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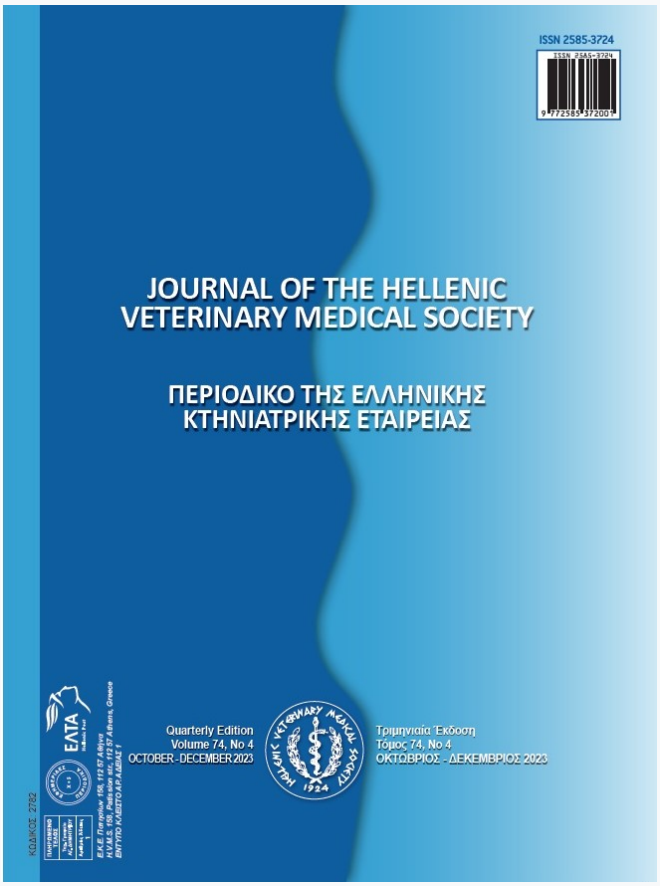

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Research article
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The Effect of Different Litter Materials on Lactate Dehydrogenase (LDH) Content and Oxidative Metabolism in Broilers

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ABSTRACT: Footpad dermatitis and hock burn are dermatological problems with similar pathologies, also known as contact dermatitis. As a result, carcass quality and the rate of water and feed utilization due to lameness decreases. It was aimed to determine serum LDH level and oxidative metabolism change in footpad dermatitis and hock skin lesions that can occur in broilers due to different litter materials, and to evaluateserum LDH activity as a potential marker of footpad dermatitis in broilers, similarly to humans. The study was conducted in 3 climate chambers, one of which was conventional poultry litter (wood shavings), the other perforated plastic floor and the last was an addition of zeolite to wood shavings (6 kg/m^2). The study followed a completely randomized design with three litter treatments replicated four times. A well ventilated house divided into 4 pans was used where each pan or a replicate contain 42 birds with a total of (n=168) birds (33 kg/m^2). Regarding broiler feet, litter had a significant influence on footpad dermatitis while hock burns were not statistically different among litter treatments. Severe lesions on the footpad were most common in the zeolite group, while no severe lesions were observed in the plastic floor. Consistent with this result, the treatment of litter supplemented with zeolite significantly increased serum LDH level and footpad MDA activity, a marker of oxidative stress. Interestingly, zeolite treatment also led an increase in antioxidant enzymes (SOD, CAT, GSH) activities in footpads. Our current findings suggest that serum LDH level may be an indicator of the severity of footpad dermatitis. Higher oxygen free radical production, evidenced by increased MDA support to the oxidative stress in footpad dermatitis. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress.

Keywords: LDH; oxidative stress; broiler; footpad dermatitis; litter material

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INTRODUCTION

Litter quality is an important factor affecting yield in broiler production. The litter material is laid under the broilers and serves to cut off the direct contact of the broilers with the ground. It prevents the negative effects of the temperature variation in the house by absorbing of water and embodying urine, feces and feathers. Wood shavings are the litter material of choice for broiler houses in most countries (Toledo et al., 2019). However, in recent years, the price of wood shavings has been increased due to the fact that it is also used for non-litter purposes (Garces et al., 2013). For the poultry industry, reducing production costs is a priority. Therefore, researchers seek alternative litter materials that will not adversely affect the performance of the animals and are less costly. Many studies have shown that zeolites, which are used as binders in animal feeds, can be used as litter additives due to their high water holding capacity, reducing gas and odor formation. (Eleroğlu and Yalçin, 2005; Turan, 2008). In addition, it has been reported that the plastic slatted floor, which has recently become widespread in broiler breeding, has more positive effects on providing ideal environmental conditions, animal health and production values compared to many litter materials (Almeida et al., 2018).The moisture content of the litter varies between 15-57% depending on the type of litter, excreta, the amount of water poured from the drinker, the ventilation in the broiler house and also the production season (Miles et al., 2011; Avcilar et al., 2018). High litter moisture increases the ammonia level in the litter (Avcilar et al., 2018). High level of ammonia causes contact dermatitis which appears first on the footpads, followed by hock burns (Kaukonen et al., 2017; Avcilar et al., 2018). All these deformities lead to decrease in carcass quality and in the rate of water and feed utilization due to lameness (Kheravii et al., 2017).

Lactate dehydrogenase (LDH), a cytoplasmic enzyme found in the body cells of almost all animals, is used as a common marker in many pathological cases such as tumors, heart diseases and hemolysis (Drent et al., 1996; Kato et al., 2006; Ding et al., 2017). In addition, previous studies have shown that serum LDH activity is increased in severe atopic dermatitis in both children and adults, and it has been suggested that serum LDH level may also be a marker of atopic dermatitis (Morishima et al., 2010; Thijs et al., 2015). However, there is no study on how the serum LDH level changes in broiler chickens is related to dermatitis that can be formed in footpads and/or hock skins due to different reasons. Therefore, the present study was conducted to determine serum LDH level and oxidative metabolism change in footpad dermatitis and hock skin lesions that can occur in broilers due to different litter materials, and to evaluate serum LDH activity as a potential marker of footpad dermatitis in broilers, similarly to humans.

MATERIALS AND METHOD

Birds, Experimental Design and Management

Approval to use of one-day-old male broiler chicks (Ross 308) from a commercial hatchery was obtained through Aydin Adnan Menderes University Animal Ethical Committee (ADÜ-HADYEK Approval no: 64583101/2020/097). Sample size calculation revealed that 504 broiler chicks allocated to3 litter groups [wood shavings (control), zeolite, and plastic floor] each containing 4 replicates (Table 1) would be sufficient, with a maximum error of 10% at 75% power and 5% Type 1 error levels. The wood shavings used in the first group were homogeneously laid at a height of 5-7 cm in each compartment with an area of 4 m^2, as described by Ramadan et al. (2013). In the second group, the zeolite material was mixed with wood shavings at a rate of 35% and laid at 6 kg/m^2, as described by Watson and Wiedemann (2019). The plastic grid used in the third group was designed in 50x50 cm dimensions, 5 cm high from the ground, with a 2 cm eye gap, and was laid on the floor of the four sub-groups, as described by Li et al. (2017).

A 23L:1D lighting program was applied up to 7 days and 18L:6D thereafter until day 42. The temperature was sustained at 32°C until day 7 followed by a reduction of 3°C per week until day 21 and a temperature of 24-26°C was sustained afterwards.

Table 1. Experimental groups

Group	Bedding Material	Replications	Birds per Replication	Total Birds
Group I	Wood shavings	4	42	168
Group II	Zeolite	4	42	168
Group III	Plastic floor	4	42	168
Total				504

Starter feed containing 3000 kcal/kg metabolic energy (ME) and 23% crude protein (CP) in the age period of 0-10 days, grower feed containing 3100 kcal/kg ME and 21.5% CP between days 11-24, and finisher feed containing 3200 kcal/kg ME and 19.5% CP between days 25- 42. Birds had free access to feed and water and the experiment's duration was 42 days.

Scoring

On the 41st day of the study, the footpad dermatitis and hock skin lesions of a total of 84 broilers, seven broilers per replicate, were evaluated. The researcher, an experienced poultry veterinarian that was blinded regarding the official results, assessed footpads and hock skins based on the example photos of WQ-assessment applied for broiler chicken (Welfare Quality, 2009). The scoring scale was based on the presence, size and severity of lesions on footpads and hock skin, as summarized in the Table 2.

Determination of LDH Level and Oxidative Metabolism

At the end of the experiment 10 ml of blood was collected from the *vena jugularis* of the total of 84 birds, seven birds per replicate. After blood collection, animals were slaughtered and the footpad and hock skin tissue samples were used to determine the oxidative metabolism.

The blood collected into serum tubes was kept at room temperature for 1 hour, then centrifuged at 3,000 rpm for 15 minutes and then serum was separated (Hettich Micro 200/200R Centrifuge, Canada). LDH concentration was determined in the obtained serum samples by ELISA (Catalog No: E-EL-Ch1372 Bioassay Technology Laboratory, Shanghai, China).

Tissue samples were first diluted 10 times with cold 150 mM PBS (pH 7.4) and homogenized for 1-2 minutes at 2,000 rpm with a tissue homogenizer (IKA WERKE Yellowline OST Basic S2 Analog Overhead Stirrer, Athy, Ireland). Homogenates were centrifuged at 12,000 rpm for 10 minutes at +4°C.Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) activities were determined in the supernatants obtained after centrifugation. The determination of MDA was made according to the method reported by Ohkawaet al. (1979). In this method, the pink colored pigment formed as a result of the reaction of thiobarbituric acid and MDA in acidic pH and hot environment was measured at 532 nm (Shimadzu UV-1601, Kyoto, Japan). Results were defined as nmol/mg protein. SOD activity was determined according to the method of Sun et al.(1998). Briefly, in this method, superoxide radicals which are produced by the xanthine-xanthine oxidase system form formazone dye in the presence of nitro blue tetrazolium. This color intensity was measured spectrophotometrically at 560 nm. The percent inhibition was calculated depending on SOD activity and the results were expressed as U/mg protein. CAT activity was measured in supernatants according to the method determined by Luck (1965). In this method, the conversion of substrate H_2O_2 to H_2O was monitored spectrophotometrically at 240 nm at 20-second intervals and the decrease in absorbance was measured. Enzyme activity was expressed as k/mg protein. GSH was measured as described by Tietze (1969). In this procedure, Ellman's reagent [5,5′-dithiobis,2-nitrobenzoic acid (DTNB)] is used that reacts with GSH resulting in a product that be measured at 412 nm for 4 min and the results were expressed as mg/g protein.

Statistical Analyses

SPSS software (Version 22.0; SPSS Inc., Chicago, IL, USA) was used to perform statistical analyses. The dependent variables were investigated using visual (histogram) and analytical methods (Shapiro-Wilk's test) to determine whether or not they are

Table 2. Description of the footpad and hock skin lesions scoring performed (8, 19)

Score	Description
0 (Healthy footpad)	Smooth skin, no lesion Small superficial lesion Discoloration on limited area
1 (Mild superficial lesion)	Superficial lesion of marked size covering several papillae Discolored or dark papillae No ulceration
2 (Severe, deep lesion)	Ulceration or crust of significant size, over 5 mm×5 mm, without existing papilla structure Ulceration on the bottom of toes > 1 cm long

normally distributed. The homogeneity of variances was determined by Levene's test. One-way ANOVA followed by post hoc Duncan test was conducted for the normal distributed data to compare the effect of the intervention among groups. The frequencies of footpad dermatitis and hock skin lesions according to score 0, 1, and 2 were determined on the basis of different bedding materials and chi-square test was used for the analysis of frequency of these variables. The results are presented as means ± SEM. $P \leq 0.05$ were considered as significant.

RESULTS

The effect of different bedding materials on footpad dermatitis was found to be significant ($P < 0.05$) (Table 3). On the other hand, there was no difference among the groups in terms of hock skin lesions (Table 4). In Group I (wood shavings), 17 broilers had no lesions despite that 6 broilers had moderate lesions and 5 broilers had severe lesions. No lesions were observed in 13 broilers in Group II (zeolite), while moderate and severe lesions were detected in 8 and 7 broilers, respectively. While no lesions were observed in 22 broilers in Group III (plastic floor), moderate lesions were formed in 6 broilers. No severe lesions were observed in this group.

Serum LDH activity differed greatly among groups using different litter materials ($P < 0.05$) (Figure 1). Serum LDH level was found to be higher in Group II (zeolite) than that of the other groups, but the difference between Group III (plastic floor) was statistically significant.

Zeolite led to an increase in MDA level of the foot-

Table 3. The effect of different bedding materials on footpad dermatitis

Group	Bedding Material	Footpad Dermatitis (%)							
		n	S: 0	n	S: 1	n	S: 2	X^2	P
Group I	Wood shavings	17	60.7	6	21.4	5	17.9		
Group II	Zeolite	13	46.4	8	28.6	7	25.0	9.246	0.045
Group III	Plastic floor	22	78.6*	6	21.4	0	0.0*		

S: Score; X^2: Chi-square value. *P < 0.05 versus Zeolite group.

Table 4. The effect of different bedding materials on hock skin lesions.

Group	Bedding Material	Hock Skin Lesions (%)							
		n	S: 0	n	S: 1	n	S: 2	X^2	P
Group I	Wood shavings	14	50.0	11	39.3	3	10.7		
Group II	Zeolite	16	57.1	7	25.0	5	17.9	1.789	0.774
Group III	Plastic floor	15	53.6	10	35.7	3	10.7		

S: Score; X^2: Chi-square value.

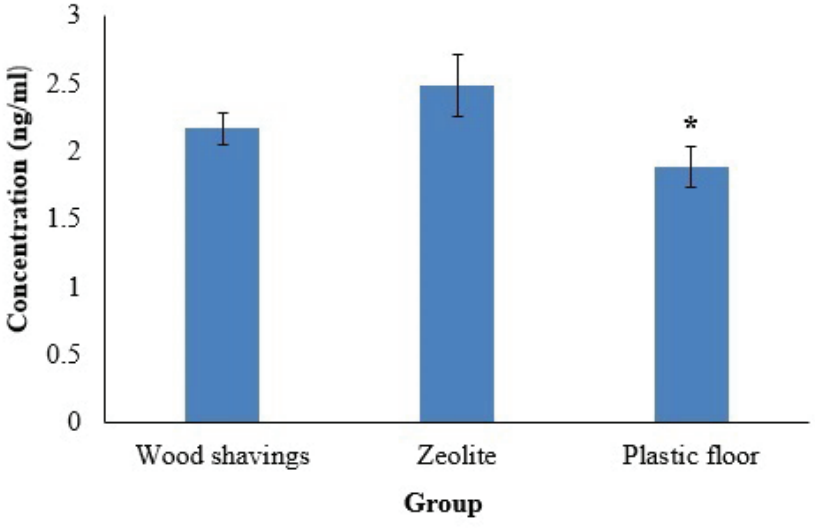

Figure 1. Serum LDH concentration according to groups. *P < 0.05 versus Zeolite group.

Table 5. Effect of different bedding materials on oxidative metabolism in broiler chickens.

Variables	Bedding Materials			*P-Value*
	Wood shavings	Zeolite	Plastic floor	
Footpad MDA (nmol/mg protein)	44.35 ± 3.10[b]	78.70 ± 6.79[a]	48.37 ± 4.69[b]	0.000
Hock burn MDA (nmol/mg protein)	37.36 ± 3.10	35.20 ± 3.04	31.10 ± 2.93	NS
Footpad SOD (nmol/mg protein)	10.13 ± 1.18[b]	17.71 ± 2.17[a]	11.98 ± 2.02[b]	0,022
Hock burn SOD (nmol/mg protein)	13.19 ± 1.05	12.03 ± 0.78	11.04 ± 0.67	NS
Footpad CAT (k/mg protein)	0.35 ± 0.06[b]	0.56 ± 0.06[a]	0.36 ± 0.05[b]	0.047
Hock burn CAT (k/mg protein)	0.29 ± 0.01	0.34 ± 0.03	0.25 ± 0.01	NS
Footpad GSH (mg/g protein)	10.26 ± 1.93[a]	17.28 ± 3.66[a]	3.02 ± 0.37[b]	0.002
Hock burn GSH (mg/g protein)	8.14 ± 1.81	4.95 ± 0.85	4.95 ± 1.73	NS

Data are expressed as means ± SEM. [a, b]The differences between the values in the litter type category with different letters in the same column are significant. NS: not significant.

pad compared to groups wood shavings and plastic floor (P = 0.000) (Table 5). However, when compared with the other groups, Zeolite significantly increased the antioxidant enzyme activities such as SOD, CAT and GSH in the footpad (P = 0.022, P = 0.047, and P = 0.002, respectively) (Table 5). There was no significant difference between the groups in hock skin regarding oxidative metabolism.

DISCUSSION

Footpad dermatitis, hock burn, and breast blisters are dermatological problems with similar pathologies, also known as contact dermatitis (Greene et al., 1985). Contact dermatitis is an ulcerative condition of the skin affecting the plantar surface of the feet, the hock and the breast (Haslam et al., 2007). Some lesions are superficial, while others progress to deep ulcers and cause significant discomfort and pain (Cengiz et al., 2011). As shown in previous studies, litter quality is an important factor affecting the severity of the footpad dermatitis and the hock burns in broiler chickens and broiler turkeys (Harms et al., 1977; Martland, 1985; Mayne et al., 2007). A higher rate of contact dermatitis develops in broilers that are in constant contact with the wet litter due to moisture (water), unidentified irritants in fecal materials, and ammonia (Greene et al., 1985; Weaver and Meijerhof, 1991; Mayne et al., 2006). Similar to present findings, Güler et al.(2021) in their study showed that the type of litter had an effect on footpad skin lesions of broilers. In the present study, broilers grown on the slatted plastic floor was never scored as 2 (severe and deep lesion). Karcheret al. (2013) also compared the effect of plastic flooring and wood shavings in duck production and concluded that lower frequency of footpad dermatitis in animals raised on plastic floors than in animals reared on wood shavings was observed. In contrast to the present results, de Almediaet al. (2017) observed that plastic floor showed lower frequency of broilers (males and females) without footpad lesions, compared to wood shavings treatment. In addition, zeolite supplemented to wood shavings at the rate of 35% (6 kg/m^2) did not have a significant effect on footpad dermatitis compared to wood shavings, as also shown by Bintaş et al. (2014). Apart from the zeolite added to the litter, the stock density and the high quality of the litter have a more pronounced effect on the footpad health (Nakaue et al., 1981).The lowest feathering scores for the footpads of broilers were obtained from the plastic floor system. The zeolite group had significantly more footpad irritations than that of the plastic floor group. The main factor contributing to the development of lesions on the footpad was the poor litter quality and closer contact with poor-quality manure (Çavuşoğlu et al., 2018). In addition, the hardness of 5-6 according to the Mohs scale classifies the zeolite as medium hard (Bankowski and Spadlo, 2018). It is well-known that zeolites used in litter material have certain structural differences (Eleroğlu and Yalçın, 2005). Depending on these factors, the zeolite may have caused damage to the footpad skin.

Lactate dehydrogenase (LDH) is an enzyme found predominantly in the liver, kidneys, striated muscle, skin and heart muscle. Therefore, LDH is widely used to monitor the change of condition of patients with lung disease, heart disease, blood disease and malignant disease (Morishima et al., 2010). The elevated serum LDH levels are correlated with epidermal cell damage in eczema associated erythroderma (Kogawa et al., 2022). For example, it has been noted that changes in serum LDH levels can serve as a clinical marker to assess the severity and clinical course of atopic dermatitis (Vestergaard et al., 1999). In this study, serum LDH level was found to be highest in the

zeolite group in which footpad dermatitis was more intense. Similar result was obtained in mice with induced allergic contact dermatitis (Balaha et al., 2022) and in children with atopic dermatitis (Morishima et al., 2010). Serum LDH level may be considered as a marker for footpad dermatitis in broiler chickens.

Free radical production in the animal cell is inevitable. Normally, there is an equilibrium among a free radical and ROS formation and endogenous antioxidant defense mechanisms, but if this balance is disturbed, oxidative stress is observed (Sivaranjani et al., 2013). ROS play a central role in the development of contact dermatitis (Kim et al., 2012). Free radicals mediate lipid peroxidation, which is considered to be main mechanism of cell membrane destruction and cell damage (Sen, 1995). MDA is used as an indicator of lipid peroxidation. Antioxidants are the substances that scavenge and suppress the formation of free radicals and which even oppose their activities (Dormandy, 1978). In this study, the MDA level in the footpad skin was significantly higher in the zeolite group, where severe footpad dermatitis was more intense, than in the other groups. Similar results were obtained in rat skins with contact dermatitis (Ayuob et al., 2022) and sera from humans with atopic dermatitis (Sivaranjani et al., 2013).In the present study, SOD, CAT and GSH enzyme activities were found to be the highest in the zeolite group where footpad dermatitis was most intense. However, in terms of enzymatic (CAT and SOD) and non-enzymatic (GSH) antioxidant activity that controls the various cellular defense mechanisms, the opposite results were obtained from those obtained by Ayuobet al. (2022). Since SOD catalyzes the dismutation of superoxide anion to H_2O_2, which is in turn the substrate of CAT, this fact could explain the observed increment of the two enzyme activities. As these enzymes have a protective role against oxygen free radical-induced damage, their induction can be understood as an adaptive response to oxidative stress.

CONCLUSION

To the best of the author knowledge, this is the first study which has been performed to evaluate the level of serum LDH and the role of oxidative stress in footpad dermatitis due to different bedding materials.

Different litter materials are associated with the occurrence of footpad dermatitis, also known as contact dermatitis. In this study, perforated plastic floor showed a better result than zeolite in terms of footpad dermatitis and oxidative metabolism. On the other hand, LDH levels can be utilized for evaluating disease severity of footpad dermatitis. The limitation of this study is that we did not compare LDH and disease severity of footpad dermatitis directly. So, more studies are needed on the role of LDH activity in footpad dermatitis directly.

In addition to the increased LDH level due to the intensity of severe footpad dermatitis, there was an increase in lipid peroxidation and antioxidant enzymes activities. The increase in antioxidant enzyme activity may be a compensatory regulation in response to increased oxidative stress.

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CONFLICT OF INTEREST

The author declares no conflict of interest.The sponsors had no role in the design, execution, interpretation, or writing of the study.

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