on the dung substrates including saponins, terpenoids, flavonoids, and alkaloids, which varied quantitatively from traceable to appreciable amounts. Fruit bodies on horse dung had the most number of mycochemicals detected in appreciable amount, followed by cow dung. Therefore, *C. comatus* is considered

coprophilous mushroom in aseptic condition which the growth and production performance and its chemical composition are substrate dependent and are thereby indicating its excellent applications in food and drug industries.

Keywords: *Coprinus comatus*, Mycochemicals, Coprophilous, Ruminants' Dung

INTRODUCTION

Central Luzon, Philippines, particularly in Nueva Ecija, is considered as the rice granary of the Philippines due to its fine climatic conditions and soil fertility. Farmers are producing rice, fruits, and vegetables and raising ruminants (e.g. cow, carabao, goat, and horse) as major sources of income. These ruminants are raised in the pasture lands and their dung drops elsewhere which supports a wide variety of dung-loving inhabitants called coprophilous fungi. This group plays an important role in biodegradation of organic materials especially dung of herbivores and remains to be the heed of many mycologists because of their fungal succession in the natural habitat. One of the most common species of coprophilous mushrooms that is being recognized by the local farmers is the *Panaeolus antillarum*. This mushroom has been successfully domesticated and propagated using formulated dung of the ruminants in an aseptic cultivation condition [1].

Previously, some species of the genus *Coprinus* such as *C. doverii*, *C. radiatus*, *C. miser*, and *C. stercorarius* were categorized as coprophilous fungi since they are usually

found growing on the horse dung and other several types of dung [2, 3]. Unlike these Agaricales, *Coprinus comatus* is naturally growing on decomposing piles of rice straw and even on the bed culture of *Volvariella volvacea*, acting as a weed fungus. This mushroom is typically collected by the farmers in the field every afternoon of the rainy season and then combined to many native Filipino delicacies to enhance umami flavor. Since this wild edible mushroom was introduced for culinary and even for nutraceutical purposes, practical production technologies have been developed using various agro-industrial substrates [4-6].

Mushrooms have been long valued as nutritional and medicinal foods. They are very rich in protein, complex carbohydrates, vitamins, and minerals. However, these properties may vary depending on several factors such as strain types, substrates used and other environmental conditions (temperature, humidity, and growing period). Therefore, the effect of the different dung of the ruminants as basal media on the production performance and nutritional and mycochemical compositions of *C. comatus*

in an aseptic controlled condition was carried out in this present study.

MATERIALS AND METHODS

Culture source and inocula

Wild fruiting bodies of *C. comatus* were collected from the rice field of Central Luzon State University, Science City of Munoz, Nueva Ecija. The collected fruiting bodies were brought in the laboratory to rescue the cell lines following the standard tissue culture protocol for mushroom. Tissues were aseptically inoculated into sterilized potato dextrose agar (PDA) plates and incubated at 30°C for 7 days to allow mycelial growth. The mycelial discs were prepared using flame sterile cork borer (10 mm diameter size) in revived culture which were used as inoculant in the evaluation of fruiting body production.

Evaluation of production performance

The production of fruiting body of *C. comatus* was evaluated on the three types of ruminants dung namely; cow dung (T1), carabao dung (T2), horse dung (T3), and rice straw (T4, control) following the aseptic cultivation protocol of Dulay et al. [5]. Each substrate was enriched with 10% rice bran and maintained to 65% moisture content. The organic carbon and nitrogen content of the four substrates were analyzed. Two hundred grams of each formulated substrate was placed in a glass container. Ten replicates were prepared each substrate.

The bottles were covered with polypropylene sheets, secure with rubber band and sterilized at 121°C, 15 psi for 45 minutes. After cooling, sterilized substrates were inoculated with mycelia discs from the *C. comatus* culture plate. The inoculated bottles were incubated at 30°C to allow full ramification of mycelia. The periods of incubation for complete ramification and fruiting body development were recorded. The emergence of fruiting bodies was allowed and non-matured fruiting bodies were harvested and weighed. The bio-efficiency was also determined. Data were analyzed using analysis of variance (ANOVA) and treatment means were compared using least significant difference (LSD) at 5% level of significance in a SAS statistical program. Fruiting bodies were air-dried for 3-4 days for nutritional and mycochemical analyses.

Nutritional composition analyses

The crude protein, crude fat, crude fiber, ash, and moisture content (MC) of the air-dried fruiting bodies of *C. comatus* harvested from the four substrates were analyzed according to the guidelines of the Association of Official Analytical Chemist [7]. The crude protein was determined by Kjeldahl method, using the conversion factor $N \times 4.38$ while crude fat was analyzed using Soxhlet apparatus. Total carbohydrate content was calculated as

follows: total carbohydrates = 100 - (protein + fiber + fat + ash + MC). Energy value was computed as follows: energy value (kcal/100 g) = 4 × (g of protein + g of carbohydrates) + 9 × (g of fat).

Mycochemical analyses

The chemical screening of the aqueous extracts of fruiting body of *C. comatus* were carried out following the procedure described by Edeoga and Gomina [8]. Triplicate tests were laid out for each parameter. Results were compared with distilled water as control, determined based on the color/intensity of the reaction and interpreted as; (+) if chemical is present in traceable amount, (++) if chemical is present in appreciable amount, and (0) if chemical is absent.

RESULTS AND DISCUSSION

Organic carbon and nitrogen content of substrates

Carbon and nitrogen are essential elemental composition of all living things, including mushrooms. They serve as the basic constituent of different substances that are synthesized by anabolic process within the hyphae of mushrooms. Thus, the carbon and nitrogen content of the substrates used for the evaluation of the growth and production performance of *C. comatus* were considered in this study. **Table 1** presents the organic carbon and nitrogen content of the different types of dung as basal substrates for *C.*

comatus production. Among the different dung types, cow dung recorded the highest organic carbon (35.40±0.1%) and nitrogen content (1.20±0.0%). On the other hand, carabao dung had the lowest organic carbon (31.40±0.0%) and nitrogen content (0.67±0.1%). These results strongly suggest that the different dung hold varying level of nutrients which could possibly affect the growth and production performance of *C. comatus*.

In most of the previous works, the influence of C/N ratio on the yield of mushrooms is always considered. Some technique of enhancing the C/N ratio of the substrate for efficient growth and high yield is by addition of some supplements. For instance, addition of complex nitrogen sources and amino acids supports the efficient growth of *Agaricus macrosporus* wherein ammonium nitrate enhanced more than minimal growth while arginine showed the rapid mycelial growth [9]. In the present study, rice bran was used as the additive to augment the levels of C/N ratio in the different dung as basal media for *C. comatus* production. Apart from the C/N ratio, the different growth response of mushroom could also be attributed to variations in the chemical composition of the substrates [10].

Cultivation phases and production of fruiting body of *C. comatus*

Incubation period is the total number of days from the time of inoculation up to the time of complete ramification of the entire substrate by mycelia. Once successfully ramified, fruit initials (primordia) emerge from the very thick mycelia mass and eventually develop to fruiting bodies. The mean number of days of incubation period, fruit body development, and production performance of *C. comatus* on the different substrates is shown in **Table 2**. Rice straw had the shortest incubation period of 8.00 ± 0.0 days which statistically differed from the dung substrates. Both cow dung and horse dung recorded the most extensive incubation period. However, in terms of the fruit body development, horse dung significantly showed fruiting body development (8.67 ± 0.6 days) even before the complete mycelial ramification of the substrate. In contrast, both cow dung and carabao dung registered the most extensive fruiting body development. These results on incubation period and fruiting body development strongly suggest that the cultivation phases of *C. comatus* are significantly affected by the nutritional compositions of the different dung substrates. Although the incubation period in horse dung was more extensive compared to rice straw, this substrate provided the most suitable nutritional requirements for growth to produce vigorous mycelia needed

for advanced development of the reproductive phase.

Interestingly, in terms of the production performance, significant difference of the substrates was found in the number of fruiting bodies, yield and biological efficiency of *C. comatus* (**Table 2**). The fruiting bodies of *C. comatus* grown on the different dung in an aseptic cultivation is shown in **Figure 1**. The highest number of fruiting bodies was recorded in carabao dung (5.67 ± 0.5) which statistically comparable with horse dung (5.33 ± 0.5). However, horse dung significantly produced the highest yield of 4.10 ± 0.4 g ($13.67 \pm 0.3\%$ BE), followed by carabao dung with 3.30 ± 0.1 g ($11.01 \pm 0.2\%$ BE). Aside from being nutritious, this favourable fruiting body production in horse dung could also be explained by the buffering capacity of this substrate and the absence of the growth inhibitory attributes. Although these properties could also exhibited by the other dung substrates evaluated, the levels of nutrients and pH vary as these are all from natural sources.

On the contrary, the cow dung which contained the highest organic carbon and nitrogen among the different dung produced the lowest number of fruiting body, yield and biological efficiency. This poor performance could possibly be explained by the presence of volatile organic compounds

(VOCs) in the cow dung that might impinge on the development of fruiting bodies of *C. comatus*. Park *et al.* [11] enumerated some toxic VOCs present in cow dung in high concentration, including acetone, methyl ethyl ketone, benzene, and toluene. Moreover, although C/N ratio is important, it is not always correlated with growth and yield of mushrooms [12]. In general, the ability of *C. comatus* to grow on the dung of the different ruminants in an aseptic condition could strongly be considered as a character of being coprophilous basidiomycetes.

Proximate nutritional composition

The proximate nutritional composition and the calculated energy value of the air-dried fruiting bodies of *C. comatus* grown on the different dung substrates is shown in **Table 3**. Proteins and carbohydrates are two major nutrient compositions of mushrooms. Fruiting bodies grown on cow dung recorded the highest amount of protein ($32.30 \pm 0.02\%$) and carbohydrates ($30.25 \pm 0.06\%$). In contrast, the lowest protein content was found in those grown on carabao dung while the lowest amount of carbohydrates was noted in those grown on horse dung. However, the protein contents obtained are within the range of 25-40% when dry [13], which suggest that *C. comatus* can be used as substitute for meat, milk, egg, and fish as rich source of protein,

thereby making its importance in tissue regeneration and cellular activities. The carbohydrate contents appeared relatively lower when compared to the carbohydrate content (68.31%) of wild *C. comatus* [14] and to the range of 43.0-50.1% when grown on the spent of different *Pleurotus* species [6]. Most of these carbohydrates present in mushrooms include polysaccharides (glucans), mono- and disaccharides, sugar alcohol, glycogen and chitin [15] which greatly contributes to the several functionalities of mushrooms.

Crude fiber and fat content are important components to be regarded that the sample is a nutritious food. Fruiting bodies harvested from the horse dung had the highest fiber content ($11.31 \pm 0.04\%$) and the lowest amount of fat (2.54 ± 0.02). High fiber and low fat contents imply an ideal food source for the reduced risk of atherosclerosis and hypertension [16, 17] and exhibition of hypocholesterolemic and anti-tumorigenic effects [18]. Similarly, the highest amount of ash was also found in fruit bodies grown on the horse dung ($13.16 \pm 0.01\%$), which indicates that this sample contains high nutritionally important minerals. The main constituents of ash in mushrooms are potassium and phosphorous, approximately about 60% [19]. Some other minerals include magnesium, sodium, calcium, iron, copper, and manganese,

which are required in metabolic processes, stimulation of action potential across nerve endings, enhance heart contractile rate, muscular activity and skeletal muscle development, and maintenance of fluid balance [20, 21]. On the other hand, the moisture contents and the calculated energy values of the fruiting bodies from the different dung were found varied ranging from 12.11 ± 0.01 to 13.41 ± 0.01 (for moisture) and from 264.86 ± 0.30 to 273.31 ± 0.27 (for energy value).

Generally, these significant results suggest that the nutrient composition of *C. comatus* may vary depending on the type of the substrate. The same is true with the previous report of Sarker et al. [22] who observed a significant variation in the nutrients of oyster mushroom grown on the different substrates. Moreover, the nutrient composition of *P. pulmonarius* is dependent on maturity state of fruiting body and the type of substrate on which they were grown [23] while the yield and nutritional content of the *P. ostreatus* on sawdust depends on the chemical constituents such as the cellulose, hemicellulose, lignin, and nitrogen content of the substrate used [24]. Other important factors that causes nutrient variation include state of maturity of fruiting bodies, type of strain, quality of the substrate, environmental conditions (e.g. humidity and temperature), and storage

conditions. However, regardless of the substrate types and some other factors, this present study demonstrated that *C. comatus* holds excellent sources of nutrients such as protein, carbohydrates, fibre, and minerals, therefore can be ranked as nutritive food source which indeed has a great potential applications in food industries.

Mycochemical component

Alike with the nutrient composition, the mycochemical attributes of *C. comatus* were also influenced by the different dung types (Table 3). These mycochemicals in the samples were varied quantitatively from traceable to appreciable amount. Among the dung substrate grown samples, fruiting bodies from horse dung had the most number of mycochemicals detected in appreciable amount, followed by cow dung. Out of six mycochemicals screened, four (saponins, terpenoids, flavonoids, and alkaloids) were found present in fruiting bodies grown on the dung substrates while only three were detected in those harvested on rice straw. These results are in correlation with the screening analysis done on the aqueous and methanol extracts of wild fruit bodies of *C. comatus* and other basidiomycetes viz. *P. ostreatus*, *V. volvacea* and *Agaricus bisporus* collected from the forest of India [25]. Moreover, *Auricularia judae*, *Pleurotus squarrosulus*, and *Russula* species contained appreciable

amounts of alkaloids, phenols, saponins, and flavonoids [26]. In the present work, both tannins and cardiac glycosides were not detected in all the samples. However, these two mycochemicals were found present in the methanol extract of wild *C. comatus* [25].

Saponins are reported to demonstrate a wide range of bioactivities such as immune stimulating, cancer risk reduction, blood sugar lowering, dental caries and platelet aggregation inhibition, cholesterol lowering, antidote against acute lead poisoning and can be used in the treatment of hypercalciuria [27]. Moreover, the four furostanol saponins isolated from the red onion (*Allium cepa*) bulbs were found to possess anti-spasmodic activity in guinea pig, which support the anecdotal report of using onion in the treatment of gastrointestinal tract disturbances [28]. Another effective chemical ingredient that demonstrates biological activities and is used in the treatment of various human diseases is the group of terpenoids. Kuttan et al. [29] reported that triterpenoids, diterpene and monoterpenoids have shown immunomodulatory and anti-tumor activities via induction of apoptosis in various cancer cells and inhibition of metastatic progression and tumor-induced angiogenesis.

Flavonoids are also important chemical nutra-components with healthful benefits. They have been reported to exhibit antiviral, anti-tumor, antibacterial, anti-inflammatory and anti-allergenic activities [30]. In addition, intake of flavonoid is reported to be associated in the reduction of risk of ischemic stroke and this epidemiological association may potentially be cardio-protective [31]. Also, intake of some flavonoids may reduce Parkinson disease risk, particularly in men [32]. On the other hand, alkaloids have been reported for its powerful effects in human physiology and its presence is an indication of pharmacological importance which is of interest in drug industries. Some of these effective physiological bioactivities of alkaloids include anti-inflammatory and anti-diabetic effects [33, 34]. The tryptophan-based indole alkaloids, psilocybin and psilocin from the genus *Psilocybe*, have been identified as most attractive bioactive alkaloids in large number of mushrooms [35]. Considering these very important attributes together, *C. comatus*, preferably grown on horse dung, could demonstrate enormous functional activities to play a vital role in promoting human health.

Substrate	Organic Carbon (%)	Nitrogen (%)
Carabao dung	31.40±0.0	0.67±0.1
Cow dung	35.40±0.1	1.20±0.0
Horse dung	35.00±0.1	1.07±0.1
Rice straw	33.40±0.2	1.30±0.1

Values are the Mean ± SD

Substrate	Incubation period (d)	Fruit body development (d)	Number of fruit body	Yield (g)	Biological efficiency (%)
Carabao dung	10.67±0.6 ^a	15.00±0.0 ^a	5.67±0.5 ^a	3.30±0.1 ^b	11.01±0.2 ^b
Cow dung	11.00±0.0 ^a	15.67±1.2 ^a	2.33±0.5 ^b	2.69±0.3 ^c	8.98±0.2 ^c
Horse dung	11.00±0.0 ^a	8.67±0.6 ^c	5.33±0.5 ^a	4.10±0.4 ^a	13.67±0.3 ^a
Rice straw	8.00±0.0 ^b	11.00±0.0 ^b	3.00±1.0 ^b	1.46±0.1 ^d	4.86±0.1 ^d

Values are the Mean ± SD. Means having the same letter of superscript in the same column are not significantly different from each other at 5% level of significance using LSD.

Composition	Fruit body of <i>C. comatus</i> grown on			
	Carabao dung	Cow dung	Horse dung	Rice straw
Nutrients:				
Protein (%)	30.89±0.02	32.30±0.02	31.79±0.01	31.22±0.01
Fiber (%)	10.56±0.01	10.15±0.03	11.31±0.04	10.00±0.03
Fat (%)	2.60±0.04	2.57±0.01	2.54±0.02	2.64±0.01
Moisture (%)	13.41±0.01	12.16±0.04	12.11±0.01	13.48±0.04
Ash (%)	13.07±0.01	12.58±0.01	13.16±0.01	12.54±0.02
Carbohydrates (%)	29.48±0.00	30.25±0.06	29.10±0.00	30.14±0.04
Energy value (kcal)	264.86±0.30	273.31±0.27	266.38±0.25	269.16±0.04
Mycochemicals:				
Saponins	+	++	++	+
Tannins	0	0	0	0
Cardiac glycosides	0	0	0	0
Terpenoids	+	+	+	0
Flavonoids	++	++	++	+
Alkaloids	+	+	++	+

Values are the Mean ± SD. In mycochemicals; (+) traceable amount, (++) appreciable amount, and (0) absent



Figure 1: Fruit body of *C. comatus* grown on (A) carabao dung, (B) cow dung, (C) horse dung, and (D) rice straw enriched with 10% rice bran in an aseptic cultivation

CONCLUSION

Taken the data together, the growth and production performance, and the nutrient and mycochemical compositions of *C. comatus* are substrate type dependent. It is established that *C. comatus* could be considered in the wide variety of coprophilous basidiomycetes in an aseptic controlled condition since it was successfully cultivated on the formulated rice bran enriched dung of the different ruminants. This mushroom holds promising nutritional constituents for the human diet which can be an excellent source of proteins, carbohydrates, fiber, and minerals. Aside from being nutritive, *C. comatus* can also be regarded as natural food with several functional activities as shown by its appreciable amount of saponins, terpenoids, flavonoids and alkaloids. Further works on the isolation and characterization of specific mycochemicals from *C. comatus* and evaluation of their bioactivities in-vitro and in-vivo assays are highly considered.

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