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# VERIFICATION OF EFFICIENCIES OF HYGIENIC SEPARATOR, AND BACTERIOLOGICAL EVALUATION OF RECYCLED MANURE MATERIAL USED AS BEDDING FOR DAIRY COWS

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**ABSTRACT**

It is well known that the bedding material commonly used in dairy cows' barns have significant impact on animal health, and welfare as well. In addition, the exact identification of the bedding materials' properties which could affect dairy cows' health is extremely important. This study mainly aims to determine the microbiological properties of recycled bedding material from dairy cow manure, as well as the impact on somatic cell count, milk production and milk quality. Microbiological evaluation was focused on the pathogen microorganisms, especially *Escherichia coli*, *Enterococcaceae*, *Enterobacteriaceae* (*Salmonella*), *Clostridiaceae*, as well as on yeasts and filamentous fungi. The somatic cells count (SCC), which could indicate the presence of clinical or subclinical mastitis, was evaluated by fluorescent optical electronic method. Our study also dealt with the effect of recycled manure solids (RMS) on milk yield production and nutrition profile. The obtained results showed a significant (P<0.0001) decrease of pathogenic microorganisms in the RMS samples compared to the input material. The results also confirmed a positive effect on the number of somatic cells, which indicates a decrease in the incidence of mastitis on the farm. At the same time, a positive trend in the milk production during the year 2023, when the RMS bedding was used, was confirmed. The achieved results confirm the fact that the process of hygienic separation of cow manure on the experimental farm is set up correctly and it could be used in the conditions of normal practice.

**Keywords:** cows, manure, hygienic separation, microbiology

## INTRODUCTION

Radically increasing costs in all segments of agriculture have forced many farmers to look for new methods that can save the cost and maintain profitability of their farms. Above all, in the area of livestock breeding, an increase in commonly available bedding materials such as straw and sawdust was confirmed. Because of this, farmers have promptly started to look for alternative bedding sources **(Bacenetti et al., 2016)**. From a practical point of view, bedding is a very important attribute in dairy farming. It has been confirmed previously that the use of a proper bedding in stalls can maintain the comfortable status of a cow for a long time, support the health status and reproduction, stimulate the life of the individual, reduce hock lesions, minimize lameness, prevent injuries, and promote udder health. there is clear evidence that proper bedding management plays a critical role in daily milk production in dairy farms **(Singh et al., 2020; Trucker and Weary, 2004)**. During the majority of the day, cows prioritize rest, and they spend 8 to 16 hours lying down. The lying behaviour of cows is affected by many factors, but one of the most important is the type of bedding material the producer chooses. It must provide the animals with thermal comfort, and the right softness. Moreover, bedding must be durable and have sufficient friction to allow for rising any lying down without slipping. At the same time, dry matter of bedding can also play a critical role in lying behaviours and cow health. While bedding significantly influences lying behaviours, it has the potential to pose a risk to the health of cows. The hygiene-related risk varies depending on the type of bedding, as different sources carry distinct bacterial loads **(van Gastelen et al., 2011; Lombard et al., 2010; Fregonesi et al., 2007; Kull et al., 2017; Trucker et al., 2009; Reich et al., 2010)**. As we have already stated, the majority of cow farmers know the importance of bedding care and rely on an adequate supply of it to ensure the sufficient hygiene and welfare of cows. At the same time, each litter is characterized by specific parameters, such as particle size and water retention capacity. This also depends on other physical properties of the bedding material, which determine the methods of handling, storage, or subsequent disposal. The mentioned physical and biochemical properties can directly support the growth of bacteria and thus affect the overall quality of the bedding **(Godden et al., 2008; Leso et al., 2018)**. In the case of using organic bedding, the risk of mastitis in cows' increases. Conversely, inorganic materials do not support the growth of bacteria and therefore the risk of udder inflammation is lower **(Leso et al., 2020; Oliveira et al., 2019)**. As has been already mentioned, the increasing costs of raising dairy cows, and the responsibility of farms to reduce the negative impact on the environment have forced farmers starting to look for alternative sources of bedding material. Dairy farm management therefore undergoes change for practical, economic reasons or for food safety **(Gagnon et al., 2020)**. For example, triticale straw has recently started to be used as a bedding material **(Ferreira et at., 2020)**. Brazilian farmers have used materials such as peanut shells, coffee shell, and rice straw, according to the availability of these materials in their regions. Besides that, using wheat husks has recorded increasing popularity, but it is still crucial to gain a complete understanding of characteristics concerning their use and handling as bedding materials **(Agnew and Leonard, 2003)**. An overview of other alternative sources as well as conventional sources of cow bedding is summarized in **Table 1.**

**Table 1** Classification of sources of alternative and conventional bedding materials

| Material | Classification | Literature |
|---|---|---|
| **Dried/Fresh sawdust** | Conventional | Reich et al., 2010 |
| **Triticale husks/straw** | Alternative | Ferreira et al., 2020 |
| **Hemp straw** | Alternative | Arango et al., 2023 |
| **Wheat straw** | Conventional | Kheravii et al., 2017 |
| **Wood chips** | Conventional | Van Dooren et al., 2018 |
| **Spelt husks** | Alternative | Vogt et al., 2021 |
| **Miscanthus grass** | Alternative | Weyenberg et al., 2015 |
| **Flax straw** | Alternative | Dufourni et al., 2018 |

One of the current trends in bedding materials is recycled manure solids (RMS). This concept was first described in the 1970s in the USA. The description of the treatments was difficult at first, but the methodical process of separating the solid and liquid fractions of manure was improved over time. Due to the growing number of dairy cattle farms and the overproduction of manure, the manure recycling method has gained popularity. This practice is widespread e.g. in United Kingdom and has been used for several years. In today's time, which has brought an increase in the costs of operating farms, saving primary resources such as straw, sawdust and others is very important. Therefore, the RMS method could save finances in bedding management, which could be used in other farming segments

**(Timms, 2008a, Feiken and van Laarhoven, 2012; Bradley et al., 2014)**. The solid fraction, mainly composed of undigested fibres, represents the basal matrix of the RMS, but gradually there were concerns about the high bacterial load that can occur when the input material is insufficiently matured **(Menear and Smith, 1973)**. Therefore, other intermediate steps of thermal treatment were gradually implemented, which were able to reduce the number of pathogenic microorganisms and thus the risk of excessive bacterial load. Different combinations of separation, composting or aerobic digestion have been established, which help to use RMS throughout the year, in different regions of the world, and at the same time RMS is sufficiently stable in the direct influence of exogenous factors **(Carrol and Jasper, 1978, Timms 2008b)**. In the field of RMS usage, the current legal regulations of many countries around the world are different. While some of them have implemented the use of RMS into common practice, other political authorities expect more massive research in this area to take a responsible position. For the above reasons, technological companies in the field of agriculture are intensively engaged in the development of high-performance machines for separating sludge from animal manure and thus producing a product of solid particles with more than 30% dry matter content **(Zähner et al., 2009)**. Detailed data from previous experimental studies dealing with the characteristics or use of RMS on dairy cow farms are significantly limited. Therefore, the aim of this study was (1) to carry out the microbiological screening of the bedding material (straw and RMS) used on the farm, (2) to determine the level of somatic cells, (3) to determine the effect of RMS on milk yield and milk characteristics. At the same time, we aimed (4) to verify the effectiveness of the hygienic separator implemented in AgroBan Batka farm.

## MATERIAL AND METHODS

### Livestock farm and study design

The AgroBan s.r.o is a farm for livestock in the southeast of the Slovak Republic (Bátka, Rimavská Sobota), which was involved in this study. The pens (150 cm x 230 cm) held from 80 to 105 dairy cows in one stall with a total number of 225 individuals. Cows were fed by feed mixture consisting of corn silage, soy extract, rapeseed scrap, molasses, brewer's spent grain and mineral supplements. The entire process of the study and analyses is shown in **Figure 1.**

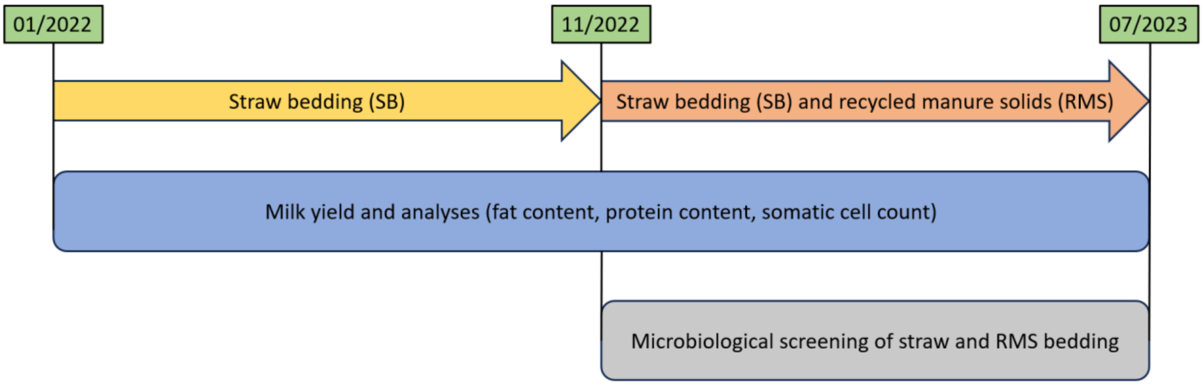


**Figure 1** Graphic display of the study process

Our study lasted from January 2022 to July 2023. In the period from January to October 2022, the farm used exclusively straw as bedding. During this period, the health status of dairy cows was monitored based on analysis of milk (fat content, protein content and somatic cell count).
From November 2023, farmers used, in addition to straw bedding (SB), also recycled manure solids (RMS), as an alternative bedding (for 50% of dairy cows). RMS has been obtained from a hygienic separator. Microbiological analyses of the used bedding were added to the analyses of milk. During the entire process of the study, of course, the total milk yield was also monitored.

### Bedding samples collection, processing, and microbiological analysis

Cows' pens were visited regularly, four times per month from November 2022 to July 2023 to collect samples and observe the biological properties of used SB and RMS bedding. Sampling was conducted throughout 9 months when cows were present or absent in the pens. To analyse the presence of microorganisms in SB and RMS, more than two thousand samples were screened. In general, three samples of SB on the floor of 10 pens were collected in a sterile Petri dish and composited into one sample for microbiological analyses. Similarly, three samples of RMS coming from a hygienic separator were collected and used for microbiological quantification. The representative samples consisted of approximately 100 g of SB material and the same amount of RMS bedding. Samples from cow pens were taken just before the addition of new bedding. Immediately after collection, samples were chilled and transported in a cooler to the laboratory where they were stored at −40°C until analysis.
Analyses focused on the microbiological evaluation of samples were carried out at the laboratory of the Institute of Applied Biology, Slovak University of Agriculture in Nitra. Gained data were supplemented by the analyses carried out by State Veterinary and Food Institute in Dolny Kubín. Frozen bedding samples were allowed to be thawed in a refrigerator before analysis. Firstly, 50 g of collected SB and RMS material was removed and placed into a sterile Petri dish. Then, 100 mL of sterile distilled water was added to the bedding material, followed by horizontal shaking for 30 min. Subsequently, a liquid part of the sample was removed by pipette, and serial 10-fold dilutions were performed. Sample dilutions were plated (200 µL) on Sabouraud agar (SA, BioRad, France) and Endo agar (ED, BioRad, France) medium. The total counts of colonies were determined for each sample after 24 h for EA or 48 h for SA agar medium after the incubation at 37°C. Screening of bacterial groups has been focused on *Escherichia coli, Enterococcaceae, Enterobacteriaceae (Salmonella), Clostridiaceae*, as well as yeasts and filamentous fungi (molds). Colonies that grew on selective culture medium – Sabouraud agar after incubation at 37 °C for 48 h were identified as yeasts and molds. Colonies showing a dark-purple colour cultured on selective culture medium – Endo agar at 37 °C for 24 h were identified as E. coli, and colonies with a yellow-pink to red colour were identified as coliforms. Bacterial counts were expressed as colony-forming units (CFU) per gram of bedding sample according to previous study by Godden et al. (2008) and Husfeld et al. (2012).

### Milk sampling and quality analyses

Dairy cows were milked regularly (three-times per day) during the entire period from January 2022 to July 2023, by trained workers. To avoid the influence of the diet on the quality parameters of the milk, the cows were fed the same feed ration as stated in the "Material and Methods: Livestock farm and study design". After teat cleaning and elimination of the first drafts of milking and just before fitting the clusters, 150 mL of milk was collected by hand into a sterile bottle then stored at 4 °C overnight. Milk samples were taken from 225 dairy cows. An automatic milking parlour was used to collect whole milk.
Somatic cell count was determined by fluorescent optical electronic method (Somatic cells counter FOSSOMATIC) following the Slovak Technical Norm (STN 13366-3). In individual milk samples, basic milk quality parameters - fat and protein, were measured by MILKOSCAN device using infrared absorption spectroscopy.

### Statistical analyses

Statistical analyses were performed using the statistical software GraphPad Prism 6.01 (GraphPad Software Incorporated, San Diego California, USA). Descriptive characteristics (minimum, maximum, mean, and standard error of the mean, etc.) were evaluated at first. One-way analysis of variance (ANOVA) and Dunnett's multiple comparison tests were used to examine differences among the number of bacteria presented in SB bedding samples and RMS samples. A P-value of <0.05 was considered to be significant.

## RESULTS

### Microbiological screening of straw and RMS bedding materials

One critical aspect in characterizing bedding materials is microbiological safety. The results obtained suggest that the average total count of *Escherichia coli, Enterococcaceae, Enterobacteriaceae – Salmonella, Clostridiaceae* in the samples of straw bedding material samples varied from <10 to 340000 CFU/g from October 2022 to July 2023.

**Straw bedding material**

The presence of Salmonella was confirmed in February 2023 (up to 10 CFU/g *Salmonella enterica ser typhimurium*) in straw bedding material. This positive sample, collected during winter, suggests contamination possibly occurredthrough rodents during straw storage. In other months, any bacteria from *Samonella* genus were not confirmed. In all collected samples, the presence of *E. coli* colonies was confirmed 9-times, ranging from4 300 CFU/g to 330000 CFU/g. The correlation of the qualitative appearance of the observed colonies with the season was not confirmed. A similar trend was observed in *Enterococcaceae* screening, where colony counts fluctuated between 2100 CFU/g to 300 000 CFU/g. Clostridiaceae presence (<10 CFU/g) was confirmed three times out of nine examinations, considered a negative result. However, *Clostridiaceae* was confirmed from 1700 CFU/g to 311636 CFU/g in 6 samples throughout the sampling period.

**RMS material**

After hygienic separation of used straw bedding, Salmonella presence was confirmed only in March and April 2023, specifically *Salmonella infantis.* The remaining samples from 9 months collection were negative. Increased incidence of *E. coli* was detected in June 2023 (65 000 CFU/g) and in July 2023 (1300 CFU/g). In the remaining samples, the total colony count was <10 CFU/g. Similarly, a higher presence of *Enterococcaceae* was observed in June 2023 (78 000 CFU/g), while in the remaining months, colonies were detected at <10 CFU/g. the same colonies were detected at <10 CFU/g in the restremaining of months. The elevated content of pathogens *E. coli* and *Enterococcaceae* may be attributed to a software fault in the hygienic separator. The capture of *Clostridia* in the output material was not confirmed in the given month, since no *Clostridial* pathogens were present in the input material either. All screened RMS samples were negative for the presence of *Clostridiaceae* at <10 CFU/g. The implementation of hygienic separator into daily practise is assumed to be useful. Clostridium infections occur in breeding regularly, which must be solved by regular vaccination of the herd and burdens breeding from a health and economic point of view. In general, the application of thermal treatment through the hygienic separator resulted in a significant (P<0.0001) reduction in total count of all monitored microorganisms throughout the entire period of experiment. **Tables 2A** and **Table 2B** summarize the amount of some types of microorganisms in straw bedding and RMS using for the subsequent bedding.

**Table 2A** Overview of average bacteria count: *Escherichia coli*, *Enterococcaceae*, *Enterobacteriaceae – Salmonella*, *Clostridiaceae* in straw bedding material

| Microorganism (CFU/g) | Straw bedding material | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Month/year | 11/22 | 12/22 | 01/23 | 02/23 | 03/23 | 04/23 | 05/23 | 06/23 | 07/23 |
| *Escherichia coli* | 20236 | 12909 | 100000 | 80000 | 4300 | 130000 | 1600 | 330000 | 340000 |
| *Enterococcaceae* | 2103 | 21272 | 300000 | 130000 | 6300 | 150000 | 28000 | 24000 | 56000 |
| *Enterobacteriaceae - Salmonella* | neg | neg | neg | pos | neg | neg | neg | neg | neg |
| *Clostridiaceae* | 311636 | <10 | 7000 | 21000 | 4200 | 4500 | 1700 | <10 | <10 |

neg – negative, pos – positive, **** - significant reduction in total bacteria count

**Table 2B** Overview of average bacteria count: *Escherichia coli*, *Enterococcaceae*, *Enterobacteriaceae – Salmonella*, *Clostridiaceae* in RMS material

| Microorganism (CFU/g) | RMS material | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Month/year | 11/22 **** | 12/22 **** | 01/23 **** | 02/23 **** | 03/23 **** | 04/23 **** | 05/23 **** | 06/23 **** | 07/23 **** |
| *Escherichia coli* | <10 | <10 | <10 | <10 | <10 | <10 | <10 | 65000 | 1300 |
| *Enterococcaceae* | <10 | <10 | <10 | <10 | <10 | <10 | <10 | 78000 | <10 |
| *Enterobacteriaceae - Salmonella* | neg | neg | neg | neg | pos | pos | neg | neg | neg |
| *Clostridiaceae* | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 | <10 |

neg – negative, pos – positive, **** - significant reduction in total bacteria count (P<0.0001)

**Yeasts and filamentous fungi**

Analyses aimed at quantifying the occurrence of yeasts and filamentous fungi were also carried out in samples of SB and RMS material during the first half of the year 2023. The results obtained indicated threshold values of yeast occurrence below100 in January. Positive presence of yeasts in SB was observed between February and May, as well as in July. For RMS material, yeast presence was confirmed only in April, underscoring the effectiveness of the hygienic separator and its substantial reduction of yeasts in the monitored months. Regarding filamentous fungi in SB samples, their presence was confirmed in almost all monitored months throughout the year, with the exception of June. The hygienic separator´s effectiveness was confirmed by the results achieved in the quantification of filamentous fungi in RMS samples. Except for February and April 2023, collected RMS samples showed no presence of filamentous fungi in individual months. In summary, the positive impact of the hygienic separator was affirmed, as the majority of samples, particularly those subjected to thermal treatment (RMS), exhibited a rare occurrence of both yeasts and filamentous fungi. The summarized data can be found in **Table 3.**

**Table 3** The presence of yeast and microscopic filamentous fungi in the monitored period of 7 months

| (CFU/g) | SB material | | | | | | |
|---|---|---|---|---|---|---|---|
| Month/year | 01/23 | 02/23 | 03/23 | 04/23 | 05/23 | 06/23 | 07/23 |
| Filamentous fungi | <100 | pos | pos | pos | pos | neg | pos |
| Yeasts | <100 | pos | neg | pos | neg | neg | NI |
| | RMS material | | | | | | |
| Filamentous fungi | <100 | pos | neg | pos | neg | neg | neg |
| Yeasts | <100 | pos | neg | pos | neg | neg | NI |

neg – negative, pos – positive, NI – not investigated,

**Somatic cell count (SCC) quantification**

To minimize the influence of the season on milk yield and milk quality as much as possible, it's important to note that we have specifically compared the same months, spanning from January to July, in both 2022 and 2023. This approach aims to provide a more accurate assessment of any observed changes.

Throughout the mentioned periods of 7 months in the year 2022 and 7 months in the year 2023, an overall reduced average somatic cell count of 20 000/mL of milk was recorded for all screened dairy cows. **Figure 2.** and **Table 4** shows changes in the SCC in individual months of both years. The obtained results indicate a slight reduction in the incidence of subclinical and clinical mastitis in breeding herd. Considering the reduced consumption of anti-mastitis drugs **(Supplementary material 1),** there is evidence to support an improvement in the health status of the mammary glands of dairy cattle. This, in turn, may signify benefits in terms of reducing antibiotic consumption for the treatment of mammary gland inflammation. These findings collectively contribute to enhancing the welfare and health of the individual cows, overall herd health, and potentially, the health of the consumer.

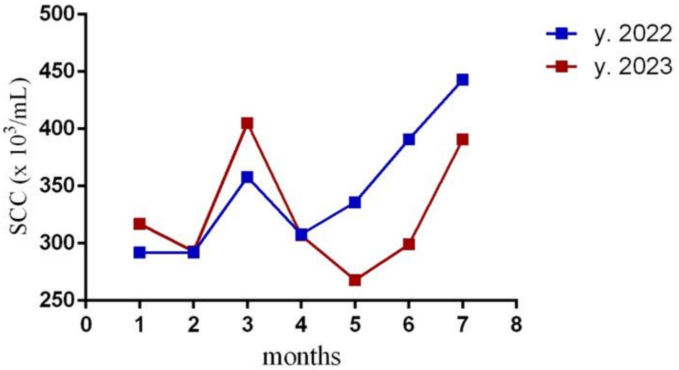


**Figure 2** Comparison of the average somatic cell count in milk during the experimental period

**Table 4** The overview of the total average SCC during individual months

| | January (SCC) | February (SCC) | March (SCC) | April (SCC) | May (SCC) | June (SCC) | July (SCC) | Ø of breeding |
|---|---|---|---|---|---|---|---|---|
| **2022** | 292 x $10^3$/mL | 292 x $10^3$/mL | 358 x $10^3$/mL | 308 x $10^3$/mL | 336 x $10^3$/mL | 391 x $10^3$/mL | 443 x $10^3$/mL | Ø 345 x $10^3$/mL |
| **2023** | 317 x $10^3$/mL | 293 x $10^3$/mL | 405 x $10^3$/mL | 307 x $10^3$/mL | 268 x $10^3$/mL | 299 x $10^3$/mL | 391 x $10^3$/mL | Ø 325 x $10^3$/mL |
| | ↑ 25 x $10^3$/mL | ↑ 1 x $10^3$/mL | ↑ 47 x $10^3$/mL | ↓ 1 x $10^3$/mL | ↓ 68 x $10^3$/mL | ↓ 92 x $10^3$/mL | ↓ 52 x $10^3$/mL | Ø ↓ 20 x $10^3$/mL |

SCC – somatic cell count, ↑ - increased, ↓ - decreased, Ø - mean.

**Milk yield and milk characteristics**

As illustrated in **Figure 3.,** milk production during the initial 7 months of 2022 varied from 35.91 kg/cow/month in January to 39.78 kg/cow/month in June. In contrast, the level of milk production fluctuated from 38.4 kg/cow/month in April to 41.5 kg/cow/month in May during the year 2023. A comparison between the years 2022 and 2023 reveals that higher milk production was recorded in 2023, with an average increase of 2.40 kg/cow/month. This indicates that the use of RMS bedding in 2023 did not negatively impact milk production in dairy cattle. Regarding selected milk parameters (fat and protein content), the following changes were observed. The percentage representation of protein content in the milk samples did not show significant differences over the monitored period (7 months in 2022 and 2023), maintaining an average value of 3.45%. In terms of quantifying the fat percentage content in the same samples, the level was 3.84% (in 2022), and increased to 3.95%in 2023. The average year-on-year difference was 0.11% in fat content. Results are summarized in **Figures 4A** and **4B.**

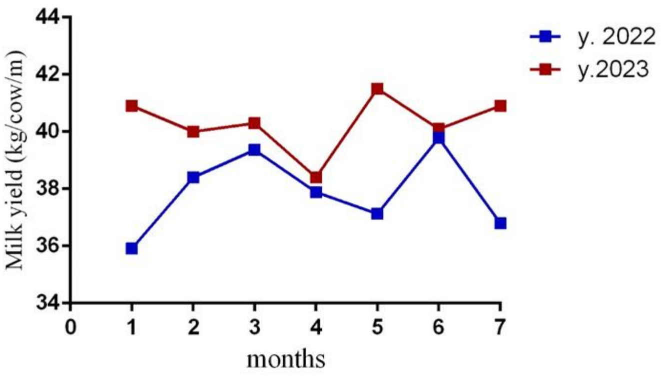


**Figure 3** Comparison of the average amount of milk produced during the experimental period of the years 2022 and 2023

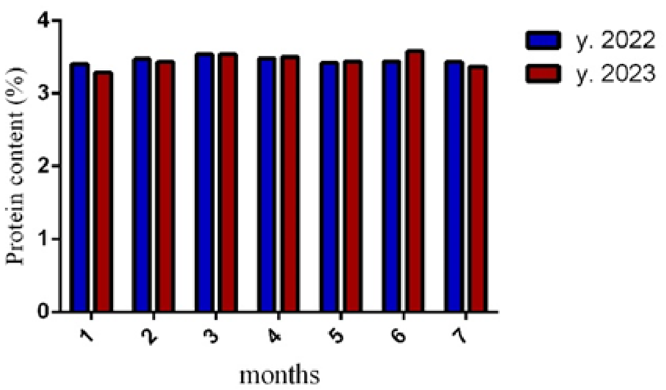


**Figure 4A** Milk characteristics: protein content in % during experimental period

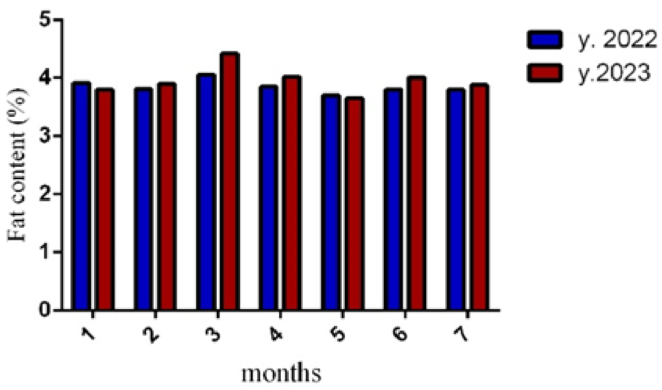


**Figure 4B** Milk characteristics: fat content in % during experimental period

**DISCUSSION**

Dairy farmers looking for new and practically usable methods for efficient handling of cattle waste to minimize the negative impact on the environment and, to save funding resources **(Bacenetti et al., 2016)**. Transformation used cow bedding through a hygienic separator for subsequent use in new cow bedding (RMS) is an alternative recycling method that meets these requirements **(Robles et al., 2020)**. Our study focused on determining pathogen microorganisms' occurrences in RMS and monitoring its impact on somatic cells counts, milk quality, and milk production. Bacterial profiling results indicate the highly effective implementation of the hygienic separator on AgroBan s.r.o. farm. For observed microorganisms *(Escherichia coli, Enterococcaceae, Salmonella, and Clostridiaceae)*, a significantly (P<0.0001) reduced number of colonies of these pathogens (<10) was demonstrated after thermal treatment in the hygienic separator compared to the input material - used straw bedding. Throughout the monitored period, there were only two instances of *Salmonella infantis* presence were detected. **Zehner et al. (1986)** monitored the occurrence of various types of pathogen microorganisms (*Eschericha coli, Klebsiella, pneumoniae, Streptococcus uberis*) in different sterilized types of bedding materials (hardwood chips, sawdust, straw, and RMS). Results showed the highest level of pathogen occurrence was detected in RMS, followed by straw and hardwood chips. In this context, it is crucial to highlight the ongoing advancements in sterilization technology over time, indicating that the current hygienic separation could be demonstrably more effective. This trend was confirmed by a study by **Leach et al. (2015)**. Our results align with the statement of their study, confirming lower bacterial counts in RMS, particularly *Klebsiella spp.* and gram-negative bacteria, compared to straw bedding. However, differences were not found in comparing RMS, recycled sand, and shavings **(Godden et al., 2008)**. The potential of RMS as bedding was evaluated by **Okamoto et al. (2017)**, showing hygienic treatment of the cows´ slurry could eliminate the presence of pathogenic microorganisms such as *Escherichia coli, Streptococci, Enterococci* and other coliform bacteria. A mutual comparing of these materials suggested that the level of monitored microorganisms was reduced on average between 15.86% (Enterococci) and 25.15% (Coliforms bacteria). Several other researchers have shown that the occurrence of pathogen bacterial colonies varies significantly between individual types of bedding **(Zdanowicz et al., 2004, Godden et al., 2008, Bradly et al., 2018)**. At the same time, he diverse results of these individual studies can also be caused by different methods of separation of cow manure intended for bedding. As reported in their studies by **Jorgensen and Jensen (2009)**, different types of separation systems significantly influence variations in the chemical, biological and biochemical properties of RMS. Despite studies, relevant proofs in RMS usage are ambiguous and therefore there are currently no unequivocal conclusions about the safe use of RMS as cow bedding.

Initial assumptions at the beginning of our study indicated that the RMS-based bedding would be correlated with a higher content of the pathogen microorganisms as well as with overall higher somatic cells count in the cow's milk. The results of our analysis suggest a positive trend in SCC changes during the monitored period of 2022 and 2023. The difference in SCC was 20 x $10^3$ cells/ml milk. This fact could have a direct impact on suppressing the occurrence of udder mastitis in dairy cows, which was also confirmed by the data on the use of anti-mastitis drugs **(Supplementary material 1).** This aligns with **Smith et al. (2017)**, indicating lower average SCC in RMS compared to sawdust, sand, shavings and hay. In some cases, measured differences were more than 30x$10^3$ cells/mL. Questions about SCC are common among dairy farmers, since it is considered as a sensitive indicator of mastitis presence and milk quality. Many preliminary studies have confirmed the direct influence of bedding type on SCC. Increased mastitis occurrence caused by *Klebsiella spp*. was detected in sawdust bedding samples mainly **(Zdanowicz et al., 2004, Dyck et al., 2009)**. On the other hand, **Dufour et al. (2011)** declare that sand-based bedding can suppress the development of pathogen bacteria compared to other types of bedding. RMS effect on SCC was examined by **Wenz et al. (2007)** with similar conclusions as in our study. RMS usage in stalls caused reduced SCC, which could refer to a lower incidence of mastitis in dairy cows. This study further underscores the importance of employing the correct method of RMS hygienic separation, affirming the feasibility of using this material as bedding for dairy cows. **Harrison et al. (2008)** analysed mastitis incidence and SCC data on six farms, where different bedding types were used. The prevalence of average SCC was not significantly different when comparing RMS to other types of bedding. Authors of the study declared that usage of RMS in breeding on the experimental farms should not affect the health and milk production. As reported by several authors, large variability has been observed in SCC due to several factors. It can also be directly influenced by the method of housing, the composition of feed rations or the hygiene of the stall environment **(Rodrigues et al., 2005, Dufour et al., 2011)**. In general, the current conclusions are diverse and affected by many factors. For this reason, it is possible to use RMS for bedding, but the hygienic management principles on farms are crucial.

As we mentioned previously, for cow manure usage as bedding, a proper hygienic separation process is essential to minimize mastitis risk, avoid reduction in milk yield, and maintain milk quality. The results of our analyses showed that RMS usage did not significantly affect milk production on the farm. Thanks to the sufficient RMS hygienic separation, a balanced level of milk production was demonstrated during the year 2023. In addition, the nutritional parameters of the milk, such as fat and protein content, were not affected, aligning with study performed by **Schwarz et al. (2010, 2011)** who monitored usage of RMS in relation to milk quality. The achieved results did not demonstrate a significant effect of RMS on the volume of milk yield or quality parameters. Similar conclusions are presented by **Husfeldt and Enders (2012)**. This study monitored use of different technological procedures for RMS preparation at 34 dairy farms during 12 months. In relation to the level of milk production, no significant differences were noted when compared to farms where RMS was not used. The production value fluctuated from 34 kg/cow/day to 45.5 kg/cow/day. At the same time, the authors emphasized that milk production may be influenced by multiple factors and using it as the sole parameter for welfare evaluation in dairy cows is not recommended.

## CONCLUSION

Results of our study indicate that the controlled hygienic separation of input cow manure could effectively eliminate the presence of pathogenic microorganisms. Moreover, the utilization of recycled manure solids as bedding material exhibited no adverse impact on somatic cell content in milk, and it also did not reduce the production and quality parameters of cow's milk. It means that the application and use of RMS could lead to a reduction in the cost of primary resources such as hay or straw and does not have a negative effect on the welfare and health of animals.

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