Aus dem Institut für Tierernährung
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

und

dem Bundesinstitut für Risikobewertung

Bedding and enrichment materials for pigs – a risk for food safety?

Inaugural-Dissertation
zur Erlangung des Grades einer
Doktorin der Veterinärmedizin
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vorgelegt von
Felicitas Koch
Tierärztin aus Dresden

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Chapter IV

List of abbreviations

ADFI	Average daily feed intake
ADG	Average daily gain
AIA	Acid insoluble ash
ALARA	As Low As Reasonable Achievable
BW	Body weight
CF	Complete feed
C_{25}	*n*-pentacosane
C_{27}	*n*-heptacosane
C_{29}	*n*-nonacosane
C_{31}	*n*-hentriacontane
C_{33}	*n*-tritriacontane
C_{36}	*n*-hexatriacontane
dl-PCBs	Dioxin-like polychlorinated biphenyls
DM	Dry matter
DPS	De-inked paper sludge
EFSA	European Feed Safety Authority
EU	European Union
FAO/WHO	Food and Agriculture Organization / World Health Organization
F/G	Feed conversion ratio
GC-FID	Gas chromatography coupled to a flame ionization detector
ICP-MS	Inductively coupled plasma mass spectrometry
KED	Kinetic energy discrimination
LOD	Limit of detection
LOQ	Limit of quantification
ME	Metabolizable energy
MI	Total material intake
MTL	Maximum tolerable level
NC	Negative control group
OM	Organic matter
OMD	Organic matter digestibility
PAHs	Polycyclic aromatic hydrocarbons
PCBs	Polychlorinated biphenyls
PCDD/Fs	Polychlorinated dibenzo-p-dioxins and dibenzofurans
PC3/5	Positive control group receiving a diet with 3 and 5% disinfectant powder
PC10/20	Positive control group receiving a diet with 10 and 20% peat
Py-FIMS	Pyrolysis field ionization mass spectroscopy
RMS	Recycled manure solids
TF	Total amount of faeces
TII	Total ion intensity
TiO_2	Titanium dioxide
TPeat/TPow	Treatment group with free access to peat and disinfectant powder
VDLUFA	Association of German Agricultural Analytic and Research Institutes

Definitions

Bedding material

Bedding material can be defined as a subgroup of enrichment material. It is of organic or inorganic origin and its use in animal stables intends to fulfil three main functions: 1) absorbing moisture from excreta to maintain a clean and hygienic environment; 2) providing lying comfort for animals and 3) enable animals to perform natural exploration behaviour using bedding material as enrichment material.

Enrichment material

Enrichment material is a material or substrate that enables animals to perform natural exploration behaviour (EU 2016) and thus attempts to improve animal welfare. Encouraging pigs to perform natural behaviour, enrichment material increases positive interaction of pigs with their environment (exploration) and decreases and prevents behavioural disorders (Mkwanazi et al. 2019). It should ideally be edible and chewable and allow investigation and manipulation activities (EU 2016). Council Directive 2008/120/EC requires that adequate enrichment material must be readily accessible for all pigs. Its use must not compromise animal health (EU 2009a). Enrichment materials to use may be straw, hay, wood, sawdust, mushroom compost, peat or a material combination (EU 2009a). Additionally, this could be all other materials, natural or synthetic, within the pen that might be investigated and manipulated from pigs, including also feed and bedding material.

Introduction

In commercial pig husbandry, pigs receive diets with high nutrient and energy concentration, reducing the time spend actually eating. Poor structure of feed (e.g. mash, liquid or homogenous pellets) and housing on slatted floors cannot fulfil the animals’ need to perform foraging and oral exploration behaviour. If these behavioural needs of pigs are not met, the behaviour could be redirected toward pen equipment or even pen mates. To prevent behavioural disorders such as ear and tail biting, enrichment materials are essential in pig farming.

The European Union (EU) requires appropriate materials accessible to all pigs. They enable pigs to perform natural rooting and exploration behaviour and thus enhance animal welfare. Straw as bedding material is generally understood as ‘gold standard‘ in animal husbandry. Additionally, a wide range of other materials is available on the market with intended use in livestock farming. This includes materials for investigation and manipulation (e.g. hay, wood, peat), bedding and absorption of moisture (e.g. sawdust, biochar) and even disinfection (e.g. disinfectant powders).

Farm animals and especially pigs explore the provided materials intensively. Thereby, they do not distinguish between the intended uses (e.g. environmental enrichment or disinfection) and investigate, manipulate, bite and chew the materials provided. Regardless of the material’s palatability, this may lead to its oral intake. However, bedding and enrichment materials are available on the market without necessarily being tested for their safety (e.g. contents of possibly toxic substances) and beneficial and adverse effects on livestock or food safety. Information on the composition and ingredients of the respective materials are not required. Accordingly, it is unknown whether the use of various bedding and enrichment materials might have an impact on food safety when ingested by pigs.

It was hypothesized that some of the above-mentioned materials contain levels of toxic metals, trace elements, dioxins and polychlorinated biphenyls (PCBs) exceeding maximum levels that are set for animal feed. Furthermore, the potential risk for food safety is relevant only if respective materials are actually consumed by the animals. Therefore, it was hypothesized that material consumption by pigs is independent of their material preference. Finally, investigations to quantify the material intake by pigs are necessary to complete the risk characterization and risk assessment. Knowledge of material composition and intake by pigs enables exposure assessment of animals and consumers. Risk characterization is then the next step in the risk assessment of potential risk sources originating from bedding and enrichment materials.

Results of these studies are supposed to increase the awareness for potential risks from bedding and enrichment materials used in pig husbandry. Thus, appropriate measures can be taken, such as labelling requirements or even a classification of bedding and enrichment materials as animal feed, whereby regulations of the feed and food laws could be applied. This enables a safe use of bedding and enrichment materials.

The results of the current thesis are presented and discussed in three consecutive manuscripts in **Chapter II, III and IV**.

Chapter I

Literature review

Importance of bedding and enrichment materials for pig welfare

Animal welfare may be defined as well-being of animals under environmental conditions where they are free to act out their natural behavioural repertoire being free of hunger and thirst, discomfort, pain, injuries and diseases as well as free from distress (Koknaroglu and Akunal 2013; EC 2022). Based on these ‘Five Freedoms’, the EU established general rules to ensure animal welfare for all farm animals (Council Directive 98/58/EC) (EU 1998). The natural behavioural repertoire of pigs has been highly conserved during domestication and includes three main categories: foraging, social interaction and resting behaviour. In free-range husbandry, foraging accounts for up to 80% of the total daily activity time, although time for actual feed intake is rather low (Wechsler et al. 1991; Mayer et al. 2006). In commercial pig husbandry, feed is offered directly in a trough in low quantity (due to an optimised nutrient and energy concentration) and its consumption takes only a few minutes (Colpoys et al. 2016; Adrion et al. 2018). Even though the diet complies with nutritional and energy requirements of pigs, feed intake itself does not meet the pigs’ need to perform extensive foraging behaviour (Holm et al. 2008).

Multiple studies reported beneficial effects of adequate bedding and enrichment materials on the well-being of animals: it positively affects behaviour of pigs by reducing aggressive and harmful behaviour towards pen mates (Blackshaw et al. 1997; Beattie et al. 2001; Bolhuis et al. 2005; Taylor et al. 2010). Studies comparing pigs in enriched compared to barren environments revealed less aggressive behaviours when adequate substrates for manipulation were provided (Blackshaw et al. 1997; Bolhuis et al. 2005). Also, D'Eath et al. (2014) concluded in a comprehensive review, that the provision of adequate materials for foraging and exploration considerably contributes to reduced tail biting. Generally, tail biting is understood as a multifactorial event but may be enhanced by aggressive behaviour. This initially redirected explorative behaviour (Taylor et al. 2010) results in painful tail lesions compromising animal welfare and inducing economic losses. The prevalence of all tail damage in pig husbandry was found to be as high as 28 and 35% and clearly related to the occurrence of abscesses and arthritis in carcasses (Valros et al. 2004; Kongsted et al. 2020). Thus, any tail damage increases the risk of economic losses due to carcass condemnation as well as medical treatments and possibly increased mortality. Niemi et al. (2011) estimated the yearly costs caused by tail biting at a prevalence level of 5 - 12% to be 4 000 - 10 000 € per 1 000 pig spaces.

Taylor et al. (2010) reviewed multiple studies and concluded that assuming all pigs within a group to be in a good health status, receiving a balanced diet and resources, such as feed and water being equally accessible to all individuals, initial tail biting (non-damaging) can be considered as part of natural exploration behaviour. Thus, appropriate materials for manipulation and investigation can reduce redirected oral exploration activities towards pen mates (Beattie et al. 2001; Taylor et al. 2010; EFSA 2022b).

Furthermore, bedding and enrichment materials effectively reduce stress levels in pigs. DeBoer et al. (2015) could show decreased blood cortisol levels in pigs in an enriched environment compared to pigs in a barren environment over a seven-day period. Long-term barren housing resulted in a cortisol depletion as seen in pigs suffering from chronic stress (de Jong et al. 2000). To comply with the pigs' behavioural needs and ensure animal welfare the EU requires appropriate materials to perform exploration activities readily available to all pigs (Council Directive 2008/120/EC) (EU 2009a).

Legal framework

Minimum husbandry standards for pigs

In the EU, the European Council has defined minimum standards for the protection of pigs (Council Directive 2008/120/EC) (EU 2009a) including regulations for enrichment and bedding materials, complemented by Commission Recommendation (EU) 2016/336 (EU 2016). Accordingly, appropriate and safe material for investigation and manipulation must be provided to all pigs in an adequate amount. Recommended enrichment materials are straw, hay, wood, sawdust, spent mushroom compost or peat, used alone or in combination (EU 2009a; EU 2016). Ideally, the materials are chewable and edible, while containing beneficial nutrients. Furthermore, they should sustain the pigs' interest to perform exploration activities (manipulation, investigation), be readily accessible as well as clean and hygienic (EU 2016). In Germany, materials are further required to be organic and rich in fibre (BMEL 2021b). Depending on how many of the recommended characteristics are met, materials can be classified as optimal, suboptimal or materials of marginal interest. Materials of the two latter categories should be complemented by materials providing missing characteristics (EU 2016) (Table 1).

Table 1 Enrichment materials listed in Annex I of the Commission Working Document accompanying Commission Recommendation (EU) 2016/336.

Materials	Level of interest[1]	Characteristics	Should be complemented
Bedding materials			
Straw, hay, silage, miscanthus, root vegetables	+++	Edible, chewable, manipulable, investigable	
Mushroom compost, peat	++	Chewable, manipulable, investigable	✓
Shredded paper	++	Chewable, manipulable, investigable	✓
Wood shaving	++	Chewable, investigable	✓
Soil	++	Manipulable, investigable	✓
Sawdust	++	Manipulable, investigable	✓
Sand and stones	++	Manipulable, investigable	✓
Rack feed or dispenser			
Straw, hay or silage	++	Edible, chewable	✓
Pellet dispenser	++	Depending on the amounts of pellets provided	✓
Objects			
Compressed straw in cylinder	++	Edible, chewable	✓
Soft, untreated wood, cardboard, natural rope, hessian sack	++	Chewable, manipulable	✓
Sawdust briquette (suspended or fixed)	++	Chewable	✓
Chain, rubber, soft plastic pipes, hard plastic, hard wood, ball, salt lick	+	Should be complemented by optimal or suboptimal materials	✓

[1] Optimal (+++), suboptimal (++) and marginal (+) level of interest as enrichment materials

Legal classification of recycled manure solids as bedding material

Challenging in terms of its use as bedding material is the legal classification of recycled manure solids (RMS). The RMS are usually produced on-farm to reduce the amount of stored manure and produce a low-cost bedding material increasing the lying comfort for cows (Green et al. 2014; Leach et al. 2014). Yet, no studies report the use of RMS in pig husbandry. Regulation (EC) No 1069/2009 on animal by-products and derived products includes manure as category 2 material (EU 2009c). Commission Regulation (EC) No 142/2011 implementing the before mentioned regulation states that category 2 material and derived products shall not be fed to farm animals with exception of fur animals (EU 2011). Further use (e.g. as animal bedding) is not specified but may be possible after an assessment by the European Feed Safety Authority (EFSA) on alternative methods of use (EU 2009c). Operators have to ensure the control of risk to public and animal health if a derived product is placed on the market (EU 2011). Considering bedding materials equal to feed as suggested by FAO and WHO (2004) the use of RMS as bedding material would be unacceptable because of its main components faeces and urine, being prohibited as feed materials (Regulation (EC) No 767/2009 on the placing on the market and the use of feed) (EU 2009b).

Risk assessment for bedding and enrichment materials

Risk assessment of a (potential) hazard includes four steps: identification of a hazard, characterisation of the hazard, assessment of exposure and finally risk characterisation (BfR 2022). In feed, any substance presenting a potential risk to animal and consumer health or the environment is defined as undesirable substance and accordingly identified as risk and characterized as hazard for feed and food safety (EU 2002a; EU 2002b). Thus, Directive 2002/32/EC on undesirable substances in animal feed sets maximum levels of contaminants in feed (EU 2002a). However, no EU legislative act on undesirable substances in bedding and enrichment materials exists, although these materials should ideally be chewable and edible (EU 2016). The Codex Alimentarius, providing international standards for food to ensure consumers' health, includes the safety of feed for food producing animals (FAO and IFIF 2010). By pointing out that livestock usually consume a part of their bedding material, the Codex encourages to treat bedding materials the same as feed ingredients (FAO and WHO 2004). Consequently, the Codex Alimentarius also includes bedding materials in its animal exposure assessment as part of the risk assessment for feed (FAO and WHO 2013).

Assuming the presence of undesirable substances in bedding and enrichment materials intended for the use in stables of food producing animals and that feed law regulations also account for bedding and enrichment materials, the following guidelines would be applicable to regulate heavy metals and environmental contaminants: Directive 2002/32/EC concerning maximum levels of undesirable substances (EU 2002a), Commission Regulation (EC) No 1334/2003 concerning maximum levels of trace elements (EU 2003), Commission Regulation (EC) No 277/2012 concerning maximum levels of dioxins and polychlorinated biphenyls (EU 2012) and Regulation (EC) No 396/2005 on maximum levels of pesticides (EU 2005). For some other environmental contaminants such as polycyclic aromatic hydrocarbons (PAHs) no regulations currently exist. Regarding PAHs, it is generally assumed that these substances are not transferred from feed to animal products as poor absorption and/or no effective metabolism occurs. Thus, the German Federal Ministry of Food and Agriculture recommends refraining from regulating maximum levels of PAHs in animal feed (BMEL 2004). However, benchmarks for PAHs in feed are available (QS 2022). Undesirable substances in feed also include natural toxins (e.g. plant alkaloids and mycotoxins) (EFSA 2022a) but are not object of the current investigations. The adoption of feed law regulations on bedding and enrichment materials remains hypothetical. However, in Germany, the Food and Feed Code (LFGB) involves a paragraph authorizing national authorities to restrict the use and market placement of materials intended for the use by food producing animals, if an uptake by animals cannot be ruled out and a risk for food safety by potentially toxic substances in those materials cannot be excluded (BMEL 2021a).

Bedding and enrichment materials

Ideal bedding and enrichment materials comply with animals' needs, farmers' requirements and consumers' expectations including an increasing interest in animal health and welfare. Appropriate materials enable animals to express natural behaviours such as foraging and exploration, nest-building, dustbathing or even chewing. Furthermore, dry, soft, deformable and slip-resistant bedding increases animal comfort. Besides animal welfare, hygienic aspects, such as material quality (low dust-burden, low initial microbial population) and water absorption capacity, are important for animal health and on-farm management. Farmers intend to use a material with high economic efficiency. This includes low costs, easy material handling, suitability for multiple animal species and further usage of bedding as organic fertilizer. All together aims at providing animal products of good quality meeting consumer expectations. Based on their composition, bedding and enrichment materials can be classified as organic or inorganic materials. Here, different advantages and disadvantages of available materials are presented.

Organic bedding materials

Straw

Straw has been a common bedding and enrichment material for centuries and its presence in stables is commonly associated with high animal welfare. It is usually considered as 'gold standard' to alleviate or prevent behavioural disorders in pigs (Van de Weerd et al. 2006; Studnitz et al. 2007; Chou et al. 2018). Studnitz et al. (2007) proposed straw as reference or baseline material in order to rank the preference of pigs for other materials above or below their preference for straw. Besides beneficial effects on pigs' behaviour, straw bedding has been demonstrated to improve pig health by reducing incidences of claw and leg injuries and lowering the prevalence of stomach and intestinal disorders due to its high fibre content, finally reducing mortality (EFSA 2014). In cereal production areas straw is a low-cost material and readily available, whereas in areas with low or no cereal production the amount of straw required for animal bedding cannot be provided. Straw can block slatted flooring and liquid manure handling systems, though alternatively chopped straw may be provided in lower quantities several times daily. Nevertheless, purchase of straw from other areas and its necessary ongoing replenishment increases labour and costs (Tuyttens 2005; Chou et al. 2018). Additionally, straw has been shown to support microbial growth in the stable due to its optimal carbon:nitrogen ratio (Zehner et al. 1986; Ward et al. 2000). With regard to dryness of the lying area, barley straw is more absorbent than other types of straw, but generally the absorptive capacity of straw is low (300%) compared to wood shavings (400%) or deinked paper sludge (up to 700%) (Beauchamp et al. 2002; Rübcke 2011). Mycotoxins were found in most samples collected from pig farms and were considerably high in some samples. Only straw of good quality should be used in animal housing as pigs are especially sensitive to mycotoxicosis (Edwards and Stewarts 2010; Dänicke and Diers 2013).

Peat and spent mushroom compost

Studies showed that pigs prefer materials with a texture and moisture content comparable to earth over straw, hay, wood or toys as enrichment material (Beattie et al. 1998; Beattie 2001; Pedersen et al. 2005; Jensen et al. 2008).

Peat is a soft material improving lying comfort and thus preventing bruises. It absorbs humidity and odorous substances (Trckova et al. 2005; Tuyttens 2005). In addition to its high qualities as bedding material, it encourages piglets to perform explorative behaviour (Vanheukelom et al. 2011). Due to its heterogeneous and chewable structure peat might be as attractive as feed (Studnitz et al. 2007; Vanheukelom et al. 2011). Indeed, peat is currently registered in the list of feed materials (Commission Regulation No 68/2013 on the Catalogue of feed materials) (EU 2013). Thus, besides straw it is the only material officially used as bedding and enrichment material and animal feed and, when intended as feed, is supposed to comply with the restrictions on the use of feed materials in accordance with the relevant legislation of the EU. Due to its content of non-saturated fatty acids, low pH (3.0 - 5.5) and high contents of humic acids, positive effects on the immune system, metabolism and intestinal microbiota as well as growth performance have been reported (Trckova et al. 2005). Furthermore, peat is often supplemented with iron in order to prevent piglets from iron deficiency anaemia in their early stages of life (Victor and Mary 2012; Yefimov et al. 2017). Knowledge about undesirable effects of peat is limited. However, peat has been identified as source of mycobacteria, causing tuberculosis in cattle and pig, and thus can be considered as a risk raw material (Matlova et al. 2005; Pavlik et al. 2005a; Pavlik et al. 2005b; Johansen et al. 2014). In addition, mining for peat raises environmental concerns (Durrell et al. 1997; Trckova et al. 2005). Carbon that is stored in peat is released as carbon dioxide, a greenhouse gas, into the atmosphere (BLE 2022).

Mushroom cropping remains, called spent mushroom compost, vary considerably in particle size. This material is pasteurized at 60°C for seven days, resulting in a low microbiological contamination (Beattie et al. 2001; Uzun 2004). Spent mushroom compost is commercially available as bedding material of heterogeneous texture, which improves lying comfort and serves as enrichment for pigs (Beattie 2001; Beattie et al. 2001; Tuyttens 2005). No disadvantages or negative effects of spent mushroom compost are mentioned in the literature. However, spent mushroom compost might be more expensive than other materials (Uzun 2004).

Wood shavings, sawdust and lignocellulose

Sawdust and wood shavings can be used as bedding and enrichment materials in pig husbandry. The provision of wood pieces, especially in fully slatted systems, has been demonstrated to reduce harmful behaviour like ear and tail biting, when loose bedding cannot be provided (Telkänranta et al. 2014; Chou et al. 2018). Softer woods, such as pine, spruce, cedar, fir and larch, are more destructible than harder woods like oak, maple or birch. They are less durable but attract the pigs' attention more often and for a longer period compared to hard woods (Van de Weerd et al. 2003; Froberg-Fejko and Lecker 2012; Chou et al. 2018). Per se untreated woods may contain substances that inhibit bacterial growth (terpenes and resin acids in softwoods) but also aromatic hydrocarbons which are possibly toxic (liver damaging effect in rodents and rabbits), if consumed over a longer period of time (Vesell 1967; Zehner et al. 1986; Froberg-Fejko and Lecker 2012). Fresh wood may not be readily available and thus recycling waste wood from construction as bedding for animals might be an alternative. However, the insufficient removal of contaminants in recycled waste wood, such as wood preservatives, organochlorine insecticides, dioxins and heavy metals, can lead to an accumulation in the animals' body and pose a potential risk to animal health and food safety of animal products (Asari et al. 2004).

Lignocellulose pellets isolated from small wood particles, are a wooden material predominantly used to reduce foot pad dermatitis in poultry as it is soft and has a higher water binding capacity than straw or wood shavings (Youssef 2011). The use in commercial pig husbandry has not been reported, possibly due to costs.

Paper materials

In areas with little cereal and straw production, an alternative for farmers is to use de-inked paper sludge (DPS) as bedding material for animals (Beauchamp et al. 2002). It is a waste material of the paper industry mostly consisting of cellulose, which does not support bacterial multiplication (Ward et al. 2000; Reneau et al. 2002). Its water absorptive capacity is higher than the absorptive capacity of straw and wood shavings. To avoid a too closely packed layer with a reduced water holding capacity and to simplify the handling, DPS should not exclusively be used, but in a combination with other materials (Beauchamp et al. 2002). Paper itself can be an appropriate material to enrich the pigs' environment, e.g. in form of cardboard, as it is chewable, rootable and can be carried around. However, paper materials quickly become soiled and only a short-term use to treat acute behavioural disorders or a continuous replenishment with clean material should be considered (Fàbrega et al. 2019). To secure animal health and welfare as well as food safety, the paper should not be printed. Ink is a source of mineral oil saturated and aromatic hydrocarbons (Raters and Matissek 2012). These substances easily accumulate in the body and cause liver toxicity, among other things.

Biochar

Biochar is charcoal produced from pyrolysis (350 - 1 000°C) of biomass in an oxygen-limited environment under environmentally sustainable conditions. This heterogeneous substance is rich in aromatic carbon and minerals (Guo et al. 2016a; EBC 2022). Use of biochar for agricultural purposes augmented notably in recent years (Man et al. 2021; EBC 2022). In animal farming, biochar is implemented as bedding material, silage additive, slurry or manure conditioner and feed and, alike peat, registered in the Catalogue of feed materials (EU 2013; Kammann and Schmidt 2014; Guo et al. 2016a; Guo et al. 2016b). In its capacity of binding ammonia, ammonium, methane emissions and other odorous or toxic substances, biochar is used for slurry treatment to retain nitrogen and improve the manure's fertilizing effect. To optimize nutrient retention and reduction of greenhouse gas emissions, it is recommended to apply biochar already in the animal bedding (Kammann and Schmidt 2014; Schmidt 2022). It helps to quickly decompose animal excretions and to limit odours in the stable. Farmers also noted a reduction of hoof problems in horses, possibly due to an increased absorptive capacity of liquids, when biochar was applied as mix with other bedding materials, e.g. straw (O'Toole et al. 2016). Generally, biochar has a good adsorption potential for hydrophilic (e.g. antibiotics such as cephalosporins) as well as lipophilic substances (PAHs, dioxins) and can therefore be used to sequester undesirable substances from the environment (Oleszczuk et al. 2012; Mitchell et al. 2015). Its adsorption capacity increases with the surface area and is greatest in biochar produced at high temperatures (Chen et al. 2012; Fang et al. 2014). Mitchell et al. (2015) reported that biochar, especially if made from pinewood and produced at temperatures higher than 500°C, is capable to sequester antibiotic residues from manure. Biochar may effectively immobilize chemical substances and numerous heavy metals making them unavailable for plant uptake and the food chain (Park et al. 2011; EBC 2012).

When used as feed, positive effects on animal performance, binding of toxic substances and reduction of methane emission, have been reviewed and reported in several studies (Toth et al. 2015; Man et al. 2021). Improved feed intake and digestibility led to a greater weight gain and better quality of meat (e.g. colour, marbling, tenderness and storage life). Schmidt et al. (2019) reviewed 112 scientific papers and concluded that feeding biochar is safe as significant negative effects on animal health were not identified. However, Kana et al. (2011) found that charcoal in feed might depress feed intake and weight gain in broiler chickens. Further, biochar is a precursor of activated charcoal (Azargohar and Dalai 2006). In veterinary medicine this carbon adsorbent it is mainly used to treat animals with diarrhoea (Volkmann 2018) or as antidote in case of intoxication (Man et al. 2021). However, Cabassi et al. (2005) identified a poor detoxifying effect of activated carbon in piglets fed a mycotoxin contaminated diet.

Recycled manure solids

Many livestock farms produce more manure than can be applied on their own farmland and alternative uses of slurry need to be established. Especially on cattle farms RMS are a possibility to produce a low-cost bedding material, readily available on-farm, which at the same time, reduces the volume of stored manure (Leach et al. 2014; Fournel et al. 2019). Although the use of RMS, commonly called 'green bedding', is controversial and is still in its early development stages, many benefits have been reported for its use in dairy cattle barns, including reduced costs, increased cow comfort and cow cleanliness, reduction of stored manure and easy handling (Green et al. 2014; Leach et al. 2014). Adamski et al. (2011) reported a preference of cows for RMS compared to sand, sawdust and straw. This 'green bedding' has a high absorptive capacity of moisture and the dust burden for farm workers decreases (Green et al. 2014; Leach et al. 2014). In addition, slatted floors and manure drains are less likely to be obstructed due to the manures' improved flow characteristics compared to other bedding materials. However, compared with other bedding materials the bacterial load was highest in unused and considerably higher in used 'green bedding', as bacteria quickly multiply once the bedding is spread in stables (Green et al. 2014; Fournel et al. 2019). As composting of RMS promotes growth of spore-forming, heat-resistant bacteria, composting or anaerobic digestion should be avoided and RMS should be used within 12 hours of its production. To prevent the transfer of pathogens (e.g. *Mycobacterium avium* ssp. *paratuberculosis*), RMS needs to be produced and spread out on the same farm and in the same unit as the originating manure. The 'green bedding' should not be used in calving areas, transition cow accommodation and in units with cattle younger than 12 months. Only excreta from adult cattle should be used for the production of RMS, excluding slurry from calving, hospital and isolation pens. In addition, the use of manure from another species should strictly be avoided, requiring a separate collection of different manures (Green et al. 2014; APHA 2017). Because little is known about the transfer of bacterial populations from bedding to milk, milk should be pasteurized before consumption and not be used in raw milk products (Green et al. 2014; APHA 2017). No studies using RMS in pig housings were found.

Inorganic bedding materials

Disinfectant powder

Regular washing and disinfection in livestock and especially pig farming is crucial to limit spread and growth of (zoo-)pathogens. To optimize stable hygiene between periodic basic cleanings, farmers can use disinfectant powders. However, data on the use of disinfectant powders in pig husbandry are very limited. On dairy farms Kristula et al. (2008) could show that hydrated limestone significantly decreased mastitis causing bacteria in organic bedding material for dairy cows but caused skin irritations at a rate of 0.5 kg limestone per mattress every 48 hours. Schou and Permin (2003) identified a disinfectant powder as a possible alternative to control parasites in poultry when used on a weekly basis. Generally, the use of disinfectant powders for different livestock (pigs, ruminants and poultry) in multiple production areas (e.g. farrowing section, fattening section, lying areas for cows) or even on the animal itself is advertised. The powders can be spread daily to improve stable hygiene or during certain periods requiring a higher hygienic standard (e.g. when piglets are suffering from diarrhoea or during insemination of sows). In Germany, quality labels for disinfectant powders are based on characteristics such as water binding capacity, corrosion properties, hygienic characteristics and skin compatibility (DLG 2022).

Main compounds of most disinfectant powders are silicates – inorganic silicon dioxide compounds, which have a high absorptive capacity. Studies of Wattanaphansak et al. (2009) and Gongora et al. (2013) have shown that these powders can reduce the number of microorganisms significantly, depending on the dosage and time of exposure. However, Rübcke (2011) could not prove a significant influence of disinfectant powders on bacteria in bedding materials of cows. In order to obtain a decreased number of bacteria, viruses, fungi and parasites over a longer time period, disinfectant powders need to be spread on a regular basis, increasing labour and costs (Wattanaphansak et al. 2009; Gongora et al. 2013). Probably the most sanitizing effect of these powders is the absorption of residual moisture. In a dry environment, growth of microorganisms is poor and air quality improves as production of ammonia is reduced (Trckova et al. 2004; Wattanaphansak et al. 2009). Furthermore, on dry floors, pigs get a better grip with their claws and the risk of slipping, as well as injuries, decreases. However, the powdery character of disinfectant powders might cause a health risk to farmers due to inhalation of small particles during handling. Trckova et al. (2004) investigated kaolin, a siliceous material, as supplement for pigs. As feed supplement it might be capable to bind mycotoxins, enterotoxins or heavy metals among other substances and thus prevent diarrhoeal diseases in pigs. However, kaolin and other clay minerals may be a source of dioxins (polychlorinated dibenzo-p-dioxins and dibenzofurans [PCDD/Fs]). For example, clay minerals used as anti-caking agent in animal feed were causal for the contamination of poultry meat, eggs and pork with dioxins (Schmid et al. 2002; Trckova et al. 2004). Although the source of dioxins in kaolin was not identified, it appears likely that the contamination was of geogenic origin because PCDDs but not PCDFs were found (Schmid et al. 2002).

Sand

Sand is a very dry (92% dry matter [DM]) material compared to straw, wood products or RMS (88 - 38% DM). Its draining efficiency is high and even used sand provides dry lying surfaces (Hill 2000; Robles et al. 2020). In dairy farming cow cleanliness increases and claw damage decrease when cows are bedded on sand. Still, straw is the cows' preferred bedding (Norring et al. 2008). Pigs' foothold is better on sand than on bare floors reducing injuries due to slipping (Garcia and McGlone 2014). Inorganic quality of sand contributes to a low bacterial load in unused sand being lower compared to straw, wood products and RMS (Hill 2000; Bradley et al. 2015; Robles et al. 2020). Thus, heat produced by composting is minimized and sand bedding preferably used in warmer regions (Hill 2000; Honeyman 2005). However, the low absorption rate (< 15% of wheat straw) of sand-based bedding aggravates air quality due to higher ammonia concentrations (Hill 2000). Further, handling of large quantities of sand and manure disposal in sand-based bedding systems is difficult (Rübcke 2011). It is not a common practice in pig farming and only few studies report the use of sand as bedding in pig farming which are limited to warmer areas of the USA (Hill 2000; Honeyman 2005).

Aims and objectives of the thesis

Bedding and enrichment materials are essential in pig farming. In the EU they need to be readily accessible to all pigs to perform exploratory behaviour, which is strongly connected with foraging and may lead to accidental or even intended oral consumption of provided bedding and enrichment materials by pigs. Although these materials are available on the market and advertised for farm animals, information on the composition and ingredients of the respective material are not required. Knowledge about beneficial or even adverse effects (e.g. due to contents of undesirable substances according to feed law regulations) on animal health and food safety as a consequence of possible material consumption by pigs is yet limited.

The aims of the present thesis were:

- Overview and chemical composition of materials available on the market with focus on contents of compounds regulated under feed law regulations
- Evaluation of possible differences in material preference of pigs
- Quantification of oral material intake in pigs by means of different markers

Results will be presented and discussed in three consecutive manuscripts in **Chapter II, III and IV**, followed by a general discussion and conclusion in **Chapter V**.

Chapter II

Chemical analysis of materials used in pig housing with respect to the safety of products of animal origin

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Chapter III

Preference and possible consumption of provided enrichment and bedding materials and disinfectant powder by growing pigs

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Chapter IV

Peat and disinfectant powder used in swine husbandry systems – quantification of oral intake using toxic metals as potential markers

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Chapter V

General discussion and conclusion

Bedding and enrichment materials are an integral part of pig husbandry to enhance animal welfare by complying with the European regulations on the protection of pigs. Its provision is meant to allow pigs to express natural exploration behaviour and thus, can prevent redirection of this behaviour toward pen mates (e.g. tail and ear biting). To date, the focus of classifying bedding and enrichment materials for its use in animal farming, has been their contribution to animal welfare and stable hygiene. Their chemical composition has been neglected so far, although it is of utmost importance as oral exploration, such as biting, chewing and eating, might result in material consumption by pigs. Thus, bedding and enrichment materials may be of concern for animal health and food safety. To better estimate the potential risk of bedding and enrichment materials the present research had two objectives: 1) identifying the potential risk for animal health and food safety originating from bedding and enrichment materials by providing an overview of materials available on the market and their levels of compounds restricted by feed law regulations; 2) characterizing the potential hazard by conducting on farm trials to verify and quantify the oral uptake of bedding and enrichment materials by pigs.

Overview of materials available on the market and their levels of compounds regulated by feed law regulations

Materials were purchased from the manufacturer, an online-shop or collected on-farm with the assistance of German Regional Offices, Agricultural and Federal Research Institutions and Universities. Samples for analyses of toxic metals and trace elements were chosen according to their relevance on the German market (**Chapter II**). Additionally, disinfectant powders were analysed for dioxins and dl-PCBs as incidences occurred were clay minerals, main component of most disinfectant powders, were the source of dioxin contamination in animal feed (Schmid et al. 2002; Trckova et al. 2004). Disinfectant powders showed the major presence on the market (51 samples), probably due to the many beneficial characteristics advertised (e.g. positive effects on animal health and stable hygiene; see **Chapter I**) and its easy application for a wide range of animal species. Earth/peat, biochar and RMS (collected on-farm) were less abundant (12, 8 and 3 samples, respectively). In 20% of the respective samples levels of toxic elements were above limits as set for animal feed (Directive 2002/32/EC). Four percent of samples of disinfectant powder were above limits as set for dioxins and dl-PCBs in feed (Commission Regulation [EU] No 277/2012). Levels of trace elements were considerably high in samples of disinfectant powder and earth/peat. Assuming material consumption, pigs are exposed to substances additional to the regular diet that might have an impact on the food safety. Considering samples of disinfectant powder and earth/peat with highest trace element levels (Table 1), ingestion of 1% disinfectant powder and 6% peat of the total diet, respectively, would be sufficient to reach maximum levels for iron or copper in the complete diet of pigs. Maximum levels of trace elements in animal feed are established with regard to requirement variations of animals and possibly decreased element bioavailability (EU 2003). Although supply recommendations based on physiological trace element requirements of animals usually include a safety margin (Jeroch et al. 2008), most compound feeds contain trace element levels that largely exceed physiological requirements and are close to legal maximum levels (Grünewald and Staudacher 2017). In the EU, maximum levels in feed follow the ALARA principle (As Low As Reasonably Achievable), but pigs can tolerate dietary levels of iron, copper and zinc that are far above those legal maximum levels (EU 2003; NRC 2005). However, ingestion of small amounts of materials with trace element levels as high as in certain samples in the first study (**Chapter II**) could be a risk for animal health by exceeding maximum levels in feed for pigs and may even reach element levels in the total diet that are close to maximum tolerable levels (MTLs) (Table 1). Additionally, element transfer to animal derived products (e.g. copper in liver) may be a concern of food safety.

Table 1 Nutritional recommendation, EU maximum level and maximum tolerable level (MTL) for trace elements in feed for pigs; calculated material intake as percentage of the daily ration of pigs to reach EU maximum level and MTL, respectively (neglecting trace element content in feed itself).

	Iron	Copper	Zinc
		mg/kg complete feed	
Nutritional requirement + safety margin[1]	50 - 60	4 - 5	50 - 60
EU maximum level[2]	750	150[4]/25	150
Maximum tolerable level (MTL)[3]	3 000	250	1 000
Highest level[5] in		*mg/kg*	
Peat (42% DM)[6]	11 736	449	1 586
Disinfectant powder (98% DM)[6]	93 287	5 110	506
Maximum intake levels to reach EU maximum level[7]		*% of the total diet*	
Peat	6.4	5.6	9.5
Disinfectant powder	0.8	0.5	30
Maximum intake levels to reach MTL[8]			
Peat	26	56	63
Disinfectant powder	3.2	4.9	-

[1] Jeroch et al. (2008)

[2] Commission Regulation (EC) No 1334/2003 (EU 2003)

[3] NRC (2005)

[4] Piglets up to 12 weeks (EU 2018)

[5] Koch et al. (2021) – **Chapter II**

[6] Koch et al. (2023) – **Chapter IV**

[7] Calculated: 1 / highest element level [mg/kg] x EU maximum level [mg/kg] x 100 = % of the total diet

[8] Calculated: 1 / highest element level [mg/kg] x MTL [mg/kg] x 100 = % of the total diet

Similar to our findings, Hou et al. (2017) found considerable levels of heavy metals in sawdust, rice husk and straw used as animal bedding in deep-litter systems. Contents of cadmium and lead exceeded Chinese standard levels for organic fertilizers already in fresh, unused bedding material. In used bedding material, that was spread in stables and contaminated with urine and faeces, the major part (> 70%) of lead, mercury, nickel, chromium and arsenic accumulation traces back to the bedding material itself. However, accumulation of iron, copper, zinc and manganese was rather caused by the diet (Hou et al. 2017). Besides toxic metals and trace elements, occurrence and transfer of persistent organic pollutants, such as dioxins, PCBs and perfluoroalkyl substances, were investigated in recycled materials used as bedding for poultry and soil amendment in pig farming (Fernandes et al. 2019). Fernandes et al. (2019) found persistent organic pollutants in all materials, but contaminant levels were higher in recycled cardboard, dried paper sludge and biosolids than in recycled wood shavings and ashes. In the present study (**Chapter II**), levels of dioxins and PCBs in disinfectant powders were generally found at lower levels than in the study of Fernandes et al. (2019) (highest levels at 3.8 and 33 ng WHO-TEQ/kg [88% DM], respectively). Dioxins were more likely of geogenic origin, whereas PCB are usually a result of anthropogenic influences such as industrial emissions (Schmid et al. 2002; Weber et al. 2018). Generally, varying levels of dioxins and PCBs in recycled materials and disinfectant powders are likely, as no guidelines exist specifying their composition or origin. Fernandes et al. (2019) thus emphasised the importance of knowledge about material ingestion by livestock, as bedding materials present a risk of exposure of livestock to considerable levels of contaminants and might affect the safety of animal derived products. Yet, studies on the consumption of bedding and enrichment materials are rare.

In vivo experiments to verify and quantify the oral material uptake for risk assessment

Exposure assessment of pigs to contaminants in bedding and enrichment materials requires actual prove and eventually quantification of material ingestion. Studies on material consumption by pigs are scarce. Here, an observational study with 12 pigs (**Chapter III**) was conducted to test the pigs' preference for disinfectant powder, peat, biochar and straw (reference material) and subsequently consumption of the respective materials. Pigs most preferred peat and biochar but consumed at least a portion of all materials. Thus, even exploration behaviour that not necessarily intends feeding leads to consumption of bedding and enrichment materials to a certain extent.

Day et al. (1996) concluded that initial exploration may lead to consumption when materials are palatable and even more, when pigs receive nutritional feedback. Accordingly, in the second study, it was found that explorative chewing behaviour led to consumption, as *n*-

alkanes, which naturally occur in peat, were detected in pig faeces. Even while exploration of the least preferred material disinfectant powder, consumption occurred and AIA originating from disinfectant powder was detected in pig faeces. Usually, *n*-alkanes and AIA are used to quantify DM-intake, digestibility and faecal output (Bachmann et al. 2016; Garrett et al. 2020; Prawirodigdo et al. 2021). These common marker techniques focus on dietary compounds which are ideally stable, inert, non-absorbable, non-toxic and have a high faecal recovery rate when passing the gastrointestinal tract (Owens and Hanson 1992; Sales 2012). They are labour intensive requiring complex or total faecal collection (van Barneveld 2012; Garrett et al. 2020). Still, such markers may also be reliably used to detect intake of bedding and enrichment materials by pigs. Van Barneveld (2012) provides quantitative bedding material intake levels for pigs kept on deep-litter bedding, that are based on *n*-alkane analysis. Also, for estimating soil intake by pigs and poultry both, *n*-alkanes and AIA, were successfully used (Fries et al. 1982; Jurjanz et al. 2015).

Material consumption was also shown as contaminants from materials were found in animal tissues of pigs and poultry and eggs of laying hens (Fernandes et al. 2019; Schulze et al. 2022). The approach to prove ingestion of a substrate by measuring contaminants originating from the respective substrate in animal tissues is rarely considered but was previously published by Hansen et al. (1981). The authors investigated soil consumption by pigs kept on sewage sludge amended soils and accordingly reported an accumulation of cadmium and pesticides in kidney, liver and spleen. Still, neither previous (Hansen et al. 1981) nor recent studies (Fernandes et al. 2019) provide quantitative material intake measurements or even estimations. However, to further characterize the risk for animal and consumer health and food safety, material intake estimations are crucial.

To quantitatively estimate material intake by pigs, the third study included a multi-marker approach (**Chapter IV**). Initially, the dietary internal markers *n*-alkanes and AIA, known to be indicative for consumption of peat and disinfectant powder (**Chapter III**), and additionally titanium dioxide as external marker added at a rate of 5 g/kg to disinfectant powder in the treatment group with free access to disinfectant powder (Jagger et al. 1992; Pieper et al. 2016), were used to verify material consumption. To eventually quantify material ingestion by pigs, the new approach presented uses contaminants (toxic metals) as dietary markers, which are known to accumulate in the animal tissues (Hansen et al. 1981; Hoogenboom et al. 2014; Fernandes et al. 2019). Therefore, voluntary consumption of peat and disinfectant powder has been investigated in a trial with 28 pigs. The toxic metals cadmium, arsenic and lead in peat and disinfectant powder were used as markers for consumption. Considering element levels in tissues of pigs with free access to peat and disinfectant powder, in comparison to tissue levels of pigs fed a known material quantity, voluntary material intake could be estimated. Results revealed a mean consumption up to 7% peat and 2% disinfectant powder of the daily ration of pigs. Maximum intake estimations even revealed intake levels up to 19.7% peat and 3.4% disinfectant powder of the daily ration. These ingestion rates are higher than intake levels of respective materials with highest trace element levels in study one to exceed maximum limits, i.e. for copper (25 mg/kg complete feed [pigs older than 12 weeks]), in the total diet: 5.6% peat (449 mg Cu/kg) and 0.5% disinfectant powder (5 110 mg Cu/kg) of the total diet (see Table 1). For the disinfectant powder sample with the highest iron content (93 287 mg/kg), the highest estimated intake level of 3.4% of the total diet may even exceed maximum tolerable levels for pigs (3 000 mg/kg complete feed; see Table 1).

Besides the toxic metals in pig tissues the multi-marker approach included the dietary internal markers *n*-alkanes, AIA, and titanium dioxide. Although used as qualitative markers, levels of the *n*-alkane C_{27} and AIA showed clear differences according to experimental groups (**Chapter IV**). Linear regression for levels of C_{27} and AIA in pig faeces across control groups (negative control group and positive control groups receiving 10 and 20% peat and 3 and 5% disinfectant powder with the diet, respectively) revealed quantitative material intake levels close to estimations based on levels of toxic metals originating from peat and disinfectant powder in pig tissues (Table 2). Titanium dioxide was only added to disinfectant powder in the treatment group with free access to the material, and comparison between groups was not applicable. However, calculating apparent DM-digestibility using AIA as marker in the experimental groups for disinfectant powder intake estimations (negative control, positive controls receiving 3 and 5% disinfectant powder within the diet and the treatment group with free access to disinfectant powder), total amount of faeces was determined. Consequently, based on faecal concentrations of the external marker titanium dioxide in the treatment group with free access to disinfectant powder, the amount of voluntarily ingested disinfect powder was calculated and in accordance with intake estimations based on toxic metal analyses and internal marker analyses (Table 2). Eventually, quantitative intake estimations based on different marker methods revealed results within the same range and thus proved the suitability of the new toxic metal approach and common marker techniques.

Table 2 Comparison of material intake estimations based on different markers expressed relative to the daily ration of pigs.

Marker	Peat	Disinfectant powder
	% of the total diet	
Pig tissues		
Toxic metals (Cd, As, Pb), mean	3.1 - 7.0	0.5 - 1.9
Toxic metals (Cd, As, Pb), min - max	1.0 - 19.7	0.2 - 3.4
Pig faeces		
n-alkane C_{27}	7.1 - 14.0	
Acid insoluble ash		0.1 - 3.8
Titanium dioxide		0.5 - 2.9

Determining material intake levels quantitatively based on material contaminant levels and accumulation of respective toxic metals (cadmium, arsenic, lead) in animal tissues is a completely new approach. Although absorption of toxic metals is incomplete, their excretion is very low to negligible (EFSA 2005; NRC 2005; Hoogenboom et al. 2014; Liao et al. 2020) and they accumulate in animal tissue proportional to material intake levels. Thus, toxic metals as markers are independent of characteristics of dietary markers, such as being non-absorbable in the intestine or their faecal recovery. Furthermore, common marker techniques reflect material intake about 24 hours prior to faecal sampling and require continuous labour-intensive faecal sampling to conclude on long-term intake levels (Bachmann et al. 2018; Garrett et al. 2020). Material intake estimations based on contaminant levels in feed, materials (peat and disinfectant powder) and animal tissues, respectively, require sampling of animal tissues only at the end of exposure after slaughter and are independent of variations between daily material intake levels. Toxic metals as markers for material intake might be more precise determining material-intake over long-term, but it cannot be identified whether the pigs' interest and consumption of materials varies within the growth period and increases, decreases or is consistent with age. Still, regarding food safety aspects, organ-contaminant-levels reflecting total long-term exposure (e.g. fattening period until slaughter) are of interest. Beside toxic metals, peat and disinfectant powder contained high levels of copper and zinc. In contrast to cadmium, arsenic and lead as toxic metals, trace elements are part of homeostatic regulations (e.g. absorption of iron and copper alters according to current animal requirements) (NRC 2005) and thus not suitable for intake estimations based on element accumulation in animal tissues.

Exposure assessment for peat and disinfectant powder

The presence of contaminants (e.g. toxic metals) and high levels of trace elements in bedding and enrichment materials for pigs represents a potential thread to animal and consumer health and may also be of environmental concern. In the present work, it was be shown that pigs consume considerable amounts of these materials through exploration (**Chapter III and IV**). In **Chapter IV** material intake levels of 1.0 up to 19.7% and 0.1 to 3.8% of the daily ration were found for peat and disinfectant powder, respectively (Table 2). Using *n*-alkanes and AIA as markers in pig faeces, material intake was estimated on a group basis. In contrast, the accumulation of toxic metals as markers in pig tissues could be evaluated for individual pigs. Thus, in addition to mean intake levels, toxic metals as markers also identified pigs with the highest material intake levels. This enables a risk assessment as a worst-case scenario (highest intake level of materials with the highest element concentrations). However, material intake varies for each individual pig and may be even higher than the currently derived intake levels. In addition to animal-specific preferences, the animal age and housing conditions might have an impact on material consumption.

With consumption of peat and disinfectant powder, possible contaminants such as toxic metals or even high doses of trace elements could enter the food chain and affect animal and consumer health. To assess the risk, the potential exposure of pigs was evaluated considering a worst-case scenario. Table 3 shows the calculation of the intake levels of toxic metals and trace elements by pigs assuming the consumption of peat and disinfectant powder with highest detected element levels (**Chapter II**) with the mean and maximum identified ingestion rates of these materials by pigs identified in **Chapter IV**. Calculated element intake was set in relation to maximum levels for complete feed according to EU regulations (EU 2002a; EU 2003) and MTLs (NRC 2005). Maximum levels for some elements have not necessarily been set considering animal health but based on the ALARA principle and environmental concerns. In contrast, the MTL represent the maximum tolerable concentration of an element in complete feed that will not adversely affect animal health. As a consequence and depending on the element, the MTLs are up to twenty times higher than the EU maximum levels.

The calculation of the oral exposure of pigs to peat with the highest respective element concentration showed that the element intake would exceed maximum levels in the total diet for trace elements according to EU regulations assuming mean and maximum intake levels of peat, respectively (Table 3). For disinfectant powder, element intake of pigs would exceed the EU maximum level for lead, iron and copper assuming mean disinfectant powder intake levels. The MTL for lead and iron would be also exceeded at the maximum intake level of disinfectant powder, which could cause adverse effects on animal health (Table 3).

Thus, peat and disinfectant powder used in the present work, may pose a risk to animal health. Since ALARA principles apply for some of these elements with regard to human toxicology, the use of these materials in animal feeding may have consequences for food safety. Applying maximum levels (similar to animal feed) to bedding and enrichment materials could be one possibility for their safe use in animal farming.

Table 3 Calculation of element intake by pigs due to material intake and comparison to EU maximum levels and maximum tolerable levels (MTLs) for pigs, assuming intake of peat and disinfectant powder as part of the total diet and considering mean and maximum material intake levels (7.0 and 19.7% and 1.9 and 3.4% of the total diet) as well as highest measured element levels in peat and disinfectant powder and neglecting element contents in feed itself; all values in mg/kg.

	Highest level[1] in		EU maximum level[2] (total diet)	MTL[3] (total diet)	Element intake (total diet)[4]			
	Peat	Powder			Peat intake [% of the total diet][5]		Powder intake [% of the total diet][5]	
					Mean 7.0	Max 19.7	Mean 1.9	Max 3.4
Toxic metals								
Cadmium	0.3	12	0.5[6]	10	< 0.1	< 0.1	0.2	0.4
Arsenic	4	24	2[6]	30	0.3	0.7	0.5	0.8
Lead	17	922	5[6]	25[7]	1.2	3.3	18*	31**
Trace elements								
Iron	11 736	93 287	750	3000	821*	2 312*	1 772*	3 172**
Copper	449	5 110	150[8]/25	250	31*	88*	97*	174*
Zinc	1 586	506	150	1000	111*	312*	10	17

[1] Koch et al. (2021) – **Chapter II**; levels adapted to original substance assuming 42% and 98% DM for peat and disinfectant powder, respectively (Koch et al. 2023)

[2] Directive 2002/32/EC (EU 2002a) and Commission Regulation (EC) No 1334/2003 (EU 2003)

[3] Maximum tolerable level (NRC 2005)

[4] Calculated: material intake level [% of the total diet] x highest material level [mg/kg] / 100 = element intake [mg/kg]

[5] Mean and maximum intake levels according to Koch et al. (2023) – **Chapter IV**

[6] Values in mg/kg referred to 88% DM

[7] Level results in decreased growth in pigs, but no MTL established (NRC 2005)

[8] Piglets up to 12 weeks (EU 2018)

* Level exceeding EU maximum level in the total diet of pigs

** Level exceeding MTL in the total diet of pigs

Feed and food safety aspects

Beside contaminant levels in materials and feed, also bioavailability, feed composition and additional contaminant exposure (e.g. *via* soil) may influence element accumulation in pig tissues (Linden et al. 2001). As an example, already 1 mg/kg cadmium (maximum level for feed of vegetable origin [EU 2002a]) as cadmium chloride and cadmium cysteine in feed fed over a period of 12 weeks resulted in cadmium kidney levels of 1.53 and 1.43 mg/kg that were above maximum levels for cadmium in foodstuffs (1 mg/kg) (EU 2006; Hoogenboom et al. 2014).

Although feed law regulations are yet not applicable for bedding and enrichment materials, the German Food and Feed Code (LFGB) includes regulations that would allow legal authorities to restrict or prohibit the use of respective materials under certain conditions. This includes materials containing compounds that may negatively affect animal health and food safety when possible consumption by the animals cannot be excluded (BMEL 2021a). The current work reveals considerable contamination as well as oral consumption of bedding and enrichment materials by pigs and an accumulation of therein contained contaminants in edible tissues. Thus, prerequisites are met to regulate e.g. the placing on the market of bedding and enrichment materials to ensure animal health and food safety.

Finally, high levels of toxic metals and trace elements in bedding and enrichment materials are also of environmental concern. Even if no consumption by animals occurs, toxic metals, trace elements and other contaminants from materials will eventually end up in farm manure, which is used as organic fertiliser on agricultural land (Linden et al. 2001; Schultheiß et al. 2004; Hou et al. 2017).

Study limitations

Investigations on the potential risk of bedding and enrichment materials revealed reliable results regarding contaminant levels of materials (**Chapter II**) and material intake levels by pigs (**Chapter III and IV**). Thus, these materials were identified as actual risk for animal health and food safety. Still, some limitations of the current research should be discussed.

In both animal experiments (**Chapter III and IV**), materials were presented either in a trough or in a tub and not on the floor. This was due to the experimental settings as quantification of residual material in the observational study was necessary and to prevent materials from being soiled. Contamination of materials with urine and faeces might not affect the pigs' interest over short-term, but over long-term (Beaudoin et al. 2019). Further, materials provided in a trough or tub might seem to the pigs like feed, although, to avoid confusion, the diet was presented in separate feeding troughs.

Either female or male pigs were used for investigations on preference and material consumption in the animal experiments. Gender-related preference or material intake levels could thus not be considered. This may be of minor importance as there is no evidence for a gender-selective explorative behaviour or contaminant intake for pigs during fattening until slaughter (Docking et al. 2008; Fernandes et al. 2019). However, object and material directed behaviour and potential consumption increases with age (sucklers versus weaners versus growers) (Docking et al. 2008; Kauselmann et al. 2021) and in the present studies only ten- and eight-week-old animals were used.

The animal experiments investigated different bedding and enrichment materials but did not consider varying housing conditions such as high and low stocking density and indoor and outdoor reared pigs. Previous studies found no significant effects of stocking density on exploration and manipulation of enrichment materials (Cornale et al. 2015; Caldas et al. 2020). The ability of outdoor reared pigs to perform foraging behaviour (e.g. rooting) on pasture does not necessarily require the provision of additional enrichment material. However, the exploration behaviour between outdoor on pasture and indoor reared pigs does not differ (Blumetto Velazco et al. 2013). Thus, bedding and enrichment materials are especially important for indoor kept pigs to live out their natural exploration behaviour under often barren housing conditions. Some housing systems provide outdoor concrete areas to pigs. However, these areas cannot compensate for access to pasture as Wei et al. (2019) found that indoor kept pigs on deep litter housing spend more time exploring than pigs with an outdoor play area out of concrete. Although exploration behaviour did not differ, total activity time was higher in outdoor than indoor reared pigs due to grazing and additional consumption of soil may occur (Fries et al. 1982; Linden et al. 2001; Blumetto Velazco et al. 2013). The soil, also possibly contaminated with substances such as toxic metals (Fries et al. 1982; Linden et al. 2001), might contribute to total toxic metal accumulation in pig tissues influencing material intake estimations based on element tissue levels.

Composition of feed may also influence material intake. In the present study, material consumption due to an unbalanced diet is unlikely, as all pigs received compound feed adopted to their nutritional requirements *ad libitum*. Food feedback (nutritional value, palatability and edible compounds) from materials increases attractiveness of the respective material to pigs but feed intake was found to be independent of material preference (Zwicker et al. 2013; Kauselmann et al. 2020; Kauselmann et al. 2021).

Conclusion and perspectives

The aims of the present thesis were 1) identifying the potential risk source for animal health and the safety of the food chain originating from bedding and enrichment materials providing an overview of materials available on the market and their contents of compounds regulated by feed law regulations and 2) characterizing the potential hazard conducting on farm trials to verify and quantify the oral uptake for risk assessment.

A risk originating from bedding and enrichments materials was clearly identified. In the first study (**Chapter II**), in three (disinfectant powder, earth/peat and biochar) out of four material categories samples contained considerably high levels of toxic metals and trace elements. Element levels in RMS were low, but the sample size (n = 3) was not representative for final conclusions. Further, characterizing the potential hazard, the second study (**Chapter III**) clearly showed material intake by pigs. Consumption was higher for materials of greater interest, but even pigs exploring the least preferred material, disinfectant powder, (intentionally) consumed a portion thereof. Thus, substances contained in bedding and enrichment materials might transfer into animal tissues and negatively affect animal health and food safety. Additionally, environmental aspects should be considered as bedding and enrichment materials also contribute to manure composition. For further hazard characterisation quantitative material intake estimations (**Chapter IV**) revealed a material intake up to 19.7% peat and 3.4% disinfectant powder of the daily ration of pigs. Mean intake levels were found at 7% peat and 2% disinfectant powder of the daily ration. However, to reliably estimate the risk and include a safety margin, highest intake levels should be considered. Thus, with maximum voluntary intake levels of almost 20% peat of the daily ration, peat can become a substantial part of the pigs' total diet. Although maximum intake levels of disinfectant powder are almost six times lower than for peat, highest element levels in disinfectant powders compared to other material categories, considerably contribute to the eventual risk. Accordingly, treating peat, disinfectant powder and generally bedding and enrichment materials like feed materials presents a way to ensure animal health and increase food safety.

Next steps in the risk assessment would now include an exposure assessment for consumers when consuming animal products of animals reared with contact to contaminated bedding and enrichment materials. Further, material intake quantification for various animal species (e.g. poultry and cattle) and various types of bedding and enrichment materials considering different feed availability and composition would expand knowledge on the potential risk of bedding and enrichment materials for animal health and food safety. Assuming differences in material preference and consumption between species, species-specific management measurements can be adopted.

The present work emphasises the potential risk for animal health and food safety using bedding and enrichment materials in pig husbandry. Results underline the need to apply legal regulations for feed on bedding and enrichments materials, to ensure the use of respective materials, which are essential to meet the behavioural needs of pigs to perform exploratory behaviour, without any risk for animal and consumer health. Applying Regulation (EC) No 767/2009 on the placing on the market and use of feed and Directive 2002/32/EC on undesirable substances in animal feed on bedding and enrichment materials can be a first step. Potentially, this would enable respective materials to be safely used for animals, farmers and consumers by limiting the entry of contaminants and high levels of trace elements into the food chain and the environment.

Summary

Bedding and enrichment materials for pigs – a risk for food safety?

Bedding- and enrichment materials are an essential part in pig husbandry to allow animals to perform natural behaviours such as exploration by rooting, biting and chewing under often barren housing conditions. Thus, behavioural disorders such as tail biting can be prevented or reduced. On the other hand, a possible intake of offered materials by pigs cannot be excluded. Legal regulations apply for feedstuffs, setting maximum levels for undesirable substances in animal feed and minimizing the entry of contaminants into the food chain and thus protecting animal health and food safety. However, these regulations do not include bedding and enrichment materials. So far, only few studies investigated the chemical composition of those materials and their consumption by farm animals. The present work aimed at identifying and characterizing the risk of bedding and enrichment materials for animal health of pigs and food safety.

To identify possible risk sources, the first study presented, analysed 74 bedding and enrichment materials of the categories disinfectant powder, earth/peat, biochar and recycled manure solids for contaminants and trace elements (**Chapter II**). In 20% of the samples, levels were found to exceed maximum levels for arsenic, lead and cadmium in mineral and complementary feed. Additionally, some samples showed very high levels of trace elements (iron, copper, zinc). Considering a possible consumption of materials by the animals, a transfer of these substances into the food chain cannot be excluded.

These high element levels are relevant for animal health and food safety when animals ingest the materials. Thus, a second, camera-assisted observational study with twelve pigs investigated the animals' preference for and intake of disinfectant powder, peat, biochar and straw (**Chapter III**). Two materials were presented at the same time for five consecutive days. All material combinations were tested and the pigs' preference evaluated on day one and five. Albeit peat and biochar were the most preferred material by pigs (mean manipulation time 50 and 63 min/d versus 39 and 24 min/day for straw and disinfectant powder, respectively; $p< 0.05$), all materials were manipulated and in part consumed. As prove of material consumption, the internal markers *n*-alkanes and acid insoluble ash, which naturally occur in the materials earth/peat, straw and disinfectant powder, could be detected in the pigs' faeces. Thus, these materials may considerably contribute to the daily ration of pigs and, when contaminated with undesirable substances, present a risk to animal and consumer health.

To quantify the actual contribution of bedding and enrichment materials to the daily ration of pigs in the context of risk assessment, the third study investigated the voluntary consumption of peat and disinfectant powder during the fattening period of 28 pigs (**Chapter IV**). As the toxic metals cadmium, arsenic and lead, originating from the materials and used as markers for material consumption, accumulated in the animal tissues of respective control and treatment groups, a mean voluntary consumption of 7% peat and 2% disinfectant powder of the total ration could be determined. Animal-specific material intake estimations revealed maximum intake levels up to 19.7% peat and 3.4% disinfectant powder of the daily ration. These intake levels in combination with high element levels in bedding and enrichment materials can lead to element levels in the feed/material intake by pigs that exceed maximum levels according to feed law regulations and a transfer of undesirable substances into food of animal origin.

Estimating the risk of bedding and enrichment materials for animal health and food safety, levels of substances relevant for animal health and food safety and highest material intake levels should be considered in future. Regulations on the composition and placing on the market of bedding and enrichment materials based on feed law regulations may enable bedding and enrichment materials, which are essential for the well-being of farm animals, to be safely used for animals and consumers.

Zusammenfassung

Einstreu- und Beschäftigungsmaterialien für Schweine – ein Risiko für die Lebensmittelsicherheit?

Einstreu- und Beschäftigungsmaterialien sind ein essenzieller Bestandteil in der Schweinehaltung, um den Tieren unter zumeist abwechslungsarmen Haltungsbedingungen das Ausführen natürlicher Verhaltensweisen wie das Erkunden durch Wühlen, Beißen und Kauen zu ermöglichen. So können Verhaltensauffälligkeiten, wie z. B. das Schwanzbeißen, vorgebeugt oder vermindert werden. Andererseits kann eine mögliche Aufnahme angebotener Materialien durch die Schweine nicht ausgeschlossen werden. Für Futtermittel gelten rechtliche Regelungen, die Höchstgehalte für unerwünschte Stoffe in Futtermitteln festsetzen und den Eintrag von Kontaminanten in die Nahrungskette minimieren und so die Tiergesundheit und Lebensmittelsicherheit schützen. Jedoch schließen diese Regelungen Einstreu- und Beschäftigungsmaterialien nicht ein. Bisher haben nur wenige Studien die chemische Beschaffenheit und Aufnahme solcher Materialien durch Nutztiere untersucht. Ziel der vorliegenden Arbeit war es, das von Einstreu- und Beschäftigungsmaterialien ausgehende Risiko für die Tiergesundheit von Schweinen und die Lebensmittelsicherheit zu identifizieren und charakterisieren.

Um mögliche Gefahrenquellen zu identifizieren, untersuchte die erste der hier präsentierten Studien 74 Einstreu- und Beschäftigungsmaterialien aus den Kategorien Einstreupulver, Erde/Torf, Pflanzenkohle und Güllefeststoffe auf Kontaminanten und Spurenelemente (**Kapitel II**). In 20% der Proben wurden Gehalte ermittelt, die Höchstgehalten für Arsen, Blei und Cadmium wie sie für Mineral- und Ergänzungsfuttermittel festgelegt sind, überschritten. Einige Proben wiesen zudem sehr hohe Gehalte an Spurenelementen (Eisen, Kupfer, Zink) auf. Bei einer möglichen Aufnahme der Materialien durch die Tiere ist nicht auszuschließen, dass diese Substanzen in die Nahrungskette gelangen.

Relevant für Tiergesundheit und Lebensmittelsicherheit sind diese hohen Elementgehalte, wenn die Materialien von den Tieren oral aufgenommen werden. Daher untersuchte eine zweite, kameragestützte Beobachtungsstudie mit zwölf Schweinen deren Präferenz und Aufnahme von Einstreupulver, Torf, Pflanzenkohle und Stroh (**Kapitel III**). Zwei Materialien wurden gleichzeitig an fünf aufeinanderfolgenden Tagen angeboten. Alle Materialkombinationen wurden getestet und die Präferenz der Schweine an Tag eins und fünf ausgewertet. Obwohl Torf und Pflanzenkohle von den Schweinen am meisten bevorzugt wurden (mittlere Manipulationszeit 50 bzw. 63 min/Tag versus 39 bzw. 24 min/Tag für Stroh und Einstreupulver; $p < 0.05$), wurden alle Materialien untersucht und zum Teil oral aufgenommen. Als Nachweis für die Materialaufnahme, konnten die internen Marker *n*-Alkane

und salzsäureunlösliche Asche, die natürlicherweise in den Materialien Erde/Torf, Stroh und Einstreupulver vorkommen, im Kot der Schweine analysiert werden. Somit tragen diese Materialien möglicherweise erheblich zur täglichen Ration der Schweine bei und können, wenn sie mit unerwünschten Stoffen kontaminiert sind, ein Risiko für die Gesundheit von Tieren und Verbrauchern darstellen.

Um im Rahmen der Risikobewertung den tatsächlichen Beitrag von Einstreu- und Beschäftigungsmaterialien zur Gesamtration von Schweinen zu quantifizieren, untersuchte die dritte Studie die freiwillige Aufnahme von Torf und Einstreupulver während der Mastperiode von 28 Schweinen (**Kapitel IV**). Durch Akkumulation der in den Materialien enthaltenen toxischen Metalle Cadmium, Arsen und Blei, die als Marker für die Materialaufnahme genutzt wurden, im tierischen Gewebe entsprechender Kontroll- und Behandlungsgruppen, konnte eine mittlere freiwillige Aufnahme von 7% Torf und 2% Einstreupulver gemessen an der Gesamtration ermittelt werden. Tierindividuelle Materialaufnahme-Schätzungen ergaben maximale Aufnahmemengen bis zu 19,7% Torf und 3,4% Einstreupulver gemessen an der Gesamtration. Diese Aufnahmemengen in Kombination mit hohen Elementgehalten in Einstreu- und Beschäftigungsmaterialien können zu Überschreitungen der futtermittelrechtlichen Höchstgehalte in den von Schweinen aufgenommenen Futtermitteln/Materialien sowie dem Transfer unerwünschter Stoffe in tierische Lebensmittel führen.

Im Rahmen der Abschätzung des Risikos von Einstreu- und Beschäftigungsmaterialien für die Tiergesundheit und die Lebensmittelsicherheit sollten zukünftig die Gehalte der für die Tiergesundheit und Lebensmittelsicherheit relevanten Substanzen und die höchsten Material-Aufnahmemengen berücksichtigt werden. Regelungen zur Beschaffenheit und das Inverkehrbringen von Einstreu- und Beschäftigungsmaterialien in Anlehnung an futtermittelrechtliche Regelungen können eine für die Tiere und den Verbraucher sichere Anwendung der für das Wohlbefinden der Nutztiere essenziellen Materialien ermöglichen.

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Interessenskonflikte

Im Rahmen dieser Arbeit bestehen keine Interessenskonflikte durch Zuwendungen Dritter.

Selbstständigkeitserklärung

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

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