RESEARCH REPORT

THE SUITABILITY OF COW MANURE AS BEDDING IN HORSE STABLES

by
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A Bachelor Thesis Report
Submitted to the Department of Business Administration and Agribusiness
AERES University of Applied Sciences
In Partial Fulfilment of the Degree
International Equine Business Management
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Wehl, January 15, 2024

Preface

The idea for this study arose out of my own need.

I have always been fascinated by horses and almost my entire life has revolved around them since early childhood. So when I was accepted to start my studies International Equine Business Management at the Aeres University of Applied Sciences in 2019, it was a great step for me to combine both hobby and knowledge. During this time, I have gained a lot of new knowledge and have also continued my education in other areas. I have always been very interested in the health and keeping of horses and when I got the chance to keep my horses myself the way I wanted to, I tried to put my knowledge into practice as much as possible.

Now it turned out that my mare was always coughing more despite all possible measures and aids. A bronchoscopy in the clinic revealed slight (stage 1) inflammation of the airways. The mare was put on medication, inhaled, hay is washed, and the stall is ventilated and they do not stand indoors for more than 7 hours. In consultation with the vet, the only way to improve the situation was to change the horse's bedding from straw to something else.

After some research I got nowhere, as all the bedding materials did not suit me due to various points. Whether it was the enormous dust formation with wood shavings or linen straw, or the mould with organic compost, the storage possibilities or the enormous prices with pellets, and also the spreading on the field, the manure removal, became more difficult with every other type of bedding straw. Since the cows have separated manure in their boxes, I suddenly had the idea of whether this would not be an alternative for horses, as I could imagine this in theory. The price is lower, the dust is less visible to the bare eye, manure removal should not be a problem for my idea as no substances are used that decompose slowly or are harmful to the environment. So I decided to take a closer look at weather separated manure could be an idea for box bedding for horses. Mari van Barneveld, my advisor for the bachelor thesis, supported me in my idea.

I am curious whether the results can play an important role in the treatment of horses with lung disease and also with regard to sustainability in horse husbandry.

The study started in August 2023 in the Netherlands and ended on 10th of January 2024.

Marnie Wilhelm

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Glossary

C:	carbon to nitrogen ratio
Cfu:	colony forming units
Cfu/g:	colony-forming unit per gram
Cfu/ml:	colony-forming unit per millilitre
cm:	centimetre
C/N ratio:	Carbon-to-nitrogen ratio
E. coli:	Escherichia coli
g:	grams
i.e.:	that is
l:	litre
Log:	Log is a mathematical expression, which we use in this case to be able to easily compare two large natural numbers. We assume a base of 10, which means whenever we write Log, it is short for Log_{10}.
2 Log:	100 to 999 cfu This expresses that 2 Log represents the values between 100 and 999 cfu. This means in detail, that values between those two numbers, taken to Log, result in a value of 2
m:	meter
m^2:	square metres
m^3:	cubic metres
Marktplaats:	sales platform in the Netherlands
N:	nitrogen
N_2O:	nitrous oxide
pH value:	usually given as 0 to 14; between 0-6 it is an acid, 7 is neutral and 8 to 14 is a base
PM:	categorises dust into particle sizes
ppm:	parts per million
RVO:	Rijksdienst voor Ondernemend Nederland
UVDL:	Universitair Veterinair Diagnostisch Laboratorium
WBVR:	Wageningen Bioveterinary Research
µg/m^3:	micrometres per cubic metres

Summary

The topic of this bachelor's thesis is an investigation of the manure solids produced during the separation of cow manure for suitability as bedding in horse stalls. The aim is to show the aspects of this material that influence the health of horses, but also with regard to the sustainability aspect and cost reduction. There is a wide variety of bedding materials in the equine sector, all of which have different strengths and weaknesses. Not only do the different properties have a major impact on horse health, such as on the respiratory tract, but the call for sustainability in equestrian sport is also growing and the procurement and quantity of bedding in particular have a major influence on this. In addition, purchasing costs are rising due to various factors, such as the harvest yields of straw, for example. A by-product of manure separation is manure solids, which is sometimes used as bedding for cows. However, the main focus is on reducing the quantity of manure and the rapid availability of nitrogen in the liquid manure. As a result, the solids are often not used, accumulate as a waste product and have to be disposed of for a fee. This is where this study comes in to investigate suitability as a bedding material for horses.

After an introduction to the topic of bedding for horses, existing problems and an explanation of separated manure and its use, the reader will find the approach and detailed questions in the second chapter. On the one hand, quantitative laboratory tests are used to investigate dust formation, the bacteria contained and liquid absorption, but also qualitative and quantitative methods are used to address sustainability and the costs associated with the material. In Chapter 3, the results are explained and illustrated using tables and graphs to provide an insight into the most important information obtained. Based on the results, the material is particularly low in dust and absorbs many times its own mass of water. The manure solids are sustainable due to their further use as bedding, as well as the other possibilities for using the manure produced. The amount of manure is significantly reduced with this bedding.

Building on this, the results obtained are critically assessed in chapter 4 and their relevance for the target group is clarified. The sustainability aspect as well as the low-dust properties are very interesting for horse owners and could arouse interest as a bedding material. The final 5th chapter draws conclusions from the study and makes a recommendation for action. The material can be an option for use as bedding in horse stalls but should only be used with caution and not for animals with weak immune systems. Moreover it should only be recommended after further research into the development of bacteria in a horse stall. In horse keeping and equestrian sport not only low-dust bedding to protect the horse's sensitive lungs, as well as cost savings are increasingly, sustainability in particular is also becoming an ever-increasing topic, which will also include the bedding materials in future, as well as the reuse of the manure produced.

Summary in Dutch

Het onderwerp van deze bachelorscriptie is een onderzoek naar de geschiktheid van meststoffen die vrijkomen bij het scheiden van drijfmest van koeien, als stalbedekking voor paarden. Het doel was om de aspecten van dit materiaal te laten zien die van invloed zijn op de gezondheid van paarden, maar ook met betrekking tot het duurzaamheidsaspect en kostenreductie. Er is een grote aanbod aan stalbedekkingsmaterialen in de paardensector, die allemaal verschillende voor- en nadelen hebben. Niet alleen hebben de verschillende eigenschappen een grote invloed op de gezondheid van paarden, zoals de luchtwegen, maar ook de vraag om duurzaamheid in de paardensport neemt toe en met name de inkoop en de hoeveelheid stalstrooisel hebben hier een grote invloed op. Daarnaast stijgen de inkoopkosten door verschillende factoren, zoals bijvoorbeeld de oogstopbrengst van stro. Een bijproduct van mestscheiding is vaste mest, die soms wordt gebruikt als strooisel voor koeien. De belangrijkste focus ligt echter op het verminderen van de hoeveelheid mest en de snelle beschikbaarheid van stikstof in de vloeibare mest. Als gevolg daarvan worden de vaste stoffen vaak niet benut, hopen ze zich op als afvalproduct en moeten ze tegen betaling worden afgevoerd. In deze studie wordt de geschiktheid als stalstrooisel voor paarden onderzocht.

Na een inleiding op het onderwerp stalstrooisel voor paarden, bestaande problemen en een uitleg over gescheiden mest en het gebruik ervan, vindt de lezer de aanpak en gedetailleerde vragen in het tweede hoofdstuk. Aan de ene kant worden kwantitatieve laboratoriumtests gebruikt om de stofvorming, de aanwezige bacteriën en de vloeistofopname te analyseren, maar ook de duurzaamheid en kosten van het materiaal komen aan bod met behulp van kwalitatieve en kwantitatieve methoden. In hoofdstuk 3 worden de resultaten toegelicht en geïllustreerd met tabellen en grafieken om inzicht te geven in de belangrijkste verkregen informatie. Op basis van de resultaten is het materiaal bijzonder stofarm en absorbeert het vele malen zijn eigen massa aan water. De vaste mest is duurzaam door het hergebruik als strooisel en door de andere mogelijkheden om de geproduceerde mest te gebruiken. De hoeveelheid mest wordt aanzienlijk verminderd met dit strooisel.

Hierop voortbouwend worden de verkregen resultaten in hoofdstuk vier kritisch beoordeeld en hun relevantie voor de doelgroep verduidelijkt. Het duurzaamheidsaspect en de stofarme eigenschappen zijn zeer interessant voor paardeneigenaren en zouden interesse kunnen wekken als stalstrooisel. Het vijfde hoofdstuk trekt conclusies uit het onderzoek en doet een aanbeveling voor actie. Het materiaal kan een optie zijn voor gebruik als bodembedekking in paardenboxen, maar moet alleen met voorzichtigheid worden gebruikt en niet bij dieren met een zwak immuunsysteem, en moet alleen worden aanbevolen na verder onderzoek naar de ontwikkeling van bacteriën in een paardenbox. Niet alleen stofarme bodembedekking om de gevoelige longen van het paard te beschermen en kostenbesparingen worden steeds vaker genoemd in de paardensport, ook duurzaamheid in het bijzonder zal een steeds groter onderwerp worden, waarbij ook de bodembedekkingsmaterialen en het hergebruik van de geproduceerde mest aan bod zullen komen.

Chapter 1 - Introduction

Research Topic

Over the last decades, the best-known bedding straw has been joined by many other bedding options. The market is constantly evolving, this is of course due to the general development of testing possibilities, but also due to the question of cheaper and continuously available material.

The bedding for a horse box must take many factors into account. The horse has both its toilet and its bed within this space, sometimes even his food. That means that the bedding must absorb everything the horse excretes and at the same time provide a good climate in the box and comfort for sleeping. There are many factors that have an effect on the ammonia and dust in the stables. So for example even with low-dust materials, attention must be paid that ammonia can be bound. Straw is still the number one most commonly used bedding, but the question arises as to whether this is still justified in view of the advantages and disadvantages of straw. Growing number of increasing lung diseases of horses. As a result, many stables are rethinking their approach and looking for other materials for horse boxes. (Koch, 2023)

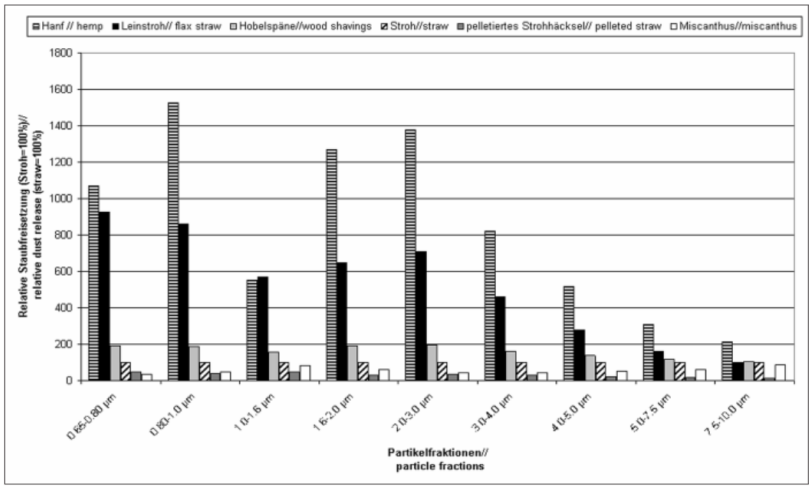
Also owners are searching for different types of bedding than straw because of various kinds of allergies or also comfort for the horse and in combination with comfort for the horse and taking a handable and also approprimate cleaning into account. Approximately 30% of unusable sport horses in Germany are attributed to chronic respiratory diseases (Schlichting, 2001).

According to a study by Salzbrunn (2005), respiratory diseases are considered the second most common cause of death in insured horses, as the respiratory system of horses is particularly sensitive to dust and noxious gases (Holcombe et al., 2001; Malikides and Hodgsen, 2003). The most common bedding material is still straw, resulting in the fact that it is not surprising that increasingly horses have respiratory problems (Borowicz et al., 2016) and horse owners are looking for a different type of box bedding than straw. Wood shavings, straw pellets or even hemp are used as alternatives. But here too, opinions differ as to which is the best alternative and different studies show different results. It is explained that hemp litter is absolutely dust-free and free of insecticides and pesticides in contrast to conventional straw litter. However, there is a risk that horses are tempted to try the litter because of its structure. To counteract this, eucaliptus oil is often used (Joschko, 2022). This ensures that the bedding is not tasty for the horse so there is less chance that the horse will try to eat it up. The opposite side is that the oil can contribute to an allergic reaction if used too much or if the horse is sensitive. So, the use of an oil is not necessarily a good option either.

Dust forming

Coming back to the importance of dust prevention and the dust generation of bedding types as already mentioned in the previous section, there are several studies. In the study *Staubfreisetzung von Einstreumaterialien in der Pferdehaltung* (Szabo et al., 2004) about the most commonly used beddings, the dust development was investigated with regard to the different particle sizes of the 6 most commonly used bedding products: straw, pelletised straw chaff, wood shavings, flax straw, hemp and miscanthus. Results showed that hemp, flax straw, shavings and straw released the most dust, whereas straw pellets and miscanthus had the least dust release.

Figure 1 Dust formation of different bedding sources in relation to straw taken over



(Szabo et al., 2004)

This is interesting to see, as also veterinarians often recommend flax straw or shavings as an alternative to straw for coughing horses. According to the study cited here, however, linseed straw produces 10 to 15 times more dust with smaller particles than straw. (Szabo et al., 2004) The research "*Generation of Airborne Particles from Different Bedding Materials Used for Horse Keeping"* investigated the particle sizes and proportions of wheat straw, dry wood shavings and wheat straw pellets as litter. In their study, the particle sizes are divided into different fractions in order to classify the extent of the size. The following applies <100 µm (micrograms) are inhalable particles that are inhaled through nostrils and mouth; 100 ≥ 10 µm are called extra thoracic particles, i.e. those that are inhaled but cannot travel further than the larynx; <10 µm are the tracheobronchial particles, which travel further than the larynx but not into the non-fibrillating area of the lungs (alveoli); and the last group is the thoracic particles <5 µm, which travel into the alveoli. Officially, the groups are roughly divided into coarse dust PM10 (<10 µm) and fine dust PM2.5 (<2.5 µm). The starting point of the assessment is the suspended particles from the environment abbreviated as TSP (Total Suspended Particles).

The average particle concentration of the litter materials varied continuously over the 14-day period, so no trend could be identified. However, the average particle concentration of wheat straw was significantly higher at 557.8 with a variation of +/- 31.7 µg /m^3 compared to wood shavings at 218.6 +/- 31.5 5 µg/m^3 or straw pellets at 185.9 +/- 38.5 5 µg/m^3.

According to Cargill (1999), respirable fractions = PM 10 are relevant to the health of horses. In larger quantities, such dust particles can have an allergising, infectious or toxic effect on the respiratory organs (Art et al., 2002). With heavy dust exposure, the self-cleaning mechanism of the bronchial tubes decreases, which means that there is no effective removal of the particles. This exposure causes non-specific inflammatory reactions in the tracheobronchial area (Deconto, 1983).

The bedding in the stable should of course not only be as dust-free as possible, but also bind the horse's urine as well as possible. This is especially important because ammonia not only smells unpleasant but is also harmful to the horse's health. It has keratolytic properties, which means that it dissolves and removes horny layers. It has an irritating effect on mucous membranes and eyes in particular (Hartung et al., 1990).

The guideline value in the horse stable should be 10 ppm, which corresponds to 6.96 mg/m^3. At a concentration of >30 ppm, ammonia can cause serious damage to the respiratory organs. (BMVEL, 2009). Furthermore, an increased concentration of ammonia in the environment has a negative effect on existing respiratory diseases (Clements & Pirie, 2007).

Absorption capacity and nitrogen absorption

"Einstreumaterialien in der Pferdehaltung" looked at litter volume and water binding capacity, daily bedding consumption and manure quantity, as well as the composting of bedding products mixed with manure and a substrate analysis of these. The results showed that fine wood shavings absorb water best in contrast to coarse wood shavings, straw, hemp and straw pellets. Straw and coarse shavings are excellent for bedding areas where a large area needs to be covered with a small amount. A mattress and thus a larger amount of litter should be used with fine shavings, straw pellets and hemp shavings.

Not only in cow husbandry, but also for horses, the nitrogen and phosphate content of manure is determined for a maximum of 350 kg per year and there is an obligation to record the quantity and removal in written form.

Tabel 1 Animal-related standards

ANIMAL CATEGORY	KG NITROGEN PER YEAR	KG PHOSPHATE PER YEAR
PONY	27,3	13
HORSE	58,8	28,6

(Rijksdienst voor Ondernemend Nederland, 2024)

At the moment there are no restrictions on how many horses can be kept in view of the CO_2 problem in the Netherlands. The question is, however, whether this will still happen. The following studies deal with the issue of manure, its removal and alternatives. (Fourages, 2023)

Forced aeration composting meant that in the case of straw manure, the wide C/N ratio of 50:1 and good carbon availability meant that atmospheric nitrogen could be microbially fixed. (Häußermann et al., 2002) This is interesting to see in the context regarding the nitrogen problems worldwide, but especially the nitrogen discussion in the Netherlands. Furthermore, if one looks at the sustainability of the bedding materials, there are big differences. Production, use, disposal, all those have an influence on sustainability. Reusement plays a big role, and it shows that the problem is that more and more manure, especially with other types of bedding than straw, is no longer readily collected and cannot be reused. It has become more difficult to get rid of it and in terms of sustainability, horse manure is often not the best solution either. Too much bedding is thrown away that could still be reused. Also, many bedding products are only produced for this purpose and also cannot be used again. This is not particularly helpful in the current situation we find ourselves in.

Problems surrounding horse manure

"*Pferdemist - Problemlösung durch mechanische Aufbereitung, Kompostierung und thermische Verwertung"* (Beck, 2005) examines the possibility of reducing the volume of manure in the different methods of shredding, but also looks at the current prices of bedding. We are experiencing more and more climate change in recent years. This of course has an importing impact on the availability and price of the bedding product. Some products are no longer available, others are partly not available for quite some time and, as in other areas, prices have also increased significantly. Both the reduction of the volume and the costs for commercial disposal should be reduced.

The bedding materials studied were straw (four different types), wood (five different types) and hemp and flax bedding (three different sorts). According to the article, € 290 to € 510 are spent annually per horse on stable bedding, and € 195 to € 427 are spent annually per horse for disposal. This prices would result from the combination of increased bedding and a small amount of horse manure but the prices are additionally depending on the bedding type.

According to the results, it would be possible to reduce the problems of manure disposal and storage, which will become even more acute in the future, by means of mechanical shredding. On the one hand, it makes it possible to reduce the volume of the manure, which facilitates transport and storage, plus reduces the costs for transport. Furthermore, unwanted by-products such as straw or bedding materials can be sorted out. This improves the quality of the end product and makes it easier to utilise. Due to a lower content of undesirable substances, the horse manure can be used as fertiliser, for example. Another advantage of mechanical processing is that shredding the manure enables faster and more efficient composting. This accelerates the decomposition of organic substances and produces valuable compost that can be used as a soil conditioner. In addition to composting, mechanically processed horse manure can also be utilised thermally. Energy can be generated through incineration or gasification, thus reducing the ecological footprint. What is relevant for this topic is that the volume of bedding and manure costs was around € 500 to € 1,000 horse per year, which could perhaps be reduced by other bedding options since the quantity and the removal could be smaller and more effective (Beck, 2005). This article by Becks is from 2005 and in the last 5 years, prices have fluctuated again and again due to, for example, poorer harvests caused by extreme weather conditions and also rising costs such as for fertiliser or energy, with a tendency towards higher prices (“Stroh in Zeiten Der Dürre: Das Macht Den Preis 2022 | agrarheute.com,” 2022).

Other options for bedding in horse boxes

In a study of Seedorf et al. (2007) a rather uncommon material in horse stalls was investigated with bio-compost bedding. The results did not show any negative health signs that could have been caused by exposure to the bio-compost material. (Seedorf et al., 2007) The step towards more sustainable bedding materials has been taken. In Germany, forest soil is increasingly being used as bedding, especially in open stables. Because it is said to have a good climate due to the bacteria it contains, it saves time when mucking out plus forms a good mattress so that the horses are happy to lie down. At the same time, it does not take up much storage space as opposed to straw.

The deeper you delve into the matter, the clearer it becomes that there are actually multiple bedding materials, but their use is limited by different factors. Does the horse tolerate the bedding, is there no incompatibility like allergies to it, what is the horse's lying behaviour, what are the conditions in the stable, how often is it mucked out. But certainly the question is, what are the storage options and how can the material be stored. How does the product have to be available, directly or for a longer period of time? Does the price make a difference, and shouldn't more attention be paid to the sustainability of the bedding?

It seems that many questions are still unanswered and if we look at literature sources, we see that different recommendations are made depending on the focus. Roughly speaking, however, it can be said that so far no perfect bedding material can be recommended and certainly not with regard to dust allergy sufferers.

Bedding in cow boxes

Picture 1 Separated cow manure in a cow box

In the case of cows, the traditional bedding materials such as straw, sand or wood shavings have been joined over time by separated manure. This type of bedding has been studied for cows with regard to various factors. As with horses, there are different types of housing for cows, but the most common is that the cows are kept in stalls where they can move freely and decide when to eat, drink, lie down and socialise.

The barns are constructed in such a way that the floor allows manure and urine to be drained into a room under the barn. A cow does not normally urinate and defecate in her box, but on the floor. A clean and comfortable lying area is important for well-being, health and milk production. A dry lying area helps to maintain hygiene in the stalls. Damp or dirty lying surfaces can encourage the growth of bacteria and fungi, which in turn can lead to infectious diseases.

Clean and dry lying areas also make it easier to maintain and clean the stable. Bedding plays a major role here, as does checking and cleaning the lying areas to ensure the health and welfare of the cows.

Separated cow manure

But what is separated cow manure? As the name suggests, separated manure is obtained from cow dung. The manure is collected, and the solid components are separated from the liquid using a press. The solid components serve as bedding in the stalls. This process is used for various reasons. Cow manure has high absorbency, which makes it a suitable material for absorbing moisture and keeping the bedding dry. The absorbent nature of cow manure helps to prevent the growth of bacteria and fungi, reducing the risk of infections or diseases in animals.

Further cow manure provides good insulation, helping to maintain a comfortable temperature for the animals. It acts as a natural regulator, retaining body heat and providing warmth in colder climates. Utilizing cow manure as bedding material can be cost-effective, as it is readily available on farms and does not require additional expenses to procure bedding from external sources. However, it should be noted that the purchase of such a press is very expensive, with prices ranging from €20,000 to €30,000 depending on the design and manufacturer.

Using separate cow manure as bedding helps in waste management within the farm. It is a sustainable approach to recycle and reuse the cow manure, reducing its disposal as waste and minimizing the environmental impact. It provides a soft and comfortable bedding surface for the animals, promoting better rest and sleep, which is crucial for their overall health and productivity. Another advantage of this separation is that less ammonia is released, and the thin manure can be applied in the field in a more targeted manner.

However, it is important to note that cow manure used as bedding should be properly managed and regularly replaced to ensure hygiene and prevent odours.

Good management practices, such as proper ventilation and regular cleaning, should be followed to maintain a healthy and clean environment for the animals. (Van Den Broek, 2010)

Cow manure in horse boxes

But isn't it now also an idea to use this type of bedding for horses? In the following sections, various studies on separated manure as bedding for cows will be used to illustrate the state of knowledge.

For cows, in addition to the usual bedding materials, separated manure is also being investigated again and again as a bedding material, with all its advantages and disadvantages.

Farmers are increasingly deciding to use separated manure in the stalls. This is a cost-effective alternative to using the separated manure as bedding and spreading it on the land at the same time. Nonetheless, the purchase price of such a manure separator must first be earned back. This fact can be offset against alternative bedding material that that is necessarily paid for. Under certain conditions, such as regular spreading of the litter so that it can dry, the bacteria density is stable and udder health is not necessarily affected.

Such are properties and necessities that are also interesting for a horse box. It is particularly absorbent, forms a mattress, is easily deformable and resilient. Nevertheless, it is not recommended to use the litter for sick or immunocompromised animals such as calves. (Bedding alternative: separated manure | Chamber of Agriculture Upper Austria, n.d.)

Bacteria content in separated cow manure

In another study of the use of separated cow manure, the number of bacteria contained in the bedding was compared with the number that multiplied during the use of the litter types such as straw, sawdust, compost and manure solids. It was found that the separated manure already contained a number of bacteria such as streptococci before use, but the other bedding materials reached the same number after only a short period of use. In contrast to the other bedding materials, the number of streptococci remained the same and did not increase. E-coli bacteria, however, were found in higher numbers in the unused and used type. (Top Agrar Online, 2018) This raises the questions 'How high is the bacterial concentration in horse boxes with what kind of bedding? Which bacteria in what quantity is dangerous for horses or has an influence on their health?'.

According to a study of Masterhorse, unused samples were tested under laboratory conditions for bacterial growth, pH, total carbon (C) content and total nitrogen (N) content. Clean sand, recycled sand, digested manure solids and wood shavings were sampled. On the digested manure solids, the bacterial population of Klebsiella pneumoniae increased the most. The bacterium also belongs to some extent to the horse's digestive tract, but it can appear as a pathogen in other parts of the body. This bacterium can be the cause of pneumonia in many cases. Therefore, the intake and the number of this bacterium in the cow dung should be clarified before the horse comes into contact with it (Masterhorse, n.d.). With a high pH, the manure also promoted the growth of Enterococcus faecium, but only in small amounts. This bacterium, Enterococcus faecium, is found in both the cow and the horse intestine, but an excessive number of these pathogens can lead to various clinical pictures such as heart muscle inflammation, an inflammation of the bile duct or even bladder inflammation (Saalschmidt, 2017). In addition, the manure solids also have a high C content, from which it can be inferred that this probably supports bacterial growth of K. pneumoniae. (Godden et al., 2008) By conducting such studies, it is known which bacteria thrive on which litter.

Still, are these groups of bacteria also harmful to horses, and if so, in what quantity, or do not other bedding materials that are frequently used also promote such growth?

Theoretical Framework and Knowledge Gap

Theoretical Framework

Theoretical Framework

At the moment it is known that there are various types of bedding, most of which have already been investigated with regard to costs of purchase and removal, as well as time for mucking out, lying comfort of the horses and also health aspects such as dust development and bacteria formation. It turns out that, for example, straw pellets, hemp or compost seem to be suitable with regard to dust formation. Miscanthus also seems to be very suitable for coughing horses due to its low dust development in the different particle sizes. (Szabo et al., 2004) There are various studies from which it can be deduced that some bedding materials are better than others, however, depending on the method of investigation, different results are obtained. If one looks at the studies listed below, it becomes clear that there are many preliminary studies with regard to the different types of bedding and its advantages and disadvantages.

The bedding materials wheat straw, hemp, straw pellets, shavings, linen straw and paper were investigated with regard to their suspended dust development, emission behaviour and ammonia compound potential. The C and N content and the C/N ratio, as well as the particle size, the water absorption capacity, the fungal and bacterial content, and the number of microorganisms contained in the source materials were also determined. The results show that the release of suspended particles is highest in hemp and flax straw than in the other materials examined. According to this study, ammonia binding is best in straw pellets. However, the N_2O load here is significantly higher than in the other types of bedding investigated.

Mechanical processing seems to increase the microbial vulnerability, that highlighted another study. It observed an increase in bacterial activity and heat development, although the potential harmfulness for horses was not assessed. In contrast to a previous study, hemp did not perform exceptionally well, and straw pellets showed less reduction in dust along with better urine absorption. However, the N_2O load was significantly higher with straw pellets compared to other types of bedding. The study also noted an increase in bacteria, although the specific types were not discussed. (Fleming et al., 2007)

Moreover, a separate investigation explored three litter materials: straw (S), peat with shavings (PS), and crushed wood pellets (CWP). It examined air pollution, dust formation, and microbial contamination, including bacteria and fungi. The results showed that PS exhibited the lowest mechanical dust contamination, while CWP had the highest. Bacterial contamination was measured in colony forming units (CFU), with crushed wood pellets performing the best and PS showing the highest bacterial development. Regarding fungal air contamination, CWP had the lowest levels, while straw and peat with shavings had higher levels.

In this study, mechanical dust contamination was lowest for PS at 1.09 mg/m^3 and highest for CWP at 4.07 mg/m^3. Bacterial contamination was measured in colony forming units (CFU). Here crushed wood pellets (4.81 log10 CFU/m^3) performed best, and bacterial development was highest in PS (5.14 log10 CFU/m^3). Fungal air contamination was lowest for CWP (4.54 log10 CFU/m^3) and highest for straw (4.82 log10 CFU/m^3) and peat with shavings (4.88 log10 CFU/m^3) (Kwiatkowska-Stenzel et al., 2017).

Tabel 2 Overview of study results of "The effect of stable bedding materials on dust levels, microbial air contamination and equine respiratory health"

	Bedding materials	**Wheat straw**	**Shavings**	**Crushed wood pellets**
Contents of the examination				
Dust development		2.43 mg/m³	1.09 mg/m³	4.07 mg/m³
Fungal content		4.82 log10 CFU/m³	4.88 log10 CFU/m³	4.54 log10 CFU/m³
Bacterial content measured in colony forming units (CFU)		-	5.14 log10 CFU/m3	4.81 log10 CFU/m³

(Kwiatkowska-Stenzel et al, 2007)

In summary, the studies mentioned highlight the varying suitability of different bedding materials based on factors such as dust formation, ammonia binding, microbial contamination, and air pollution.

As written in the previous paragraph, the concentration of harmful substances such as dust or bacterial and ammonia content is important for assessing suitability as bedding, and the following study "Suitability of biocompost as a bedding material for stabled horses: respiratory hygiene and management practicalities" (Seedorf et al., 2007) analyses this aspect with the content of NH_3, dust, endotoxins, and fungi in wood chips and bio compost, which are used as bedding in horse stalls.

The concentrations of NH_3, dust, endotoxins and fungi were significantly higher with wood chips than with bio compost during the study period. In contrast, the concentrations of mesophilic bacteria, mesophilic actinomycetes and thermophilic actinomycetes were highest with bio compost. Yet the water content of loose bio compost was significantly higher than that of wood chips. This means that the uptake of liquids is lower. Particles with a size of ≤ 0.4 mm were not detectable in bulk wood chips. The concentration of thermophilic actinomycetes was 639 times higher in raw bio compost than in raw wood chips. No significant differences were found with regard to the time in which the horses lay in their stable. The bio compost material tended to adhere more to the hair coat of the animals. The research revealed that the horses did not show any clinical signs indicating negative effects of the bio compost material during the trials. (Seedorf et al., 2007) This study is therefore not only concerned with the usual bedding material, but also investigates the possibility of spreading compost. With this material a step towards more sustainable litter materials is taken.

For sustainability, not only the material is important, but also how much manure is produced. Häußermann et al. (2002) looked at litter volume and water binding capacity, daily bedding consumption and manure quantity, as well as composting of litter products mixed with manure and a substrate analysis of these. The results showed that fine wood shavings absorb water best in contrast to coarse wood shavings, straw, hemp and straw pellets. Straw and coarse shavings are excellent for bedding areas where a large area needs to be covered with a small amount. A mattress and thus a larger amount of bedding should be used with fine shavings, straw pellets and hemp shavings. With regard to the amount of manure, fine shavings proved to be the winner with an average of only 5.3 kg of manure per horse per day.

For straw, this amount averaged 10.8 kg, but was closely followed by hemp with 10 kg, coarse shavings with 10.6 kg and wood pellets with 9 kg. In forced aeration composting, the straw manure showed an increase in absolute total nitrogen content of 13 % on average. According to the studies, the broad C/N ratio of 50:1 and the good carbon availability led to the microbial fixation of atmospheric nitrogen. (Häußermann et al., 2002)

The idea for using separated cow manure in the stable was already exposed in the introduction but is filled in by the following articles.

The possible alternative is examined with regard to its bacterial development and influence on the health of the cows. It deals with the frequency of pathogens in the litter and whether this can be reduced by treating the litter. It turns out that the pathogens responsible for mastitis, E. coli and Klebsiella ssp. were found above all. Warm, damp litter provides a pleasant environment for pathogens. Various measures, such as heating the separated manure and also different storage methods, should ensure that the number of bacteria decreases. In practice, however, it turned out that after a short time the number had increased again. The question that arises is whether the bacteria contained in the separated manure harm the horse and if so in what quantity. Furthermore, it was shown in practice and in studies that the udder health of cows in cubicles littered with separated manure did not differ significantly in comparison with other bedding. (Berkemeier, 2021)

The following article of "Landwirtschaftskammer Oberösterreich" (Einstreualternative: Separierte Gülle | Landwirtschaftskammer Oberösterreich, n.d.) discusses whether the bedding of separated manure is an alternative to other bedding methods such as sand or shavings in cubicles for cows. What was particularly striking in this article was that it basically is described as a good alternative, but certain conditions are attached to it. Such as that the dry matter of the separated manure should ideally be 32%, that there must be good ventilation in the barn in order to dry out the bedding again and again, that it should be regularly re-spread or that it should also be treated with chalk, if the pH value should be higher than 10. The question is, of course, to what extent this is required for horses.

The bedding is recommended, but not for sick animals and calves, as there is a higher risk of infection for these groups with the separated manure. Again, the question is whether and in what quantity the bacteria and germs contained are harmful to horses or a particular group of horses like foals, sick horses or pregnant mares.

Advice was given to keep the litter as good as possible so as not to endanger health with the following tips. According to recommendations from Germany for cow boxes, the organic bedding material in the udder area of a cow should reach a dry matter content of about 70 %. However, this requires appropriate ventilation of the stall. In practice, more attention should be paid to the following points:

- Dry matter content of the separated manure: at least 30 % (32 % is better)
- Sufficient stable ventilation: so that the bedding material can dry out
- Regularly (ideally every 12 to 24 hours) re-spread in thin layers
- Chalk if necessary (pH value >10)
- Paying attention to hygiene and biosecurity measures

Separated manure has a high germ content and also offers good growth conditions for various pathogens. In order to keep the germ content as low as possible, the material should therefore always be spread freshly. Furthermore, the moister the material, the higher the germ growth.

Therefore, the dry matter content should be at least 30 %. A dry matter content of approx. 30 to 35 % is suitable for the upper layer of the construction of a lying mattress - with higher dry matter contents it is difficult to form a firm lying mattress. The top layer of bedding should be as dry as possible. Dry matter contents of the upper layer of approx. 40 % should be the aim. In any case, the top layer should be dry in the boxes.

- The use in the special needs area and in sick pens is not recommended due to the increased risk of infection.
- Use in the calf or young cattle area is possible - but not advisable for calves due to a higher risk of disease transmission (e.g. Klebsiella, etc.).

(Einstreualternative: Separierte Gülle | Landwirtschaftskammer Oberösterreich, n.d.)

The researchers of the study *"'Gülle-Einstreu': Bleiben die Euter gesund ?"* (Top Agrar Online, 2018) investigated the germ load of bedding materials show of compost and manure solid. In order to determine the germ load before and after use, the scientists examined the bedding materials for streptococci, Klebsiae and E. Coli. In addition to compost, straw and manure solids, sawdust was also examined. In the unused state, sawdust and straw had the lowest germ contents. Compost and manure solids, on the other hand, had significantly higher total germination contents even in the starting material. Compared to sawdust, the content of manure solids was 125 times higher, compared to straw it was 15 times higher. In the used material, the germ contents also increase rapidly in straw and sawdust due to the input of faeces, urine and milk. Overall, an equally high level was thus reached. Looking at the individual germ groups, the scientists found high levels of streptococci in unused manure solid compared to the other materials. Nonetheless, the content remained relatively stable even after the material was used in the cubicles. In the case of compost and sawdust, on the other hand, the streptococci content exploded in the course of use. For Klebsiae, the highest levels were measured in the compost, both in the used and unused variant. The highest number of E. coli bacteria was found in the unused and used manure solids variant. (Top Agrar Online, 2018)

Knowledge gap

As described above, there have been extensive studies on almost all types of bedding materials, including dust development, bacteria formation, intake volume, muck quantity, sleeping behaviour, composting, etc. The most varied studies are available. However, opinions differ as to which type of bedding would be the most suitable for horses in view of rising prices and the limited availability of other bedding materials, as well as allergies and sensitivity to dust. Separated manure is always used more in cow stables than other beddings such as straw, as the advantages clearly outweigh the disadvantages. But until now this material has not yet been studied with the aspect of horses, such as harmful bacteria, dust release, absorption of fluids, composting in connection with horse manure, disposal and further use.

Delimitation

In this study of the use of separated cow manure is researched on the topics of: dust development, the bacteria contained, liquid absorption, sustainability of the material - moreover costs are discussed. At the end of the study, depending on the results, it is recommended or not recommended to carry out a further study.

Neither the opinion of horse owners on the use of separated manure is investigated as well as the will of cow farmers to sell it. If the study is positive, these above-mentioned issues could be clarified in a subsequent study. Due to the limitations of the study, the use for lactating mares will not be investigated.

Main question and sub-questions

Which features of separated cow manure make it an option to commercially available materials such as straw, straw pellets, flax straw and shavings for use as bedding in horse stalls?

1. What is the impact of separated cow manure as bedding on the health of horses?
2. What impact would this bedding material have on sustainability (in the Netherlands)?
3. What is the total cost of the bedding material?

Objectives

The aim of the study is to answer the main question through the following objectives. To find out whether separated manure can have positive or negative effects on the health of horses when used as bedding in horse stalls. To find out whether separated manure is a bedding alternative to conventional materials. To investigate whether this type of bedding would be a sustainable alternative to conventional bedding materials. To provide an overview of the costs associated with purchase, storage and disposal.

Chapter 2 – Material and methods

The following chapter describes the materials and methods that were used to write the bachelor thesis. The secondary research method, also called desk research, was used. At the beginning, research papers, studies, and other literature sources on the subject of box bedding for horses and also currently known research results on separated manure -as bedding in cow boxes- were reviewed. As a second method, an investigation was carried out in the laboratory on the bacteria contained and the development of bacteria, as well as the behaviour of horse manure in connection with separated manure.

The research question is "Which features of separated cow manure make it an option to commercially available materials such as straw, straw pellets, flax straw and shavings for use as bedding in horse stalls?".

The intention is to word the research topic out. For this purpose, the following parts will be investigated:

1. The impact on health of the horse
    a. Dust development
    b. Bacteria formation
    c. Fluid absorption
2. The impact on the sustainability
    a. Amount of bedding
    b. Amount of manure
    c. Disposal
3. The costs of separated manure in horse stables
    a. Costs of purchasing
    b. Costs of storage
    c. Cost of disposing of the material

Collection of data

The required data is collected using the following databases: GREEN-I, ScienceDirect, Google Scholar, JSTOR, PubMed, Springer and Tylor & Francis. Relevant scientific articles in books containing references, journals, technical papers, reports from research institutes and conference proceedings are searched for. Secondary sources are overall peer-reviewed references, such as journals, publications of governmental settings or even institutes. The search is based on various search terms such as "horse box bedding", "bedding material", "horse box bedding", "sustainability horses", "cost bedding", "health horse bedding", "horse bedding material", "horse bedding", "bedding material", "horse manure disposal", "separated manure", "cow box bedding", "cow manure", "solid cow manure", "solid manure bedding", "solid manure bacteria", "gescheiden mest koe", "gescheiden mest instrooi", "paardenbox instrooimateriaal", “bacteria horse manure”, “bacteria bedding horse”, “lying behaviour bedding”, “health bedding”, “air horse bedding”, “Pferdeboxeneinstreu”, “Bakterien Einstreu Pferdebox”, . This literature research will be carried out in the months of August 2023 until January 2024.

The laboratories Universitair Veterinair Diagnostisch Laboratorium (UVDL) Utrecht (*Universitair Veterinair Diagnostisch Laboratorium (UVDL)*, n.d.) and Wageningen Bioveterinary Research (WBVR) (*Contract Research Organisatie (CRO)*, n.d.-b) were involved in the examination of the samples for dust formation, bacteria, and fluid absorption.

Publications and other literary sources in languages other than German, Dutch and English are not used. Also, mainly literature younger than 20 years is used to illustrate the urgency of the topic and to use the latest state of knowledge.

Reliability

This study is both qualitative and quantitative. The information obtained from the literature sources used are evaluated qualitatively. The conclusions are reconstructed based on the indicated sources used. The examinations and tests that of the bacteria formation, dust formation and absorption of fluids which were carried out are quantitative in nature.

The bacterial content was determined by culturing on agar-based culture media. To do this, the test material was spread on the culture media and then incubated under controlled conditions. The colonies that formed were counted to determine the bacterial content.

To assess the dust formation the test material was dumped into a plastic box and then assessed using a dust collection device such as a dust meter. To determine the particle sizes, the test material was analysed using sieve analysis, the most commonly used method for particle measurement. The particles are separated in size by the filter in the corresponding size and the collected material then is weighed.

A common method for studying the uptake of liquid from materials is to immerse the test material in a precise amount of liquid and then measure the amount of liquid absorbed. To test how much liquid the separated manure can absorb, the test was set up as follows: The specific amount of solids were weighed, then a measured amount of liquid was slowly poured onto the liquid. The collected quantity was then measured. The amount of water collected was used to determine the amount absorbed by subtracting the water poured over it. This was done using a volumetric instrument.

The tests described for bacterial development, dust development and absorption of liquids were submitted to the certified laboratories Universitair Veterinair Diagnostisch Laboratorium (UVDL) Utrecht (*Universitair Veterinair Diagnostisch Laboratorium (UVDL)*, n.d.) and Wageningen Bioveterinary Research (WBVR) (*Contract Research Organisatie (CRO)*, n.d.-b) and carried out in co-operation with Bart van Heugten, veterinarian at Graafschap Dierenartsen, together.

It can be said that the results of the investigation are reproducible, which should not be equated with all results being valid. This can only be produced on the examinations and tests, the quantitative procedure.

Analysis

In order to obtain answers to sub-questions 1 to 3 and their sub-questions, the tests of the dust formation, contained bacteria and liquid absorption were conducted, and literary sources were also consulted. In co-operation with Bart van Heugten, veterinarian at Graafschap Dierenartsen, as well as the UVDL, the dust development and fluid absorption were tested. The bacteria contained were analysed by the WBVR. An attempt was made to combine the results of these sources of information and to form an overlapping answer. Sub-question 1 is primarily supported by the laboratory research.

The characteristic of the research is the investigation of health effects on the horse, sustainability and reduction of costs of bedding in horse stalls.

Evaluation criteria

All sources of information used are individually assessed for inaccuracies or confounding factors. The results of the analysed data and information are checked against various criteria. These criteria consist of relevance, efficiency, effectiveness, impact of the research and finally the sustainability of the research. Starting with relevance, how were the results relevant to the topic of the research and is it applicable. The criterion of efficiency is that the results provide clear answers to the main and sub-questions. The criterion effectiveness examines whether all the objectives mentioned in chapter 1 had been achieved. Sustainability of the research was the last criterion, and it is analysed whether the result of this research is beneficial and whether it is necessary to continue the research. The penultimate criterion indicates whether the results have a benefit for the equine sector and whether they are as expected.

Chapter 3 – Results

This chapter presents the results of the investigation and research. The sub-questions are answered with the research methods used. Various graphics are used to visualize the results and illustrate possible correlations. These graphics have all been specially created and marked accordingly.

3.1. Subquestion 1

What is the impact of separated cow manure as bedding on the health of horses?

In order to be able to answer this sub-question regarding the influence on horse health, the separated cow manure was analysed for various factors which have an effect on the health of horses when used as bedding:

a. Dust development
b. Bacteria formation
c. Liquid absorption

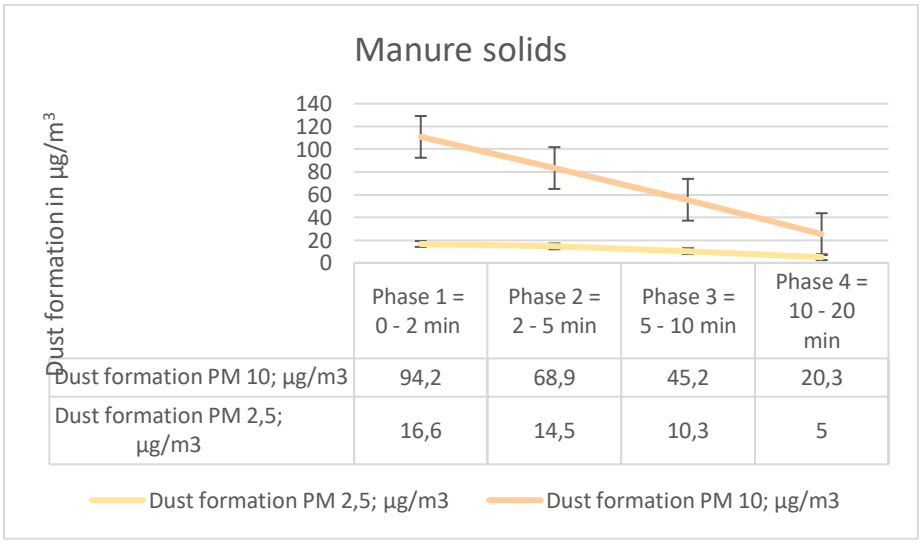
a. Dust development

The dust development was measured with a dust meter. For this purpose, the separated manure was placed in a plastic box and then measured using a dust sensor type SDS011 from Inovafit. The fine dust particles were measured using a fine dust laser, with continuous recording. In addition, the respective particle sizes were measured using filters and the number of particles was then determined based on the particles collected behind the filter.

The particle sizes <2mm and <0.84mm were determined as a percentage. The analysed samples yielded values between 31 and 74% below <2mm and 6 to 37% <0.64mm. The wide range between the results is due to the processing and the fact that it is a natural product that does not continuously have the same values. The dust development of PM 10 developed from 0-2 min with a value of 94.2 µg/m^3 to minute 10-20 to 20.3 µg/m^3. This is a reduction of -73.9 µg/m^3 within 8-18 min.

For the particle size of PM 2.5, the dust load in the air decreases from 16.6 µg/m^3 in minute 0-2 to 5 µg/m^3 in minute 10-20. This is also a reduction of -11.6 µg/m^3.

Figure 2 Dust formation of manure solids



It turns out that the dust particles that are in the air decrease significantly within the test phase of 20 minutes.

b. Bacteria formation

The bacterial concentration is given in log = colony forming units per millilitre (cfu/ml) of the litter. The bacteria were measured using a volume of 120ml, which corresponds to a weight of 40.6g. This results in cfu/ml = (cfu/g) * (weight of material/volume of material) of 169.250cfu/ml.

Tabel 3 Bacteria formation of manure solids

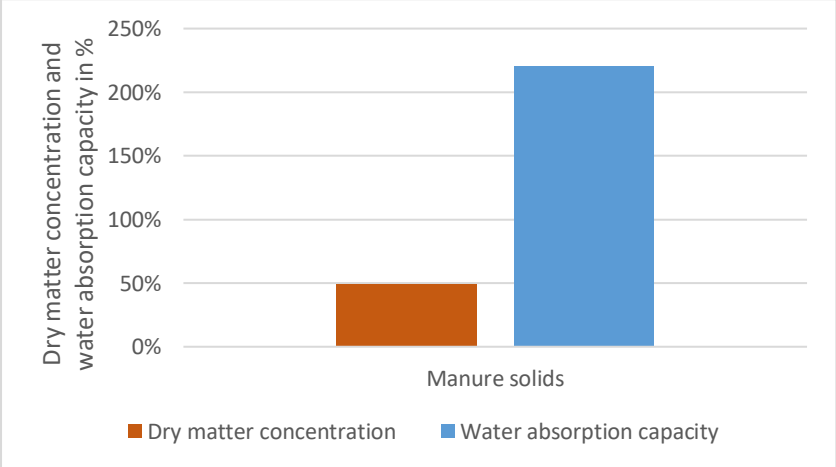
Bacteria	**2 log10 = cfu/ml**
Staph spp	0.8
Enterococcus	0.3
Enterobacter	0.2
Proteus	0.9
E. coli	0.7
Klebsiella	3.8
Streptococcus spp	9.9
Corynebacterium	3.7

The bacteria found are not particularly unusual for cow dung. The exact number of these bacteria in a horse box is difficult to determine as contamination depends on various factors. Regular cleaning and disinfection of the stall, good ventilation and hygiene measures such as the removal of waste products can help to keep the number of bacteria at a low level. However, it is important to note that the presence of some bacteria in low numbers is normal and not necessarily harmful to health. The bacteria mentioned above that are also normally found in a horse box are Streptococcus equi, E. coli, Staphylococcus and Enterococcus (Wolny-Koładka, 2018). In addition, mould spores were found in one of the 3 samples tested. However, it was not possible to reconstruct when these were created. Whether they were already present when the samples were taken or whether they were created during storage until the analysis, as the lowest sample was slightly moister than the average moisture content of the other samples.

c. Liquid absorption

The measured dry matter content of the substance, averaged from the samples, was 49%. The water absorption capacity of the fresh mass was 220% with a volume of 120 ml.

Figure 3 Dry matter concentration and water absoption capacity



Furthermore, the tests showed that a thicker layer dries worse than a thinner layer. The larger the surface area, the faster the bedding will dry.

It has not been tested how the material binds ammonia, but after studying the absorption capacity of liquids, it can be said that the urine released should be bound.

3.2 Subquestion 2

What impact would this bedding material have on sustainability (in the Netherlands)?

3.2.1 The impact on the sustainability

The results of the analysis of the moisture content, as well as the absorption capacity of liquids and dust formation, show that the manure solids can be compared in terms of the amount of bedding required and the manure produced.

a. Amount of bedding

The general guideline for bedding the box should be the lying area in m^2 x 0.2, as this gives the minimum volume of 20 cm in m^3 for this area. It is normal for the bedding to sink to around 5 cm after a few days. The time depends on the ambient humidity and the time the horse spends on the surface.

For a box with the standard dimensions of 3 x 3.5 metres, which corresponds to the 'Sectorraad Paard' recommendation of at least 10 m^2 for a horse, this would result in a basic bedding quantity of

1. 3 x 3.5 m = 10.5 m^2
2. 10.5 m^2 x 0.2 = 2.1 m^3
3. 2.1 m^3 ≈ 2,100 l

This 2,100 l, is equivalent to a mass of 525kg, should also be placed in the box to ensure that urine can be absorbed and a mattress can be created so that the horse can lie comfortably.

Less bedding can be used if only the horse's lying area is to be bedded, if the horse has the opportunity to excrete in another place, such as in a group stable. However, it should be ensured that there is always a layer of 15 – 20 cm thick bedding in the box to guarantee the above-mentioned characteristics.

b. Amount of manure

A horse excretes an average of 10 to 20 kilograms of manure and 5 to 10 litres of urine per day, depending on its size. At the beginning after the new bedding in the stalls, only a small amount of bedding is removed by mucking out the horse's droppings. The formation of a mattress after a few days reduces the amount of manure removed to the horse's faeces.

Soaked areas of urine should be removed and allowed to dry at the side. It is not necessary to completely remove the wet patches from the box, as the ammonia is bound and dries off.

The amount of manure produced in the box therefore depends on the size and feed intake of the horse, as well as the time spent in the box.

However, the amount of manure does not add up due to the additional amount of bedding material and is limited to the manure that is produced by the horse anyway.

It should be considered that the bedding should be completely replaced after some time. An absolute time limit cannot be given, as this depends on the conditions on site. With optimum humidity between 60 and 70% (Sectorraad Paarden, 2019) and daily removal of faeces, as well as the opportunity to dry off wet areas, it is possible that the bedding does not need to be replaced for up to six months. This is based on the study by Sorter et al. (2014), in which the development of bacteria in the box in the cowbarn was investigated over time, but it should be secured that in any case a weekly topping of separated manure should be added to ensure a sufficient amount and reduction of an infection by bacteria in the box. It can also be helpful to loosen the top layer with a hay fork, for example, to make it more comfortable for the horse.

c. disposal

As with all types of manure, the manure can be removed by various parties. In terms of sustainability, the manure can be used as fertiliser, in a biogas plant, for own use with the help of a fermenter for conversion into methane gas or, as in a pilot project of the Frauenhofer Institute for Environmental, Safety and Energy Technology, to produce CO_2-neutral electricity and heat.

The substances contained in horse manure, also in combination with cow manure, are of interest to various recipients. The mixture of horse manure with manure solids contains the nutrients and minerals listed below. Horse manure contains a natural proportion of ~ 1.2 to 1.8% nitrogen in fresh matter. Phosphorus, magnesium and calcium content are lower than in cattle manure, but horse manure contains more potassium. The ammonium content is also lower in horse manure. In the separated solids of the cow manure, more phosphate is bound to it through separation, but less nitrogen and potassium than in the liquid manure.

The combination of pure horse manure and the solids of the separated manure without volume-filling bedding materials means that the manure takes up less space, allowing it to remain lying around for longer and giving it the opportunity to compost.

The advantages of this composted fertiliser are:

- Stimulation of soil life: the quantity and activity of microorganisms in the soil increases, which has a positive effect on soil fertility, soil structure and water retention capacity
- Recycling of nutrients: the nutrients absorbed are returned to the soil, which also helps to reduce the use of other fertilisers.
- Replacement of humus: the decomposition of organic matter by soil organisms produces substances from which humus is formed over a longer period of time

A disadvantage of the mixture of horse and manure solids is the low nitrogen content due to the composting of the horse manure and the fact that with a dry matter content of 25% of the solids there is about 15-25% nitrogen, 15-35% phosphorus and 10-15% potassium in it. This is relatively low for a fertiliser, which means that more nitrogen should be added to optimally nourish the soil. This in turn leads to the problem that the soil should be fertilised. In addition, solid manure must not be applied to the land throughout the year.

Compilation and decomposition is also a step towards sustainable disposal in the biogas plant. In the biogas plant, the horse manure is stored together with other organic materials in special containers or digesters. There, the materials are broken down under anaerobic conditions and converted into biogas by microorganisms. The biogas produced can then be used to generate heat and electricity.

After the decomposition process, a residue remains, which is known as fermentation residue. This digestate still contains nutrients and can be used as fertiliser on fields and in gardens to improve soil quality and reduce the use of chemical fertilisers.

The company Pöttinger *(pferde.fermenter-technologies.com | Pferdemist Verwerten Mit PÖTTINGER Fermenter, n.d.*) offers a way for larger farms to become more climate-neutral. This is achieved with a fermenter in which the horse manure is fermented into biogas and can then be used on the farm as required. Dry fermentation plants are well designed for horse manure and cattle solids, as they can work with dry matter contents. The digesters come in various sizes and can generate "electricity" from as few as 10 horses. Cyclically operated fermenter boxes are often used here. These can be filled and emptied using a wheel loader.

In order for fermentation to take place, the manure, also known as substrate, is sprinkled with thin percolate. Furthermore, it is sometimes necessary to mix ¼ to ⅓ of the fermented material into the new substrate as a starter culture when the material is placed in the following container. The process depends on the type of fermenter.

The table below is an example of how much product is produced from one tonne of horse manure.

Tabel 4 Profits of horse manure of a fermentation process

HORSE MANURE IN T	M3 BIOGAS	M3 METHANE	KWH ENERGY OUTPUT
1	90	45	450

(pferde.fermenter-technologies.com | Pferdemist Verwerten Mit PÖTTINGER Fermenter, n.d.)

Another option for utilising horse manure is to convert it into heat and energy using thermocatalytic reforming, or TCR for short. This process uses thermochemical conversion to convert biogenic residues into electricity and heat. A stable oil and hydrogen-rich gas is produced and then converted into the end products electricity and heat with the help of a dual-fuel combined heat and power station. A secondary product of the production process is a soil-stable biochar, which could be used for soil improvement in the future.

The fermentation unit and the TCR system offer users the opportunity to cover part of their electricity, energy, heat, etc. consumption.

3.3 Subquestion 3

‘What is the total cost of the bedding material?’

In this case, the total cost of the bedding material for horse boxes is made up of the purchase price, the storage costs and the disposal costs of the solid cow dung.

3.3.1 The costs of separated manure in horse stables

a. Costs of purchasing

The cost of purchasing the solids is currently very dependent on the area in which this type is to be used. Are there many cow farmers here, do they have a manure separator, how far is the approach distance, would it be possible to collect the required quantity yourself and how much can be stored or is required?

A purchase price cannot be determined across the board, as several factors play a role here. On the one hand, whether the solids are not used, and on the other hand, the manure accounting regulations. Solid manure accounting is an administrative method used to track the amount and use of solid manure on a farm. The purpose of manure accounting is to understand the use of fertilisers, the amount of nitrogen and phosphate input and output, and the balance between them.

To keep manure accounts for solid manure, a farmer should follow the following steps:

1. Recording the amount of manure supplied: The farmer should keep accurate records of the amount of solid manure supplied on the farm. This can be done, for example, by using a weighbridge when loading or unloading manure, or by measuring the contents of manure storages.
2. Record of the amount of manure applied: The farmer must also keep track of how much solid manure is applied to the land. This can be done by accurately measuring how much manure is applied, for example by using a manure spreader with a built-in weighing system.
3. Nutrient balance calculation: based on the recorded amounts of manure landed and applied, the farmer can calculate the nutrient balance. This involves comparing the amount of nitrogen and phosphate supplied through manure with the amount of nitrogen and phosphate removed through crops. The difference between nutrient inputs and outputs gives insight into how much fertiliser is being used on the farm.
4. Reporting: Finally, the farmer has to report the results of manure accounting to the government. This can be done through a digital system, such as the I&R (Identification and Registration) system for livestock farming in the Netherlands.

Keeping correct manure accounts for solid manure is important to comply with laws and regulations regarding the use of fertilisers on farms. Moreover, it provides insight into the efficiency of manure management and can help optimise the use of fertilisers.

Prices for cow dung solids can vary depending on the region and demand. Though, they are usually traded at kilogramme or tonne prices. It is also possible that prices depend on the quality of the manure, the proportion of straw or other materials and the fertiliser content. For gardens in particular, 10 litre bags to 1,000 litre big bags are traded online. (*Home | Heihoeve Natuurproducten*, 2023) These prices vary between €2.66 - €3.75 and €165.00 – €169.99. The larger the quantity purchased, the lower the price. (BrabantseKoemest.nl, 2022)

These interrelationships mean that it is not possible to give a generalised purchase price. This would require further research or an over-coupling unit that connects the producers (farmers) of the separated manure, for example, as well as the end users.

b. Costs of storage

The costs for storage are either a covered barn, a silo/manure slab, or a hose silo. These costs depend on the conditions, the size, the materials to be used and the effort involved.

A covered storage area is often available for storing the usual bedding materials, such as straw or bales of shavings. This could be used for the storage of separated manure.

In any case, it is advisable not to allow too much moisture to reach the material, as this can cause it to clump together, and mould can form by trapping air. Too much air should also not be added, as the material will then start to compost and become finer and firmer. In itself, this is not particularly bad, as this also happens in the box, but in its compacted form it is then no longer so easy to scatter.

Tabel 5 Storage influences

INFLUENCE	PROCESS	EFFECT*
AIR	Start of composting	0/-
HUMIDITY	Chance of mold growth	-
LIGHT	No proven change	0

**-: bad; 0: neutral*

c. *Cost of disposing of the material*

The cost of disposing of the material depends on the frequency, the location and the recipient of the manure. There are different types of collectors:

- Specialised manure disposal by a company
- Biogas plant
- Farmer
- Spreading on your own land
- Collection by private individuals

These are the most common types of manure removal in the equine sector.

The top customer has to pay for the transport to the biogas plant and the farmer is often only charged for the transport. Depending on the volume of manure, special handling may be required, such as a permit or licence to take over or transport the manure. The manure accounting of the Netherlands also plays a role here, especially the nitrogen and phosphate rights must be taken into account.

The costs for manure disposal by a specialised company amount to between €80 and €140 for a 6m^3 container including collection per month, for example. (*Paardenmest Afvoer Prijzen - Bing*, n.d.)

In the best case, spreading on your own land costs no money if you have the right machinery. If this is carried out by a contractor, there are also costs for labour, fuel and machine use. It is also important to consider how much manure can be spread on the land and at what time. This type of manure is solid matter, which may only be applied to land registered with the RVO between 1 February and 31 October and then only in the permitted quantity as laid down in the manure regulations. Companies are generally allowed to apply between 50 and 80kg of phosphate and private individuals up to 80kg per hectare. The permitted quantity also differs from region to region and the local conditions, so no more precise general statement can be made.

The collection of manure by private individuals is free of charge, but the question is whether all the manure that is produced is collected.

Tabel 6 Overview manure disposal

TYPE OF MANURE DISPOSAL	ADVANTAGES	DISADVANTAGES	COSTS
SPECIALIZED COMPANY	Regular disposal	High costs	High
BIOGAS PLANT	Transportation costs only	High transportation costs (depending on the distance)	Average (depending on distance)
FARMER	On-demand disposal	Disposal doesn't always work	Average
USE ON OWN LAND	No secondary companies	Costs time, risk of too much manure with too many horses on too little available space	Low
PRIVATE ‚CUSTOMERS'	No costs	Danger of not finding a buyer	None

It should be noted that the amount of manure decreases significantly as already answered in sub-question 2b. This also reduces the frequency with which the manure has to be collected.

Manure accounting must be done by a horse farmer if the animals produced more than 350kg of nitrogen per year. This can be calculated via the in chapter 1 section absorption capacity and nitrogen absorption mentioned table of the RVO.

For private individuals, up to 5 horses in the Netherlands, an allowed size of 3 m^3 for manure storage applies without request. If this size is not sufficient, a permit must be requested.

Chapter 4 – Discussion of results

The aim of this study is to analyse the solids produced during the separation of manure, which is partly used as bedding for cows, with regard to its use in horses. The focus here is on the harmlessness for the health of the horses, the sustainability aspect and the cost savings with regard to the utilisation of the by-product.

In this chapter, the methods chosen to carry out this work, examinations and tests with regard to the bacteria contained, dust formation and liquid absorption, as well as with the help of desk research, are critically assessed per sub-question. In general, it should be noted that these methods were largely expedient and delivered sound results, which underpins the validity of the work as a whole. Carrying out the tests for the first sub-question was more difficult than everyone involved had anticipated. The dust measurement tests in particular required some rethinking and rescheduling. Furthermore, it was more difficult than expected to find or provide concrete figures. Discussions with experts and additional literature sources revealed that it would be more accurate to name the points that have an influence on pricing instead of concrete figures that cannot be generalised.

4.1 Subquestion 1

The first subquestion is: ‚What is the impact of separated cow manure as bedding on the health of horses?'. This aspect includes dust generation, bacterial populations and fluid uptake of the separated manure.

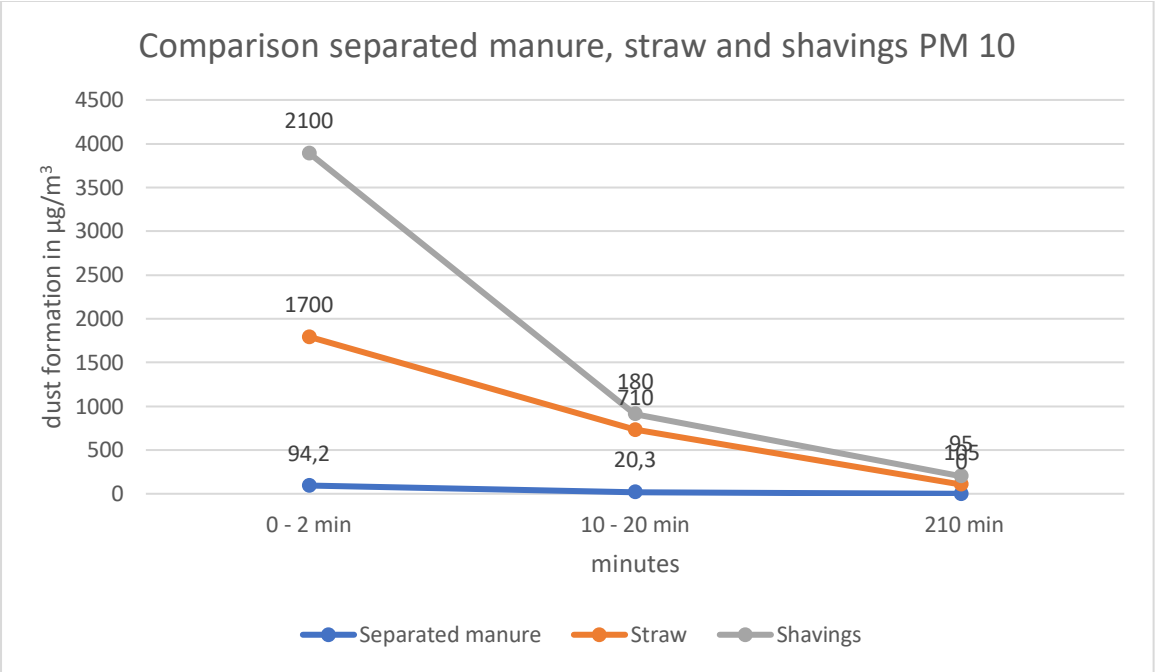
a. Dust formation

The results of the dust measurement show that within these 20 minutes the pollution in the air with the particle size PM 10 decreases by -73.9 µg/m^3 and between minutes 10 -20 it is at the value of 20.3 µg/m^3. The particle size PM 2.5 is 5 µg/m^3 at minute 10-20 and has been reduced by -11.6 µg/m^3. The dust evolution of PM 10 developed from 0-2 min with a value of 94.2 µg/m^3 to minute 10-20 to 20.3 µg/m^3. This is a reduction within 8-18 min.

With the particle size of PM 2.5 the dust load in the air drops from 16.6 µg/m3 in minutes 0-2 to 5 µg/m3 in minutes 10-20. Again, this is a reduction of -11.6 µg/m^3. Labie et al. (2019) recommended for horses a maximum of 30µg/m^3 for the particle size PM 10 after 30 minutes and for PM 2.5 a maximum of 15µg/m^3. The values resulting from the tests for the solids are well below the recommended values of Labie et al. (2019) with a difference of 9.7 µg/m^3 for PM 10 and 10 µg/m^3 for PM 2.5. The sizes PM 10 and 2.5 were examined, as these two groups are the most dangerous for horses. The size of <10 µm can reach the tracheobronchial and the other smaller group can reach the alveoli. These two groups, especially in larger quantities, have a dangerous effect on the health of horses (Art et al., 2002).

A test conducted by the Georg-August University in Göttingen (Engel Hessel, 2013) showed the values of wheat straw PM 10 at 1 minute rotation and 14 revolutions per minute a concentration of around 1700 µg/m^3, whereas this was ~ 2100 µg/m^3 for chips. With a particle size of 2.5 the dust concentration of wheat straw was ~140 µg/m^3 and of shavings around 210 µg/m^3. In addition, the concentration was measured during and after mucking out. A concentration of PM 10 was found in wheat straw with a peak of around 710 µg/m^3 and in shavings of 180 µg/m^3. The time for the values to rise and fall was about 3 ½ hours. Then they were back at values between 110 and 100 µg/m3 and chips around 90 to 100 µg/m^3.

Figure 4 Comparison dust formation PM 10



(Own results and Engel Hessel, 2013)

Comparing these values with the values from the study and the guide values of Labie et al. (2019) the values of the manure solids of PM 10 are significantly lower than the values of the average dust concentration under laboratory conditions of wheat straw and shavings, as well as under practical conditions. The practical conditions provided 'resting results' for both shavings and wheat straw is at 3 times the recommended concentration of Labie et al (2019). These resting results appeared only after about 3 ½ hours, with Labie recommending them after 30 minutes.

The results thus show that the manure solids would be a significant step towards dust-free bedding in terms of dust generation. The dust generation of the hazardous particles is below the recommended values and well below the values of current bedding materials.

b. *Bacteria count*

The bacteria found in the samples examined are not particularly uncommon for cow manure. The following bacteria were found: *Staphylococcus, Enterococcus, Enterobacter, Proteus, E.coli, Klebsiella, Streotococcus spp* and *Cornybacterium.*

Tabel 7 Bacteria formation in cow manure solids

Bacteria	**2 log10 = cfu/ml**
Staph spp	0.8
Enterococcus	0.3
Enterobacter	0.2
Proteus	0.9
E. coli	0.7
Klebsiella	3.8
Streptococcus spp	9.9
Corynebacterium	3.7

The quantity of bacteria present in bedding materials can be measured and reported using different criteria, such as wet weight, dry weight, or volume. However, reporting on a wet weight basis is not particularly meaningful as it heavily relies on the moisture content of the material. Therefore, when comparing bacterial counts among different bedding materials, it is more appropriate to do so based on the dry weight. However, since the solids of the separated manure have a higher volume due to the moisture content than other bedding types, this was converted to volume basis for easier comparison and not with the dry weight.

The table in 3.1.b shows the values of the bacterial colonies found from a volume of 120 ml. Bacterial concentrations are reported as log 10 colony forming units per milliliter (cfu/ml) of bedding material.

That is, a value of 2.0 log 10 is equal to 102 or 100 cfu/ml, while a value of 6.0 log 10 is equal to 106 or 1,000,000 cfu/ml.

It is particularly noticeable in the table that Klebsiella, Streptpcoccus spp and Cornyebacterium seem to be very high. There are different types of Streptococcus spp and a particularly well-known sort is Streptococcus equi qui feared in horses especially for the disease strangels. Klebsiella occurs normally in the intestines of horses and can lead to infection if there is an increased number or, in particular, if the immune system is weakened. This also applies to the corny bacterium, which is found on the skin and mucous membranes of horses and can be pathogenic if the immune system is weakened. The specific bacteria and their number in a horse box can vary greatly depending on factors such as hygiene practices, ventilation, number of horses, feed and waste management. In a typical horse box, however, the following bacteria may be present: Streptococcus equi, Escherichia coli, Staphylococcus aureus, as well as Enterococcus faecalis.

The exact number of these bacteria in a horse box is difficult to determine as the contamination depends on several factors. Regular cleaning and disinfection of the box, good ventilation and hygiene measures such as the removal of waste products can help to keep the number of bacteria at a low level. However, it is important to note that the presence of some bacteria in low numbers is normal and not necessarily harmful to health. In the study by Kwiatkowska-Stenzel et al (2007), the airborne gram-negative bacteria were investigated. Here only the colonies were determined and not the groups. Therefore, shavings showed 5.14 log10 CFU/m^3 and crushed wood pellets 4.81 log10 CFU/m^3. This was not determined in our test, but it can be said that Enterobacter, Proteus and E. coli are gran-negative bacteria that can occur in the air and can be transmitted via droplet infection or aerosols. This happens especially at high humidity.

Tabel 8 Overview of the study 'The effect of stable bedding materials on dust levels, microbial air contamination and equine respiratory health'

Bedding materials	**Wheat straw**	**Shavings**	**Crushed wood pellets**
Contents of the examination			
Dust development	2.43 mg/m^3	1.09 mg/m^3	4.07 mg/m^3
Fungal content	4.82 log10 CFU/m^3	4.88 log10 CFU/m^3	4.54 log10 CFU/m^3
Bacterial content measured in colony forming units (CFU)	-	5.14 log10 CFU/m^3	4.81 log10 CFU/m^3

(Kwiatkowska-Stenzel et al 2007)

In the following, all bacteria found in the samples are briefly summarized in the order as shown in the table of manure found in separated manure and explained in connection with their effects in horses.

Staphylococcus aureus (Staph spp) is also found normally on horse skin. S. aureus, Staphylococcus hyicus subsp. hyicus and Staphylococcus delphini as the main staphylococcal species associated with bacterial pyoderma in horses, with S. aureus being the most common. Staphylococcus also belonged to the 5 bacterial genera with the highest relative prevalence in equine fetal dermatitis. Most commonly, these staphylococci are extracted from soft tissues, such as skin and hair samples, ankle biopsies, wounds, surgical and excision sites, as well as from the respiratory system.

Methicillin resistance was detected in slightly more than half of the isolated bacteria. This bacterium is involved in various diseases affecting different systems, such as bacterial pyoderma, respiratory infections, intestinal pocket infections, abscesses, surgical wound infections, etc. (Sauvé, 2021)

*Enterococcu*s are gram-positive bacteria that are very resistant to environmental conditions. They can survive under a wide variety of environmental conditions, such as high salt concentrations. Enterococci are known for their ability to develop and spread resistance to various antibiotics. This makes it difficult to treat infections caused by these bacteria. Usually these bacteria are harmless and benign and can also be found in the intestinal flora of the horse, where they also help to regulate it. However, in certain situations, enterococci can become opportunistic pathogens and cause various infections in the equine organism, particularly in the gastrointestinal tract, urinary tract and wound infections. (Lauková et al., 2008) It is important to note that enterococci in horses are not usually primary pathogens but rather cause secondary infections, especially when the horse's immune system is weakened. Thorough hygiene and the avoidance of potential sources of infection are therefore important to minimise the risk of enterococcal infections in horses.

Enterobacteria (Enterobacter) are gram-negative bacteria belonging to the family Enterobacteriaceae. In horses, different types of enterobacteria can cause infections. One of the main characteristics of enterobacteria in horses is the risk of contagion, as they can be transmitted to other horses through direct contact with infected animals or through contaminated environments. Some species of enterobacteria, such as Escherichia coli, can produce enterotoxins, which can lead to severe diarrhoea in horses. (Uzal et al., 2021) The bacteria are highly resistant and can survive in the environment and in the digestive tract of humans and animals and are often resistant to a variety of antibiotics. In addition, they cause a wide range of diseases. They can cause a variety of diseases in horses, including diarrhoea, respiratory tract infections, urinary tract infections and wound infections.

The identification of enterobacterial infections in horses requires specific diagnostics such as culture and resistance testing to select the optimal antibiotic for treatment. (Vetlexicon, 2023) These characteristics of enterobacteria in horses underline the importance of appropriate hygiene and responsible antibiotic use to prevent and control infections.

Proteus bacteria are gram-negative, mobile bacteria that may play an important role in infections in horses. They are peritrically whipped and therefore highly mobile. They can actively move through the horse's tissue and thus cause infections. In addition, they can produce a capsule of polysaccharides, which gives them a certain protective function. This capsule may help the bacteria in the horse's body to hide from the immune system and cause infections. Some strains of Proteus may have natural resistance to certain antibiotics, making it difficult to treat infections. It is important to consider the antibiotic resistance profiles of Proteus strains in order to select the most effective treatment. These bacteria can produce various virulence factors, such as enzymes and toxins, which can damage horse tissue and cause inflammation. These virulence factors contribute to the pathogenicity of the bacteria. Proteus bacteria are often opportunistic pathogens, which means that they normally occur in the animal flora and become pathogenic under certain conditions. In weakened horses, they can cause infections, particularly in the urinary and respiratory system. It is important to note that the characteristics of Proteus bacteria in horses may vary and depend on various factors such as strain and environmental conditions. (Drzewiecka, 2016) Accurate identification and characterisation of bacterial properties is essential for the proper diagnosis and treatment of infections in horses.

Escherichia coli (E. coli) is one of the most common bacterial species in the digestive tract of mammals, including horses. E. coli are gram-negative, facultatively anaerobic bacteria that occur in the intestines of horses. They are part of the normal intestinal flora of horses and contribute to the digestion and absorption of nutrients. However, there are also pathogenic strains of E. coli that can cause disease in horses. Some of these strains produce toxins such as Shiga toxin, which can lead to severe diarrhea. (Van Duijkeren et al., 2000) The transmission of the bacteria can be transmitted by contact with contaminated surfaces, water or food. Unhygienic conditions and lack of cleanliness may increase the risk of E. coli infection. Different symptoms may indicate an E-Coli infection, depending on which strain is involved. Common symptoms are fever, diarrhoea, abdominal pain and general malaise. It is important to maintain good hygiene and cleanliness in order to minimise the risk of E. coli infection.

Klebsiella bacteria are known to be pathogens in horses and can cause a number of serious diseases. Klebsiella bacteria are opportunistic pathogens that normally occur in the digestive tract of horses but can lead to infection under certain conditions. This happens, for example, if the horse's immune system is weakened or if the bacterium reaches other parts of the body. Grade respiratory infections can cause these bacteria in horses, especially foals. This manifests itself in symptoms such as cough, fever, runny nose and difficulty breathing. They are also a common cause of urinary tract infections in horses. This is manifested in symptoms such as frequent urination, pain when urinating, and cloudy or bloody urine. Infection with Klebsiella bacteria can also lead to inflammation of the colon (colitis). This manifests itself in symptoms such as diarrhoea, abdominal pain and weight loss. Another important feature of Klebsiella bacteria is their ability to develop antibiotic resistance. These bacteria can be resistant to a variety of antibiotics, making it difficult to treat infections. It is important to detect Klebsiella infections in horses early and treat them appropriately to avoid complications. Diagnosis is made by clinical examinations, laboratory tests and sometimes bacterial cultures. Treatment usually involves the administration of highly effective antibiotics that are effective against the specific bacterium. (Estell et al., 2015)

Streptococcal bacteria (*Streptococcus spp)* are gram-positive, spherical bacteria that multiply by cell division and often form long chains or clusters. Some streptococci species can destroy red blood cells and are therefore haemolysin-producing. The three types of hemolysis: alpha, beta and gamma have specific antigens on their surface that help in classification and identification. For example, group A streptococci (Streptococcus equisimilis, Streptococcus zooepidemicus) are among the most important pathogens of respiratory and wound infections in horses. Some species of streptococci can penetrate body tissue and cause infections. These may manifest as fever, swelling, inflammation and secretions. This bacterium can cause diseases such as strangles, an infectious respiratory disease in horses. The bacteria produce various virulence-promoting factors such as hemolysis enzymes, proteases, capsules and adhesion molecules. These factors contribute to the pathogenicity of the bacteria and allow them to penetrate into tissues and cause infections. (Boyle et al., 2018) Some streptococcal species have developed resistance to common antibiotics, making it difficult to treat infections. It is important to note that there are many different types of streptococci, which may have different characteristics and effects on horses. Therefore, the exact characteristics may vary depending on the species and strain.

Corynebacterium are gram-positive bacteria, which means that they have a thick layer of peptidoglycan in their cell wall. In addition, they are anaerobic bacteria, which means that they can live without oxygen. They are natural inhabitants of the skin and mucous membranes of horses and cannot cause disease in a healthy state. However, they can cause disease when the immune system is weakened or when injured.

Corynebacterium diseases in horses can affect various organ systems, such as the skin (e.g. dermatitis), the respiratory tract (e.g. rhinitis) or the urogenital tract (e.g. urinary tract infections). The diagnosis of Corynebacterium infections in horses is made by clinical examination, microscopic examination of tissue samples and culture methods to identify the pathogen. It is important to remember that there are different species of Corynebacterium that can cause different clinical pictures in horses. (Woolcock et al., 1980) More detailed information on the different species and their specific characteristics and clinical pictures should be obtained from a vet or microbiologist if required.

All this information shows that bacteria can be dangerous but are always present in the environment. This is not only due to the manure solids, but also to other bedding materials or the environment. It can be summarised that these bacteria in themselves are no more dangerous than bacteria found on straw, hemp or linen bedding or shavings. The only important thing is how this certainty is handled. Care should be taken to ensure that the humidity is not too high and that this bedding is not used for animals with weak immune systems.

c. Liquid absorption

The tested sample of separated manure was still somewhat wet at 49% dry matter and could have been drier. However, the water absorption capacity of the fresh mass was 220%. "Bedding materials in horse husbandry" tested the water absorption capacity of cereal straw as well as fine and coarse wood shavings and obtained values of 305%, 266% and 242%. However, if this is considered in connection with the litter volume, the quantitative water absorption of fine wood shavings is about ten times higher than that of cereal straw, and about seven times higher for wood granulate and hemp hurds. The volume of the solids was 880 ml less than that of the bedding materials tested in the experiment by Häußermann et al. (2002). From the tests, as from other sources regarding separated manure as bedding for cow stalls, it was found that it is important to spread a thick layer so that enough liquid can be absorbed and the top layer can dry. The larger the surface area, the faster it dries. It is also very important that not only the faeces are removed daily, but also the humidity in the stable, which according to 'Sectorraad Paarden' should be 60 - 70%. Good ventilation ensures that the top layer of the material can dry out, creating a compact and mouldable mattress. This also smells neither of ammonia nor of cow dung, but of peat. The danger of ammonia lies in its keratolytic properties, which have an irritating and particularly irritating effect on the mucous membranes and eyes (Hartung et al., 1990). This can result in damage to the cellular surface of the respiratory tract and a decrease in mucociliary clearance (Katayama et al., 1995). The guideline value for ammonia in horse stables is 10 ppm (6.96 mg/m³) (BMVEL, 2009), as serious damage to the respiratory organs can occur at 30 ppm. Of course, existing respiratory diseases (RAO) can also be adversely affected by increased ammonia concentrations. This is why it is so important that excreted urine is absorbed and cannot volatilise into the air.

The absorption quality of the potential litter should be seen in connection with the litter thickness of at least 20 cm, which drops to 15 cm after some time. This thickness guarantees that no ammonia is released and that the horses can lie softly.

4.1.2 Conclusion Subquestion 1

Sub-question 1 answers the question: 'What is the impact of separated cow manure as bedding on the health of horses?'.

Picture 2 Deformability of separated manure

Picture 3 Residues of the material

The influence on the health of horses can be influenced by the use of separated solids as bedding. This is positive due to the low dust formation of the material, as well as the good fluid retention. It contains bacteria that can be harmful to horses, but these bacteria are also present in the environment. It is recommended not to use the bedding for animals with weak immune systems or foals and nursing mares. The own use and the examination of the absorption properties yielded further results, such as the formation of mattresses, a good deformability of the material and the ability to damping. In addition, it turned out that the material can easily discolor a light hair dress with too much moisture. However, if the specified dry percentage is followed, no residues remain in the fur.

4.2.1 Subquestion 2

With sub-question number two, "What impact would this bedding material have on sustainability (in the Netherlands)?", the bedding savings, reduction of manure and disposal options were analysed via desk research.

a. Amount of bedding
b. Amount of manure
c. Disposal

a. Amount of bedding

A height of 20 cm is recommended for bedding with separated manure. This is based on the experience of farmers and the results of studies for cows. A deep bedding box is recommended for cows or a mattress underneath to provide comfort and minimise lying and standing up injuries. The mass will settle after some time, depending on use and length of time in the box. This can allow up to 5cm.

In any case, the lying and manure area should be littered well enough. This means that, depending on the size of the box, either the entire box or a bed in the centre is created. The amount of bedding for the first time is calculated by multiplying the size of the box in m^2 by 0.2 in order to have enough bedding. The mattress should be carefully raised piece by piece so that it does not become too wet and start to compost. For a currently averaged box in the Netherlands of around 10 m^2, a volume of 2,100 l is required as initial bedding. This seems a lot, in comparison the needed amount of straw is shown at the end of the page, but it should be noted that a maximum of 70 to 100 l of bedding must be added per week.

To keep the stall clean and the amount of bedding as low as possible, it should be mucked out daily and the wet areas should be put to one side so that they can dry. Research on cows, for example Leach et al. (2015), also shows that it can be helpful to scrape the top layer a little loose to keep it softer and give the material a chance to dry even better. If this is adhered to, a springy, soft, mouldable mattress is formed, which can remain in the box for up to 6 months. (Sorter et al., 2014) After that, everything should be replaced, ideally with a wheel loader to save labour.

It should not only be re-spread, as litter is taken out and carried out by the horses despite the removal of manure. In addition, the bedding settles over time as the bottom layers become compacted.

To ensure a comfortable lying surface, additional bedding can be added. At 70 to 100 litres per week, which is ~ 25 kg and, depending on the size of the bedding area, 5 to 6 cm. (Valacon-Dairy, 2014)

The amount of straw bedding is calculated to be between 7 - 10 kg per day per horse. As a rule of thumb, a farm calculates with one round bale of straw (220 -300kg) per month. (Gregg, 2021) A minimum bedding depth of 15 cm is also recommended here, as the study "The effects of night light and bedding depth on equine sleep duration and memory consolidation" showed that horses sleep better on such a quantity than on 5 or 10 cm thick bedding. (Clark, 2023) Depending on the size of the box, between 1 and 2 bales are recommended as basic bedding. This would be a quantity of 220 – 600 kg per horse. Personally, this seems a bit much to me.

If you compare these two quantities of bedding, both the basic bedding of straw 220 - 600 kg, and the manure solids of around 2,100 litres, this equates to around 525 kg. This is roughly comparable. As seen in literature, the amount of bedding is also the same. In a small field study, the results where that was both scatter and spread less bedding as told in theory. It was about one wheelbarrow of 80 litres per box every 2 weeks. This corresponds to a quantity of 40 litres per week and in kilograms 10 -12 kg. The initial bedding was 4-5 wheelbarrows of 80 litres each. This is therefore 320 - 400 litres in a box of 12 -14 m^2 as initial bedding. For the first 2 weeks 1 - 2 wheelbarrows of the manure were used to build up the mattress. After 3 weeks, the amount of mattress in the boxes was 160 - 320 litres of bedding and therefore a total of 480 - 720 litres, which corresponds to 180 kg. There was a significantly higher consumption of straw, both when bedding and when re-bedding. It was used 220 kg of bedding per box and added ~ 20 kg every 2 days. After 3 weeks, this amounts to around 420 kg of bedding. This with a box size of between 12 - 14 m^2. However, the manure solids were spread as a mattress over an area of ~ 9m^2 and the straw bedding was spread over the entire box, as with less straw bedding the horses slip faster. (Kitchener, 2016)

If you compare the two bedding quantities, as well as the daily scattering, it is noticeable that at first glance they are not that different from each other. If taken a closer look at the data, especially in comparison with the practical test, it is noticeable that significantly less bedding in kg is required for the separated manure - around 240 kg less. Beyond that, it should not be forgotten that the test horses spend a maximum of 10 hours in the box and therefore the minimum bedding quantity of 0.15 - 0.2 m was not always present.

If you now look at the poor harvest of recent years in connection with the consumption of straw, as well as the associated imports and rising prices, then it becomes clear that the consumption and use of straw for horses is not really environmentally conscious. More and more straw has to be imported from other countries, such as Germany and France. Even these countries are not spared from the weather. Either it is too hot and the crop cannot grow well or withers before it can be harvested, or it is too wet, causing the soil to compact and the plants to drown or not germinate at all, and the farmers cannot get to the land, which means that the crops can only be harvested very late, resulting in very poor quality. Usually 5 tonnes per hectare are harvested, but in the last 3 years this has been revised to a maximum of 3 tonnes per hectare. This has caused a price increase and supply problems. (Stijgende Stro- En Ruwvoerprijzen, 2023) It is also important to ensure that the quality of the straw is good enough not to jeopardise the horse lung, as mentioned in chapter 1.

These factors, together with the CO_2 that is produced when importing straw from other countries, as well as the whole process of climate change, which is making straw harvesting increasingly difficult, show that this is not the most sustainable way to procure stable bedding. As manure solids are a by-product, which is partly seen as a waste product, it can be given a second 'life' by being used as animal bedding before being spread on the land again or used for energy production.

b. *Amount of manure*

The amount of manure produced daily in horses depends on factors such as the size of the horse, the amount of feed and the horse's metabolism. On average, a healthy horse produces around 10-20 kilograms of manure per day and 5-10 litres of urine per day. Depending on how the horse is kept, most of this faeces is excreted in the stall.

As a rule, horse boxes are mucked out once or twice a day. With a daily muck-out, only the moist and soiled area of the litter is usually removed, while the remaining area stays in the box for a while. With a double daily muck-out, the litter is completely removed and replaced with new litter. The amount of straw manure produced per box can vary greatly, but it is usually estimated to be around 4 to 10 kilograms of pure straw manure per day. This is on top of the 10 - 20 kg of manure. Depending on the type of bedding, this will be less as with separated manure. The manure is mucked out according to the type of mattress. The bottom layer remains in the box, whereby the solid manure and very wet urine spots are removed. The top layer is then loosened up and spread out so that the bedding can dry out. The urine spots can also dry on the side and do not need to be removed.

When using separated manure on average only these 10 - 20 kg of manure per day per horse are removed for up to 6 months.

After up to 6 months, after all it should be noted that the entire mattress should be removed, resulting in the 2100 litres already mentioned in 4.2.1. a., i.e. 525 kg, with a box size of 3 x 3.5m and a mattress 0.2m thick.

In a comparison between straw manure and manure solids, 14 - 40 kg of straw manure and 10 - 20 kg of excreted manure without bedding are produced per day with manure solids. This makes a difference of 4 to 20 kg per day per horse.

In my case, a complete emptying of a straw box with a box size of 12 - 14 m^2 results in 420 kg of pure straw manure and 180 kg of pure bedding with separated manure solids. (Admin, 2019) The difference of 240 kg of pure bedding is relatively large, whereby it should also be noted that a straw mattress should be removed every 2 - 3 weeks due to the bacterial population and the release of ammonia. (Horse husbandry - manure storage - dung heap, n.d.) In comparison, the manure solids can be disposed of every 6 months. This corresponds to a straw manure disposal of 8 x 420 kg of straw manure and therefore a total amount of straw manure of 3,360 kg after 6 months. This calculation clearly shows how much can be saved by using separated manure as bedding for the manure produced.

c. *Disposal*

As with all types of manure, the manure produced from separated solids can be handled by various parties. It can be spread on your own land, collected by a farm or a company specialising in manure disposal, sent to a biogas plant or sold to private individuals. The manure can be spread on your own land, collected by a farm, sent to a biogas plant or sold to private individuals.

The further utilisation of manure is much more sustainable than burning it, which is what mainly happens when horse manure is mixed with bedding materials other than straw. In particular, the use of a digester or a biogas plant helps with the sustainable disposal of the manure produced. The manure can be converted into methane gas or CO_2-neutral electricity and heat. The advantages of selling it to private individuals or spreading it on the land are that the nutrients taken from hay, straw, grass, etc. can be returned to the cycle.

The mixture of horse manure in combination with the manure solids results in a mixture of potassium-rich horse manure and the magnesium, phosphate, ammonium and calcium-rich manure solids. The nitrogen content is relatively low in the mixture, as approximately 25% nitrogen is lost during the storage of horse manure and 80% of the nitrogen content of the separated manure is separated in the liquid manure. (Top Agrar Online, 2022)

Composting in the biogas plant can produce heat and electricity, leaving a residue that can also be reused. This residue is called digestate and contains nutrients, which means it can be used as fertiliser in the garden and on the field, reducing the use of chemical fertilisers.

Fermentation plants can also increase the sustainability of horse manure. By adding an admixture to promote fermentation, it is possible for the manure to be fermented into biogas in these plants and thus used as electricity on the farm. Table 4 in chapter 3.2.1 c. shows what can be produced from 1 tonne of horse manure.

A new option using TCR (thermocatalytic reforming) is currently being tested. Here, electricity and heat are generated from the biomass using thermochemical conversion. A waste product is also produced here, a soil-stable biochar, which could be used as a fertiliser after further tests.

As in 4.2.1 b., the amount of manure produced is reduced, which also extends the time of disposal. Not only are the possible uses for the manure produced greater, but the amount of manure that can affect the organisms is also lower, which is an advantage for the sustainability of the manure solids.

4.2.2 Conclusion Subquestion 2

The sustainability of the separated manure solids is clearly demonstrated by the total amount of bedding, the amount of manure produced and the possibilities for further utilisation. Not only is the amount of bedding used less than straw, for example, but it is also actually a waste/by-product of manure separation.

The amount of manure is also reduced to the amount of the horse's faeces and approximately every six months to the box bedding, which corresponds to about half of the straw manure produced in a month. This also reduces the amount of manure that needs to be removed, the time that is spent mucking out and offers further opportunities to reuse it.

4.3.1 Subquestion 3

The last sub-question was "What is the total cost of the bedding material?" and is made up of the sub-categories costs of purchasing, costs of storage and costs of disposing of the material. As with the previous sub-question, this question was answered in general terms and not with specific figures, as the costs depend on various factors.

a. Costs of purchasing
b. Costs of storage
c. Costs of disposing of the material

a. Costs of purchasing

Picture 2 Separator for cow manure

It is not possible to give an exact cost breakdown of the purchase costs, as these costs depend on various factors such as the environment, labour hours, delivery costs, number of cows, self-use, purchase and maintenance costs or even rental costs for such a separator. A separator costs between €20,000 and €30,000. (Van Den Broek, 2010) Farmers who do not use the manure solids themselves sell them to a company that resells the manure, such as arable farmers or champion breeders. An overcoupling unit that brings both sides into contact with each other, such as a company that offers separation, would be advantageous here.

Good quality straw is becoming more and more expensive, rising from €45 per tonne 2/3 years ago to over €100 per tonne, which corresponds to a price increase of around 122.22%. (*"Met Deze Stroprijzen Is De Champignonteelt Nauwelijks Rendabel,"* 2023)

It is currently possible to collect manure solids cheaply or free of charge via farmers in the neighbourhood or via Marktplaats. This is certainly possible for small quantities, but all manure that normally leaves the farm must be declared. This is in connection with the 'Mestboekhouding' in the Netherlands, where the manure produced and what happens to it is recorded. This has to do in particular with the limitation of the effect of the nitrogen and phosphorus content on the environment. Dried cow dung is offered online with a price difference of €2.66 - €3.75 for 10 l up to €165.00 - €169.99 for 1,000 l. This may be a guide value, but is not decisive, as these are designed for smaller quantities in particular. (*Home | Heihoeve Natuurproducten*, 2023)

b. Costs of storage

The storage costs for separated manure amount to a covered, paved shelter, a silo slab or an area for a silage hose. These two storage options are often already available, as other bedding materials, especially the straw that is predominantly used, should also be stored dry. Ideally, the humidity should not be too high and water or too much air should not get to the material if it is to be stored for long periods of time. Humidity or water can lead to mould and water contamination. Air can cause the manure solids to start composting if stored for long periods. This has no known side effects so far, but in combination with some moisture it becomes solid and more difficult to spread.

The option of storing the manure solids in a tube is more expensive, as they are placed in the tube on site. These costs depend on the amount of manure separated, the length and size of the hose, as well as the time required and the journey. The advantage of such a hose is that neither light nor direct water can reach the solids.

Depending on the conditions on site, either the option of a hose can be advantageous or the use of an existing storage.

c. *Costs of disposing of the material*

As already mentioned in section 4.2.1 c., manure can be removed or spread in various ways. The usual ways are:

- Specialised manure disposal by a company
- Biogas plant
- Farmer
- Spreading on your own land
- Collection by private individuals

The advantages and disadvantages of the manure disposal techniques listed above are listed below:

Tabel 9 Advantages and disadvantages of manure disposal

POSSIBILITY TO GET RID OF THE MANURE	ADVANTAGES	DISADVANTAGES
SPECIALISED MANURE DISPOSAL BY A COMPANY	- Efficient and professional handling - Compliance with environmental regulations - Timesaving: normally on a regular basis	- Costs €80 - €140 for 6m^3 container per month (*Paardenmest Afvoer Prijzen - Bing*, n.d.) - Lack of control
BIOGAS PLANT	- Environmental sustainability - Waste reduction	- Infrastructure requirements - High costs of transport
FARMER	- Nutrient enrichment for crops - Cost-saving - Pick-up on call	- Storage and handling challenges - Potential nutrient imbalances - Not always when needed
SPREADING ON YOUR OWN LAND	- Nutrient recycling	- Space and time limitations - Time consumption - Odour and nuisance
COLLECTION BY PRIVATE INDIVIDUALS	- Free or low-cost	- Limited customers - No guarantee for quality

The amount of manure is significantly reduced by the bedding used in contrast to conventional bedding materials such as straw. This also reduces the storage space required and the frequency of emptying the container or manure tray. So costs are reduced usually compared to straw manure. The most cost-effective way of disposing the manure for private individuals is to collect it free of charge. Spreading manure on the land is very cost-effective too, compared to other methods if you have enough space and your own machinery, as you only have to pay for labour, machine wear and fuel. This also includes the possible use of a fermentation plant, which is of course more expensive at first. On the other hand, it pays for itself once the purchase has been paid off if there is enough manure.

The delivery to a farmer might cost money, but does not have to. Delivering the manure to a biogas plant often produces transport costs to the plant. Depending on the distance, this can also be relatively expensive. The most expensive option, however, is to have the manure picked up by a specialised company that collects the manure regularly and which takes care of the registration of the 'mestboekhouding' for more than 5 horses.

4.3.2 Conclusion Subquestion 3

The cost situation is somewhat more difficult to explain, as no specific details can be given due to the various factors involved. The purchase is currently very cheap to free, but certainly only up to a certain quantity. It should also be noted that the legal situation regarding the sale of manure as bedding has not yet been clarified. It could be that this is not permitted due to nitrogen and phosphate regulations. Moreover, it could also be that the advantages of further utilisation outweigh the disadvantages and this would be permitted. Legal enquiries are required for this.

The costs for the storage of bedding often amount to an existing storage facility for straw, for example. These are often covered, have a concrete floor and are sometimes closed or lockable all round. If this is not available, it is possible to store the solids in a silo or a hose. Sufficient space is required for this, depending on the quantity. The purchase of a hose costs money with every purchase of bedding.

The cost of removing the manure also varies depending on the method of disposal. The cheapest option is definitely collection by private individuals, the simplest is disposal via a specialised company, although this is also the most expensive. In addition, it can also be inexpensive to dispose of the waste on your own land or have it collected by a neighbouring farmer. Transport to a biogas plant is often relatively expensive due to the distance, but this disposal method is otherwise also very advantageous.

4.1 Reflection

The results shown here were not particularly easy to obtain, as not everything has been researched, even for cows, and it is a legal grey area, such as the use as bedding or the sale of the solids with regard to the 'mestboekhouding' or that the laboratory tests were more difficult than expected. Some of the laboratories I contacted were unable to help me, particularly with regard to dust formation. It took many phone calls, consultations with a very dedicated vet from the Graafschap-Dierenartsen, as well as his contacts and endless literature research to develop an experimental setup that could be carried out and produced comprehensible results.

Summarizing the efforts right now, I’m still convinced of the topic cow manure might be a recommended alternative for the bedding of horses. But working on the theme was really challenging, very complex and demanding in both – time and thought.

Even after completing my bachelor’s thesis I am not sure whether this project comes to an end for me. Perhaps I will try to find someone to support me in research and continue to develop this idea further. I think this topic has a lot of depth remaining.

4.2 Research influence

I think that this research is an interesting approach for further research into the continued use of manure solids, as the results do not generally rule out this substance as bedding for horses. It should be noted that these investigations and researches are a starting point and should not be seen as a general recommendation to use separated cow manure as bedding for horses without hesitation. The recommendation is issued to further investigate the effects of bacteria, such as the development of the use as bedding for horses, but also the legal situation regarding the purchase, use and disposal of these manure solids.

The very low dust generation and the sustainability aspect of this potential bedding material should be emphasised in particular. These characteristics indicate that further research should be indicated as a bedding option for horses.

4.3 Results and correlation theory

The results are predominantly positive. There are limitations in terms of the legal situation and the cost of procuring and disposing of the possible bedding material. The results that emerged from the tests are in line with those already carried out as bedding for cows. The results of the tests show that the values for dust formation and liquid absorption are significantly below or above the recommended values for bedding for horses. The properties such as odourless, deformable and slightly damping were also shown to be secondary results of the tests and are confirmed by literature on research for cows. The results basically give a go for further research as bedding for horses.

Chapter 5 – Conclusions and recommendations

This chapter deals with the conclusions and recommendations for action drawn from the whole of the preceding study for the sub-questions, as well as the main question above: *„Which features of separated cow manure make it an option to commercially available materials such as straw, straw pellets, flax straw and shavings for use as bedding in horse stalls?“.*

5.1 Conclusion

Choosing the right bedding depends on several factors, such as the horse's behaviour, the health and sensitivity of the horse's hooves, the owner's budget and available resources. The right bedding should be absorbent to bind moisture, be odourless, be easy to clean, provide adequate cushioning to reduce pressure on the hooves, and protect the horse from injury. It is also important that the bedding does not contain too many dust particles to avoid respiratory problems.

Picture 5 Separated manure as bedding

The analysis of the subquestion ‘What is the impact of separated cow manure as bedding on the health of horses?’ demonstrated multiple positive effects:

Dust development: The Values determined (PM 10 in minutes 10-20 at 20.3 µg/m^3 and PM 2.5 at 5 µg/m$^{3)}$ were significantly lower than the recommended values of PM 10 30 µg/m^3 and PM 2.5 15 µg/m^3 after 30min in horse stables.

Bacterial population: Most of the bacteria found in the separated cow manure also exist in the body of a horse or in its surroundings. Remark: the number of these populations is questionable and therefore might cause diseases in immunocompromised animals.

Liquid absorption: Fluid uptake was examined for an uptake of 220 % (straw 305 %). With a layer of 20 cm, it should be guaranteed that the excreted urine is absorbed in the lowest layers and not released.

Comfort: The formation of a soft mattress is possible; the material shows a high cushioning effect and is odourless.

These facts do not have negatively affect health or even support health of the horse.

‘What impact would this bedding material have on sustainability (in the Netherlands)?’ was investigated in the second sub-question with the help of the desk research method.

The amount of straw with a minimum thickness of 15 – 20 cm was compared with the amount of straw cow manure.

Quantity: Measured over a period of 3 weeks, 240 kg less bedding was required.

Residence time: Material can remain in the box for up to 6 months in contrast to a straw mattress, that has to be removed after a maximum of 2 to 3 weeks due to bacterial formation and ammonia content.

Sustainability: Separated cow manure is a by-product that can be used close to production for a second purpose. Especially with regard to import of straw from other countries in case of demand quantity is greater than production quantity this is much more sustainable. The amount of manure produced is also significantly lower than with conventional bedding agents.

Removal and Working time: Horses produce about 10 – 20 kg of excrement and 5 – 10 l of urine per day that have to be removed. With manure solids only these faeces have to be taken away and the surface should be roughened to dry better. With straw it occurs that 4 up to 10 kg is additionally removed per manure and has to be refilled.

Requirements: A humidity in the barn of 60 – 70% ensures the dryness of the separated cow manure and prevent mould.

Further advantages: The resulting manure has not only a smaller volume which allows it to lie longer and also begins to compost, as well has the benefits of the nutrient and mineral mixture of horse manure and cattle solids. The low nitrogen content of this mixture should be noted, which may be advantageous in terms of the nitrogen regulation and discussion in the Netherlands. However, it should be noted that this is not sufficient for all plants when fertilising and should be supplemented accordingly. The possibilities for further use offer especially in terms of sustainable energy such as through a fermentation plant convince on a sustainability level.

The last question answers the costs of acquisition, storage and disposal. These are vague and more theoretical, since a specific cost statement depends on many factors, some of which are not yet clarified. The legal status of the disposal or sale of manure as bedding has not been clarified, nor has the question of the cost of disposal or the declaration. The accommodation costs are not necessarily higher than for straw, since the same conditions apply. The possibilities for using the manure are greater by mixing the two types of manure and the volume is smaller by the spreading behaviour, which opens up more possibilities to use the manure and to reduce costs.

In summary, the question **"Which features of separated cow manure make it an option to commercially available materials such as straw, straw pellets, flax straw and shavings for use as bedding in horse stalls?"** can be answered that in particular, the low dust development of the material, as well as the sustainable possibilities resulting from the reduced manure volume and the continued use it could be a great option as a bedding material in horse boxes. This under the condition that further investigation and research regarding the bacterial development and legal conditions, as well as availability for horse owners are looked after.

5.2 Recommendation

Separated manure may be both: an equine- and environmentally friendly alternative to other bedding materials, but further studies and testing should be carried out before it is made available on the market as a bedding agent. Due to the content of bacteria, it is not recommended in boxes for immunocompromised or sick horses, as well as lactating mares. This material can offer many advantages, such as low dust generation and good absorbency, as well as springy mattress formation. The procurement of the material is not yet sufficiently researched at the present time of the investigation and there is no general body from which information on procurement can be requested. In addition, the removal and notification of the manure is not legally secured, as questions arise about the phosphorus and nitrogen content. These legal requirements should be developed on the basis of further research and with the help of a lawyer.

The manure solids may offer an alternative, but they should not be used indiscriminately and without further research regarding the risk of infection. Particularly important is the dryness of the fabric, as well as the humidity in the barn, as well as regular dunging to prevent mould development and unusual bacterial formation.

List of references

Ackerman, J. N., Khafipour, E., & Levin, D. B. (2018). Sustainable re-use of dairy cow manure as bedding and compost: Nutrients and self-heating potential. *Canadian Biosystems Engineering*, *60*(1), 6.1-6.7. https://doi.org/10.7451/cbe.2018.60.6.1

Admin, L. (2019, March 5). Stall Waste Production and Management – Livestock and Poultry Environmental Learning Community. Retrieved July 6, 2023 from https://lpelc.org/stall-waste-production-and-management/#:~:text=How%20Much%20Manure%20Will%20a%20Horse%20Produce%3F%20A,produce%20approximately%20nine%20tons%20of%20manure%20per%20year.

Anzeige von Staubfreisetzung von Einstreumaterialien in der Pferdehaltung. (n.d.). Retrieved April 2, 2023 from https://www.landtechnik-online.eu/landtechnik/article/view/2004-59-6-336-337/2004-59-6-336-337-de-pdf

Bambi, G., Rossi, G., & Barbari, M. (2018). Comparison between different types of bedding materials for horses. *Agronomy Research*, *16*(3), 646–655. https://doi.org/10.15159/ar.18.124

Beck, J. (2005). Pferdemist - Problemlösung durch mechanische Aufbereitung, Kompostierung und thermische Verwertung. *Landtechnik*, *60*(1), 40–41. https://doi.org/10.15150/lt.2005.1161

Berkemeier, C. S. K. (2021, June 15). Elite Magazin. Elite Magazin. https://www.elite-magazin.de/heftarchiv/technik/separierte-gulle-einstreuen-kein-problem-13928.html

BrabantseKoemest.nl. (2022, March 7). Koop (gedroogde) koemest direct online! - Brabantsekoemest.nl. https://www.brabantsekoemest.nl/winkel/page/3/

Borowicz, H., Kubiak, K., Nikolovski, G., & Niedźwiedź, A. (2016). Impact of Recurrent Airway Obstruction (RAO) on Selected Antioxidants in Horses. Macedonian *Veterinary Review*, *39*, 5–14. https://doi.org/10.1515/macvetrev-2016-0074

Boyle, A. G., Timoney, J. F., Newton, J. R., Hines, M. T., Waller, A. S., & Buchanan, B. R. (2018). Streptococcus equi Infections in Horses: Guidelines for Treatment, Control, and Prevention of Strangles—Revised Consensus Statement. Journal of Veterinary Internal Medicine, 32(2), 633–647. https://doi.org/10.1111/jvim.15043

Clark, A. (2023, November 5). Horse bedding: types to choose from, best thickness level and money-saving tips - Your Horse. Your Horse. https://www.yourhorse.co.uk/horse-care/horse-bedding/

Clements, J., & Pirie, R. S. (2007). Respirable dust concentrations in equine stables. Part 1: Validation of equipment and effect of various management systems. Research in Veterinary Science, 83(2), 256–262. https://doi.org/10.1016/j.rvsc.2006.12.002

Contract Research Organisatie (CRO). (n.d.-a). WUR. https://www.wur.nl/nl/onderzoek-resultaten/onderzoeksinstituten/bioveterinary-research/cro.htm

De Rijksdienst voor Ondernemend Nederland. (2022). Mestbeleid 2022 Tabellen : Tabel 4 Diergebonden normen. In De Rijksdienst Voor Ondernemend Nederland. RVO. Retrieved August 26, 2023, from https://www.rvo.nl/sites/default/files/2021/12/Tabel-4-Diergebonden-normen-2022.pdf

Drzewiecka, D. (2016). Significance and Roles of Proteus spp. Bacteria in Natural Environments. Microbial Ecology, 72(4), 741–758. https://doi.org/10.1007/s00248-015-0720-6

Engel Hessel. (2013, November 12). Stäube und Schadgase in der Pferdehaltung [Slide show]. Georg-August-Universität Göttingen, Außenstelle Vechta, Department für Nutztierwissenschaften, Abteilung Verfahrenstechnik. https://alb-bw.uni-hohenheim.de/fileadmin/einrichtungen/alb-bw/Archiv/2013/Fachgespraech/Hessel.pdf

Einstreualternative: Separierte Gülle | Landwirtschaftskammer Oberösterreich. (n.d.). https://ooe.lko.at/einstreualternative-separierte-g%C3%BClle+2400+2726388

Estell, K. E., Young, A. D., Kozikowski, T. A., Swain, E. A., Byrne, B. A., Reilly, C. M., Kass, P. H., & Aleman, M. R. (2015). Pneumonia Caused by Klebsiella spp. in 46 Horses. Journal of Veterinary Internal Medicine, 30(1), 314–321. https://doi.org/10.1111/jvim.13653

Fleming, K., Hessel, E. F., & Van Den Weghe, H. (2007). Gasbildung und Staubentwicklung aus verschiedenen Einstreumaterialien für die Pferdehaltung. *Landtechnik*, *04/2007*, 238–239. https://doi.org/10.15150/lt.2007.996

Fleming, K., Hessel, E. F., & Van Den Weghe, H. F. (2008). Generation of Airborne Particles from Different Bedding Materials Used for Horse Keeping. Journal of Equine Veterinary Science, 28(7), 408–418. https://doi.org/10.1016/j.jevs.2008.05.004

Fourages, N. (2023, May 19). Regels: Waar moet een particuliere mestopslag wettelijk aan voldoen? Mestcontainer.com - Nijssen Fourages. https://www.mestcontainer.com/moet-particuliere-mestopslag-wettelijk-aan-voldoen/

Gregg, S. (2021, May 18). Reiterrevue International. Reiterrevue. https://www.reiterrevue.de/ausbildung-und-praxis/stall-und-weide/10-fakten-und-tipps-zum-misten-12035414.html

Häußermann, A., Beck, J., & Jungbluth, T. (2002). Einstreumaterialien in der Pferdehaltung. Landtechnik, 50–51. https://doi.org/10.15150/lt.2002.1560

Home | Heihoeve Natuurproducten. (2023, September 14). Heihoeve Natuurproducten. https://heihoevenatuurproducten.nl/

Joschko, L. (2022, January 12). *Hanf, Späne oder Stroh – Alternativen zur Einstreu*. Uniqhorse. https://uniqhorse.com/blogs/uniqblog/alternatives-to-litter#:~:text=Verschiedene%20Einstreu%20im%20%C3%9Cberblick%201%20-%20Stroh%20Stroh,Sp%C3%A4ne%20. . .%205%20-%20Einstreu%20aus%20Hanf%20

Kitchener, N. (2016, April 28). The Ultimate Guide to Choosing Bedding: Choose the best bedding for your horse, taking into consideration their health, safety and comfort, as well as affordability and storage. Horse Canada. Retrieved January 13, 2024, from https://horse-canada.com/magazine/farm-management/the-ultimate-guide-to-choosing-bedding/

Koch, A. (2023). Einstreu jenseits von Weizenstroh. Oldenburger International, 5/2023, 58–65.

Kwiatkowska-Stenzel, A., Witkowska, D., Sowinska, J., & Stopyra, A. (2017). The effect of stable bedding materials on dust levels, microbial air contamination and equine respiratory health. Research in Veterinary Science, 115, 523–529. https://doi.org/10.1016/j.rvsc.2017.09.022

Lauková, A., Simonová, M. P., Strompfová, V., Štyriak, I., Ouwehand, A. C., & Várady, M. (2008). Potential of enterococci isolated from horses. Anaerobe, 14(4), 234–236. https://doi.org/10.1016/j.anaerobe.2008.04.002

Leach, K. A., Archer, S. C., Breen, J., Green, M. J., Ohnstad, I., Tuer, S., & Bradley, A. J. (2015). Recycling manure as cow bedding: Potential benefits and risks for UK dairy farms. The Veterinary Journal, 206(2), 123–130. https://doi.org/10.1016/j.tvjl.2015.08.013

Marquardt, S. (2023, July 24). Biogas aus Pferdemist: Trockenvergärung. Bauernzeitung. https://www.bauernzeitung.de/agrarpraxis/erneuerbare-energien/biogas-aus-pferdemist-trockenvergaerung/

Masterhorse. (n.d.). Lungenentzündung beim Pferd. Masterhorse. https://www.masterhorse.de/expertentipps/pferd/atemwegserkrankungen/lungenentzuendung-beim-pferd

“Met deze stroprijzen is de champignonteelt nauwelijks rendabel.” (2023, June 9). https://www.agf.nl/article/9536150/met-deze-stroprijzen-is-de-champignonteelt-nauwelijks-rendabel/

paardenmest afvoer prijzen - Bing. (n.d.). Bing. https://www.bing.com/search?pglt=41&q=paardenmest+afvoer+prijzen&cvid=60ac290e119247faacf914936a9850db&gs_lcrp=EgZjaHJvbWUyBggAEEUYOdIBCDU0MTNqMGoxqAIAsAIA&FORM=ANNTA1&PC=HCTS

pferde.fermenter-technologies.com | Pferdemist verwerten mit PÖTTINGER Fermenter. (n.d.). https://pferde.fermenter-technologies.com/

Pferdehaltung- Mistlagerung-Misthaufen. (n.d.). http://www.pferdeschuppen.de/pferdehaltung_mistlagerung.htm#:~:text=Als%20Faustformel%20k%C3%B6nnen%20pro%20Pferd%20und%20Tag%20eine,Monates%20unterzubringen%2C%20wenn%20dieser%202m%20hoch%20gestapelt%20wird.

Prišenk, J., Grgić, I., Rozman, Č., Pažek, K., & Janžekovič, M. (2018). Feasibility analysis of different bedding materials for horses. *Journal of Applied Animal Research*, 798–803. https://doi.org/10.1080/09712119.2017.1403919

Rijksdienst voor Ondernemend Nederland. (2024). Mestbeleid 2024 Tabellen. In Rijksdienst Voor Ondernemend Nederland. Retrieved January 5, 2024, from https://www.rvo.nl/onderwerpen/mest/tabellen#mestproductie-oudere-tabellen

Rowe, S. P., Godden, S., Royster, E., Timmerman, J., Crooker, B. A., & Boyle, M. H. (2019). Cross-sectional study of the relationships among bedding materials, bedding bacteria counts, and intramammary infection in late-lactation dairy cows. *Journal of Dairy Science*, *102*(12), 11384. https://doi.org/10.3168/jds.2019-17074

Saalschmidt, F. L. (2017). Untersuchungen über verdauungsphysiologische Effekte eines Probiotikums aus Enterococcus faecium und Lactobacillus rhamnosus bei Pferden: Inaugural-Dissertation zur Erlangung des Grades eines Doktors der Veterinärmedizin an der Freien Universität Berli. Refubium, 3856, 11–33. https://refubium.fu-berlin.de/bitstream/handle/fub188/8302/Diss_online_Saalschmidt.pdf

Sauvé, F. (2021). Staphylococcal cutaneous infection in horses: From the early 2000s to the present. The Canadian Veterinary Journal = La Revue Veterinaire Canadienne, 62(9), 1001–1006. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8360305

Schwintzer, C. R., Tjepkema, J. D., & Seekins, B. (2002). Nitrogenase activity in composting horse bedding and leaves. *Plant and Soil*, *242*(2), 277–282. https://doi.org/10.1023/a:1016254029856

Sectorraad Paarden. (2019). Gids voor Goede Praktijken: Richtlijnen voor het houden van en de omgang met paarden. In Sectorraad Paarden. SRP Sectorraad Paarden.

Seedorf, J., Schröder, M., Köhler, L., & Hartung, J. (2007). Suitability of biocompost as a bedding material for stabled horses: respiratory hygiene and management practicalities. *Equine Veterinary* Journal, 39(2), 129–135. https://doi.org/10.2746/042516407x170085

Stijgende stro- en ruwvoerprijzen. (2023, December 4). FNRS. https://www.fnrs.nl/nieuws/425-stijgende-stro-en-ruwvoerprijzen

Stroh in Zeiten der Dürre: Das macht den Preis 2022 | agrarheute.com. (2022). Agrarheute. https://www.agrarheute.com/markt/stroh-zeiten-duerre-macht-preis-2022-596020

Siegers, E., Anthonisse, M., Van Eerdenburg, F., Van Den Broek, J., Wouters, I. M., & Westermann, C. (2018). Effect of ionization, bedding, and feeding on air quality in a horse stable. *Journal of Veterinary Internal Medicine*, *32*(3), 1234–1240. https://doi.org/10.1111/jvim.15069

Sorter, D., Kester, H., & Hogan, J. (2014). Short communication: Bacterial counts in recycled manure solids bedding replaced daily or deep packed in freestalls. Journal of Dairy Science, 97(5), 2965–2968. https://doi.org/10.3168/jds.2013-7814

Szabo, E., Schäfer, I., & Büscher, W. (2004). Staubfreisetzung von Einstreumaterialien in der Pferdehaltung. *Landtechnik*, 336–337. https://doi.org/10.15150/lt.2004.1371

top agrar online. (2018, September 4). Top Agrar Online. https://www.topagrar.com/rind/aus-dem-heft/guelle-einstreu-bleiben-die-euter-gesund-9674610.html

top agrar online. (2022, January 3). Top Agrar Online. https://www.topagrar.com/rind/aus-dem-heft/was-die-guelle-separation-in-der-praxis-bringt-9657088.html

Universitair Veterinair Diagnostisch Laboratorium (UVDL). (n.d.). Universiteit Utrecht. Retrieved August 26, 2023, from https://www.uu.nl/uvdl

Uzal, F. A., Arroyo, L., Navarro, M. A., Gómez, D. E., Asín, J., & Henderson, E. E. (2021). Bacterial and viral enterocolitis in horses: a review. Journal of Veterinary Diagnostic Investigation, 34(3), 354–375. https://doi.org/10.1177/10406387211057469

Valacon-Dairy. (2014). De vaste fractie van gescheiden mest als biobedding in ligboxen van melkvee: Aandachtspunten en tips voor de praktijk. In Nizo food research, Agriconnect.

Van Den Broek, W. (2010). Scheiden van drijfmest Afzet bepaalt succes: Afzet bepaalt succes. Veehouderij Techniek, 67, 8–11. https://edepot.wur.nl/67

Vetlexicon. (2023, August 14). Enterobacter / Aerobacter spp. https://www.vetlexicon.com/equis/microbiology/articles/enterobacter-aerobacter-spp/

Woolcock, J. B., Mutimer, M. D., & Farmer, A. T. (1980). Epidemiology of Corynebacterium equi in horses. Research in Veterinary Science, 28(1), 87–90. https://doi.org/10.1016/s0034-5288(18)32778-4

Yarnell, K., Bon, M. L., Turton, N., Savova, M., McGlennon, A., & Forsythe, S. M. (2017). Reducing exposure to pathogens in the horse: a preliminary study into the survival of bacteria on a range of equine bedding types. Journal of Applied Microbiology, 122(1), 23–29. https://doi.org/10.1111/jam.13298