Are recycled manure solids an appropriate bedding material
 for dairy cattle compared to traditional materials?

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#### 6 Abstract:

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8 This review explores the literature regarding the use of Recycled Manure Solids (RMS) as a bedding 9 material for dairy cattle, in the UK. Recent papers, which used robust statistical analysis of data and 10 were peer reviewed were accessed using commonly available search engines (Pub Med, Google 11 Scholar and Science Direct). The effects of RMS use on cow welfare (particularly lameness and 12 pressure lesions), milk hygiene, the environment and human health are explored. The practice of 13 composting (whether intentional or inadvertent) to produce RMS is prohibited in the UK as the 14 evidence for its benefits is not yet convincing and there are risks of increasing the growth of 15 thermoduric organisms. There are benefits to cow welfare and comfort when RMS is used in cubicle 16 housing. Some data regarding bacterial load and the risks to milk hygiene and human health are promising, in that RMS may be comparable with other bedding materials, but the issues of parlour 17 18 routine and farm husbandry confound the effects of bacterial load in any chosen bedding. Research 19 to date demonstrates that RMS may have a place in dairy cattle housing, but that the lack of studies, 20 particularly those on a large scale, limits the data available and the ability to draw safe conclusions. 21 There are significant uncertainties regarding associated risks to animal and human health from using 22 recycled manure solids making it difficult to establish whether recycled solids can meet 23 requirements and be deemed as safe to use. A structured review of literature highlighted that the 24 management of the bedding material itself is more important especially regarding diseases and that 25 there are substantial gaps within the research yet to be addressed.

26

## 27 Introduction:

Virtually all dairy cows at some point during production are housed, during this period the use of safe, comfortable, dry bedding is imperative for good health and animal welfare. Increased cost and a reduced availability of traditional bedding materials such as sand, straw, and wood shavings has provoked farmers to search for more accessible and sustainable options for example, recycled manure solids (Ball, 2016). The production of recycled manure solids comes from a method first established in the USA in the 1970's and is now being adopted by <u>some</u> UK dairy farmers (Timms, 2008). Physical separation of the solids from liquids in cattle manure through mechanical and
gravitation force, leaves behind a solid fraction consisting of mainly undigested fibres (Leach, et al.,
2015) which can then be processed to produce a suitable bedding material. The act of composting
the material, permitted elsewhere, is prohibited in the UK.

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39 Livestock manures are classed as Category Two Animal By-Products, as defined by EC Regulation 40 1069/2009 (Ball, 2016). Their use as a 'technical product', for example as animal bedding is only 41 permitted if strict conditions are applied to minimise potential health risks (Leach , et al., 2015). 42 Examples include only using recycled manure solids as bedding for cattle which are in the same 43 epidemiological unit as those cattle from which it is generated and not using recycled manure solids 44 produced from herds which are subject to official restrictions for notifiable diseases such as TB (Ball, 45 2016). The risk of spreading disease can be particularly high in herds using recycled manure solids 46 therefore manure from animals in quarantine, from animals which have aborted and manure from 47 calving areas must not be used in the production of recycled manure (Ball, 2016). The production of 48 the recycled manure solids is closely monitored and provided a sufficiently high dry matter content 49 can be achieved, the solid fraction of manure can be considered usable as bedding, but only if it is 50 correctly made, stored and used within twelve hours (Red Tractor Farm Assurance). The minimum 51 dry matter recommendation is between 32-34% to minimise pathogen growth as a lower value 52 (increased moisture) will support more rapid growth of pathogens and fail to provide a sufficiently 53 hygienic bedding material for dairy cattle (Bradley, et al., 2014). The Red Tractor Farm Assurance 54 defines the strict terms under which RMS may be used by farms seeking Red Tractor Assurance and 55 these may be accessed via https://assurance.redtractor.org.uk/contentfiles/Farmers-56 5409.pdf? =636262273844028704

57 Despite the accompanying risks, recycled manure solids have the potential to offer benefits to dairy 58 units such as providing lower dust levels, thus promoting better respiratory health and reducing the 59 transmission of pathogens via dust particles within houses (Bradley, et al., 2014), alongside a 60 noticeable reduction in hock lesions and lameness cases (Husfeldt & Endres, 2012).

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Along with the production of the bedding material, the management of both the bedding itself and cattle during milking is also of utmost importance to maintain good herd health (Rowbotham & Ruegg, 2015). Cattle housing should be well ventilated regardless of the bedding material but particularly when using recycled manure solids and adequate drainage should be installed to ensure a drier environment to discourage pathogen growth (Bradley, et al., 2014). Weather conditions can also have a profound effect on the dry matter content of the recycled manure therefore it should be

- 68 stored indoors before being used as bedding; the need for appropriate buildings for storage should
- 69 be considered before farmers attempt to adopt the use of recycled manure solids (Timms, 2008).
- 70 This analysis of peer reviewed literature summarises the potential opportunities and challenges
- 71 presented by recycled manure solids as a potential source of bedding material.
- 72

## 73 Methodology:

- 74 Initially, several publication search engines were used to give a comprehensive view of the literature
- 75 on recycled manure solids deemed appropriate to be used in this review. Many papers were
- returned which concentrated on certain major topics, namely diseases such as Johne's disease and
- 77 Bovine Tuberculosis and cow welfare. The search criteria were subsequently refined to limit the
- 78 literature to papers addressing these topics.
- 79

80 Databases searched were PubMed, Science Direct and Google Scholar (being commonly used search

- 81 engines, available to readers) and search terms included 'recycled manure solids', 'Johne's disease'
- 82 'green bedding' 'bovine tuberculosis' 'bacteria' and combinations thereof. A summary of the
- 83 keywords used and papers returned is shown in table 1a.
- 84

85 As shown a large number of papers were returned which prompted concise refining. Duplicate

- 86 papers were discarded, as were those not relevant to the UK dairy industry, those not scientifically
- 87 proven and those published more than 10 years ago.
- 88 Additional search refinements included refining to recent papers and those from higher impact
- 89 factor journals in the field of veterinary and dairy publications, to return a sensible number of papers
- 90 to be read and analysed. The papers returned from each search determined the keywords used for
- 91 the next search by highlighting which topics had the largest availability of literature and should
- 92 therefore be investigated further. Detailed repeatable searches are listed in Appendix 1 (tables 1-
- 93 27). All papers were critically reviewed; papers used within this review were considered scientifically
- 94 relevant and their main conclusions are documented. Conflicting findings are included to identify
- 95 potential areas of additional research.
- 96
- 97

#### 98 Results:

99 The flow diagram (Figure 1) shows how the literature was screened and the evidence each paper100 provided is listed in tables 1-27.

101 Discussion:

## 102 **Cow comfort and welfare:**

103 Cows can spend between 12 and 14 hours resting in cubicles between milking sessions therefore 104 providing a comfortable and clean place for them to rest is imperative (Rowbotham & Ruegg, 2015). 105 Recycled manure solids are described as soft, easily deformed and non-abrasive, all which can be 106 described as beneficial traits (Harrison, et al., 2008). Decreased cow comfort when lying down and 107 resting can be associated with increased lameness as the cows spend less time lying down and more 108 time standing or using the cubicles incorrectly. Given that lameness is one of the greatest animal 109 welfare concerns in the dairy industry there should be continual efforts made to improve cow 110 comfort (Husfeldt & Endres, 2012).

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112 Cow comfort can be measured by looking at cow preference, standing and lying behaviour and 113 looking at the prevalence of lameness and hock lesions. A study conducted in upper Midwest US 114 looking at 34 dairy farms with herds ranging from 130 to 3,700 lactating Holstein cows using 115 recycled manure solids investigated the effect of bedding material on locomotion scores. The 116 cattle's locomotion was scored out of five, with score one (normal) being used for cows standing and 117 walking normally with a level back, to score three (moderately lame) for cows standing and walking 118 with an arched back and short strides with one or more legs, to score five (severely lame) for cattle 119 with pronounced arching of the back, reluctance to move with almost complete weight transfer off 120 the affected limb (Sprecher, et al., 1997). The results demonstrated that lameness prevalence was 121 significantly lower in deep-bedded freestalls (14.4%) compared to freestalls with mattresses (19.8%) 122 and severe lameness prevalence (when cows had a locomotion score equal to or above 4) was also 123 significantly higher in mattress freestalls (5.9%) compared to deep bedded free stalls (3.6%). Cows 124 bedded on deep bedded recycled manure solids also had a statistically significant lower prevalence 125 of hock lesions (49.4% versus 67.3% for cows on mattresses) (Husfeldt & Endres, 2012). Lameness is 126 a multifactorial problem and confounding factors such as a breed lameness prevalence should be 127 considered; the study only used Holstein dairy cattle therefore the effect of genetic makeup as a 128 confounding variable is possibly reduced and the lameness prevalence is more attributable to the type of bedding material; however, the wide genetic base of the Holstein does cause there to be 129 130 genetic variation within a herd of one breed and the influence of genetics cannot be dismissed. 131 Deeper bedding is therefore a way of reducing lameness and hock lesions in dairy cattle. The

132 increased costs and reduced availability of other bedding materials such as sand and sawdust often 133 deters farmers from applying copious amounts of bedding. Recycled manure solids are freely 134 available as a raw material encouraging a more liberal application to mats and mattresses hence 135 achieving a deeper bed; however, considerable investment in machinery and possibly storage is 136 required for the processing of the "free" resource and these costs should be weighed against the 137 "saving" of not buying in sand or sawdust or straw. The study's results highlight the fact the choice 138 of recycled bedding materials does not overcome the problem but the reduction in prevalence of 139 lameness and hock lesions is a successful starting point.

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# 141 Cleanliness:

142 Another aspect of cow comfort and welfare that has been considered is cow cleanliness. This was 143 particularly investigated in a study conducted by Hippen et al. (2007) which compared the hygiene 144 and comfort of dairy cows bedded on dolomite limestone versus those on recycled manure solids on mattresses. The study involved four pens using 104 cows and fresh bedding was applied every two 145 146 days, there was a noted improvement in cow cleanliness (cows measured on average 1.4 out of 2 on 147 the AHDB cleanliness scoring system compared to cows scoring an average of 2 when bedded on 148 limestone) and a decrease in hock lesions when using recycled manure solids was also discovered in 149 this study. These conclusions were supported in another study by Timms (2008) which also claimed 150 that cow comfort, cleanliness and feet and leg health were "excellent" on bedded manure solids. 151 Although the appearance of cows looking clean sometimes does not correspond to an absence of 152 pathogenic microorganisms, a study by Zadoks (2011 cited by Bradley, et al., 2014) claimed that 153 bacterial counts are lower when there is a reduced faecal burden and increased cleanliness of the 154 legs.

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#### 156 Disease:

157 Studies of the bacterial load of recycled manure solids are often conflicting, with results highlighting 158 areas that need more exploration before conclusions can be drawn. It is supposed that freshly 159 separated manure solids contain relatively high levels of pathogenic microorganisms, especially if 160 there was a large microorganism burden in the slurry before separation (Bradley, et al., 2014). 161 Harrison et al., (2008) however found that certain pathogens such as Escherichia coli were not found 162 in recycled solids after they had undergone treatment (in this study composting) prior to being used 163 as bedding but significantly higher levels were found in the bedding after use. This highlights the 164 possibility that bacterial levels in used bedding are more likely to be a result of bacteria in the fresh 165 manure of the animal, how well the stalls are cleaned along with how frequently the bedding is

166 changed. Harrison et al., (2008), along with showng that composting reduced bacterial numbers in 167 recycled manure solids (apart from Klebsiella which was able to survive), also compared other 168 commercial bedding materials and demonstrated that sometimes even the commercial materials 169 can contain a relatively high bacterial load and can reach similar levels of environmental mastitis 170 pathogens as recycled manure solids when being used as bedding. Recycled manure solids contain a 171 high pathogen load for a consistent period of time compared to commercial bedding materials which 172 have a reduced pathogen load when first applied to the stalls but can rapidly exhibit similar 173 pathogen levels after time. This provides an area of potential investigation exploring whether the 174 steep increase in pathogen numbers seen in commercial beddings presents a bigger challenge to 175 teat ends than the more stable level of pathogens which may be seen in some recycled solids. 176 However, there will be variation in pathogen loads between farms and with seasonal/daily variations 177 in weather which have not been investigated as confounding factors.

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179 The literature investigating pathogens other than mastitis pathogens in recycled manure is limited. 180 There have been some studies investigating Mycobacterium avium spp paratuberculosis (MAP), 181 responsible for Johne's Disease but more studies are needed before conclusions can be made. MAP 182 is shed in faeces therefore there is the possibility that using recycled manure solids can spread 183 Johne's disease if the bacteria remains viable. There is every chance this could happen as the 184 pathogen is not always killed by composting, separation or drum composting (Gooch, et al., 2006). 185 Bonhotal et al., (2011) provided conflicting evidence that composting unseparated manure to 186 temperatures of 55°c was effective in reducing MAP to undetectable levels within five days. A study 187 was conducted investigating MAP survival further and results showed after composting for three 188 days at 55°c there was no evidence of Escherichia coli, Salmonella and Listeria monocytogenes in the 189 bedding even when it was present prior to composting. MAP was detected on day zero of 190 composting but was undetectable on day three and day seven (Grewal, et al., 2006). The study 191 however did identify MAP DNA on day 56 in all methods of treating the manure. With these 192 conflicting results in mind it should be suggested that recycled manure solids should not be used for 193 bedding in calves as they may be more inclined to eat it than adult cows (Harrison, et al., 2008). 194 Considering the study's findings and the knowledge of high risk of transmission in early life, recycled 195 manure solids should also not be used where cows are kept for the late dry period or for calving to 196 minimise the chance of Johne's disease transmission to young stock (Leach, et al., 2015). 197

198 Clinical Mastitis:

199 Mastitis is considered the most economically important disease seen in dairy cattle and is a very 200 common problem farmers have to deal with (Rowbotham & Ruegg, 2016b). The pathogens that 201 cause mastitis can be categorized as environmental or contagious depending on their primary 202 reservoir and their point of exposure (Smith & Hogan, 2006). Contagious mastitis pathogens are 203 often picked up during the milking process when the teats of healthy cattle are infected with 204 pathogens from infected teats by either defective milking machines, inappropriate hygiene practices 205 or the presence of carrier cows within the herd. The prevalence of contagious mastitis within herds 206 is seen to be decreasing due to incorporation of good milking hygiene and the selective culling of 207 chronically infected cows. (Rowbotham & Ruegg, 2016b).

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209 Environmental mastitis pathogens are prevalent in the cow's housing environment and exposure can 210 occur when teats encounter these micro-organisms either through bedding or in the manure itself. 211 Teats can be in contact with bedding material for 40-65% of the day, therefore keeping 212 environmental pathogens in the bedding to a minimum is imperative (Rowbotham & Ruegg, 2016a). 213 Many studies have been conducted investigating the prevalence of environmental pathogens such 214 as Streptococcus spp, Coliform bacteria and Klebsiella spp within different bedding materials. 215 Rowbotham and Ruegg (2016b) concluded that large numbers of Streptococci species were 216 recovered from all bedding types in all seasons however were greatest in shallow bedded recycled 217 manure solids and recycled sand and least in deep bedded recycled manure. Total counts of gram 218 negative Coliform bacteria and Klebsiella spp were greatest in deep bedded manure solids and least 219 in new sand as expected. The study therefore displayed there was not a single bedding material that 220 provided low levels of all mastitis pathogens indicating there is a risk of environmental pathogen 221 contamination present with any bedding. The incidence rate of mastitis was also studied in the 222 population of cows to try and determine if there was a correlation between pathogen load in the 223 bedding and clinical cases of mastitis. The low prevalence of clinical mastitis detected in this study 224 however could not be attributed to bedding materials as the population was made up of 225 primiparous cattle and therefore not characteristic of cows on commercial dairy farms. Primiparous 226 cows are known to have a lower risk of both clinical and subclinical mastitis and can withstand a 227 greater exposure to environmental pathogens (Rowbotham & Ruegg2016a), the study therefore 228 highlighted that recycled manure solids can be used in this population with a minimal effect on 229 udder health. 230

As bedding can be a reservoir for bacterial growth and can create a heightened issue for teat
exposure to environmental pathogens (Godden, et al., 2008), many studies have been conducted to

233 investigate whether certain techniques could reduce bacterial load within recycled manure solids. 234 The ability of composting to effectively reduce numbers of Coliform bacteria has been discovered in 235 many studies (Carroll & Jasper, 1978; Cole & Hogan, 2016). Cole and Hogan (2016), also discovered 236 that composting for four weeks reduces counts of gram negative bacteria and *Streptococcal* counts 237 associated with environmental mastitis. Bishop et al., (1981) conducted a study which further 238 supported this conclusion as results showed bacterial counts decreased in dairy waste solids by 239 composting over 14 days. This study also attempted to detect a direct relationship between bacterial 240 load within bedding and bacterial count on teats and within the milk. When comparing recycled 241 manure to rubber mats, there was no statistically significant difference in bacterial counts on teats 242 or in milk between the two types of bedding thus indicating there is no relationship between 243 microflora in bedding and microflora on teats and in milk. More recent studies conducted have 244 supported Bishop et al., (1981) and have discovered the high level of bacteria seen in recycled 245 manure solids does not correspond to an increased incidence of mastitis, teat end bacteria count or 246 increased cell count in milk (Driehuis, et al., 2012). Driehuis, et al., (2012) also highlighted that once 247 in the cubicles, pathogen concentrations increase in a relatively short space of time, independent of 248 the bedding material therefore there is no evidence to highlight an increased risk of mastitis when 249 using recycled manure solids provided they are composted and managed correctly. However, the 250 practice of composting is currently not permitted in the UK as there is a risk of selection for 251 thermoduric organisms which are associated with milk and milk product spoilage, therefore the 252 perceived benefits of the process are not yet applicable in the UK.

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### 254 Somatic Cell Count:

255 Bulk milk somatic cell count is associated with intramammary infections and can be used to 256 determine premium payments by milk purchasers (Rowbotham & Ruegg, 2015). It is therefore 257 important to consider any potential effects of using recycled manure solids on somatic cell count. 258 The consensus after analysing numerous studies is that using recycled manure solids is not 259 associated with an increased somatic cell count and that bacterial levels within bedding do not 260 correspond to more animals presenting with an increased cell count (Harrison, et al., 2008; Hippen, 261 et al., 2007; Timms, 2008). Studies also highlighted that although bedding bacterial counts were 262 important to manage, excellent cow preparation, cow hygiene, bedding/stall and alley management 263 appeared to be more important in maintaining a low somatic cell count (Husfeldt & Endres, 2012; 264 Timms, 2008).

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### 266 Bovine Tuberculosis:

267 With Bovine Tuberculosis being a current notifiable disease causing major economic impacts 268 through culling and movement restrictions preventing sales, the potential for recycled manure solids 269 to spread the disease is a concern for dairy farmers. There is limited literature investigating the 270 excretion of Mycobacterium bovis through faeces, urine and milk however these routes of spread 271 are considered to be rare in the UK (Menzies & Neill, 2000). Literature studying the shedding 272 patterns of Bovine Tuberculosis describes that a heavy load of bacteria in faeces and urine is unlikely 273 to occur until the disease is at an advanced stage, even then the shedding can be sporadic (Neill, et 274 al., 1988). As regular testing is mandatory in most of the UK and in all areas not declared Officially TB 275 free, the chance of reaching this late stage is reduced. However, information on shedding patterns 276 as the disease progresses are limited and more research is needed before conclusions can be 277 achieved.

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279 If the causal microorganism were to contaminate slurry from infected animals, then it is unlikely to 280 be reduced by physical separation meaning it may be present in recycled manure. Hence, why using 281 recycled manure from herds under tuberculosis movement restrictions due to the finding of test 282 reactors is currently prohibited (Red Tractor). Phillips, et al., (2003) discovered in their study that 283 survival of Mycobacterium bovis in slurry can range from ten weeks to six months depending on 284 temperature. The study revealed aerobic digestion or heat treatment is needed to kill the bacteria. 285 With a paucity of literature available in this field also, more studies are needed to decipher exact 286 conditions needed to ensure complete removal of the pathogen to make recycled manure solids a 287 safe bedding option for herds testing positive for Bovine Tuberculosis.

288

### **Digital Dermatitis:**

290 Digital dermatitis is a painful skin condition seen in dairy cattle and is considered a major cause of 291 infectious lameness (Stokes, 2011). Wet and unhygienic conditions underfoot along with dirty feet 292 and legs have been highlighted as risk factors for the disease (Stokes, 2011). Considering the dry 293 matter of recycled manure solids can increase to between 60-80% after being placed in cubicles and 294 spread out allowing for more drying (Bradley, et al., 2014), the risk of digital dermatitis whilst using 295 this bedding can be assumed to be reduced. Cows bedded on recycled manure solids are reportedly 296 cleaner (Timms, 2008) and alley ways are often markedly drier (Bradley, et al., 2014) further 297 suggesting that the risk of digital dermatitis could potentially be reduced. Treponemes are the major 298 pathogens concerned causing digital dermatitis and are notably abundant in the dairy cow's housing 299 environment (Evans, et al., 2010). The specific phylotype linked with digital dermatitis has not yet 300 been detected in slurry samples however has been recovered from the recto-anal junction of cattle

(Evans, et al., 2010). Therefore, the inability to detect the causal *Treponeme* in slurry could be due to
detection techniques and methods or due to intermittent shedding. The growth of *Treponemes* is
promoted by damp environmental conditions (Bradley, et al., 2014) thus there is no evidence to
suggest recycled manure solids would enhance pathogen numbers. Although the specific dry matter
content to prevent *Treponeme* growth is currently unknown, the dry and clean environmental
conditions promoted by using recycled manure solids as a bedding could mean *Treponeme* numbers

- to be at a minimum; however, specific research is needed before this can be assumed.
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# 309 Antibiotic resistance:

310 There is a relative paucity of research investigating antibiotic resistant bacteria specifically in 311 recycled manure solids but there is more readily available literature looking at antimicrobial 312 resistant organisms and their genes in livestock manures in general. A study was conducted 313 comparing antibiotic resistant microbes in manure from livestock treated with antibiotics and those 314 with control medication. As expected higher levels of antibiotic resistant microbes were identified in 315 manure from livestock which had been treated with antibiotics (Sharma, et al., 2009). This trial also 316 investigated treating the manure and the effect this had on the organisms. The study which lasted 317 for 18 weeks explored the survival total Escherichia coli, Escherichia coli resistant to ampicillin and 318 tetracycline and select tetracycline and erythromycin resistance methylase genes. The results 319 showed that even though the compost temperatures did not reach the recommended temperature 320 of 55°c for 15 days, the effect of composting was still apparent and reduced high initial levels of total 321 Escherichia coli resistant to ampicillin and tetracycline after two weeks. However even after 322 composting, tetracycline and erythromycin resistant methylase genes were still detected. Despite 323 composting being an economical and environmentally friendly approach to stabilising livestock 324 organic matter, optimum conditions are needed for the process to remain efficient, any deviation 325 from these conditions can lead to reduced efficiency and the potential for microbial growth (Selvam 326 & Wong, 2017). Considering this and the lack of literature regarding antimicrobial resistant 327 organisms, a cautious approach to discard excreta from animals under antimicrobial treatment 328 instead of using them to provide bedding materials should be recommended. 329

## 330 Ammonia emissions:

Dairy cattle barns are a major source of gaseous ammonia emissions. The negative impacts of elevated ammonia levels on human and animal health are well documented and highlight the irritation caused to lungs and eyes (Bradley, et al., 2014). Studies have been conducted which investigate whether the type of bedding can influence the emissions of ammonia within the

- 335 livestock buildings. Emissions demonstrated a linear increase with the absorbance capacity of
- bedding material and were inversely related to bulk density of the bedding material (Misselbrook &
- Powell, 2005). The properties of recycled manure solids therefore advocate that ammonia emissions
- may be slightly higher compared to other bedding materials. Thus, recycled manure solids should
- only be considered in well ventilated cattle buildings to prevent the build-up of emissions which
- could potentially impinge on animal and human health.
- 341

## 342 Attempts to reduce bacterial load within the recycled manure solids:

343 There is an evident element of risk when using recycled manure solids as a bedding material. Smith 344 and Hogan (2006) stated that when using recycled manure solids there was a "significant risk factor 345 for exposure to environmental pathogens that cause mastitis in dairy herds" but also commented 346 that this was the case when using most other commercial organic bedding materials too. Harrison et 347 al (2008) went on to discover that the management of the bedding is more crucial to controlling 348 microbial populations compared to what bedding material is used in the first place. Management of 349 bedding particularly the use of conditioners and changing bedding frequency has been investigated 350 in a number of studies.

- 351 The use of "conditioners" in recycled manure solids has been explored as this can lead to the
- alteration of the pH of the bedding which can subsequently help to control microbial populations. A
- 353 study was conducted which compared levels of common environmental mastitis pathogens in
- 354 recycled manure treated with a commercial bedding conditioner containing 93% sodium
- 355 hydrosulphate, to bedding which received no treatment and remained as a control. The results
- 356 showed that the effects of adding chemical conditioners to the organic bedding material were

relatively short lived (Hogan, et al., 2007). This study also revealed that alkaline conditioners were

- 358 more effective in recycled manure solids and had a more profound effect on the microbial
- 359 population. Gram negative bacterial and streptococcal counts were reduced in recycled manure
- 360 solids immediately after application of the conditioner on day one. Bacterial counts however did not

differ between treated and untreated recycled manure bedding on day two and six after application.

- 362 The antibacterial activity of the conditioner was found to be related to the pH of the bedding and
- 363 was diminished by day two after application. The short period of action is thought to be linked to
- the bedding being contaminated with manure as the cows enter the stall and removal of the
- bedding along with the conditioner as the cows leave the stalls. The practice of adding a conditioner
- to the bedding can therefore be described as ineffective.
- 367

368 Frequent removal and replacement of any bedding material is recommended to maintain good cow 369 hygiene and to reduce the transmission of a variety of diseases. Studies have shown management 370 factors can help reduce the impact of recycled manure solids on mastitis and other disease risks, 371 these include maintaining adequate bedding in stalls, frequent stall grooming, excellent ventilation 372 and parlour hygiene (Godden, et al., 2008). Harrison et al., (2008) also conducted a comprehensive 373 study of pathogens in bedding materials and concluded that individual farm factors and 374 management had a strong influence alongside that of the underlying bedding type. Sorter, et al,. 375 (2014) compared deep beds with shallow layers which were replaced daily. Conclusions were that 376 coliform and Klebsiella spp. bacterial counts were lower in daily replaced bedding compared with 377 deep packed bedding across the experiment on day zero, one, two and six but there was no 378 measurable effect on Streptococcus spp. counts. Bradley et al., (2014) argued that applying less 379 bedding and keeping a thin layer of recycled manure solids offered the advantage that the bedding 380 material could dry out further and maintain a lower dry matter content. The study investigated this 381 potential advantage and saw an increase from an initial 29% dry matter to a range between 45-62% 382 dry matter. Although the bedding material was less likely to stick to the cows and contaminate milk 383 there was no significant reduction in bacterial numbers. Furthermore, Rowbotham and Ruegg (2015) 384 supported this conclusion and commented that bulk milk somatic cell score for farms using organic 385 bedding was reduced when bedding in the back of stalls was replaced regularly.

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387 One study has also been performed which investigated the effect of alley- floor scraping frequency 388 on environmental bacterial counts which were regularly involved in mastitis cases. Lowe, et al,. 389 (2015) conducted a small study lasting six weeks from which results indicated that increasing alley-390 floor scraping to a frequency of two times a day decreased all bedding pathogen counts, teat end 391 coliform, Escherichia coli, Streptococcus species and milk coliform counts. Increased scraping did not 392 however correlate to a profound effect on milk Escherichia coli, Streptococcus, Klebsiella or somatic 393 cell count. More studies are therefore needed to accredit these results and to highlight other 394 potential mechanisms which may reduce the bacterial burden within dairy cow housing.

395

## 396 **Consumer perception and risks:**

Consumer perception is often the largest battle for dairy farmers when using recycled manure solids
as the customer often assumes recycled bedding is dirty with a higher bacterial burden which may
have an impact on milk and food quality (Bradley, et al., 2014). Perception that recycled manure
solids have high bacterial counts means some dairy producers are sceptical about using manure
solids as bedding for cows (Husfeldt, *et al.*, 2012). The main risk identified when considering the

402 impact of recycled manure solids on food quality is of coliforms, bacterial spores, yeast and fungi in
403 the milk all which could potentially increase the risk of food spoilage, particularly in cheeses
404 (Bradley, et al., 2014).

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406 As food safety is of uppermost importance, numerous studies have been conducted considering the 407 effect of recycled manure solids on potential risk pathogens. One study conducted compared 408 recycled manure solids with sawdust and demonstrated that all bedding materials are potential 409 sources of contamination for raw milk (Driehuis, et al., 2012). The study commented that bacteria 410 can be killed by pasteurisation of milk however some spores of certain bacteria can survive 411 pasteurisation and high temperatures and high concentrations of these pathogens can lead to 412 production losses of cheese and reduced shelf life of milk. Driehuis, et al,. (2012) demonstrated that 413 spore levels of *B.cereus*, butyric acid bacteria and mesophilic aerobic spore formers in milk were no 414 higher on farms using recycled manure solids than those farms using straw or sawdust.

415

416 Leach, et al., (2015) discussed that other important pathogens to consider in regards to human 417 health are *Escherichia coli* and *Salmonella*. The risk of increased levels of these pathogens in recycled 418 manure solids is not well defined or investigated, but mitigation is straight forward and affordable 419 through pasteurisation. Leach, et al., (2015) also went on to discuss mesophilic, thermophilic, heat 420 resistant and aerobic spores and concluded freshly separated manure solids did not show elevated 421 levels to raise concern. More recent work (Bradley et al. 2018) compares bacterial load in sand, 422 sawdust and RMS bedded cubicles, finding substantial differences in bacterial counts both within 423 and between bedding materials; there were no significant differences between bedding types in the 424 counts in milk for any of the organisms studied, and no significant correlations between bacterial 425 load in the bedding used and the milk. Higher levels of bacterial load in RMS were found but did not 426 necessarily correlate with an increase of bacteria in milk. However, it is very important to note that 427 teat preparation did have an effect upon reduction of milk bacterial load and that the parlour 428 routine offers control points for the minimisation of bacteria in milk. The choice of a bedding 429 material is not a substitute for correct procedures in the parlour, nor does the choice of bedding 430 negate the need for good management practices within the housing.

Importantly, some zoonotic bacteria were found, not specifically relating to the use of RMS, but
their presence demonstrates the importance of pasteurisation in the production of milk for human
consumption.

#### 434 Conclusion

It is evident that recycled manure solids have the potential to offer a safe and sustainable alternative
to more traditional bedding materials, but that appropriately high standards of hygiene and
husbandry in the cattle environments must be maintained. There is a lack of evidence to suggest
whether composting is an appropriate means of processing the product and the procedure remains
prohibited in the UK at the time of writing.

440 Using recycled manure solids provides cows with a welfare friendly resting surface and reduces 441 lameness and hock lesions potentially reducing the burden of lame dairy cattle that dairy producers 442 currently face. Considering other diseases, recycled manure solids have shown there is no positive 443 correlation between the high bacterial count sometimes seen in recycled solids and the incidence of 444 mastitis within herds. As reiterated in many studies, the management of cows in the milking parlour 445 and the bedding itself is more important in managing disease prevalence. Daily removal of recycled 446 manure solids and keeping the bedding dry and clean is imperative to keeping bacterial levels to a 447 minimum. Being as the teats often look cleaner as the recycled solids do not stick to the animal, 448 farm workers can become complacent when it comes to teat preparation. The failure of teats to be 449 cleaned correctly before milking can predispose dairy cows to mastitis and an increased somatic cell 450 count, independent of the bedding material used. Thus, pre-milking teat disinfection should be 451 compulsory on any farm but particularly those using recycled manure solids to ensure an increase in 452 either somatic cell count or mastitis cases cannot be attributed to using recycled manure.

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454 There are still significant uncertainties regarding any detrimental effects on human health due to 455 using recycled manure solids and customer perception is a massive problem faced by dairy 456 producers. Consumers can be reassured that bacterial counts in milk however are not affected by 457 using recycled manure and pasteurisation is an effective way of making milk products safe for 458 consumption. The strict controlled conditions that must be followed by all participating farmers 459 reduces the possible detrimental effects of using recycled solids on milk products; but only if farmers 460 follow the correct procedures for composting and RMS management, as well as maintaining high 461 standards of hygiene in the housing and milking parlour, thus minimising all risk factors for microbial 462 growth and infection.

464 Despite the uncertainties there are also apparent benefits of using solids as bedding both financial 465 and in terms of animal welfare. Further research is warranted into the relative economic advantages 466 of using recycled manure solids once initial costs for the set up and acceptable storage buildings are 467 considered. Concerning animal welfare, provided excellent milking preparation is adopted along 468 with adequate bedding management, recycled manure solids have no increased associated risks for 469 common diseases.

There are still substantial gaps within the research regarding recycled manure solids which should prompt future investigations. The paucity of research into the effect of manure solids on antibiotic resistance highlights the need for additional research. Potential investigations may include determining whether different antibiotic resistant pathogens can survive composting and whether an increased level of antimicrobial resistant pathogens within the bedding material itself pose any substantial threat to the cattle and potentially consumers. As mastitis is an economically important disease in the dairy industry and has been identified as a multifactorial disease, this too poses an opportunity for further investigations. Different risk factors, for example genetic predisposition and anatomical positioning of teats and their effect on mastitis prevalence should be investigated as it can be assumed it is more than just different bedding materials that influence a herd's mastitis prevalence. 

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