

Supplementation of High Nitrogen *Agaricus* Compost: Yield and Mushroom Quality

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ABSTRACT

Supplementation to increase the quality and yield of white button mushrooms (*Agaricus bisporus*) consists of the addition of nutrients, particularly proteins, to the compost. In this study, the effect of mushroom cultivation on applying a delayed-release nutrient supplement to Phase II composts was evaluated. Two application dosages and composts with high nitrogen contents were used. Depending on the compost and supplement dosage used, increases in biological efficiency ranging between 6.2 and 22.3% were obtained. An excellent response to supplementation can be obtained even when using composts with high nitrogen contents (in our case, 2.45 and 2.61% sms) during spawning. It is additionally conceivable that the application of supplements to composts with lower N contents or the application to spawned compost would provide an even better yield, without adversely affecting the quality of the harvested mushrooms. This practice is of considerable interest because of the economic benefit.

Keywords: *Agaricus bisporus*, Business category, C/N ratio, Mushroom technology, Yield.

INTRODUCTION

The yield obtained from the cultivation of *Agaricus bisporus* (Lange) Imbach depends on many factors, the most important of which is the quality of the compost, the base substrate used for cultivation (Sharma and Kilpatrick, 2000). The availability of nutrients is one of the factors directly related to the productivity of compost (Saharan and Guleria, 1993). Improving the nutritional quality of the compost is therefore of great importance to increase profit (Sánchez and Royse, 2001).

Supplements are materials that are added to the compost after the composting process for direct use by the mushroom and are not intended, unlike compost activators, to be

integrated into the substrate by the activity of other organisms (Randle, 1985).

Most modern supplements based on vegetable materials and agricultural wastes, such as soybean meal, are rich in protein, which is the most important ingredient (Carroll and Schisler, 1976). These supplements require a chemical or heat treatment to create a slow-release effect and limit temperature increases after their application (Peeters, 2008).

The practice of nutritionally supplementing compost for mushroom cultivation at the time of spawning or casing to maximize crop yield is widely recognized and accepted but is in limited use worldwide. The correct application of this technique is an essential condition for obtaining the expected results, with several

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important culture aspects, such as the preparation of the compost, the control of temperature for mycelial growth, hygiene measures, the choice of supplement and its application time and, especially, the uniform distribution of the product used (Desrumaux *et al.*, 1999).

When the characteristics of the facilities and growing rooms permit (such as air-conditioned and mechanized shelves for the crop), supplements are added to the colonized compost just before applying the casing layer; thus, excessive compost temperatures are avoided during spawning, and the incidence of fungal competitors is minimized (Bano *et al.*, 1993). However, cropping in bags, the primary system used in many parts of the world, requires supplementation during spawning primarily because of mechanical restrictions.

The aim of this study was to determine the effect of the application during spawning of a commercial delayed-release nutrient supplement on crop yield and quality for mushroom cultivation (*A. bisporus*) which is performed using commercial composts with high nitrogen contents.

MATERIALS AND METHODS

Physical, Chemical and Biological Analyses

To determine the physical, chemical and biological characteristics of composts and casing, the following measurements were taken: moisture (Mapa, 1994), organic matter and ash (Ansorena, 1994; Mapa, 1994), pH (Ansorena, 1994; Aenor, 2001a), electrical conductivity (Aenor, 2001b), total N content (Foss, 2003), C/N ratio and crude fiber (Msc, 1985a; Ankom, 2008), crude fat (Msc, 1985b; Ankom, 2009), nitrogen-free extract (González *et al.*, 1987), particle real density (Aenor, 2001c), bulk density, total porosity and water-holding capacity (Ansorena, 1994; Aenor, 2001c), mushroom pathogenic nematodes (Nombela and Bello,

1983), mites (Brady, 1969) and competitor molds (Krantz, 1986).

Supplement Use and Application Rate

A commercial delayed-action product based on soybean meal with a protein content of 48% (Promycel[®]480, Amycel Europe, Vendôme, France) was evaluated as a nutritional supplement for mushroom cultivation. Two independent experiments were conducted, both using supplement doses of 0.6 and 0.8% by weight, calculated using the fresh weight of compost. These doses correspond to the extremes of the range recommended by the supplement manufacturer.

Compost

Two commercial composts, whose main characteristics are presented in Table 1, were used as cereal straw for growing the mushrooms. All the observed values were adjusted to the range considered optimal for cultivating mushrooms (Zied *et al.*, 2011a). The nitrogen contents of these composts were 2.45 and 2.61% on a dry matter basis.

Casing Layer

A mixture widely used in the production area of Castilla-La Mancha (Spain) of mineral soil and coconut fiber (4:1, v/v) was used as the casing layer (Pardo-Giménez *et al.*, 2010). The analytical characteristics of this layer are given in Table 2. The casing was applied at a thickness of 3 cm in both experiments.

Spawn

The commercial strain of mycelium used was Fungisem H-25 (Micelios Fungisem SA, Calahorra, La Rioja). This strain belongs to the white hybrid "halftime," which is especially recommended for spring, autumn and winter "spawn". Its optimal culture conditions include a fruiting

Table 1. Analytical properties of the commercial composts used.

| | Experiment 1 | Experiment 2 |
|--------------------------|--------------|--------------|
| pH (1:5, p/v) | 7.81 | 7.87 |
| Moisture (%) | 72.4 | 72.1 |
| Total N (% , sms) | 2.45 | 2.61 |
| Ash (% , sms) | 25.18 | 26.35 |
| Organic matter (% , sms) | 74.82 | 73.65 |
| C/N ratio | 17.7 | 16.4 |
| Crude fiber (% , sms) | 38.34 | 36.65 |
| Crude fat (% , sms) | 0.57 | 0.27 |
| N-Free extract (% , sms) | 20.60 | 20.42 |
| Nematodes | Absent | Absent |
| Mites | Absent | Absent |
| Competitor molds | Absent | Absent |

temperature of 15-20°C, relative humidity of 85-90%, and average ventilation, providing medium-sized, thick mushrooms for both the fresh market and cannot (Pardo-Giménez *et al.*, 2011a; Pardo-Giménez *et al.*, 2012). The spawning rate was 10 g kg⁻¹ of fresh compost.

Experimental Design

Two cropping trials, in which two supplement doses were applied, were evaluated relative to unsupplemented compost. A completely randomized design with six replicates was used in both cases (Experiments 1 and 2). Experimental trays

(plastic boxes) were each filled with 6 kg of Phase II compost, which was compacted at a rate of 450 kg m⁻³ and presented a surface of 870 cm² to be covered (69.0 kg filling weight m⁻²). The same variety of mycelium (Fungisem H-25) was used in both experiments. A total of 18 trays were used in each experiment, corresponding to the two doses applied (0.6 and 0.8% by weight) and the control without supplement, with six replicates per treatment. Trays were placed randomly at two levels on both sides of the growth chamber.

Cultivation Facilities

The crop cycles were performed in an IBERCEX chamber with dimensions of 3.70×2.10×2.60 m (20.2 m³). The chamber was provided with humidification, heating/cooling and recirculating/outside ventilation systems to allow automatic control of the relative humidity, temperature and carbon dioxide concentration.

Crop Cycles

The crop cycles were conducted according to the conditions recommended for the strain used (Pardo-Giménez *et al.*, 2011b), with the

Table 2. Analytical properties of the casing layer.

| Characteristic | Value |
|---|-------|
| pH (1:6, v/v) | 8.03 |
| Electrical conductivity (1:6, v/v) (μS cm ⁻¹) | 191 |
| Bulk density (g cm ⁻³) | 0.626 |
| Particle real density (g cm ⁻³) | 2.710 |
| Total porosity (%) | 74.5 |
| Percent saturation | 54 |
| Organic matter (%) | 2.02 |



same schedule of operations in both experiments. The casing layer was applied 14 days after compost inoculation, after which the insecticide and fungicide treatments were applied as usual (3.6 g m^{-2} of 25% diflubenzuron and 0.62 g m^{-2} of prochloraz 46%, respectively). The casing was raked 7 days after application, inducing fruiting two days later. Harvesting began 34 days after the compost inoculation. During the harvest period, the casing was kept wet at a level between 60 and 70% of its maximum water-holding capacity by regular, uniform watering with between 0.5 and 1.5 L m^{-2} , as needed in accordance with the usual practice of cultivation. The total duration of the crop cycle was 62 days in both experiments, and the crop was harvested in four flushes.

Harvesting and Production Parameters

Mushroom harvesting was performed daily at the commercially optimal stage of development, corresponding to the morphogenetic states 2, 3 and 4 as defined by Hammond and Nichols (1976).

For calculating the yield per unit area, the weight and number of mushrooms produced daily by each experimental unit was determined. In addition, the mushrooms were separated into two groups according to size: large ($\geq 40 \text{ mm}$) and medium ($15\text{-}40 \text{ mm}$). The biological efficiency, expressed in kg dt^{-1} of compost (dry matter), was established from the yield per unit area, considering the density of the compost and its moisture content. The unit weight of the mushrooms, in grams, was determined from the total weight yield obtained and the number of harvested mushrooms (Zied *et al.*, 2013).

A second estimate of size, expressed as cap diameter in mm, was determined from the unit weight of the mushrooms, using a previously established, nonlinear regression curve. This curve is given by Equation (1), which provided the highest R^2 values as a result of adjusting the regression equation to

fit the various data patterns obtained for the calculation.

$$d = 6.84525 + 7.1942 p^{1/2} \quad (1)$$

In this equation, d is the diameter of the cap (mm), and p is the unit weight of the mushrooms (g).

Earliness, or days to first harvest, was expressed as the number of days between casing and harvesting the first flush. On the day of maximum harvest for each of the four flushes, mushrooms of uniform size and maturity were chosen for quality assessment. The color of the mushrooms and their dry matter content was determined.

The surface color of the mushrooms was measured by reflection using a colorimeter that had been previously calibrated with a D65 illuminant and CR-A43 calibration plate [With the Lightness (L^*)= 96.12, The red-green component (a^*)= -0.11 and The yellow-blue component (b^*)= +2.66].

A total of twenty measurements were taken on uniformly sized, disease-free mushrooms in each of the three flushes for each experimental unit, with four measurements performed on each of five mushroom pilei: one in the center of the pilei and three at distances between 1 and 2 cm off their centers, depending on the size of the fruit bodies.

For the description of the color values of L^* , b^* and ΔE^* , a parameter that measures the degree of deviation in comparison with the values of an ideal sporophore, the following values are used: $L^*= 97$, $a^*= -2$ and $b^*= 0$ (Roy *et al.*, 1995). Because the red-green tones are practically uninvolved in the final color of the mushroom, a^* was not taken into account. ΔE^* was obtained from Equation (2).

$$\Delta E^* = \left[\{L^* - 97\}^2 + \{a^* - (-2)\}^2 + \{b^*\}^2 \right]^{1/2} \quad (2)$$

Dry matter levels were determined by measuring weight loss after oven drying at 105°C for 72 hours. For determination, a universal stove with forced air circulation was used.

Statistical Analysis

Statistical analysis was performed with statistical package program (Statistical Graphics Corp., Princeton, NJ, USA). Technical analysis of variance was used to evaluate the data. For the establishment of significant differences between the means, the Tukey HSD test ($P= 0.05$) was applied. Multiple-variable analysis was used to establish the Pearson product moment correlations coefficients between production parameters and the P -values which tests the statistical significance of the estimated correlations.

RESULTS AND DISCUSSION

The results obtained for the quantitative production parameters recorded in Experiment 1 are shown in Table 3. The supplementation produced no significant differences in the precocity or the number or size of the harvested mushrooms. However, differences in the yield per unit area and biological efficiency were registered. The best results occurred when the highest dose of supplement (0.8%) was used, with a yield of 27.95 kg m^{-2} and a biological efficiency of 146.8 kg dt^{-1} , significantly higher than the values provided by the unsupplemented compost (25.31 kg m^{-2} and 133.0 kg dt^{-1}). The values for the low-dose supplementation (0.6%) fell between those for the high-dose-supplemented and unsupplemented composts. No significant differences were observed for any of the qualitative production parameters evaluated in Experiment 1 (Table 4).

Experiment 2 presented a quite similar situation (Table 5), with no significant differences in earliness, the number of fruiting bodies harvested or size. The best yields were again provided by the compost supplemented at the highest dose, with a yield of 28.14 kg m^{-2} and biological efficiency of 146.3 kg dt^{-1} , significantly higher than the values obtained with the other treatments. In this case, however, the

low-dose-supplemented compost produced a yield of 25.30 kg m^{-2} and a biological efficiency of 131.5 kg dt^{-1} , values that proved significantly greater than the yield of 23.01 kg m^{-2} and biological efficiency of 119.6 kg dt^{-1} produced by the unsupplemented compost. In this case, the main differences were due to different behavior in the 1st and 3rd flushes. For the qualitative production parameters evaluated in Experiment 2, significant differences were observed only for the color difference, with the lowest value (9.09) associated with the mushrooms harvested from compost supplemented at the high dose (Table 6).

The results obtained for the Pearson product moment correlation coefficients between the main production parameters recorded in Experiments 1 and 2 are shown in Tables 7 and 8, respectively. In both experiments, similar results were obtained in general for the correlation coefficients. Thus, obtaining a high number of mushrooms per unit area is associated with higher productivity (higher yield and biological efficiency) and to a smaller size of harvested fruiting bodies (unit weight and diameter). High values of the coefficients have been logically recorded between the total yield and the biological efficiency on the one hand, and between the unit weight and the diameter of fruiting bodies on the other. The parameters defining the color are significantly correlated with each other, but only the Lightness (L^*) (Experiment 1) and ΔE^* (Experiment 2) are correlated with productivity parameters (yield and biological efficiency). The dry matter content of harvested mushrooms only shows significant correlation with yield and biological efficiency in Experiment 2.

The expected increase in biological efficiency with this type of supplementation is between 5 and 20% (Zied *et al.*, 2011b). In our case, a comparison of the supplementation at the lower dose with the unsupplemented compost showed that the biological efficiency increased 6.2% in Experiment 1 and 9.9% in Experiment 2, whereas the higher dose increased the

Table 3. Results for the quantitative production parameters in Experiment 1. ^a

| Treatments | Number of mushrooms (m ⁻²) | Unit weight (g mushroom ⁻¹) | Yield (kg m ⁻²) | | | | Total | Biological Efficiency (kg dt ⁻¹ compost) | Earliness after (Days casing) |
|---------------|--|---|-----------------------------|-----------|-----------|-----------|----------|---|-------------------------------|
| | | | 1st Flush | 2nd Flush | 3rd Flush | 4th Flush | | | |
| Control | 2268 | 11.2 | 8.59 | 9.23 | 4.63 | 2.85 | 25.31 b | 133.0 b | 20.7 |
| Promycel 0.6% | 2249 | 12.1 | 9.74 | 8.14 | 5.18 | 3.83 | 26.90 ab | 141.3 ab | 20.7 |
| Promycel 0.8% | 2286 | 12.3 | 9.32 | 9.47 | 5.33 | 3.82 | 27.95 a | 146.8 a | 20.5 |
| Average | 2268 | 11.9 | 9.22 | 8.95 | 5.04 | 3.50 | 26.72 | 140.4 | 20.6 |
| F-Ratio | 0.04 | 1.36 | 2.59 | 3.74 | 0.34 | 1.79 | 5.52 | 5.48 | 0.44 |
| P-Value | 0.9626 | 0.2870 | 0.1083 | 0.0502 | 0.7203 | 0.2001 | 0.0160 | 0.0163 | 0.6529 |

^a Values within columns followed by different letters are significantly different from each other (P≤0.05, Tukey test).

Table 4. Results for the qualitative production parameters in Experiment 1. ^a

| Treatments | Business category (kg m ⁻²) | | | Color | | | Dry matter (%) |
|---------------|---|-----------------|-------------------|--------|--------|--------|----------------|
| | Large (≥40 mm) | Medium (<40 mm) | Cap diameter (mm) | L* | b* | ΔE* | |
| CONTROL | 5.28 | 20.02 | 30.9 | 93.40 | 8.88 | 9.88 | 7.43 |
| Promycel 0.6% | 5.44 | 21.45 | 31.9 | 92.98 | 9.04 | 10.20 | 7.14 |
| Promycel 0.8% | 6.65 | 21.29 | 32.1 | 93.04 | 9.04 | 10.18 | 7.22 |
| Average | 5.79 | 20.92 | 31.6 | 93.14 | 8.99 | 10.09 | 7.26 |
| F-Ratio | 1.37 | 0.63 | 1.36 | 2.22 | 0.27 | 0.76 | 2.87 |
| P-Value | 0.2871 | 0.5457 | 0.2866 | 0.1430 | 0.7695 | 0.4851 | 0.0878 |

^a Values within columns followed by different letters are significantly different from each other (P≤0.05, Tukey test).

Table 5. Results for the quantitative production parameters in Experiment 2.^a

| Treatments | Number Mushrooms (m ⁻²) | Unit weight (g mushroom ⁻¹) | YIELD (kg m ⁻²) | | | | Total | Biological efficiency (kg dt ⁻¹ compost) | Earliness (Days after casing) |
|---------------|--|--|-----------------------------|-----------------------|-----------------------|-----------------------|---------|--|----------------------------------|
| | | | 1 st Flush | 2 nd Flush | 3 rd Flush | 4 th Flush | | | |
| Control | 1767 | 13.4 | 8.43 b | 8.38 | 3.38 b | 2.83 | 23.01 c | 119.6 c | 21.2 |
| Promycel 0.6% | 1933 | 13.3 | 10.55 a | 8.51 | 3.97 b | 2.27 | 25.30 b | 131.5 b | 21.1 |
| Promycel 0.8% | 2205 | 13.1 | 10.34 a | 8.99 | 5.84 a | 2.98 | 28.14 a | 146.3 a | 21.1 |
| Average | 1968 | 13.3 | 9.77 | 8.63 | 4.40 | 2.69 | 25.48 | 132.5 | 21.1 |
| F-Ratio | 2.82 | 0.04 | 18.61 | 0.42 | 7.24 | 0.83 | 18.00 | 18.05 | 0.11 |
| P-Value | 0.0914 | 0.9620 | 0.0001 | 0.6640 | 0.0063 | 0.4547 | 0.0001 | 0.0001 | 0.8965 |

^a Values within columns followed by different letters are significantly different from each other (P ≤ 0.05, Tukey test).

Table 6. Results for the qualitative production parameters in Experiment 2.^a

| Treatments | Business category (kg m ⁻²) | | | Color | | | |
|---------------|--|---------------------|-------------|----------------|--------|---------|-------------------|
| | Large (≥ 40 mm) | Medium (< 40 mm) | Cap (mm) | Diameter L* | b* | ΔE* | Dry matter (%) |
| Control | 7.67 | 15.34 | 33.1 | 93.19 | 8.55 | 9.68 ab | 7.18 |
| Promycel 0.6% | 8.19 | 17.12 | 33.0 | 93.52 | 8.85 | 9.82 a | 7.65 |
| Promycel 0.8% | 9.09 | 19.06 | 32.8 | 93.78 | 8.21 | 9.09 b | 7.54 |
| Average | 8.31 | 17.17 | 33.0 | 93.50 | 8.54 | 9.53 | 7.46 |
| F-Ratio | 0.37 | 2.52 | 0.03 | 1.65 | 3.36 | 4.59 | 1.91 |
| P-Value | 0.6972 | 0.1136 | 0.9697 | 0.2243 | 0.0621 | 0.0278 | 0.1822 |

^a Values within columns followed by different letters are significantly different from each other (P ≤ 0.05, Tukey test).

Table 7. Pearson product moment correlations between the main production parameters in Experiment 1.^a

| | Number of mushrooms | Unit weight | Total yield | Biological efficiency | Earliness | Cap diameter | Color <i>L</i> * | Color <i>b</i> * | Color ΔE^* | Dry matter |
|-----------------------|---------------------|---------------------|---------------------|-----------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Number of mushrooms | | | | | | | | | | |
| Unit weight | -0.8047 (0.0001) | | | | | | | | | |
| Total yield | 0.2143 (0.3932) | 0.3961 (0.1037) | | | | | | | | |
| Biological efficiency | 0.2150 (0.3915) | 0.3955 (0.1043) | 1.0000 (0.0000) | | | | | | | |
| Earliness | 0.6592 (0.0029) | 0.0162 (0.9492) | 0.0174 (0.9455) | | | | | | | |
| Cap diameter | -0.8012 (0.0001) | 0.9991 (0.0000) | 0.4063 (0.0943) | 0.4057 (0.0949) | | | | | | |
| Color <i>L</i> * | -0.1114 (0.6599) | -0.2133 (0.3954) | -0.5140 (0.0291) | -0.5144 (0.0290) | 0.3395 (0.1681) | -0.2224 (0.3751) | -0.3902 (0.1095) | 0.2665 (0.2851) | 0.2628 (0.2921) | 0.0237 (0.9256) |
| Color <i>b</i> * | 0.2665 (0.2851) | -0.3139 (0.3954) | -0.1829 (0.4677) | -0.1983 (0.4303) | -0.1541 (0.6592) | 0.0650 (0.9455) | 0.0650 (0.9455) | 0.0650 (0.9455) | 0.0650 (0.9455) | 0.0650 (0.9455) |
| Color ΔE^* | 0.0650 (0.9455) | 0.0650 (0.9455) | 0.0650 (0.9455) | 0.0650 (0.9455) | 0.0650 (0.9455) | 0.0650 (0.9455) | 0.0650 (0.9455) | 0.0650 (0.9455) | 0.0650 (0.9455) | 0.0650 (0.9455) |
| Dry matter | 0.0237 (0.9256) | -0.1983 (0.4303) | -0.2725 (0.2740) | -0.2721 (0.2747) | -0.1726 (0.4934) | -0.2080 (0.4076) | -0.1550 (0.5390) | -0.0532 (0.8340) | -0.1550 (0.5390) | -0.1550 (0.5390) |

^a Values in parentheses correspond to the *P*-value which tests the statistical significance of the estimated correlations. *P*-values below 0.05 indicate statistically significant non-zero correlations at the 95% confidence level.

Table 8. Pearson product moment correlations between the main production parameters in Experiment 2.^a

| | Number of mushrooms | Unit weight | Total yield | Biological efficiency | Earliness | Cap diameter | Color L* | Color b* | Color ΔE* | Dry matter |
|-----------------------|---------------------|---------------------|---------------------|-----------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Number mushrooms | | | | | | | | | | |
| Unit weight | -0.8122 (0.0000) | | | | -0.2981 (0.2296) | -0.8107 (0.0000) | 0.2591 (0.2991) | -0.2576 (0.3020) | -0.3111 (0.2089) | -0.1180 (0.6410) |
| Total yield | 0.5044 (0.0000) | | | | 0.4849 (0.0414) | 0.9993 (0.0000) | -0.0923 (0.7158) | 0.0065 (0.9795) | -0.0044 (0.9863) | 0.4090 (0.0919) |
| Biological efficiency | 0.0713 (0.0328) | 0.0713 (0.7785) | | | 0.0899 (0.7229) | 0.0798 (0.7530) | 0.2975 (0.2305) | -0.4147 (0.0871) | -0.5120 (0.0298) | 0.4801 (0.0438) |
| Earliness | 0.5040 (0.0330) | 0.0719 (0.7766) | 1.0000 (0.0000) | | 0.0912 (0.7188) | 0.0804 (0.7512) | 0.2982 (0.2293) | -0.4152 (0.0866) | -0.5130 (0.0295) | 0.4793 (0.0442) |
| Cap diameter | -0.2981 (0.2296) | 0.4849 (0.0414) | 0.0899 (0.7229) | 0.0912 (0.7188) | | 0.4671 (0.0506) | 0.0249 (0.9219) | -0.0522 (0.8369) | -0.0977 (0.6996) | 0.0150 (0.9530) |
| Color L* | 0.5040 (0.0330) | 0.0719 (0.7766) | 1.0000 (0.0000) | 0.0804 (0.7512) | 0.4671 (0.0506) | | -0.0989 (0.6962) | 0.0015 (0.9952) | -0.0037 (0.9883) | 0.4241 (0.0795) |
| Color b* | -0.2576 (0.3020) | 0.0065 (0.9795) | -0.4147 (0.0871) | 0.2982 (0.2293) | 0.0249 (0.9219) | -0.0989 (0.6962) | 0.0067 (0.9789) | 0.0067 (0.9789) | -0.5012 (0.0341) | -0.2638 (0.2902) |
| Color ΔE* | -0.3111 (0.2089) | -0.0044 (0.9863) | -0.5120 (0.0298) | -0.4152 (0.0866) | -0.0522 (0.8369) | 0.0015 (0.9952) | 0.0067 (0.9789) | 0.8555 (0.0000) | 0.8555 (0.0000) | -0.0704 (0.7815) |
| Dry matter | -0.1180 (0.6410) | 0.4090 (0.0919) | 0.4801 (0.0438) | 0.4793 (0.0442) | 0.0150 (0.9530) | 0.4241 (0.0795) | -0.2638 (0.2902) | -0.0704 (0.7815) | 0.0771 (0.0000) | 0.7610 (0.7610) |

^a Values in parentheses correspond to the P-value which tests the statistical significance of the estimated correlations. P-values below 0.05 indicate statistically significant non-zero correlations at the 95% confidence level.



efficiency up to 10.4% in Experiment 1 and 22.3% in Experiment 2.

Gerrits and Amsing (1996) demonstrated that the increased yield obtained with supplementation depends on the nitrogen content of the unsupplemented compost; thus, the positive effect of supplementation was greater using compost poor in nitrogen. According to these authors, a positive effect of supplementation is still observed with high-nitrogen composts, but the extra yield is much lower. In addition, the mycelium in compost supplemented with high nitrogen levels may be too active, hindering temperature control.

In an evaluation of different types of supplements, Desrumaux *et al.* (1999) also verified that supplementation produces minor increases in performance (7.5 to 15.1%, depending on the type of supplement) in compost with high nitrogen content. The claim that high nitrogen content can decrease the effect of supplementation cannot be generalized, especially considering the results of our present experiments, which demonstrated major increases in performance in composts with high nitrogen content (2.61% in experiment 2 and 2.45% in Experiment 1) and certain contradictions found in other studies (Desrumaux *et al.*, 1999).

Another parameter to consider regarding the compost used for the cultivation of *A. bisporus*, related to the nitrogen content of the compost, is the C/N ratio, whose recommended range in Phase II compost is between 16/1 and 22/1, with an optimum of 19/1 (Zied *et al.*, 2011a). Composts with high nitrogen contents generally have low C/N ratios, which in our case were found to be 17.7 and 16.4.

The additional nitrogen provided by the supplement implies an added decrease in the C/N ratio, placing the ratio below the recommended range given its low initial value, but this decrease did not appear to influence the effectiveness of supplementation. Other aspects related to the preparation of compost, such as its formulation, the type of activators used and

the microbial biomass and selectivity may condition the response to supplementation (Randle, 1985).

On the economic side, Randle and Smith (1986) established that an increase in the performance level of 1.5 kg m⁻² is required to offset the cost of supplementation. This increase was exceeded in both of our experiments with both doses used, which demonstrates the potential of this practice even when mushrooms are grown on compost with high nitrogen contents.

In our experiments, the supplement was applied at the time of spawning. This is the only practical time of application for the culture system using bags (compost blocks) in Phase II compost. However, according to Gerrits (1988), the addition of supplements to well-colonized compost immediately before applying the casing layer is the best method of supplementation. When Phase III compost is used, this operation can be performed either at the time of packaging or in the bulk-filling growth room (chamber of cultivation).

Given the need for a good system to control the substrate temperature during mushroom cultivation, the application of supplements is a technique to consider in temperature-controlled rooms to improve mushroom yields and increase the commercial profitability of the crop.

The results obtained in our study demonstrate that an excellent response to supplementation can be obtained even when using composts with high nitrogen contents (in our case, 2.45 and 2.61% sms) during spawning. It is additionally conceivable that the application of supplements to composts with lower N contents or the application to spawned compost would provide an even better yield, without adversely affecting the quality of the harvested mushrooms.

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REFERENCES

1. Aenor. 2001a. Mejoradores de Suelos y Sustratos de Cultivo. Determinación del pH. Norma Española, Madrid, 11 PP.
2. Aenor. 2001b. Mejoradores de Suelos y Sustratos de Cultivo. Determinación de la Conductividad Eléctrica. Norma Española, Madrid, 13 PP.
3. Aenor. 2001c. Mejoradores de Suelos y Sustratos de Cultivo. Determinación de las Propiedades Físicas. Densidad Aparente Seca, Volumen de Aire, Volumen de Agua, Valor de Contracción y Porosidad Total. Norma Española, Madrid, 25 PP.
4. Ankom. 2008. *Crude Fiber Analysis in Feeds by Filter Bag Technique*. ANKOM Technology, Macedon, 03 PP.
5. Ankom. 2009. Rapid Determination of Oil/Fat Utilizing High Temperature Solvent Extraction. ANKOM Technology, Macedon, 02 PP.
6. Ansorena, J. 1994. *Sustratos. Propiedades y Caracterización*. Mundi-prensa, Madrid, 172 PP.
7. Bano, Z., Shashirekha, M. N. and Rajarathnam, S. 1993. Improvement of the Bioconversion and Biotransformation Efficiencies of the Oyster Mushroom (*Pleurotus sajor-caju*) by Supplementation of Its Rice Straw Substrate with Oil Seed Cakes. *Enzyme Microb. Tech.*, **15**: 985-987.
8. Brady, J. 1969. Some Physical Gradients Set up in Tullgren Funnels during the Extraction of Mites from Poultry Litter. *J. Appl. Ecol.*, **6**: 391-402.
9. Carroll, A. D. and Schisler, L. C. 1976. Delayed Release Nutrient Supplement for Mushroom Culture. *Appl. Environ. Microbiol.*, **31**(4): 499-503.
10. Desrumaux, B., Seydeyn, P., Werbrouck, A. and Lannoy, P. 1999. Supplémenter dans la Culture du Champignon de Couche: Experience Comparative Avec Quelques Produits de Supplémentation du Commerce. *Bull. FNSACC*, **81**: 789-802.
11. Foss. 2003. The Determination of Nitrogen According to Kjeldahl Using Block Digestion and Steam Distillation. Foss Application Note AN 300, Högan, 12 PP.
12. Gerrits, J. P. G. 1988. *Nutrition and Compost. The Cultivation of Mushrooms*. (Ed.): van Griensven, L. J. L. D. East Grinstead, Sussex, UK., PP. 29-72.
13. Gerrits, J. P. G. and Amsing, J. G. M. 1996. Milli Champ 3000 en 6000. *De Champignoncultuur*, **40**(10): 397-400.
14. González, J., Alvira, P. and González, G. 1987. La Cascarrilla de Arroz en la Alimentación Animal. II. Composición Químico-Bromatológica. *Rev. Agroquím. Tecnol. Aliment.*, **27**:139-149.
15. Hammond, J. B. W. and Nichols, R. 1976. Carbohydrate Metabolism in *Agaricus bisporus* (Lange) Sing.: Changes in Soluble Carbohydrates during Growth of Mycelium and Sporophore. *J. Gen. Microbiol.*, **93**: 309-320.
16. Krantz, G. W. 1986. *A Manual of Acarology*. 2nd Edition (Emended), Oregon State University Book Stores, Corvallis, 509 PP.
17. Mapa. 1994. *Métodos Oficiales de Análisis*. Ministerio de Agricultura, Pesca y Alimentación, Madrid, 532 PP.
18. Msc. 1985a. Determinación del Índice de Materias Celulósicas (Método de Weende). In: "Análisis de Alimentos. Métodos Oficiales y Recomendados por el Centro de Investigación y Control de la Calidad". Ministerio de Sanidad y Consumo, Servicio de Publicaciones, Madrid, Spain, PP. 346-348.
19. Msc. 1985b. Grasa. In: *Análisis de Alimentos. Métodos Oficiales y Recomendados por el Centro de Investigación y Control de la Calidad*. Ministerio de Sanidad y Consumo, Servicio de Publicaciones, Madrid, Spain, PP. 354-355.
20. Nombela, G. and Bello, A. 1983. Modificaciones al Método de Extracción de Nematodos Fitoparásitos por Centrifugación en Azúcar. *Bol. Sanid. Veg., Plagas*, **9**: 183-189.
21. Pardo-Giménez, A., Pardo-González, J. E., de Juan, J. A. and Zied, D. C. 2010. Modelling the Effect of the Physical and



- Chemical Characteristics of the Materials Used as Casing Layers on the Production Parameters of *Agaricus bisporus*. *Arch. Microbiol.*, **192**: 1023–1030.
22. Pardo-Giménez, A., Zied, D. C. and Pardo-González, J. E. 2011a. Using Spent Mushroom Substrate as Casing Layers in New Growing Cycles. *Pesq. Agropec. Bras.*, **45(10)**: 1164-1171.
23. Pardo-Giménez, A., Pardo-González, J. E. and Zied, D. C. 2011b. Evaluation of Harvested Mushrooms and Viability of *Agaricus bisporus* Growth Using Casing Materials Made from Spent Mushroom Substrate. *Int. J. Food Sci. Technol.*, **46(4)**: 787-792.
24. Pardo-Giménez, A., Zied, D. C., Álvarez-Ortí, M., Rubio, M. and Pardo-González, J. E. 2012. Effect of Supplementing Compost with Grape Seed Meal on *Agaricus bisporus* production. *J. Sci. Food Agric.*, **92(8)**: 1665-1671.
25. Peeters, J. 2008. The Art of Supplementing. *Mushroom Business*, **30**: 24-25.
26. Randle, P. E. 1985. Supplementation of Mushroom Composts: A Review. *Mushroom J.*, **151**: 241-249.
27. Randle, P. E. and Smith, J. F. 1986. Economic Aspects of Compost Supplementation. *Mushroom J.*, **165**: 297-305.
28. Roy, S., Anantheswaran, R. C. and Beelman, R. B. 1995. Sorbitol Increases Shelf Life of Fresh Mushrooms Stored in Conventional Packages. *J. Food Sci.*, **60(6)**: 1254-1259.
29. Saharan, M. S. and Guleria, D. S. 1993. Effect of Oilseed Cakes Supplementation on Yield Parameters of Mushroom [*Agaricus bitorquis* (Quel.) Sacc.]. *Crop Res.*, **6(1)**: 59-63.
30. Sánchez, J. E. and Royse, D. J. 2001. Adapting Substrate Formulas Used for Shiitake for Production of Brown *Agaricus bisporus*. *Bioresour. Technol.*, **77(1)**: 65-69.
31. Sharma, H. S. and Kilpatrick, M. 2000. Mushroom (*Agaricus bisporus*) Compost Quality Factors for Predicting Potential Yield of Fruiting Bodies. *Can. J. Microbiol.*, **46**: 515-519.
32. Zied, D. C., Pardo-Gonzalez, J. E., Minhoni, M. T. A. and Pardo-Gimenez, A. 2011. A Reliable Quality Index for Mushroom Cultivation. *J. Agr. Sci.*, **3(4)**: 50-61.
33. Zied, D. C., Savoie, J. M. and Pardo-Giménez, A. 2011b. *Soybean the Main Nitrogen Source in Cultivation Substrates of Edible and Medicinal Mushrooms. Soybean and Nutrition*. InTech Open Access Publisher, Rijeka, Croatia. PP. 433-452.
34. Zied, D. C., Pardo-Giménez, A., Pardo-González, J. E., Souza Dias, E., Carvalho, M. A. and Minhoni, M. T. A. 2013. Effect of Cultivation Practices on the β -Glucan Content of *Agaricus subrufescens* Basidiocarps. *J. Agric. Food Chem.*, **62(1)**: 41-49.

افزودن کمپوست *Agaricus* نیتروژن بالا: عملکرد و کیفیت قارچ

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