1

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

Abstract:

7 8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

5 6

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

26

27

28

29

30

31

32

33

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 some 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.

38

39

40

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

34

35

36

37

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

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

60 61

62

63

64

65

66

67

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

Methodology:

Initially, several publication search engines were used to give a comprehensive view of the literature 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 Bovine Tuberculosis and cow welfare. The search criteria were subsequently refined to limit the literature to papers addressing these topics.

Databases searched were PubMed, Science Direct and Google Scholar (being commonly used search engines, available to readers) and search terms included 'recycled manure solids', 'Johne's disease' 'green bedding' 'bovine tuberculosis' 'bacteria' and combinations thereof. A summary of the keywords used and papers returned is shown in table 1a.

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

papers were discarded, as were those not relevant to the UK dairy industry, those not scientifically proven and those published more than 10 years ago.

Additional search refinements included refining to recent papers and those from higher impact factor journals in the field of veterinary and dairy publications, to return a sensible number of papers to be read and analysed. The papers returned from each search determined the keywords used for the next search by highlighting which topics had the largest availability of literature and should therefore be investigated further. Detailed repeatable searches are listed in Appendix 1 (tables 1-27). All papers were critically reviewed; papers used within this review were considered scientifically relevant and their main conclusions are documented. Conflicting findings are included to identify potential areas of additional research.

Results:

98

101

102

103

104

105

106

107

108

109

110

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

Discussion:

Cow comfort and welfare:

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

111112

113

114

115

116

117

118

119

120

121

122

123

124

125

126

127

128

129130

131

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

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

Cleanliness:

Another aspect of cow comfort and welfare that has been considered is cow cleanliness. This was particularly investigated in a study conducted by Hippen *et al.* (2007) which compared the hygiene 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 days, there was a noted improvement in cow cleanliness (cows measured on average 1.4 out of 2 on the AHDB cleanliness scoring system compared to cows scoring an average of 2 when bedded on limestone) and a decrease in hock lesions when using recycled manure solids was also discovered in this study. These conclusions were supported in another study by Timms (2008) which also claimed that cow comfort, cleanliness and feet and leg health were "excellent" on bedded manure solids. Although the appearance of cows looking clean sometimes does not correspond to an absence of pathogenic microorganisms, a study by Zadoks (2011 cited by Bradley, *et al.*, 2014) claimed that bacterial counts are lower when there is a reduced faecal burden and increased cleanliness of the legs.

Disease:

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

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

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

Clinical Mastitis:

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

208209

210

211

212

213

214

215

216

217

218

219

220

221

222

223

224

225

226

227

228

229

199

200

201

202

203

204

205

206

207

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

230231

232

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

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

252253

254

255

256

257

258

259

260

261

262

263

264

233

234

235

236

237

238

239

240

241

242

243

244

245

246

247

248

249

250

251

Somatic Cell Count:

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

265266

Bovine Tuberculosis:

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

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

Digital Dermatitis:

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

308 309

310

311

312

313

314

315

316

317

318

319

320

321

322

323

324

325

326

327

301

302

303

304

305

306

307

Antibiotic resistance:

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

328329

330

331

332

333

334

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

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.

340341

342

343

344

345

346

347

348

349

350

351

352

353

354

355

356

357

358

359

360

361

362

363

364

365

366

335

336

337

338

339

Attempts to reduce bacterial load within the recycled manure solids:

There is an evident element of risk when using recycled manure solids as a bedding material. Smith and Hogan (2006) stated that when using recycled manure solids there was a "significant risk factor for exposure to environmental pathogens that cause mastitis in dairy herds" but also commented that this was the case when using most other commercial organic bedding materials too. Harrison et al (2008) went on to discover that the management of the bedding is more crucial to controlling microbial populations compared to what bedding material is used in the first place. Management of bedding particularly the use of conditioners and changing bedding frequency has been investigated in a number of studies. 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 study was conducted which compared levels of common environmental mastitis pathogens in recycled manure treated with a commercial bedding conditioner containing 93% sodium hydrosulphate, to bedding which received no treatment and remained as a control. The results 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 more effective in recycled manure solids and had a more profound effect on the microbial population. Gram negative bacterial and streptococcal counts were reduced in recycled manure 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. The antibacterial activity of the conditioner was found to be related to the pH of the bedding and 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.

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

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

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

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

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

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

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

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

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

501 Bibliography

502

- 503 Ball, D., 2016. *AHDB Dairy*. [Online]
- 504 Available at: https://dairy.ahdb.org.uk/technical-services/knowledge-exchange-team/technical-
- 505 knowledge-exchange-team/#.WNOWFBicZAY
- 506 [Accessed 23 March 2017].

507

Bishop, J. R. et al., 1981. Dairy Waste Solids as a Possible Source of Bedding. *Journal of Dairy Science*, April, 64(4), pp. 706-711.

510

Bonhotal, J. M. S. a. S. M. S., 2011. How Mycobacterium avium paratuberculosis is affected by the composting process.. *Trends in Animal and Veterinary Sciences*, 2(1), pp. 5-10.

513

Bradley, A. J. et al., 2014. *Scoping Study on the Potential Risks (and Benefits) of using Recycled Manure Solids as Bedding for Dairy Cattle*, Warwickshire: DairyCo.

516

517 Carroll, E. J. & Jasper, D. E., 1978. Distribution of Enterobacteriaceae in Recycled Manure Bedding on Calfornia Dairies. *Journal of Dairy Science*, October, 61(10), pp. 1498-1508.

519

Cole, K. J. & Hogan, J. S., 2016. Short communication: Environmental mastitis pathogen counts in
 freestalls bedded with composted and fresh manure solids. *Journal of Dairy Science,* February, 99(2),
 pp. 1501-1505.

523

Driehuis, F., Lucas-van den Bos, E. & Wells-Bennik, M. H., 2012. Risks of the use of cattle manure
 solids as bedding material for milk quality: Bacillus cereus and butyric acid bacteria spores, Ede: NIZO
 Food Research BV.

527

Evans, N. J. et al., 2010. Characterisation of Novel Bovine Gastrointestinal Tract Treponema Isolates
 and Comparison with Bovine Digital Dermatitis Treponemes. *Applied and Environmental Microbiology*, 77(1), pp. 138-147.

531

Godden, S. et al., 2008. Ability of organic and inorganic bedding materials to promote growth of environmental bacteria.. *Journal of Dairy Science*, January, 91(1), pp. 151-159.

534

Gooch, R. C., Hogan, J., Glazier, N. & Noble, R., 2006. *Use of post-digested separated manure solids* as freestall bedding: a case study. New York, Cornell University, pp. 151-160.

537

Grewal, K. S., Rajeev, S., Sreevatsan, S. & Michel, F. C., 2006. Persistence of Mycobacterium avium
 subsp. paratuberculosis and Other Zoonotic Pathogens during Simulated Composting, Manure
 Packing, and Liquid Storage of Dairy Manure. *Applied and Environmental Microbiology*, January,
 72(1), pp. 565-574.

542

Harrison, E., Bonhotal, J. & Schwarz, M., 2008. *Using Manure Solids as Bedding- Final Report,* New
 York: Cornell Waste Managment Institute.

545

Hippen, A., Garcia, A. D., Hammink, W. & Smith, L. J., 2007. Comfort and Hygiene of Dairy Cows Lying
 on Bedding Limestone vs. Separated Solids, Michigan: American Society of Agricultural and Biological
 Engineers.

Hogan, J. S., Wolf, S. L. & Petersson-Wolfe, C. S., 2007. Bacterial Counts in Organic Materials Used as
 Free-Stall Bedding Following Treatment with a Commercial Conditioner. *Journal of Dairy Science*,
 90(2), pp. 1058-1062.

553

Husfeldt, A. W. & Endres, M. I., 2012. Association between stall surface and some animal welfare measurements in freestall dairy herds using recycled manure solids for bedding.. *Journal of Dairy Science*, October, Volume 95, pp. 5626-5634.

557

Husfeldt, A. W., Endres, M. I., Salfer, J. A. & Janni, K. A., 2012. Management and characteristics of recycled manure solids used for bedding in Midwest freestall dairy herds.. *Journal of Dairy Science*, April, 95(4), pp. 2195-2203.

561

Leach, K. A. et al., 2015. Recycling manure as cow bedding: Potential benefits and risks for UK dairy farms. *The Veterinary Journal*, November, 206(2), pp. 123-130.

564

Lowe, J. L. et al., 2015. Effect of alley-floor scraping frequency on Escherichia coli, Klebsiella species,
 environmental Streptococcus species, and coliform counts. *The Professional Animal Scientis*, June,
 31(3), pp. 284-289.

568

Menzies, F. D. & Neill, S. D., 2000. Cattle-to-Catte Transmission of Bovine Tuberculosis. *The Veterinary Journal*, September, 160(2), pp. 92-106.

571

572 Misselbrook, T. H. & Powell, J. M., 2005. Influence of Bedding Material on Ammonia Emissions from Cattle Excreta. *Journal of Dairy Science*, December, 88(12), pp. 4304-4312.

574

575 Moher, D., Liberati, A., Tetzlaff, J. & Altman, D. G., 2009. Preferred Reporting Items for Systematic 576 Reviews and Meta-Analyses. The PRISMA Statement. *Open Medicine*, 3(3), pp. 123-130.

577

Neill, S. D., Hanna, J., O'Brien, J. J. & McCracken, R. M., 1988. Excretion of Mycobacterium bovis by experimentally infected cattle.. *The Veterinary Record*, 123(13), pp. 340-343.

580

Phillips, C. J., Foster, C. R., Morris, P. A. & Teverson, R., 2003. The transmission of Mycobacterium bovis infection to cattle. *Research in Veterinary Science*, 74(1), pp. 1-15.

583 584

Rowbotham, R. F. & Ruegg, P. L., 2015. Association of bedding types with managament practices and indicators of milk quality on larger Wisconsin dairy farms.. *Journal of Dairy Science*, November, Volume 98, pp. 7865-7885.

586 587

585

Rowbotham, R. F. & Ruegg, P. L., 2016a. Associations of selected bedding types with incidence rates of subclinical and clinical mastitis in primiparous Holstein dairy cows. *Journal of Dairy Science*, June, 99(6), pp. 4707-4717.

591

Rowbotham, R. F. & Ruegg, P. L., 2016b. Bacterial counts on teat skin and in new sand, recycled sand, and recycled manure solids used as bedding in freestalls.. *Journal of Dairy Science,* August, 99(8), pp. 6594-6608.

595

Selvam, A. & Wong, J. W., 2017. Degradation of Antibiotics in Livestock Manure During Composting.
In: M. G. D. A. P. Christian Larroche, ed. *Current Developments in Biotechnology and Bioengineering*.
London: Elsevier, pp. 267-292.

600 Sharma, R. et al., 2009. Selected antimicrobial resistance during composting of manure from cattle 601 administered sub-therapeutic antimicrobials. Journal of Environmental Quality, February, 38(6), pp. 602 567-75. 603 604 Smith, K. L. & Hogan, J. S., 2006. Bedding Counts in Manure Solids. Tampa, National Mastitis Council, 605 pp. 161-167. 606 607 Sorter, D. E., Kester, H. J. & Hogan, J. S., 2014. Short communication: Bacterial counts in recycled 608 manure solids bedding replaced daily or deep packed in freestalls.. Journal of Dairy Science, May, 609 97(5), pp. 2965-2968. 610 611 Sprecher, D. J., Hostetler, D. E. & Kaneene , J. B., 1997. Locomotion Scoring of Dairy Cattle. [Online] 612 Available at: http://www.zinpro.com/lameness/dairy/locomotion-scoring 613 [Accessed 18 April 2017]. 614 615 Stokes, J. E., 2011. Investigating novel and existing methods of preventing, detecting and treating 616 digital dermatitis in cattle. Bristol: The University of Bristol. 617 618 Timms, L. L., 2008. Characteristics and Use of Separated Manure Solids (following composting) For 619 Dairy Freestall Bedding, and Effects on Animal Health and Performance in a Iowa Dairy Herd, Iowa: 620 Animal Industry Report. 621

https://assurance.redtractor.org.uk/contentfiles/Farmers-5409.pdf? =636262273844028704

622