Bedding Material and Stocking Density Influence the Performance, and the Occurrence of Footpad Dermatitis in Turkey Hens

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Abstract

The turkey industry needs to continuously monitor the economics of production as well as the welfare of the animals in their care. To that end, turkey industry continuously examines new production systems that will meet consumer demands. Understanding factors like stocking densities and bedding material characteristics can give the turkey producers tools to adjust management to keep production sustainable. Footpad dermatitis (FPD) is a common problem in commercial turkey houses, which is characterized by presence of hyperkeratosis, discoloration, necrotic lesions and ulcers that develop on the footpad of turkeys. Development of FPD has multifactorial causes, although high litter moisture is usually a direct or underlying cause. FPD is associated with pain for birds and thus, is a welfare concern. Many footpad scoring systems are available to classify the footpad lesions of poultry and have been used as indices of flock welfare. Although footpad scoring systems exist, none meet all the needs of the turkey industry alone. The ideal system should objectively evaluate footpad health on commercial farms by lay persons using easily understandable terms, which classifies the footpad lesions easily. Farm FPD assessment is important, as the litter moisture can be improved by management techniques, thus improving turkey welfare during their life span. Both the type of bedding material and turkey stocking density can affect both performance and welfare. A study was conducted to investigate two bedding materials and three stocking densities on turkey hen performance, FPD and litter characteristics. Turkey hens were kept on two bedding materials (pine shavings, PS, and Giant miscanthus grass, MG), and three stocking densities (8.5, 10.7, and 14.2 hens/m^2 from d 1 until 2 wk of age, then 4.3, 5.4, and 7.1 hens/m^2 between 2 and 14 wk of age, as low, medium, and high density, LD, MD, and HD, respectively). Density affected turkey performance, and improved indices including BW, FI, FCR were observed at LD. LD was associated with lower footpad scores, and lower litter moisture content. Giant miscanthus grass resulted in lowered FCR in the first two weeks, however, litter moisture, and footpad scores were higher, which may impair turkey welfare. Correlation also indicated the positive relationship between larger particle size (> 4.76 mm, sieve size 4) of MG bedding and increased incidence of FPD ($r = 0.81$, $P < 0.05$). Therefore further studies needed to investigate the effects of finer particle size on litter moisture and footpad health to evaluate MG, and using other welfare indices in addition to the FPD might be beneficial to see a broader welfare aspect.

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CHAPTER I.

LITERATURE REVIEW

Introduction

Production and Consumption of turkey in the US and worldwide

The consumption of turkey meat is about 7.2 kg/person in the United States of America (2014), and has been at the same level since 1990 (between 7.9 and 7.2 kg/person). The goal of the National Turkey Federation (NTF) is to have consumption reach 9.2 kg (20 pounds) per person by 2020 in the US (National Turkey Federation, 2016). Consumption is seasonal with 65.2 million turkeys eaten between October and December in the USA, fewer (51.1 million turkeys) from July to September, and 44-46 million birds in the first half of the year. In 2014, 237.5 million turkeys were produced, 2.5 million less than were produced in 2013. In 2017 the US total turkey production was 242.5 million turkeys (USDA, 2018a).

The USA is not only the largest global market for turkey meat, it is also the largest turkey producer. In 2012, global turkey meat production was 5,634 million metric tonnes, and the majority originated from the American continent (3.45 M tonnes) (National Turkey Federation, 2016). 2,645 M tonnes, comes from the the US (47% of global production), 510 M tonnes from Brazil, 144 M tonnes from Canada and 97 M tonnes from Chile, respectively (Turkey Federation 2016). Europe produced 1.92 M tonnes of turkey meat followed by Africa, Asia and Oceania which produced 0.12, 0.11 and 0.02 M tonnes, respectively.

Minnesota is the leading turkey producer state in the US. One hundred-one million turkeys, which is 41% of the total turkey production in the USA in 2017 originated from three states: Minnesota (42 M), North Carolina (32.5 M) and Arkansas (26.5 M) (USDA, 2018b). In 2012, Minnesota-produced turkey was valued at $839 million (1.16 billion pounds), however, North Carolina realized even more: $849 million from 1.18 billion pounds of turkey (Laux, 2013). Although Minnesota raises more turkeys, it conversely realizes less value, compared to North Carolina, which might suggest, that more female turkeys are raised in Minnesota. Hen production, increases the number of turkeys being raised, but not the amount of turkey meat produced since female turkeys weigh less than males.

Footpad Dermatitis

FPD is a common welfare and economic problem worldwide in intensive broiler and turkey production systems (Mayne et al., 2007c). It is a condition of necrotic lesions on the footpad and toes (Shepherd and Fairchild, 2010), and is characterized by lesion size, features of discoloration, and inflammation (Greene et al., 1985). Clark et al. (2002) simply describe FPD as inflammation of the footpad, and claim that FPD in turkeys is common. FPD research has been done mostly on broilers and thus, we have less information about the prevalence and severity of FPD in turkeys, especially in commercial flocks. Many different synonyms for FPD have been used in the literature including pododermatitis, foot burn, footpad burns (Clark et al., 2002), ulcerative dermatitis, plantar dermatitis, scabby foot pads, and ammonia burns (Martland, 1984).

FPD is a global problem in commercially-raised turkeys. A study evaluating FPD in commercial turkey flocks in the US reported that 95% of market toms developed footpad lesions (Da Costa et al., 2014). German field studies reported FPD occurrence of 21.1% (males) and 29.5% (females)(Krautwald-Junghanns et al., 2011), with only 2.8% of commercial turkey toms having no footpad lesions at marketing (20-21 wk) (Große Liesner, 2007). Only 2% of Swedish turkeys at market age had no footpad lesions while 78% showed mild (discoloration, erosion), and 20% severe (ulcers) lesions (Berg, 1998). The frequency of low FPD scores (without lesions) in turkeys was similar in Swedish and German studies with 2% and 2.80% respectively (Große Liesner, 2007)

FPD has economic implications, which can have many aspects. Severe lesions can affect: 1.) the quantity of broiler feet that can be marketed, 2) audits at the slaughter house may result in fines for growers, 3) live bird performance and 4) additional costs to remove the lesions, and avoid potential bacterial contamination in processing plants.

Marketable broiler feet. FPD can directly affect the sale of chicken paws, which are saleable products in Asia. The revenue from this commodity was worth $280 million in 2008 to US broiler production (Shepherd and Fairchild, 2010). Currently there is no export demand for turkey paws, although this may change in the future.

Audits in plants. In order to describe the changes in the turkey footpad due to FPD, many scoring systems have been developed. At first, external signs were classified, including a 3-point scale (1 – mild, 3 – severe) (Jensen and Martinson, 1969) and a 4-point scale by Martland (1984) (0-no lesions, 3-“severe large punched-out ulcers”). In order to measure the welfare of European turkey flocks in processing plants a 5 point external FPD scale system was proposed (Hocking et al., 2008) ranging between 0 ("No external signs of FPD…") and 4 ("…more than half the footpad covered by necrotic cells").

In addition to the external footpad scoring systems, a few histological scoring systems were developed to categorize cellular changes, such as the 8-point scale of Mayne et al. (2006), ranging between 0 ("no change, sample normal") and 7 ("split epidermis – 1 + lesion or 1 very large lesion, more than 1/3 of total sample").

In the late 90s, FPD became an animal welfare concern in Europe. Since that time FPD measurements have become part of welfare audits, which are routinely used in processing plants, such as the Welfare Quality® Assessment (Butterworth et al., 2009).

Reduced performance. Severe lesions can affect live bird performance. A broiler study in the Netherlands reported a total loss of $0.11 loss per broiler placed due to reduced live body weight (1.95 vs. 2.09 kg, d 37) (DeJong et al., 2014). Broilers raised on wet litter had lower body weight gain (1.90 vs. 2.06 kg), feed intake (3.20 vs. 3.31 kg), FCR (1.68 vs. 1.61), higher water intake (5.01 vs. 5.49 L), and lower water-to-feed ratio (1.57 vs. 1.66) when compared to birds kept on dry litter.

Cost and impact of trimming lesions. At the slaughterhouse, broilers raised on wet litter were associated with reduced live weight (1.98 vs. 2.17 kg), carcass weight (1.31 vs. 1.43 kg), and more downgrades of commercial parts (0.38 vs. 0.14%).

Although some broiler studies have calculated the economic impact of FPD, little is known about the losses associated with severe FPD lesions in turkeys. There are indications of poorer performance, such as reduced feed intake and body weight for turkeys on wet litter although no direct relationship to FPD has been established (Mayne et al., 2007a; Youssef et al., 2010; Hocking and Wu, 2013).

Factors influencing FPD

Litter

The origin of FPD is usually multifactorial (Mayne et al., 2006). However, high litter moisture (above 35%) can induce FPD in the absence of other factors. Husbandry and management, such as drinker design, relative humidity, litter source and quality, stocking density, specific feed components (Martland, 1984, 1985), body weight, age, gender or strain of the bird may contribute to the incidence of FPD (Shepherd and Fairchild, 2010). Any factor that adds moisture to litter can play an important role in the occurrence and severity of FPD.

Excess moisture in the litter is the factor that most authors agree is a primary cause of FPD in chickens (Martland, 1985) and turkeys (Martland, 1984; Mayne et al., 2007a; Wu and Hocking, 2011; Youssef et al., 2012). Breast and hock skin may also be affected by wet litter resulting in contact dermatitis (Greene et al., 1985). Although authors agree on the importance of excess litter moisture in FPD pathogenesis, some see it as a primary cause (Mayne et al., 2007a) while others see it as a contributing factor (Martland 1984).

Since wet litter has such an important role in FPD (Martland, 1984; Mayne et al., 2007a), researchers have developed wet litter treatments to induce this condition in order to observe FPD under controlled conditions. Martland (1984) kept male and female turkeys in an experiment from 6 weeks of age until their 20th week, and treated the bedding by spraying it with water to keep the litter moisture between 60 and 70%. As a result of wet litter, impaired walking, lameness, shaky legs, and discomfort were observed. Turkeys showed discomfort when walking and sat down frequently suggesting this was a reaction to pain (Martland, 1984).

Since FPD has been associated with high litter moisture, bedding materials and the management of bedding sources in poultry houses were studied. Clark et al. reported (2002) that in the United Kingdom the commonly used litter materials are straw or wood shavings. In the United States, wood shavings are the most common bedding type. In the southern part of the United States rice or peanut hulls are sometimes used, as is the practice in tropical countries (Mayne et al., 2007a). Bedding in the US, is commonly reused several times cleaning barns and changing the litter completely only every 2 to 5 years (Navarra, 2015). For footpad health, the ideal litter or bedding should work to absorb or wick moisture away from contact with the birds, but should also contribute to drying by ventilation. The use of wood shavings is recommended due to its great absorptive and non-clumping characteristics. Many farmers may try different litter materials, depending what is affordable and available for them at the time. For example in Canada and Europe, cereal straw is available (Clark et al., 2002). Some authors recommend using wood shavings over straw bedding (Ekstrand et al., 1998) to improve litter quality and thus lower the incidence of FPD.

Recommendations for litter depth vary between countries. In the US, litter is recommended to be a deep layer, and kept between 3-5 inches (7.62 cm- 12.7 cm).

However, in Sweden broiler farmers are told to keep thin litter depths (2-3 cm), which will encourage the birds to scratch in the bedding and keep it friable (Ekstrand et al., 1998).

Youssef et al. (2010) examined the effects of four different bedding materials on footpad scores, using dry and wet litter treatments on various bedding types. External footpad scores were lower for lignocellulose than wood shavings, chopped straw and dried maize silage. For wet litter, scores were lower for turkeys housed on dried maize silage compared to chopped straw, lignocellulose or wood shavings. Histologically, less severe FPD was seen on wood shavings and dried maize silage compared to lignocellulose for the dry litter treatment. Lignocellulose bedding, when compared to wood shavings, yielded lower footpad scores in a wet litter treatment, while chopped straw and dried maize silage were not different from them. (Youssef et al., 2010)

The Youssef et al (2010) study shows the importance of bedding material, as its characteristics might affect footpad scores externally and histologically. Thus, the selection of appropriate bedding material can directly impact the incidence of FPD. Their study examined the two most frequently used bedding types, straw and wood shavings. No differences were seen in the external footpad scores although lower histological footpad scores were reported on wood shavings compared to straw in the dry treatment on d 28.

Mayne et al. (2007) found that the litter moisture plays an important role in both the development and healing of FPD lesions. An 8-point scale was used to assess footpad lesions, ranging 0 (normal footpad) to 7 (split epidermis – 1+ lesion or 1 very large lesion, more than 1/3 of total sample). Turkey hens were kept on wet litter for two days beginning at 21 d of age, resulting in an average footpad score of 6.7. Then turkeys were then transferred to dry litter, and after 15 days, re-scored, resulting in a 0.9 average footpad score ($p<0.001$). The authors also noted that turkeys with severe lesions on wet litter were reluctant to walk which they attributed to the painful necrotic lesions on the footpads. The findings indicate, that even in the most severe lesions, significant healing can occur in a relatively short time period (15 days), which emphasizes the importance of litter moisture in the development and maintenance of FPD.

Gender and FPD

In observations of FPD the contribution of gender to the development of this condition remains a question. There are many contributing gender-related factors, which might affect the incidence of FPD, such as differences in skin tissue, market ages, and body weights. There are differences between male and female skin tissue, which might contribute to the incidence of FPD. Female broilers are reported to have higher insoluble collagen levels than do males. Although total collagen and insoluble collagen was higher in males (Smith et al., 1977). Male birds are heavier than females of the same species, so males put more pressure on their footpads, than do females (Garcia et al., 2012). Additionally, in the case of turkeys, toms are usually kept longer (20 weeks) compared to females (14 weeks) to optimize meat yield. Considered collectively, these facts may explain why many studies have found higher incidences of FPD in males than in females (Harms and Simpson, 1975; Greene et al., 1985; Garcia et al., 2012).

However, there are also studies, where no differences in FPD incidence was found between male and female broilers (Martland, 1985), and some that show there is a higher incidence of FPD in females than in males (Kjaer et al., 2006).

Martland (1985) did not find differences between male and female broilers, when footpad lesions in birds on wet litter were monitored. By the 7^{th} week, both female and male broilers on wet litter reached peak FPD severity, the third level on a 4-point scale (0 (no lesions) and 3 (severe large punched-out ulcers) and developed large ulcers. Then the broilers with severe lesions were transferred to dry litter, and two weeks later the author observed that among the transferred birds, females recovered more rapidly than the males. He concluded that broiler chicken gender has no impact on the affected area of the plantar surface regarding the FPD (Martland, 1985).

Kjaer et al. (2006) reported higher incidence of FPD in female broilers (49%) compared to males (36%) in the first replicate of a trial where the second replicate showed incidences of 52% and 33%, respectively (Kjaer et al., 2006).

Bilgili et al. (2006) reported mixed results when evaluating the footpad lesions of male and female broilers. A 3-point footpad scoring system was used (no lesions, mild = lesion < 7.5 mm, severe = lesions > 7.5 mm). A higher proportion of male broilers had normal footpads (no lesions) compared to females at d 42, however, more females than males had mild footpad scores at d 49 and d 56.

Clark et al. (2002) claimed that the incidence of FPD is higher in male turkeys than hens both in the USA and the UK in commercially-raised turkeys. In fact, little is known about the relationship of gender differences and FPD in turkeys. A field study

(Krautwald-Junghanns et al., 2011) investigated the prevalence of FPD in male and female turkeys in commercial flocks in Germany. This study found that turkey hens had more and injuries of greater severity than males, however, this difference is may be due to the fact that in Germany, the average density of turkeys is 2.8 toms/m^2 and 5.1 hens/m^2, which is 58 and 52 kg/m^2 respectively. Comparing these values currently the recommended stocking density is 4.5 hens/m^2 and 2.5-3 toms/m^2 at the end of rearing period in the United States (Aviagen Turkeys, 2015). Given that 10 kg as body weight of an average hen, and 20 kg as an average market tom, the stocking density is between 45 kg and 60 kg/m^2. In case of female turkeys, the 60 kg/m^2 density might suggest that the fact that there are more turkeys which will deteriorate the litter. Further, there may be other confounding facts as a result of stocking density which contribute to the FPD differences between the two genders. Krautwald-Junghanns et al. (2011) reported in the 16th week of clinical evaluation of turkey flocks, 0.3% and 0.4% of hen footpads (left and right footpad, respectively) were normal, while 3.4% and 3.9% of toms had normal feet. At the same time, 33% of toms had lesions, and 51-52% showed epithelial necrosis, while 60% of hens had lesions and 36% showed epithelial necrosis. In an external evaluation of 7,800 male and 8,400 female turkeys, approximately 60% of the footpads were scored as 2. Twenty-one percent of toms and 29% of hens received footpad scores of 3. Seventeen percent of tom, and 12% of hen footpads were scored as 1. The 2.11% of tom and 0.62% of turkey hen had healthy footpads without lesions. The most severe score of 4 was given to 0.1% of the birds (Krautwald-Junghanns et al., 2011).

The mixed results from studies investigating gender differences of FPD suggests that it is not directly a factor (Shepherd and Fairchild, 2010). It is more likely that the confounding factors related to gender differences, such as various stocking densities used in commercial production, market ages, and body weights contribute to the differences in FPD prevalence of males and females.

Nutrition and FPD

Many studies have been conducted to evaluate the possible nutritional factors for developing FPD. In the 1940's Patrick et al. reported typical symptoms of biotin deficiency, which were later associated with dermatitis. The clinical signs of biotin deficiency include skin and bone lesions and depressed growth (Whitehead, 1964). Many authors agree that the biotin deficiency is a possible factor in the development of FPD (Jensen and Martinson, 1969; Harms and Simpson, 1975). Biotin is also called Vitamin H or Vitamin B7 or B8 (Nutri-Facts, 2014). Although biotin is found in most feed ingredients, its bioavailability in feed components varies: 40-50% in cereal (wheat or barley), 100% in corn. The first signs of biotin deficiency occur at an early age, even as early as in the hatchery, where hatchability will be decreased. Later, the skin of the feet and toes may become cracked and scaly with scabs and hemorrhages (Jensen and Martinson, 1969). Biotin deficiency includes other lesions including dermatitis at the corner of the beak or eyelids, leg problems often arise because leg bones are shortened, tendon slippage or perosis of the hocks is typical, all of this resulting in birds that are reluctant to walk (Whitehead, 1964).

In addition to the link to biotin deficiency, others have suggested a relationship between FPD and zinc. The zinc is a trace mineral, an essential factor for various enzymes and contributes to immune function, wound healing, carbohydrate and protein synthesis, cell division, and growth (National Research Council, 1994). Symptoms of zinc deficiency are similar to those of biotin deficiency and may include abnormal scales on the feet, hock enlargement, perosis, shorter and thicker leg bones, and feather problems (National Research Council, 1994).

Youssef et al. (2012) found that the severity of FPD was reduced by higher levels of dietary biotin or zinc when birds were kept on dry litter but not on wet litter (73% moisture). Mayne et al. (2007) also found that higher levels of biotin did not prevent FPD under wet litter conditions (Mayne et al., 2007b). Jensen et al. similarly found that biotin injections did not impact FPD, and biotin was not a factor in the FPD (Jensen et al., 1970).

In the research of Abd El-Wahab et al. (2011) the impacts of electrolyte contents and floor heating was compared as risk factors for the development of FPD. It was found that normal dietary electrolyte content was more important than floor heating or even litter moisture. Youssef et al. (Youssef et al., 2011b) found that footpad scores were similar between groups of birds fed different diets.

Although, deficiencies of biotin or zinc could be confounding factors in the development of FPD, nutrient deficiencies are rare in commercial turkey production and thus cannot explain the widespread and frequent appearance of FPD in poultry. These studies demonstrate that although deficiencies can contribute to footpad lesions, they emphasize the importance of litter moisture in the etiology of FPD.

Genetic lines, different production systems and FPD

Kjaer et al. (2006) studied the incidence of FPD in fast and slow growing chicken lines. Ross 308 (fast growing) and LB (slow growing) broilers were kept in an experiment lasted from d 8 until slaughter. Very low footpad scores were found in the traditional birds at 10 weeks of age, while the prevalence of FPD was 44% in the fast growing birds slaughtered at 6 weeks of age.

Hocking and Wu (2013) conducted a research to study the FPD in four genetic lines of turkeys: two Large White female lines (UK), an F1 cross between Large White female lines (USA), and traditional Narragansett toms in order to study the differences between modern and traditional turkey strains. When the experiment ended, there was no significant difference between the lines, all developed severe FPD when exposed to wet litter and had normal footpads on dry litter (Hocking and Wu, 2013).

Pagazaurtundua and Warriss (2006a) attempted to identify the prevalence of FPD among five different broiler rearing systems: standard intensive, free-range (outside access), organic (outside access), Freedom Food (RSPCA, Royal Society for the Prevention of Cruelty to Animals) and corn-fed. The prevalence of FPD ranged between 9.6% (Freedom Food) and 89% (organic), birds with a frequency of severe lesions ranging between 1% and 89% for the Freedom food and organic systems, respectively. However, due to the many different confounding factors (age, body weight, feed, stocking density, different management practices, etc) the results of this study cannot be used to draw definitive conclusions (Pagazaurtundua and Warriss, 2006a).

Hatchery conditions and FPD

The effects of incubation temperatures and eggshell conductance in Pekin ducks on footpad skin development was studied (Da Costa et al., 2015). The authors found that dermal thickness at hatch and at 35 days of age was significantly different in the normally incubated vs. those incubated at elevated temperature. Although the pathogenesis of FPD in ducks and other poultry may differ, this study provided insights that were pursued in a follow up study conducted in broilers.

To explore factors for improving footpad skin, Da Costa et al. (2016) investigated the relationship between the hatch temperature and trace minerals on the live performance of Ross 708 broiler chickens and the development of footpad skin (Da Costa et al., 2016). The authors found significant correlations ($P<0.05$) between the thickness ($r = 0.24$) and the area ($r = 0.26$) of the stratum corneum and body weight at d 7 and 21. The same correlation was found between the area of epidermis ($r = 0.20$) and body weight. Interestingly on the last day of the study (d21), the birds receiving inorganic trace minerals had thinner dermis than those receiving the chelated diet (Da Costa et al. 2016).

Although this study found weak correlations ($0.2 < r < 0.4$) between footpad skin and body weight, high variability in the results leave open to question, whether changes in the skin as a result of hatchery condition would affect the incidence of FPD.

Housing Conditions and Environment - Season and Humidity Impacts on FPD

The incidence of FPD lesions increases during the winter in broilers (Greene et al., 1985; McIlroy et al., 1987; Martrenchar et al., 2002; Bassett, 2009), and in poultry generally (Shepherd and Fairchild, 2010; Da Costa et al., 2014). McIlroy et al. (1987) concluded from field studies conducted with broilers that the incidence of FPD lesions in the Northern hemisphere peaked between October and April. Many sources attribute the seasonal incidence of FPD to weather variables such as relative humidity (McIlroy et al., 1987; Shepherd, 2008). During cold months, incoming air contains more moisture and this factor contributes to the development of wet litter (Dowsland, 2008). From the farmers' practical point-of view, ventilation is reduced in winter to maintain barn temperatures. This lowered rate of ventilation does not adequately reduce air and litter moisture in the barn resulting in wet litter conditions (Meluzzi et al., 2008).

The appropriate drinker type, ventilation rate and the resultant control of air humidity can improve litter quality, which will result in a lower incidence of FPD (Mayne, 2005). Ekstrand (1997) examined 101 broiler flocks coming from 23 farms in Sweden and found that the prevalence of FPD was significantly higher ($p < 0.05$) on those farms where small cup drinkers were used rather than nipple or bell drinkers. Tucker and Walker (1999) refer to studies conducted in the United Kingdom (ADAS Gleadthorpe) where researchers studied the effect of different drinker styles on broiler growth rate, feed and water usage and litter friability. The nipple drinker without a drip cup had the best associated litter quality, but mean broiler body weight was also the lowest compared to the other drinker types. The next two best for litter quality, were the nipple with a drip cup and auto-cup drinkers.

The environmental effects, such as drinker design, or humidity are confounding factors in the etiology of FPD, which can indirectly influence litter moisture. If the litter moisture is kept low, then the incidence of FPD will likely be low.

Inflammatory or allergic response

Mayne and coauthors (2007) studied whether the FPD is an allergic response to wet litter or inflammatory reaction. They analyzed the level of pro-inflammatory cytokines and chemokines, because these are chemical messengers drive the immune system and regulate inflammatory reactions.

Real-time quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) on cytokine transcripts confirmed changes. The mRNA transcripts of interleukin IL-1β (+635 fold increase over baseline), IL-6 (+65) and CXCLi2 (+1924) and IFN-$^{\gamma}$ (interferon-gamma) increased by 32 fold, while Th2 (IL-13) was only increased two fold. Treg (IL-10) levels were not altered. Birds kept on wet litter had an increased level of macrophages (85%), and CD4+, CD8+ T lymphocytes (90%). However, only 25% of the birds had elevated T-lymphocytes and none of them macrophages on dry litter conditions (Mayne et al., 2007c). These findings suggest that the birds on wet litter had inflammatory reactions, but not induced by pathogens.

Micro-, and macroscopic findings

Normal skin is subdivided into three layers: epidermis, dermis and hypodermis as we move from surface to the inner layers. The epidermis is further divided into three strata: 1) the outermost layer is called stratum corneum, or the horny cell layer, 2) next is the stratum spinosum (transitional layer) and 3) the innermost layer of the epidermis is the stratum germinativum, where epidermal cells are formed via mitosis, and then continuously move to the outer surface progressing through the strata as they age (PoultryHub, 2018).

At early stages of FPD, mild hyperkeratosis and superficial lesions have been observed. As the condition progresses, horned pegs may be evident as the scales of the footpad become thickened and enlarged. Finally, the strata corneum and germinativum became ulcerated and necrotic. Inflammation will be evident in the tissue surrounding the ulcer (Martland, 1984; Mayne et al., 2007c). FPD is not a primarily bacterial condition, but bacteria may invade wounds when present (Greene et al., 1985. Martland, 1984; Ekstrand et al., 1997; Shepherd and Fairchild, 2010). With an opening in the protective layers of the skin, microorganisms can access the bloodstream, and settle in joints, which may lead to arthritis, synovitis (Clark et al., 2002) and lameness (Jensen et al., 1970).

Even though the description of lesions at certain time points during the turkey's lifespan has been reported in the literature, little is known about the progression of the lesions, e.g., how and when the lesions start and develop. Field studies have shown in Germany and the United Kingdom that at the age of 3 weeks fully developed lesions can be found, which means the epidermis have been lost (Mayne et al., 2006; Bergmann et al., 2013). Some authors observed reddening as the first sign of FPD at 24-48 hours in turkey poults exposed to wet litter (Clark et al., 2002). Mayne et al (2007) were among the first scientists, who studied the gradual progression of FPD in turkeys in a field study examining the lesions micro- and macroscopically.

Mayne et al. (2006) sampled commercial turkeys and monitored the gradual development of footpad alterations, and examined both externally and histologically. In the first week, normal footpads, hyperkeratosis and discoloration were observed externally. Histologically, normal epidermis and minor abnormalities were reported, such as compressed keratin, horned pegs, and increase in blood vessel. No inflammation.

Starting at week 2 and 3, necrotic discoloration could be seen externally. Histologically, necrotic cells were observed, along with ruptured epidermis, and inflammatory cells.

In weeks 4 and 5, the hyperkeratotic stratum corneum developed into horned pegs, and few inflammatory cells (macrophages and heterophils) were reported.

By the 6th and 7th wk, 2/3 of the turkeys had lesions. Histologically, the lesions were similar and only varied in their sizes. An abundance of inflammatory cells were described in the surrounding tissues.

During the 8th and 10th wk, the lesions became more severe and eroded, and signs of chronic inflammation were reported.

At market age, wk 21, the lesions developed earlier, such as chronic inflammation characterized by an abundance of macrophages and heterophils, and necrosis progressed.

The novelty of this work was the longitudinal observations of external and histological changes in the turkey footpad due to FPD from a commercial setting. Further, the description of lesion progression revealed, that even though in the early phase there were no external signs present on the footpads, histological evidence of disease was obvious.

Welfare and FPD

In developed countries, animal welfare is an important topic. To measure an animal's well-being, different indicators are audited, such as FPD, hock or shank burns, cleanliness and scratches on the body (DeJong et al., 2014). Many authors agree that FPD may cause pain to birds with these lesions (Mayne et al., 2007b), impacting their ability to walk and gain access to food and water (Shepherd and Fairchild, 2010).

Researchers have reported pain reactions after handling turkeys with FPD during an experiment (Martland, 1984). Other researchers state that turkey toms with FPD move less than normal birds matched by age and weight (Da Costa et al., 2014). Martland reported that male broiler breeders could not mate because of severe FPD which made locomotion difficult. Additionally, he observed that birds on wet treatment treatments sat huddled and moved only if driven, and connected those signs to pain in affected birds. He added that birds on dry litter were active (Martland, 1985).

In a behavioral study by Hocking and Wu (2013), turkeys on wet litter spent more time sleeping (35-45%) and standing (18-35%) than did birds on dry litter, which spent only 15-30% of their time sleeping, and 18-28% standing. Birds on dry litter walked (9-

15%) and sat (5-15%) more, compared to birds on wet litter which walked (2-6%) and sat (1%) for much shorter times. This less active behavior in the wet litter treatment group was attributed to pain (Hocking and Wu, 2013). On d 6 of the experiment, no birds on dry litter had evidence of FPD while the birds on wet litter had FPD average scores of 5-6 on the Mayne et al.'s footpad scale (Mayne et al., 2006).

Dawkins et al. (Dawkins et al., 2004) investigated the welfare of commercial broiler chickens with a large scale study representing 2.7 million broilers in the United Kingdom. The study was done in different houses of the ten major broiler producing companies. For evaluating the welfare of the flocks the authors investigated many indices, including mortality, gait, FPD, hock burn. FPD was evaluated with a 3 point scale externally, where score 0 indicated the healthy pad without lesions, score 1 is given if the size of the lesion is less than 5 mm, and score 2 used if the lesion exceeds 5 mm. 81.2% of the broilers had healthy footpad with no lesions, 16% had moderate lesions (score 1), and only 2.8% showed lesions over 5 mm on their footpads (score 2). The authors concluded that the welfare of chickens was influenced by the housing and not by other factors like stocking density.

Overall, many confounding factors exist, which can contribute to the incidence of FPD, including housing conditions, stocking density and production system, however, if the litter moisture is kept under 30%, then lower FPD incidence can be expected.

CHAPTER II.

COMPARISON OF FOOTPAD SCORING SYSTEMS: DO WE SCORE WHAT WE NEED TO?

Summary

Footpad dermatitis (FPD) is an animal welfare indicator that is widely used in many countries in poultry production. It has been used to characterize the welfare of a population alone or in a combination with other welfare indices, such as hock burns. There are several footpad scoring systems that classify the footpad lesions externally, and a few histological classifications. None are ideal and many have the same or similar weaknesses. Most of these schemes use highly technical terms understandable only by trained professionals. Additionally, the terminology is subjective, for example it might be difficult to distinguish between brown and black discoloration, or evaluate what percentage of the footpad is affected, when a system refers to lesions covering between 25 and 50% of the footpad, or 1/3rd of the footpad surface. Most footpad scoring systems are intended for use in processing plants, when the lesions are visible after scalding, and not intended for field settings and use by lay people. Severe footpad lesions can be healed within two weeks if litter moisture is reduced therefore, monitoring footpad health in a turkey flock should be done so that management changes, such as adding dry litter can be done when needed. In this review, we describe existing footpad scoring systems and compare them to an ideal scoring system, one which is easily understandable and easy to use in commercial turkey farms.

Introduction

Footpad dermatitis (FPD) is common condition in the commercial turkey and boiler industry worldwide. FPD is associated with pain, and thus is a welfare concern that has attracted the attention of the poultry industry and the interest of consumers in the US and Europe (Shepherd and Fairchild, 2010). Chicken feet are a valuable export commodity, with a revenue of $280 million (Shepherd and Fairchild, 2010) in the US in 2008. Export markets are not currently interested in turkey feet, but this could change in the future. The economic aspects of FPD may be realized through its potential effects on the supply chain as well as on bird performance. FPD is also a food safety concern due to the open lesions on the footpad, which can be an entry opportunity for pathogens (Martland, 1984). Severe footpad lesions can also affect broiler performance, resulting in reduced body weight (DeJong et al., 2014).

To measure the footpad health of poultry, it is essential to evaluate the footpads objectively and reproducibly so scores can be reliably compared. Several scoring schemes have been established for broilers and some for turkeys to evaluate footpads externally (Berg, 1998; Hocking et al., 2008). A few scoring systems have also been established for histological (internal) assessment, mostly for broilers (Michel et al., 2012) and some in turkeys (Mayne et al., 2006).

The scoring schemes are based on various criteria such as depth and the extent of the lesion, which makes the comparison of footpad scores difficult. To further complicate comparisons, some turkey footpad scoring systems use three levels (Martland, 1984), some use 8-point scales (Mayne et al., 2007a; Da Costa et al., 2014), and one uses a 10-point scale to evaluate footpad alterations.

The industry, farmers and consumers are all interested in animal welfare, but ensuring welfare for animals can be ambiguous. What does “better welfare” mean? How can animal welfare be measured? The assessment of footpads and scoring FPD is a widely-used index for animal welfare, but it can be a difficult since there are many scoring systems, most of which are not comparable. In addition, footpad scores may be assessed on farms or in processing plants; however, it is unknown if scores taken in these different settings agree with each other. For example, are footpad lesions evaluated accurately so that they reflect animal welfare? Lund et al. (2017) compared footpad scores of broiler chickens assessed in the processing plants and found agreement when footpads had no or mild lesions. However, for medium and severe lesions, there was no agreement in the scores. Cleaned broiler footpads classified as having medium lesions, were later evaluated as severe lesions after validation. This study indicates that even if clean footpads are scored, broiler welfare may not be as advanced as indicated by the scores. If that is the case, then what is the agreement between not cleaned (farm/live birds) and scalded (postmortem) footpads? In addition, how well do the footpad scores represent welfare if they are not accurate?

An ideal footpad score should be objective and authentic, and should accurately represent the welfare status of the birds measured. Observers should be able to continuously monitor the footpad status in the flock and help farmers make decisions regarding management changes. Therefore, there is a need for an accurate footpad scoring system that includes easily understandable terms and identifiable signs, be easily associated with the classification of different levels, and be not time or labor intensive. Optimally, a footpad scoring system should be intuitive, requiring less education for the scorer. The goal is for scorers to have less hesitation when giving footpad scores for turkeys that are restrained and waiting for a footpad assessment. Ideally, there should be little preparation using gentle manual rubbing when evaluating footpads. It should be sufficient to gently clean the footpads without the use of brushes, water, and buckets, as these might increase the footpad scoring time. Using brushes can also be a biosecurity issue, if equipment is moved between turkey houses. In addition, clear, realistic pictures should be linked to the scoring system to effectively communicate the differences between the scoring levels. These pictures should show dirty footpads and footpads of younger birds rather than only very clean, scalded market-age turkey footpads. Another important aim for the external scoring systems is to be universal so that the prevalence and severity of FPD in different flocks, seasons, farms, management practices and even countries can be compared.

Although many footpad scoring systems exist, a comprehensive review is needed to compare the advantages and disadvantages of the schemes, and systematically evaluate against an ideal. Understanding the footpad status of turkeys is essential to address the animal welfare. Therefore the aim of this chapter is to a) review the available footpad scoring systems for assessing footpad lesions in turkeys, and in some cases in broilers, if the scoring system has a unique feature; b) compare the existing schemes to identify any research gaps; and c) elucidate previous results comparing footpad scores assessed on farms and in the processing plants. To characterize and classify the footpad lesions different scoring systems have been used in experiments, processing plants and the field. Many times the scoring system is designed for one specific purpose (e.g., use in a processing plant when the footpads are clean after scalding and thus easier to assess) but it would be difficult to use the equipment in other settings (e.g., in the field).

External scoring systems

External scoring systems were first developed to assess what is seen on the plantar surface and sometimes on the digits, and to describe the lesions, mostly based on their size. These scoring systems emerged in the 60s and 70s when researchers and the industry recognized footpad lesions. However, FPD was not considered a welfare problem. For these reasons, footpad scoring arose as a by-product of other research, mostly on nutritional, such as biotin. When FPD became economically important (late 80s), and concerns were raised about animal welfare (late 90s), studies purely focusing on FPD (2000s) were conducted. FPD is now routinely part of the welfare assessment used in many studies. In commercial production settings, welfare audits are routinely done on farms and in processing plants.

Jensen et al. (1970) studied the effect of nutrition (biotin, and soybean meal) and antibiotic use on FPD in unsexed turkey poults in the late 60s and early 70s on mesh wire floors and flooring systems bedded with wood shavings. This was one of the first studies to create wet litter treatments by adding water to bedding to induce FPD. A 5-point scale system was used (Table 1) to measure FPD, which was reported in a footnote as: “0 = no dermatitis and 4 = most severe" (Jensen, Martinson, & Schumaier, 1970). An earlier study by the first author used a 3-point scale (Table 2) to assess turkey footpads (Jensen and Martinson, 1969). However, the Jensen et al.'s (1970) footpad scoring system used provides very limited information on the assessment of footpad lesions, and is difficult to use in the field because it does not provide scoring metrics in easily understandable and clear terms. For example, it does not describe what the most severe category means. Further, it is not known how scores of 1, 2, and 3 are defined and no pictures are included to provide examples.

Martland (1984) studied male and female white turkeys between 6 and 20 wk of age. The wood shaving bedding was sprayed with water to induce FPD (wet litter). The footpads were cleaned with water and a brush, then assessed on a 4-point scale (Table 3) and the most severe footpad scores were used in the statistical analyses. Although Martland's footpad scoring system includes objective and subjective elements to assess footpad lesions, it would be difficult to use in the field. He distinguishes between footpad lesions described as scabs and ulcers, however, scabs are not defined in this system. Martland's scoring system does include, however, a clear and objective definition of ulcers. However, since, most ulcers will also have scabs there is significant overlap of the categories creating confusion for scoring. Another limitation is that although size indications for lesions are included, the scale is subjective making it difficult to determine the difference between small scabs or larger scabs. Pictures included in the publication showed turkey footpads without lesions and with severe ulcers (Probably scores of 0 and 3, respectfully) but not for intermediate scores where the scale is more ambiguous. This scoring system is an improvement over Jensen et al.'s (1968, 1970) schemes, but is not intuitive and lacks clear definitions.

In the late nineties in Sweden, animal welfare concerns were raised regarding FPD. To understand the prevalence and severity of FPD in commercial broiler flocks, Ekstrand et al. (1997) developed a 6-point footpad scoring system for broilers in processing plants (Table 4). For analytical purposes, the six categories were reassigned into three groups (Table 5): no lesions, mild lesions, and severe lesions to potentially make the scale more intuitive. They also observed that most broilers presented the same degree of damage on each foot resulting in the same footpad scores on left and right feet, which therefore indicated that assessing one foot per broiler would be sufficient to get an impression of the foot health status of a flock (Ekstrand et al., 1997).

Ekstrand, Algers, and Svedberg's scoring system is novel and an improvement over previous systems in that it uses new terms, characterizes skin layers involved in FPD, and attempts to provide in-depth descriptions of footpad changes in FPD for veterinarians inspecting carcasses in processing plants. However, this system would be difficult to use on farms because it has six lesion classes, which might be time consuming to identify, and the categories are described in terms that non-veterinarians would not understand such as papillae, hyperkeratosis, erosions, and signs of inflammatory reactions. The term discoloration, in contrast, is easy for lay people to understand, and is a novel term in this scheme. Unfortunately, the rest of the terminology will not likely be useful for a poultry producer.

Another unique characteristic of this scoring system is that the evaluation distinguishes between the skin layers (epidermis, dermis), which would be difficult to use for live bird assessments. This system classifies the scores into larger categories, such as no lesions, mild/superficial lesions and severe ulcerations, which might be easier to identify, but it still lacks simple definitions to distinguish between mild/superficial and severe lesions. The fact that the authors described six categories of FPD, but used only three groups for statistical analyses might indicate that the shorter scheme is easier to use even for veterinarians and even more so for farmers. However, no pictures are included in the scoring descriptions, which would be a help for using this scoring system.

Ekstrand et al. (1997) used the same extensive 6-point footpad scoring system they had developed for commercial broilers in Sweden (1997) to assess the footpad health of 53 turkey flocks in processing plants (Ekstrand and Algers, 1997). They found that most of the turkeys had developed mild/superficial lesions and papillae (score 4), and 14% of turkeys exhibited severe ulcerations and papillae. Only 2% of the marketed turkeys had no lesions. Specifically, when lesions were grouped, 78% of the turkeys belonged to category B (mild lesions only), 20% were in category C (severe lesions), and only 2% of the turkeys were in category A (no visible lesions). Footpad lesions from broilers and turkeys can be compared in terms of similar tissues and responses to FPD. However, it is more common for turkeys to have severe lesions than for broilers (Clark et al., 2002). This might be because turkeys spend more time on the litter over their lifetime and thus they spend significantly more time in close contact with wet litter (turkey toms live 19-21 weeks compared to broilers that live 5-6 weeks), which can increase the likelihood of FPD.

Ekstrand et al. (1998) established the modified Swedish system, by simplifying their earlier 6-point scale to the 3-point scale used in analysis. This modified system was used in broilers in the processing plant, and trained inspectors evaluated 100 individual feet per load using the scoring system shown in Table 5.

The scoring system by Ekstrand et al. (1998) has only three levels compared to the first version with six classes, which is an improvement. However, more clarification would be needed for lay persons to evaluate things like hyperkeratosis, and to distinguish between lesions described as mild or superficial. The system does not provide clear distinctions between mild and severe lesions, and whether the size of the lesions is important. There is no information about digital lesions. Three pictures are included, which help with the identification of lesions.

Footpad scores might not represent the entire flock well, therefore a flock score was introduced by using footpad scores and indices. Furthermore, this assessment is part of the Swedish and Danish (Lund et al., 2017) broiler welfare surveillance program. In this system, after evaluators examine the feet in processing plants, the proportion of feet with scores of 0, 1, and 2, are multiplied by 0, 1 and 2, respectively. Based on the results, flocks are classified into 3 levels: (1) a total score less than 40 is classified as without remark for the entire flock; (2) a score between <41 and <80 is classified as remark low level; and (3) a score of 81 or above is remark high level. Producers are informed of the footpad scores given in the processing plants and receive warnings if the flocks have more than 20% severe footpad lesions. Advisors contact farmers to discuss how to reduce severe footpad lesions, and if production conditions do not improve on the farm, then farmers must decrease stocking density to meet expectations (Ekstrand et al., 1997).

Another new system was introduced by Allain et al. (2009) who proposed a 10-point scale for assessing broiler footpad lesions macroscopically based on the severity and size of lesions (Table 6) in French processing plants. They used four lesion categories, which were further divided based on the affected area on the footpads (<25, 25-50%, >50%). A score of 0 was given if no lesion is seen on the pad. Scores 1-3 were given if hyperkeratosis/papillae was seen without discoloration on less than 25% of the footpad area (Score 1) or between 25-50% of the footpad (Score 2), or over 50% of footpad (Score 3). For scores 4-6, brown discoloration and erosion were seen, and the difference between these scores was based on the size of the lesions on the footpad: score 4 was given, if lesions appeared <25%; score 5, if 25-50%; and score 6, if <50% of the footpad. The more severe scores (scores of 7-9) were assigned to black, deep lesions and split epidermis, based on the size of the lesions (<25%, 25-50%, >50% of footpads).

Allain et al.’s (2009) footpad scoring system aims to characterize lesions very accurately in the processing plant both by the depth and extent of the lesions. However, although the system is useful in the plant, it would be difficult to use on farms. There are easily understandable terms in the scale, such as identifying the categories based on the color of the discoloration (i.e., no discoloration, brown and black) in the footpad scoring system. In addition to the color codes, there are other terms to determine the categories, such as keratosis/papillae, deep lesions, and split epidermis, which are useful for veterinarians and trained raters in the processing plant, but it may be difficult for lay people to use since there are no clear explanations. This scoring system is intended for use in processing plants after scalding when the footpads are clean. In contrast, in farm settings, it may be difficult for farmers to distinguish the categories and the extent of the lesions, such as identification of lesions covering 25-50% of the footpads or greater proportion. Footpad lesions are frequently dark brown and black colored, making the colors difficult to distinguish in the processing plant, but even more difficult on farms where the litter is also dark brown to black and lighting may be dim. Footpad scoring can also be time consuming. To carefully select one of the ten categories, very thorough footpad cleaning is needed. Pictures of cleaned footpads are attached to this scoring system, but the difference in the pictures of a foot with a score of 5 versus one with an 8, for example, highlights the difficulty of correctly identifying when the lesions cover 25-50% of the footpad surface. This footpad scoring system was later simplified into a 5-point scale ranging from 1 (no lesion or enlargement of scales and erythema, whatever the size) to 5 (depressed lesion, loss of substance [= ulceration], with or without dark thick adherent crust, size >50% footpad) (Michel et al., 2012).

In addition to developing a new footpad scoring system for broilers in the processing plant, Allain et al. (2009) attempted to find the relationship between footpad score categories, which was a unique aspect of the study, since until the late 2000s, footpad scoring systems were developed mostly just for the identification and categorization of lesions. For the correlation, a moderate positive correlation was found ($r = 0.51$ $P<0.05$) between minor and intermediate footpad lesions (FPD1 and FPD2). A very high negative ($r = -0.95$ $P < 0.05$) correlation was found between minor, and deeper lesions (FPD1 and FPD3). A negative strong correlation ($r = -0.63$, $P < 0.001$) was also detected between “intermediate” and “deeper lesion” categories (FPD2 and FPD3).

In 2013, Allain et al. (2013) developed a new footpad scoring system for turkeys in processing plants (Table 8), which had previously been established only for broilers (Allain et al., 2009). The aim of the study was to evaluate contact dermatitis, which was considered a major poultry welfare problem. A total of 60 turkey flocks were sampled between April and July 2006, with 100-300 observations made per load based on the flock size and speed of the processing line. The footpad scoring system developed is based on the severity and extent of lesions on the right feet of the turkey, and also includes the scoring of hock burns and breast buttons. In addition, to assessing turkey footpads, the presence or absence of footpad swelling, and toe deviation (one of predominant leg and foot abnormalities) were also noted (Buffington et al, 1975, as cited in Allain et al. 2013).

Allain et al. (2013) intended to establish a turkey footpad scoring system for use in processing plants, where experts, veterinarians, or trained raters would understand the technical terms such as erythema or depressed lesion. In addition, determining the portion of the footpad surface area that is affected by FPD is not straightforward and probably takes considerable time to distinguish among the footpad scores. There are some descriptions of the color coding, such as yellowish to brownish exudates, and dark, adherent scab, which may help with lesion characterization, but there is overlap between the categories and no objective measures. Thus, it would still be difficult to put lesions into distinctive scoring categories even though pictures of footpads are included in the scale, and to illustrate the abundance of scores. The biggest challenge, however, is the need to clear and scald the pads. To assess the footpad surface and evaluate FPD based on this scoring system, accurate cleaning or scalding is necessary, which would require time to clean and score. Thus, the practicality of this system on farms is questionable. In particular, based on the description and pictures, it would be difficult for farmers to differentiate between scores 4-6, and 7-9, as they appear to be very similar in color.

In Europe, particularly, experts expressed a need to evaluate turkey footpads using a uniform system. Therefore, in 2000, at the 4th International World's Poultry Science Association meeting, they decided that a footpad scoring system for turkeys should be developed. This system would allow farmers to identify footpad health and make it possible to decrease the FPD by decreasing stocking density on turkey farms. This approach is similar to what already existed on broiler farms in Europe, particularly in Swedish turkey and broiler processing plants. The Council of the European Union (Document DS 564/05) had already declared that the "prevalence of footpad dermatitis in broilers should be used as one of the main criteria of welfare on which future stocking density for specific farms would be based". Although the intent of the Council of the European Union was to reduce FPD prevalence in turkey flocks, it is known, that reducing stocking density may not decrease FPD (Hocking, Mayne, Else, French, & Gatcliffe, 2008). Thus, a unified, easily understandable and reproducible scoring system was needed, which could be used in processing plants, and would make different farms, management systems and countries comparable in Europe.

To answer this call, Hocking et al. (2008) developed an external scoring system ranging from 0 (no signs of FPD) to 4 (over 50% of footpad is covered by necrosis), as shown in Table 9. The authors' purpose was to assess turkey flocks rather than individual turkeys with the help of a simple footpad scoring scheme. Modifying the current scoring system was proposed based on feedback of raters who were already scoring in processing plants and farms. They did not specifically include an evaluation of the toes because based on their observations, toe lesions are similar to those on the footpad. Only the right footpad was assessed in processing plants, and since the feet are cleaned and discarded before they are examined, lesions can be better visualized. Postmortem examinations are not traumatic for the bird and therefore it preserves the welfare of live birds. Although the footpad assessment is done in processing plants, the authors claimed it provides good feedback for farmers to help them monitor flock foot health.

Hocking et al. (2008) noted that the scoring system contains novel elements such as use touch and colors, but it is not easy to use in field settings due to the use of many subjective and undefined terms. The scheme contains terms describing how the skin of the footpad feels (e.g., soft or hard) and is highly subjective. Description of colors, such as "small black necrotic areas" or "black areas may have turned white" are also subjective evaluations. Terms intended to characterize size, such as "small black necrotic areas" or "the area of necrosis is less than one quarter of the total area of the footpad" may be difficult to distinguish. Identification of footpad lesions using multiple senses (touch, vison) may also be biased (Tuyttens et al., 2014). In addition, this scoring system contains a few special terms that only trained evaluators may understand, such as “reticulate scales” and “necrosis”. Although the descriptions are improved over previous systems, the categories of lesions are overly broad and leave room for interpretation. Scoring systems that are not precise are difficult to use and may add restraint time for turkeys waiting for their footpads to be scored on the farm, or in the processing plant when time is also a limiting factor. Although the pictures of the clean footpads are intended to help with classification, human interpretation may commonly misclassify terms, such as “a quarter”, “less than half”, and “more than half” of the footpad surface. Although the aim of this scoring system was to improve the welfare of turkey flocks, it is not clear how post mortem evaluations can impact the welfare of live turkey flocks, and how the welfare of subsequent turkey flocks would be measured.

An extensive field study was done in Germany, assessing footpads of young male and female turkeys (Krautwald-Junghanns et al., 2013). A total of 5531 turkey poults (3131 males and 2400 females, B.U.T. 6) were assessed on 24 farms at two time points between 3-5 and 22-35 days of age in order to evaluate the footpads in the early production phase (Krautwald-Junghanns et al., 2013). A 5-point footpad scoring scheme was established ranging from 0 (no abnormalities) to 4 (“deep lesions”) (Table 10.) Footpads were assessed at the market age in the processing plant. Shortly after the turkeys arrived in the brooding facilities, at 3-5 days of age, the right footpad of most turkey poults (72.7%) had no alterations (score 0), 17.7% of turkeys exhibited hyperkeratosis, 9.5% hyperkeratosis with adhered materials and 0.1% necrosis. The same categories for the left legs had 72.5, 17.3, 10, and 0.2%, of the same lesions respectively. Shortly before the ending of brooding period, and moving the turkeys into a finisher house (between days 22 and 35 of age), normal footpads were seen in 36.7 (right) and 35.7% (left), a score of 1 was given in 17.4 (left) and 16.9% (right leg), a score of 2 in 33.6 (right) and 35.7% (left), and the worst lesions were detected in 12.3 (right) and 11.7% (left) of the feet (Krautwald-Junghanns et al., 2013).

The terms in the scoring scheme established by Krautwald-Junghanns et al. (2013) are intended to help a veterinarian or a well-trained rater, but a lay person would not understand terms, such as ablation of the epidermis. Color descriptions are included in this system, such as “no discoloration”, or “reddish-brown” discoloration. Terms, such as “elevation in the center”, and “high-grade hyperkeratosis” are somewhat difficult to identify. Terms, such as “reticulate scales arranged actinomorphic symmetrically” could be explained more simply. The pictures complement the verbal description, however, it is somewhat difficult to distinguish between categories 2-4 based on the pictures. A unique feature of this footpad scoring system is the description of “crusts of adhesive dirt that are not detachable without damage of the plantar skin, increased tendency to bleed on manipulation”, which is a common phenomenon in field settings. Besides the description of footpad, scale photos taken were taken in the field to show example footpads which is rarely seen, since usually pictures are taken in the processing plants after scalding, when the footpads are clean.

A field study (Da Costa et al., 2014) evaluated the footpad health of 41 commercial tom flocks (Hybrid converter and Nicholas 85) in the United States. The footpads of 50 toms per flock were examined between 16 and 20 weeks of age. Both footpads were assessed, but only the score from the more severely affected footpad was used. The authors developed an 8-point footpad scale ranging between 0 (no lesion) to 7 (“All of the footpad and toes severely affected with lesions”) (Table 11). Most turkeys (22.13%) received a score of 5, 19.70% a score of 4, and 18.26% a score of 3. Collectively, 60.09% of the turkeys assessed developed lesions on at least one of their footpads of 1.6 to 2.5 cm. Only 4.72% of birds had no lesions on their footpads (Da Costa et al., 2014).

Da Costa et al.’s (2014) scoring system is intended to be objective, because the authors measured the size of the lesions with a bursometer (Fort Dodge Animal Health, Overland Park, KS.); but did not include a measure of the depth of the lesions. Multiple lesions can develop on a footpad, and the lesions might not be circular. Therefore measuring the diameter of the lesion(s) with a bursometer may not be accurate. In addition, it might be important to distinguish the lesions on the footpads that fall between the scores of 0 and 1 (no lesion and lesions less than 1.3 cm diameter), since it is a significant jump, and many changes can be overlooked. To assess lesions on the footpads, thorough cleaning is needed, which assumes the time spent with cleaning in the turkey houses. Since this system uses a measuring tool (bursometer) to assess the lesions, without this tool the footpads cannot be assessed. In addition, it may be difficult to distinguish between lesions that are 1.3 and 1.6 cm, or 1.6 and 1.9 cm. This scoring system uniquely includes the toes. Ultimately, although this scoring scheme is meant to be objective, it still might have too many levels, and shortcomings, such as how to evaluate multiple lesions exhibiting different depths.

Internal (histological scoring systems)

Few studies exist to describe histopathological lesions of FPD. Although the histological changes in the footpad tissues due to FPD were described in the literature for broilers (Greene et al., 1985; Martland, 1985), and in turkeys (Martland, 1984), these papers only reported the changes. To date, only a few footpad scoring systems were established to make histopathological changes comparable, and to validate the macroscopic and microscopic changes due to FPD in broilers (Michel et al., 2012; Heitmann et al., 2018), and turkeys (Mayne et al., 2007a).

Mayne et al. (2006) established a histopathological FPD scoring system based on commercial turkey samples (Table 12). A total of 20 turkeys (Large White Broad Breasted) were sampled at ages of 1, 2, 3, 4, 5, 6, 7, 8, 10 and 21 weeks to study the gradual development of FPD and to ascertain whether this condition, seen only at older age begins earlier than detected and to identify potential mechanisms for FPD. Two turkeys were selected weekly representing normal footpad and two turkeys with affected feet, a total of 40 birds (80 feet) were examined over the course of the study. All birds were males, except those sampled on the 4th week, and the birds were kept under the same management, although they were raised on four different farms within the same company. All birds had physical (upper body) injuries and were found dead or culled, which was not related to their footpad lesions according to the authors. The authors concluded, that FPD may occur earlier than originally thought, even at one week of age abnormal cellular changes were detected and by three weeks of age fully developed lesions were seen microscopically. Even if footpads appeared to be normal macroscopically, microscopic changes had already started by 4 weeks of age. Mayne et al simultaneously monitored the development of external and internal footpad lesions (Mayne et al., 2007a), and found that the early signs of FPD could be detected histologically, although if no macroscopic evidence was found on the footpad surface.

Mayne et al. (2006) used an 8 point scale, with five categories, such as none, mild, medium, medium-severe, and severe. Skin lesions were categorized by the skin layer affected (epidermis, dermis). Although their intent was to correctly monitor and describe FPD changes, it might not be easy to use this histological scheme. They used terminology that is not explained and even the term FPD is not defined. The authors enumerated inflammatory signs of FPD, and distinguished between acute and chronic signs, although they note that at older ages (21 wk of age) chronic inflammation is present, while acute inflammation is found in younger birds. The paper contains pictures of two histological slides, turkeys that were sampled at 1, and 6 wk of age and exhibiting histopathological scores of 3, and 7 illustrating mild and severe lesions, respectively. As the authors noted, footpads from injured and dead turkeys were used for this study, which might have affected overall health and also their footpad status.

Michel et al. (2012) worked on the histologically validated FPD classification in broilers at processing plants in order to compare micro-, and macroscopic findings of footpad lesions (Table 13). Two hundred ten chicken feet were sampled from seven flocks in four processing plants, and of these 54 feet were randomly selected and examined microscopically. The basis of macroscopic footpad scoring was an earlier system, which was modified from a scale with 10 points (Allain et al., 2009) to one with five (macroscopic differences) (Table 14) and then three (macro-, and microscopic footpad lesions) (Table 13). Footpad alterations were classified into three categories (Table 13), merging macro-, and microscopic changes, summarizing and synchronizing both the macro- and microscopic findings as a validation. Type I describes the mildest lesions, where enlarged scales and mild hyperkeratosis could be seen and macroscopically besides the latter phenomenon also hyperplasia in the epidermis, and edema, mild inflammatory signs were present. Type II lesions describes poorly adhered (yellowish-brownish) crusts over scales showing signs of hypertrophy and hyperkeratosis. The histological findings also confirmed these two features, in addition to inflammatory cellular infiltration. The most severe, Type III category, is assigned if dark colored (brown-black) thick adhered crusts cover the pads and crater (ulcer) and/or depressed lesions are present macroscopically. These findings are confirmed as ulcers, where the epidermis is lost and covered with necrotic debris. There is no description of inflammation found in Type III category lesions (Michel et al., 2012).

Based on the meanings and characterization of histological findings, the authors proposed a three point scale for external footpad assessment (Table 13.). Scars and healed lesions were not included, because they were noted only very rarely by the authors. The authors concluded by the histological characterization, in the cases of type II and III lesions “i.e. inflammation or marked ulcerative dermatitis suggest that pain is induced”, and “pain level is certain to increase from type II to type III lesions, and also probably with increasing size of lesions”, therefore this is an animal welfare concern and these footpad lesion categories should be monitored in flocks (Michel et al., 2012).

The advantage of the histopathologic scoring system developed by Michel et al. (2012) is its simplicity, there are only 3 levels (Table 13). The classification of categories was based on the extent of hyperplasia, hyperkeratosis, inflammation, and ulceration. This evaluation system is unique in the way that it compares macroscopic findings with histological. In addition, it identifies two different crust types by color (yellowish-brownish vs. dark brownish-black) and other characteristics (poorly or massively adhered to the pads). It is not known, how the feet were randomly selected for the study: whether pairs of feet or only right or left feet were chosen, and at what point the feet were selected at the processing plant (probably after scalding, although it is unknown how the authors assessed the adhered materials on the broiler footpads). This system does not classify the sizes of lesions, however, it does consider crusts, demonstrating their important role and attempts to explain some of the terminology used in scoring.

Comparing these two histological scoring systems, Mayne et al. (2006) were the first to develop a microscopic assessment of FPD. Their goal was to follow the weekly progress of FPD in turkey males (and 4 turkey females at 4 weeks of age), from weeks 1 to 9, then again at 21 and compare the histological changes even in externally healthy footpads. They sampled from commercial farms in the United Kingdom, all had a very similar management techniques. Forty total turkeys were sampled (Large White Broad Breasted), and 80 footpads examined.

Michel et al.’s (2012) aim was to develop an external footpad scoring system which aligns with histological findings. They worked with broilers, 210 birds were selected in French processing plants and 54 feet chosen for histology. Michel et al.

developed a three point scale for characterizing histopathology: Mild/early, moderate/superficial and severe/deep lesions. In addition to the histological findings, macroscopic signs are also included.

Mayne et al. (2006) established an eight point histological scoring scale, where the levels could be re-assigned to four main categories: No lesion, Mild lesions, Medium, Medium/severe, and Severe lesions. Mayne et al. distinguish the no lesion category (normal sample, no changes), while Michel et al. (2012) starts with the mild/early stage of FPD. Both methods differentiate mild lesions with hyperkeratosis and hyperplasia, Mayne added increased dermal blood vessel density and necrotic debris in epidermis, while Michel included dermal congestion and edema, and mild inflammatory infiltration. Swelling, increased blood flow to the affected area and edema are mostly signs of acute inflammation. Evaluating moderate lesions, both groups noted the presence of immune cells in the dermis: heterophils, macrophages and lymphocytes, which are signs of both acute and chronic inflammation. In the case of severe/deep lesions the authors agree that the presence of necrotic debris in the epidermis (and dermis according to Mayne et al.). Mayne et al distinguish a split epidermis with one or more lesions, and information on the extent of the lesions (over 1/3 of total sample). The French authors noted necrotic ulcerations of the epidermis.

An automated footpad scoring system was developed to minimize the footpad assessment costs of human raters, and perhaps to decrease inherent human bias. A Dutch company, Meyn Food Processing Technology B. V. (Noordeinde, The Netherlands) invented an image analyzing system to automatically assess footpad lesions of every broiler going through a processing plant without slowing processing. The goal of automatic footpad scoring was to continuously monitor broiler flocks and record the data automatically, fulfilling the requirements of the Danish and Swedish broiler surveillance systems described earlier. To verify the accuracy of the automatic system, Vanderhasselt et al. (2013) compared footpad scores from an automated footpad assessment system to human evaluators in both field and slaughterhouse settings. At first a trained expert selected 20 Ross 308 broilers and 20 Cobb 500 birds (38 d) based on the five point footpad evaluation system from Welfare Quality® (2009), which was converted to a three point scale. The experiment was repeated twice. On day 40, the selected broilers were sent to the slaughterhouse and subjected to the automated footpad scoring. This system made an assessment based on the dark area (lesions) of the footpad and toes, and was set to use the modified Ekstrand three point scale and recording the higher score of the two feet. Photographs were also taken of every footpad. After this process, the same evaluator who assessed the birds on the farm re-scored the feet using also the modified Ekstrand footpad scoring system.

The automatic system was supposed to score both footpads, but it was only successful 43.7% of the time; one footpad was scored in 41.1% of the broilers, and 15.2% of the broilers were not recognized and thus not scored. When lesions on both feet were recognized, the feet agreed in 70.5% of cases. The result of the pairwise correlation showed that the scores given for the entire broiler population (n=200) by the automatic system and the expert were moderately correlated (r = 0.54 on-farm and r = 0.59 in processing plant). When both feet were successfully assessed by the automatic system, there was a higher correlation between automatic and human scoring (r = 0.68 on-farm and r = 0.74 in processing plant). Although the automatic scoring was supposed to provide data on every footpad in the flock, approximately 40% of the broilers received footpad scores for both feet, and a similar proportion received only one score. Since both feet exhibit very similar lesions, it can be assumed that even scoring one foot of the same broiler would receive a very similar score for the other leg. Overall, the automatic footpad scoring system may be comparable to the results of the Danish/Swedish system where 100 broiler feet are selected per load and an index method is used to evaluate the flock FPD (Vanderhasselt et al., 2013).

Further research is needed to validate the footpad scores of the automated system, and to establish a correlation between the automated system and human raters. The aim is to be able to use an objective system to improve the reliability of FPD scoring by eliminating human bias, including bias on the origin of flocks or miscalculations.

Welfare audits – FPD is one of the welfare indices

It is well-established that in order to ensure animal welfare in poultry production, there is a need for a reliable measurement of the welfare of broilers and turkeys. Several welfare audit systems have been developed to evaluate flock welfare based on a complex system, with FPD being one of the indices monitored. Welfare audit systems evaluate poultry flocks on farm and consecutively in the processing plants (Butterworth et al., 2009) assessing footpad lesions, or only in processing plants (Humane, 2014).

The European Animal Welfare Indicators Project (AWIN) created a welfare assessment program for commercial turkeys which does not measure of any type of contact dermatitis (Ferrante et al., 2015). European experts collaborated in 2009 to develop the Welfare Quality® Assessment (WQA) Protocol for Poultry (Broilers, Laying Hens), which intended to evaluate farm animal welfare on farms, at slaughterhouses, and during transportation in a standardized way. The authors noted that "There is no gold standard measure of overall animal welfare…," and that the assessment should be improved later based on experience. Besides measurements taken on individual broilers, including plumage cleanliness, lameness, hock burns, and FPD, it is necessary to assess the broiler house environment such as litter quality and dust. Flock assessments such as observations of signs of clinical disease or stress such as panting or huddling, and bird behavior are part of the assessment. Information provided by farmers such as the mortality rate as well as floor and drinker space is also included. Based on the results of the welfare assessment, farms are assigned to one of four categories ranging from 1 - not classified, unacceptable welfare to 4 - excellent, highest welfare level. The WQA is based on measurements taken on the animals, such as body condition, injuries, and their behaviors. The on-farm assessment should be executed by project trained evaluators within five days of slaughter.

In the WQA, FPD is assessed on a five point scale (Butterworth et al., 2009), which assigns scores in a three point classification ("No evidence," "Minimal evidence," "Evidence of FPD") based on five pictures taken of cleaned broiler feet, as shown in Table 16. The WQA defines FPD as "…a contact dermatitis found on the skin of the foot, most commonly on the central pad, but sometimes also on the toes. The skin is turned dark by contact with litter and consequently deep skin lesions can result”; without further description. Measurements are taken first on farms five days prior to market, then again in the processing plant. FPD, plumage cleanliness, and hock burns should also be examined in 100 broilers in the house, from 10 locations (10 broilers/location). The estimated time for evaluation is 60 minutes to collect these three measures. The total estimated time for the entire WQA protocol is approximately 195 minutes per flock.

Measurements taken at the slaughterhouse complement the results of on the farm assessments, and should be evaluated together as part of the WQA. In the case of FPD two 5- minute time periods (a total of 10 minutes) should be spent on footpad assessment, representing 500-1000 broilers. After recording how many broilers passed the observational point per minute, the observers count the footpads with scores of 2-4, then calculate the percentage of broilers with footpad scores of 1-4/ observation period, and classification (category of a, b, c). The estimated time for FPD assessment in the slaughterhouse is 10 minutes per flock. Based on the percentage of broilers in the 1 and 2 categories (moderate and severe lesions), an index calculation (Ip) should be used, with the help of I-spline (Sp) functions, which supports a representative curve. There are also many other measures to assess welfare in processing plants, such as absence of disease (e.g., ascites, dehydration, septicemia, hepatitis) or absence of injury (e.g., breast blister, broken bones). The estimated time for the slaughterhouse assessment is 40 minutes per flock. The authors also considered the time and circumstances of transportation as part of the welfare assessment toolkit.

The WQA refers to a five point footpad scoring scheme developed by the University of Bristol. However, no description of this scale is available in the WQA protocol and only five pictures are included. Other studies have used a four point footpad assessment, referring to the University of Bristol (Pagazaurtundua and Warriss, 2006b; Haslam et al., 2007). However, there is no explanation of what “no evidence”, “minimal evidence”, or “evidence of FPD” mean in the descriptions making it unclear how this scale would be used. Based on this evaluation, it is also not clear which foot or feet should be sampled and whether the mean or worst footpad score should be considered, and whether footpads were cleaned on the farm. To assess the footpad lesions in the slaughterhouse, it is not clear how to select the footpads within the required two 5-minute intervals, and if the footpads should be collected and then scored, or scored on the processing line.

The American Humane Certified Society developed the “Animal Welfare Standards Audit” in 2000, which is a practical welfare assessment for turkeys on farms, during transport, and in processing plant audits (Humane, 2014). The farms in this program are audited by a trained assessor annually to maintain an acceptable welfare standard. The basis for the footpad assessment is a five point scoring system ranging from 0 –Healthy footpad, “No lesions” to 5 – The most severely ulcerated (i.e., burned) lesions; Severe, according to Poultry Intellimetrics Inc. (Clark et al., 2002) (Table 17). The five point scale was simplified to a two point audit scale (0- not severe, 1 –severe) according to the National Turkey Federation (National Turkey Federation, 2007), with footpad audit scores being either 0 (not severe) or 1 (severe) based on the reassigned five point methodology: footpad scores of 1-3 for an audit score of 0, and more severe scores of 4-5 for an audit score of 1.

During the audit on the farm, "… the auditor must also examine producer records for the following elements: bird health; nutrition plan; mortality; ammonia; lighting; and processing plants to score at shackling; broken leg; broken wings; and foot pads" , which indicates that footpads are not evaluated on the farm.

The American Humane Society states that footpad evaluations should be done at processing, preferably after hock cutting or when the feet are still on the carcass after scalding to avoid unnecessary stress for the turkeys (National Turkey Federation, 2007) and likely also to improve the accuracy and ease of scoring. Other measurements, such as broken legs, stunning rate, missed turkeys at auto knife and broken wings are evaluated in addition to the footpad scoring. According to the American Humane Certified Society footpad scores should be given for 300 turkeys in the processing plant, and a maximum of 10% (30 turkeys) may receive higher scores (3-5) on a five point scale and still be considered acceptable. The rest of the turkeys must have lower footpad scores (i.e., scores 1-2).

The National Turkey Federation also recommends sampling both feet from 100 turkeys or 200 feet in a minimum of two different loads, preferably in the processing plants to avoid causing stress in live animals. Over 40% of turkeys presenting an audit score of 1 is unacceptable, so "If one sample indicates unacceptable percentage of '1's', then another sample should be examined" (National Turkey Federation, 2007). The goal of the American Humane Society, as well as the National Turkey Federation for turkey welfare audits is to score animal welfare, which uses footpad scoring as one of the indices. However, if the FPD assessment is done only in processing plants, it will not allow any management changes to improve animal welfare during the long life span of turkeys (14 wk, females, and 19-20 wk, males). The five point scale of the Poultry Intellimetrics Inc. does not provide a description of lesions, potentially impeding the correct identification and classification of lesions. There are five pictures included with the scale, which can support the footpad scoring on farm, however, there is no differentiation of lesion severity in the system.

The description provided by the NTF contains information that is not fully supported by the scientific evidence, such as "the incidence of FPD in toms is higher than hens" (National Turkey Federation, 2007). Further, instructions are unclear such as when the sample size is to be 300 turkey feet, but they do not indicate if they should be from 150 turkeys or 300 feet from different turkeys. Additionally, it is not clear which foot should be scored. Finally, the guidelines contain pictures of turkey feet (after scalding) and the associated scores of the lesions, but there is no description of how to evaluate the depth of lesions, and based on the pictures, the only criterion for scoring is their size.

DISCUSSION

A gap in the current literature regarding the comprehensive evaluation of existing footpad scoring systems, and the need for an objective and easily understandable scoring system was identified by this review. The overall goal of the footpad scoring systems is to reflect the footpad health, and the welfare, however, this intention cannot be implemented by the existing systems.

Several footpad scoring systems are in use for broilers and turkeys. Many schemes are intended to be used in processing plants to avoid the stress of animal handling (Hocking et al., 2008; Humane, 2014). However, continuously monitoring the animal welfare on farms is essential to ensure animal welfare and decrease the incidence of FPD by providing immediate measurements on FPD so farmers can make decisions to improve litter conditions.

FPD has been found to be a relevant indicator of poultry welfare; therefore, an objective and intuitive scoring system is needed to classify FPD lesions and interpret the correlated welfare conditions of the flock. However, existing footpad scoring systems often lack descriptions of the lesions and extensively use highly technical terms. Lesions can also be difficult to identify if the color (dark brown vs. black) or the size (small or larger, covering "25-50% of the footpad area") is specified in the scheme but without an objective standard against which to measure. In addition, lay persons may rely entirely on illustrations of FPD scales to classify lesions and many systems do not provide any illustrations (Da Costa et al., 2014), or only images from some lesion categories (Martland, 1984), and may not have illustrations that accurately represent the differences among the classes particularly when there are 9 (Allain et al., 2009) or 10 lesion categories in the scale (Allain et al., 2013). Most pictures were taken after scalding (Clark et al., 2002), and few papers have included photographs taken in field settings where FPD can look quite different (Martland, 1984; Krautwald-Junghanns et al., 2013).

Furthermore, most systems illustrate the feet of adult broilers and turkeys at market age, with only one scheme shows the feet of turkey poults at a younger age (Krautwald-Junghanns et al., 2013). Without knowing what early FPD looks like, a farmer may not be able to make timely decisions about litter conditions that could help to address an emerging welfare problem.

The ideal footpad scoring system has not yet been developed but the features are clear. It should be intuitive, lesions should be easily identifiable as a category or score, and there should be just a few classes to save time and effort in assigning scores. To reflect the footpad status in all settings, little cleaning should be needed. A recent study (Lund et al., 2017) indicated a significant misclassification when broiler footpads were assessed in processing plants implying that even greater differences may exist in field settings. This suggests that the existing footpad scoring systems may need a revision, because we might still not identify the footpad lesions accurately and consistently

Table 1. Footpad scoring system in a turkey experiment (Jensen et al., 1970)

Score	Description
0	No dermatitis
4	Most severe

Table 2. Footpad scoring system in a turkey experiment (Jensen and Martinson, 1969)

Score	Description
1	Mild
2	Medium
3	Severe

Table 3. Footpad scoring system in a turkey experiment (Martland, 1984)

Score	Description
0	No lesion
1	Small scabs or scabs
2	Larger scabs occupying up to ¼ of pad areas
3	Severe large punched-out ulcers

Table 4. Footpad scoring system for broilers used in processing plant (Ekstrand et al., 1997)

Score	Category	Description
1	No lesion	No visible lesions: smooth epidermis, no discoloration
2	Mild lesion	Papillae only: hyperkeratosis but no discoloration
3	Mild lesion	Mild/superficial lesions: discoloration or erosions in the epidermal layer
4	Mild lesion	Mild/superficial lesions and papillae: hyperkeratosis and discoloration or erosions in the epidermal layer
5	Severe lesion	Severe ulcerations: discoloration, ulcers and signs of inflammatory reactions, and

Table 5. Footpad scoring system for broilers used in processing plant, “Modified Ekstrand” or “Modified Swedish scoring” (Ekstrand et al., 1998)

Score	Description
0	No remark; no lesions, only mild hyperkeratosis, no discoloration or scars
1	Mild lesions; superficial lesions, erosions, papillae and discoloration of the foot- pad
2	Severe lesions; deep lesions, ulcers, and scabs

Table 6. Footpad scoring system for broilers in processing plant (Allain et al., 2009)

Description	Area (% of footpad affected)		
Lesion observed	<25	25-50	>50
No lesions	Score 0	-	-
Keratosis/papillae, no discoloration	Score 1	Score 2	Score 3
Brown discoloration, erosion	Score 4	Score 5	Score 6
Black discoloration, deep lesions, split epidermis	Score 7	Score 8	Score 9

Table 7. Modified footpad scoring system for broilers in processing plant (Allain et al., 2009)

Score	Description	Footpad scores, Table 2.6.
FPD1	No or minor lesions	0-4
FPD2	Intermediate lesions	5-7
FPD3	Deeper lesions	8-9

Table 8. Footpad scoring system for turkeys in processing plant (Allain et al., 2013)

Description	Area (% of foot affected)		
Lesion observed	<25	25-50	>50
No lesion	Score 0	-	-
Enlargement of scales and erythema	Score 1	Score 2	Score 3
Hypertrophic and hyperkeratotic scales covered by a yellowish to brownish exudates	Score 4	Score 5	Score 6
Depressed lesion, loss of substance (=ulceration), with or without thick dark, adherent scab	Score 7	Score 8	Score 9

Table 9. Footpad scoring system for use in turkey processing plants in Europe (Hocking et al., 2008)

Score	Description
0	No external signs of FPD. The skin of the footpad feels soft to the touch and no swelling or necrosis is evident
1	The pad feels harder and denser than a non-affected foot. The central part of the pad is raised, reticulate scales are separated and small black necrotic areas may be present.
2	Marked swelling of the footpad. Reticulate scales are black, forming scale shaped necrotic areas. The scales around the outside of the black areas may have turned white. The area of necrosis is less than one quarter of the total area of the footpad.
3	Swelling is evident and the total footpad size is enlarged. Reticulate scales are pronounces, increased in number and separated from each other. The amount of necrosis extends to one half of the footpad.
4	As score 3, but with more than half the footpad covered by necrotic cells.

Table 10. Footpad scoring system for use on turkey farms (Krautwald-Junghanns et al., 2013)

Score	Description
0	No abnormality detected, surface of the plantar skin shows no alterations, reticulate scales arranged actinomorphic symmetrically, covering the whole plantar surface
1	Hyperkeratosis, moderate hypertrophy of the plantar skin, reticulate scales are elongated and/or separated, but not discolored, elevation in the center of the metatarsal foot pad
2	High-grade hyperkeratosis with crusts of adhesive dirt that are not detachable without damage of the plantar skin, increased tendency to bleed on manipulation
3	Epithelial necrosis, superficial lesions, reddish-brown discoloration of the reticulate scales, extensive necrotic areas
4	Deep lesions of the plantar skin, ablation of the epidermis with crater formation

Table 11. Footpad scoring system for use on turkey farms (Da Costa et al., 2014)

Score	Description
0	Footpad skin with no lesion
1	Lesion with diameter ≤1.3 cm
2	Lesion with diameter >1.3 and ≤1.6 cm
3	Lesion with diameter >1.6 and ≤1.9 cm
4	Lesion with diameter >1.9 and ≤2.2 cm
5	Lesion with diameter >2.2 and ≤2.5 cm
6	Lesion ≤2.5 cm of diameter; all footpad area is affected
7	All footpad and toes severely affected with lesions

Table 12. Histopathological footpad scoring system for turkeys (Mayne, Hocking, & Else, 2006)

Score	Description	Definition
0	None	No change, sample normal
1	Mild	Hyperkeratosis; 'Horned pegs' of keratin on surface; Epithelial hyperplasia; Compressed keratin on foot pad surface
2	Mild	Epidermal acanthosis; Increased dermal blood vessel density
3	Mild	Vacuoles in dermis/epidermis; Necrotic debris in keratin/epidermis
4	Medium	Presence of heterophils, macrophages and lymphocytes in dermis
5	Medium—severe	Increased density of heterophils, macrophages and lymphocytes; Congested/necrotic blood vessels; Necrotic debris of cells in dermis/epidermis
6	Severe	Split epidermis—1 lesion
7	Severe	Split epidermis—1 lesion or 1 very large lesion, more than 1/3 of total

Table 13. Histopathological footpad scoring system for broilers according to Michel et al. (2012)

Description	Macroscopic	Microscopic
Type I (mild/early-stage lesions)	Enlargement of scales, erythema +/- hyperkeratosis	Mild to moderate hyperplasia and/or hyperkeratosis of the epidermis; superficial dermal congestion and edema, variable and mild inflammatory infiltration
Type II (moderate/ superficial lesions)	Hypertrophic and hyperkeratotic scales covered by a yellowish to brownish exudate (poorly adherent crust)	Marked hyperplasia and hyperkeratosis of the epidermis, abundant heterophilic epidermal exocytosis with pustule formation; congestion in the superficial dermis with inflammatory infiltration
Type III (severe/ deep lesions)	Depressed lesion, loss of substance, crater (ulceration), with dark (brown or black) thick adherent crust	Ulceration (full-thickness necrosis of the epidermis, replaced by a necrotic and suppurative material), underlying granulation tissue; type II lesions at the ulcer margins

Table 14. External footpad scoring system according to Michel et al. (2012)

Score	Description
1	No lesion or enlargement of scales and erythema, whatever the size
2	Hypertrophic and hyperkeratotic scales covered by yellowish to brownish exudates, size <50% footpad
3	Hypertrophic and hyperkeratotic scales covered by yellowish to brownish exudates, size >50% footpad
4	Depressed lesion, loss of substance (=ulceration), with or without dark thick adherent crust, size <50% footpad
5	Depressed lesion, loss of substance (=ulceration), with or without dark thick adherent crust, size >50% footpad

Table 15. Welfare Quality® Assessment - Welfare principles and criteria (Butterworth et al., 2009)

	Welfare criteria		Measures
Good feeding	1	Absence of prolonged hunger	*This criterion is measured at the slaughterhouse*
	2	Absence of prolonged thirst	Drinker space
Good housing	3	Comfort around resting	Plumage cleanliness, litter, dust sheet test
	4	Thermal comfort	Panting, huddling
	5	Ease of movement	Stocking density
Good health	6	Absence of injuries	Lameness, hock burn, foot pad dermatitis
	7	Absence of disease (hygiene and care)	On farm Mortality, culling
	8	Absence of pain induced by management procedures (e.g. castration, dehorning)	NA
Appropriate behavior	9	Expression of social behaviors (for example grooming)	No measurement developed yet
	10	Expression of other behaviors (only in free range system) (for example foraging)	Cover on the range
	11	Good human-animal relationship (handlers' ethical behavior)	Avoidance distance test (ADT)
	12	Positive emotional state (avoid fear, distress, apathy, promote security and contentment)	Qualitative behavioral assessment (QBA)

Table 16. Modification of a footpad scoring system for use on broiler farms and processing plants as part of a welfare audit system Welfare Quality® Assessment

Level	Description	Footpad scores.
a	No evidence of FPD	0
b	Minimal evidence found	1-2
c	Evidence of FPD	3-4

Table 17. Footpad scoring system for use in turkey processing plants, used as a part of a welfare audit, Certified Humane, National Turkey Federation, based on Clark et al. (2002), Poultry Intellimetrics

Score	Description
1	Healthy, footpad; No lesion
5	The most severely ulcerated (i.e., burned); Severe

Formula 1. Calculation of flock footpad index scoring (Butterworth et al., 2009)

$Ip = (100- 2 (\% \text{ moderate lesion}) + 7 (\% \text{ severe lesion})) / 7$

If $Ip < 70$, then $Sp= (0.50686 \times Ip) - (0.0072409 \times Ip^2) + (0.000081315 \times Ip^3)$

If $Ip> 70$ then $Sp= -513.33 + (22.507 \times Ip) – (0.32152 \times Ip^2) + (0.0015779 \times Ip^3)$

CHAPTER III.

Bedding material and stocking density influences turkey hen performance

Summary

In this study, we examined the impact of bedding material type and bird stocking density on turkey hen performance in pen trials. Turkey hens were raised until 14 wk of age housed on Giant miscanthus grass (GM) or Pine wood shavings (PS), and stocked at low (LD), medium (MD) and high (HD) densities: 8.5, 10.7 and 14.2 hen/m^2 in 12 pens between 0 and 2 wk of age, then reassigned to 24 pens with densities of 4.3, 5.4 and 7.1 hen/m^2. Individual BW and feed intake (FI) were measured and feed conversion ratio (FCR) calculated by pen. Mortality and culling rates were recorded for each pen. Data were analyzed by two-way ANOVA, determining the effects of B, D, and B x D. The experimental unit was the pen. Tukey HSD All-Pairwise Comparisons test separated the means. The bedding type affected FI and FCR. Hens housed on MG exhibited lower FCR compared to PS (1.14 vs. 1.20) at 2 wk of age. FI was higher in turkeys housed on PS (4.95 kg) bedding than MG (4.72 kg) between 12 and 14 wk of age. The lower FI in hens housed on MG bedding in the last two weeks can be concerning and might relate to more severe footpad lesions, as the inflammation can impact the bird performance (Chapter IV). BW was improved at LD (0.39 kg) compared to MD (0.36 kg) at 2 wk of age, however, was not different from HD (0.38 kg). At 14 wk of age, turkeys held at LD (11.20 kg) had higher BW compared to those at HD (10.47 kg), MD was intermediate (10.85 kg). The overall (2-14 wk) FCR was lower at LD (1.86) as compared to HD

(1.98), and MD was intermediate (1.91). Similar production performance was obtained for hens housed on MG when considering performance through 14 wk of age compared to those on PS indicating that it is a suitable bedding type. Overall, turkey hens housed at LD exhibited improved performance indices at market age compared to HD, including overall FCR, or BW. Thus, further research needed to better understand how stocking density impacts performance.

INTRODUCTION

Good quality bedding is needed for animal housing. Bedding should be suitable throughout the production cycle to give comfort and also be economical. The most commonly used bedding material in poultry production in the United States is wood shavings (Koon et al., 1992; Clark et al., 2002; Dunkley and Ritz, 2017) because it generally meets those criteria although both the supply and price may fluctuate (Malone, 2009).

The selection of a bedding material for poultry production should consider that it: a) absorbs moisture originating from waterers and bird excreta, b) works as a cushion and protects the skin and breast area from damage (Garcês et al., 2013), c) provides insulation from the ground, and; d) has characteristics of being non-toxic, not dusty, and is lightweight to ensure better friability (Watkins, 2001; Ritz et al., 2005). Other important characteristics for bedding materials include physical and chemical properties such as size and distribution of bedding particles, their water releasing capacity (Bilgili et al., 2009), thermal conductivity and drying rates (Garcês et al., 2013), bulk density, and compressibility (Garcês et al., 2013). It is important to select a bedding substrate of appropriate particle size to ensure that birds won't consume it, because consuming bedding material can lead to poorer performance and increased mortality (Hester et al., 1997).

The greatest impact that bedding and litter (litter defined as bedding mixed with excreta, feathers and other materials generated during the production cycle or its re-use (Tabler and Wells, 2012) has on bird performance is its role in moisture control. For broiler chickens, the recommended moisture content of litter ranges from 20 to 30% (Watkins, 2001). Poultry litter is considered to be wet, when the rate of water addition (urine/feces/spillage) exceeds the rate of its removal (evaporation), and this occurs when litter moisture exceeds 25% (Collett, 2007). A simple test for litter moisture is to take a handful and squeeze. If the material remains friable, the litter moisture content is acceptable (Fairchild and Czarick, 2011). If it compacts, moisture is most likely in excess of 30%. Litter moisture impacts litter friability with higher moisture resulting in caked litter. Caking is the state, when the litter clumps together, since it retains moisture (Tabler and Wells, 2012). Caking is an important and frequent problem in poultry houses, because as litter clumps it can develop into a sealed layer, which does not support any moisture absorption and release.

Wet litter can affect bird performance. Lowered body weights (BW), feed and water intakes, and a higher feed conversion ratio (FCR) were reported in broilers kept on wet litter by Bilgili (2009) and DeJong et al. (2014).

Bedding materials need to be consistently available and fit within production budgets. For those reasons, alternative materials need to be studied for their characteristics as bedding, effects on poultry performance and well-being. Of recent interest are annual warm season grasses, such as Giant miscanthus grass (MG), Bermuda grass, and Switchgrass because of their use in the biofuel industry and for sustainable land use. Farmers can grow warm season grasses on their land, and be less dependent on other bedding sources, which can be in short supply (Patterson, 2014).

While not much has been reported on the use of warm season grasses for poultry/animal bedding, MG showed promising results as an alternative bedding for turkey toms (Evans et al., 2013), broilers, dairy cows, and swine (DeBruyn, 2015; VanWeyenberg et al., 2015). Studies have shown, that poultry may perform equally well on warm season grasses as on pine wood shavings (PS) bedding. When MG was compared to PS, no differences were found in BW for 19 wk old commercial turkey toms (Evans et al., 2013). Likewise, no differences in BW were detected for 13 wk old commercial turkey hens raised on PS or Bermuda grass (Smith, 2002). Feed conversion rates were improved for turkeys raised on MG compared to turkeys raised on PS during the growing period (Evans et al., 2013). Broiler studies showed no differences in BW when raised on MG or PS either in pen trials (Frank et al., 2014; Dunkley and Ritz, 2016) or in commercial settings (Patterson et al., 2014).

Stocking density may also affect turkey performance. A recent Canadian study investigating body weight in turkey toms stocked on 30, 40, 50, and 60 kg/m^2 reported increasing BW as density decreased at 16 wk of age (18.78, 18.71, 18.55, and 18.13

kg/m^2, respectively, P=0.01) (Beaulac et al., 2019). Higher BW were measured in turkey toms housed at low stocking density (LD, 0.46 m^2/bird) compared to turkeys kept at high stocking density (HD, 0.21 m^2/bird) at 16 and 20 wk of age (Noll et al., 1991). Similarly, improved BW and better average feed intakes were reported in turkey toms kept at LD (0.36 m^2/bird) than at HD (0.29 m^2/bird) between 5 and 18 wk of age (Jankowski et al., 2015). However, Perkins et al. (1995) did not find difference between the BW of turkey toms kept at LD (0.36 m^2/bird) and at HD (0.18 m^2/bird).

Recommendations or requirements for the stocking density (SD) of turkeys vary by organization making the recommendation, production system and location. In particular, in the US, the turkeys can be stocked at 15 lbs/ft^2 (73.24 kg/m^2) according to the guideline of the National Turkey Federation (NTF), noting that this density may be higher under various production environments depending on the gender and life span of the turkeys (National Turkey Federation, 2012). Stocking densities for turkeys are not currently specified for organic production (USDA Agricultural Marketing Service , and National Organic Program 2017) or other certified production organizations such as American Humane (2017).

Current research on stocking density is needed due to changes in genetics and bird performance indices (Erasmus, 2017). In addition, rising consumer demand have pushed for lower stocking density to improve animal welfare in general (Dawkins, 2018).

Thus, the present study investigated the effects of various bedding materials and stocking density on turkey hen performance.

MATERIALS AND METHODS

Birds, Housing and Management

The Institutional Animal Care and Use Committee (IACUC) at the Pennsylvania State University reviewed and approved the animal use protocol (IACUC# 45605) for this study.

The experiment was conducted at the Pennsylvania State University (Pennsylvania State P4 Poultry Unit) between July 28 and November 17, 2015. A total of 1,096 one day-old commercial turkey hen poults (Hybrid Converter) were obtained from a commercial hatchery (Cooper Turkey Hatchery, Oakwood, OH). The eggs were incubated in a Jamesway single stage incubator (Cambridge, ON, Canada) and hatched. Trimming of beaks and three toe nails was conducted at the hatchery. The turkey hens were fed commercial multiphase diets (Starter, Grower I, Grower II and Finisher with analyzed crude protein levels of 27.22, 27.24, 22.97, and 17.10%, respectively) (Wenger Feed Mill, LLC, Rheems, PA). The first phase diet was fed starting at placement and subsequent phases fed starting at 6, 8, and 12 wk of age. Each pen was equipped with one feeder and a bell drinker. Feeder and drinker space was adequate across all stocking densities (see Experimental Treatments and Design section below) according to management standards in the industry (Hybrid Turkeys, 2008). A bell type drinker is sufficient for 100-150 turkeys while one feeder is needed for 60 hens (Aviagen Turkeys, 2015). Feed and water were made available *ad libitum*. The lighting program and target house temperatures were as recommended by Hybrid (Turkeys Hybrid, 2008).

Experimental Treatments and Design

There were two factors to study, bedding and stocking density. A completely randomized block design with a 2 x 3 factorial arrangement was used.

Two types of bedding material, pine wood shavings (PS) and Giant miscanthus grass (MG), were evaluated. Either fresh PS or dried MG were added to a depth of 10 cm to each of the pens (8.2 m^2). For the MG treatment, the grass was chopped into 5 cm lengths. The bedding was not manipulated and fresh bedding was not added during the study period.

During 0-2 wk the turkeys were kept in 12 pens, then at 2 wk of age, turkeys were split evenly among 24 pens. Of note, the litter was not manipulated in the 12 pens where the hens were kept for the first two weeks of life, therefore the litter was used for 14 wk in those 12 pens and 12 wk in the other 12 pens. Birds were individually identified with numbered wing bands. At placement, poults were weighed and randomly assigned to 12 of the 24 pens in the facility with an assigned bedding material and stocking density. Stocking densities were designated as low (LD), medium (MD), and high (HD) and were 70, 88, and 116 poults per pen during 0 to 2 wk of age (8.5, 10.7 and 14.2 hen/m^2), respectively (Table 1). After 2 wk of age, the stocking density was 35, 44, and 58 hens per pen (4.3, 5.4, and 7.1 hen/m^2), respectively. At 2 wk of age, the poults were weighed and one-half of the birds from each pen were placed into another pen with the same assigned density (LD, MD, or HD) and bedding treatment. The trial concluded when the birds reached 14 wk of age with stocking density adjusted for bird loss presented in Table 2.

Data Collection

Body weights were taken on a pen basis when the birds were 1 day, 2 wk, 4 wk, 6 wk, 8 wk, and 11 wk and 6 days (hereafter referred to as 12 wk) wk of age. Individual hen weights were taken at 13 wk and 6 days (hereafter referred to as 14 wk) of age. Average feed intake was recorded when body weights were taken. Intake per pen was adjusted for any birds removed due to death or removal. Feed conversion (kg feed/kg BW gain) for each pen was calculated for each feeding period and on a cumulative basis for the trial.

Mortality and culling were recorded on a daily basis and then summarized for the feeding period. Reasons for culling included bad legs, pendulous crops, and other miscellaneous causes such as wrong gender for the trial (toms).

Data Analysi**s**

To examine effects of bedding (B) and stocking density (D), two (two wk brooding) or four (post two wk brooding) replicates per combination of B and D were assigned. Brooding period was added as a block to the statistical analysis for measurements after 2 wk of age, in order to account for the differences in litter condition and starting poult density when poults were distributed to all 24 pens. One pen was not included in the analysis due to an error in BW taken at 12 wk of age. BW data was averaged on a pen basis. Average feed intake for each feeding period was calculated per bird using the number of birds in the pen at the end of the feeding period. Feed conversion ratio was calculated based on pen feed consumption and average body weight gain (BWG). Percentage mortality and culling rates were calculated per pen.

A two-way ANOVA (Factorial ANOVA, Software: Statistix10 Analytical Software, Tallahassee, FL USA) was used to determine effects of bedding, density, and their interactions ($P < 0.05$) on live performance, with the pen being the experimental unit. If significant, a Tukey HSD All-Pairwise Comparisons test was conducted to separate the means at a probability level of $P < 0.05$. After the ANOVA the plotting the residuals and the Shapiro-Wilkinson Normality Test were used to verify a normal distribution.

Linear and quadratic polynomial contrast tests (Polynomial Contrast Testing, Software: Statistix10 Analytical Software, Tallahassee, FL USA) were performed, Scheffe statistics was conducted after ANOVA, to assess the relationship between D treatments and dependent variables for turkey performance if D was significant ($P < 0.05$).

RESULTS

Turkey Brooding Performance (0 to 2 wk of Age)

Density affected hen BW at 2 wk of age (Table 3). Hen poults raised at MD were lighter ($P = 0.006$) compared to hen poults at LD and HD. Quadratic relationship was observed for density in BW ($P = 0.007$) during the first two weeks.

Bedding material type affected FCR from 0 to 2 wk of age. Hen poults brooded on MG had a lower FCR than those started on PS (1.14 vs. 1.20, $P = 0.049$). No differences were observed for average feed intake per bird. Mortality and culling rate averaged 0.9 and 0.3%, respectively, and were not affected by B or D. Nor were there any interactions of B and D noted for live performance during the brooding period.

Turkey Growing Performance (2 to 14 wk of Age)

Hen BW was not affected by bedding material at any age (Table 4). No interactions between B and D were found for hen BW. Average feed intake was influenced by B between 12 and 14 wk of age (Table 5), when turkey hens kept on MG consumed less feed (4.72 vs. 4.95 kg/bird; $P = 0.023$) than turkeys raised on PS.

Density affected hen BW at 14 wk of age ($P = 0.046$). Hens raised at LD presented the highest BW (11.20 kg) and was different for HD hens with lowered BW (10.47 kg) (Table 4). Body weights of hens at MD was intermediate and not significantly different from those held at the other two stocking densities. The BW of hens decreased as density increased linearly ($P = 0.048$) at 14 wk of age, as shown with the polynomial contrast test. The feed conversion ratio (FCR) was affected by stocking density (Table 6). The overall FCR was lower for turkeys at LD compared with turkeys at HD (1.86 vs. 1.98, $P = 0.049$), with the FCR for turkeys at MD intermediate (1.91) and not significantly different from either LD or HD groups. In addition, the overall FCR increased linearly, as stocking density increased ($P = 0.049$).

Mortality (%) was not affected by either bedding material or stocking density during the experiment (all $P>0.05$; Table 7). The culling rate overall was not affected by bedding or density (Table 8) with one exception. During 4 to 6 wk, density had a significant effect on culling when expressed on a percentage basis. However, the average number of birds culled per pen per density was equivalent to the same number of birds across all stocking density treatments. This was approximately one hen per pen or four hens per density rate that were culled. When culling rate was examined on based on the number of birds removed per treatment, similar numbers of birds were removed across all B and D treatment combinations on an overall basis (Table 9).

DISCUSSION

The turkey industry faces many challenges, such as reducing the costs of production while improving production environments for the turkeys, which can impact bird performance, including BW, FCR and feed intake. Thus, finding more economical but effective bedding sources is essential, as it can impact turkey performance. In addition, turkey production has to be responsive to consumer demands by exploring new production systems such as antibiotic free or organic production, while measuring their impact on animal welfare and monitoring how turkeys perform under new requirements, such as lower stocking density. Few studies have examined the stocking density of turkey hens in the last decade. Therefore examining MG as an alternative bedding material at various stocking densities of turkey hens fills a significant gap in the current literature.

The current study examined the influence of bedding material and stocking density, and observed that turkey hens raised on MG performed as well as their counterparts on PS bedding. Overall improved performance was detected at LD compared to HD, such as BW at 14 wk of age.

Bedding and Turkey Hen Performance

A variety of different grasses have been examined as bedding material for poultry including turkeys (Grimes et al., 2002; Evans et al., 2013; Barkley, 2017). In the current study, turkey hens housed on MG gained BW as well as the birds on PS bedding. The lack of an effect of bedding is in line with other studies examining grass bedding used with turkeys and broilers. Because only a few studies were available that examined turkeys raised on warm season grass bedding, broiler trials have been included in the discussion. Evans et al. (2013) reported no difference in BW for turkey toms housed on MG vs. PS bedding over 19 wk. Smith et al. (2002) compared PS and chopped Bermuda grass hay bedding used for turkey hens during a 13-wk trial, and did not find any difference in BW between the treatments. Davis et al. (2010) studied chopped switchgrass and PS in mixed sex broilers over a 49-day research course with no difference in BW. Davis et al. (2015) examined chopped Bermuda grass, chopped switchgrass and PS over three heavy broiler flocks (each growing cycle ended at 48 d) and observed greater BW during the first 14 days in broilers raised on PS compared to switchgrass and Bermuda grass.

In the current study, 4.65% greater average feed intake was observed in turkey hens raised on PS vs. MG bedding between 12 and 14 wk of age. Numerically the BW, and BWG was higher in hens kept on PS compared to MG, which yielded improved FCR by 12 point in turkeys kept on PS. This might indicate, that turkeys which exhibited higher footpad scores and more severe lesions on MG bedding (1.41 on a 3-point footpad scoring scale, which ranges between 0, no lesion and 2, severe lesions, according to Berg,

2015) compared to hens raised on PS bedding. The severe footpad lesions can result is lower BW (Martland, 1984; Mayne et al., 2007a), and associated with potential immune system stimulation, therefore the growth is less effective (see Chapter IV.). The present study found differences in feed intake (12-14 wk) which contrasts with previous findings. Two previous studies found no difference between bedding types on average feed intake in 19 week old turkey toms (Evans et al. (2013) and 14 week old turkey hens (Smith et al. 2002). Davis et al. (2015) reported greater feed consumption in broilers kept on PS compared to switchgrass or Bermuda grass during the first 14 days of the study. During days 15 to 28 d greater feed consumption was noted for broilers kept on PS than Bermuda grass, but was not different from the broilers raised on switchgrass (Davis et al., 2015).

In the present study, improved FCR was seen in turkeys raised on MG bedding vs. PS during the first two wk of age but not overall. The higher FCR for poults from 0 to 2 wk on PS as compared to MS (15%) might be the result of the fact that turkey poults tend to consume bedding and prefer fine particles (Hester et al., 1997). Litter characteristics are presented in Chapter IV., and demonstrated finer particle size for the shavings although these measures were done later at 6 and 14 wk of age. This behavior might lead to poorer FCR as a result of reduced body weight gain due to litter consumption (Malone et al., 1983; Smith et al., 2002). Small but numerically larger values for BWG and lower values for feed intake for the MG bedding resulted in the improved FCR for MG as compared to PS. Little information is available on FCR as related to bedding material in turkey poults. Youssef reported numerically lower FCR in turkey hens housed on dry wood shavings bedding compared to wet litter treatment between 15 and 36 days of age

(P>0.05) (Youssef et al., 2011a). To examine whether poults tended to consume bedding, the gastrointestinal track could have been investigated, such as necropsy to describe the content and measurement of proventriculus, and gizzard, as it varied in broilers raised on different bedding floor types (Xu et al., 2017). Unfortunately the cecal microbiota colonization, and development were not examined in the current study, which was found to be different in broilers housed on different bedding (Torok et al., 2009).

The present study found an improved FCR in hen poults between 0-2 wk of age, which was limited only for this short period of time at early age, and did not affect the overall performance. The comparison of this early improvement on FCR with other studies is somewhat difficult, as those tend to report differences in older age. Other studies with turkey hens (Smith et al. 2002) or broilers (Davis et al. 2010) did not find an improvement in FCR with the exception of Evans et al. (2013) who reported lower FCR in male turkeys at 6, 9, and 15 wk of age (2.61 vs. 2.67 ±0.27 at 15 wk of age) on MG vs. PS. Another possible explanation for improved FCR of hen poults raised on MG, might be related to litter temperature in the comfort range for hens for MG and thus better BW gain. This appears not to be supported in the study by Barkley (2017) who reported higher litter temperatures for PS vs. MG in a broiler experiment at 5 wk of age (28.15 vs. 24.81 °C, P = 0.015), but no differences at 1 week of age.

In the current experiment the mortality rate was not different between PS and MG, which agrees with the findings of Hester et al. (1997). In that trial, tom turkeys were raised on fine or course particle board residue and hardwood shavings up to 123 d with no differences in mortality rate. Bedding material was found to have no impact on mortality rates in broiler studies as well. Davis et al. (2002) examining Bermuda grass and PS in broilers at 49 d, or Davis et al. (2015) comparing Bermuda grass, switchgrass and PS bedding of three heavy broiler flocks.

Density and Turkey Hen Performance

In the current study, stocking density influenced the body weight of turkey hens at 2 wk of age and at the end of the experiment (14 wk of age). At 2 wk of age a 7.7% greater BW was seen in birds at LD by compared to those at MD. At the end of the trial (14 wk of age), again the highest BW was seen in the LD treatment group by 6.5% compared to HD. These findings are consistent with the conclusions of papers by others studying male turkeys under research conditions. Martrenchar et al. (1999) noted greater BW in toms kept at LD than HD (0.40 vs. 0.25 m^2/tom) at 12 wk of age by 2%, and at 16 wk of age greater BW was observed in birds at LD than MD or HD by 2%. Noll et al. (1991) found greater BW (13.5 vs. 12.8 kg) in male turkeys kept at LD than at HD (0.46 vs. 0.21 m^2/bird) at 20 wk of age. Beaulac (2018) reported increased BW (18.78 vs. 18.13 kg) in turkey toms at 16 wk as stocking density decreased (0.56 vs. 0.29 m^2/bird). In contrast, Perkins et al. (1995) did not find differences in BW between toms kept at LD or at HD (0.54 vs. 0.27 m^2/bird) at 16 wk of age. Martrenchar et al. (1999) reported 3% higher BW in turkey hens kept at LD compared at HD (0.16 vs. 0.10 m^2/hen), MD (0.12 m^2/hen) was intermediate. Martrenchar (1999) observed 3% higher BW of turkey hens raised at LD (0.16 m^2/hen) over either MD or HD (0.12 or 0.10 m^2/hen) at 12 wk of age.

In this study, we found improved overall feed conversion rate in birds raised at LD (by 6 %) compared to the HD group. In contrast, Beaulac (2018) did not find differences in FCR between LD and HD (0.56 vs. 0.28 m^2/bird) over the course of a 16 wk trial in turkey toms (Beaulac, 2018). Similarly, Perkins et al. (1995) reported no FCR differences between LD and HD groups (0.36 vs. 0.29 m^2/bird).

The rate of mortality was not different among stocking density treatments in the present study, which agrees with the findings of Beaulac and Schwean-Lardner (2018) who also did not find evidence of relationship between mortality rate and stocking density (0.56 vs. 0.28 m^2/bird) on heavy toms raised to 16 wk of age. Likewise, Moran et al. (1985) reported no difference in the rate of mortality of toms raised at LD or HD (0.89 vs. 0.44 m^2/bird) up to 16 wk of age. However, Noll et al. (1991) found that increasing stocking density (0.46 vs. 0.21 m^2/bird) tended to increase mortality ($P < 0.10$). Overall culling was not different among densities, which is in agreement with the findings of Beaulac et al.'s (2019) who studied heavy toms stocked at LD and HD (0.56 vs. 0.28 m^2/bird) up to 16 wk of age.

In summary, we found that turkey hens raised on MG performed equally well as compared to turkeys housed on PS bedding, however had lower average feed intake during 12-14 wk of age compared to those raised on PS, which might be concerning.

The turkey hens housed at LD presented higher BW (at both 2 and 14 wk of age), and had an improved overall FCR Stocking density did not affect the average feed intake or the overall mortality rate of turkey hens.

In the present study turkey hens performed well when measured against the performance goals of the breeder company (Hybrid Turkeys, 2019). The average BW of hens during the entire study period with the exemption of 8-12 wk of age was superior to the industry standards, exhibiting at least the same BW, and exceed it by 1-9%. In particular, at 14 wk of age the average BW of the hens was higher by 9% than the standards suggesting the good performance throughout the study. The only time point, when hens performed worse than expected was at 12 wk of age, when the BW of the hens in the current study was lower by 4% than the industry standard (Hybrid Turkeys, 2019).

When feed intake was considered, hens in the present study consumed less feed on average by 1-10% than predicted by industry standards, except from 6 to 8 wk of age when turkeys consumed more feed than suggested by 1% (Hybrid Turkeys, 2019).

The limitation of this study design was the relatively few replications (two in the first two weeks, and four during weeks 2-14), which might have resulted in failing to detect small differences between the performance indices. The selection of the discrete stocking densities of 35, 44, 58 hens/pen lacks equally gradual differences resulting in overly generalized conclusions on density categories. Due to numerically higher culling rates in certain pens, there were variable final stocking densities. This could have been corrected by using space occupying blocks added to the pen, to physically decrease the pen size and thus equalize the space per bird.

Measuring other potentially confounding factors might have helped in understanding the observed improved turkey performance on MG or at LD, such as the water intake, ammonia levels, ambient temperatures, including those of the litter surface.

Observations of foraging behavior might have helped better understand the FCR differences as a result of increased feed intake, although as the litter tends to cake that might limit the foraging.

This study supports the current needs of the turkey industry by providing information related to bedding sources (PS vs. MG), and stocking densities. However, further research is needed to clarify the advantages of MG on FCR, and lowered feed intake.

Table 1. Description of targeted stocking density (hens/m^2) levels from 0 to 2 wk of age, 2 to 14 wk of age, and at 14 wk of age.

DENSITY	LOW (LD)		MEDIUM (MD)		HIGH (HD)	
Age (wk)	Hens/m^2	Hens/pen	Hens/m^2	Hens/pen	Hens/m^2	Hens/pen
0 to 2	8.54	70	10.72	88	14.2	116
2 to 14	4.27	35	5.36	44	7.1	58

Table 2. Description of final stocking density levels at 14 wk of age (kg/m^2)

DENSITY	LOW (LD)		MEDIUM (MD)		HIGH (HD)	
Age (wk)	kg/m^2	Hens/pen	kg/m^2	Hens/pen	kg/m^2	Hens/pen
14	43.02	31.50	49.13	37.13	65.55	51.34

Table 3. Influence of bedding material and stocking density on the body weight (BW), feed intake (FI), and feed conversion ratio (FCR) of hen turkeys to 2 wk of age.

	Bedding[1,2]		Pooled	Density[3, 4]			Pooled	P-value		
	PS	MG	SEM[5]	LD	MD	HD	SEM[5]	Bedding	Density	B × D
BW (kg)	0.3718	0.3782	0.0045	0.3875[a]	0.3588[b]	0.3786[ab]	0.0059	0.3337	0.0059+	0.3105
BWG (kg)	0.3165	0.3336	0.0077	0.3432	0.3042	0.3278	0.0094	0.1677	0.0692	0.1826
FI (kg)	0.3788	0.3797	0.0076	0.3955	0.3623	0.3798	0.0093	0.9371	0.1150	0.6156
FCR	1.1996[a]	1.1397[b]	0.0172	1.1587	1.1912	1.1590	0.0210	0.0486	0.4953	0.1602

[a,b] Different superscripts within the same column and factor designate significant differences ($P<0.05$)
[1] Bedding: PS (Pine wood shavings) and MG (Miscanthus grass);
[2] Number of replicates (n): n=5 for MG and n=6 for PS
[3] Stocking density: LD 4.2, MD 5.3 and HD 7 hens/m^2
[4] Number of replicates (n): n=4 for LD and MD; n=3 for HD
[5] SEM - the largest SEM value was selected due to the unbalanced design, which did not allow for one SEM value for the factors
+ Polynomial contrast, quadratic relationship among densities (Scheffe, P = 0.0070, quadratic)

Table 4. Influence of bedding material and stocking density on turkey hen body weight (BW) from 4 to 14 wk of age (kg)

Item	Bedding[1,2]		Pooled SEM[5]	Density[3, 4]			Pooled SEM[5]	P-value		
	PS	MG		LD	MD	HD		Bedding	Density	B × D
Week 4	1.13	1.17	0.0209	1.19	1.12	1.14	0.0263	0.1837	0.1918	0.5410
Week 6	2.45	2.54	0.0384	2.54	2.45	2.48	0.0483	0.1067	0.3503	0.5941
Week 8	4.27	4.35	0.0427	4.36	4.26	4.30	0.0537	0.1994	0.3771	0.1027
Week 12	7.88	7.98	0.1196	8.16	7.88	7.75	0.1503	0.5997	0.1534	0.6934
Week 14	10.94	10.74	0.1552	11.20[a]	10.85[ab]	10.47[b]	0.1951	0.3501	0.0456+	0.5540

[a,b] Different superscripts within the same column and factor designate significant differences ($P<0.05$)

[1] Bedding: PS (Pine wood shavings) and MG (Miscanthus grass);

[2] Number of replicates (n): n=5 for MG and n=6 for PS

[3] Stocking density: LD 4.2, MD 5.3 and HD 7 hens/m^2

[4] Number of replicates (n): n=4 for LD and MD; n=3 for HD

[5] SEM - the largest SEM value was selected due to the unbalanced design, which did not allow for one SEM value for the factors

+ Polynomial contrast, linear relationship among densities (Scheffe, P = 0.0479, linear)

Table 5. Influence of bedding and stocking density on turkey hen feed intake from 2 to 14 wk of age

Item	Bedding[1, 2]		Pooled	Density[3, 4]			Pooled	P-value		
	PS	MG	SEM[5]	LD	MD	HD	SEM[5]	Bedding	Density	B × D
2 to 4 wk	1.03	1.06	0.0280	1.10	1.00	1.03	0.0351	0.4737	0.1113	0.5606
4 to 6 wk	1.97	2.04	0.0416	2.01	1.99	2.03	0.0523	0.2129	0.8401	0.6715
6 to 8 wk	3.17	3.19	0.0389	3.23	3.13	3.18	0.0488	0.7633	0.2867	0.1391
8 to 12 wk	8.62	8.41	0.1145	8.47	8.65	8.42	0.1439	0.1924	0.4463	0.6258
12 to 14 wk	4.95[a]	4.72[b]	0.0670	4.84	4.82	4.83	0.0841	0.0229	0.9811	0.7980
Overall[6]	20.12	19.79	0.1927	20.05	19.95	19.87	0.2421	0.2348	0.8611	0.3588

[a,b] Different superscripts within the same column and factor designate significant differences ($P<0.05$)

[1] Bedding: PS (Pine wood shavings) and MG (Miscanthus grass);

[2] Number of replicates (n): n=5 for MG and n=6 for PS

[3] Stocking density: LD 4.2, MD 5.3 and HD 7 hens/m^2

[4] Number of replicates (n): n=4 for LD and MD; n=3 for HD

[5] SEM - the largest SEM value was selected due to the unbalanced design, which did not allow for one SEM value for the factors

[6] Overall data between 2 and 14 wk of age

Table 6. Influence of bedding and stocking density on turkey hen feed conversion ratio (FCR, f:g) from 2 to 14 wk of age

Item	Bedding[1, 2]		Pooled	Density[3, 4]			Pooled	P-value		
	PS	MG	SEM[5]	LD	MD	HD	SEM[5]	B	D	B × D
Week 2-4	1.36	1.36	0.0256	1.40	1.31	1.36	0.0322	0.9051	0.1220	0.4246
Week 4-6	1.51	1.51	0.0284	1.48	1.53	1.52	0.0357	0.9284	0.6030	0.4480
Week 6-8	1.75	1.77	0.0072	1.78	1.74	1.74	0.0091	0.5488	0.5701	0.0748
Week 8-12	2.42	2.34	0.0362	2.25	2.44	2.44	0.0454	0.5634	0.3858	0.7180
Week 12-14	1.67	1.79	0.1269	1.64	1.69	1.86	0.1594	0.4924	0.5721	0.7481
Overall[6]	1.92	1.93	0.0257	1.86[b]	1.91[ab]	1.98[a]	0.0323	0.7873	0.0491+	0.8880

[a,b] Different superscripts within the same column and factor designate significant differences (P<0.05)
[1] Bedding: PS (Pine wood shavings) and MG (Miscanthus grass);
[2] Number of replicates (n): n=5 for MG and n=6 for PS
[3] Stocking density: LD 4.2, MD 5.3 and HD 7 hens/m^2
[4] Number of replicates (n): n=4 for LD and MD; n=3 for HD
[5] SEM - the largest SEM value was selected due to the unbalanced design, which did not allow for one SEM value for the factors
[6] Overall data between 2 and 14 wk of age
+ Polynomial contrast, linear relationship among densities (Scheffe, P= 0.0497, linear)

Table 7. Influence of bedding and stocking density on turkey hen mortality (%) from 2 to 14 wk of age

Item	Bedding[1, 2]		Pooled	Density[3, 4]			Pooled	P-value		
	PS	MG	SEM[5]	LD	MD	HD	SEM[5]	B	D	B × D
Week 2-4	0.74	3.22	0.0186	4.04	1.01	0.89	0.0233	0.5302	0.3507	0.2855
Week 4-6	0.81	0.90	0.0089	0.37	1.91	0.30	0.0112	0.3874	0.9312	0.7022
Week 6-8	1.41	1.45	0.0070	1.16	2.17	0.96	0.0088	0.5248	0.9658	0.4214
Week 8-12	1.83	1.57	0.0115	1.19	3.05	0.87	0.0144	0.2298	0.8128	0.3104
Week 12-14	1.47	1.74	0.0107	0.83	2.02	1.98	0.0134	0.3580	0.7298	0.2189
Overall[6]	6.27	8.89	0.0279	7.58	10.03	5.12	0.0350	0.5346	0.4608	0.7721

[a,b] Different superscripts within the same column and factor designate significant differences ($P<0.05$)
[1] Bedding: PS (Pine wood shavings) and MG (Miscanthus grass);
[2] Number of replicates (n): n=5 for MG and n=6 for PS
[3] Stocking density: LD 4.2, MD 5.3 and HD 7 hens/m^2
[4] Number of replicates (n): n=4 for LD and MD; n=3 for HD
[5] SEM - the largest SEM value was selected due to the unbalanced design, which did not allow for one SEM value for the factors
[6] Overall data between 2 and 14 wk of age

Table 8. Influence of bedding and stocking density on turkey hen culling (%) from 2 to 14 wk of age

Item	Bedding[1, 2]		Pooled	Density[3, 4]			Pooled	P-value		
	PS	MG	SEM[5]	LD	MD	HD	SEM[5]	B	D	B × D
Week 2-4	0.19	0.39	0.0023	0.00	0.87	0.02	0.0029	0.5360	0.0607	0.7106
Week 4-6	2.35	2.34	0.0002	2.90[a]	2.37[b]	1.76[c]	0.0003	0.7155	0.0000+	0.8514
Week 6-8	0.25	0.84	0.0039	0.76	0.31	0.57	0.0049	0.2921	0.7785	0.6960
Week 8-12	3.77	4.44	0.0109	3.09	5.93	3.29	0.0137	0.6578	0.2449	0.7149
Week 12-14	0.52	0.03	0.0028	0.45	0.33	0.05	0.0035	0.2182	0.6926	0.5699
Overall[6]	7.08	8.04	0.0148	7.19	9.80	5.68	0.0186	0.6423	0.2786	0.9140

[a,b] Different superscripts within the same column and factor designate significant differences ($P<0.05$)

[1] Bedding: PS (Pine wood shavings) and MG (Miscanthus grass);

[2] Number of replicates (n): n=5 for MG and n=6 for PS

[3] Stocking density: LD 4.2, MD 5.3 and HD 7 hens/m^2

[4] Number of replicates (n): n=4 for LD and MD; n=3 for HD

[5] SEM - the largest SEM value was selected due to the unbalanced design, which did not allow for one SEM value for the factors

[6] Overall data between 2 and 14 wk of age

+ Polynomial contrast, linear relationship among densities (Scheffe, $P < 0.0001$, linear)

Table 9. Distribution of culling in bedding and density treatments (%) from 2 to 14 wk of age

	Culled hens (count)				Culled hens (%) based on total culling			
Treatments	Legs	PC	Other	Total Culls	Legs	PC	Other	Total Culls
PS-LD	3	0	6	9	4%	0%	8%	13%
PS-MD	2	4	8	14	3%	6%	11%	20%
PS-HD	1	0	11	12	1%	0%	15%	17%
MG-LD	0	1	9	10	0%	1%	13%	14%
MG-MD	2	3	12	17	3%	4%	17%	24%
MG-HD	0	0	9	9	0%	0%	13%	13%
Total	8	8	55	71	11%	11%	77%	100%
Bedding								
PS	6	4	25	35	8%	6%	35%	49%
MG	2	4	30	36	3%	6%	42%	51%
Total	8	8	55	71	11%	11%	77%	100%
Density								
LD	3	1	15	19	4%	1%	21%	27%
MD	4	7	20	31	6%	10%	28%	44%
HD	1	0	20	21	1%	0%	28%	30%
Total	8	8	55	71	11%	11%	77%	100%

Chapter IV.

Bedding material and stocking density influence on footpad dermatitis in turkey hens and litter characteristics

Summary

Bedding characteristics, and increased stocking density may influence the litter moisture resulting in higher incidence of footpad dermatitis (FPD), a frequently observed condition that may impact welfare and bird performance. A pen study investigated the effects of turkey hen density (D; 4.3, 5.4 and 7.1 hens/m2, referring to low, medium and high density, LD, MD, and HD, respectively) and 2 bedding (B) materials (pine shavings, PS and chopped Giant miscanthus grass, MG) in a factorial arrangement on severity and prevalence of FPD in turkey hens, and litter characteristics. Hybrid Converter poults (1056) were placed into 24 pens for rearing. At 6 and 14 wk of age, a composite footpad score (increasing severity 0, 1 or 2) for each hen in the study was obtained without cleaning the pads, and averaged. ANOVA determined significance ($P < 0.05$) of B, D, and BxD. Spearman correlation determined the relationship between footpad scores and litter characteristics. Pine shavings bedding was associated with lower footpad scores compared to MG (0.14 vs. 0.38) at 6 wk, and (0.71 vs. 1.43) at 14 wk. Lower average footpad scores were observed at LD (0.77) as compared to either MD (1.16) or HD (1.27). Bedding affected Mn content of litter at 6 wk, and at 14 wk the litter moisture, total N, and iron content of the litter. Density influenced ammonium-N, Cl, Na at 6 wk, and moisture, pH, total N, and organic N content of the litter at 14 wk. Litter particle size was larger (> 4.76 mm) for MG, and finer particle (<3.36 mm) size was associated with PS. Correlation revealed that larger particle size, and higher litter moisture is associated with more severe footpad scores at 6 wk ($r = 0.81$, $P < 0.0001$), and 14 wk ($r = 0.44$, $P = 0.030$). In conclusion, hens reared at lowered density and PS had better footpad condition. Rearing turkey hens on MG was associated with more severe footpad lesions, higher litter moisture, and larger particle size compared to PS. Reduction in MG particle size should be investigated as a means to reduce FPD severity in future research.

INTRODUCTION

Footpad dermatitis (FPD) is a condition characterized by hyperkeratosis, scabs and ulcers on the footpads of poultry (Chen et al., 2016). Footpad dermatitis is an animal welfare and economic concern in broilers and turkeys (Chen et al., 2016) and has been used as a welfare indicator in many countries (Clark et al., 2002). In some European countries footpad lesions of broilers and turkeys are monitored at processing plants, and used as the sole index of flock welfare (Lund et al., 2017).When the incidence of severe footpad lesions is excessive at processing, farmers can be required to reduce stocking density (Ekstrand et al., 1997; Mayne et al., 2007b). Since the condition of the footpad is primarily influenced by litter moisture, litter moisture should be kept under 30% (Mayne et al., 2007b) to keep the prevalence and severity of FPD low in turkeys. However, FPD is multifactorial, and in addition to the selection of bedding material, other management factors, such as stocking density, drinker design, genetics, and/or gender of the turkeys can influence the severity of FPD.

The type of bedding material used in turkey barns is a very important factor in turkey production, since the birds may spend 14 to 20 weeks in turkey houses on litter. The bedding becomes litter when mixed with other materials generated in the production cycle, including excreta, feathers, spilled feed and water (Tabler and Wells, 2012), and all these components may affect the nature of the litter, such as increasing its moisture content, changes in nutrient content, and ammonia production. Litter becomes wet litter when it’s moisture content exceeds 25% which surpasses the amount of moisture that can be evaporated under normal barn conditions (Collett, 2007). Wet litter negatively affects bird performance, and multiple welfare indices, including FPD, feather cleanliness, breast irritation scores, hock burns and gait (DeJong et al., 2014). For these reasons, litter moisture should be maintained below 30% throughout the production cycle.

The moisture content of litter is influenced by many components besides bedding material. In addition to the bedding characteristics, management practices, such as preventing water spillage or leakage from the water system, and using proper ventilation and heating systems, are needed to control litter moisture. Other factors affecting the amount and/or moisture content of excreta include water consumption, gender, age and species of poultry, diet composition, intestinal health, and stocking density (Collett, 2007; Collett, 2012). While the moisture content of poultry excreta is similar among broilers, layers, and turkeys at 75%, the moisture content of litter removed from turkey houses tends to be higher than that removed from broiler houses (34% vs. 24%) (Mukhtar, 2005), indicating that moisture control of the litter may be more difficult in turkey production systems.

Characteristics associated with the litter may influence the FPD lesions. These include particle size, ammonia, minerals. It was shown, that bedding consisting of smaller particle size lowered the litter moisture, therefore FPD prevalence in a turkey experiment (Hester et al., 1997), and a broiler field trial (Barkley, 2017). Litter characteristics, such as greater water holding capacity can influence the litter moisture content, therefore the FPD lesions (Barkley, 2017). The high litter moisture supports the microbial activity in the litter, which will result in greater ammonia production (Bessei, 2006). Ammonia can be detrimental for poultry health, such as FPD in broilers, (Nagaraj et al., 2007), and the respiratory, and the immune system can be affected. Ammonia is supplied by uric acid and urea in turkeys (Nahm, 2003). Many factors can affect the ammonia volatilization. The activity of microbial enzymes, especially uricase and urease are influenced by the basic pH (above 8), high litter moisture, temperature, and the presence of oxygen, which will enhance the production of ammonium-hydroxide, and finally the ammonium (Nahm, 2003). Wet litter and pH play an essential role in the formation of ammonia, therefore keeping the litter moisture and the pH low is critical in terms of poultry health.

The most frequently used bedding type in poultry houses is pine wood shavings (PS) in the United States (Clark et al., 2002). Giant miscanthus grass (MG) is a relatively new bedding alternative, and there is a lack of information in the current literature on the impact of MG on flock performance (see Chapter III), and welfare indices, such as FPD in turkey hens. Few research studies have examined MG as bedding for poultry, therefore, trials using Bermuda grass, which has similar physical properties, are referenced here. Smith et al. (2002) studied Bermuda grass and PS bedding on market turkey hens and found neither differences in footpad scores nor litter moisture between the two treatments. Similarly, Dunkley and Ritz (2016) found no differences in footpad scores between boilers raised on MG and PS. In contrast, Patterson et al. (2014) and Frank et al. (2014) reported that footpad scores were greater in boilers kept on MG compared to PS. No difference in litter moisture was detected between MG and PS litter in the study by Frank et al. (2014), while litter moisture was not assessed in the study by Patterson et al. (2014).

Limited, and sometimes mixed information is available about the characteristics of MG as bedding. DeBruyn (2015) reported a higher rate of water absorption for MG compared to PS (2.97 vs. 2.55 g water held per g of dry bedding). Barkley (2017) noted a lower moisture holding capacity for MG than PS (2.07 vs. 4.33 g water held per g of dry bedding). Rauscher and Lewandowski (2016) found lower water absorption capacity for MG, when compared to wheat straw or PS (3.28, 4.60 vs. 5.52 g water/g DM). Based on this limited information, moisture absorption for MG is estimated to be approximately 67% of that of PS, which indicates that more moisture may accumulate when MG is used for bedding. More studies are needed to ascertain whether MG can be a satisfactory bedding material.

High stocking density (HD) can exacerbate wet litter conditions because of the increased output of excreta per area (Haslam et al., 2007). In addition, HD is associated with increased litter moisture and nitrogen which can create an ideal environment for microbial growth (Bessei, 2006). Feddes et al. (2002) reported elevated water consumption in broilers held at high density, which can consequently increase the moisture content of excreta and thus, litter moisture. Furthermore, HD might be associated with increased water spillage in open style drinkers, as the increased number of birds may increase the amount of water spilled during drinking. Turkey studies have reported mixed results, or haven't monitored the effects of stocking density on litter moisture. Noll et al. (1991) found lower litter moisture in pens with turkey toms kept at LD (0.46 m^2/bird) compared to pens with turkeys kept at HD (0.21 m^2/bird) at 12, 16 and 20 wk of age. In contrast, a recent Canadian study (Beaulac and Schwean-Lardner, 2018) found no difference in litter moisture between pens of turkey toms held at LD (0.56 m^2/bird) vs. those with toms at HD (0.28 m^2/bird). Martrenchar et al. (1999) studying turkey toms raised at LD (0.4 m^2/bird) and HD (0.25 m^2/bird) did not find differences in litter moisture due to the densities. Many studies evaluating turkey stocking density did not determine litter moisture (Moran, 1985; Perkins et al., 1995; Jankowski et al., 2015).

Stocking density may affect welfare indices, such as footpad scores (Moran, 1985; Martrenchar et al., 1999). A recent study (Beaulac and Schwean-Lardner, 2018) reported higher average footpad scores in turkey toms housed at HD (0.28 m^2/bird), than in those at LD) (0.56 m^2/bird) at 10 and 16 wk of age, as well as poorer average feather scores at 10, 12 and 16 wk of age. Some researchers found a relationship between increased stocking density and a greater incidence of footpad lesions in broilers (Haslam et al., 2007; Krautwald-Junghanns et al., 2013) and turkeys (Martrenchar et al., 1999; Beaulac and Schwean-Lardner, 2018). However, others reported that stocking density had no association with FPD in broilers (Dawkins et al., 2004; Sirri et al., 2007; Meluzzi et al., 2008) and turkeys (Martrenchar et al., 2002). Dawkins concluded (2004, 2018) that

FPD is not related to stocking density directly but rather due to problems that arise with increased bird density. Management techniques such as good ventilation and selecting appropriate bedding material, which keeps moisture away from birds, are crucial for maintaining lower litter moisture and providing birds with an environment where they can perform well and also fulfill welfare criteria (Dawkins, 2018).

In conclusion, low litter moisture is a key component of providing good welfare conditions for turkeys in production. Litter conditions can be compromised by increased stocking density. Thus, turkey production requires an excellent bedding material, which will help keep litter moisture under 25%. Based on the available information from the literature, MG is a viable bedding alternative for poultry houses, although there is little information on its physical properties and few analyses. With this in mind, more studies are needed to test MG bedding under conditions of increased stocking density, which may challenge the capacity of MG bedding to absorb added moisture. In addition, few studies have been conducted studying the footpad health of turkey hens stocked at various densities. Thus, the objective of this study was to study the effects of bedding and stocking density on FPD occurrence and litter characteristics.

MATERIALS AND METHODS

Bird, Housing and Management

Animal procedures for this study were approved by The Institutional Animal Care and Use Committee (IACUC), Pennsylvania State University (IACUC# 45605).

Day of hatch female turkey poults (N=1096, Hybrid Converter) were obtained from a local commercial hatchery (Cooper Turkey Hatchery, Oakwood, OH) and used in an experiment conducted between July 28 and November 17, 2015 in the Pennsylvania State University, PA. Poults were beak and toe trimmed (three toe nails) at the hatchery. The turkeys had *ad libitum* access to feed and water. The photoperiod and target temperatures followed Hybrid Turkey guidelines (Hybrid Turkeys, 2008). Detailed housing and management practices are described in Chapter III.

Experimental Treatments and Design

The experiment was a completely randomized block design with a 2 x 3 factorial arrangement. Two experimental treatments were evaluated: bedding materials and stocking density.

Turkeys were individually identified by wing bands and raised in pens (8.2 m^2) on either pine wood shavings (PS) or Giant miscanthus grass (MG) bedding. Dried MG bedding was chopped into 5 cm lengths. Both types of bedding material were applied to a depth of 10 cm, and were not manipulated during the experiment.

Three stocking densities were used. Poults were weighed and randomly distributed to 12 of 24 pens on the day of placement. Three stocking densities, low (LD), medium (MD) and high (HD) were achieved by assigning 70, 88, and 116 turkeys to each pen, respectively (Table 1). At two weeks of age, the poults were reassigned into 24 pens and kept at 35, 44 and 58 hens per pen for LD, MD, and HD categories, respectively (Table 1), and raised until 97 d of age (hereafter referred as 14 wk of age; Table 2).

Data Collection

Footpad Dermatitis Scoring. At 6 and 14 wk of age, the surfaces of both footpads of each turkey were gently cleaned by hand to remove adhered litter material without the use of water or a brush. The left and right footpads were then scored on a three point scale (Berg, 2015) by two trained persons. A composite footpad score was assigned for each bird. Prevalence was calculated for the proportion of turkeys in each pen with scores of 0 (FS0), 1 (FS1), and 2 (FS2).

Litter Sampling. At 6 and 14 wk of age, litter samples were collected from each pen. The surface area of the pen was divided into quarters (excluding the feeders and drinkers, corners and edges). A gallon of litter material was collected and pooled from each quarter of the pen. Litter samples were stored in a -30 °C freezer at the Pennsylvania State University, and later transferred on ice to the University of Minnesota, where the samples were replaced into a -30C freezer for storage. Litter samples were sent for analyses on ice.

Litter Characterization. Samples were analyzed for the following components at a commercial laboratory (Minnesota Valley Testing Laboratory, New Ulm, MN) for: moisture, nitrogen (N), ammonium-N, organic nitrogen, phosphorus (P), potassium (K), sulfur (S), zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), calcium (Ca), magnesium (Mg), sodium (Na), pH, soluble salts, and chloride (Cl). For analyzing P, K, Na, Zn, Fe, Cu, Mn, Mg and Na content of the litter samples, a microwave digestion / ICP detection method was used. The total N was analyzed by using the Kjeldahl method. For ammonium-N, an ion selective electrode method was used. The pH of the samples was determined by suspending five grams of the sample in 40 ml water and then tested with a pH meter

(Thermo Fisher Scientific, Waltham, MA). For determining soluble salt content, 20 grams of litter sample was suspended in 40 ml water and analyzed using an electrical conductivity meter (Peters et al., 2003). Chloride content (%) was measured by using the potentiometric method. The reference for all the analyses can be found in Methods of Manure Analysis, A3769 University of Wisconsin Extension (Peters et al., 2003), with the exception of Cl (%), which was analyzed based on the Official Methods of Analysis by the Association of Official Analytical Chemists, AOAC 969.10 (AOAC, 1990). Analyses was conducted on an as is basis and then converted to dry matter basis where appropriate.

Particle size measurements were conducted at the University of Minnesota Biosystems Engineering Laboratory. Duplicate subsamples weighing between 40 and 70 g on average were used for analyses. During the dry matter analysis, litter moisture (%) was calculated after the samples were placed in a drying oven (60°C) for 24 hours. Briefly, litter moisture (%) was calculated by comparing the net weight of samples before and after drying. To analyze the particle size of the dried litter, 10 different sieves with pore sizes ranging between 4.76 and 0.149 mm, (Table 3; U.S. Standard Sieve) were placed on top of each other in descending pore size order on a shaker (Soiltest Model CL-305 Ro-tap vibrating shaker) (Koon et al., 1994). The dried litter sample was placed in the sieve with the largest pores on top. After 5 minutes of shaking, the net retained sample weight was calculated for each sieve.

Data Analysis

The effects of bedding material and stocking density were examined with the pen as the experimental unit. The brooding period was considered as block. A two-way ANOVA (Factorial ANOVA, Software: Statistix10 Analytical Software, Tallahassee, FL USA) was conducted to analyze the main effects of bedding, stocking density and their interaction on footpad score and litter characteristics. Differences were considered significant at $P < 0.05$. When the treatment effect was significant, Tukey HSD ALL-Pairwise comparisons separated the means ($P \leq 0.05$). After ANOVA the residuals were plotted and a Shapiro-Wilkinson test was performed to verify the normal distribution of residuals. Proportional data for footpad score prevalence and particle size distribution data yielding proportions were arcsine transformed to achieve normality, and transformed data were used for the ANOVA, and Tukey HSD All-Pairwise Comparison test.

When effect of density was significant ($P \leq 0.05$) as tested by ANOVA, linear and quadratic polynomial contrast test (Polynomial Contrast Testing, Software: Statistix10 Analytical Software, Tallahassee, FL USA) was performed (Scheffe) for footpad scores and litter moisture, components and particle size data.

A Spearman correlation analysis (Software: Statistix10 Analytical Software, Tallahassee, FL USA) was performed to ascertain the degree of relatedness between footpad scores and litter characteristics based on pen means. The strength of the correlation was judged as very weak (r between 0, and 0.19), weak (r between 0.20 and 0.39), moderate (r between 0.40 and 0.59), strong (r between 0.60 and 0.79) or very strong (r between 0.80

and 1.00) in a negative and a positive direction (Evans, 1996). Only moderate to very strong correlations are reported and considered in the discussion.

RESULTS

Footpad Dermatitis Scores at 6 and 14 wk of Age

Average footpad scores were lower for turkeys raised on PS compared to turkeys raised on MG bedding at 6 wk of age (0.14 vs. 0.38, P = 0.0019; Table 4) and at 14 wk of age (0.71 vs. 1.43, P ≤ 0.0001). Density influenced footpad scores at 14 wk of age, with the lowest average scores in turkeys at LD (0.77), which differed significantly from turkeys at MD (1.16) and HD (1.27) (P = 0.0057). No bedding by density interaction was detected.

The prevalence of each of the footpad scores at 6 and 14 wk of age is presented in Table 5. At 6 wk of age, only scores of 0 and 1 were observed. More birds were found with a footpad score of 0 (FS0, no or mild lesions) on PS (86.39%) than on MG (61.77%, P = 0.0009). More birds with a footpad score of 1 (FS1, moderate lesions) were found on MG (38.23%) than on PS (13.61%, P = 0.0009) at 6 wk of age.

At 14 wk of age, bedding material and stocking density impacted the number of birds with no or mild footpad lesions (FS0, Table 5). The prevalence of FS0 was greater among birds on PS bedding compared to birds on MG (39.53 vs. 2.74%, P ≤ 0.0001). Density affected prevalence with birds at LD having a greater prevalence of FS0 compared to either MD or HD held birds (37.98 vs. 15.78 or 9.65%, P = 0.0094).

A bedding by density interaction (P = 0.0114) was detected for FS1 at 14 wk of age. The prevalence of FS1 was lowest for birds on PS housed at LD (28.89%) while the prevalence was similar among the remaining treatment combinations.

At 14 wk of age, bedding and density both affected the prevalence of deep and severe footpad lesions (FS2, Table 5). The most severe and deep lesions were associated with MG bedding (45.53%) compared to PS (10.24%, P = 0.0001). Prevalence of FS2 was greatest for hens housed at HD (36.55%) compared to LD (15.23%, P = 0.0267), while MD was intermediate (31.88%) and not different from either LD or HD.

Litter Characteristics

6 wk of age. Litter moisture (Table 6) tended to be influenced by bedding, whereas higher moisture content was detected on MG (33.74%) litter compared to PS (28.41%, P = 0.096). Density tended to influence moisture content as well with HD exhibiting the highest moisture content numerically (P<0.071). Bedding material and stocking density did not affect either soluble salts or the pH of litter samples at 6 wk of age.

Of the nitrogen measurements (Table 7), bedding tended to influence total N (P<0.082) and with a significant difference in ammonium-N (P<0.051) with MG having greater content as compared to PS. No difference in organic-N content between bedding materials. Stocking density tended to influence total N (P<0.061) and significantly affected ammonium-N (P<0.012). Total N increased numerically as stocking density increased. Ammonium-N content was higher for HD (0.56%) bedded pens as compared to either LD or MD (0.41 and 0.44%, respectively, P = 0.012).

For the macro-minerals presented in Table 7, no differences existed for bedding material. Sulfur tended to be higher on MG bedding (P = 0.077). For stocking density, Cl and Na content increased in a linear pattern with increasing density. Chloride content was higher at HD (0.50%) compared to either LD or MD (0.37 and 0.38%, respectively) (P = 0.003; Table 7). The sodium content was increased on HD (1806 mg/kg) compared to LD (1398 mg/kg), whereas MD (1629 mg/kg) was intermediate (P = 0.037).

Manganese was the only other mineral component of litter samples (Table 8) that was affected by bedding material, with lower Mn in MG than for PS litter (341 vs. 439 mg/kg; P = 0.001) at 6 wk of age. Stocking density did not affect any micro-mineral components of the litter.

14 wk of age. Bedding affected moisture but not soluble salt content or pH (Table 9). Greater litter moisture was detected in MG than PS (55.72 vs. 48.95%, P = 0.0008). The soluble salt content of litter averaged 12.12 and 10.84 mmhos/cm for MG and PS litter, respectively, while respective pH averaged 7.24 and 6.99. Linear trends for the effect of density were observed for litter moisture and litter pH. As stocking density increased, litter moisture increased, which was greatest for HD pens (57.61%) compared to either LD or MD pens (48.35 or 51.05%, P = 0.0009). Litter from LD or MD pens was more basic in pH (7.44 or 7.57) compared to HD (6.32, P = 0.0007).

Of the nitrogen measurements (Table 10), bedding significantly influenced total N (P<0.0436) and ammonium-N (P<0.0511) with MG having greater content as compared to PS. The total nitrogen content was higher on MG compared to PS (7.15 vs. 6.32%, P =

0.044) while ammonium nitrogen was also higher for MN as compared to PS (2.27 vs 1.89%, P=0.0511). No difference in organic-N content was detected for bedding materials.

Stocking density affected total-N (P<.0436) and organic-N (P<0.0174) but not ammonium-N. Total-N was lowest for MD litter (6.07%) and was different in content from HD litter (7.43%). A quadratic trend was observed for the organic nitrogen content due to a lowered content with MD litter (4.00%) as compared to HD litter (5.18%).

Mineral content (Tables 10 and 11) was not affected by bedding or stocking density with the exception for iron. Greater iron level (Table 11) was detected on MG litter than PS (902 vs. 699 mg/kg, P = 0.048).

Litter Particle Size. At 6 wk of age, bedding material influenced the retained mass of litter with the exception of sieve pore size 6 (Table 12). At the largest pore size sieve (size 4, 4.76 mm), almost twice as much litter was retained when MG was sieved than PS (52.32% vs. 26.14%, $P \leq 0.0001$). Litter mass retained in the smaller pore sieves starting at size 8 (less than 2.38 mm particles) contained significantly more PS bedding particles than MG at 6 wk of age. Density did not affect the particle size of litter at 6 wk of age (Table 12).

At 14 wk of age, bedding material influenced the litter mass that remained in all of the sieves excepting the smallest size material (Table 13). The largest pore sieve (size 4, 4.76 mm) retained more MG than PS litter (61.72 vs. 49.51%, P = 0.0002). For the smaller pore sieves, more PS litter was retained compared to the MG litter ($P \leq 0.05$) at 14 wk of age.

At 14 wk of age, stocking density did not affect litter particle size (Table 13).

Correlation of Footpad Score with Body Weight or Litter Characteristics

6 wk of Age. Average BW or pH were not correlated with footpad score on a pen basis at 6 wk of age. A positive correlation was, however, observed for footpad score and litter moisture (r =0.55, P = 0.006), and ammonium-N (r = 0.41, P= 0.047). A negative correlation between footpad score and litter Mn content (r = -0.47, P = 0.021) was also detected.

There was a strong positive correlation between average footpad score and the amount of litter retained in the largest pore sieve (size 4, r =0.81, P ≤ 0.0001) (Table 14). For smaller particle sizes, negative correlations between litter particle size and footpad scores were observed, with the exception of sieve size 6.

14 wk of Age. Average footpad score and BW were correlated moderately (r = -0.47) at 14 wk of age. Litter components that were correlated (all P ≤ 0.05) with footpad score were litter moisture (r = 0.68), N (r = 0.45), and ammonium-N (r = 0.55).

When the relationship between footpad scores and the distribution of litter particle by size was considered, the average footpad score correlated (all P ≤ 0.05) moderately with the amount of litter retained by sieve size 4 (r = 0.44; Table 14), and there was a negative correlation by sieve sizes 6 (r = -0.44), 8 (r = -0.55) and 12 (r = -0.42).
Other measures such as mineral content, pH were not correlated with footpad score.

Correlations among Litter Characteristics

6 wk of Age. Total moisture (%) was negatively correlated with the amount of litter material held back by sieve size 12 (r =-0.41, P = 0.0464), and the remainder (r = -0.50, P

= 0.0148). A positive association was found between moisture content of the litter and ammonium-N (r = 0.79), potassium (r = 0.47), chloride (r = 0.75), sulfur (r = 0.41), iron (r = 0.48), and calcium (r = 0.43, $P < 0.05$).

The pH of the litter was not correlated with particle sizes but was moderately associated with phosphorus (r = 0.48), potassium (r = 0.47), chloride (r = 0.49), sulfur (r = 0.44), and manganese (r = 0.50, $P < 0.05$). The pH was not correlated with litter moisture at 6 wk of age.

14 wk of Age. The moisture content of the litter was correlated negatively (all $P \leq 0.05$) with average BW (r = -0.46), pH (r = -0.69), and amount of litter retained by sieve size 8 (r = -0.50). There was a positive correlation (all $P \leq 0.05$) between moisture content, and Cl (r = 0.45), I (r = 0.68), Ca (r = 0.58), soluble salt (r = 0.70), total N (r = 0.70), ammonium-N (r = 0.72), organic-N (r = 0.48), and the amount of litter retained by the largest pore sieve (sieve size 4, r = 0.44).

Except for the positive correlation with average BW (r = 0.48), litter pH was correlated (all $P \leq 0.05$) negatively with litter moisture (r = -0.69), total N (r = - 0.75), and organic N (r = -0.69).

DISCUSSION

In the present study, lower average footpad scores were found in turkeys raised on PS bedding than turkeys on MG at 6 and 14 wk of age. The difference between footpad scores at 6 wk of age was not biologically significant, as the scores were 0.14 and 0.38 (P = 0.0019), both belonging to the no lesion category on the three point scale used in the current study. The low footpad scores are in agreement with results of a previous study with turkey hens (Abd El-Wahab et al., 2011), where an average footpad score of 1.50 on an eight point scale was observed (0 - "normal skin", 7 – "over half of the footpad is covered with necrotic lesions") at 5 wk of age. For scores of 1 and 2, footpads may have reddening and swelling similar to a score of 0 in the scoring system used in this study. At 14 wk of age, the average footpad scores in birds housed on different bedding materials diverged, with birds kept on PS having a lower footpad score (0.71) than turkeys raised on MG (1.43). Evaluating these scores, hens on PS bedding developed mild lesions, and turkeys kept on MG had lesions between medium and severe. In addition, when the prevalence of the footpad lesions were considered, turkeys reared on PS bedding had better footpad condition compared to MG at 6 and 14 wk of age.

The average footpad scores from the hens on MG bedding at 14 wk of age in this study are less severe compared to the findings of Smith et al. (2002), who assessed footpad lesions in turkey hens at 13 wk of age raised on PS or Bermuda grass hay (1.33 vs. 1.29, $P>0.05$) on a five point scale, where 0 score is given if no lesions were found, a score 1, if "25% burn", score 2, if there is a "50% burn", a score 3 if "75% burn", and a score 4 if there is "100% burn" on the footpad surface. Unfortunately, the footpad scores of Smith et al. (2002) and the current study are difficult to compare, since the basis for footpad scoring in the current study was the depth and severity of lesions, and not the size of the lesions. Mayne et al. (2007a) also found that the footpad lesions were affected by bedding materials under research conditions. Mayne et al. (2007a) compared wood shavings, straw, recycled cardboard and recycled paper as turkey bedding and found the lowest external and histopathological footpad scores were from birds on wood shavings at the end of experiment (d 6). In contrast, lower scores were reported for commercial toms on MG grass compared to PS (Grimes, 2015). Smith et al. (2002) did not find significantly different footpad scores in turkey hens at 13 wk of age among PS, chopped Bermuda hay and a 50-50% mixture of these two litter material treatments**.**

Density affected the FPD scores in the present study. Most birds with low footpad scores were kept at LD, and the most severe footpad scores were observed in birds kept at HD, which agrees with previous studies (Martrenchar et al., 1999). A recent study, (Beaulac and Schwean-Lardner, 2018) reported that average footpad scores of turkey toms increased as the stocking density increased (at 30, 40, 50 and 60 kg/m^2) at 10 and 16 wk of age. Increasing severity might be because as more birds are housed in a pen, more excreta is produced, which will likely increase litter moisture. Also, a broiler study, (Feddes et al., 2002) reported that broilers increased water intake at higher stocking densities, which could contribute to increased litter moisture. In the current study, water consumption was not recorded.

The bedding and the density affected the mineral content and litter characteristics, such as litter moisture, and pH of the litter. Bedding affected the manganese content of the litter at 6 wk of age, and moisture, total nitrogen, and iron content at 14 wk of age. At 14 wk of age, higher moisture content was found in MG than PS litter. Our findings contradict those of Dunkley and Ritz (2017) who refer to a study where the MG bedding had lower moisture content compared to PS. Another source found MG to be more absorbent, with 5% less moisture than PS (Wurzbacher, 2017). In a 5 wk broiler study, litter moisture tended to be lower in the MG litter than PS (32.15 vs. 36.17%, P = 0.0720), however, because the lifespan of broilers is shorter than turkeys and the moisture content of their manure is lower (24 vs. 35% moisture, as manure removed) (Mukhtar, 2005) these studies are difficult to compare.

Litter nutrient composition was different by density, several elements including ammonium-nitrogen, chloride, sodium at 6 wk of age, and total nitrogen, organic nitrogen at 14 wk of age, and all these increased as the density increased. At 6 and 14 wk of age, there were differences between the lower density treatments compared to the HD, e.g. either LD or MD compared to HD, but LD and MD was not different from one another throughout the study period. The final density was 43, 49 and 66 turkeys/sq. m, representing LD, MD and HD, respectively. The smaller difference seen between LD and MD might be explained by the lack of difference in space between the two lowest densities.

In addition to the nutrient composition, density also directly affected the litter moisture content, and the pH at 14 wk of age, likely as a result of greater amount of excreta produced by more birds. The excreta produced by the turkeys housed at LD was calculated as 16.5 kg per pen, and 25.1 kg per pen in the HD groups. This estimate is based on the assumptions that 1 kg live turkey produces 0.05 kg total manure (Lorimor et al., 2004), and calculating with the final stocking density at the end of the current study at 32 and 52 turkeys per pen. The moisture content of turkey excreta is estimated to be 75% (Mukhtar, 2005), which taken together with the amount of excreta produced emphasize the impact of excreta quantity on the litter moisture. In the current study, litter moisture increased as stocking density increased at 14 wk of age and a similar trend was observed at 6 wk of age.

These results are in agreement with a previous study (Noll et al., 1991) where litter moisture also rose as space decreased from 0.46 to 0.21 m^2/bird when turkey toms were 12, 16 and 20 wk of age. In contrast, Beaulac and Schwean-Lardner (2018) reported no difference in litter moisture among stocking density treatments (0.56, 0.42, 0.34 and 0.28 m^2/bird).

Density only affected Cl, Na and ammonium-N content at 6 wk of age, and not at 14 wk of age. For 14 wk, density did not affect ammonium-N, P, K, Cl, S, Ca, Mg, Na, Zn, Fe, Cu and Mn level of the litter samples. These results were expected to change as density increased as excreta and spilled feed good sources of these elements. Interestingly, these elements were higher numerically as stocking density increased at 6 wk of age, however, was higher on LD and HD as compared to MD at 14 wk of age with the exception of ammonium-N. It might be due to the sampling and litter analysis, as only one samples were sent to analysis, and the caked litter might influence the element content of the litter samples.

When the particle size distribution was analyzed in the current study, it was affected by the bedding both at 6 and 14 wk of age, and B x D interaction was found at 14 wk of age, when sieve pore size 6 (between 3.36 and 4.76 mm) was different. In general, MG bedding was associated with larger particle sizes (>4.76 mm, sieve size 4), than the PS bedding which had finer particles (<2.38 mm, sieve size 8). Samson et al.(2018) indicated that warm season grasses (such as MG) best absorb and release moisture if their particle sizes are between 20 and 30 mm. Furthermore, there are recommendations that MG should be ground in order to use it as animal bedding because it may contain large woody pieces

(VanWeyenberg et al., 2015). Bedding with small particle sizes have a larger surface area, which impacts water holding and release capacity of the litter, therefore the litter moisture and consequently the footpad health. Hester et al. (1997) concluded that finer particle size bedding material decreases the incidence of footpad lesions in turkey males kept to 17 wk of age, compared to larger particle size bedding materials. Similar to our results, Barkley (2017) found MG bedding had larger particle size compared to PS and was associated with a higher rate of caking. In a broiler study comparing large particle MG bedding (larger than 2 mm) and small particle MG bedding (smaller than 2 mm) Barkley (2017) found greater moisture content (10.96 and 7.55%, $P \leq 0.0001$) and moisture holding capacity (1.77 vs. 2.37, $P = 0.0004$) in the larger particles compared to the smaller particles.

Taken together the effects of bedding and density on FPD, and on litter characteristics, MG bedding and HD were associated with higher litter moisture, due to the larger particle size, and the amount of excreta produced by more turkeys housed in a pen. The pH (above 8) and the temperature play important role in ammonia formation (Nahm, 2003), which can be detrimental for turkey health. The pH was around 8.4 at 6 wk of age, then decreased to 7 at 14 wk of age in the current study, which is neutral, therefore the temperature and litter moisture may create an ideal environment for the ammonia formation compared to the contribution of pH. Thus, the increased litter moisture and the ammonia production can increase the incidence and severity of FPD lesions, which contribute to worsening welfare. In the current study the litter pH increased as density and litter moisture increased at 14 wk of age, which may related to the presence of cations in the water and excreta, such as Ca, which can influence the pH. A turkey pen study (Wu and Hocking,

2011) investigated whether chemicals in the PS bedding may change the pH, and therefore influence the footpad lesions. The scientists reported pH 4 at the study period for PS bedding, and pH 5 for tap water, which was added to the litter to establish wet litter treatment and study the footpad health. At the end of the experiment, the litter pH was 5.7, 7.4, and 6.8, when the litter moisture reached 28, 47, and 73%. The authors suggested, that the buffering capacity of tap water, and cations in the excreta, such as Ca may alter the pH in the experiment, and concluded that the litter moisture was the primary factor in the development of footpad lesions, and not the pH per se, or any other chemicals, which can alter the pH (Wu and Hocking, 2011).

Similar to our study, a field study also found a linear relationship between litter moisture and FPD lesions, examining 41 commercial tom flocks between 16 and 19 wk of age (Da Costa et al., 2014), and the prevalence of FPD and litter scores were directly correlated ($r = 0.33$, $P \leq 0.05$).

Footpad dermatitis has been linked to increased litter moisture. Excess moisture in the litter softens the footpad epithelium, as the skin dries, it becomes more susceptible to damage. As the condition progresses, erosions, and severe ulcers can develop on the footpad and toes (Mayne, 2005). Litter moisture in half of the pens, especially those of the HD in the current study exceeded the recommended 30% at 6 wk of age averaged 31.08% in the study, and exceeded 50% moisture by 14 wk of age averaged 52.34%.

Based on the recommendations regarding litter moisture, turkey hens in the present study spent the last 8 weeks on wet litter, which provided an ideal environment for developing footpad lesions. However, the pen average footpad scores were low at 6 wk of age (0.26), and was higher at 14 wk of age, averaged 1.07. When PS bedding was considered, these footpad scores were even lower, 0.14 at 6 wk of age, and 0.71 at 14 wk of age. These footpad scores might indicate that even on higher than 30% litter moisture the footpad lesions were not as severe as it could have been expected based on the literature data.

In the current study characteristics of the bedding, were not analyzed at the outset of the study making the comparison of bedding, then the litter characteristics difficult without baseline values. While baseline analyses of bedding, feed, and water could have helped the understanding of the litter nutrient analyses conducted at 6 and 14 wk of age, and could have explained whether the changes in nutrient composition were due to the original characteristics of the bedding. If not, then nutrient changes might were modified during the study period. Other materials, including spilled feed could have change nutrient composition of litter related to the turkeys, either in their output or behavior. In addition, the crude protein level in the Grower I feed (27.24%) was slightly higher than NRC recommendations (National Research Council 1994. Nutrient requirements of turkeys. Pages 36-39. in Nutrient requirements of poultry: Ninth revised edition, 1994. Washington, DC: The National Academies Press.,) (26% protein, 4 to 8 wk of age) in the current study, which were provided for the hens between 6 and 8 wk of age. High protein content in the diet is related to the higher uric acid and ammonia production, which may contribute to the FPD lesions (Youssef et al., 2011a). Jensen (1970) reported that feed high in soybean meal caused pasty vents of turkeys, and the excreta was sticky, which adhered to the footpad of turkeys resulting in more severe FPD lesions.

The study would have been more informative, if it had included additional measures, such as litter depth at various weeks of age to see how much the bedding materials compacted, litter scores (as an indication of caking and moisture), evaluation of dust (Barkley, 2017 observed that MG bedding was dusty), and a measure of water consumption. The current study only used FPD to measure welfare, which can be characterized by multiple aspects. Therefore other welfare indices, such as feather cleanliness, hock burn, and breast irritations could have been relevant to measure, which evaluate the housing conditions, such as litter moisture. Measuring indices in the slaughter house, such as scratches and meat yield could have added more information about the welfare and production status of the turkeys related to the treatments. For example, as a study found lower carcass weight in broilers with higher footpad scores, and less thigh scratches, compared to the broilers exhibiting lower footpad scores, which was thought to be due to the increased activity of broilers kept in the dry litter treatments as compared to the wet litter treatment (DeJong et al., 2014).

In conclusion, the incidence and severity of footpad scores were higher for turkeys raised on MG compared to turkeys raised on PS in the current study, which indicates that the welfare of the turkey hens housed on MG was poorer compared to their counterparts kept on PS bedding material. The current study that MG bedding chopped into 5 cm length was associated with higher moisture on MG bedding, and larger particle size compared to PS, which most likely contributed to the more severe footpad lesions. Therefore further research is needed to identify strategies to improve footpad scores of turkeys raised on MG, more specifically MG chopped into smaller particle lengths might improve the litter moisture, thus the footpad lesions. Related to stocking density, results of the current study suggest that low stocking density improved performance and welfare condition as indicated by lower footpad scores of turkey hens.

Table 1. Description of targeted stocking density levels during 0 to 2 and after 2 wk (hens/m^2)

DENSITY	LOW (LD)		MEDIUM (MD)		HIGH (HD)	
Age (wk)	Hens/m^2	Hens/pen	Hens/m^2	Hens/pen	Hens/m^2	Hens/pen
0 to 2	8.54	70	10.72	88	14.2	116
2 to 14	4.27	35	5.36	44	7.1	58

Table 2. Description of final stocking density levels at 14 wk (kg/m^2)

DENSITY	LOW (LD)		MEDIUM (MD)		HIGH (HD)	
Age (wk)	kg/m^2	Hens/pen	kg/m^2	Hens/pen	kg/m^2	Hens/pen
14	43.02	31.50	49.13	37.13	65.55	51.34

Table 3. Sieve numbers and pore size used in particle size analysis (UMN)

Sieve number	Pore size (mm)
4	4.76
6	3.36
8	2.38
12	1.68
16	1.19
20	0.841
30	0.595
50	0.297
70	0.210
100	0.149
Remainder (R)	Less 0.149

Table 4. Influence of bedding material and stocking density on footpad dermatitis score of turkey hens at 6 and 14 wk of age

Factor	Level	Week 6	Week 14
Bedding[1, 2]	PS	0.14[b]	0.71[b]
	MG	0.38[a]	1.43[a]
Density[3, 4]	LD	0.22	0.77[b]
	MD	0.28	1.16[a]
	HD	0.28	1.27[a]
P-value	B	0.002	0.000
	D	0.681	0.006+
	B × D	0.686	0.240
SEM	B	0.035	0.080
	D	0.043	0.098

[a,b] Different superscripts within the same column and factor designate significant differences ($P<0.05$)
[1] Bedding: PS (Pine wood shavings) and MG (Miscanthus grass);
[2] Number of replicates (n): n=5 for MG and n=6 for PS
[3] Stocking density: LD 4.2, MD 5.3 and HD 7 hens/m^2
[4] Number of replicates (n): n=4 for LD and MD; n=3 for HD
BW measurements were taken for all turkeys in the pens, then BW was averaged
+ Polynomial contrast, linear relationship among densities (Scheffe= 0.0117, linear)

Table 5. Influence of bedding material and stocking density on the prevalence and severity of footpad dermatitis scores of turkey
hens at 6 and 14 wk

	Prevalence of footpad lesions, at week				
	6		14		
Variable	FS0	FS1	FS0	FS1	FS2
Bedding[1,2]					
PS	86.39[a]	13.61[b]	39.53[a]	50.22	10.24[b]
MG	61.77[b]	38.23[a]	2.74[b]	51.73	45.53[a]
Density[3,4]					
LD	78.28	21.72	37.98[a]	46.79	15.23[b]
MD	72.03	27.97	15.78[b]	52.33	31.88[ab]
HD	71.93	28.07	9.65[b]	53.80	36.55[a]
B × D					
PS-LD	94.72	5.28	68.84	28.89[a]	2.27
PS-MD	81.86	18.14	30.92	64.40[a]	4.68
PS-HD	82.60	17.40	18.84	57.39[a]	23.78
MG-LD	61.84	38.16	7.12	64.70[a]	28.18
MG-MD	62.21	37.79	0.64	40.27[a]	59.09
MG-HD	61.26	38.74	0.46	50.22[a]	49.33
Source of variation			*P*-values		
B	0.001	0.001	0.000	0.854	0.000
D	0.355	0.355	0.009+	0.678	0.027++
B × D	0.355	0.348	0.178	0.011	0.233
			SEM		
B	0.059	0.059	0.0651	0.113	0.059
D	0.072	0.072	0.0797	0.069	0.073
B × D	0.102	0.102	0.1127	0.069	0.103

[a,b] Different superscripts within the same column and factor designate significant differences (P<0.05)

[1] Bedding: PS (Pine wood shavings) and MG (Miscanthus grass);

[2] Number of replicates (n): n=6 for MG, and PS

[3] Stocking density: LD 4.2, MD 5.3 and HD 7 hens/m^2

[4] Number of replicates (n): n=4 for LD, MD, and HD

Footpad scores were given according to Berg, 2015 (0 – no lesions, 1 - mild lesions, 2 - deep/severe lesions)

Footpad scores were assigned as the average of footpad scores given for both footpads of individual hens in a pen basis,

ANOVA and Tukey HSD comparisons were performed on arcsine-square root transformed data to equalize variance, however, for ease of interpretation, original values are reported so that the total proportion of data sums to 100%.

+ Polynomial contrast, linear relationship among densities (Scheffe= 0.0197, linear)

++ Polynomial contrast, linear relationship among densities (Scheffe= 0.0431, linear)

Table 6 Influence of bedding and turkey hen stocking density on litter - Litter analyses after 6 wk of use on a DM basis

Factor	Level	Litter moisture %	Soluble salts (mmhos/cm)	pH
Bedding[1,2]	PS	28.41	3.17	8.59
	MG	33.74	3.11	8.19
Density[3, 4]	LD	28.98	2.79	8.17
	MD	27.88	2.97	8.45
	HD	36.37	3.66	8.54
P-value	B	0.096	0.882	0.179
	D	0.071	0.172	0.542
	B × D	0.447	0.895	0.817
SEM	B	2.14	0.27	0.20
	D	2.62	0.33	0.24

[a,b] Different superscripts within the same column and factor designate significant differences (P<0.05)
[1] Bedding: PS (Pine wood shavings) and MG (Miscanthus grass);
[2] Number of replicates (n): n=6 for MG, and PS
[3] Stocking density: LD 4.2, MD 5.3 and HD 7 hens/m^2
[4] Number of replicates (n): n=4 for LD, MD, and HD

Table 7 Influence of bedding and turkey hen stocking density on litter macro nutrient analyses after 6 wk of use on a DM basis

Factor	Level	Nitrogen, Total %	Ammonium, N%	Nitrogen, Organic %	Phosphorus, as P2O5%	Potassium, as K2O %	Chloride %	Sulfur mg/kg	Calcium mg/kg	Magnesium mg/kg	Sodium mg/kg
Bedding[1,2]	PS	2.88	0.43	2.45	2.14	2.14	0.40	2978	14103	2999	1560
	MG	3.29	0.51	2.78	2.24	2.36	0.44	3498	15394	3173	1663
Density[3,4]	LD	2.74	0.41^{b}	2.34	1.93	2.01	0.37^{b}	2839	13104	2672	1398^{b}
	MD	3.06	0.44^{b}	2.63	2.18	2.24	0.38^{b}	3247	14335	3155	1629^{ab}
	HD	3.45	0.56^{a}	2.89	2.46	2.50	0.50^{a}	3627	16805	3431	1806^{a}
P-value	B	0.082	0.051	0.133	0.615	0.184	0.188	0.077	0.408	0.499	0.393
	D	0.061	0.012+	0.131	0.101	0.065	0.003++	0.095	0.160	0.072	0.037+++
	B × D	0.409	0.621	0.293	0.279	0.284	0.861	0.578	0.594	0.245	0.331
SEM	B	0.16	0.03	0.15	0.13	0.11	0.02	195	1076	178	83
	D	0.19	0.03	0.18	0.16	0.14	0.03	239	1318	219	102

[a,b] Different superscripts within the same column and factor designate significant differences (P<0.05)

[1] Bedding: PS (Pine wood shavings) and MG (Miscanthus grass);

[2] Number of replicates (n): n=6 for MG, and PS

[3] Stocking density: LD 4.2, MD 5.3 and HD 7 hens/m^2

[4] Number of replicates (n): n=4 for LD, MD, and HD

+ Polynomial contrast, linear relationship among densities (Scheffe= 0.014, linear, Ammonium-N)

+ +Polynomial contrast, linear relationship among densities (Scheffe= 0.005, linear, Cl)

+ ++Polynomial contrast, linear relationship among densities (Scheffe= 0.042, linear, Sodium)

Table 8 Influence of bedding and turkey hen stocking density on litter micro nutrient analyses after 6 wk of use on a DM basis (mg/kg).

Factor	Level	Zinc	Iron	Copper	Manganese
Bedding[1,2]	PS	314	189	266	439[a]
	MG	322	235	289	341[b]
Density[3,4]	LD	279	213	231	351
	MD	313	199	292	395
	HD	362	225	310	425
P-value	B	0.789	0.063	0.409	0.001
	D	0.065	0.656	0.070	0.094
	B × D	0.321	0.695	0.250	0.413
SEM	B	314	16	19	18
	D	322	20	24	22

[a,b] Different superscripts within the same column and factor designate significant differences (P<0.05)
[1] Bedding: PS (Pine wood shavings) and MG (Miscanthus grass);
[2] Number of replicates (n): n=6 for MG, and PS
[3] Stocking density: LD 4.2, MD 5.3 and HD 7 hens/m^2
[4] Number of replicates (n): n=4 for LD, MD, and HD

Table 9 Influence of bedding and turkey hen stocking density on litter - Litter analyses after 14 wk of use on a DM basis

Factor	Level	Litter moisture %	Soluble salts (mmhos/cm)	pH
Bedding[1,2]	PS	48.95[b]	10.84	7.24
	MG	55.72[a]	12.12	6.99
Density[3,4]	LD	48.35[b]	11.43	7.44[a]
	MD	51.05[b]	10.82	7.57[a]
	HD	57.61[a]	12.19	6.32[b]
P-value	B	0.001	0.226	0.310
	D	0.001	0.558	0.001++
	B × D	0.708	0.901	0.657
SEM	B	1.18	0.72	0.17
	D	1.44	0.88	0.20

[a,b] Different superscripts within the same column and factor designate significant differences ($P<0.05$)
[1] Bedding: PS (Pine wood shavings) and MG (Miscanthus grass);
[2] Number of replicates (n): n=6 for MG, and PS
[3] Stocking density: LD 4.2, MD 5.3 and HD 7 hens/m^2
[4] Number of replicates (n): n=4 for LD, MD, and HD
+ Polynomial contrast, linear relationship among densities (Scheffe= 0.0009, linear, Litter moisture)
++ Polynomial contrast, linear relationship among densities (Scheffe= 0.0023, linear, pH)

Table 10 Influence of bedding and turkey hen stocking density on litter - Litter macro nutrient analyses after 14 wk of use on a DM basis

Factor	Level	Nitrogen, Total %	Ammonium, N%	Nitrogen, Organic %	Phosphorus, as P2O5 %	Potassium, as K2O%	Chloride%	Sulfur mg/kg	Calcium mg/kg	Magnesium mg/kg	Sodium mg/kg
Bedding[1,2]	PS	6.32[b]	1.89	4.44	4.95	3.67	0.94	6785	28298	5743	4336
	MG	7.15[a]	2.27	4.89	5.15	3.96	1.00	7135	30460	5949	4421
Density[3,4]	LD	6.71[ab]	1.91	4.81[ab]	5.24	3.94	0.98	7295	29887	6060	4510
	MD	6.07[b]	2.07	4.00[b]	4.66	3.56	0.93	6429	27256	5528	4171
	HD	7.43[a]	2.26	5.18[a]	5.24	3.94	1.00	7156	30994	5950	4455
P-value	B	0.044	0.051	0.158	0.563	0.251	0.220	0.388	0.342	0.623	0.711
	D	0.031	0.315	0.017+	0.283	0.350	0.401	0.189	0.386	0.549	0.439
	B × D	0.969	0.814	0.902	0.923	0.757	0.755	0.750	0.993	0.981	0.855
SEM	B	0.27	0.13	0.22	0.24	0.17	0.03	280	1562	291	160
	D	0.33	0.16	0.26	0.29	0.21	0.04	343	1913	356	196

[a,b] Different superscripts within the same column and factor designate significant differences (P<0.05)
[1] Bedding: PS (Pine wood shavings) and MG (Miscanthus grass);
[2] Number of replicates (n): n=6 for MG, and PS
[3] Stocking density: LD 4.2, MD 5.3 and HD 7 hens/m^2
[4] Number of replicates (n): n=4 for LD, MD, and HD
+ Polynomial contrast, linear relationship among densities (Scheffe= 0.032, quadratic, Nitrogen, Organic %)

Table 11 Influence of bedding and turkey hen stocking density on litter - Litter micro nutrient analyses after 14 wk of use on a DM basis (mg/kg)

Factor	Level	Zinc	Iron	Copper	Manganese
Bedding[1,2]	PS	572	699[b]	304	593
	MG	594	902[a]	336	584
Density[3,4]	LD	604	785	346	607
	MD	531	697	265	548
	HD	615	920	348	611
P-value	B	0.591	0.048	0.521	0.815
	D	0.199	0.183	0.312	0.354
	B × D	0.990	0.672	0.726	0.943
SEM	B	28	67	35	27
	D	34	82	43	34

[a,b] Different superscripts within the same column and factor designate significant differences ($P<0.05$)
[1] Bedding: PS (Pine wood shavings) and MG (Miscanthus grass);
[2] Number of replicates (n): n=6 for MG, and PS
[3] Stocking density: LD 4.2, MD 5.3 and HD 7 hens/m^2
[4] Number of replicates (n): n=4 for LD, MD, and HD

Table 12. Influence of bedding and turkey hen stocking density on litter particle size distribution after 6 wk of use on a DM basis (% total mass)

Factor	Level	4	6	8	12	16	20	30	50	70	100	>100, Remainder
Bedding[1,2]	PS	26.14^{b}	14.05	14.23^{a}	12.34^{a}	12.96^{a}	6.87^{a}	5.80^{a}	5.14^{a}	0.93^{a}	0.80^{a}	0.74^{a}
	MG	52.32^{a}	12.15	9.51^{b}	7.33^{b}	7.44^{b}	4.21^{b}	3.15^{b}	2.59^{b}	0.44^{b}	0.38^{b}	0.48^{b}
Density[3,4]	LD	40.88	12.43	11.00	9.48	9.99	5.46	4.55	4.09	0.77	0.66	0.68
	MD	38.15	13.84	13.14	10.20	9.83	5.22	4.20	3.63	0.65	0.56	0.59
	HD	38.65	13.04	11.46	9.82	10.78	5.94	4.68	3.88	0.65	0.54	0.56
Bedding × Density	PS-LD	21.02	14.52	14.52	13.05	13.98	7.42	6.42	5.99	1.17	0.98	0.94
	PS-MD	29.13	13.68	14.34	12.21	11.98	6.22	5.34	4.82	0.87	0.76	0.64
	PS-HD	28.27	13.96	13.82	11.74	12.93	6.98	5.64	4.72	0.76	0.65	0.64
	MG-LD	60.75	10.35	7.48	5.91	6.00	3.51	2.69	2.19	0.38	0.33	0.43
	MG-MD	47.18	13.99	11.94	8.19	7.69	4.21	3.06	2.43	0.42	0.36	0.53
	MG-HD	49.03	12.12	9.11	7.90	8.63	4.91	3.72	3.14	0.53	0.44	0.49
P-value	B	0.000	0.140	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.00	0.007
	D	0.875	0.583	0.098	0.673	0.590	0.458	0.727	0.831	0.787	0.727	0.736
	B × D	0.075	0.338	0.088	0.130	0.094	0.270	0.341	0.281	0.193	0.201	0.143
SEM	B	0.031	0.015	0.010	0.009	0.010	0.009	0.009	0.010	0.004	0.005	0.004
	D	0.037	0.019	0.012	0.011	0.012	0.011	0.011	0.013	0.005	0.006	0.005
	B × D	0.053	0.026	0.017	0.016	0.017	0.015	0.016	0.018	0.008	0.008	0.007

[a,b] Different superscripts within the same column and factor designate significant differences ($P<0.05$)

[1] Bedding: PS (Pine wood shavings) and MG (Miscanthus grass);

[2] Number of replicates (n): n=6 for MG, and PS

[3] Stocking density: LD 4.2, MD 5.3 and HD 7 hens/m^2

[4] Number of replicates (n): n=4 for LD, MD, and HD

Table 13. Influence of bedding and turkey hen stocking density on litter particle size distribution after 14 wk of use on a DM basis (% total mass)

		Sieve size										
Factor	Level	4	6	8	12	16	20	30	50	70	100	>100, Remainder
Bedding[1,2]	PS	49.51^{b}	10.16^{a}	9.11^{a}	8.14^{a}	9.29^{a}	4.90^{a}	4.19^{a}	3.22^{a}	0.56^{a}	0.45^{a}	0.47
	MG	61.72^{a}	8.33^{b}	6.45^{b}	6.10^{b}	6.90^{b}	3.79^{b}	3.17^{b}	2.46^{b}	0.41^{b}	0.32^{b}	0.36
Density[3,4]	LD	56.33	9.68	7.59	6.96	7.58	4.18	3.59	2.82	0.50	0.37	0.40
	MD	53.81	9.38	8.36	7.36	8.62	4.49	3.88	2.86	0.46	0.38	0.39
	HD	56.72	8.67	7.39	7.04	8.07	4.36	3.56	2.84	0.50	0.41	0.45
Bedding × Density	PS-LD	51.77	9.48^{abc}	8.41	7.75	8.78	4.83	4.22	3.34	0.59	0.42	0.41
	PS-MD	47.69	10.80^{a}	10.16	8.67	9.63	4.78	4.07	2.90	0.49	0.41	0.41
	PS-HD	49.08	10.20^{ab}	8.78	8.01	9.45	5.08	4.28	3.43	0.62	0.50	0.58
	MG-LD	60.88	9.88^{ab}	6.77	6.17	6.38	3.54	2.96	2.30	0.41	0.31	0.40
	MG-MD	59.93	7.96^{bc}	6.57	6.07	7.62	4.20	3.70	2.83	0.44	0.34	0.36
	MG-HD	64.36	7.14^{c}	6.00	6.06	6.69	3.63	2.84	2.25	0.39	0.32	0.32
P-value	B	0.000	0.001	0.000	0.001	0.001	0.009	0.015	0.015	0.031	0.028	0.160
	D	0.612	0.227	0.187	0.795	0.401	0.763	0.740	0.975	0.881	0.799	0.823
	B × D	0.646	0.016	0.209	0.773	0.878	0.576	0.407	0.212	0.484	0.593	0.393
	B	0.019	0.006	0.005	0.007	0.008	0.007	0.007	0.006	0.003	0.003	0.004
SEM	D	0.023	0.007	0.007	0.008	0.010	0.008	0.009	0.007	0.004	0.004	0.005
	B × D	0.032	0.010	0.009	0.012	0.014	0.012	0.012	0.011	0.006	0.005	0.007

[a, b] Different superscripts within the same column and factor designate significant differences ($P<0.05$)
[1] Bedding: PS (Pine wood shavings) and MG (Miscanthus grass);
[2] Number of replicates (n): n=6 for MG, and PS
[3] Stocking density: LD 4.2, MD 5.3 and HD 7 hens/m^2
[4] Number of replicates (n): n=4 for LD, MD, and HD

Table 14. Spearman correlation coefficient (r) between average FPD score and the amount of litter retained at each sieve size (particle size of turkey litter) at 6 wk

Sieve size	FPD score	P-value
4	0.81	<0.001
6	-0.02	0.937
8	-0.59	0.003
12	-0.81	<0.001
16	-0.80	<0.001
20	-0.79	<0.001
30	-0.77	<0.001
50	-0.77	<0.001
70	-0.79	<0.001
100	-0.76	<0.001
Remainder	-0.81	<0.001

Table 15. Spearman correlation coefficient (r) between average FPD score and the amount of litter retained at each sieve size (particle size of turkey litter) at 14 wk

Sieve size	FPD score	P-value
4	0.44	0.0304
6	-0.4365	0.0341
8	-0.5530	0.0058
12	-0.4165	0.0440
16	-0.2991	0.1554
20	-0.1939	0.3622
30	-0.1713	0.4218
50	-0.1113	0.6033
70	-0.2470	0.2436
100	-0.1200	0.5751
Remainder	-0.0035	0.9886

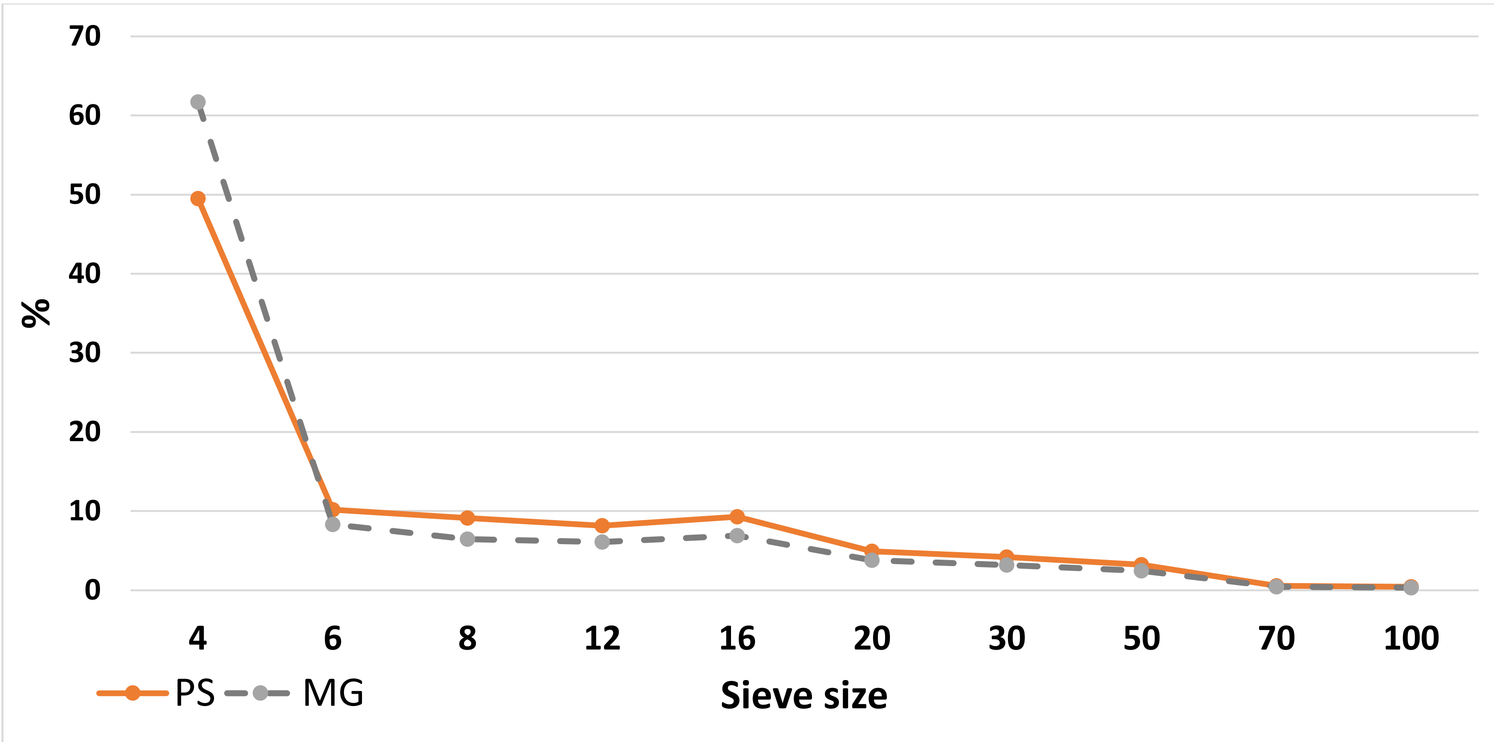

Figure 1. Bedding and particle size (Sieve size) distribution at 14 wk of age.

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