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A short-term comparison of wheat straw and poplar wood chips used as litter in tiestalls on hygiene, milk, and behavior of lactating dairy cows

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ABSTRACT

A short-term study was conducted to compare the effect of using poplar wood chips (PWC) instead of wheat straw (WS) litter in dairy cows. A total of 38 lactating Holstein cows (204 ± 119 days in milk, 26.9 ± 6.5 kg of milk yield [MY]) were housed in a tiestall farm for a 10-d trial including 5 d of adaptation followed by 5 sampling days (from d 5 to 10). Cows were divided into 2 homogeneous groups: one group was bedded with WS, and the second with PWC. Both litter materials were provided in the amount of 7 kg/stall per d. Each group was composed of 3 subgroups of 6 or 7 cows; the subgroups were physically separated along the feeding line by wooden boards. During the sampling days, fecal composition, used litter composition, and bacterial count (*Clostridium* spp., *Salmonella* spp., *Escherichia coli*, *Lactobacillus*, and total bacterial count) were analyzed by subgroup twice a day. On d 1 and from d 5 to 10, udder hygiene score and cow cleanliness score were also evaluated individually twice a day. Meanwhile MY, milk hygiene (total bacterial count [TBC], coliform bacterial count [CBC], and spore-forming units [SFU]) and quality were measured and analyzed from 9 animals per group. Moreover, individual animal behavior (body position and behavioral traits) and subgroup dry matter intake were measured on d 9 and 10. Fecal dry matter did not differ between groups, PWC had the lowest used litter moisture and N content favoring the highest clean cow frequency, but also gave rise to the greatest used litter microbial contamination. The MY, milk quality, TBC, SFU, and CBC were similar. The lying behavior frequency was similar between groups. However, the PWC group showed the lowest sleeping frequency, the highest frequency of other behaviors (including discomfort signs), and the lowest dry matter intake. However, despite this apparent reduction in cow comfort, no biologically important differences were observed in this short-term study between cows on PWC and WS in milk production or hygiene.
Key words: milk composition, total bacterial count, bedding materials, organic

INTRODUCTION

The organic production system is looking for new alternative litter materials that can fulfill the organic husbandry production rules and phase-out the use of conventionally-produced wheat straw (**WS**) litter that is, currently, still accepted in organic production (European Union, 2018). However, bedding materials and resting areas have a huge effect on dairy cows' welfare and productivity (Bewley et al., 2017; Mondaca, 2019; Singh et al., 2020). It is, thus, fundamental to test the adequacy of every new litter product with the potential to be certified as organic before it is proposed to farmers as an alternative to WS.

When assessing a potential new litter material, the sustainability of its production process, its availability, and its local cost (Kour, 2017; Singh et al., 2020) should be considered, along with how it is going to be used (e.g., resting area characteristics and type). These aspects become even more important within the context of organic production. The use of by-products as bedding material on dairy farms—organic or not—is a good option, as they are generally inexpensive and can contribute to reduction of industry waste (Kuipers et al., 2022). Indeed, WS is commonly used as litter material in southern Europe because it is a by-product of wheat production. In contrast, in regions with limited grain harvest (e.g., in parts of northern Europe), wood chips are more commonly used (Johanssen et al., 2018), as they are either available as a by-product from the wood industry or obtained from dedicated crops (Kunttu et al., 2020).

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If properly managed, the ideal litter should provide a comfortable resting area and optimize general health status, particularly hoof, hock and udder health and hygiene, while simultaneously supporting high milk production and quality (Singh et al., 2020; Alanis et al., 2021; Li et al., 2021a). Used litter is the main source of pathogens responsible for environmental mastitis and a key source of bacteria and spores that contaminate milk and lead to high bacterial counts in bulk milk (Hogan et al., 1989; Godden et al., 2008; Patel et al., 2019; Cheng and Han, 2020; Alanis et al., 2021). This bacterial contamination and high microbiological load can occur because of poor physical and biochemical characteristics of the litter material.

Among alternative bedding materials, wood products have been reported to be widely used in organic farms (Smith et al., 2017) and specifically in organic tiestall farms (Andrews et al., 2021) in the United States. Among wood products, few studies have tested the effects of wood chips as a bedding material for dairy cattle. Johanssen et al. (2018) evaluated the effects of wood chip bedding material from broadleaf trees on heifers, and Ferraz et al. (2020) studied their effects on lactating cows. However, Ferraz et al. (2020) did not report the plant from which wood chips were produced. It should be considered that the plant of origin and its structural anatomy may affect the physico-chemical (Munir et al., 2019) and antibacterial properties (Reneau et al., 2002) of the bedding material. Additionally, the available litter studies often focused on a specific aspect (e.g., behavior, milk contamination, or bedding characteristics) and consequently did not provide a general overview of the effects of the litter. Among suitable wood bedding materials, untreated poplar wood chips (**PWC**)—a mixture of bark, sawdust, and postpeelings—have the potential to be a useful litter material based on the criteria proposed by Niraula et al. (2018). It is, in fact, characterized by good availability, easy handling and storage, cost-effectiveness, absence of bacteria or unhealthy and toxic products, high DM level, modular particle size, and good cleanliness.

In this context, our short-term study aimed to compare the use of PWC as an alternative litter material to conventional WS in terms of used litter composition and microbial load, cow milk hygiene, milk quality and yield (**MY**), and animal behavior.

MATERIALS AND METHODS

This study was approved by the Ethical Committee for the Care and Use of Experimental Animals of the University of Parma, Italy (PROT. N.16/CESA /2021) and was conducted in accordance with Italian law (Decreto legislativo no. 26/2014) and Directive 2010/63/EU on the protection of animals used for scientific purposes (European Parliament and Council. 2010).

Animals and Housing

The study was conducted in July 2021 in a tiestall farm located in the Parmigiano-Reggiano cheese production area (Po Valley, northern Italy: Lat. 44.693080; Lon. 10.367579). The dairy barn included 2 rows of 19 face-to-face stalls separated by a 5.5-m drive-through feed alley. Each tiestall had a smooth concrete floor with a 2% slope toward the rear end, covered by a layer of bedding material, which was cleaned and partially renewed daily. A channel fitted with flap scrapers for manure evacuation was also present. A 50-cm-wide concrete feed bunk was located in the front end, 10 cm over the floor level. The feed bunk was separated from the floor of the stall by a 12-cm-high border. An automatic drinker was placed at every other stall. The individual stalls were not separated. A total of 38 lactating Holstein dairy cows (630 ± 102 kg BW; 3.03 ± 0.43 BCS; 204 ± 119 DIM; 26.9 ± 3.5 kg/d MY), originally housed on long wheat straw, were allocated into 2 homogeneous (for productivity and parity) litter groups corresponding to the 2 separated stall rows, namely WS and PWC. The WS was self-produced and harvested as a wheat by-product by the farmer in the summer of 2020 and stocked in the barn as round bales. The WS was long WS (>50 cm). The PWC was produced and provided by the Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB; Potsdam, Germany). The geometric mean particle size of the PWC was 1.44 cm (ranging from <0.1 to 4.5 mm). The PWC was packed into waterproof plastic big-bags and transported to the farm in January 2021, where it was stored in the barn together with the straw until the beginning of the trial. The commercial values of the products at the time, and in the area where the trial was performed, were approximately 75 and 50 €/t for WS and PWC, respectively. The trial lasted 10 d and included an adaptation period of 5 d followed by a sampling period of 5 d. Each litter group was further divided into 3 subgroups of 6 or 7 animals. The feeding line was divided through wooden boards into 3 sections per group, corresponding to the subgroups, for an accurate measurement of the related feed intake. No separation was created in the bedding area between the subgroups, to avoid effects on the behavioral traits. Due to difficulties with on-field individual measurements for some traits and sampling procedures, the subgroup instead of the individual animal was considered as the experimental unit. The dirty and wet used litter was

Table 1. Chemical composition of the wheat straw (WS) and poplar wood chips (PWC) tested as litter materials

Item	PWC	WS
Chemical composition,[1] % DM		
DM, % as fed	99.8	90.7
CP	2.01	3.58
Ether extract	0.34	0.72
aNDFom	87.3	75.7
CP-NDF	0.97	1.24
ADFom	71.3	47.0
Lignin	18.5	6.9
Ash	8.7	11.8
pH	6.3	6.5
LTBC,[2] $\log_{10}$ (cfu × 10^3)/g	2.1	1.8
Escherichia coli, $\log_{10}$ (cfu + 1)/g	ND[3]	ND
Lactobacillus spp., $\log_{10}$ (cfu + 1)/g	ND	ND

[1]aNDFom = amylase-treated NDF expressed as exclusive of residual ash; CP-NDF = CP bound to NDF; ADFom = ADF expressed as exclusive of residual ash.

[2]LTBC = litter or used litter total bacterial count.

[3]ND = not detected.

removed daily from the back of the stall and placed in the rear channel for automatic removal. Each stall was provided with a constant amount of 7 kg/d of the corresponding clean litter material, whose volumes were 5.43 and 22.72 dm^3/kg for PWC and WS, respectively.

Before the beginning of the trial, both litter materials and feed ingredients were sampled and chemically analyzed. The chemical composition analysis panel followed the analyses described by Simoni et al. (2021a) and Manuelian et al. (2021). It included DM, ash, ether extracts (**EE**), aNDFom, ADFom and lignin, CP, CP bound to NDF (**CP-NDF**) and ADF (**CP-ADF**), and starch. The CP, CP-NDF, and CP-ADF were calculated as N × 6.25, and starch was determined only on feedstuffs. The pH of the litter was determined using a pH meter Checker (HANNA instruments), and an aliquot was used to test microbiological contamination. The analytical results of the litter and feed, expressed on a DM basis, are reported in Table 1 and Table 2 respectively. Animals were sequentially fed grass hay, alfalfa hay, and mixed hay twice a day (starting at 0700 and 1900 h). The concentrate was provided separately from the forage every 3 h by automatic feeder. The estimated chemical composition of the whole diet is shown in Table 3 and was calculated using the software NDS professional (version 3.9.10.01, Rumen S.a.s., Reggio Emilia, Italy, based on the CNCPS model, version 6.55, Cornell University, Ithaca, NY), based on the average amount of each ingredient supplied daily to the cows.

Measurement and Sampling

On the first day of the experiment (d 1) and every day during the 5 d of sampling (d 6–d 10), fecal (**FS**), overall cleanliness (**CS**), and udder hygiene (**US**) scores of each cow were evaluated before each milking (0600 and 1800 h). The FS was evaluated as described by Hutjens (2010), with a 5-point scoring system where 1 indicated a pea soup-like consistency and 5 indicating fecal balls. The CS and US were evaluated according to Reneau et al. (2002) and Schreiner and Ruegg (2003) also using a 5-point scoring system, where 1 signified a very clean and 5 a very dirty body or udder.

At the same time as the recording of FS, CS, and US, 1 kg of feces and 500 g of used litter were collected from each stall per cow and pooled by subgroup. Feces were collected directly from the rectum of each cow, mixed thoroughly, stored at room temperature, and transported to the University of Parma. Used lit-

Table 2. Chemical composition (mean ± SD) of the feeds supplied to the dairy cows

Item[1]	Grass hay	Alfalfa hay	Mixed hay	Feedstuff[2]
DM, % as fed	91.88 ± 3.26	86.26 ± 0.54	87.90 ± 4.45	92.67 ± 0.33
% DM				
CP	6.91 ± 0.09	17.54 ± 0.05	15.07 ± 5.81	16.85 ± 0.73
EE	0.95 ± 0.03	1.76 ± 0.15	1.38 ± 0.29	1.80 ± 0.63
aNDFom	67.74 ± 3.93	43.66 ± 5.26	59.87 ± 7.54	33.56 ± 0.86
CP-NDF	3.72 ± 0.21	6.32 ± 0.73	6.61 ± 1.94	9.93 ± 0.00
ADFom	43.61 ± 4.50	30.55 ± 2.84	39.58 ± 5.10	13.75 ± 0.74
CP-ADF	2.75 ± 0.17	3.64 ± 0.61	3.70 ± 0.75	6.31 ± 0.00
Lignin	7.61 ± 2.82	6.48 ± 0.39	8.97 ± 0.45	1.74 ± 0.43
Ash	8.06 ± 0.17	13.50 ± 1.54	9.96 ± 1.43	6.24 ± 0.19
Starch				28.79 ± 0.15
Crude fiber				12.63 ± 1.11

[1]aNDFom = amylase-treated NDF expressed exclusive of residual ash; CP-NDF = CP bound to NDF; ADFom = ADF expressed as exclusive of residual ash; CP-ADF = CP bound to ADF.

[2]Feedstuff: beet pulp 43%, corn meal 28%, soybean meal 11%, sorghum meal 10%, soybean hulls 3%, barley malt 1%, calcium carbonate and sulfate 0.8%, wheat middlings 0.8%, sodium bicarbonate and chloride 0.8%, dicalcium and monocalcium phosphate 0.7%, cane molasses 0.4%, magnesium sulfate, oxide, and carbonate 0.4%, vitamin 0.1%.

Table 3. Proportion of feeds delivered to the animals and estimated chemical composition of the whole diet

Item[1]	Amount
Ingredient proportion, % DM	
Grass hay	16
Alfalfa hay	30
Mixed hay	20
Feedstuff	34
Estimated chemical composition, % DM	
DM, % as fed	89.5
CP	15.1
Ether extract	1.6
aNDFom	47.3
ADFom	28.7
Lignin	5.5
Ash	9.5
Starch	10.0
Sugar	6.0

[1]Feedstuff: beet pulp 43%, corn meal 28%, soybean meal 11%, sorghum meal 10%, soybean hulls 3%, barley malt 1%, calcium carbonate and sulfate 0.8%, wheat middlings 0.8%, sodium bicarbonate and chloride 0.8%, dicalcium and monocalcium phosphate 0.7%, cane molasses 0.4%, magnesium sulfate, oxide and carbonate 0.4%, vitamin 0.1%. aNDFom = amylase-treated NDF, expressed exclusive of residual ash; ADFom = ADF, expressed exclusive of residual ash.

ter samples were obtained by pooling 3 similar aliquots collected with a scoop in the front, middle, and rear areas of each stall. A different scoop was used for each subgroup. The used litter samples were further divided into 2 composite samples, labeled, and stored at room temperature to investigate chemical composition, or at −20°C to be transported to the microbiology laboratory for bacterial culture determination.

Nine cows per group were chosen so that the mean and standard deviation of MY per milking at the beginning of the trial were similar and representative of the entire herd (PWC = 13.6 ± 2.3 kg/milking per cow; WS = 13.3 ± 1.9 kg/milking per cow) and allocated 3 per subgroup. On the first day of the experiment (d 1) and every day during the 5 d of sampling (d 6–10), MY was measured on the selected cows twice daily using a 42-kg milk meter (Waikato MKV, Waikato Milking Systems, Hamilton, New Zealand), and 2 aliquots of 150 mL were sampled per cow and kept at 4°C until analysis. In the first milk aliquot, 0.3 mL/dL Bronopol were added as a preservative for chemical composition determination. The second aliquot was used to determine total bacterial count (**TBC**), coliform bacterial count (**CBC**), and spore-forming units (**SFU**).

Feces and Used Litter Analysis

Chemical Analysis. The DM content of feces and used litter samples was determined by drying the samples at 103°C overnight (European Commission, 2009). Ash content was obtained by ignition at 550°C for 4 h. Nitrogen was determined following the Dumas method (AOAC 968.03; AOAC International, 2005) by combustion digestion (Dumatherm, Gerhardt GmbH and Co., Königswinter, Germany), and CP was calculated as percentage of N × 6.25. The aNDFom was analyzed according to Van Soest et al. (1991) and Mertens et al. (2002) with the use of α amylase, but without the use of sodium sulfite, and corrected for ash. A semi-automated system was used for the boiling and filtering phase (FIWE Raw Fiber Extractor, VELP Scientifica, Usmate Velate, Italy).

Used Litter Bacterial Count. Aliquots of 25 g each, of fresh PWC and WS and each used litter sample, were withdrawn and stored at −20°C. For the analysis, each aliquot was thawed and suspended in 225-mL sterile saline solution (NaCl 0.9%) in a stomacher bag and homogenized with a stomacher (Laboratory Blender 400, Seward Medical, London, UK) for 60 s at medium speed (230 rpm).

From this 1/10 suspension, 1 mL was withdrawn and resuspended in 49-mL sterile saline in a sterile tube to achieve a 1/50 dilution. This was thereafter further diluted to reach a final dilution of 1/25,000. From this final dilution, 20 μL was plated onto MacConkey agar, Columbia blood agar and de Man, Rogosa, and Sharpe (**MRS**) agar plates (Oxoid, Basingstoke, UK). All 3 plates were incubated for 24 h at 37°C in an aerobic environment, except MRS agar, which was incubated under microaerophilic conditions. For each sample, an additional blood agar plate was incubated for 48 h at 37°C under anaerobic conditions to evaluate the growth of *Clostridium* spp. To evaluate the presence of *Salmonella* spp., 50 μL of the final dilution were inoculated in 5 mL of peptone water and incubated aerobically for 24 h at 37°C. Subsequently, 1 mL of peptone water was transferred into Rappaport-Vassiliadis broth (BD Difco, Franklin Lakes, NJ) and incubated aerobically for 48 h at 42°C. Thereafter, an aliquot of 20 μL was plated onto a MacConkey agar plate and incubated for 24 h at 37°C in aerobic conditions. Lactose nonfermenting colonies were tested through a micro-agglutination test with anti-*Salmonella* serum (*Salmonella* O Poly-Gp A-S; Thermo Fisher Scientific, Waltham, MA). Then, the positive lactose nonfermenting colonies were identified with API 20E biochemical test systems (bioMérieux, France) and conventional biochemical tests (Quinn, 1994). *Escherichia coli* and *Lactobacillus* spp. were identified through the morphology of bacterial colonies. Colony-forming unit counts were performed directly on MacConkey agar plates for *E. coli* and on MRS agar plates for *Lactobacillus* spp., and *E. coli* hemolytic activity was assessed on Columbia blood agar. The presence of *Clostridium* spp.

Table 4. Descriptions of body positions and general behaviors recorded during direct observations

Observed variable	Definition[1]
Body position	
Lying	Without support of any leg and with the belly in contact with the floor
Standing	Standing with all 4 feet on the ground
General behavior	
Ruminating	Regurgitating or chewing on bolus that had been regurgitated
Eating	Head down close to forages or feedstuff, taking bites or chewing without regurgitating
Drinking	Cow engaged in water ingestion
Sleeping	Lying down on the side or on the belly, not ruminating, head up or turned to the side or stretched forward, eyes closed or half-closed
Other	All other behaviors not listed above, including grooming, licking itself or other animals, pushing other cows, observing, social interactions, vocalizing, stepping or kicking, urinating and defecating

[1]The classification was based on the description reported by Pavlenko et al. (2011), with minor modifications.

was evaluated through microscopic examination of the suspected colonies grown in an anaerobic environment and stained with Gram staining. The sample was considered positive for *Clostridium* spp. when oval, bulging spores located in terminal or subterminal positions were detected.

For each sample, 50 µL from the 1/10 suspensions were withdrawn and resuspended in 50 mL of sterile saline in a sterile tube to determine the litter or used litter total bacterial count (**LTBC**). This step was repeated until it reached a dilution of 10^{-3} for clean litter material and 10^{-7} for used litter material. One milliliter was withdrawn and plated onto a sterile Petri dish filled with plate count agar medium (PCA, BD Difco) and incubated for 24 h at 37°C. After incubation, the colony-forming units were counted.

Milk Quality and Hygiene

Milk composition was analyzed with Milkoscan FT7 (Foss Electric A/S, Hillerød, Denmark) to determine fat, protein, casein, and lactose percentages according to ISO 21543:2020 (ISO, 2020). Cheese coagulation properties were analyzed by FT6000 (Foss Electric A/S, Hillerød, Denmark; rennet coagulation time [**RCT**], curd firmness 30 min after rennet addition to milk [**A30**], curd firming time [**K20**]; De Marchi et al., 2013) in the laboratory of the Breeders Association of Veneto Region (Padova, Italy). Somatic cell count was determined using a Fossomatic (Foss Electric A/S, Hillerød, Denmark) according to ISO 13366-2:2006 (ISO, 2006).

The TBC was determined through flow cytometry with BactoScan FC (Foss Electric A/S). The CBC was determined on polystyrene Petri dishes with VR-BA Agar ground after incubation at 37°C for 24 h (ISO 11866-1/IDF 170-1; ISO, 2005). The SFU was determined as the most probable number using the Weinzirl method modified by Annibaldi (1969)

Calculations

Energy-corrected milk (3.140 MJ/kg) was calculated as described by Sjaunja et al. (1990) using the following equation:

$$\text{ECM yield (kg)} = \text{MY (kg)} \times [(38.3 \times \text{fat g/kg} + 24.2 \times \text{protein g/kg} + 15.7 \times \text{lactose g/kg} + 20.7)/3{,}140].$$

Behavior and Feed Intake

Individual cow behavior was monitored on d 9 and 10 (the last 2 sampling days) by 4 groups of paired trained operators (switched every 6 h) for each litter group. Every 15 min, for a time span of 48 h, the operators recorded the frequency of standing or lying and behavioral traits, including ruminating, eating, drinking, sleeping, or other (grooming, licking itself or other animals, pushing other cows, observing, social interactions, vocalizing, stepping or kicking, urinating, or defecating). The definitions adopted to describe the body positions and behaviors are those given by Pavlenko et al. (2011) with minor modifications (Table 4)

On d 9 and 10, the amount of forage and concentrate fed to the cows, as well as the feed refusals, were weighed by subgroup on a daily basis and multiplied by their DM content to calculate the DMI. The latter was calculated as the difference between hay and concentrate supplied (kg of DM) and the refusals (which were also analyzed for DM content), divided by the number of cows of the subgroup.

Statistical Analysis

The data were analyzed using the software SPSS v28 (IBM SPSS Statistics for Windows, Version 28.0; IBM Corp., Armonk, NY). Normality of continuous variables

Table 5. Mean chemical composition of feces and litter from the dairy cows of the 2 litter groups (PWC = poplar wood chips, n = 30; WS = wheat straw, n = 30)

Item[1]	PWC	WS	SEM	*P*-value
Feces				
Moisture, %	86.0	85.2	0.191	0.086
CP, % DM	15.1	15.0	0.121	0.869
aNDFom, % DM	62.9	61.0	1.128	0.445
Ash, % DM	14.1	14.5	0.128	0.041
Litter				
Moisture, %	59.1	67.9	1.736	0.006
CP, % DM	7.13	8.99	0.254	≤0.001
aNDFom, % DM	79.6	68.8	0.471	≤0.001
Ash, % DM	8.55	13.49	0.436	≤0.001
pH	8.22	8.29	0.062	0.593

[1]aNDFom: NDF obtained using heat-stable α amylase and expressed as exclusive of residual ash.

was assessed by evaluating kurtosis, skewness, and the Q-Q plot. When necessary, data were transformed to ensure a normal distribution: a $\log_{10}$-transformation was applied to SCC and a $\log_{10}$ (cfu + 1) per gram of litter or used litter was applied to bacterial count (Rowbotham and Ruegg, 2016). Data on chemical composition of fecal and used litter samples, used litter pH, milk composition, milk clotting ability, ECM, and $\log_{10}$ SCC were analyzed by generalized linear models (**GLM**) using the least significant difference post hoc test. Differences between used litter bacterial counts were tested by ANOVA. The MY was analyzed using repeated measures GLM with the value recorded on d 1 as a covariate. The FS frequency was analyzed by a chi-squared test. Multinomial logistic regression was used to evaluate the effect of the bedding typology on CS and US. Scores were divided into a multinomial response. In addition to bedding typology, the FS effect was tested as further explanatory variable in the model. Because the FS variable had a significant effect ($P < 0.05$) was included in the final model. The frequency of each score was calculated and reported also as a percentage. The TBC, CBC, and SFU milk data were normalized through a log-transformation and analyzed using repeated measure GLM with the $\log_{10}$ transformed value collected before the start of the study as a covariate. The number of observations of animals standing or lying as well as the number of observations of each behavioral trait was compared between groups using the Chi-squared test. The DMI of both groups was compared by a t-test. The statistical significance was set a $P \leq 0.05$.

RESULTS

Fecal and Used Litter Composition

Feces and litter mean chemical composition are shown in Table 5, and FS score is displayed in Figure

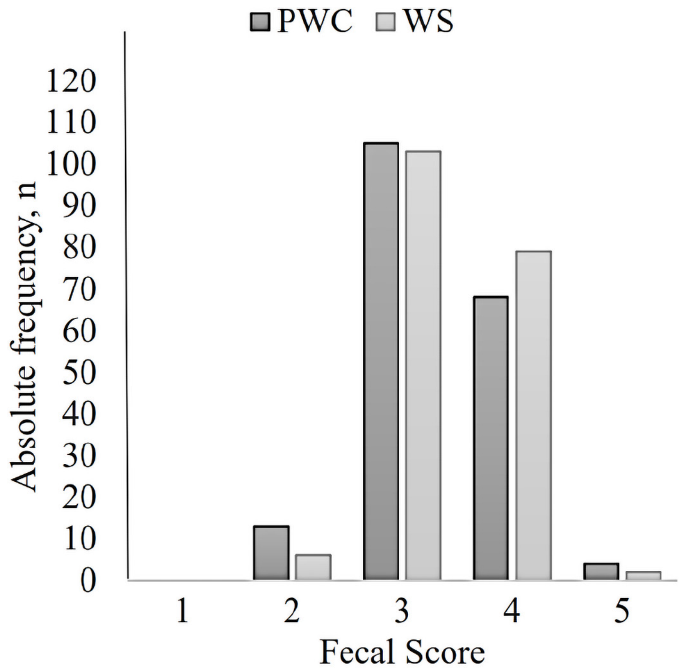

Figure 1. Effect of the litter used (PWC = poplar wood chips; WS = wheat straw) on the absolute fecal score frequency evaluated with a 5-point scoring system, where 1 indicated a pea soup-like consistency and 5 was descriptive of fecal balls.

1. Moisture, CP, and aNDFom content, as well as fecal consistency were similar between the groups. Mean fecal ash content was greater in the WS than the PWC group ($P = 0.041$).

The WS used litter had a greater moisture, CP, and ash content than the PWC ($P = 0.006$, $P \leq 0.001$, and $P \leq 0.001$, respectively) but a lower aNDFom content ($P \leq 0.001$). The pH was similar between the groups.

Used Litter Bacterial Count. Higher counts of *E. coli* ($P \leq 0.001$), *Lactobacillus* spp. ($P = 0.017$), and LTBC ($P \leq 0.001$) were observed in the PWC than WS used litter (Table 6). Moreover, *E. coli* hemolytic activity, *Clostridium* spp., and *Salmonella* spp. were not present.

Udder and General Cleanliness. Table 7 displayed the relative score frequency of the US and CS. Most of the cows were scored in categories 1 and 2. Tables 8 and 9 report the results of the multinomial logistic regression for the US and CS respectively. The FS was demonstrated to inversely affect the CS, with higher fecal score leading to cleaner cows. The probability of having dirtier animals (for both CS and US) was higher for animals housed on WS than PWC.

Milk Yield, Quality, and Hygiene

Milk yield, milk composition, $\log_{10}$ SCC, and clotting ability were unaffected by the litter material as

Table 6. Used litter microbiology according to the 2 litter groups (PWC = poplar wood chips, n = 30; WS = wheat straw, n = 30)

Item	PWC	WS	SEM	*P*-value
Escherichia coli, $\log_{10}$ (cfu + 1)/g	3.488	0.863	0.417	≤0.001
Lactobacillus spp., $\log_{10}$ (cfu + 1)/g	5.185	2.922	0.481	0.017
LTBC,[1] $\log_{10}$ (cfu + 1)/g	10.157	9.106	0.150	≤0.001

[1]LTBC = litter or used litter total bacterial count.

shown in Table 10. The CBC tended to be greater in milk from cows on WS than PWC litter ($P = 0.053$), whereas SFU and TBC were unaffected (Table 11).

Behavior

The body position frequency occurrences were similar between groups (Table 12). Similarly, drinking, eating, and ruminating frequencies were not affected by the litter material. Inversely, the WS group exhibited a greater sleeping frequency compared with PWC ($P \leq 0.001$). Meanwhile, the PWC group showed a higher frequency of "other" behaviors ($P \leq 0.001$), which included several types of nervousness or discomfort behaviors such as stepping, vocalizing, licking themselves or other animals, and kicking.

Dry Matter Intake

A 13.72% DMI reduction was observed in cows housed on PWC in comparison to WS (19.5 vs. 22.6 kg; $P = 0.008$).

DISCUSSION

Our study aimed to compare the short-term effect of PWC as an alternative litter material to conventional WS in terms of used litter composition and microbiology, cows' milk hygiene, milk quality and MY, and behavior. Considering the characteristics of the experimental setup, it should be noted that the results obtained can be considered specific to the tiestall barn type and are not necessarily transferable to other barn types. Additionally, it needs to be highlighted that tiestall animals are in contact with the bedding material 24 h a day, and the trial was performed in the summer season, with possible effects on moisture and microbial proliferation. The experimental period was relatively short because the trial was conducted as a small-scale exploratory field study, testing a bedding material whose effects on animal welfare and productivity were substantially unknown. Despite the limited trial duration, all the measured outcome results were stable and consistent among the sampling days (data not shown), indicating the adequacy of the adaptation period length. The only exception could be milk production, whose evaluation deserves further studies.

Fecal and Used Litter Composition

Fecal composition and consistency were similar between the 2 groups and in line with values reported in literature for dairy cows (Thomsen et al., 2013; Righi et al., 2017). In the present short-term study some differences were detected in the ash content, which was higher in the WS group. Even if not quantitatively measured, cows of the WS group were occasionally observed eating some litter material, and this could have increased the fecal ash content of these animals. Part of this effect may be related to the greater DMI of these animals, which may have led to variations in the digestion process.

Although the animals of the 2 groups showed similar fecal composition and consistency, a lower moisture content was found in the PWC used litter, probably in relation to the higher lignin content of PWC compared with WS litter (Table 1), as observed also by Larney et al. (2008). Those authors reported that, due to its physico-chemical characteristics, lignin makes wood chips less biodegradable and more hydrophobic. This may have improved urine percolation through the bedding, facilitating its flowing on the smooth and sloped surface of the stall, or reduced urine retention of the bedding itself. This would also have facilitated evaporation of water. Indeed Ferraz et al. (2020) showed that WS have a water-holding capacity more than double that of wood chips. Additionally, a lower N concentration was observed in the PWC used litter. This could

Table 7. Frequency of udder and cleanliness scores (4-point scale) of the animals bedded on the 2 litter groups (PWC = poplar wood chips; WS = wheat straw)

	Udder score, %		Cleanliness score, %	
Score	PWC	WS	PWC	WS
1	67.5	37.2	48.6	18.3
2	16.9	33.3	21.6	38.3
3	8.4	14.1	25.7	23.3
4	7.2	15.4	4.1	20.1

Table 8. Results of the multinomial logistic regression describing the factors influencing the udder cleanliness of cows housed on the 2 litter groups (PWC = poplar wood chips; WS = wheat straw)

						CI 95% per OR	
Udder score	Factor	Coefficient	SE	*P*-value	OR[1]	Lower limit	Upper limit
1 vs. 2	Intercept	0.949	1.064	0.372			
	Bedding						
	WS	Referent					
	PWC	−1.130	0.360	0.002	0.323	0.159	0.654
	FS[2]	−0.119	0.326	0.714	0.887	0.468	1.682
1 vs. 3	Intercept	0.394	1.432	0.783			
	Bedding						
	WS	Referent					
	PWC	−1.174	0.484	0.015	0.309	0.120	0.798
	FS	−0.248	0.445	0.578	0.781	0.326	1.867
1 vs. 4	Intercept	1.146	1.660	0.490			
	Bedding						
	WS	Referent					
	PWC	−1.402	0.574	0.015	0.246	0.080	0.758
	FS	−0.593	0.527	0.261	0.553	0.197	1.553
2 vs. 3	Intercept	−0.555	1.428	0.698			
	Bedding						
	WS	Referent					
	PWC	−0.044	0.480	0.927	0.957	0.374	2.450
	FS	−0.128	0.447	0.775	0.880	0.366	2.114
2 vs. 4	Intercept	0.197	1.647	0.905			
	Bedding						
	WS	Referent					
	PWC	−0.272	0.570	0.633	0.762	0.249	2.327
	FS	−0.474	0.527	0.369	0.623	0.222	1.749
3 vs. 4	Intercept	0.752	1.900	0.692			
	Bedding						
	WS	Referent					
	PWC	−0.228	0.654	0.727	0.796	0.221	2.870
	FS	−0.346	0.606	0.568	0.708	0.216	2.320

[1]OR = odds ratio.
[2]FS = fecal score.

partially support the previous hypothesis of a more pronounced urine percolation, even if this result can be affected by the initial difference in litter's CP content. The lower moisture content of the PWC used litter contributed to maintaining the animals drier and cleaner. These results are, however, inconsistent with findings showing a higher ability of the wood litter materials in lowering N losses, when composting, compared with straw litter materials (Larney et al., 2008). However, our short-term study was focused on used litter's performance over a limited time span without considering the composting phase that usually follows used litter removal and lasts several months. Some differences were observed for aNDFom and ash content between the 2 materials, even if they are more likely due to their initial differences. Concerning used litter pH, no differences were found in this study between the 2 materials tested (Table 5), in agreement with Spiehs et al. (2013), who reported a similar used litter pH when WS and wood chips were compared. Overall, these results suggest that PWC does not exert an acidic effect, in contrast to the results on other wood chip materials (e.g., pine products; Miller et al., 2003).

Used Litter Microbiology

The microbial count was expressed in this study on a weight basis because, considering the nature of the tested bedding materials, the mass (weight) is more representative of the quantity of bedding material that is in contact with the animal body when the animal is lying. Furthermore, the weight is a more objective measure than volume because in general the volume of these materials may be affected by the length of the fiber, the particle size, and the pressure exerted on the mass while measuring the volume itself.

The higher LTBC found in PWC is partially explained by the initially higher microbial contamination of this material, which also appeared dustier. However, contamination of the litter material may be affected by the humidity, temperature, and managing conditions during storage (Bramley and Neave, 1975). In the

Table 9. Results of the multinomial logistic regression describing the factors influencing the general cleanliness of cows housed on the 2 litter groups (PWC = poplar wood chips; WS = wheat straw)

						CI 95% per OR	
Cleanliness score	Factor	Coefficient	SE	*P*-value	OR[1]	Lower limit	Upper limit
1 vs. 2	Intercept	3.402	1.394	0.015			
	Bedding						
	WS	Referent					
	PWC	−1.699	0.464	<0.001	0.183	0.074	0.454
	FS[2]	−0.619	0.409	0.130	0.538	0.242	1.199
1 vs. 3	Intercept	4.842	1.443	<0.001			
	Bedding						
	WS	Referent					
	PWC	−1.273	0.483	0.008	0.280	0.109	0.723
	FS	−1.210	0.430	0.005	0.298	0.128	0.692
1 vs. 4	Intercept	5.249	1.732	0.002			
	Bedding						
	WS	Referent					
	PWC	−2.197	0.586	<0.001	0.111	0.035	0.350
	FS	−1.439	0.532	0.007	0.237	0.084	0.673
2 vs. 3	Intercept	1.439	1.212	0.235			
	Bedding						
	WS	Referent					
	PWC	0.427	0.391	0.275	1.532	0.712	3.299
	FS	−0.591	0.380	0.120	0.554	0.263	1.167
2 vs. 4	Intercept	1.847	1.495	0.217			
	Bedding						
	WS	Referent					
	PWC	−0.498	0.510	0.329	0.608	0.224	1.650
	FS	−0.820	0.478	0.086	0.440	0.172	1.125
3 vs. 4	Intercept	0.407	1.498	0.786			
	Bedding						
	WS	Referent					
	PWC	−0.925	0.517	0.074	0.397	0.144	1.092
	FS	−0.229	0.484	0.636	0.795	0.308	2.052

[1]OR = odds ratio.

[2]FS = fecal score.

present study, the tested litter materials were stored in the same conditions, but PWC underwent a different process, had a longer transport duration, and were packed in waterproof plastic big-bags. These factors could have affected microbial growth in the biomass before its usage.

Despite the absence of *E. coli* and *Lactobacillus* spp. in the clean litter materials (Table 1), which was expected, these bacteria were present in the used litter and were found to be greater in the PWC than in the WS. Variable results can be found in literature concerning this topic, and in general the reported LTBC are higher in comparison to our findings. Spiehs et al. (2013) found initial differences in coliform and *E. coli* contamination of WS and wood chips, which disappeared after 6 wk of use. However, some authors reported similar total bacteria, coliforms, and *E. coli* counts between WS and wood chips (Miller et al., 2003; Ferraz et al., 2020). Differences across studies may be attributable to the

Table 10. Milk yield (MY) and quality according to the 2 litter groups (PWC = poplar wood chips, n = 90; WS = wheat straw, n = 90)

Item[1]	PWC	WS	SEM	*P*-value
MY, kg/cow per d	27.5	26.4	0.24	0.483
ECM, kg/cow per milking	12.0	12.7	0.427	0.123
Fat, %	4.11	4.13	0.101	0.932
Protein, %	3.48	3.68	0.052	0.465
Lactose, %	4.65	4.63	0.042	0.568
Casein, %	2.74	2.93	0.047	0.517
A30, mm	24.7	27.3	2.054	0.719
K20, min	4.65	4.01	0.335	0.856
RCT, min	20.5	20.3	0.707	0.952
Urea, mg/dL	35.9	35.0	0.698	0.549
Log_{10} SCC × 10^3 cells/mL	1.827	1.974	0.157	0.657

[1]A30 = curd firmness 30 min after rennet addition to milk; K20 = curd firming time; RCT = rennet coagulation time.

Table 11. Milk hygiene parameters according to the 2 litter groups (PWC = poplar wood chips; WS = wheat straw)

Item[1]	PWC	WS	SEM	*P*-value
CBC, log_{10} cfu/mL	2.87	3.07	0.083	0.053
SFU, log_{10} MPN/L	2.09	2.07	0.030	0.658
TBC, log_{10} cfu/mL	1.64	1.77	0.040	0.102

[1]CBC = coliform bacterial count; SFU = spore-forming unit; TBC = total bacterial count; MPN = most probable number.

Table 12. Body position and general behaviors (number of observation and frequency) of the cows according to the 2 litter groups (PWC = poplar wood chips; WS = wheat straw)[1]

Item	PWC, no. of observations	PWC, %	WS, no. of observations	WS, %	*P*-value
Body position					
Standing	1,946	53.6	1,971	54.3	0.556
Lying	1,683	46.4	1,658	45.7	
General behaviors					
Ruminating	1,069	29.5	1,098	30.3	0.457
Eating	1,077	29.7	1,074	29.6	0.938
Drinking	169	4.7	158	4.4	0.533
Sleeping	267	7.4	375	10.3	≤0.001
Other	1,047	28.9	924	25.5	≤0.001

[1]No. of observations per group = 3,629. Behaviors were defined in Table 4 following the instruction of Pavlenko et al. (2011) with minor modifications. Behavior other than those cited were classified as "other" (including grooming, licking itself or other animals, pushing other cows, observing, social interactions, vocalizing, stepping or kicking, urinating, and defecating).

plant species used to produce the wood-based materials, with specific reference to their potential content of antimicrobial substances (Miller et al., 2003; Spiehs et al., 2013). Furthermore, because *Lactobacillus* spp. are usually isolated in cow feces (Lin et al., 2020), the highest contamination of the PWC used litter detected in this study, can be considered as indicative of a greater fecal contamination. Additionally, these results suggest greater bacterial proliferation in the used litter as result of more favorable environmental conditions, including higher bulk density (Ferraz et al., 2020) with decreased aeration. The higher bulk density also justifies the increased *E. coli* presence in PWC used litter because in this case it is associated with a smaller particle size and consequently with a higher surface area for bacterial colonization per weight unit of material. However, according to Lin et al. (2020) autochthonous lactic acid bacteria isolated from dairy cow feces seem to express antibacterial activity against enteric bacterial pathogens of cattle. This could provide an explanation for the decreased values observed for milk CBC and TBC found in cows housed on PWC. This is consistent with some evidence relating to the presence of *Lactobacillus* spp. to a reduction of *E. coli*-induced mammary gland infections in both experimental animals (Zhao et al., 2021) and dairy cattle (Li et al., 2021b, 2022) as well as in dairy cattle reproductive trait (Genís et al., 2016).

Udder and General Cleanliness Score

As previously mentioned, the PWC used litter increased animal cleanliness. Indeed, cows housed on this litter material were found to be more frequently clean considering both US and CS (Tables 8 and 9). Similar results were found by Johanssen et al. (2018) testing wood chip (from broadleaf trees) and straw (from barley) as litter for heifers. Dirty used litter can exacerbate the potential for environmental mastitis by exposing teats to the high level of bacteria present in the litter material. However, due to the diet type, the animals in our study had firm feces, and this was beneficial to maintaining stalls and keeping animals clean (Ward et al., 2002).

Milk Yield, Quality, and Hygiene

The mean MY and composition observed (Table 10) are both similar to or higher than data obtained in the same geographic area and productive context in other studies (Comino et al., 2015; Franceschi et al., 2020; Simoni et al., 2021b). The mean of MY in the present study was close to the lowest productivity (29.0 kg/d) reported by Simoni et al. (2021b), which may be due to our study having hay and concentrates administered separately instead of using a total mixed ration, which is a feeding system that notoriously improves ruminal fermentation and productivity (Schingoethe, 2017). Despite the short duration of the present trial, the similar MY and composition between the litter groups is consistent with findings from Tucker et al. (2009). Those authors observed that MY was unaffected by the different litters used (straw and wood shavings) after 7 d of treatment, and they argued that litter and time devoted to lying behavior should not be considered limiting factors for MY. Moreover, the same authors claimed that a 1-wk trial is too short to observe MY variations, which are more related to DMI. Because this latter parameter was negatively affected by PWC in our study, we speculate that changes in MY could have occurred over a longer time span. Thus, any economic evaluation should be performed only after a follow-up confirmatory experiment on this parameter.

The literature shows a direct relation between coliform count in litter and incidence of coliform mastitis in bovines. Bramley and Neave (1975) concluded that sawdust, which is also included in wood chips, can produce high levels of contamination even if those levels are different among materials and year of production. In agreement with this finding, our wood product showed a greater coliform contamination. However, due to the lower moisture of the PWC used litter, and the consequent greater cleanliness of the cows, PWC led to a trend of lower CBC of the milk, which was, along with the TBC of the milk, lower than values reported by Franceschi et al. (2020) under the same productive conditions.

For proper dairy product processing and quality, it is essential to reduce the contamination of spores in raw milk (Cook and Sandeman, 2000). In our study, similar milk SFU were found between the WS and PWC groups. To the best of our knowledge, there are no data available in literature regarding the milk spore content related to cattle housed on PWC. However, studies comparing straw with other litter typologies highlighted inconsistent results for this parameter. For example, Gagnon et al. (2020) found no difference between recycled manure and straw, whereas Driehuis et al. (2014) obtained higher numbers of thermophilic spore-forming bacteria in the bulk milk from farms using biodegradable litter from composted materials compared with farms using straw. According to Gagnon et al. (2020) the discrepancy between studies may be due to other external factors, including season or feeding plans. Indeed, a study focusing on *Bacillus cereus* reported an increase in milk spore levels during summer compared with other seasons (Vissers et al., 2007).

In our study the TBC of milk was not affected by litter materials, in analogy to the findings of Robles et al. (2020), who conducted a survey on 70 farms in Ontario (Canada) testing wood products and straw. However, it should be considered that the latter study was performed in the colder months, whereas our study was set in the middle of the summer, which potentially exacerbated the used litter microbiological load (Hogan et al., 1989).

Behavior

When optimal litter and welfare conditions are reached, dairy cows lie down between 8 and 16 h per day (Tucker et al., 2009), but inappropriate litter conditions could reduce the lying time by 48% (O'Connor et al., 2019). Depriving cows of the possibility to lie down represents a stressor that leads cows to prioritize resting over other activities (Cooper et al., 2007; Norring et al., 2008) and increases plasma cortisol concentration (Fisher et al., 2002). Tucker et al. (2009) found no differences in the lying behavior time of animals bedded with straw or wood shavings provided in a similar amount. Accordingly, we found no differences in the lying behavior frequency. It should be highlighted here that, although different quantities of litter were not tested in the present study, the amount of litter provided per stall per day was comparable to the middle–high levels tested by the latter-mentioned authors, who also found a positive correlation between quantity of litter material offered to the animals and lying behavior time. Norring et al. (2008) observed that animals spent a longer time lying when housed on straw than on sand, because cows prefer to lie down on soft litter materials (Singh et al., 2020). Therefore, the results of this study could indicate that PWC is comparable to WS. However, looking into other behavioral parameters such as sleeping frequency and "other behaviors" reported in Table 12, it appears that WS is preferred over PWC. In our case, a higher sleeping frequency was in fact seen in animals housed on WS than PWC litter, suggesting a greater level of comfort. It is well known that the comfort level affects DMI in livestock animals. The reduction of DMI has, in fact, been addressed as one of the most important indicators of reduced welfare (Leliveld and Provolo, 2020). Both the reduction in DMI and reduced sleeping frequency indicate impaired welfare attributable to the bedding type. In contrast to our results, Fukasawa et al. (2019) indicated that sleeping parameters were unaffected by farm management practices including litter type (i.e., rice husk, straw, and sawdust) when 12 dairy farms were evaluated. Additionally, we found a higher frequency of behaviors classified as "other" in animals housed on PWC. Indeed, these animals devoted more time to stepping, kicking, licking the other animals or their own legs and bellies, as well as observing the other cows or the litter per se. As explained by Hedlund and Løvlie (2015), increased stepping behavior, which is positively correlated with milk cortisol and heart rate, along with other behaviors such as vocalization and facing the herd, reflect nervousness or discomfort in cows. In the latter study those traits were negatively related, although not strongly, with MY. Conversely, a rested cow produces more saliva, which improve ruminal health and increases both udder blood supply and MY (Tucker et al., 2009; Sadiq et al., 2017; Ferraz et al., 2020)

Dry Matter Intake

The overall DMI found in the present trial is consistent with those reported in other studies performed in the Parmigiano-Reggiano area, where animals were also fed hay-based diets (Cavallini et al., 2021; Simoni et al., 2021b). Moreover, it is similar to the one reported by Umphrey et al. (2001) under analogous managerial and environmental conditions, namely tiestalls and summer season (DMI between 16.7 and 18.5 kg/d). However, in this short-term study, the PWC group showed a lower DMI than the WS group, probably due to greater discomfort or impaired welfare related to the former litter material. The reduction of DMI has, in fact, been addressed as one of the most important indicators of reduced welfare (Leliveld and Provolo, 2020). This hypothesis is supported also by the lower sleeping frequency observed, along with the higher frequency of behaviors classified as "others" in the present study, which, according to literature (Rousing et al., 2006; Pavlenko et al., 2011; Hedlund and Løvlie, 2015), includes manifestations of discomfort (e.g., stepping, vocalizing, licking themselves or other animals, and kicking behaviors). Additionally, based on the resource allocation theory, if cows invested more time licking, kicking, observing, interacting, and stepping, then they had less time for other activities (Hedlund and Løvlie, 2015).

CONCLUSIONS

Based on the results of this trial, productive performance of the cows housed on WS and PWC litter materials are comparable in the short-term period. The PWC used litter showed lower moisture, leading to a higher frequency of cleaner animals, and even though the used litter microbial contamination was higher, milk contamination was similar. However, PWC litter reduced the sleeping frequency, induced the frequency of behaviors including discomfort signs, and reduced the DMI of the cows in the short term. Larger-scale, confirmatory studies are needed to evaluate the use of PWC litter material over a longer period, also considering different physical forms of the poplar wood materials.

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