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RESEARCH ARTICLE

Influence of substrates on the nutritional quality of *Pleurotus pulmonarius* and *Pleurotus ostreatus*

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Abstract: This investigation is aimed at determining the influence of agro-wastes as substrates on the nutritional quality of *Pleurotus pulmonarius* and *Pleurotus ostreatus.* Cassava peels, banana leaves and amended sawdust (sawdust mixed with rice bran in ratio 4:1) were used as growth substrates. Proximate and mineral analyses were carried out using DA 7250 NIR Analyzer and Atomic Absorption of Spectrophotometer machine (AA320N). Both species in amended sawdust had the highest stipe length and pileus diameter of the fruiting body. Starch was the most abundant proximate constituent in the two species. The starch was the most abundant proximate constituent in the two species but highest starch contents were observed in. *P. pulmonarius* grown in cassava peels substrate and *P. ostreatus* raised in banana leaves substrate. Fat appeared to be the lowest proximate constituent in the two species. Potassium was predominant among the minerals in both *P. pulmonarius* and *P. ostreatus* and the highest value (68.204 mg/L) was observed in *P. ostreatus* cultivated in amended sawdust. Other minerals varied considerably and as they were differential affected by the substrates. The mushrooms contained useful nutritional constituents however their quantities were influenced by the substrates used in raising them.

Keywords: Incubation, mineral element, proximate composition, spawn running, substrates.

INTRODUCTION

The genus *Pleurotus* contain edible mushrooms belonging to Class Basidiomycetes and Order Agaricales. Mushrooms are widely distributed in the temperate and tropical regions (Chang and Miles, 2004). The fruit-body of the *Pleurotus* is oyster-shaped hence 'Oyster Mushroom' is used as the common name. Cultivation of mushroom is seen as alternative ways by which poverty in developing countries like Nigeria can be reduced due to low cost of production with high profit. *P.ostratus*gains ground among the mushroom farmers (Rosado *et al.*, 2002) and this may be because it is easy to grow and economically viable. The substrates used in mushroom farming are usually agricultural remnants such as rice straw, banana leaves, sawdust, wheat straw, bamboo leaves, sugarcane tops and maize stover (Dlamini *et al.*, 2012; Kumari and Achal, 2008).

Generally, mushrooms are very palatable and are used in the preparation of intercontinental dishes. They are very nutritious as they contain appreciable amounts of both starchy and non-starchy carbohydrates, protein including most essentialamino acids, vitamins B, C and E,unsaturated fatty acids and dietary fibre (Chang and Miles, 2004; Croan, 2004).Oyster mushroom contains essential minerals such as potassium, phosphorous, calcium, magnesium, sodium, iron and selenium (Ezeibekwe, 2009).

Recent pharmacological research has confirmed the medicinal efficacy of mushroom in reducing the risk of disease such as epilepsy, wounds, skin diseases, heart ailments, rheumatoid, arthritis, fever, diaphoretic, diarrhea, dysentery, cold, anesthesia, liver disease, gall bladder diseases and used as vermicides (Bahl, 1983). Also, it has antifungal, antibacterial, antitumour, anti-inflammatory, antihypertensive, antihyperglycaemic, immunomodulatory and antioxidant properties (Gregori *et al*,. 2007; Wani *et al*., 2010). Species of the genus *Pleurotus* are good sources of lovastatin and as such, *Pleurotus* could be considered as a functional food with natural cholesterol-lowering ability (Gunde-Cimerman, 1999).

One of the major plans of Nigeria government is to diversify the nation's economy. Mushroom cultivation is a promising alternative agribusiness as it has the benefits of being land

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and labour savings, requires low startup capital and is eco-friendly. Besides, the agricultural remnants and industrial wastes used as substrates are readily available. The awareness of mushroom farming has been already made but nutritional benefits from the produce needs to be identified as well. Substrates played an indispensable role in the nutritional composition of oyster mushroom and astounding imbalances have been observed in both proximate and mineral compositions of *Pleurotus* raised in different substrates (Bhattacharjya *et al.,* 2015; Sarker *et al.,* 2007).This investigation was aimed at finding out the substrates that will be most suitable for improving the fruiting bodies, proximate composition and minerals of *Pleurotus pulmonarius* and *Pleurotus ostreatus.*

MATERIAL AND METHODS

Preparation of Substrates

The substrates used were cassava peels, banana leaves and sawdust. All the substrates were soaked in water for 12 hours to moisten them thoroughly and were stalked on the steep cemented floor so as to remove the excessive moisture from the substrates (Shah *et al.,* 2004).Amended sawdust was prepared by mixing the sawdust with rice bran in ratio 4:1. Each substrate was weighed, wetted to about 65-75% moisture content and 200g of calcium carbonate ($CaCO_3$) was added. The squeeze method of Dlamini *et al*. (2012) was used to determine the moisture content. When squeezing the substrate between fingers, small amounts of water oozed from the substrate. Thereafter, 1kg of each substrate was packed into an autoclaving bag and fastened using rubber bands and sterilized at 15 psi, 121 °C for 90 min (Kumari and Achal, 2008).

Isolation and Maintenance of Pure Cultures of Desired Mushroom

Freshly harvested *P.pulmonarius* and *P.ostreatus*were obtained from Federal Institute of Industrial Research, Oshodi (FIIRO). Potato Dextrose Agar (PDA) was poured into petri dishes and allowed to solidify. The mushroom fruits were dissected longitudinally with a sterile razor. Small tissues from the fruits were aseptically inoculated into petri dishes containing PDA and they were incubated at 28^{o}C for 7 days (Girmay *et al*., 2016).

Spawn production

The *Sorghum bicolor* grains were washed and the chaff were sorted out. It was parboiled with $CaCO_3$ until gelatinized. After draining excess water, it was mixed with 1% $CaCO_3$ and gypsum ($CaSO_4$) to prevent adhesion of grains and for optimizing the pH for spawn. After cooling, it was bottled and covered with aluminum foil, thereafter autoclaved for 1hr at 121^{o}C. Pure cultures each of *P. pulmonarius* and *P. ostreatus* growing on the petri dishes were picked with a sterilized corkborer and inoculated aseptically on the grains and incubated at room temperature. The spawn was inoculated into the substrates and kept in the dark to allow the substrates to ramify.

Fruiting and Harvesting

After ramification of the substrates, the polythene bags were loosened to allow air, light and daily watering till the pin heads appeared. Matured mushrooms were harvested after about 3-4days of fruiting. Thereafter, the height of fruiting bodies, stipe length and pileus diameter were measured with calibrated ruler. The fruiting bodies were oven dried and separately packed for analyses.

Proximate and Mineral analyses

The proximate analysis of *P. pulmonarius* and *P. ostreatus* was carried out using DA 7250 NIR Analyzer. This machine incorporates Near Infra-Red (NIR) technology with solid state optics and has specially designed software for its operation. The samples were pulverized prior to analysis. The samples were poured into an open dish for automatic analysis. The results were displayed on the touch-screen. FOS KJeltec ™ 8200 Auto Distillation Machine was used to verify the protein content in the samples. For mineral analysis, digestion and analysis was carried out using the method of Khan *et al*. (2011).A Flame Atomic Absorption Spectrophotometer (AA320N) was used to quantify the minerals present in the samples. Zinc (Zn), Manganese (Mn), Iron (Fe), Copper (Cu), Magnessium (Mg), Calcium (Ca), Selenium (Se) and Potassium (K) were determined. Each of the analyses was replicated three times.

Data Analysis

The data collected were analyzed using Statistical Package for Social Science (SPSS version 16.0) software. Analysis of Variance was carried out and the means were separated using Dunca Multiple Range Test (DMRT) at 5% level of significance. Also, Origin (7.0) was used to plot the bar charts.

RESULTS

Running of spawn and formation of fruiting bodies of *P. pulmonarius* and *P. ostreatus*

Banana leaves showed the fastest rate of mycelium growth (Table 1) during the spawn running and takes about 17 days in *P. pulmonarus* and 15days in *P. ostreatus* for full ramification. There were significant differences in the mean number of days taken for the completion of spawn running between the substrates in the two species. Appearance of pin heads and formation of fruiting bodies were delayed most in amended sawdust substrates.

Effects of the substrates on the morphology of the *P. pulmonarius* and *P. ostreatus*

P. pulmonarius and *P. ostreatus* cultivated in amended sawdust had the highest stipe length, pileus diameter and height of fruiting body. There were no significant differences in stipe lengths of the two species grown on different substrates ($p \leq 0.05$). Also, theamended sawdust showed a significantly higher pileus diameter, while other measurements did not show significant differences (Table 2).

Table 1: Effects of different substrates on growth and yield performance of Pleurotus pulminarius and Pleurotus ostreatus.

Mushroom species	Substrates	Spawn running	Pin head appearance Days	Fruiting body formation
Pp	Banana leaves	17.00 ± 0.00^{c}	22.33 ± 0.02^{c}	25.00 ± 0.00^{b}
	Cassava peels	29.33 ± 0.01^{b}	34.00 ± 0.01^{b}	37.33 ± 0.33^{a}
	Amended Sawdust	30.00 ± 0.00^{a}	35.33 ± 0.33^{a}	39.33 ± 0.33^{a}
	Mean	25.44±0.00	30.55±0.12	33.89±0.22
Po	Banana leaves	15.33 ± 0.67^{a}	20.33 ± 0.67^{c}	23.33 ± 0.33^{c}
	Cassava peels	25.67 ± 0.33^{b}	30.67 ± 0.33^{b}	33.67 ± 0.33^{b}
	Amended Sawdust	28.30 ± 0.33^{c}	33.33 ± 0.33^{a}	37.33 ± 0.33^{a}
	Mean	23.10±0.44	28.11±0.44	31.44±0.33

Means followed by the same letter along the same column for each species of mushroom are not significantly different at $p \leq 0.05$. Pp = *Pleurotus pulmonarius* Pp = *Pleurotus ostreatus*

Table 2: Effects of substrates on the morphology of *Pleurotus pulminarius* and *Pleurotus ostreatus.*

	Pleurotus pulminarius			*Pleurotus ostreatus*		
Substrates	**Stipe (cm)**	**Pileus(cm)**	**Height of fruiting bodies (cm)**	**Stipe (cm)**	**Pileus (cm)**	**Height of fruiting bodies (cm)**
Banana leaves	5.33 ± 0.33^{a}	4.50 ± 0.00^{b}	5.73 ± 0.33^{a}	5.33 ± 0.33^{a}	4.50 ± 0.00^{b}	5.70 ± 0.33^{a}
Cassava peels	7.00 ± 1.00^{a}	5.67 ± 0.17^{ab}	7.10 ± 0.70^{a}	6.00 ± 1.00^{a}	4.67 ± 0.17^{ab}	6.250 ± 0.70^{a}
Amended Sawdust	8.00 ± 1.00^{a}	6.33 ± 0.33^{a}	8.47 ± 0.64^{a}	7.00 ± 1.00^{a}	5.33 ± 0.33^{a}	7.46 ± 0.64^{a}
Mean	6.78±0.78	5.50±0.17	7.10±0.56	6.11±0.78	4.83±0.17	6.47±0.56

Means followed by the same letter along the same column for each substrate are not significantly different at $p \leq 0.05$.

Effects of substrates on proximate composition of *P. pulmonarius* and *P. ostreatus*

The percentage moisture in *P. pulmonarius* in cassava peels was the highest and significantly different from the quantity present in those harvested from banana leaves and amended sawdust. The percentage protein in *P. pulmonarius* grown in cassava peels was significantly higher than the amounts present in those harvested from banana leaves. Also, the amount of fat in *P. pulmonarius* was very infinitesimal compared to other proximate constituents regardless of the substrates used. The percentage fibre in *P. pulmonarius* in all three substrates ranged from 5.31% to 5.83%. The highest percentage starch was observed in *P. pulmonarius* grown in cassava peels but ash in the same sample was the lowest as summarized in Figure 1.

The moisture in *P. ostreatus* grown in banana leaves was the highest and significantly different from *P. ostreatus* grown in both amended sawdust and cassava peels. The protein in *P. ostreatus* harvested from amended sawdust was significantly different from the amount obtained in the mushroom raised in banana leaves. The fat in *P. ostreatus* in cassava peels substrate was found to be highest. In banana leaves, the *P. ostreatus* harvested from the substrates had the highest fibre and starch contents. The ash in *P. ostreatus* harvested from all the substrates ranges from 7.99% to 11.21% as fully represented in Figure 2.

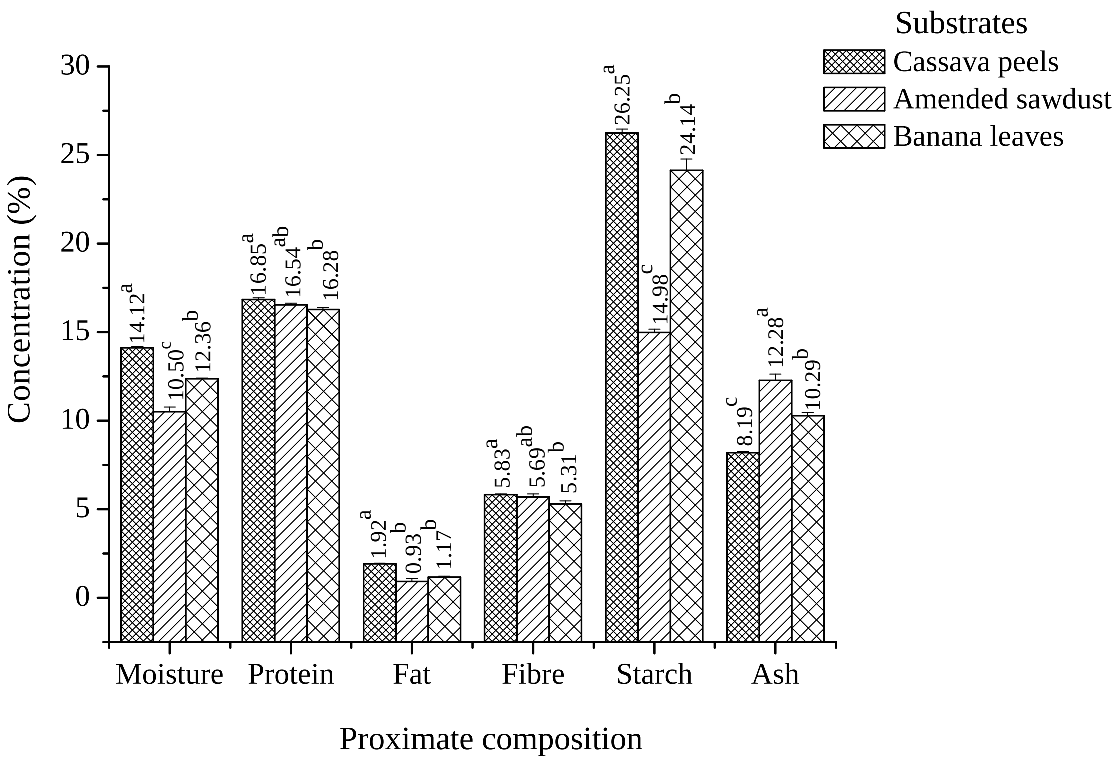

Figure 1: Proximate analysis of *Pleurotus pulmonarius* as influenced by substrates.

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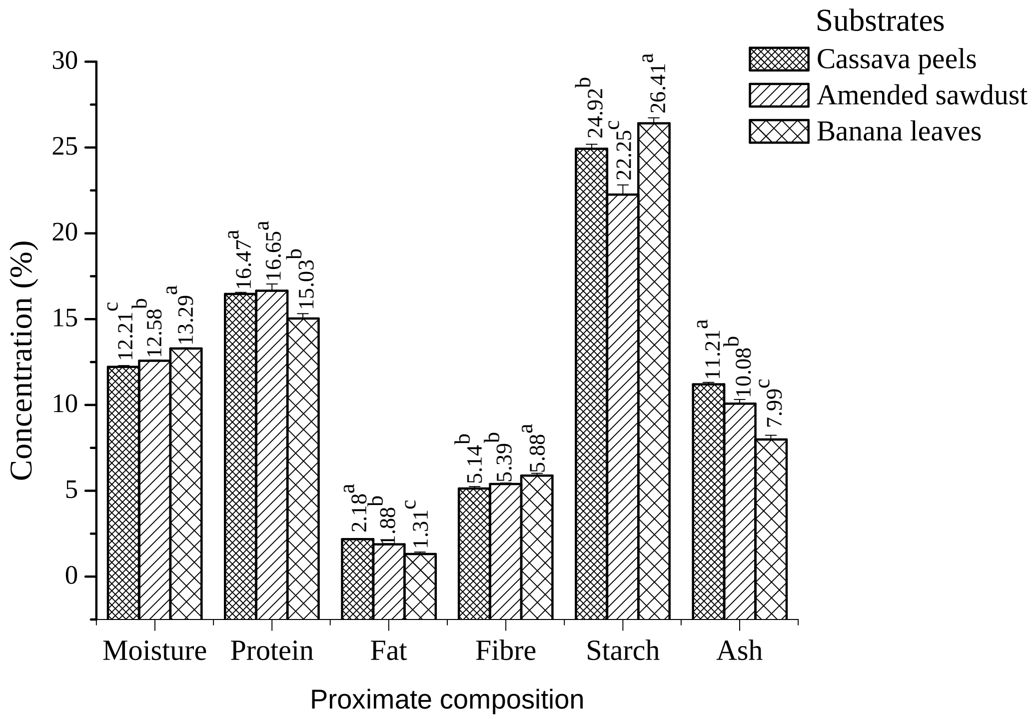

Figure 2: Proximate analysis of *Pleurotus ostreatus* as influenced by substrates.

Effects of substrates on minerals in *P. pulmonarius* and *P. ostreatus*

There were no significant differences in the Zn and Mg contentsin *P. pulmonarius* in all three substrates.The amounts Mn, Fe and Cu contents in *P. pulmonarius* were significantly higher in the banana leaves substrates. Mn and Cu in *P. pulmonarius*cultivated in cassava peels and amended sawdust showed no significant difference. The level of Ca was found to be highest in the *P. pulmonarius* grown in cassava peels and the lowest level was recorded in those raised in banana leaves. *P. pulmonarius* in banana leaves hadthe lowest Se. The K in *P. pulmonarius* grown in cassava peels was the highest and was significantly different from that of banana leaves and amended sawdust. There was no significant difference in K contents of *P. pulmonarius* raised in both banana leaves and amended sawdust (Table 3).

As in *P. pulmonarius,* the Zn and Mg in *P. ostreatus* showed no significant difference from one another along the three substrates used.The Mn and Fe in the mushroom raised in sawdust werefound lowest. *P. ostreatus* grown in amended sawdust was the richest in Ca and was significantly different from the amounts obtained in thoseraised in other two substrates (Table 3).The highest amount of K was observed in *P. ostreatus* harvested from amended sawdust (Table 3).

DISCUSSION

Mushroom cultivation is a very profitable agribusiness as it can be raised in botanical wastes such as wheat straw, plant leaves and sawdust (Shah *et al.*, 2004). Spawn running was fastest in banana leaves substrates but the highest pileus diameter and stipe height were observed in mushroom from amended sawdust which took about four weeks for complete spawn running. Differences in rate of spawn running may be attributed to the size of the grains (Pathmashini *et al*., 2008).The suitability of amended sawdust agreed with the work of Shah *et al* (2004) who both advocated the effectiveness of sawdust in mushroom cultivation. Wheat straw substrate is one of the best substrates in *Pleurotus ostreatus* cultivation (Kumari and Achal, 2008) and the produce has average pileus diameter that is higher than the one obtained from amended sawdust.

Table 3: Mineral analysis of *Pleurotus pulmonarius* and *Pleurotus ostreatus* as influenced by substrates.

		Zn	Mn	Fe	Cu	Mg	Ca	Se	K
					mg/L				
Pp	Banana leaves	0.028±0.00[a]	0.025±0.00[a]	0.039±0.00[a]	0.016±0.00[a]	0.038±0.00[a]	0.009±0.00[c]	0.003±0.00[c]	57.120±0.85[b]
	Cassava peels	0.022±0.00[a]	0.014±0.00[b]	0.017±0.00[c]	0.007±0.00[bc]	0.055±0.00[a]	0.041±0.00[a]	0.007±0.00[b]	63.941±1.48[a]
	Amended sawdust	0.022±0.00[a]	0.012±0.00[b]	0.026±0.00[b]	0.004±0.00[c]	0.049±0.01[a]	0.036±0.01[b]	0.009±0.00[a]	58.826±0.00[b]
	Mean	0.024±0.00	0.017±0.00	0.027±0.00	0.009±0.00	0.047±0.00	0.029±0.00	0.006±0.00	59.962±0.78
Po	Banana leaves	0.100±0.07[a]	0.023±0.00[a]	0.022±0.00[a]	0.007±0.01[ab]	0.032±0.01[a]	0.036±0.00[b]	0.001±0.00[a]	59.678±0.85[b]
	Cassava peels	0.100±0.07[a]	0.006±0.00[b]	0.013±0.00[b]	0.005±0.00[b]	0.024±0.19[a]	0.007±0.00[c]	0.001±0.00[a]	61.383±0.00[b]
	Amended sawdust	0.030±0.00[a]	0.002±0.00[b]	0.010±0.00[b]	0.008±0.00[a]	0.024±0.00[a]	0.158±0.00[a]	0.002±0.00[a]	68.204±0.85[a]
	Mean	0.077±0.05	0.010±0.00	0.015±0.00	0.007±0.00	0.027±0.07	0.067±0.00	0.001±0.00	63.088±0.57

Means followed by the same letter along the same column for each species of mushroom are not significantly different at $p \leq 0.05$.

Pp = *Pleurotus pulmonarius* Po = *Pleurotus ostreatus*
Zn = Zinc Mn = Manganese
Fe = Iron Cu = Copper
Mg = Magnesium Ca = Calcium
Se = Selenium K = Potassium

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P. pulmonarius and *P. ostreatus* are very rich in carbohydrate notably starch. The bulk of mushroom fruiting bodies are made of carbohydrate (Wani *et al*, 2010). Chang *et al.* (1996) reported that the fruit bodies of mushrooms contained 40.3-50.7% of carbohydrates. The quantity of fat in the species of *Pleutorus* was very low and was expected as mushrooms generally have low fat content (Wani *et al*, 2010). Oleic acid and linoleic acid are the major monounsaturated fatty acid and polyunsaturated fatty acid respectively in *P. ostreatus* (Deepalakshmi and Mirunalini, 2014). The percentage protein in the two *Pleurotus* species ranged from 15.03% to 16.85% but was below 27.30% to 25.35% as reported by Bhattacharjya *et al.* (2015). Chang (1980) reported that the crude protein in mushrooms rank below animal meats but well above most other foods. Protein content of *Pleurotus* depends on the composition of the substrates and species of mushrooms (Erjavec *et al*., 2012).It is reported that mushroom is generally becoming a reliable source of protein (Deepalakshmi and Mirunalini, 2014). Proteins of *Pleurotus* sp. mushroom have superior quality because some of the members of this genus contain complete proteins with a good distribution of essential amino acids and non-essential amino acids (Wang *et al.,* 2001). Oyster mushrooms are rich in dietary fibres containing about 5-6% and this quantity is within range as reported by Kalac (2009).

Minerals such as Fe, Cu, Zn, Mn and K are essential metals since they play an important role in biological systems. Heavy metals such as Mn and Cu were present. Turkekul *et al*. (2004) reported that mushrooms have effective mechanisms to absorb heavy metals from their substrates. High Zn and Fe are contained in the *Pleurotus* especially in the fruiting body (Ijeh *et al.*, 2009). Deficiency of Zn in diet may result to growth retardation, loss of appetite and impaired immune function (Prassad, 2004). Both *P. pulmonarius* and *P. ostreatus* can be taken as diet supplement. Cu, like any other microelement are needed by the body in small quantity (Araya *et al*., 2006) and banana leaves substrate produce *Pleurotus* with highest amount of Cu. Potassium was found to be highest in two species of *Pleurotus* harvested from all the substrates. This mineral element is very essential and plays a vital role in the body. Wang *et al.* (2001) reported that K is a co-factor of several enzymatic reactions and this is available in the mushroom in large quantities.

CONCLUSION

P. pulmonarius and *P. ostreatus* grew in the three substrates used in varying degree. However, banana leaves and amended sawdust substrates were found to be suitable for growth of the mushrooms. Potassium (K) was found to be highest among all minerals tested in the two species. *P. pulmonarius* and *P. ostreatus* are rich in carbohydrate with low fat. Both species can be taken for dietary requirement as well as supplements to cater for deficient minerals in the body.

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