Original Research Article

Chemical characterisation and *in vitro* assessment of two mushroom stalks as prebiotics for *Clarias gariepinus* (Burchell, 1822)

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Abstract

The potentials of mushroom stalks as supplements in aqua-feeds have been grossly underutilised. Stalk meals of two *Pleurotus* species were analysed for proximate composition, fibre fractions, mineral and phytochemical constituents. *In vitro* digestibility and fermentability were assessed using caecal digesta from *Clarias gariepinus* (n = 108; weight: 138 ± 10.8 g). Stalks of *Pleurotus pulmonarius* and *Pleurotus ostreatus* were air-dried at ambient room temperature and milled. *Pleurotus ostreatus* contained higher ($P < 0.05$) moisture, crude protein, ether extract and crude fibre than *P. pulmonarius* stalks which had higher ($P < 0.05$) nitrogen-free extract. *Pleurotus ostreatus* had higher ($P < 0.05$) neutral detergent fibre, acid detergent fibre, acid detergent lignin and cellulose but lower ($P > 0.05$) hemicellulose than *P. pulmonarius*. Except in manganese and iron content, *P. ostreatus* contained higher ($P < 0.05$) sodium, calcium, magnesium, potassium, phosphorus, copper and zinc than *P. pulmonarius*. Both stalks contained flavonoids, phlobatannin, terpenoid, cardiac glycosides, steroids and antraquinone. Substrate loss was higher ($P < 0.05$) in *P. pulmonarius* (0.20 g) than *P. ostreatus* (0.15 g). Maximum rate of gas production was more ($P < 0.05$) for *P. pulmonarius* (0.16 mL/h) at 4.96 hours compared to 0.04 mL/h at 6.04 hours for *P. ostreatus*. Both stalk meals were partially resistant to *in vitro* digestibility and were fermentable. Hence, they possess favourable prebiotics characteristics and can be used as supplement in aqua feed.

Keywords: Proximate composition; fibre fraction; caecal digesta; fermentability; digestibility.

INTRODUCTION

Pleurotus species of mushrooms are white-rot fungi which are wood-decay saprotrophs that principally decompose lignin (Bari et al., 2015). They grow naturally in the wild in temperate and sub-tropical regions of the world and can be artificially cultivated on a broad variety of wastes generated from forest, food processing and agricultural industries in a wide range of temperatures (Hoa and Wang, 2015). *Pleurotus* species are commercially important and are extensively grown for food due to their taste, exceptional flavour and nutrients as well as the medicinal properties they possess (Deepalakshmi and Mirunalini, 2014).

Pleurotus species have extremely varied proximate composition depending on the growth substrate and the species (Hoa et al., 2015). Fresh *Pleurotus* mushroom

commonly have high moisture content which ranges from 85% to 95%. They are rich in protein, dietary fibre, carbohydrates, minerals and vitamins but low in fats and calories (Khan, 2010). According to Alam et al. (2008) and Chou et al. (2013), the fruiting body (cap and gills) of *Pleurotus* species are rich in protein and lipid while the stalks are rich in carbohydrate and fibre. *Pleurotus* species of mushrooms possess high level of well assailable mineral constituents (Mattiala et al., 2001). They also bio-accumulate numerous secondary bioactive phytochemicals from the substrates on which they grow (Khatua et al., 2017).

The stalks of mushrooms are considered to be waste products from the harvest of mushrooms. They are generated as by-products of low economic value from the rapidly growing mushroom cultivation and processing industry. According to Chou et al. (2013), the stalks of fresh mushrooms constitute 25 to 32% of its weight. Also, Ahmed et al. (2013) stated that 161 g to 502 g of mushroom stalk waste are generated to produce one kilogram of Oyster mushrooms. The increasing production of mushrooms has led to increasing quantities of mushroom stalk wastes being disposed which may lead to environmental pollution (Ahmed et al., 2015), hence the need to search for alternative ways to utilise it.

Mushroom stalk wastes are rich in fibre and carbohydrates and have great potential to act as a prebiotic in aqua feeds. According to Slavin (2013), prebiotics are partially or wholly resistant to gastric acidity, mammalian enzymes hydrolysis and absorption in the gastrointestinal tract. They are also fermentable by the microbiota of the gastrointestinal tract. *In vitro* digestion and fermentation methods for aquatic organisms are mostly developed from terrestrial animals (ruminants and monogastrics) and human studies (Moyano et al., 2015). The cumulative gas production method is a unique method of the batch *in vitro* fermentation system which is simple to analyse and generally used for routine assessment of food and fermentation products (Coles et al., 2005).

Previous research investigated some nutrient composition of the stalks of some species of mushrooms (Buwjoom et al., 2004; Alam et al., 2008; Oboh and Shodehinde, 2009; Nasiri et al., 2013 and Camay, 2016). However, the chemical composition of the stalks has not been adequately studied and documented for its utilisation in aquafeed. There is a dearth of information on the *in vitro* prebiotic potential of the stalk meals of *P. pulmonarius* and *P. ostreatus* in fish. This study therefore evaluates the proximate composition, dietary fibre fraction, mineral composition and phytochemical constituents of *P. pulmonarius* and *P. ostreatus* stalk meals and also examines its resistance to *in vitro* gastric conditions and fermentability using the caecal digesta of *Clarias gariepinus*.

MATERIALS AND METHODS

Procurement and processing of mushroom stalks

Pleurotus pulmonarius spawn was obtained from the Waste Utilization and Fermentation Division, Federal Institute for Industrial Research, Oshodi (FIRRO), Lagos while *Pleurotus ostreatus* spawn was obtained from the Mushroom Research and Cultivation Laboratory, Yaba College of Technology, Yaba, Lagos. The two *Pleurotus* species of mushrooms were grown separately on the same formula of sawdust substrate (Formula: 10 kg sawdust, 1.5 kg rice bran, 200 g chalk, 30 g gypsum and 15 litres of water) according to the method of Oei (2005) for Oyster mushrooms. The stalks of cultivated mushrooms (*P. pulmonarius* and *P. ostreatus*) were cut off from the fruiting body using a pair of sharp scissors and air-dried at room temperature (25 °C) for two weeks. They were milled to powder in a blender, bagged and stored in the refrigerator at 4 °C pending analysis.

Proximate analysis of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meal

Proximate analyses of *P. pulmonarius* and *P. ostreatus* stalk meals were performed according to the standard method of AOAC (2002).

Characterisation of dietary fibre of *Pleurotus* species stalk meals

The determination of the dietary fibre was done according to the method of AOAC (2002) and Van Soest et al. (1991). The hemicellulose and cellulose were computed as follows:

Hemicellulose = Neutral Detergent Fibre – Acid Detergent Fibre

Cellulose = Acid Detergent Fibre – Acid Detergent Lignin

Mineral analysis of *Pleurotus* species stalk meal

The digestion of the samples was done using nitric acid and perchloric acid. Aliquots obtained were used to evaluate the mineral composition using the atomic absorption spectrophotometer with suitable hallow cathode lamp according to the method of AOAC (1990).

Phytochemical screening of *Pleurotus* species stalk meal

The preliminary quantitative phytochemical screening was carried out on ethanolic extracts of the mushroom stalk meal as described by Harborne (1984), Sofowara (1993) and Trease and Evans (2002).

Quantitative determination of phytochemical constituents of *Pleurotus* species stalk meal

Flavonoid was determined quantitatively using the method of Bohm and Kocipai-Abyazan (1994). Phenol was determined using the spectrophotometric method (Folin-Ciocalteu reagent) according to the procedure of Singleton et al. (1999). Alkaloid was determined according to the procedure of Obadoni and Ochuko (2001). Tannins was evaluated using the Folin-Denis colorimetric method by as elucidated by Kirk and Sawyer (1998). Cardiac glycoside content was determined using spectrophotometric method of Solich et al. (1992). Phlobatannin was determined according to the method of AMC-RSC (2003).

Gastric acidity hydrolysis

The stalk meals were pre-digested in an *in vitro* 2-step pepsin and pancreatin digestion to simulate digestion in the fore gut by the dialysis bag method of Gauthier et al. (1986). Triplicate samples (500 mg) were each suspended in 16 ml of 0.01 N hydrochloric acid; pH was adjusted to 1.9 at 37 °C. Pepsin (1 mg) (> units/mg; porcine mucosa, Sigma-Aldrich, USA) was weighed into each solution for enzyme hydrolysis for 30 minutes. Digestion was stopped by raising the pH to 7.5 with 1N sodium hydroxide. Digesta obtained were transferred into a dialysis bag and then 10 mg pancreatin (P3292, porcine pancreas, Sigma-Aldrich, USA) was added. The dialysis bag was steeped in a beaker containing 100 mL of 0.01 M sodium phosphate buffer for 6 hours at pH of 7.5. Dialysate and residues obtained were analysed for ammonia-nitrogen and substrate loss, respectively. Residues were used in the cumulative gas production incubations.

In vitro cummulative gas production technique (fermentability analysis)

Fresh caecal digesta were collected and pooled from post-juvenile *C. gariepinus* (138.89 ± 10.8 g) fed a commercial diet that does not contain antibiotics and the test ingredient. A buffer medium was prepared as done by William et al. (2005). About 30 mL of the prepared buffer medium was added to 20 g of caecal digesta of *C. gariepinus* to get the slurry. The slurry was sifted using a double-folded cheese cloth to obtain the inoculum. The entire preparation of the inoculums was done maintaining an anaerobic condition under a continuous flow of CO_2 gas. It was then made up to 200 mL volume with the buffering media.

Pre-digested samples (100 mg) were measured into 50 mL syringes. About 20 mL of buffered inoculums was added and the syringes were closed using a metal clip to prevent the gas from escaping. Each test ingredient was done in triplicate and incubated at 39 ± 1 °C. The reading of the gas production was recorded every 3 hours for a period of 60 hours.

The profiles of the cumulative gas production were fitted to the monophasic model of Groot et al. (1996) as:

$$G = A/(1 + (C/t)^B)$$

Where:

G = Total gas produced; A = asymptotic gas production; B = switching characteristic of the curve; C = time at which half of the asymptote was reached ($T_{1/2}$); t = time (h).

The maximum rate of gas production (R_{max}) and time of maximum rate of gas production (T_{max}) were computed using the following equations by Bauer et al. (2001).

$$R_{max} = (A(C^B)B(T_{max}^{(-B-1)}))/(1 + (C^B)(T_{max}^{(-B)}))^2$$

$$T_{max} = C(((B - 1)/(B + 1))^{1/B})$$

Where A = asymptotic gas; B = switching characteristics; C = time at which half of the asymptote reached $T_{1/2}$

Post-fermentation analyses

When incubation was completed, 4 mL of 10 M NaOH solution was introduced into the syringe to measure the methane and carbon IV oxide gas production, as elucidated by Fievez et al. (2005). Residues of fermentation were rinsed with hot water and filtered. Dry matter and ash content of the residues were determined according to AOAC (2002). The pH within each fermentation vessel was recorded using a pH meter while ammonia-nitrogen of the filtrate was determined using the distillation method using the Markham apparatus according to AOAC (2002).

Statistical analysis

Data obtained were analysed using T-test at $p < 0.05$ level of significance using the statistical package for Windows (SPSS 17.0). Version 6.6 of NLREG(1992) was used to compute data for fermentability analysis. All values are presented as mean ± S.D (standard deviation).

Table 1. Proximate composition of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meals

Parameters	*P. pulmonarius* stalk meal (%)	*P. ostreatus* stalk meal (%)
Moisture	6.04 ± 0.03[a]	6.70 ± 0.04[b]
Crude protein	9.24 ± 0.53[a]	10.84 ± 0.05[b]
Ether extract	2.41 ± 0.04[a]	3.58 ± 0.04[b]
Crude fibre	10.36 ± 0.03[a]	10.81 ± 0.07[b]
Ash	3.85 ± 0.28	4.17 ± 0.07
Nitrogen Free Extract	68.11 ± 0.34[b]	63.85 ± 0.10[a]

Mean ± SD with different superscripts within a row are significantly different ($P < 0.05$).

Table 2. Fibre fractions (%) of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meals

Fibre fraction (%)	*P. pulmonarius* stalk meal	*P. ostreatus* stalk meal
NDF	65.45 ± 0.65[a]	70.60 ± 0.40[b]
ADF	46.40 ± 0.90[a]	59.35 ± 0.25[b]
ADL	23.25 ± 0.65[a]	30.89 ± 0.67[b]
Hemicellulose	19.05 ± 1.55[b]	11.58 ± 0.33[a]
Cellulose	23.15 ± 1.55[a]	28.47 ± 0.42[b]

Mean ± SD with different superscripts in a row are significantly different ($P < 0.05$).
NDF – Neutral detergent fibre; ADF – Acid detergent fibre; ADL – Acid detergent lignin; Hemicellulose = NDF – ADF and cellulose = ADF – ADL.

RESULTS

Proximate composition of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meals

The proximate composition of air-dried *P. pulmonarius* and *P. ostreatus* stalk meals is shown in Table 1. The most predominant nutrients were nitrogen-free extract and crude fibre while the least constituent was ether extract. *Pleurotus ostreatus* stalk meal had a higher ($P < 0.05$) crude protein, ether extract, crude fibre and moisture content than *P. pulmonarius*. However, *P. pulmonarius* stalk meal contained a higher ($P < 0.05$) nitrogen free extract than *P. ostreatus*. The ash content of both stalk meals was not significantly different ($P > 0.05$).

Fibre fractions of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meals

The fibre fractions of *P. pulmonarius* and *P. ostreatus* stalk meals are presented in Table 2. All the fibre fraction parameters analysed were significantly different ($P < 0.05$). *Pleurotus ostreatus* stalk meal had higher Neutral detergent fibre, Acid detergent fibre, Acid detergent lignin, and cellulose while *P. pulmonarius* stalk meal was more in hemicelluloses content.

Mineral composition of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meals

The macro and micro mineral contents of *P. pulmonarius* and *P. ostreatus* stalk meals are as shown in Table 3. The most abundant macro-mineral was sodium, followed by calcium, for both species; whereas the least macro-mineral was phosphorus for both species. The most predominant micro-mineral was copper for both *Pleurotus* species. The least micro-mineral was iron for *P. pulmonarius* stalk meal and manganese for *P. ostreatus* stalk meal. However, the iron content in the stalk meal of both species were not significantly different ($P > 0.05$). *Pleurotus pulmonarius* stalk meal had a higher quantity of manganese but had lower quantities of sodium, calcium, magnesium, potassium, phosphorus, copper, iron and zinc compared to *P. ostreatus*. *Pleurotus ostreatus* stalk meal had a higher content of sodium, calcium, magnesium, potassium, copper, iron, and zinc, with a lower quantity of manganese compared to that of *P. pulmonarius*.

Quantitatively, the mineral elements when arranged in descending order for *P. pulmonarius* stalk meal was sodium > calcium > magnesium > potassium > phosphorus > copper > manganese > zinc > iron; whereas *P. ostreatus* stalk meal was sodium > calcium > magnesium > potassium > phosphorus > copper > zinc > iron > manganese.

Table 3. Mineral composition of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meals

Minerals (mg/100g)	Mineral constituents	*P. pulmonarius* stalk meal	*P. ostreatus* stalk meal
Macro-minerals	Sodium	144.63 ± 1.05[a]	275.61 ± 0.78[b]
	Calcium	85.53 ± 0.18[a]	98.09 ± 0.41[b]
	Magnesium	78.34 ± 0.11[a]	93.22 ± 0.24[b]
	Potassium	54.11 ± 0.26[a]	65.23 ± 0.16[b]
	Phosphorus	24.69 ± 0.17[a]	37.05 ± 0.57[b]
Micro-minerals	Iron	2.29 ± 0.16	2.33 ± 0.08
	Zinc	4.30 ± 0.04[a]	8.44 ± 0.16[b]
	Manganese	7.02 ± 0.32[b]	0.11 ± 0.02[a]
	Copper	9.92 ± 0.28[a]	35.97 ± 0.18[b]

Mean ± SD with different superscripts within a row are significantly different ($P < 0.05$).

Table 4. Qualitative characterisation of the ethanolic extracts of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meals

Phytochemicals	*P. pulmonarius* stalk meal	*P. ostreatus* stalk meal
Flavonoids	+	+
Phenol	+	–
Alkaloid	–	+
Tannin	+	–
Saponin	–	–
Phlobatannin	+	+
Terpernoid	+	+
Cardiac glycoside	+	+
Steroid	+	+
Antraquinone	+	+

Keys: + : Present, – : Absent

Table 5. Quantitative composition of ethanolic extracts of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meals

Phytochemicals	*Pleurotus pulmonarius* (mg/100g)	*Pleurotus ostreatus* (mg/100g)
Flavonoid	33.06 ± 0.18^{a}	39.74 ± 0.23^{b}
Phenol	21.78 ± 0.23	ND
Alkaloid	ND	19.39 ± 0.35
Tannin	18.74 ± 0.16	ND
Cardiac glycoside	24.19 ± 0.74^{b}	20.18 ± 0.33^{a}
Phlobatannin	15.81 ± 0.09^{a}	16.96 ± 0.33^{b}

Mean ± SD with different superscripts within a row are significantly different ($P < 0.05$).
ND = Not detected.

Table 6. *In vitro* digestibility of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meals

Indicators analysed	*P. pulmonarius* stalk meal	*P. ostreatus* stalk meal
Weight before digestion (g)	0.50 ± 0.00	0.50 ± 0.00
Weight after digestion (g)	0.30 ± 0.01^{a}	0.35 ± 0.05^{b}
Substrate loss after digestion(g)	0.20 ± 0.01^{b}	0.15 ± 0.05^{a}
Ammonia nitrogen from filtrate after digestion (%)	1.93 ± 0.09^{a}	2.53 ± 0.11^{b}

Mean ± SD within the same row with different superscripts are significantly different ($P < 0.05$).

Table 7. *In vitro* fermentibility of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meal

Indicators analysed	*P. pulmonarius* stalk meal	*P. ostreatus* stalk meal
Weight before fermentation (g)	0.10 ± 0.00	0.10 ± 0.00
Weight after fermentation (g)	0.028 ± 0.01	0.027 ± 0.06
Substrate loss after fermentation (g)	0.072 ± 0.01	0.073 ± 0.01
pH	6.19 ± 0.07	6.07 ± 0.02
Methane (mL)	5.50 ± 0.5^{a}	6.50 ± 0.5^{b}
CO_2 production (mL)	11.00 ± 0.01	10.00 ± 0.01
Ammonia nitrogen from filtrate after fermentation	0.02 ± 0.01^{a}	0.03 ± 0.01^{b}
Ash from residue after fermentation (g)	0.11 ± 0.002^{b}	0.07 ± 0.002^{a}
G (mL)	16.5 ± 0.5	16.5 ± 0.5
A	20.84 ± 1.48	21.29 ± 0.69
B	2.33 ± 0.19^{a}	3.06 ± 0.12^{b}
C	29.09 ± 1.25^{a}	36.39 ± 2.62^{b}
T_{max}(h)	4.96 ± 0.19^{a}	6.04 ± 0.50^{b}
R_{max}(mL/h)	0.16 ± 0.04^{b}	0.04 ± 0.01^{a}

Mean ± SD within the same row with different superscripts are significantly different ($P < 0.05$). CO_2 – Carbon IV oxide, G – total gas produced, A – asymptotic gas production, B – switching characteristics of the curve, C – time at which half of the asymptote was reached ($T_{1/2}$), R_{max} – maximum rate of gas production, T_{max} – time of maximum rate of gas production.

Qualitative and quantitative analyses of ethanolic extracts of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meals

The qualitative and quantitative analyses of the ethanolic extracts of *P. pulmonarius* and *P. ostreatus* stalk meals are presented in Tables 4 and 5. The preliminary phytochemical screening of both stalk meals indicated the presence of flavonoids, phlobatannin, terpenoid, cardiac glycoside, steroid and anthraquinone. Phenol and tannin were present in *P. pulmonarius* stalk meal but were not detected in *P. ostreatus* stalk meal. Alkaloid was present in *P. ostreatus* but was not detected in *P. pulmonarius* stalk meal, while saponin was not detected in both stalk meals.

Quantitatively, the predominant phytochemical was flavonoids for both *Pleurotus* stalk meals. The least phytochemical was phlobatannin for both *Pleurotus* species. Quantitative analysis of the ethanolic extracts of *P. pulmonarius* and *P. ostreatus* stalk meals were significantly different at $P < 0.05$. *Pleurotus ostreatus* stalk meal had higher flavonoids, alkaloid and phlobatannin, whereas *P. pulmonarius* stalk meal had higher cardiac glycoside. *Pleurotus pulmonarius* stalk meal had phenol and tannin, which were not detected in *P. ostreatus* stalk meal. Alkaloid was present in *P. ostreatus* stalk meal but was not detected in *P. pulmonarius* stalk meal.

Invitro digestibility of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meal

The *in vitro* digestibility of *P. pulmonarius* and *P. ostreatus* stalk meal are shown in Table 6. Substrate loss and ammonia nitrogen from the filtrate of both stalk meals were significantly different ($P < 0.05$). *Pleurotus pulmonarius* stalk meal had a higher substrate loss of 0.20 ± 0.01 g and less ammonia nitrogen content of 1.93 ± 0.01% in the filtrate; while *P. ostreatus* had a lower substrate loss of 0.15 ± 0.05 g and higher ammonia nitrogen content of 2.53 ± 0.11%.

In vitro fermentibility of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meal

The *in vitro* fermentation of *P. pulmonarius* and *P. ostreatus* stalk meals is shown in Table 7. The substrate loss, pH, carbon IV oxide gas production, total gas produced, and asymptotic gas production were not significantly different ($P > 0.05$). However, there were significant differences ($P < 0.05$) in methane gas production, ammonia nitrogen from filtrate after fermentation, ash residue, switching characteristics of the curve, time at which half of the asymptote was reached ($T_{1/2}$), maximum rate of gas production (R_{max}) and the time of maximum rate of gas production (T_{max}). The two stalk meals had a cummulative gas production of 16.50 ± 0.5 mL at the end of 60 hours. *Pleurotus ostreatus* stalk meal had a significantly higher methane gas production of 6.5 ± 0.5 ml and ammonia nitrogen (0.03 ± 0.01) from filtrate after fermentation. *Pleurotus ostreatus* stalk meal had a significantly ($P < 0.05$) lower R_{max} of 0.04 ± 0.01 mL/hour and took a longer time ($P < 0.05$) of 6.04 ± 0.5 hours to reach its T_{max}; while *P. pulmonarius* had a significantly higher ($P < 0.05$) R_{max} of 0.16 ± 0.04 mL/hour and took a significantly ($P < 0.05$) shorter time of 4.96 ± 0.19 hours to reach its T_{max}.

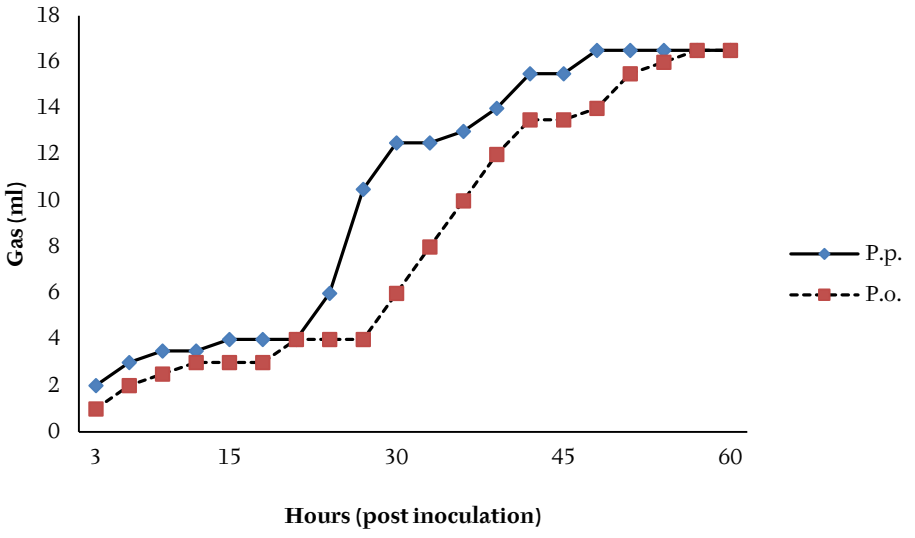

Figure 1. Cummulative gas production profile of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meals at 3 hours intervals for sixty hours; P.p. *Pleurotus pulmonarius* stalk meal; P.o. *Pleurotus ostreatus* stalk meal

Cumulative gas production profile of *Pleurotus pulmonarius* and *Pleurotus ostreatus* at 3-hour intervals for sixty hours

Figure 1 captures the cummulative gas production profile of *P. pulmonarius* and *P. ostreatus* stalk meals at 3-hour intervals for sixty hours. It was observed that there was a gradual production of gas until after 21 hours for *P. pulmonarius* stalk meal and 27 hours for *P. ostreatus* stalk meal when gas production increased. However, gas production became constant at 48 hours for *P. pulmonarius* and 51 hours for *P. ostreatus* stalk meal.

DISCUSSION

Proximate composition of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meals

Proximate composition is essential in estimating the nutrient content of a sample material. It was observed that both stalk meals had the basic food nutrients. The higher crude protein, ether extract, crude fibre and moisture observed in the air-dried stalk meal of *P. ostreatus* observed in this study when compared with *P. pulmonarius* stalk meal may be due to specie variation as they were grown on same cultivation substrate and under same ecological and processing (drying) conditions.

The crude protein of the air-dried *P. pulmonarius* and *P. ostreatus* stalk meals did not follow the trend as described by Buwjoom et al. (2004); Oboh and Shodehinde (2009); Nasiri et al. (2013) and Camay (2016). They recorded higher crude protein content in Shiitake stalk meal, stalks of three edible mushrooms (*Termitomycetes robustus*, *Coprinus* species and *Volvariella estulenta*), Botton mushroom stalks and *P. ostreatus* waste powder (mushroom stalks and rejects), respectively. The protein content may vary due to factors such as species, texture, freshness of mushroom before experiment, mushroom part analysed and humidity (Barros et al., 2007).

The ether extracts of the air-dried *P. pulmonarius* and *P. ostreatus* stalk meals in our study revealed that they had a low fat content. This is in agreement with the observation of Nasiri et al. (2013) on Botton mushroom stalks. It did not follow the trend reported by Buwjoom et al. (2004) and Camay (2016) who observed a much lower ether extract in Shiitake stalk meals and *P. ostreatus* waste powder, respectively. However, Oboh and Shodehinde (2009) reported higher ether extract content than the one obtained in this study in the stalks of three edible mushrooms. These differences may be due to variation in cultivation substrate for the mushrooms, ecological conditions, and the species of mushroom.

The crude fibre of the air-dried *P. pulmonarius* and *P. ostreatus* stalk meals was within the range obtained by Oboh and Shodehinde (2009) on the stalks of three mushrooms (*T. robustus*, *Corprinus* species and *V. esculenta*). The result obtained in this study was also similar to that obtained by Camay (2016) for *Pleurotus ostreatus* waste powder. However, it did not follow the trend observed by Buwjoom et al. (2004) and Nasiri et al. (2013). They reported higher content of crude fibre in Shiitake stalk meal and Botton mushroom stalks, respectively. The crude fibre obtained in this study suggests that *P. pulmonarius* and *P. ostreatus* stalk meals could be potential sources of dietary fibre and prebiotics.

The ash content of air-dried *P. pulmonarius* and *P. ostreatus* stalk meals did not follow the trends reported by Buwjoom et al. (2004); Oboh and Shodehinde (2009); Nasiri et al. (2013) and Camay (2016). They recorded higher ash content in Shiitake stalk meals, stalks of three edible mushroom (*T. robustus, Coprinus species* and *V. esculenta*), Botton mushroom stalks and *P. ostreatus* waste powder, respectively. The difference in ash content may be due to the species of mushroom as well as the substrate used in the cultivation of the mushrooms, according to Wang et al. (2015), mushrooms bioaccumulate minerals from their substrates.

The moisture content of the air-dried *P. pulmonarius* and *P. ostreatus* stalk meals were within the permissible limits of less than 10%. This implies that the stalk meals may be kept for a longer period of time and it will be less prone to microbial spoilage, as the content of moisture in a sample is an important indicator of its shelf life. In mushrooms, the moisture content depends on the species, maturity of fruiting body, growing environment, postharvest environment and processing method (Guillamon et al., 2010).

The nitrogen-free extract, also known as carbohydrates of the air-dried *P. pulmonarius* and *P. ostreatus* stalk meals were relatively high and was the most abundant nutrient. The result obtained did not follow the trend reported by Buwjoom et al. (2004), Oboh and Shodehinde (2009), Nasiri et al. (2013) and Camay (2016). They recorded lower carbohydrate content in Shiitake stalk meals, stalks of three edible mushroom (*T. robustus, Coprinus* species and *V. esculenta*), Botton mushroom stalks and *P. ostreatus* waste powder, respectively. These differences could be due to the variation in cultivation substrate, environmental factors and the species of mushroom. The elevated

carbohydrate in the stalks shows that they could be good energy source and act as prebiotic in fish diets.

Fibre fractions of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meals

Mushrooms are rich in non-digestible fibre. The differences in the fibre fractions of *P. pulmonarius* and *P. ostreatus* stalk meals may be due to the difference in specie of mushroom because they were grown on same substrate composition and under same ecological and processing conditions. The stalk meal of *P. ostreatus* had higher quantity of neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and cellulose (chitin); whereas *P. pulmonarius* stalk meal had a higher content of hemicellulose. Goyal et al. (2015) studied the dietary fibre fraction of the fruiting bodies of *Agaricus bisporus* and *Pleurotus sajor-caju*. They reported much lower contents of NDF (41.17% and 43.52%), ADF (15.72% and 17.20%), ADL (6.34% and 7.22%), hemicelluloses (25.47% and 26.32%) and cellulose (8.71% and 10.17%), respectively, which were not within the range of the values recorded in this study. Rangunathan et al. (1996), however, reported 28.4–44.8% cellulose, 28–41.2% hemicelluloses and 13.0–17.0% lignin in the fruiting body of *P. sajor-caju*. The range obtained for cellulose (chitin) in their study was within the value obtained from *P. ostreatus* stalk meal in this study. However, the value they reported for hemicelluloses and cellulose were higher, while lignin content was lower than the one obtained in this study. The variation in the result of this study compared to the other published works may be attributed to difference in the part of the mushroom from which the dietary fibre analysis was done as they analysed the fruiting body, while this study analysed the stalks. Also, the stalks of mushrooms have been reported to be more fibrous than the fruiting body of mushrooms (Chou et al., 2013).

Mineral composition of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meals

Quantitatively, the mineral elements obtained in this study were not in consonance with the report of Egwim et al. (2011) and Olagbemide and Ogunnusi (2015) on ten selected mushroom and *Cortinarius* species, respectively, who reported that potassium was predominant in the mushrooms they analysed. Egwim et al. (2011) found that manganese and sodium were in minimal amount in their study. Also Kalac (2009) stated that potassium was the prevailing element followed by phosphorus, while calcium and sodium were the least in mushrooms. According to Odoh et al. (2017), calcium was predominant and trailed by magnesium, potassium, and sodium in ten edible mushrooms, while Adebiyi et al. (2016) observed phosphorus to be the predominant mineral, followed by potassium in *T. robustus*. In agreement with the findings of this study, Vetter et al. (1994) reported that the stalks of mushrooms have greater content of sodium than the pilei. The mineral concentrations of mushrooms may be influenced by factors such as the species, age, cultivation substrate and the mushroom part analysed. The large differences in manganese and copper content of *P. pulmonarius* and *P. ostreatus* stalks may implies that their ability to extract micro-minerals from substrate differ as the cultivation substrate was identical.

Qualitative and quantitative analyses of ethanolic extracts in *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meals

Phytochemicals are chemically bioactive compounds that are non-nutritive in nature and have disease averting properties (Murugan et al., 2013). Mushrooms generally contain secondary metabolites that are pharmacologically active in diet (Soetan and Oyewole, 2009). The most abundant phytochemical observed in both species was flavonoid and the lowest was phlobatannin. Flavonoids are water-soluble polyphenolic complexes that elicit several biological functions, such as antioxidative, antiviral, antibacterial, immune stimulant and vasodilatory properties (Cushnine and Lamb, 2011). The high content of flavonoids in *P. pulmonarius* and *P. ostreatus* stalk meals indicates that they may possess antioxidant activity and may also act as biological response modifiers (Tullanithi et al., 2010). In accordance with this study, some wild edible mushrooms possessed some phytochemicals as reported by Egwim et al. (2011), who observed the presence of saponins, flavonoids, tannins and alkaloids but did not detect anthraquinones and steroids in ten selected wild edible Nigerian mushrooms. Moglad and Saadabi (2012) also reported that flavonoids, alkaloids, saponins and tannins were observed in wild mushrooms used for antimicrobial activities. Also, Sasidhara and Thirunalasundari (2014) found that *Pleurotus djamor* had anthraquinones, flavonoids, saponins, tannins and terpenoids but no cardiac glycosides and steroids. However, saponins were not observed in both stalk meals of this study. This may be due to the difference in substrates or the part of the mushroom analysed (stalk) in this study, as the authors analysed the fruiting body of the mushrooms.

In vitro digestibility of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meals

The use of the enzymes pepsin and pancreatin, to pre-digest *P. pulmonarius* and *P. ostreatus* stalk meals resulted in the partial digestion of both stalk meals. This may be due to the other nutrient constituents of the stalk meal. It may also indicate that the carbohydrate would resist gastric acidity and enzymatic hydrolysis *in vivo*. The stalk meals consequently met the initial condition for the classification of a feed ingredient as a potential prebiotic (Roberfroid, 2007).

In vitro fermentibility of *Pleurotus pulmonarius* and *Pleurotus ostreatus* stalk meals

Gas production is a function of degradable carbohydrates and microbial fermentation. *Pleurotus pulmonarius* and *P. ostreatus* stalk meals were fermentable by the intestinal microbiota of post-juvenile *Clarias gariepinus*. The non-significant difference ($P > 0.05$) in the pH may be due to the high buffering capacity of the buffer used in the *in vitro* fermentation process. The metabolites produced in terms of methane and carbon IV oxide gases may be as a result of carbohydrate fermentation to short chain fatty acids. *Pleurotus ostreatus* stalk meal produced more methane gas, which indicates that it may favour more methanogenic bacteria growth which may account for more energy loss (Bhatta et al., 2007). The minute ammonia nitrogen observed may be due to the pre-digestion of the stalk meals in which protein had been digested before fermentation. Hence, it could be inferred that the bacteria fermentation process was by saccharolytic fermentation process and not proteolytic/putrefactive. The total gas produced reflects the activity of anaerobic and methanogenic microorganisms' activity in the inoculum and the potential of the test ingredient to stimulate fermentation in the gut of the fish.

The fermentation kinetics (R_{max} and T_{max}) is used to assess the fermentability of feed ingredients *in vivo*. The rate of fermentation of the test ingredient is an indicator of its ease of access to microbial enzymes as well as growth of microorganisms. The result of this study followed the same trend as the observation of Guo et al. (2003), who worked on the *in vitro* fermentability of two mushrooms (*Lentinus edodes* and *Tremella fuciformis*) and their polysaccharide fractions using microflora from chicken ceca. The fermentation kinetics in this study indicated that *P. pulmonarius* stalk meal may have better prebiotics activity and may be fermented at an earlier and more proximal part of the small intestine with significant microbial population, while *P. ostreatus* stalk meal may be fermented at a more distal part of the gut of the fish, because feeds that are fermented in a slow manner are more liable to be fermented in the later part of the intestinal tract (Williams et al., 2005). The fermentation kinetics profile showed that *Pleurotus* species stalk meal may sustain saccharolytic fermentation as a prebiotic and may boost the growth of beneficial microorganism in the gut of *C. gariepinus*.

CONCLUSION

The two *Pleurotus* species stalk meals possessed basic nutrients and bioactive constituents. *P. pulmonarius* and *P. ostreatus* stalk meals had prebiotic activity as observed in the *in vitro* digestibility and fermentability analysis. Both stalk meals could act as a low-economic value source of prebiotics in aqua feed due to the high Nitrogen free extract (carbohydrate), high fibre content, partially digestible and fermentability. Hence, its use as supplement or prebiotics in aqua feeds should be encouraged.

CONFLICT OF INTEREST

The authors declared no conflicts of interest with respect to research, authorship and publication of this article.

ETHICAL COMPLIANCE

The authors have followed the ethical standards in conducting the research and preparing the manuscript.

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