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Wool Agro-Waste Biomass and Spruce Sawdust: Pellets as an Organic Soil Amendment

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Abstract: The production of wool is an economic burden and an issue for sheep breeders in many countries of the European Union because shorn greasy wool is defined as an animal byproduct (category 3) and must be sent to landfill as a special waste if not addressed in the textile supply chain. Nevertheless, wool is an important source of nitrogen, with high potential as agricultural renewable and sustainable organic fertilizer. To apply wool to soil, any contamination from harmful bacteria (e.g., *Listeria monocytogenes* and *Salmonella* spp.) should be excluded. In this study, we developed sheep wool pellets to test their suitability for use as an organic fertilizer. Wool was rich in N (12% of dry material) and was mixed to spruce sawdust at sawdust: wool ratios of at 2:1; 1:1 (*v*/*v*) to increase soil organic carbon. Despite the different mix of wool and sawdust, pellets were similar in size (diameter and length), and the content of the elements suited the requirements of fertilizers and did not present harmful bacteria after pelletization. Therefore, wool pellets may represent a feasible solution to provide sheep wool with an added value, introducing it in a circular economy process. However, further study is needed to test the effects of the produced fertilizing pellets in real cropping systems.

Keywords: wool pellet physical characteristics; hygroscopic properties of wool; wool chemistry; sheep wool

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1. Introduction

Agriculture strongly impacts the global nitrogen (N) and carbon (C) cycles through land-use change and agronomic management. Currently, there is a great interest in studying the possible recovery of byproducts materials, to be applied in agriculture, that can minimize C and N losses while improving technical efficiency and productivity. N is the main nutrient that limits the growth and development of plants after carbon, hydrogen, and oxygen have been involved in the photosynthetic process, phyto-hormonal activities, and proteomic changes. N is crucial for plants to complete their lifecycle [1–3]. N management is closely related to, at least, nine Sustainable Development Goals, including those focusing on food supply and pollution [2]. In 2020, the global demand for N in crop production was 152 million tons and it is expected to increase to almost 178 million tons in 2030 [4,5]. The Farm to Fork strategy of the EU's Green Deal aims to reducing chemical pesticide usage by 50% and fertilizer usage by 20%, plus a decrease in nutrient losses by at least 50% [6]. In

this respect, high organic C soil amendments are beneficial as they promote microbial N immobilization by stimulating microbes to take up N from soil [7]; C constitutes an energy source, and sufficient available N and P fulfill stoichiometric requirements and avoid nutrient mining of original soil organic matter [8]. Sheep wool, containing, on average, 44% C and 10–11% of N, can be an important resource of C and N, so it can be considered both as soil amendment and nutrient source. Additionally, the EU-27 sheep population is the second largest in the world [9]. In 2021, it amounted to about 59 million livestock heads, most of them in Spain (25.50%), followed by Romania (17.06%), Greece (13.01%), France (11.83%), Italy (11.38%), and Ireland (6.75%) [10]. The production of wool represents an economic burden, especially for famers who produce wool of scarce quality for the fashion clothing textile industry. According to European Commission Regulations 1069/2009 and 142/2011 [11], the shorn wool—in case it is not addressed in the industrial processing steps—is defined an animal byproduct (category 3) and must be sent to landfill as a special waste. This is both an economic issue for farmers, as the expenses of sheep shearing, which is a physiological need for sheep care, are not covered by the sales price of wool [12], and an environmental threat if wool is not properly disposed (i.e., buried or burnt) [13]. However, the recent EU Regulation (2019/1009) [11] on organic fertilizers and the EU Decision (2022/591) [14] on general action programme in the field of the environment up to 2030 provide two great opportunities for the enhancement of wool and its applications, both in open field and greenhouse productions in real cropping systems. However, as unprocessed (greasy) wool may be contaminated with pathogens (e.g., *Listeria monocytogenes* and *Salmonella* spp.) [15], causing transmissible diseases, it must be subject to the risk mitigation measures provided for in Regulation (EC) No 1069/2009 [11]. Sheep wool has been tested as a fertilizer in various forms, e.g., washed wool fibers, wool residues from industrial washing, and hydrolyzed wool [16–22], with positive results on crop productions and soil moisture retention. Bradshaw and Hagen [23] reported how wool pellets are a viable alternative to commercial fertilizers for organic vegetable production as they showed very similar growth and mineral uptake as compared to commercial fertilizers. In our study, we developed pellets mixing wool and sawdust to also increase the concentration of carbon in the soil and find an alternative use to sawdust biomass. We, thus, added spruce sawdust, byproduct available in the study area, characterized by a C:N ratio of 400:100, and leading to lower degradation rates and prolonged times of decay [24].

The use of sawdust in agriculture is not burdened by regulatory provisions and, because of the high content of total organic carbon [7], it can improve the chemical–physical characteristics of the soil. The aim of the study was to evaluate the use of biobased alternatives (sheep wool) in producing pellets for agricultural applications to replace nonrenewable resources. Particularly, this research is aimed at (i) developing pellets made of greasy wool and sawdust; (ii) describing the physical and chemical characteristics of the resulting pellets for their technical and commercial use in the agricultural sector; (iii) assessing the inhibitory effect of the pelletizing process on the microbiological load of the greasy wool.

2. Materials and Method

2.1. Raw Materials, Grinding, and Preparation of the Pelleting Tests

2.1.1. Wool

Henceforth, the word "wool" will refer to greasy wool, that is, wool shorn from sheep. The wool was obtained from Pomarancina sheep breed at an experimental farm, University of Florence, Italy (43°78′48.7″ N and 11°22′20.0″ E). Pomarancina is a local, traditional breed of the western inland of Tuscany, usually raised for meat in permanent semi-wild management and therefore adapted to marginal areas. The average wool production ranges from 2.5 kg (rams) to 1.5 kg (sheep). Pomarancina wool is a coarse wool that is suitable for making mattresses [25].

2.1.2. Spruce Sawdust

Fine spruce sawdust was produced from virgin spruce wood. The sawdust particle size was determined using a programmed shaker equipped with a <0.25 mm sieve. The moisture content of wool and sawdust was determined using a Kern DAB 100-3 moisture analyzer (Kern & Sohn Gmbh, Balingen, Germany).

2.1.3. Material Humidity, Density and Weight

Five repeated moisture measurements were conducted for each material and the mean value of the moisture content was calculated, resulting 11% ± 1.41 (wool) and 15.5% ± 0.71 (fine spruce sawdust).

The wool bulk density was estimated by placing and gently compressing an amount of wool in a container of known volume until filling the container. As wool is a compressible material, this operation was performed three times to identify the average standard amount of wool completely fitting the volume of the container. The same method was used for the sawdust. The three weights of each material per volume unit were recorded and the average weights both for wool and sawdust were calculated, resulting in 58.8 g ± 7.6 g per liter and 161.4 g ± 9.7 g per liter, respectively. This information was necessary to identify a standard weight of raw materials (i.e., 60 g/L and 160g/L for wool and spruce sawdust, respectively) to calculate the optimal ratios for the pellet production.

2.1.4. Wool Grinding

The wool was ground using a cutting mill (Retsch SM 200, Retsch Company, Düsseldorf, Germany) to obtain wool swabs (2 mm), according to two methods: (i) inserting the wool previously cut into 2 cm swabs; (ii) inserting the sheared wool as it was without any precutting and assess the feasibility and efficacy of the cutting mill. Three mixtures were prepared by weighing the appropriate masses of fine spruce sawdust and wool and mixing them by hand, according to the following ratios (volume on volume): 1:1 and 2:1. The preparation of the raw materials and the following pelleting tests were conducted at the laboratory of the Institute of BioEconomy (IBE), National Research Council in San Michele all'Adige, Trento, Italy, in July and September 2023.

2.2. Pelletizing Process

The pelletizing process was performed using a three-phase electric wood pellet machine Smartwood PLT 100 (Agrieuro S.r.l., Perugia, Italy) (Figure 1). The following parameters were considered in all the tests: diameter of die holes, do = 6 mm; feedstock mass flow, Qm = 50 kg · h^{-1}; compacting roll rotational speed, nr = 270 rpm; gap between rollers and die, hr = 0.35 mm. To facilitate the palletization, the three mixtures of sawdust and wool were humidified before pelletizing by spraying about 100 mL of water in each mix. Three different pelletizing tests were conducted. The test PT-1 was performed using a mixture of sawdust and wool (ratio 1:1) and the tests PT-2 and PT-3 with a mixture of fine spruce sawdust (ratio 2:1). In the PT-3, the mixture with precut wool was used.

During the pellet production, the pellet temperature was measured by placing a thermocouple surface probe in the bucket where all pellets were collected (Orbis TD50, Cassina De' Pecchi, Milano, Italy).

2.3. Physical Characterization of the Pellets

For each pelletizing test (Figure 2), the fresh weight of 100 pellets was recorded. Afterwards, the pellets were oven-dried at 105 °C to remove any moisture until constant weight and to estimate the dry weight. Pellets were characterized by weight, size (proximal, distal diameters, diameter ratio and length), density, potential water absorption, and particle size determination of produced pellets. The 1.000-pellets weight was measured using a precision scale; proximal and distal diameters were detected by using a digital caliper on two axial replicates; diameter ratio was calculated by dividing the distal/proximal value; and length was measured with a ruler. For the potential water absorption, the samples

were stored in a room (27.0 °C ± 2.1 and 69.0 RH% ± 5.7) and weighed every day from T0 to 438 h, at the same time of the day, to assess the potential water absorption. The bulk density was calculated using the following equation:

$$\rho_{n,p} = \frac{m_p}{V_n}\left(Kg \cdot m^{-3}\right) \quad (1)$$

where m_p—sample mass (kg) and V_n—cylinder volume (m^3). A glass cylinder with a volume of 0.001 m^3 (Duran™ Measuring Cylinder, Wurttemberg, Germany) and a laboratory balance were used to test the bulk density of the pellets [26]. Each time, the cylinder was filled in with the sample until a possibly even plane was obtained. The analysis was performed over four repetitions.

Figure 1. Cutting mill on the **left** and pellet machine at the **center**. Up on the **right**, the matrices: wool and sawdust. On the **bottom right**, an example of mixture (1:1) and pellets (1:1).

The particle size determination of the pellets was carried out using a programmed shaker equipped with a set of sieves, (Control, Milan, Italy). During the analysis, a set of 7 sieves with square mesh side dimensions was used: 8.0 mm; 4.0 mm; 2.8 mm; 2.0 mm; 1.40 mm; 0.710 mm; and 0.425 mm. The particle size distribution was determined in accordance with PN-89/R-64798 [27]. The analysis was carried out according to the procedure described by Obidzinski et al. [28], and the result was the arithmetic mean of three tests performed.

2.4. Microbiological and Chemical Analysis

The microbiological analyses of raw materials (wool, wool and sawdust mixture) and pellets were performed at the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Florence, Italy [29]. The samples were subjected to a comprehensive microbiological investigation to assess health risks according to the UNI EN ISO quality standards, as described in Table 1. Culture media (produced at in-house laboratory), and investigation procedures were chosen following the UNI EN ISO standards, as described in Table 1.

Figure 2. Pellets produced by the three tests: (PT-1) ratio spruce sawdust: wool = 1:1; (PT-2) ratio spruce sawdust: wool = 2:1; and (PT-3) ratio spruce sawdust: cut wool = 2:1.

Table 1. List of investigated microbiological agents and methods.

Agent	Method
Clostridium perfringens (bacterial count)	ISO 15213-2:2023 [30]
Escherichia coli (B-glucuronidase producing strains)	UNI ISO 16649-2: 2010 [31]
Listeria monocytogenes	UNI EN ISO 11290-1: 2017 [32]
Total bacterial count (30 °C)	UNI EN ISO 4833-1:2022 [33]
Salmonella spp.	ISO 6579-1:2017/Amd 1:2020 [34]
Coagulase positive *Staphylococcus*	ISO 6888-2:2021 [35]

Briefly, test portions were collected under aseptic conditions and processed as follows. For *Clostridium perfringens*, coagulase-positive *Staphylococcus* and total bacterial count, 10 g of material was mixed with 90 mL of peptone tryptone water (PTW) medium and serially diluted; 1 mL of each dilution was included in sulfite cycloserine agar and incubated at 37 °C for 20 h. Suspected colonies were confirmed biochemically, according to ISO 15213-2:2023 [30]. For total bacterial count, 1 mL of each dilution was included in plate count agar (PCA), aerobically incubated at 30 °C for 72 h [36]; *E. coli* count was performed using Tryptone–Bile—X-GLUC Agar (produced in house Lab) aerobically incubated at 44 °C for 24 h [37]; for enumeration of coagulase-positive *Staphylococci*, 1 mL of each dilution was inoculated on Baird Parker agar with RPF supplement incubated at 37 °C for 48 h [38]. The results were reported as log CFU (colony-forming units)/g of raw materials. The averages were calculated using the countable values.

For *Salmonella* spp., a 25 g test portion was mixed in 225 mL of sterile buffered peptone water (BPW) and homogenized for 60 s at room temperature (24 °C) in a Stomacher 400 device (Stomacher 400 circulator; Seward Ltd., Norfolk, UK). Homogenates were incubated at 37 °C for 24 h. BPW cultures were inoculated in enrichment in Rappaport Vassiliadis Soy Broth (RVS) at 42 °C for 24 h and then further inoculated further on

Salmonella-Shigella Agar (SS) and Xylose-Lysine-Desoxycholate Agar (XLD) at 37 °C for 24 h [39].

The presence of *Listeria monocytogenes* was also tested. Briefly, 25 g of each sample were mixed with 225 mL of Half Fraser Broth and incubated at 30 °C for 24 h for the primary enrichment. Then, 10 mL of Fraser Broth inoculated with 0.1 mL of pre-enrichment broth were incubated at 37 °C for 24 h for the secondary enrichment. After that, 1 mL of secondary enrichment was surface-plated on Agar Listeria according to Ottaviani and Agosti (ALOA) Agar and subsequently incubated at 37 °C for 24–48 h.

Total organic carbon (TOC) and N were evaluated through dry combustion using a FlashSmart elemental analyzer (Thermo Fisher Scientific, Milan, Italy). Micro- and macronutrients (Ca, Mg, Na, K, Fe, Mn, Cu, Zn, Ni, Cr, Pb, Cd) were measured by an inductively coupled plasma—optical emission spectrometer (ICP–OES 5900, Agilent, Santa Clara, CA, USA) after microwave-assisted acid digestion with H_2O_2/HNO_3 1:3 *v*:*v* (EPA 3051A, 6010C). Total phosphorus (TP) was determined by the colorimetric method [40] in acid extracts (H_2O_2/HNO_3 1:3 *v*:*v* in microwave) and measured spectrophotometrically using a Thermo Spectronic Unicam UV, Milan, Italy.

2.5. Data Analysis

Prior to analysis, the data population normality was verified using the Kolmogorov–Smirnov test and SPSS software (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY, USA: IBM Corp.). Data were subjected to statistical analysis (one-way ANOVA) using the SAS/stat package version 8.0 (SAS Inst. Inc., Cary, NC, USA), considering the effect of the pelletizing test. Means were compared using Duncan's test at $p \leq 0.05$. A significance threshold of $p = 0.05$ was considered. The linear correlation analysis was applied to determine the relationship between relative humidity (%RH), and moisture content (MC, %) dynamic of the three types of pellets produced.

3. Results

3.1. Raw Materials, Grinding, and Pellet Characterization

The moisture content of the three mixtures of sawdust and wool was 14.3% ± 2.45 (ratio 1:1) and 15.4% ± 3.01 (ratio 2:1). The pellet temperature, immediately recorded at the beginning of the process, was 57.01 °C ± 3.9, and increased after 2 min, stabilizing up to 71.05 °C ± 2.7.

PT-1 to PT-2 and PT-3 pellets were characterized by a moisture content of 90.80%, 89.61%, and 88.12%, respectively, as reported in Table 2. Bulk density showed a decreasing trend from PT-1 to PT-2 and PT-3, although differences were not significant, and the bulk density values of the pellets were inversely proportional to the increase in their length. The pellet length varied from 16.95 mm (PT-2) to 20.87 mm (PT-3), the latter significantly differing from the other two.

Regarding the pellet diameter, no significant differences were found between the three types of pellets. Figure 3 shows the particle size distribution for the three types of pellets. The most common particles obtained by the pellet sieving process were the 8.00 mm and 4.00 mm fractions. The 8.00 mm fraction was 13.56% in (PT-1), 20.99% in (PT-2), and 53.95% in (PT-3). The 4.00 mm fraction was, instead, 83.49% in (PT-1), 70.47% in (PT-2), and 44.5% in (PT-3). In the distribution of the 8.00 mm particle, the fraction amount of PT-3 was significantly higher than PT-1 and PT-2. In the distribution of the 4.00 mm particle, PT-1 and PT-2 were significantly higher than the PT-3 group. The particle size distribution of the produced pellets sized 2 mm, 1.40 mm, 0.71 mm, and <0.425 mm showed significant differences between groups; the sum of these fractions can be considered as a component of nonaggregated pellets, and the percentage value was higher in PT-2 (13.07% of the total amount).

Table 2. Physical pellets characteristics.

Items	PT-1 (Ratio 1:1)	PT-2 (Ratio 2:1)	PT-3 (Ratio 2:1)	Mean	
	Mean (SE)	Mean (SE)	Mean (SE)		*p*-Value
DM, %	90.80	89.61	88.12	89.51	-
Bulk density (a.p.p.), (Kg·m^{-3})	475.67 (12.89)	458.12 (13.22)	397.42 (16.90)	444.07	n.s.
1.000-pellet weight, g	464.14 (1.87) ^{b}	496.40 (1.08) ^{a}	501.05 (1.07) ^{a}	487.20	0.01
Proximal diameter, mm	3.64 (0.08)	3.70 (0.09)	3.82 (0.09)	3.72	n.s.
Distal diameter, mm	3.71 (0.10)	4.21 (0.09)	3.91 (0.09)	3.94	n.s.
Diameter, ratio	1.02 (0.41)	1.13 (0.13)	1.02 (0.01)	1.06	n.s.
Length, mm	17.06 ^{a} (0.71)	16.95 ^{a} (0.99)	20.87 ^{b} (0.99)	18.28	0.04

DM = dry matter; bulk density; proximal diameter = diameters measured in the proximal part of the pellet; distal diameter = diameters measured in the two proximal parts of the pellet and expressed as an average of the 2 values; diameter ratio = distal/proximal value; length = pellet length. Means (standard error in parentheses). Different letters within each row indicate significant differences (within a variable) between pelleting tests according to Duncan's range test ($p \leq 0.05$); n.s. = not significant.

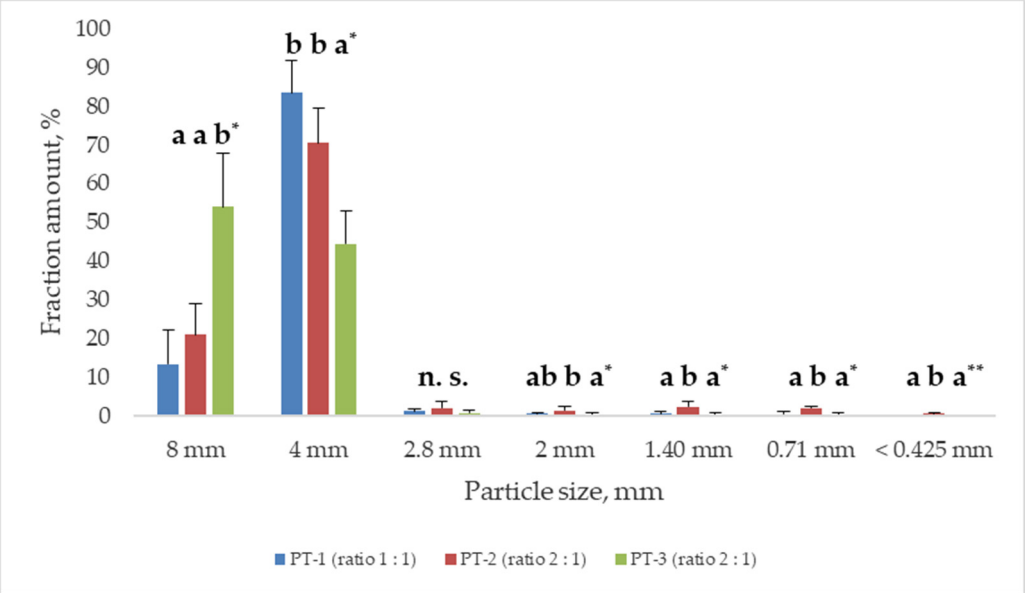

Figure 3. Particle size distribution of pellets. Different letters indicate significant differences (within a variable) between particle size distribution of pellets according to Duncan's range test (* $p \leq 0.05$; ** $p \leq 0.001$).

Figure 4 shows the results related to the moisture content measured as a percentage of the dry weight of the pellet samples at the beginning of the test (T0) as well as the dynamics of water absorption at known room conditions (56.4 ± 8.8%RH, and 20.2 ± 2.3 °C). PT-1 absorbed +12.5% of water from the storage site during the 438 h test, PT-2 +8.0%, and PT-3 +3.3%. These results were confirmed (Figure 5) by the correlations between room relative humidity (%RH) and moisture content (MC, %) dynamic after (T0) being oven-dried at 105 °C. PT-1 and PT-2 pellets showed the same trend, with high correlation in PT-1 (r^2 = 0.915), and more contained overall correlations were estimated in PT-2 (r^2 = 0.726). Moreover, Figure 5 shows a different dynamic for PT-3, with a low negative correlation (r^2 = 0.483) between room relative humidity (%RH) and moisture content (MC, %), but with a limited loss of moisture of the pellets (−0.03%) compared to T0.

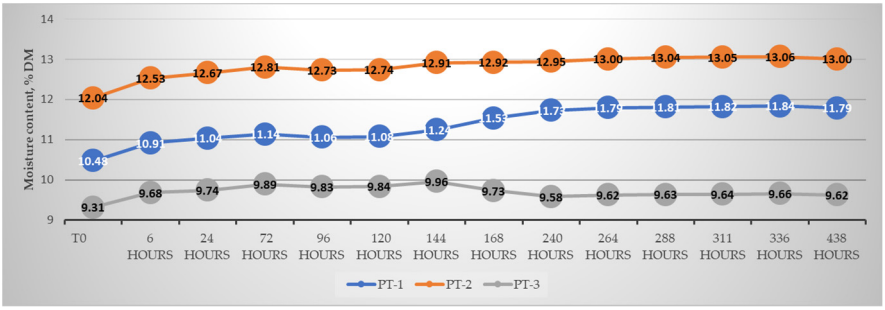

Figure 4. Dynamics of oven-dried (105 °C) pellet moisture content, measured as water content relative to the dry matter expressed as percentage (%DM), for 438 h.

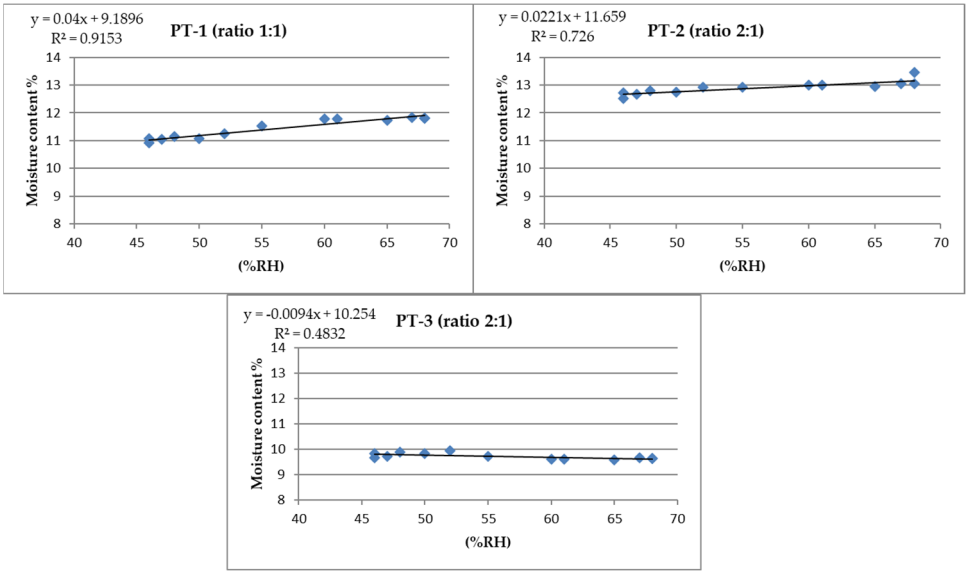

Figure 5. Statistical correlation between room relative humidity (%RH) and moisture content (MC%) dynamic of the three types of pellets oven-dried at 105 °C.

3.2. Microbiological and Chemical Analysis

The results concerning the microbiological analysis are reported in Table 3. *Salmonella* spp. and *Listeria monocytogenes* were not detected in the analyzed samples. *E. coli* in wool was limited, ranging from $<5.70 \times 10^4$ CFU/g to $<5.90 \times 10^2$ CFU/g in the mixtures prepared prior to pelletization. The total count of viable microorganisms was $<3.20 \times 10^5$ in wool and ranged between $<1.20 \times 10^7$ CFU/g and $<2.20 \times 10^4$ CFU/g in the mixtures; only in PT-1, was the value of total viable count found to be $<3.50 \times 10^3$ CFU/g. The comparison between the number of bacteria tested in the raw and pelletized materials showed a significantly lower value in the latter (*Clostridium perfringens*, *E. coli*, total viable count, and coagulase-positive *staphylococcus*).

Concerning the chemical characterization (Table 4), higher values of total N (as percentage of dry matter) were detected in PT-1 (1.79%) and PT-3 (1.71%), which were statistically different to PT-2 (1.26%). The three types of pellets showed similar values of TOC (as percentage of dry matter). Higher Ca values were detected in PT-2 and PT-1: 1225.5 mg kg^{-1} and 1011.5 mg kg^{-1}, respectively, both significantly different from the lower value of PT-3 (962.0 mg kg^{-1}). Higher Cu values were found in PT-2 (2.49 mg kg^{-1}) compared to PT-3 (1.71 mg kg^{-1}), whereas no Cu was detected in PT-1. The Fe values differed significantly in the three samples: 144.5 mg kg^{-1}, 293.5 mg kg^{-1}, 173.50 mg kg^{-1} in PT-1, PT-2, and PT-3, respectively. The higher K values detected in PT-1 and PT-2 pellets (6689.0 mg kg^{-1} and 6687.0 mg kg^{-1}, respectively) were significantly different from the PT-3 value (5769.50 mg kg^{-1}). Mg values were significantly higher in PT-3

(258.0 mg kg^{-1}) in comparison with PT-1 (231.0 mg kg^{-1}) and PT-2 (222.0 mg kg^{-1}). Mn values in PT-2 (81.40 mg kg^{-1}) were higher than values in PT-1 (70.70 mg kg^{-1}) and in PT-3 (69.10 mg kg^{-1}). With regard to Na, higher values were detected in PT-2 (72.30 mg kg^{-1}) in comparison with PT-1 (58.20 mg kg^{-1}) and PT-3 (58.80 mg kg^{-1}). The Zn values were higher in PT-2 (18.30 mg kg^{-1}) than in PT-1 (15.80 mg kg^{-1}) and PT-3 (14.50 mg kg^{-1}). Finally, the P values of the three samples significantly differed, with PT-1 having the highest values (95.80 mg kg^{-1}) followed by PT-3 (51.40 mg kg^{-1}) and PT-2 (33.30 mg kg^{-1}). No content of Cd, Cr, Ni, Pb, Mo, and B was found in the three types of pellets.

Table 3. Microbiological analysis (raw materials and pellets).

Items	Wool	Mixture 1:1	Mixture 2:1	PT-1	PT-2	PT-3
Clostridium perfringens, CFU	$<1.00 \times 10^1$	$<1.50 \times 10^2$	$<1.00 \times 10^1$	$<1.00 \times 10^1$	$<1.00 \times 10^1$	$<1.00 \times 10^1$
E. coli beta-glucuronidase positive test, CFU	$<1.00 \times 10^1$	$<5.90 \times 10^2$	$<5.70 \times 10^4$	$<1.00 \times 10^1$	$<1.00 \times 10^1$	$<1.00 \times 10^1$
Listeria monocytogenes	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total viable count (TVC), CFU	$<3.20 \times 10^5$	$<2.20 \times 10^4$	$<1.20 \times 10^7$	$<3.50 \times 10^3$	$<1.00 \times 10^1$	$<1.00 \times 10^1$
Salmonella spp., CFU	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Coagulase-positive *staphylococcus* (CPS), CFU	$<1.00 \times 10^1$	$<1.00 \times 10^1$	$<1.00 \times 10^1$	$<1.00 \times 10^1$	$<1.00 \times 10^1$	$<1.00 \times 10^1$

Each sample was analyzed in duplicate and the results are reported as CFU (colony-forming units)/g of raw materials; n.d. = not detected in 25 g.

Table 4. Pellets chemical analysis.

Items	PT-1 (Ratio 1:1)	PT-2 (Ratio 2:1)	PT-3 (Ratio 2:1)	Mean	
		Mean ± s.d. (CV)			*p*-Value
Total N, % DM	1.79 ± 0.05 (2.64) [a]	1.26 ± 0.02 (1.85) [b]	1.71 ± 0.04 (2.37) [a]	1.58	0.002
TOC, % DM	44.43 ± 2.58 (5.82)	44.04 ± 3.20 (7.28)	43.89 ± 1.23 (2.81)	44.11	n.s.
Ca, mg kg^{-1}	1011.5 ± 33.23 (3.28) [b]	1225.5 ± 33.23 (2.71) [a]	962.00 ± 28.28 (2.94) [b]	1066.33	0.007
Cu, mg kg^{-1}	n.d.	2.49 ± 0.08 (3.41) [a]	1.71 ± 0.42 (3.67) [b]	1.40	<0.001
Fe, mg kg^{-1}	144.50 ± 3.54 (2.45) [c]	293.00 ± 11.31 (3.86) [a]	173.50 ± 6.36 (3.67) [b]	203.67	<0.001
K, mg kg^{-1}	6689.00 ± 117.38 (1.75) [a]	6687.00 ± 120.21 (1.76) [a]	5769.50 ± 116.67 (2.02) [b]	6428.50	0.005
Mg, mg kg^{-1}	231.00 ± 2.83 (1.22) [b]	222.00 ± 5.66 (2.55) [b]	258.00 ± 9.90 (3.84) [a]	338.00	0.027
Mn, mg kg^{-1}	70.70 ± 2.40 (3.40) [b]	81.40 ± 1.56 (1.91) [a]	69.10 ± 3.11 (4.50) [b]	73.73	0.027
Na, mg kg^{-1}	58.20 ± 3.96 (6.80) [b]	72.30 ± 3.11 (4.30) [a]	58.80 ± 2.26 (3.84) [b]	63.10	0.035
Zn, mg kg^{-1}	15.80 ± 0.28 (1.79) [b]	18.30 ± 0.71 (3.86) [a]	14.50 ± 0.42 (2.93) [b]	16.20	0.011
P, mg kg^{-1}	95.80 ± 1.84 (1.92) [a]	33.30 ± 2.40 (7.22) [c]	51.40 ± 1.31 (2.20) [b]	60.17	<0.001

DM = dry matter, s.d. = standard deviation, CV = coefficient of variation; n.d. = below detection limit. Different letters within each row indicate significant differences (within a variable) between pelleting tests according to Duncan's range test ($p \leq 0.05$); n.s. = not significant.

4. Discussion

Pellet Characterization

The pelletizing tests performed in this work showed that it is possible to produce three different types of pellets (PT-1, PT-2, and PT-3) from mixtures of sawdust and greasy wool in various proportions.

The morphology of the pellets was examined in terms of diameter, length, and bulk density. PT-3 pellets were longer than PT-1 and PT-2, while the diameter values did not differ between the three types of pellets, with average values of the distal and proximal diameter equal to 3.94 and 3.72 mm, respectively. This can be explained by the fact that PT-3 pellets were more compact compared to the other two types and did not break. The higher mechanical strength of PT-3 pellets could be due to a more homogeneous mixture obtained by grinding sawdust with precut wool; this indicates that the biomass preparation process plays a crucial role in pelletization [41]. The well-formed pellets were mostly collected in

the 8 mm (PT-3, 53.95%) and 4 mm (PT-1 83.49% and PT-2, 70.47%) sieves. The significant difference between PT-3 and PT-1 and PT-2 was probably related to the length and bulk density of the pellets. Even though the fraction of not-well-formed pellets was found in low percentages (3.15% PT-1, 8.53% PT-2, 1.52% PT-3), in terms of product characteristics, this could represent a significant loss of material that could affect the technical and commercial value of the possible fertilizer.

Pellet solidity can be a positive characteristic from a commercial perspective as it affects the regularity of the shape and size of the pellets which, in turn, can contribute to a more even distribution of the pellets on the ground using a traditional fertilizer spreader. Moreover, moisture is important because it is necessary for a successful pelletizing process, especially in small and simplified production systems. Moisture content is one of the most important parameters that negatively influence the properties of pellets, such as bulk density and mechanical durability during transportation and storage. Greasy wool, by its nature, is a hygroscopic material and is very susceptible to moisture uptake from the surrounding environment. Therefore, quality pellets must have low water impermeability to be stored for a long period without water absorption [42]. Lower relative humidity levels in PT-3 pellets compared to PT-1 and PT-2 may be beneficial in storage and transportation, but pellet transportation systems require more specialized design strategies to prevent breakage and abrasion [43], especially for hygroscopic matrices such as those studied. Bulk density can also influence handling, transport, and storage efficiency [44]. In this regard, the bulk density of the pellets was found to be inversely proportional to the length of the pellets. The three types of pellets showed slightly different dynamics of water absorption over 20 days, but they showed different water content. This result can also be explained by the different procedures used to prepare the three sawdust–wool mixtures. Because of its compactness, PT-3 absorbed less water, while in the PT-1 and PT-2 mixtures the wool was not precut and most likely took the form of lint-like formations that could potentially absorb more moisture. However, it is unclear why PT-2 pellets have a higher moisture content despite a lower wool content compared to PT-1 pellets. The contents of certain chemical elements in the composition, such as total N, are responsible for the high hygroscopicity [45] and could explain the capacity of PT-1 pellets to retain moisture. However, these values were comparable to those of PT-3 pellets, which had the lowest moisture content, suggesting that further research on the role of the physical status of wool is required. Regarding chemical analysis, the three theses of pellets showed similar values of TOC calculated as percentage of dry matter (PT-1 44.43, PT-2 44.04, PT-3 43.89), as reported by several authors [46,47], while a significant difference was found for N. A significantly lower content was observed in PT-2.

Mixing wool with sawdust led to a clear decrease in N, notoriously high in wool [16], bringing the C/N ratio between 25 (PT-1 and PT-3) and 35 (PT-2); a TOC/TN ratio lower than 33-30 will provide net N mineralization. Furthermore, in contrast to some animal-based organic fertilizers, such as poultry manure or pig manure with comparable N contents, the pellets made of sawdust also contain cellulose and lignin, which support the development of humus and organic matter in the soil and boost microbial diversity [48]. In fact, one of the objectives in the near future research will be to assess the level of pellets degradation into the soil, and the speed with which nitrogen is available to the plants, in addition to evaluating the carbon stock and plant response to such organic fertilization. Overall, the analysis of the microelements was comparable to those of Gallico [47], indicating that a representative range of produced pellets was included in this study. The variability of macro- and microelements in the three types of pellets may also be related to the presence of organic matrices (plants and manure) in the greasy wool: this aspect also needs to be further investigated in future studies. Specifically, the inclusion of elements like iron, copper, manganese, and zinc in the pellet, which are mostly derived from wool, is very important for plant development [20].

The research of wool contaminants has been focused on chemical contaminants [49,50], and to our knowledge there are no articles on microbiological aspects. In our work, attention

is focused on a broad range of transversal microbiological indicators (aerobic and anaerobic bacterial counts), and specific pathogen agents. Among the latter, we investigated the presence of potentially zoonotic bacteria, such as *Listeria* spp. and *Salmonella* spp., according to EU Directive 2003/99/CE [51] on the monitoring of zoonoses and zoonotic agents. The presence of such bacteria was excluded, suggesting high safety standards both for human health and environmental contamination. The pelletization process reduced the bacterial loads, as already observed by other authors [23,52]. This reduction ensures the efficacy of the heat treatment during pelletization and its positive impact on the control of sanitary risks. However, although the total bacterial counts in the 1:1 and 2:1 mixtures were reduced by the pelletization process, they were higher compared to wool, meaning that may have come into contact with sources of contaminants.

5. Conclusions

The analysis of the pellets showed that they can be used as an organic soil amendment in compliance with the DNSH (Do No Significant Harm) principle and can contribute to achieving the additional environmental objectives of the ecological transition (Regulation (EU) 2020/852 of the European Parliament and of the Council of 18 June 2020).

The characterization of the pellets performed in this study can provide preliminary information about the best combination of materials and can be used in relation to the values of pellets from both a commodity and an agricultural perspective.

The results obtained from the analysis of the pellets show high content in total N and TOC and interesting dynamics of water retention. All of these factors can promote the use in crop production of pellets made of sawdust and greasy wool. Moreover, the profile of the pellets drawn in this work, even though not complete, can contribute to highlight some features in terms of physical properties that, from a commodity point of view, could be useful for the future development of a technical textile product that meets both the demand of farmers in terms of handling and the logistic requirements in terms of transportation and storage.

The drastic reduction in the content of harmful microorganisms in the pellets produced in this work in comparison with the microorganism load found in the raw materials proves that the pelletizing process used is a valid and feasible methodology to avoid the costly industrial wool scouring phase, which is required by law and regulations, to use wool as an additive to the soil.

To the authors' knowledge, this is the first study to investigate the suitability of mixed raw wool and wood materials for pellet production.

Although further research is needed, the use of raw wool for pellet processing looks promising, and encouraging circular economy actions in the agriculture sector could contribute to the economic viability of sheep farming.

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