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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2018.29.37

Research Article
Effects of Adding Chicken Blood Meal and Fishmeal to Sludge Biogas as White Oyster Mushroom Media

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Abstract

Background and Objective: The biogas sludge is generally not used optimally and people even pour it directly into the river that it cause environmental pollution. One of the ways to get the benefit from sludge and chicken blood meal or fishmeal is to use it as a substitute for bran in mushroom media. This study aimed to improve the nutrient content of biogas sludge with the addition of chicken blood meal (CBM) and fishmeal (FM) as substitute material for bran in white oyster mushroom media. **Materials and Methods:** The biogas sludge was dried in the sun for 3 days until its form resembled the soil. The treatment consisted of dried biogas sludge without CBM (BP0), dried biogas sludge with 1% CBM (BP1) and dried biogas sludge with 3% CBM (BP2). Each was added to the media of white oyster mushroom as much as 15% as substitute material for bran. The other treatment consisted of dried biogas sludge without FM (FP0), dried biogas sludge with 2% FM (FP1) and dried biogas sludge with 4% FM (FP2). Each was added to the media of white oyster mushroom as much as 5% as substitute material for bran. BP0 and FP0 created from white oyster mushroom media were commonly used by farmers. Each treatment was analyzed of nutritional and biological contents. All the data were tested using completely randomized design one-way ANOVA. **Results:** The results of the research on the use of chicken blood meal and fishmeal showed that the best treatments were BP2 and FP2. Their nutrient content increased, including the organic-C, organic matter, nitrogen, P_2O_5 and K_2O. The productivity of the oyster mushroom increased, shown by the increase of fresh weight and diameter of caps in treatment BP2. The replacement of bran by biogas sludge with 4% FM addition (FP2) in oyster mushroom media increased the fresh weight, the number of caps and the length of oyster mushroom stalks. **Conclusion:** The best treatments for mushroom media were BP2 and FP2 to be used as substitute material for bran in white oyster mushroom media.

Key words: Biogas sludge, chicken blood meal, fishmeal, nutrient content, white oyster mushroom media

Citation: Ambar Pertiwiningrum, Dianita Juli Hapsari, Palupi Ratnaningrum, Asih Kurniawati, Rochijan and Ramdhan Dwi Nugroho, 2018. Effects of adding chicken blood meal and fishmeal to sludge biogas as white oyster mushroom media. Pak. J. Biol. Sci., 21: 29-37.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Indonesia has very diverse farm business. One example is beef cattle fattening that produces animal waste that can become a source of pollution if not managed properly. Hapsari[1] stated that one of the waste of cattle farming is solid waste that can be utilized as biogas as alternative energy source. Most farmers use fecal matter as raw material in biogas production process. Faecal matter produces methane gas (CH_4) and waste in the form of mud. In addition to biogas as its main product, the installation also produces biogas sludge that is rich in organic material. Sludge from cows contains high crude fiber and various minerals needed by plants, such as phosphorus, magnesium, calcium, kalium, copper and zinc[2]. The biogas sludge is generally not used optimally and people even pour it directly into the river that it cause environmental pollution.

Ratnaningsih[3] stated that besides sludge, another livestock waste that can be used as a source of plant nutrients is blood, which is the waste of abattoirs (slaughter houses) that can be processed into blood meal. According to Aladetohun and Sogbesan[4], blood meal contains 34.35% crude protein, 1.49% ether extract, 9.85% fibre, 9.00% ash, 9.50% moisture and 35.85% nitrogen free extract (NFE). One of the ways to get the benefit from sludge and chicken blood meal (CBM) or fishmeal (FM) is to use it as substitute for bran in mushroom media. According to Sukimin[5], bran is usually used for animal feed because it contains 11.3-14.4% protein, 15-19.7% fat and 34.1-52.3% carbohydrate. Fishmeal is the waste of fish processing or the leftover pieces of fish, with or without the extraction of fish oil. It contains very high crude protein, reaching 55-72%[6]. Fishmeal or fish powder is usually used as plant fertilizer. The use of fishmeal as fertilizer varies between 4-8%. Fishmeal reacts quickly and is good for all kinds of plants and soil[7]. The percentage of fishmeal's component, according to Harris *et al*.[8], is as follows: 12.21% water content, 47.15% crude protein, 2.52% crude fiber, 26.09% ash, 11.02% fat, 5.47% calcium (Ca), 3.48% phosphorus (P) and 2.47% NaCl. Based on the literature, sludge contains the same level of crude fiber as bran and chicken blood meal has higher protein level than bran.

Pleurotus species requires a short growth time, compared to other mushrooms. Its fruiting body is not often attacked by diseases and pests and it can be grown in a simple and cheap way, with high yield, wider substrate utilization, sporelessness, wide temperature and chemical tolerance as well as environmental bioremediation. It is an edible mushroom and also has several biological effects as it contains important bioactive molecules[9]. This genus degrades cellulose, hemicellulose and lignin of wood, whereas brown rot fungi only degrade cellulose and hemicellulose[10]. According to Velioglu and Urek[11], in basidiomycete fungi, extracellular laccases are constitutively produced in small amounts and the lignocellulolytic enzymes are affected by many typical fermentation factors, such as medium composition, pH, temperature, aeration rate, etc.

This study aimed to determine the effect of chicken blood meal (CBM) and fishmeal (FM) addition on the productivity of oyster mushroom and on the nutrient content of the media. The CBM and FM were separately added to biogas sludge from cattle faeces as substitution for bran in oyster mushroom media.

MATERIALS AND METHODS

Materials: The study was conducted for 5 months from June-November, 2017 at the farm of Center of Agro-Technology Innovation, Universitas Gadjah Mada (PIAT-UGM). The main materials used in this study were chicken blood meal (from the abattoir of Faculty of Animal Science UGM), fishmeal (waste from Kranggan Market, Yogyakarta), salt, water, sludge (of biogas from beef cattle's faeces), sawdust, bran, limestone, gypsum and the spawn of white oyster mushroom (*Pleurotus florida*) (from the Center of Agro-Technology Innovation, UGM). The tools used in the field were pans, stove, shovel, barrels, press tool, bucket, thermometer, sprayer, stirrer (spatula), pH papers, plastic, cotton, pipe rings, analytical balance, manual scales, glass bottles, hoe, hygrometer and polypropylene plastic sheet.

The tools used in the laboratory included porcelain dishes, analytical balance, beaker glass, oven, desiccators, furnaces, Kjeldahl flask, destroying tool, distillation equipment, Erlenmeyer, vacuum pump, filter paper, laboratory glassware, pipettes, vortex mixer, water bath, glass wool, Gooch crucible, spectrophotometers and flame photometer. The chemicals included $K_2Cr_2O_7$, H_2SO_4, H_3PO_4, diphenylamine indicator, methyl red indicator, methyl blue indicator, distilled water, $FeSO_4$, NaOH, selenium mixture, boiling stones, H_3BO_3, HCl, HNO_3 and vanadate-molybdate solution. All chemicals used in this study were of analytical grade.

Methods

Making of blood meal: Chicken blood as the material for blood meal was obtained from the abattoir of Faculty of Animal Science, Universitas Gadjah Mada. A total of 1 L of fresh blood was poured into a pan, added with 10 g of salt (1% of the blood volume), cooked over a medium heat and stirred constantly until it thickened and blackened (15-20 min). The

boiled blood was then left for a while at room temperature, then thinly sliced and dried in the sun until its moisture level reduced to 20% (±2 days). The dried blood was then mashed or crushed using a grinding machine. The blood meal was filtered to obtain the fine powder.

Making of fishmeal: The fish waste as the material for fishmeal was obtained from Kranggan Market, Yogyakarta. The raw material obtained from the market was washed until it was clean and steamed for 1 h at the temperature of 100°C so it was perfectly cooked. Then, it was crushed and dried in the sun until its moisture level reduced to 8% for 1-2 days. Using a grinding machine, it was then milled and sieved to obtain fish flour with quite smooth texture that was ready to be used on the media.

Addition of chicken blood meal (CBM) to biogas sludge: The biogas sludge from cattle manure was dried in the sun for 3 days until its form resembled the soil, then divided into 3 parts. The media with 0% CBM was called BP0 (which is commonly used by mushroom growers), the one with the addition of 1% CBM was BP1 and the media with the addition of 3% CBM was BP2. Each treatment had 3 replicates and was tested to determine the nutrient content, including water content, crude fiber, organic matter, organic carbon, total-N, C/N ratio, total-P and total-K. The mix of sludge with 1 and 3% blood meal was then added into the ingredients of mushroom media and made three replicates. The composition of each white oyster mushroom medium with the addition of chicken blood meal is shown in Table 1.

Addition of fishmeal (FM) to biogas sludge: The dried biogas sludge was divided into 3 parts and each was added with 0, 2 and 4% FM, respectively. The media with 0% FM was called FP0 (which is commonly used by mushroom growers), the one with the addition of 2% FM was FP1 and the media with the addition of 4% FM was FP2. Each treatment consisted of three replicates and was tested for the nutrient level, including water content, crude fiber, organic matter, organic carbon, total-N, C/N ratio, total-P and total-K. The composition of white oyster mushroom medium is presented in Table 1.

Making of oyster mushroom media: In the process of wrapping the media with heat-resistant plastic (polypropylene), both ends of the plastic were bent inward. Each weighed about 800 g and was pressed down with a glass bottle to make it more dense. The plastic bags were filled with media as much as ¾ parts, then the ¼ part was bent inward and sealed with pipe ring. The ring hole should be covered with cotton and small-sized plastic that was tightened with rubber bands. This would make white oyster mushroom media in plastic (baglog). Each ready-to-use baglog should be marked P0, P1 and P2 with a marker pen, then put into sterilization barrel. After that as much as ¼ of the sterilization barrel was filled with water, sealed and heated. The sterilization carried out for 5 h at the temperature of 90°C.

Once the proper sterilization was completed, the mushroom media was taken out and left to cool for 15 h until its temperature became about 40°C. The next step was the inoculation. This study used the seeds of white oyster mushroom *Pleurotus florida* that were grown on the corn medium and obtained from the farm of the Center of Agro-Technology Innovation, Universitas Gadjah Mada (PIAT-UGM). The seeds in the form of corn grains were sown inside the upper part of the grow-bag (5 grains for each grow-bag) with the help of a spatula which had been sterilized over frame before hand. The grow-bags were resealed with cotton and plastic, then tied together with rubber bands to avoid contamination. The grow-bags of white oyster mushrooms were placed on the shelves in the incubation

Table 1: Composition of oyster mushroom media with addition of chicken blood meal and fishmeal

Constituents of media (g)	Treatments					
	BP0	BP1	BP2	FP0	FP1	FP2
Sawdust	664	664	664	1,162	1,162	1,162
Bran	120	80	80	210	140	140
Sludge	-	32	16	-	42	14
Chicken blood meal	-	8	24	-	-	-
Fishmeal	-	-	-	-	28	56
$CaCO_3$	8	8	8	14	14	14
Gypsum	8	8	8	14	14	14
Total	800	800	800	1,400	1,400	1,400

BP0: Mushrooms on commercial media, BP1: Sludge+1% CMB, BP2: Sludge+3% CBM, FP0: Mushrooms on commercial media, FP1: Sludge+2% FM, FP2: Sludge+4% FM

room. The temperature was maintained at 24-29°C with a humidity of 65-70% until the grow-bags were covered in mycelium. The temperature and humidity measurements were performed daily using a hygrometer. Once the entire grow-bags were all covered in white mycelium, they were moved to "kumbung" or mushroom hut. The grow-bags' cover were opened, the room temperature was maintained at 21-27°C and the humidity at 80-85% by watering them twice daily, in the morning and in the afternoon until the mushrooms grow and could be harvested approximately 60 days after inoculation. Then white oyster mushrooms were harvested and calculated their wet weight, the number of mushroom caps, the diameter of mushroom caps and the length of the mushroom stalk.

Nutritional analysis: The nutrient level in biogas sludge, the mixture of sludge and blood meal, the mixture of sludge and fishmeal and oyster mushroom media can be determined by a test to obtain moisture content, level of organic-C, organic matter content, level of crude fiber, N content, level of C/N ratio, level of P_2O_5 and K content[12].

Biological analysis: Biological quality was measured by observing the environment of white oyster mushroom's growth and measuring the macroscopic morphology of white oyster mushroom at the time of the first harvest. The environmental conditions of white oyster mushroom growth was determined by measuring the temperature and humidity once a day at noon. The measurement of macroscopic morphology included the wet weight, the number of mushroom caps, the diameter of mushrooms caps and the length of the mushroom stalk taken at the time of the first harvest.

Statistical analysis: The data obtained were processed with completely randomized design one-way ANOVA (Analysis of Variance) and the difference existed between the means ($p<0.01$) would be analyzed with Duncan's new multiple range test.

RESULTS AND DISCUSSION

Nutrient content of biogas sludge: To determine the nutrient content of the mixture materials (sludge with chicken blood meal and sludge with fishmeal), a laboratory analysis was conducted to examine some chemical elements including the water content, crude fiber, organic carbon, organic matter, nitrogen, C/N ratio, P_2O_5 and K_2O. The results of the tests are shown in Table 2 and 3.

Biogas sludge with the addition of 1% CBM (BP1) lowered the moisture content, crude fiber and C/N ratio. Biogas sludge with the addition of 3% CBM (BP2) also lowered the moisture content, crude fiber and C/N ratio (Table 2). The cause was the addition of blood meal that had low water

Table 2: Average nutrient content of biogas sludge with addition of chicken blood meal

Variables	BP0	BP1	BP2
Water content (%)	45.52±0.09^{c}	23.36±0.38^{b}	21.62±0.32^{a}
Crude fiber (%)	34.23±0.09^{c}	31.91±0.21^{b}	28.99±0.06^{a}
Organic carbon (%)	33.10±0.11^{a}	34.17±0.13^{b}	35.08±0.43^{c}
Organic materials (%)	57.08±0.19^{a}	58.91±0.22^{b}	60.49±0.74^{c}
Nitrogen (%)	0.39±0.00^{a}	1.08±0.05^{b}	1.33±0.04^{c}
C/N ratio	88.05±5.10^{b}	31.93±1.38^{a}	26.29±1.19^{a}
P_2O_5 (%)	0.31±0.00^{a}	0.41±0.01^{b}	0.48±0.00^{c}
K_2O (%)	0.21±0.00^{a}	0.24±0.01^{b}	0.26±0.01^{c}

BP0: Mushrooms on commercial media. BP1: Sludge+1% CBM, BP2: Sludge + 3% CBM, ^{a,b,c}: Different superscripts in the same row indicate differences ($p<0.01$)

Table 3: Average nutrient content of biogas sludge with addition of fishmeal

Variables	FP0	FP1	FP2
Water content (%)	13.69±0.40^{b}	11.40±0.14^{a}	11.26±0.12^{a}
Crude fiber (%)	37.55±0.13^{c}	34.23±0.09^{b}	33.52±0.37^{a}
Organic carbon (%)	31.75±0.10^{a}	36.04±0.33^{b}	36.48±0.17^{b}
Organic materials (%)	54.74±0.18^{a}	62.14±0.56^{b}	62.68±0.10^{b}
Nitrogen (%)	1.46±0.02^{a}	1.77±0.04^{b}	1.81±0.05^{b}
C/N ratio	21.38±0.78^{ns}	19.57±0.75^{ns}	19.47±0.69^{ns}
P_2O_5 (%)	0.59±0.00^{a}	0.73±0.44^{b}	0.78±0.39^{b}
K_2O (%)	0.25±0.00^{a}	0.29±0.02^{b}	0.31±0.02^{b}

FP0: Mushrooms on commercial media, FP1: Sludge+2% FM, FP2: Sludge+4% FM, ^{a,b,c}: Different superscripts in the same row indicate differences ($p<0.01$), n: Non-significant

Table 4: Average nutrient content of mushroom media with chicken blood meal

Variables	BP0	BP1	BP2
Water content (%)	51.69±0.10^{c}	28.03±0.14^{b}	25.64±0.01^{a}
Crude fiber (%)	33.60±0.24^{c}	30.67±0.05^{b}	27.77±0.05^{a}
Organic carbon (%)	28.32±0.11^{a}	30.73±0.10^{b}	31.19±0.10^{c}
Organic materials (%)	48.84±0.19^{a}	52.99±0.18^{b}	53.78±0.17^{c}
Nitrogen (%)	0.34±0.00^{a}	0.57±0.01^{b}	0.98±0.01^{c}
C/N ratio	81.44±0.45^{c}	53.87±0.39^{b}	31.53±0.39^{a}
P_2O_5 (%)	0.35±0.00^{a}	0.46±0.00^{b}	0.51±0.00^{c}
K_2O (%)	0.06±0.00^{a}	0.10±0.00^{b}	0.10±0.01^{b}

BP0: Mushrooms on commercial media, BP1: Mushrooms on media with sludge+1% CBM, BP2: Mushrooms on media with sludge+3% CBM, ^{a,b,c}: Different superscripts in the same row indicate differences ($p<0.01$)

Table 5: Average nutrient content of mushroom media with fish meal

Variables	FP0	FP1	FP2
Water content (%)	51.70±0.10^{c}	47.53±0.14^{b}	42.66±0.12^{a}
Crude fiber (%)	33.60±0.24^{c}	32.42±0.27^{b}	30.40±0.25^{a}
Organic carbon (%)	28.39±0.11^{a}	31.73±0.12^{b}	34.51±0.10^{c}
Organic materials (%)	48.95±0.19^{a}	54.74±0.19^{b}	59.39±0.18^{c}
Nitrogen (%)	0.35±0.01^{a}	0.38±0.02^{b}	0.41±0.00^{b}
C/N ratio	80.43±2.23^{ns}	82.11±0.26^{ns}	83.11±1.11^{ns}
P_2O_5 (%)	0.35±0.00^{a}	0.43±0.48^{b}	0.49±0.00^{c}
K_2O (%)	0.06±0.00^{a}	0.10±0.00^{b}	0.12±0.00^{c}

FP0: Mushrooms on commercial media, FP1: Mushrooms on media with sludge+2% FM, FP2: Mushrooms on media with sludge+4% FM, ^{a,b,c}: Different superscripts in the same row indicate differences ($p<0.01$), ns: Non-significant

content so it could reduce the water content and crude fiber in the sludge. Manyi-Loh *et al.*[13] stated that the moisture content of fresh dairy cow's manure is 86.6%. Divakaran[14], stated that the moisture content in chicken blood meal is 9-10% and crude fiber is 0.8%. The increase of C/N ratio is influenced by the level of organic-C. The lower the C/N ratio, the higher the amount of organic-C[15].

Tamara[16] stated that C/N ratio in dairy feces is 22.12%. The levels of organic-C, organic matter, nitrogen, phosphorus and kalium increased significantly ($p<0.01$) in biogas sludge with the addition of 1% CBM (BP1) and in BP2 (biogas sludge with 3% CBM addition), the levels of organic-C, organic matter, nitrogen, phosphorus and kalium increased in (Table 2).

Biogas sludge with the addition of 2% FM (FP1) lowered the moisture content, crude fiber and C/N ratio, while biogas sludge with the addition of 4% FM (FP2) lowered the moisture content, crude fiber and C/N ratio (Table 3). The decrease of C/N ratio in biogas sludge with the addition of 2 and 4% FM was not significant. The levels of organic-C, organic matter, nitrogen, phosphorus and kalium increased significantly ($p<0.01$) in biogas sludge with the addition of 2% FM (FP1), while in FP2 (biogas sludge with 4% FM addition), the levels of organic-C, organic matter, nitrogen, phosphorus and kalium increased (Table 3). Also, because chicken blood meal contained carbon and organic material in large quantities, the addition of it in biogas sludge can increase the carbon content and organic material[14], while the increase of N-content is due to high protein[17] contained in blood meal. Gombo *et al.*[18] stated that the level of protein, fat, crude fiber, ash, kalium and phosphorus in fishmeal is 58.52, 13.90, 2.95, 25.11, 7.04 and 3.67%, respectively.

Nutrient content of mushroom media: To determine the nutrient content of mushroom media, a laboratory analysis was conducted. The chemical elements in the media included water content, crude fiber, organic carbon, organic matter, nitrogen, C/N ratio, P_2O_5 and K_2O. The results obtained from these tests are shown in Table 4 and 5.

BP1 mushroom media had low level of moisture content, crude fiber and C/N ratio, while BP2 had even lower ones. On the other hand, the level of C-organic, organic matter, nitrogen, phosphorus and kalium in BP1 was high but BP2 media had higher ones (Table 4).

The water content in treated media was lower than in control media (BP0) because the microorganism activities in the mixture of blood meal and sludge made a big decrease in water content in BP1 and BP2. Kristiawati[19] stated that the water content in the substrate greatly affects the growth and development of oyster mushroom mycelium. If the moisture level is too low, which was less than 45%, the growth and development of mushroom mycelium will be disrupted or

Table 6: Average parameter of oyster mushrooms on media with chicken blood meal treatment

Variables	BP0	BP1	BP2
Harvesting age (days)	58.33±1.15^{ns}	58.33±1.15^{ns}	59.66±0.57^{ns}
Fresh weight (g)	55.33±5.80^{a}	78.33±5.68^{b}	83.40±1.57^{b}
Number of caps	9.00±2.00^{ns}	11.33±3.21^{ns}	6.33±2.08^{ns}
Length of stalks (cm)	4.07±0.48^{ns}	4.66±0.16^{ns}	4.68±0.06^{ns}
Diameter of caps (cm)	9.66±1.08^{a}	9.19±1.23^{a}	11.67±0.41^{b}

BP0: Mushrooms on commercial media, BP1: Mushrooms on media with sludge+1% CBM, BP2: Mushrooms on media with sludge+3% CBM, ^{a,b,c}: Different superscripts in the same row indicate differences ($p<0.01$), ns: Non-significant

Table 7: Average parameter of oyster mushroom son media with fish meal treatment

Variables	FP0	FP1	FP2
Fresh weight (g)	55.33±5.80^{a}	61.50±6.02^{a}	76.56±1.33^{b}
Number of caps	9.00±2.00^{b}	5.67±1.15^{a}	10.67±1.15^{b}
Length of stalks (cm)	4.07±0.48^{ns}	4.23±0.30^{ns}	4.48±0.63^{ns}
Diameter of caps (cm)	9.66±1.08^{ns}	9.17±0.50^{ns}	9.59±0.60^{ns}

FP0: Mushrooms on commercial media, FP1: Mushrooms on media with sludge+2% FM, FP2: Mushrooms on media with sludge+4% FM, ^{a,b,c}: Different superscripts in the same row indicate differences ($p<0.01$), ns: Non-significant

even stopped altogether. On the other hand, if there is too much water, the mycelium will rot and die. Based on the analysis, the water content in control sample was in a range of water level that was needed for the mushrooms to grow optimally, while the water content in BP1 and BP2 media was less than 45% so it needed intensive watering to increase its level in the media.

The low level of crude fiber in BP1 and BP2-lower than in control media (P0) was because the blood meal in the sludge had low level of crude fiber as the result of microorganism activities. The crude fiber contained in the media affected the growth of mushroom. Crude fiber, cellulose and lignin are needed for the mushroom to grow. Mushrooms need a source of carbon in the form of cellulose compounds, hemicellulose and lignin as a source of nutrition[20]. FP1 media had a low level of moisture and crude but FP2 had even lower ones (Table 5). However, FP1 had the increasing level of organic-C, organic matter, nitrogen, C/N ratio, phosphorus and kalium, while FP2 media had even higher level (Table 5).

High nitrogen content and C/N ratio in FP1 and FP2 were due to the addition of blood meal containing a large amount of nitrogen so mushroom media with the treatment had higher nitrogen content than the media control (FP0). Nitrogen as a source of protein is needed in the formation of living tissues that are actively growing and it affects the diameter of mushroom caps[21]. According to Li *et al.*[22], the high C/N value was beneficial for high level of crude protein, amino acids, 5′-nucleotides and equivalent umami concentration, while lower C/N value was beneficial to carbohydrate, polysaccharides and trehalose production. Sutanto[23] stated that the percentage of C/N is determined by the arranged components of basic material. The research by Febriansyah[24] stated that C/N ratio treatment of 20.31 in white oyster mushroom media can increase the production of oyster mushroom by 73.14% compared to C/N ratio treatment of 40.55. Based on the results of previous studies, the lower C/N ratio of mushroom media, the higher productivity of white oyster mushroom. The addition of sludge and chicken blood meal can lower C/N ratio so the mushrooms could reach their optimal productivity compared to the ones in media that used bran only.

Biological quality of white oyster mushroom's growth: To observe biological parameters of the mixture of sludge with chicken blood meal and sludge with fishmeal, the oyster mushrooms were cultivated. The purpose of the cultivation was to determine the effect of both mixtures so the quality of the nutrient content and the best mixture would be known for cultivating oyster mushrooms. The variables being observed in oyster mushrooms are shown in Table 6 and 7.

The analysis of the first harvest showed that BP1 and BP2 media had a significant influence ($p<0.01$) on the fresh weight. However, BP1 and BP2 media did not have any real effect on the harvesting age, number of caps and length of stalks. The percentage of fresh weight and length of the mushroom stalks in BP1 increased, while the number of the mushroom caps and their diameter in BP1 decreased (Table 6). The BP2 media had the increased wet weight, the diameter of mushroom caps and the length of mushroom stalks, while the number of caps and the diameter of mushroom caps in BP2 decreased. The harvesting age of mushrooms in BP0 and BP1 was faster than

the ones in BP2. Parlindungan[25] stated that the entire surface of grow-bag will be covered by white mycelium within 40-60 days. In this research, it happened within 40 days and the plugs of the grow-bag should be opened then. The results of the study showed that the time required for the growth of the mycelium was in accordance with the literature.

The grow-bags should be opened between 1-2 weeks after the incubation for the mushrooms to begin to sprout and within 2-3 days, they will turn into fruit bodies. In this study, the shoots began to appear between 14-16 days after the grow-bags' plugs were opened and 3 days later, became fruit bodies. The time required for the shoots to grow was not in accordance with the literature for a variety of reasons, including temperature and humidity that were less supportive.

The temperature during the incubation and maintenance ranged between 25.2-31.2°C and 47-69% for humidity[25]. Leong[26] stated that the optimum temperature for the formation of fruiting bodies ranges between 21-27°C and the humidity is 70-80%. It was influenced by the environmental conditions including dry season, so the watering was done twice to maintain the temperature and humidity to make the mushroom grow optimally. According to Djarijah[27], the weight of oyster mushrooms at the first harvest ranges between 50-75 g. The fresh weight of the mushrooms is an indicator of their increased productivity. The fresh weight of oyster mushrooms in this study was in accordance with the literature and in the media with the mixture of sludge and 1% CBM, the fresh weight was even higher than in the literature.

The analysis of the first harvest showed that FP1 and FP2 media had a significant influence ($p<0.01$) on the fresh weight and the number of oyster mushroom caps. However, FP1 and FP2 media did not have any real effect on the length of stalks and the diameter of the mushroom caps. The percentage of fresh weight and length of the mushroom stalks in FP1 media increased, while the number of the mushroom caps and their diameter in FP1 decreased (Table 7). FP2 media had the increased fresh weight, number of caps and length of mushroom stalks, while the diameter of mushroom caps in FP2 media decreased (Table 7). This suggested that the composition of FP1 media was optimal for the productivity in terms of the number of caps when compared to the composition of FP2 media. The number of mushrooms caps is an indicator of mushroom's increased productivity[28]. Gunawan[29] stated that oyster mushrooms talks have the length between 0.5-4 cm. Based on the results of this research, the length of mushroom stalks in the media with the addition of sludge and chicken blood meal was longer than the one mentioned in the literature.

Buchanan[30] stated that white oyster mushroom has cap diameter of about 10-13 cm. The diameter of mushroom caps in FP2 media was in accordance with the literature, while the diameter of caps in FP0 and FP1 were not. It was caused by the lack of nutrients in mushroom media substrate that was used for oyster mushroom's physiological needs. Soenanto[28] stated that essential nutrients, especially cellulose from media substrate, will be absorbed by the spores to grow into mycelium and to develop into adult mushrooms.

CONCLUSION

Based on the results of this study, the best treatment to mushroom media was BP2 (3% CBM) and FP2 (4% FM) to be used as a substitute material for bran in white oyster mushroom media. BP2 with the addition of sludge and 3% CBM increased nutrient content (organic carbon, organic matter, nitrogen, $P_2O_5$and K_2O). The productivity of the oyster mushrooms increased in fresh weight, length of stalks and the diameter of the caps. FP2 with the addition of biogas sludge and 4% FM to oyster mushroom media could increase the fresh weight, the number of mushroom caps and the length of mushroom stalks.

SIGNIFICANCE STATEMENT

This study discovers the possible effect of using sludge combination of waste with chicken blood meal and fishmeal as bran substitute material on white oyster mushroom media which can be useful for the quality of mushroom produced. This study will help researchers to find the right composition for bran substitution in the fungus media to improve the quality of mushrooms. Thus, a new combination of waste utilization can produce high-quality white oyster mushrooms.

ACKNOWLEDGMENT

We gratefully acknowledge the funding from USAID through the SHERA program-Centre for Development of Sustainable Region (CDSR).

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