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Influence of C/N ratio on productivity and the protein contents of *Pleurotus ostreatus* grown in differents residue mixtures

Influencia de la relación C/N sobre la productividad y contenido proteico del *Pleurotus ostreatus* cultivado en diferentes mezclas de residuos

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Abstract

The influence of the composition of different agricultural substrates standardized C/N in the productivity and protein content of the fruiting bodies of *Pleurotus ostreatus* harvested in different mixtures was determined. Six mixtures were designed using the Mixtures of Agricultural Waste for Cultivation of *P. ostreatus* program, the nitrogen content varied from 0.5 to 1.4%. In three of them, soy flour with a composition of 3, 4 and 6% was used as nitrogen source. The mixtures were categorised according to their physiochemical characteristics, the substrates were adapted and inoculated with the strain 768/12. The best results, 177.37% biological efficiency, 31.13% protein and 2.64 day^{-1} production rate, this results were achieved with the mixture of 1% nitrogen and 47.99 of C/N form of 15% of rice husks, 40% of lentil stubble, 40% of sugar cane bagasse, 3% of soybean meal and 2% of calcium carbonate, confirming the high dependence of the C/N ratio in the productivity and protein content of *P*. *ostreatus*.

Keywords
Pleurotus ostreatus • nitrogen • biological efficiency • production rate • composition of agricultural waste

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Resumen

Se determinó la influencia de la composición de diferentes sustratos agrícolas estandarizados en C/N sobre la productividad y contenido proteico de los cuerpos fructíferos de *Pleurotus ostreatus* cosechados en diferentes mezclas de residuos. Se diseñaron seis mezclas utilizando el Programa de Mezclas de Residuos Agrícolas para el Cultivo de *P. ostreatus,* se varió el contenido de nitrógeno de 0,5 a 1,4%. En tres de ellas se utilizó como fuente nitrogenada harina de soya en un 3, 4 y 6%. Las mezclas fueron caracterizadas fisicoquímicamente, se adecuaron los sustratos y se inoculó con la cepa 768/12. Los mejores resultados, 177,37% de eficiencia biológica, 31,13% de proteína y 2,64 dia^{-1} de tasa de producción, fueron alcanzados con la mezcla con 1% de nitrógeno y 47,99 de relación C/N compuesta por 15% de cascarilla de arroz, 40% de rastrojo de lenteja, 40% de bagazo de caña de azúcar, 3% de harina de soya y 2% de Carbonato de calcio, confirmando la alta dependencia de la relación C/N en la productividad y contenido proteico de *P. ostreatus.*

Palabras clave

Pleurotus ostreatus • contenido de nitrógeno • eficiencia biológica • tasa de producción • composición de residuos agrícolas

Introduction

The *pleurotus* species has culinary, nutritional and medicinal properties (4, 10). The oyster mushroom, *Pleurotus ostreatus* is grown worldwide, and it is cheap and easy to implement. It is also one of the most popular because of its high nutritional value (19), the substrate left after harvesting the mushroom can be exploited as an organic fertilizer for soil remediation (22).

For a good growth of *P. ostreatus*, it is necessary that in the substratum sources of carbon and nitrogen can be found as well as other minerals such as: S, Ca, Mg, P, K and some lower concentrations of minerals such as: Fe, Zn, Mn, Cu and Mo (5, 24), with an ash content between 2.5 to 15.7% (28). Mushrooms are known as decomposers of organic matter in general and particularly of cellulose, for which it produces a series of enzymes (17, 27).

The species of *Pleurotus* degrades a complex lignin-cellulose-hemicellulose structure and takes nutrients for their development, growing in a wide range of woody materials such as agricultural wastes and forestry, which are mainly composed of polymers present in the walls of the plant cells, such as cellulose 40 to 60%, 15 to 35% hemicellulose and lignin 10 to 30%, being the main source of carbon and nitrogen (16, 18, 30).

Several studies have shown the need to supply the substrates poor in nitrogen with richer sources of this element (bran cereal, flour, soybeans, alfalfa, sunflower, etc.) (6) and the combination of different materials for the grow of *Pleurotus* (33, 37), which will impact the quality and yield of the culture (3, 9, 21, 29).

Forero *et al.* (2008) have reported that the waste mixture, in addition to providing more appropriate nutritional characteristics also helps increase the substrate structure (grain size and porosity) to facilitate the interchanges of gases for proper colonization of the substrate.

Curvetto *et al.* (2002) show results by using sunflower husk' flour as nitrogen supplement, achieving an increase of 50% or more in the production of *P. ostreatus*. According to these authors, enhancing the substrates with nitrogen is also important to increase the protein content in the Mushrooms. By raising the nitrogen content in the substrate from 0.65 to 1.3% promote an increase of the protein content from 17.1 to 28%; however, when nitrogen concentrations from 1.75 to 2.2% were used the substrates did not colonize. Therefore, despite it being an important strategy to allow the use of certain low waste nitrogen locally available, enrichment of substrates from certain values can lead to negative effects, in addition it can increase production costs (32, 33, 37). As a result, it is important to determine the nitrogen content in each raw material used in the formulation of the substrates for the cultivation of the mushrooms; to then determine the appropriate supplementation or combination with other materials. In the Bolivar Province-Ecuador, a variety of agricultural products are cultivated in cold and temperate climates, which leave considerable amounts of waste, used only partially as fodder while the rest is deposited on the field to be burned or thrown into landfills without any treatment, contributing to environmental pollution (26).

The purpose of this research was to evaluate the influence of the composition of different substrates standardized C/N on productivity and protein content of *Pleurotus ostreatus* grown in standardized mixtures residues.

Materials and methods

The materials used were corn and lentil stubble, barley and wheat straws; sugarcane bagasse and rice husks, from the province of Bolivar-Ecuador which were already categorized based on their physicochemical composition (11, 26). For each of the residues, approximately 20 kg at random were collected and moved to a covered warehouse for natural drying (18 to 22°C and RH between 70-75%) until further use.

Mixtures of residues standardized in C/N

For the mixture design the Mixtures of Agricultural Waste for Cultivation Program was used combining different proportions of residues from: corn stubble, rice husks, barley straws, lentil stubble, wheat straws, sugarcane bagasse and soybean meal. Each mixture was composed of three different residues, varying the nitrogen content in intervals from 0.5 to 1.4%. Six mixtures were designed (M1, M2, M3, M4, M5, M6), in three of them (M4, M5 y M6) soybean meal was used as nitrogen source in 3, 4 y 6% concentrations, searching for balance in the C/N relation, leaving the mixtures standardized in this indicator. All mixtures were added 2% of calcium carbonate.

The physicochemical characterization of each mixture was realized following the methods indicated in table 1 (page 334).

For the design of the mixtures a Mixtures Program (11) was used, varying the nitrogen content in ranges from 0.5 to 1.4%. Six mixtures were designed, in three of them soybean meal in a 3, 4, and 6% was used as nitrogen source looking for a balance in the C/N relation. A physicochemical characterization of each of the mixtures was performed by the methods outlined in table 1 (page 334).

Table 1. Components and methods used in the characterization of mixtures of lignocellulosic waste.

Tabla 1. Componentes y métodos utilizados en la caracterización de mezclas de residuos lignocelulósicos.

Parameters	Methods
Humidity	Gravimetric method, AOAC (2005) 925.10
Nitrogen	TOC Method - 4 -110.TN - 4110
Lignin	Van Soest, P. (1967).
Cellulose	By difference between the A.D.F. and the lignin
Hemicellulose	By difference between N.D.F. and A.D.F.
pH	By a Potentiometric method according to AOAC (2005)
Carbon	From organic matter, Wakley method (1996)
Calcium	Method by Spectrophotometry of atomic absorption adapted by the nutrition and quality department (MO-LSAIA-03.01.01) of the INIAP-Ecuador
Phosphorus	Colorimetry method adapted by the nutrition and quality department (MO-LSAIA-03.01.01) of the INIAP-Ecuador
Magnesium	Method by Spectrophotometry of atomic adapted by the nutrition and quality department (MO-LSAIA-03.01.01) of the INIAP-Ecuador
Potassium	Method by Spectrophotometry of atomic absorption adapted by the nutrition and quality department (MO-LSAIA-03.01.01) of the INIAP-Ecuador
Cupper	Method by Spectrophotometry of atomic absorption adapted by the nutrition and quality department (MO-LSAIA-03.01.01) of the INIAP-Ecuador
Iron, manganese y zinc	Method by Spectrophotometry of atomic absorption adapted by the nutrition and quality department (MO-LSAIA-03.01.01) of the INIAP-Ecuador

The strain selection

The *P. ostreatus* strain used in this study was collected by Ruilova (2015) of Ecuador and it was deposited at the Biotechnology Research Institute (IIB-INTECH), collection of fungal cultures (WFCC 826) from the University of San Martin, Argentina (25).

The preparation of the inoculum and suitability of substrates for cultivating the mushroom *P. ostreatus* was performed following the same methodology employed by Ruilova (2015).

Spawn production

Wheat grains were used for seed production as they are the cereal produced in the area. The wheat grains were selected, washed and cooked at boiling temperature for an approximate time of 45 min, then high density polypropylene bags were filled with 500 g of the grain to which 1.5% $CaCO_3$ was added. The bags were sterilized using autoclave for 30 min at 121°C. The humidity obtained was about 45%.

Once the bags were cold, the bags were inoculated into a laminar flow chamber with mycelium (1 cm of diameter) multiplied on PDA medium (Petri dish) and incubated at 25°C in darkness with periodic agitation to achieve a complete colonization of the mushroom mycelium (17 to 21 days). By the end of this process the seed was ready to be used.

Adequacy of the substrates for growing the *P. ostreatus* mushroom

The agricultural wastes were mechanically fragmented into a size of 2 to 5 cm long according to their nature, they were hydrated using water immersion for periods of time of about 24 h, time required to achieve a humidity between 75 to 76%. The substrates were sterilized via thermal treatment using an autoclave at 121°C for 30 min and were cooled down to room temperature (18 to 20°C).

We proceeded with the planting (3 bags per mix) inoculating 4% of the seed based on the wet substrate, for this transparent polyethylene bags 40 x 60 cm were used. The bags were drilled longitudinally and at the bottom with the tip of sterile steel, to promote a state of semi-anaerobiosis required in the initial invasion stage of the mycelium, then closed the bags with rubber bands and finally were incubated in the dark at a temperature of 25°C. Subsequently, the incubation period, the bags fully colonized by the mycelium mushroom are moved into the fructification climate room, where favorable conditions of relative humidity (80-85%), temperatures 15 to 16°C and photoperiod of 12 propitiated h light/12 h dark also ventilation to induce better sprouting. In which, the presence of primordia was detected and larger cuts in the bags were made to facilitate the development of the mushroom; these conditions were maintained for 4 to 5 days. In the production stage the temperature between 18 to 22°C and relative humidity between 85 to 90% remained. The facilities for the mushroom cultivation allowed to do an automatic control of temperature and humidity. The two measuring parameters were recorded digitally in the command board of a temperature control room.

The production was constantly monitored and mushroom were manually harvested with the help of a sterile knife, in their adult stage, when the pileus was fully extended. Mushrooms were weighed on a digital scale and the weight was recorded in grams.

Evaluation of the production

In order to evaluate the production of each substrate, the carpophores produced in three batches were harvested, in a production period of 62 to 74 days, time passed from the start of planting to last harvest. Production data was recorded: First harvest (days), Period of harvesting (days), considering the initial culture time and the last harvest time and weight of harvested fresh mushrooms. Biological efficiency defined as the ability of fungi to convert a substrate into fruiting bodies (BE) and the production rate (PR) was assessed according to equations 1 and 2 (page 336), considering the production time as the production period from planting to last harvest and the protein content (Kjeldhal Method, AOAC (2005) 2001.11), of the fruiting bodies. The weight of the dry substrate corresponds to the constant weight achieved during the determination of humidity by gravimetric method (AOAC (2005) 925.10).

$$BE = \frac{weight\ of\ fresh\ mushrooms}{weigh\ from\ dry\ subtrate} x100 \quad (1)$$

$$PR = \frac{Biological\ efficiency}{Production\ time} x100 \quad (2)$$

Statistical analysis

For each one of the variables response an analysis of variance (ANOVA) was performed, according to a completely randomized design and comparison of data by using a Tukey test with a significance level of 0.05. A regression analysis was used to determine the correlation between the C/N and the variables biological efficiency and protein content.

Results and discussion

Design of mixtures of substrates based on the carbon nitrogen ratio

Six mixtures (table 2) were designed using the Mixtures of Agricultural Waste for Mushroom Cultivation *P. ostreatus* program (11), each consisting of three residues.

The mixtures were very similar in the carbon content, the variation in this variable among the six mixtures was only 10.98%, based on the highest value, while the N% varied in 64.29%, which caused a C/N ratio, which ranged from 33.21 to 104.63 with 68.26% variation among the lowest of this variable and higher value.

Chang y Miles (2009), recommend a C/N ratio of 32-150 as the most appropriate for the production of *Pleurotus spp*.

Ruilova (2015) found best results in a C/N range of 37 to 53 using the strain 768/12. All mixtures are rich in carbon with a range of variation of less than 11%.

The manipulated variable was nitrogen in a range from 0.5 to 1.4, by mixing different proportions of residues or by using soy meal as a supplement from 0 to 6%, to adjust the ration of nitrogen to carbon.

Table 2. Mixtures designed for growing the *P. Ostreatus* mushroom.

Tabla 2. Mezclas diseñadas para el crecimiento del hongo *P. ostreatus.*

	Residues composing the mixture (%)								Indicators for standardization		
Mixture	**CS**	**RH**	**BS**	**RL**	**WS**	**BS**	**FS**	**CC**	**C (%)**	**N (%)**	**C/N**
M1	40	20	0	0	38	0	0	2	52.22	0.5	104.63
M2	0	0	0	30	40	28	0	2	50.75	0.7	72.40
M3	30	20	0	48	0	0	0	2	50.72	0.9	57.81
M4	0	15	0	40	0	40	3	2	47.95	1.0	47.99
M5	0	16	38	0	0	40	4	2	46.46	1.2	38.72
M6	0	17	0	53	22	0	6	2	46.49	1.4	33.21

CS (corn stover), RH (rice husk), BS (Barley straw), SL (stover Lentil), WS (wheat straw), BS (bagasse sugarcane), FS (flour soy), CC (calcium carbonate), C (carbon), N (nitrogen) and C/N carbon nitrogen ratio.

CS (rastrojo de maíz), RH (cascarilla de arroz), BS (Paja de cebada), SL (rastrojo de lenteja), WS (paja de trigo), BS (bagazo de caña de azúcar), FS (harina de soya), CC (carbonato de calcio), C (carbono), N (nitrógeno) y C/N (relación carbono nitrógeno).

Table 3 shows the behavior of the different components in each mixture. According to the results obtained by various authors (15, 28), the mushroom *Pleurotus* has been cultivated successfully in barley straw, corn stubble, sugar cane bagasse and soybean stubbles, all containing cellulose between 39 and 45%, hemicellulose 16-22% of lignin and 11 to 16%, so these components in the mixtures are in the ranges reported in the literature.

Table 4 (page 338), shows the mineral content in the mixtures. As shown, potassium exhibits the highest variation (0.41 to 1.20%), calcium ranged from 0.12 to 0.22%, phosphorus from 0.04 to 0.48% and magnesium between (06-.16%). Ruilova *et al.* (23) obtained positive results in the ranges of concentrations of these elements in the cultivation of *Pleurotus*.

Among the group of minor minerals, the highest value reported was for iron in the mixture 1 while the lowest was reported for copper the values for zinc varied between 9.71 and 36.42 mg/kg. Meanwhile, manganese fluctuated between 82.29 and 104.43 mg/ kg. These micronutrients are required by the mushroom in the indicated ranges, for a better development, and it is possible that they are supplied by the substrate itself (1).

Evaluation of production of the *Pleurotus ostreatus* mushroom

In table 5 (page 338), it is observed for each of the variables response on results

First harvest

The first harvest from all of the treatments was performed between the 25th and 37th days after inoculating of substrates, it could be observed that the shortest time (25 days) was obtained for the M4 (1% N) mixture and the longest time for the mixture M1 (0.5% N). It was inferred that the variation over time of fruiting was due to the lower nitrogen content which resulted in a higher ratio of C/N in these mixtures, the higher the ration of C/N the longer it took to start the harvest, except for the mixture M4. These results are comparable to those obtained by Getahun (2011), who using a commercial strain of *P. ostreatus* reported the appearance of primordia in soybean and corn stubble in a period of time of 21 to 25 days and 41 days for sawdust, which by its low nitrogen content has a high C/N ratio (459.9). Sharma *et al.* (2013) when they worked with mixed wheat and rice straw obtained an average of 22 days.

Table 3. Means values of the composition of agricultural waste.

Tabla 3. Valores medios de la composición de mezclas de desechos agrícolas.

Composition	M1	M2	M3	M4	M5	M6
Cellulose (%)	43.74 (1.56)	44.51 (1.62)	39.22 (2.06)	44.60 (1.57)	42.41 (1.16)	40.16 (1.18)
Hemicellulose (%)	22.10 (1.24)	18.60 (2.02)	16.71 (1.72)	18.55 (2.02)	18.96 (1.38)	14.81 (1.68)
Lignin (%)	11.39 (1.18)	11.09 (0.88)	14.30 (1.22)	13.83 (1.14)	13.92 (0.98)	15.52 (1.18)

Values ( ) refer to the standard deviation.

Los valores entre ( ) se refieren a la desviación estándar.

Table 4. Means value of mineral content in agricultural residue mixtures.

Tabla 4. Valores medios del contenido de minerales de mezclas de residuos. agrícolas.

Values ( ) refer to the standard deviation. / Los valores entre ( ) se refieren a la desviación estándar.

Minerals		M1	M2	M3	M4	M5	M6
Potassium	(%)	1.12 (0.02)	1.20 (0.02)	1.06 (0.03)	0.41 (0.02)	1.16 (0.04)	0.74 (0.03)
Calcium	(%)	0.16 (0.02)	0.22 (0.02)	0.22 (0.01)	0.12 (0.01)	0.17 (0.02)	0.19 (0.02)
Phosphorus	(%)	0.04 (0.02)	0.48 (0.03)	0.05 (0.02)	0.04 (0.02)	0.10 (0.03)	0.05 (0.02)
Magnesium	(%)	0.08 (0.02)	0.12 (0.02)	0.16 (0.03)	0.06 (0.02)	0.07 (0.02)	0.13 (0.01)
Iron	(mg/kg)	272.49 (3.46)	272.42 (4.28)	170.87 (3.22)	260.55 (5.34)	270.03 (4.34)	210.91 (5.22)
Zinc	(mg/kg)	13.83 (0.54)	12.88 (0.62)	9.71 (0.48)	6.42 (1.18)	37.74 (3.34)	9.95 (2.14)
Manganese	(mg/kg)	94.53 (1.72)	90.00 (0.66)	104.43 (1.22)	88.94 (0.64)	86.99 (0.86)	82.29 (0.48)
Copper	(mg/kg)	9.16 (0.53)	9.18 (0.57)	7.00 (0.44)	15.53 (0.74)	13.06 (0.36)	7.56 (0.22)

Table 5. Range of days for the first harvest and period of harvesting and mean values fresh weight, biological efficiency, protein content and production rate by mixture.

Tabla 5. Tiempo en días para la primera cosecha, período de cosecha, valores medios del peso del hongo fresco, eficiencia biológica, contenido de proteína y tasa de producción por mezcla.

Mixture	Humidity	First harvest (days)	Period of harvesting (days)	Weight of the fresh mushroom (g)	Biological efficiency (%)	Protein (%)	Production rate (day^{-1})
M1	76.14	34-37	64-77	713.78b (13.41)	149.79b (9.12)	16.51c (0.68)	2.14c (0.046)
M2	74.59	33-36	64-77	768.96b (32.58)	151.31b (6.41)	17.26c (1.70)	2.16c (0.042)
M3	75.37	30-33	62-77	858.57a (26.78)	174.96a (4.22)	25.25b (2.06)	2.50ab (0.06)
M4	75.46	25-28	60-75	869.29a (5.06)	177.37a (2.45)	31.13 a (1.32)	2.64a (0.031)
M5	75.39	31-33	62-76	855.35a (18.09)	173.74a (3.46)	31.26 a (2.37)	2.52ab (0.051)
M6	74.48	32-34	62-77	851.17a (30.45)	165.22ab (8.28)	29.23ab (3.55)	2.36b (0.12)

* Different letters indicate significant differences ($p < 0.05$). Values ( ) refer to the standard deviation.

* Letras diferentes indican diferencias significativas ($p < 0,05$).
Los valores entre ( ) se refieren a la desviación estándar.

Weight of the fresh mushroom

The weight in fresh mushrooms obtained in each mixtures were grouped into two statistically different groups. For mixtures M3, M4, M5 and M6 the highest average values achieve were (858.57; 869.29; 855.35; 858.57; 851.17 g respectively), showing a higher average for the M2 mixture and among the remaining three little variations; while for the M1 and M2 mixtures the average values were lower (731.78 and 768.96 g).

The mixtures that allowed to obtain the highest weight for the fresh mushrooms were supplemented with soybean meal (3, 4 and 6%), giving the best value for the ratio C/N of 47.99 with 1% N. Very high nitrogen values cause heating of the substrate in the incubation period, which may have prevented the development of fruiting bodies. Works carried out with other types of substrates have shown similar results (7, 14, 21).

Biological efficiency

When performing the ANOVA test this indicator gave significant differences among the mixtures and by applying the Tukey test the mixtures were grouped into three groups. The highest BE was attained by mixtures M4 (177.37%), M3 (174.96%) and M5 (173.74%) within a range of nitrogen content of 0.9 to 1.2%, using as a supplement nitrogenised soybean meal (0, 3, and 4%) and significantly differ from the M1 (149.79%) and M2 mixtures (151.31%) without supplement, however the results are greater than 100% so it may be considered good. As shown, the tendency to decrease was the same as presented in the variable analyzed before, a result that was expected by the ratio of weight with this indicator.

By subjecting the results of biological efficiency (BE) to a regression analysis a significant dependence on the C/N ratio was found, obtaining equation 3, with a correlation coefficient of 0.9849.

As shown in figure 1 (page 340), there is an area in the C/N ratio where BE became higher in the range from 38 to 58, which coincides with the M3, M4 and M5 mixtures. There is no significant effect of addition of nitrogen supplement to modify the C/N, on the BE in the mixture, since the C/N ratio 47.99 (M4) gave the highest BE with only 1, 36% above the best value obtained for a mixture no supplemented.

The BE decreases 15.5% on the mixture with the highest C/N ratio, without supplementation with soy flour.

The results of BE obtained in this study are higher than those reported by Sharma *et al.* (2013), who by studying several residues found that most biological efficiency (95.46%) corresponded to higher nitrogen content and less for lower nitrogen (66%). Upadhyay *et al.* (2002) for *P. ostreatus* obtained the highest BE (73.2 and 83.2%) when they used wheat straw with the addition of 5% and 10% of soybean meal.

Protein content

The analysis of variance done to this variable (table 5, page 338) showed significant differences between the mixtures and by applying the Tukey test between the average values four groups were formed. By subjecting these results to a multiple regression analysis using as variables in mixtures the nitrogen content and nitrogen to carbon ratio resulted that the carbon nitrogen ratio was the most significant variable.

$$\mathbf{EB(\%) = -67.910411 + 12.744959\ (C/N) - 0.206126\ (C/N)^2 + 0.009957\ (C/N)^3} \quad (3)$$

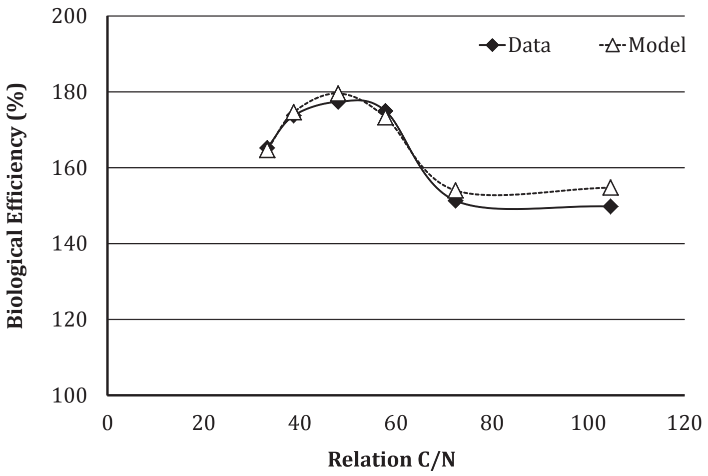

Figure 1. Biological efficiency behaviour of the mushroom in C/N ratio in the mixture.

Figura 1. Comportamiento de la eficiencia biológica en el hongo con respecto a la relación carbono nitrógeno en la mezcla de sustrato.

Equation 4 (*) it was obtained to express the protein content (P) depending on the C / N, with a correlation coefficient of 0.9946.

Figure 2 (page 341), shows that the protein content increased as the nitrogen content increased in the mixtures, the highest value (31.26%) was for M5 (1.2% nitrogen) with 4% soybean meal, without significant difference from the M4.

For M1 and M2 mixtures with higher C/N ratio and thus the N content lower (0.5 and 0.7%) manifesting a clear tendency to a decrease in protein content, evidencing that there is a wide range in the carbon nitrogen ratio (38 to 48) where the protein content of mushroom was maximum, which coincides with the M4 and M5 mixtures.

In general, these results are consistent with those reported by Sharma *et al.* (2013) who noted that the C/N ratio significantly influences the values obtained from the chemical composition of the fungus *P. ostreatus*.

Getahun (2011) reported the highest value of protein (28.9%) for oyster mushroom when used soybean stover (C/N 62.35), a value that is higher than optimal found in this investigation.

The C/N ratio of the substrate is critical to the initial development of the fungus, given the value of carbon for the formation of new cells; a low ratio C/N in the substrate will influence negatively during mycelial growth stage (11, 13, 25).

$$* \ \mathbf{P(\%) = -39.940407 + 4.05112701\ (C/N) - 0.071028\ (C/N)^2 + 0.000358\ (C/N)^3} \quad (4)$$

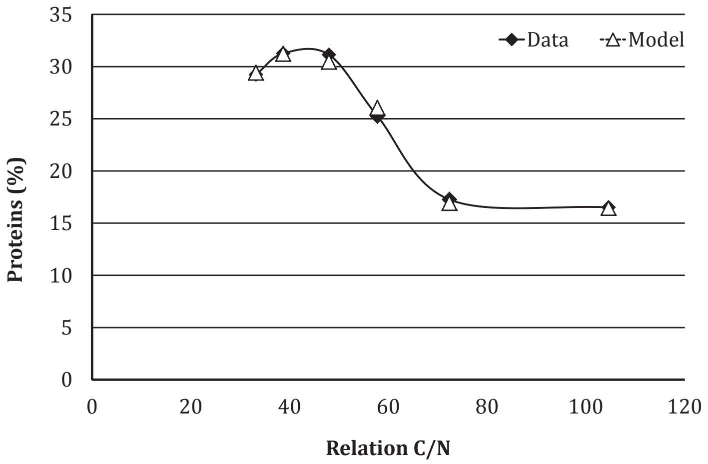

Figure 2. Fungus protein content in relation to the carbon nitrogen ratio in the mixture.

Figura 2. Contenido de proteína en el hongo con respecto a la relación carbono nitrógeno en la mezcla.

Production rate

The evaluation of the production rate is the indicator that reflects in a better way the performance of mushroom production, since it includes the time from inoculation to the last day of harvest. Assuming that the shorter period of time, production costs also will be lower as well as the risks of contamination.

The mixture M4 was higher and statistically different from the other mixtures. The production rate obtained (2.14 to 2.64%) indicates that the production of mushrooms in mixtures used was obtained in less time than recorded by Pérez-Merlo and Mata (2005), when they worked with different strains, with which they obtained a production rate of between 1.53% and 2.46% with barley straw and between 0.68 and 1.13% with pine shavings.

When working with standardized mixtures of lignocellulosic residues in the C/N ratio to grow the fungus *Pleurotus* it was found that the it is possible the addition of soy flour to up to 6% to supply nitrogen in poor substrates in this element, higher values of addition mycelial growth can difficult the grow possibly due to an increase in the substrate temperature by more accelerated metabolic activities.

So when poor substrates are used in nitrogen and is necessary to add soybean meal or any other nitrogen supplement (bran cereal, etc.), it is essential that the C/N ratio is considered as an important factor for optimal development of the fungus oyster (20).

Conclusions

The results achieved during this research while working with lignocellulosic substrates constitute by a mixtures of waste in order mushroom growing *P. ostreatus*, confirmed a high dependency with the C/N relation, with a peak area for biological efficiency within the range of 38 to 58% of C/N ratio, with a Correlation of 98.49% and a maximum area between 38 and 48 C/N ratio for the protein, with a 99.46% correlation.

The use of soybean meal as nitrogen supplement allowed the adjustment of the nitrogen content in the mixture and therefore the balance in the C/N ratio. In this case, the best results were obtained with the addition of soybean meal 3%.

It was found that by using the mixture of 15% of rice husks, 40% lentil stubble, 40% sugarcane bagasse, supplemented with 3% soybean meal and 2% of calcium carbonate, which translated 1% of the N and C/N ratio of 47.99 gave the best results for the variables: time of the first harvest (25-28 days), harvest period (60-75 days), weight from fresh fungus (869.29 g), biological efficiency (177.37%), protein content (31.13%) and production rate (2.64 days-1).

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