



The Histological Characteristics of Footpad Dermatitis in a Fast-Growing Broiler Chickens Raised on Different Floor Housing Systems

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Abstract: Broiler housing and management have become important for both manufacturers and researchers, with the increasing commercial demand over the years. The most important factor that creates the management conditions is floor housing system selection. The reason floor housing systems are important is that they allow broilers to reduce contact with the excreta and help excess moisture to be absorbed and controlling ammonia level. Poor litter conditions trigger painful pododermatitis that will affect animal welfare. Footpad dermatitis is a form of contact dermatitis affecting skin and has most commonly been associated with poor litter condition or irritating and unsuitable materials in broilers. This study was made to investigate the histopathologic characteristics of footpad lesions in fast-growing broiler chickens raised in different floor housing systems. There were four groups in the experiment as conventional deep litter flooring, fully slatted plastic flooring, fully slatted wood flooring, and partially slatted plastic flooring. After the slaughter process, random samples were taken from the birds representing each macro score (if occurred, from 0 to 4) from each group. For histopathologic examinations, footpad samples were fixed, and routine histological examinations were applied. Microscopic examination has been defined in five different scores, from the non-lesion score 0 to the score 4 with severe necrosis. As a result of macroscopic and microscopic evaluations, it has been determined that plastic and wooden slat materials have positive effects on foot health. Focusing future studies on the correlation between the size and depth of macroscopic lesions and the size and depth of histological lesions would be beneficial to determine a reliable correspondence between antemortem and postmortem lesion score of footpad in broiler.

Keywords: Broiler, footpad dermatitis, histological examination, housing systems

Farklı Altlık Sistemlerinde Yetişen Hızlı Gelişen Broilerlerde Footpad Dermatitinin Histolojik Olarak İncelenmesi

Öz: Broiler barınması ve yönetimi, yıllar içinde artan ticari talep ile hem üreticiler hem de araştırmacılar için önemli hale gelmiştir. Barınma koşullarını oluşturan en önemli faktör altlık seçimidir. Özellikle amonyak seviyesini kontrol edilmesinde, dışkı ile teması azaltılmasında ve fazla nemin emilmesine yardımcı olduğundan altlık sistemleri oldukça önemlidir. Kötü altlık koşulları, hayvan refahını etkileyecek ağırlı pododermatitlerin oluşumunu tetikler. Footpad dermatiti, cildi etkileyen bir kontakt dermatit türüdür ve broilerlerde kötü altlık koşulları veya tahriş edici ve uygun olmayan altlık malzemelerinin sonucu olarak ortaya çıkabilir. Bu çalışma, farklı altlık sistemlerinde yetiştirilen, hızlı büyüyen broilerlerin ayak tabanı lezyonlarının histopatolojik özelliklerini araştırmak için planlanmıştır. Deneyde, geleneksel derin altlık, tamamı plastik ızgaralı altlık, tamamı tahta ızgaralı altlık ve kısmi plastik ızgaralı altlık olmak üzere dört grup vardı. Kesim işleminden sonra, makro skoru (0'dan 4'e kadar) temsil edecek, her gruptan rastgele örnekler alındı. Ayak tabanı örnekleri tespit işlemi takiben rutin histopatolojik yöntemle incelendi. Mikroskopik değerlendirmeler; lezyon olmayan skor 0'dan, derin nekrozların görüldüğü skor 4'e kadar beş farklı skorda tanımlandı. Makroskopik ve mikroskopik değerlendirmelerin sonucunda, plastik ve tahta altlığın ayak sağlığına olumlu yönde etkilerinin olduğu anlaşılmıştır. Ayrıca bu çalışma, makroskopik lezyonların boyutu ve derinliği ile histolojik lezyonların boyutu ve derinliği arasındaki korelasyon incelendiğinde, broilerlerdeki ayak tabanının antemortem ve postmortem lezyon skoru arasında güvenilir bir uygunluk olduğunun gösterilmesi açısından oldukça önemlidir.

Anahtar kelimeler: Altlık sistemleri, Broiler, footpad dermatitis, histolojik değerlendirme

Introduction

The occurrence of footpad dermatitis in a broiler flocks can lead a significant welfare and economic

problems in commercial production (Christensen, 1996; US Poultry and Egg Export Council, 2009; De Jong et al., 2015). Poor litter quality is the main cause of contact dermatitis in different body regions of broiler chicken (Kaukonen et al., 2016). Litter quality can be worsened by many factors, such as diet,

nutritional deficiencies, wet litter, litter material types, genetic strain, sex, bird weight, stocking density and management (Petek et al., 2014; Swiatkiewicz et al., 2016). The housing condition and indoor climate can be improved thanks to partially or fully slatted flooring which may be a practical solution to keep animals out of contact with the litter (Chuppava et al., 2018; Cavusoglu and Petek, 2019; Adler et al., 2020). Slats can be made from different material depending on price and availability. Wooden slats are more sustainable and can be cheaper in some part of the world. But it might be absorbing the moisture and thus creates a wet litter, which accelerates the formation of lesions, especially in the feet. Plastic slats have been developed recently to provide a nonabrasive and soft surface and have recently been used in commercial broiler meat production in some part of the world (Bilal et al., 2014). It is cheap, and it can have a long service life (Almeida et al., 2018). The partially slatted system has not been studied much in broiler production.

External evaluation of foot pad in broiler chicken can sometimes be misleading due to manure adhesion and personnel mistake. The depth and width of the lesions can be different, and no lesions can be seen on the soles of the feet after slaughter (Kristensen et al., 2007; Michel et al., 2012). Histological features of the lesions can be an efficient way of determining lesion severity and it would be beneficial to improve the reliability of the scoring systems in terms of animal welfare (Louton et al., 2020). This study was carried out to reveal the histopathological conditions of footpad lesions of broiler occurred into different floor housing systems.

Materials and Methods

The experiment was conducted in the Research and Experimental Farm of Bursa Uludag University in Turkey. In accordance with the Regulation Regarding the Working Procedures and Principles of Animal Experiments Ethics Committees, since slaughterhouse material is used in the study, the permission of the ethics committee is not required (Turkey-Legal Gazette 28914, 2014). 10 legs for each group (n=40) samples were collected from the birds raised on conventional floor pens with litter (control, fully-deep litter), fully-slatted plastic flooring, fully-slatted wood

flooring and partially-slatted plastic flooring (litter and slat, 50%, each) with standard condition for broiler production (North and Bell, 1990) and slaughtered at 42 days of age. After slaughter, the severity of footpad lesion development were measured with an experienced person by visual observation using the scoring method reported by Pagazaurtundua and Warris (2006), Welfare Quality Consortium (2009) (Table 1): a score of 0 indicated no lesion, 1 indicated a very small or superficial lesion (5-25 % of the footpad is covered with a lesion), 2 indicated a mild lesion (minor superficial lesion; 25-50 % of the footpad is covered with a lesion), 3 indicated a medium-severity lesion (moderate hyperkeratosis; more than 50 % of the footpad is covered with a lesion), and 4 indicated a severe lesion (more than 50 % of the footpad is covered with a lesion with deep and large epithelial necrosis). Then, each of phenotypically affected feet was photographed with a digital camera and their type of severity was recorded. For microscopic evaluation, the feet were placed in 10% buffered formalin which was used for fixation of the samples. After washing under running tap water, specimens were dehydrated through increasing concentrations of alcohol, cleared with xylene and embedded in paraffin. Sections were cut at 5 µm, mounted on slides and dried overnight. After dewaxing and rehydration, and triple (Crossmon's) stain was applied for histopathological examination to the sections for footpad dermatitis (Crossmon et al., 1937). Micrographs were taken with Nikon 80i microscope. Every macroscopic score was determined 1 or 0 according to presence or absence of the lesion in the groups. Total and average score were defined as sum of or average of all scores determined in each group. The normality of the data was determined by the Shapiro-Wilk test. Housing differences for total and average scores were analyzed using nonparametric tests as Kruskal-Wallis and Mann-Whitney U tests (Snedecor and Cochran, 1989). The statistical analyses were performed using SPSS® computer software 13.00 (IBM SPSS, 2013) (P<0.05).

Results

The scoring criteria for footpad dermatitis and a sample of phenotypically affected foot pads of broiler were presented in Figure 1 and Table 1.

Table 1. Definition of footpad dermatitis scores

Score	Definition by Pagazaurtundua and Warris (2006), Welfare Quality Consortium (2009)
A; Score 0	healthy skin, no lesion
B; Score 1	a very small or superficial lesion, with less than 25% of the foot pad is covered with a lesion
C; Score 2	a mild lesion/minor superficial lesion with between 25% and 50% of the foot pad is covered with a lesion
D; Score 3	a medium-severity lesion/moderate hyperkeratosis with more than 50% of the foot pad is covered with a lesion
E; Score 4	a severe lesion with more than 50% of the foot pad is covered with a lesion deep and large epithelial necrosis

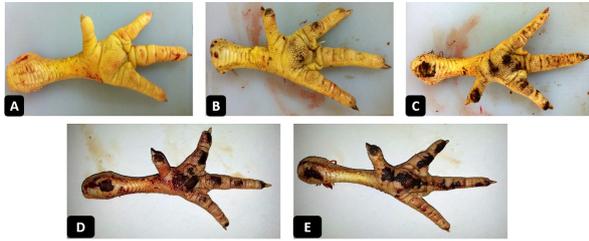


Figure 1. The scoring criteria and macroscopic evaluation of footpad lesions in broiler.

The samples for each score were taken from the birds raised in conventional deep litter housing because their legs represent all scores. Histological examination of footpad lesions in deep litter flooring and partially slatted plastic flooring were presented in Figure 2 and Figure 3.

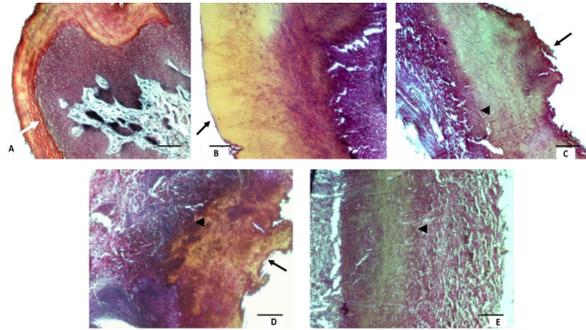


Figure 2. Histological characteristics of different footpad lesion types (hyperkeratosis [black arrow]; vascular keratin cells [white arrow]; epidermis loss [arrowhead]) from broiler raised on conventional deep litter flooring. Score 0 (A; Bar: 100 µm), score 1 (B; Bar: 100 µm), score 2 (C; Bar: 200 µm), score 3 (D; Bar: 200 µm), score 4 (E; Bar: 100 µm).

In association with the histological examination of footpad of broiler in all groups; score 0 were defined healthy skin structure with no pathological changes. They had normal histological features; regular thickness of keratin layer, epidermis, and dermis (Fig 2a, 3a, 4a, 5). In score 1; keratin layer was enlarged in all groups, but epidermis structure had different results between the groups (Fig 2b, 3b, 4b). Score 2 which is observed in broiler in partially slatted plastic flooring, had hyperkeratosis, inflammation area in epidermis and epidermis loss was seen in some part of the lesion area (Figure 3).

The score 2 examined in broiler of in conventional deep litter flooring, had no epidermis and diffuse inflammation area was observed in dermis. In score 3 and score 4 they had same structure in both two groups of partially plastic slats and conventional deep litter. Hyperkeratosis and epidermis loss were observed and marked diffuse inflammation area in der-

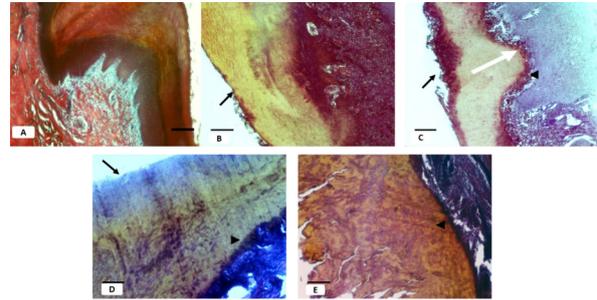


Figure 3. The skin features (epidermis and dermis) of broiler footpad (hyperkeratosis [black arrow]; vascular keratin cells [white arrow]; epidermis loss [arrowhead]) raised on partially plastic slatted flooring (litter and slat). Score 0 (A; Bar: 100 µm), score 1 (B; Bar: 100 µm), score 2 (C; Bar: 100 µm), score 3 (D; Bar: 100 µm), score 4 (E; Bar: 100 µm).

mis in both groups. In score 4, in addition to hyperkeratosis, vascular keratin cells were seen in conventional deep litter and partially slatted flooring. Microscopic features of footpad score 0 and score 1 (epidermis and dermis) from broiler raised on fully slatted wood flooring were showed in Figure 4.

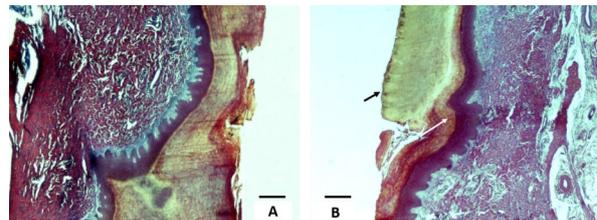


Figure 4. Microscopic features of footpads (epidermis and dermis), hyperkeratosis (black arrow); vascular keratin cells (white arrow); epidermis loss (arrowhead) from broiler raised on fully slatted wood flooring. (3a) score 0; footpad, Bar 200. (3b) score 1; footpad, Bar 100 µm.

The features of healthy footpad (score 0) of broiler raised on fully plastic slatted flooring was presented in Figure 5.

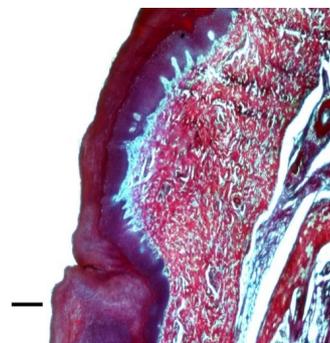


Figure 5. Features of footpad (epidermis and dermis) from broiler raised on fully plastic slatted flooring. Score 0 of footpad dermatitis. Bar: 200 µm.

As like the others they had regular thickness of keratin layer. The presence of every footpad lesion score according to macroscopic evaluation in each group was presented in Table 2. There were differences for total and average score among the groups ($P<0.05$). The conventional deep litter and partially plastic slatted floor groups had all level of footpad scores and significantly greater total and average scores than fully wood or plastic slatted floor groups.

pad of broiler. Brownish-colored skin and hyperaemic abrasions were seen in focal spots on footpads in some of the broilers (Score 1). Also hyperemia and mild hyperkeratosis were observed in score 1 of histopathological sections in fully slatted wood flooring. No more severe lesions exist in both of these slatted flooring. Chuppava et al. (2018) raised broilers in wooden slat and revealed the similar results, ≤ 1 scores were observed the 100% of the broilers, since

Table 2. Presence of every footpad lesion scores according to macroscopic evaluation in each group

Groups/Score	Score 0	Score 1	Score 2	Score 3	Score 4	TS	AS
Conventional deep litter	1	1	1	1	1	5 ^a	1 ^a
Partially plastic slatted floor	1	1	1	1	1	5 ^a	1 ^a
Fully wood slatted floor	1	1	0	0	0	2 ^b	0.4 ^b
Fully plastic slatted floor	1	0	0	0	0	1 ^b	0.2 ^b

1. If lesion was presence, 0, if lesion was absence, TS.Total score, AS.Average score
^{a-b}, within the same columns, values with different superscript letters differ significantly ($P<0.05$)

Discussion and Conclusion

Footpad dermatitis is characterized by inflammation and necrotic lesions, from superficial to deep, on the plantar surface of footpads and toes of broiler chickens (Shepherd and Fairchild, 2010). In the early stages, footpad dermatitis shows itself as small erosions and discoloration of the skin. When the condition progress, the diagnosis can be easier with inflammation and necrosis areas in different layer of the epidermis and dermis of toes and footpads. A normal footpad of a broiler chicken was defined as having no visible lesion, no discoloration, and histologically normal scales without significant inflammatory changes (Michel et al., 2012).

In present study, we evaluate the effects of the different flooring systems as fully slatted plastic or wood flooring, partially slatted plastic flooring and conventional deep-litter flooring on histopathologic characteristics of footpad lesion in a fast growing broiler. In the study, the histological analysis of foot pad was performed from the healthy and phenotypically affected legs from each group if occurred. The samples of each lesion in each housing group corresponded to a typical macroscopic appearance. The macroscopic score 2, 3 and 4 were seen in conventional deep litter and partially plastic slatted flooring. When flooring systems are examined distinctly; the footpad skin of the broilers grown on fully slatted plastic flooring was observed healthy and there was neither a macroscopic nor microscopic lesion. But Kaukonen et al. (2016) used plastic slats on average 38% of the floor and observed that poorer food pad conditions were associated with increasing slat area. Whereas, in fully slatted wood flooring, score 0 and 1 were observed in footpad of chickens. The thickness of keratin layer, epidermis and dermis were regular in score 0 of foot-

the wood material absorbs moisture more, broilers feet contact with the wet floor and excreta are minimized. On the contrary, Sander et al. (2003) reported that it would cause severe foot injuries due to the irritant substances in the wooden litter system. However, in this study, the finding of only hyperkeratosis in the use of wooden litter in broiler breeding reveals the positive side of the wooden litter.

Histologically mild lesion reported previously as by hyperplasia and hyperkeratosis of the epidermis, superficial dermal congestion, and oedema while moderate lesions by a prominent pustular and crust-forming dermatitis (Michel et al., 2012). The most severe foot pad lesions are scored score 3 and score 4 which means more than 50% of foot is covered with lesion. Although it was observed with loss of epidermis or replace with inflammation in partially slatted flooring and conventional deep litter, footpad of broiler in fully slatted wood flooring had local hyperkeratosis. Similar with our results Michel et al. (2012) described the severe lesion as histologically by extensive ulceration.

There could be an important correlation between macro and micro scores of foot pad in broiler raised in different floor system (Louton et al., 2020). Martins et al. (2016) showed that the lesion scores assigned were compatible with the histopathological results, showing that the higher the score, the more severe were the dermal and epidermal lesions. Lesions affecting the deeper layers of the skin as histopathologically were observed more in partially slatted plastic flooring and conventional deep litter (Score from 0 to 4). In these two group, discolouration, hyperaemic areas, superficial lesions were more diffuse in score 2. In microscobic view of the score 2; hyperkeratosis, inflammation area in epidermis and epidermis loss was seen in some part of the lesion area. Deep ulcerative areas with thick crust, yellow or brownish exu-

dates on the ulcerative lesions were seen score 3 and 4. The macroscopic features were more diffuse and severe in score 4. Hyperkeratosis, erosive and ulcerative epidermis or epidermis loss were observed and marked diffuse inflammation with heavy infiltration areas in dermis. In addition to hyperkeratosis, vacuolar keratin cells were seen in score 4.

The fact that footpad dermatitis is seen so severe in deep litter systems is due to the poor litter quality. Based on data given in the literature (Hashimoto et al., 2013; Nicol et al., 2017), litter quality and litter moisture are very important for the prevalence and severity of foot pad dermatitis. Therefore, minimizing contact with wet litter by using slatted flooring can be expected to lead to significant improvements in broiler dermatitis. Slatted floors that allow for temporary separation of the feet from the litter could lead to improvements in foot pad health (Cavusoglu and Petek, 2019).

As conclusion it can be said that the severity of the changes in the dermal and epidermal layers of the footpad increased with greater lesion score. The microscopic scores observed in this study showed that plastic or wood slatt materials have positive effects on footpad health probably due to no contacts with manure on slats. Focusing future studies on the correlation between the size and depth of macroscopic lesions and the size and depth of histological lesions would be beneficial. According to the level of possible correlation the existing visual scoring system can be re-developed.

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