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Effect of Litter on Development and Severity of Foot-Pad Dermatitis and Behavior of Broiler Chickens

ABSTRACT

This study was conducted to evaluate the impact of litter type and litter treatment with enzymatic-bacterial production incidence and severity of footpad dermatitis and consequently on behavior of broiler chickens. A total of 1,200 one day-old Ross 308 broilers were randomly allocated to 4 treatments with 4 replicates in 2 x 2 factorial design. The first factor was the physical form of the straw (chopped on approximately 2 cm or un-chopped) and the second factor was addition of enzymatic-bacterial product applied directly on the straw. Each replicate consisted of 75 as-hatched birds per pen. Occurrence and severity of the footpad dermatitis and histological evaluation of the scores was done at the end of the trial (42 days of age). During the trial, at 3 and 6 weeks of age, broiler behavior was observed by Scan Sampling Method. The results showed that chopped straw significantly lowered the incidence of footpad dermatitis. Chopped straw in combination with enzymatic-bacterial product showed the lowest footpad dermatitis score in broilers. Histological procedures confirmed the macroscopic evaluation of the footpad dermatitis severity. The chopped straw had a significant effect on some behavioral patterns of broiler chickens. Differences were observed for dust bathing and scratching. Correlation between footpad dermatitis and birds locomotor activity was not confirmed.

INTRODUCTION

One of the most important factors in broiler production is the litter quality. Litter plays several very important roles such as moisture absorption, thermal insulation and allows bird-scratching (Grimes *et al.*, 2002; Bilgili *et al.*, 2009; Shepherd *et al.*, 2010). It affects general health status, productive parameters, carcass quality, and welfare of broilers (Eichner *et al.*, 2007; Bilgili *et al.*, 2009; Garcês *et al.*, 2013). Animal welfare audits in Europe often use foot, hock and breast burns lesions as an indicator of housing conditions and general welfare of the birds (Haslam *et al.*, 2006). In fact, the footpad condition is an important aspect of poultry welfare that in severe cases can cause pain (Berg, 2004) resulting in unsteady walk (Hester *et al.*, 1997) and reduced weight gain due to pain-induced decreases in feed intake (Martland, 1984; Martland, 1985).

A wide range of materials could be used as broiler litter: rice hulls, ground corncobs, stump chips, pine sawdust, white wood shavings, bark and chips, pine bark, clay sand, coconut husk, Guinea grass, newspaper, corn cob, wheat straw, ground rapeseed straw and silage maize (Grimes *et al.*, 2002; Sirri *et al.*, 2007; Meluzzi *et al.*, 2008; Garcês *et al.*, 2013). Each of these materials has advantages and disadvantages, but different particle size of these materials was seen to be one of the most important factors and has been examined as a contributing factor in the development of footpad dermatitis (FPD) (Shepherd *et al.*, 2010).



Various litter amendments such as chemicals - aluminum sulfate (Madrid *et al.*, 2012), sodium bisulfate (Nagaraj *et al.*, 2007; Li *et al.*, 2012), zeolite (Li *et al.*, 2008), microbiological preparation (Iwaczuk-Czernik *et al.*, 2007), or a commercial ammonia binding agent (Lazarevic *et al.*, 2014) are used to reduce litter moisture, pH and NH₃ emission in broiler houses. Some authors documented that such litter treatments might have positive effects on the occurrence of FPD (Nagaraj *et al.*, 2007). Since the FPD is recognized as a welfare problem of broilers, a scoring method, that is fully validated histologically, is required in order to accurately define lesions and to establish a clear link between the macroscopic appearance of the lesion and its putative impact on chicken welfare (Michel *et al.*, 2012).

The purpose of this study was to evaluate the influence of litter on the occurrence and severity of FPD in broiler chickens by valid histological methods of scoring and to examine the influence of bedding material on behavior and locomotor activity of broiler chicks.

MATERIAL AND METHODS

Animals and experimental design

The experiment was conducted at the Experimental farm of Faculty of Agriculture in Novi Sad. A total of 1,200 as-hatched one day-old Ross 308 broilers were randomly allocated to 4 treatments with 4 replicates in 2x2 factorial design. Each replicate consisted of a floor pen with 75 birds with 2.5 kg of straw per floor 1 m². The first factor was the form of wheat straw used for the bedding – chopped (C) or un-chopped (U). The straw chopping was performed mechanically, and the length of the cut was about 20 mm. The second factor was the litter treatment with enzymatic-bacterial product (Micropan®Simplex, Eurovix USA) in powder form which was applied on both chopped (CMP) and un-chopped straw (UMP). Micropan product (MP) contains active microorganisms and enzymes for the deodorization and sanification of poultry bedding and manure. Micropan was applied to the surface of the litter at the rate of 1 kg /100 m². It was applied three times during the production cycle – at the days 1, 15 and 35.

Broilers were reared to 42 days of age on a 3-phase commercial feeding program consisting of starter (1-21 days), grower (22-35 d) and finisher (36-42 d) diets. The composition of the basal diet is shown in Table 1.

Table 1 – Composition of experimental diets

	Starter (0-21 d)	Grower (22-35 d)	Finisher (36-42 d)
Diet composition (g/kg)			
Corn	488.3	526.1	578.4
Full fat soybean	169.9	228.0	220.3
Soybean meal (44% CP)	199.3	122.5	5.97
Wheat feed flour	90.0	49.9	40.7
Sunflower meal (33% CP)	0.00	30.0	60.0
Monocalcium phosphate	11.8	10.3	9.40
Limestone	15.9	12.6	12.0
Salt	2.1	2.7	2.7
Sodium bicarbonate	1.8	8.0	7.0
L-Treonine	1.2	0.20	-
L-Lysine HCl	3.7	2.0	1.8
DL-methionine	4.0	2.9	2.3
Captex	2.0	2.0	2.0
Vitamin and mineral premix	10	10	10
Calculated nutrient composition*			
AME (MJ/kg)	12.65	13.20	13.40
Crude protein (g/kg)	220.0	210.0	190.0
Lysine (g/kg)	14.3	12.4	10.9
Methionine (g/kg)	7.2	6.1	5.7
Ca (g/kg)	10.5	9.0	8.5
p total (g/kg)	8.2	7.5	7.1
p available (g/kg)	5.0	4.5	4.2

CP-crude protein; Captex – mycotoxin binder, AME – apparent metabolizable energy

* According nutrition requirements for ROSS 308 (<http://en.aviagen.com/>)

Feed and water were available *ad libitum*. Birds were vaccinated against Newcastle disease (NCD) and infectious bursal disease (IBD) as required.

All procedures were conducted according to ethical norms proposed by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, confirmed by Serbian authorities.

Evaluation of footpad lesions

The incidence and severity of FPD were measured at 42 days of age using the scoring method described by Eichner (2007): no lesions (score 0), lesions cover less than 25% of the footpad (score 1), lesions in wide areas, covering between 25% and 50% of the footpad (score 2), more than 50% lesion on the footpads (score 3) (Figure 1). The scoring was done by two independent observers on all birds from the trial. The evaluation was performed on both feet. The average score was used for statistical analyses.

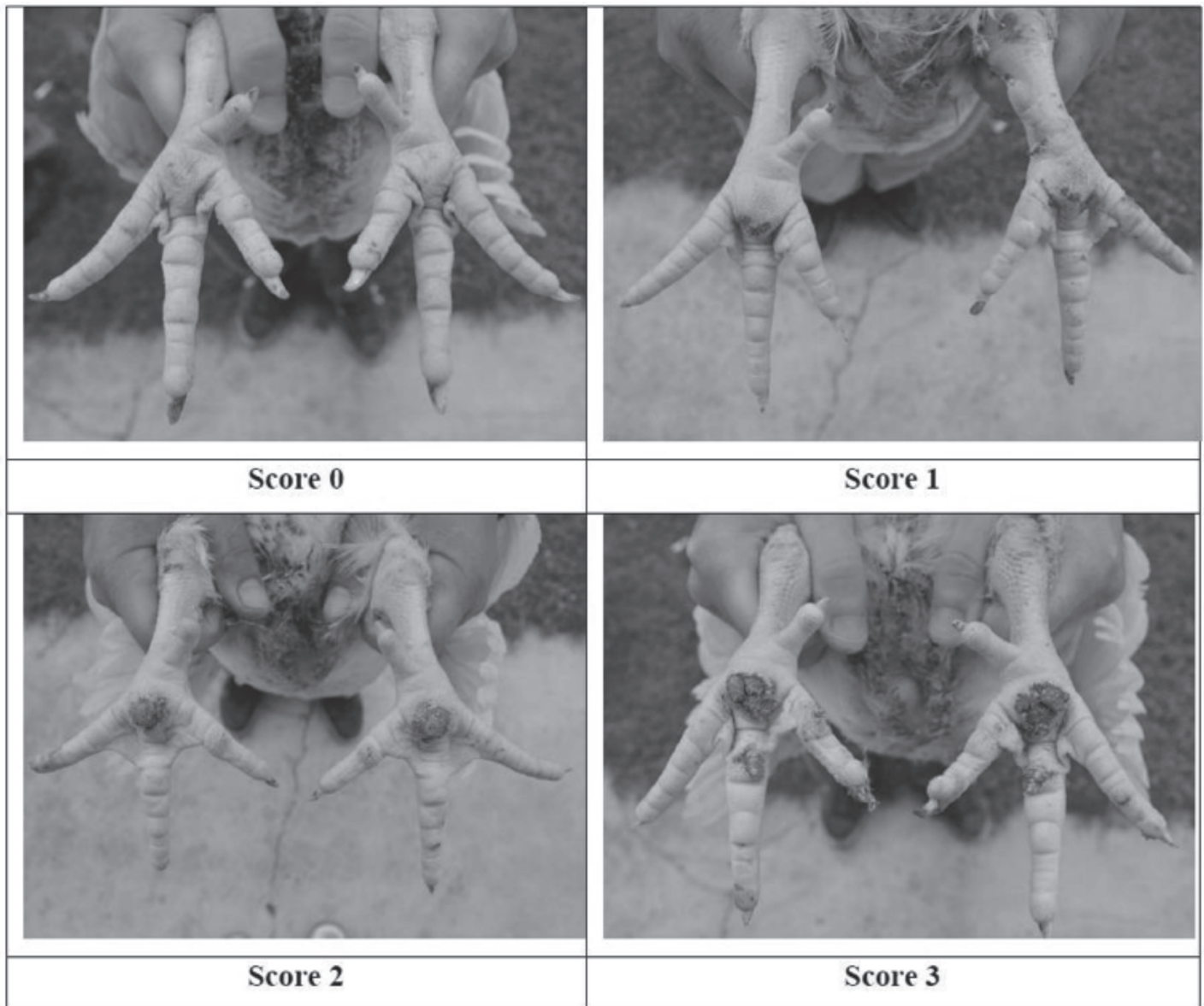


Figure 1 – Macroscopic evaluation of the footpad lesions

Histological procedures

The chicken's feet were sampled in the slaughterhouse. Random samples were taken from the birds representing each score from 0 to 3. Five birds per each score were randomly sampled and 5 feet were taken for analyses. Feet were placed in 10% buffered formalin. The histological analyses were performed at the Faculty of Agriculture in Novi Sad. Each foot was cut from the epidermis of the footpad cushion and embedded in paraffin wax. Sections (5µm thick) were obtained for each sample and stained with haematoxylin and eosin. Histology parameters were determined using light microscope and pictures were taken with digital camera Leica DF-350.

Behavioural observations

At 3, and 6 weeks of age, a scan sampling method at the pen floor level was used for behavioral data. In scan sampling method the behavior of all the individuals in a group of animals are recorded at predetermined time intervals (Martin, 2007). Observations were made in the period from 10 AM to 2 PM over 1 day of each week by eight observers. Each pen floor was scanned every 20 minutes.

The following behavioral parameters were measured: resting (birds resting and not performing any other activities), walking (moving), feeding (at the feeder with head into the lip of the feeder), drinking (standing with head directly under the drinker line), dust bathing (bird forced the litter into the plumage by squatting on the



ground and making appropriate movements with the body, wings and legs and scratching (bird moved the litter backwards with its feet). The frequency of each behavior in broilers was expressed as the percentage rate of daily collected data.

Statistical analyses

Data were analyzed by factorial ANOVA using the GLM procedure. Means were separated by Dunnett's

post hoc test using StatSoft computer package (STATISTICA 11, 2012).

RESULTS

The footpad evaluation was performed at the end of the trial (42 days) and the incidence and severity of footpad lesions are presented in Table 2.

Table 2 – Effect of the litter treatment on the incidence of foot pad dermatitis (FPD) in broilers at 6 weeks of age

Score	Treatments								p value of the main effects		
	Un-chopped (U)		Un-chopped with MP(UMP)		Chopped (C)		Chopped with MP (CMP)		Size of the straw	Treatment with MP	Interaction
	No.	%	No.	%	No.	%	No.	%			
0	5	2.69	3	1.48	3	1.50	26	14.44	0.000	0.000	0.530
1	57	30.65	86	42.36	65	32.50	77	42.78			
2	57	30.65	70	34.48	66	33.00	47	26.11			
3	67	36.02	44	21.67	66	33.00	30	16.67			
Total	186	100.00	203	100.00	200	100.00	180	100.00			
Average	1.94 ^c		1.76 ^b		1.71 ^b		1.47 ^a				

^{a-c}Means within the same row with different superscript differs significantly (p<0.05)

MP – micropan product

Results indicate that the inclusion of MP treatment in both chopped and un-chopped straw severity of food pad lesions decreased and the average score was lower. That means higher incidence of score 1 lesions in MP groups and lower incidence of scores 2 and 3. It is true that phase 1 will probably progress to the scores 2 or 3 in the future, but at the age of 6 weeks birds from MP untreated groups have already developed scores 2 and 3. Upon that we based our statement that MP treatment reduces the severity of FPD in broilers.

Physical form of the straw as well as the application of enzymatic-bacterial product (MP) had a statistically significant effect on incidence of FPD (p<0.01). Reducing the size of the straw on approximately 2 cm showed decreased significantly occurrence of FPD in broilers. The similar effect was established with the addition of MP (p<0.01). When comparing all four treatments the smallest average score (1.47) was found in the group CMP (chopped straw with MP) and the highest average score (1.94) was established in group raised on un-chopped non-treated straw (U).

Analysis of the severity of foot pad lesions showed that the highest number of birds with no lesions (score 0) was found in the group CMP (chopped straw with MP) and the number of birds with the worst score representing the deep epithelial lesions of foot pad (score 3) was in groups without MP treatment of straw (U – 67 birds and C-66 birds).

Results of the histological analyses of footpads with different severity of FPD are illustrated on the Figure 2. Footpad without damage (score 0, Figure 2A) has a normal skin structure with no pathological changes. There is a clear division of keratin layer, epidermis and dermis and all layers are of normal thickness. On Figure 2B (score 1) mild changes of the footpad skin can be noticed. This is the first phase in development of footpad dermatitis. Scales are prominent, enlarged with mild epidermal hyperplasia and hyperkeratosis. Dermal superficial congestion edema and mild perivascular infiltrates were present on some sections. Histological changes of FPD score 2 (Figure 2C) are characterized by moderate to marked hyperplasia and hyperkeratosis of the epidermis, abundant heterophilic epidermal exocytosis with pustule formation and congestion in the superficial dermis with inflammatory infiltration. The most severe FPD is scored by 3 and that means that more than 50% of the foot is covered with lesions. On the histological sections (Figure 2D) ulceration (full-thickness necrosis of the epidermis, replaced by a necrotic and suppurative material) and underlying granulation tissue could be seen.

Obtained results clearly indicate a strong correlation between macroscopic and microscopic evaluation of FPD.

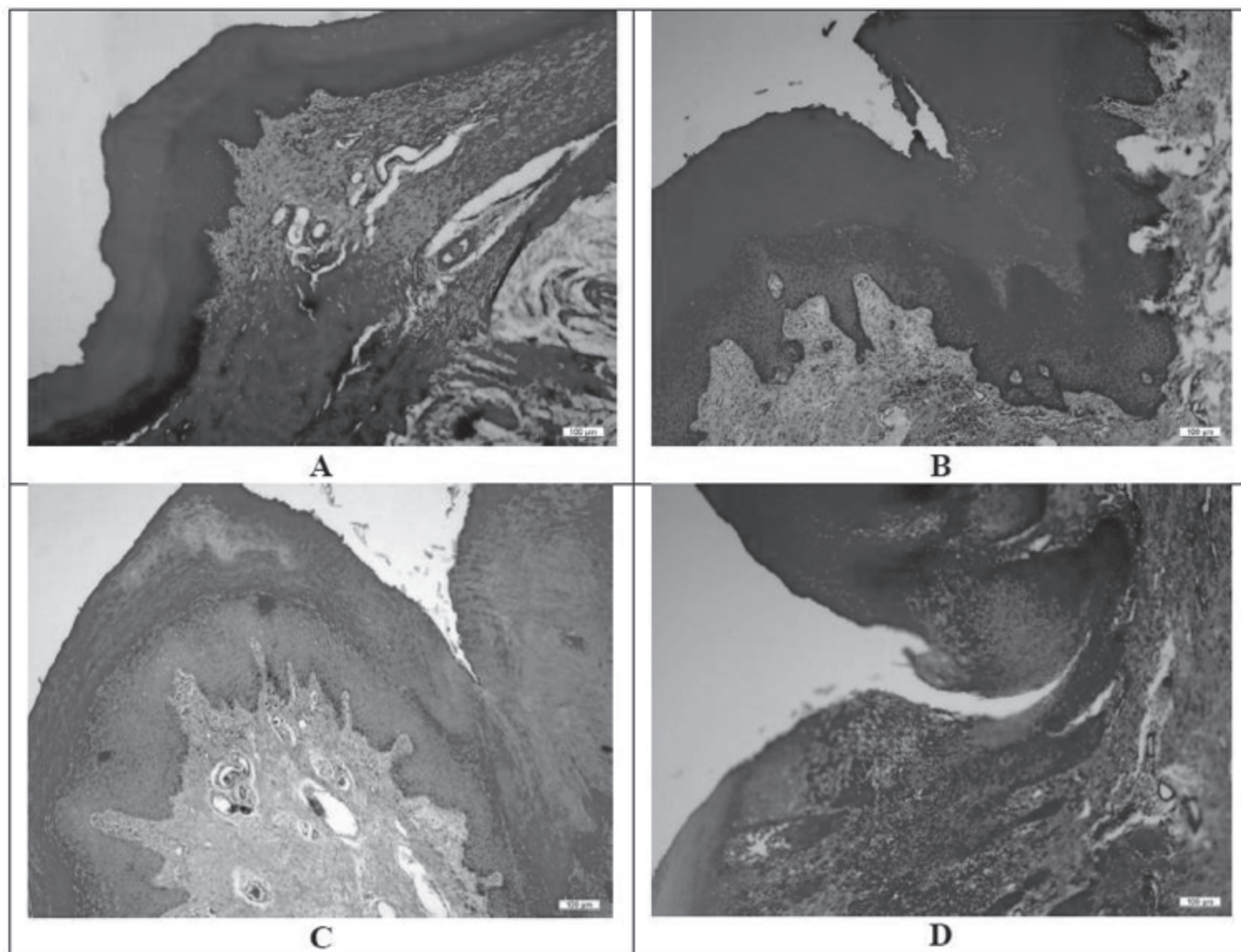


Figure 2 – Histological characteristics of different footpad lesion types A) no lesions, B) lesions -score 1, C) lesions – score 2, D) lesions - score 3 (H&E, UV 100x)

The results presented in Table 3 show that the broilers reared on chopped straw spent significantly

more time in feeding and had significantly higher scratching activity in the 3rd week of age. However, no

Table 3 – Behavior of birds at 3 and 6 weeks of age

Week	Treatment	Resting (%)	Moving (%)	Feed intake (%)	Water intake (%)	Dust bathing (%)	Scratching (%)
3	Chopped (C)	65.64	4.3	3.66 ^b	5	2.05	5.40 ^a
	Un-chopped (U)	59.83	4.09	7.58 ^a	5.75	1.26	1.80 ^b
	Chopped with MP (CMP)	61.47	3.9	5.2	3.34	2.95	3.4
	Un-chopped with MP (UMP)	61.31	4.37	6.62	3.91	2.06	3
p value	Litter	0.923	0.560	0.028*	0.967	0.089	0.001**
	MP	0.887	0.823	0.543	0.089	0.765	0.987
6	Chopped (C)	79.32	3.58	4.01	5.2	3.1 ^a	1.1
	Un-chopped (U)	79.94	3.61	4.65	6.2	0.4 ^b	0.8
	Chopped with MP (CMP)	72.62	3.42	6.22	8.6	1.2	1.3
	Un-chopped with MP (UMP)	75.6	2.35	5.89	7.4	1.6	0.9
p value	Litter	0.856	0.382	0.693	0.658	0.001**	0.487
	MP	0.231	0.830	0.324	0.255	0.589	0.523

^{a,b}Means with different superscript in the same column within the same age differs significantly (p<0.05)

*Statistically significant effect of the factor (p<0.05) ** (p<0.01)

MP – micropan product



difference was found in resting, walking, drinking and dust bathing between birds reared on chopped and un-chopped straw ($p > 0.05$). At the 6th week of age the most remarkable differences were obtained for dust bathing. Only for this parameter, we documented statistically significant difference ($p < 0.05$) between groups of broilers reared on chopped and un-chopped straw. Addition of MP did not lead to significant differences in broiler's behavior reared on chopped and un-chopped straw at both moments of observation.

DISCUSSION

There are many studies which pointed out a clear connection between litter type and incidence of FPD in broilers (Berg, 2004; Bilgili *et al.*, 2009; Shepherd *et al.*, 2010). In the present study, chickens reared on chopped straw showed lower rates of FPD occurrence compared to broilers raised on un-chopped straw probably because of the better condition of the litter and lower ammonia emission. This assumption is in line with findings that long straw had higher ammonia emission compared to chopped straw (Slobodzian-Ksenicz *et al.*, 2002). There was a strong tendency at long straw litter for caking with eventually close to 100 % of flooring being covered by almost impermeable layer. It was suggested that long straw should be particularly avoided for negative environmental impact in broiler housing (Slobodzian-Ksenicz & Kuczyński, 2002). There are results confirming that particle size of litter material has an important role in the development of FPD (Bilgili *et al.*, 2009; Shepherd *et al.*, 2010). The highest incidence of FPD was found with the coarse particle board treatment (Hester *et al.*, 1997).

The addition of enzymatic-bacterial product (MP) significantly lowered the incidence of FPD. Crust formation at the surface of the litter treated with MP was observed from the 4th to the 6th week of the trial. The formed crust was dry, thus the footpads were cleaner and it was assumed that this was a reason for lower FPD with chickens reared on un-chopped straw. Litter amendments are often used in poultry production to reduce litter pH to control ammonia and as an intervention method in houses with a recurring disease issue such as gangrenous dermatitis (Shepherd *et al.*, 2010). Results of other authors showed that litter treatments with chemical or microbiological products have positive effects on litter condition by lowering pH value (Madrid *et al.*, 2012), reducing ammonia emission (Kim & Patterson, 2003; Iwaczuk-Czernik *et al.*, 2007; Li *et al.*, 2008; Madrid *et al.*, 2012), or litter

moisture (Iwaczuk-Czernik *et al.*, 2007; Lazarevic *et al.*, 2014). Such litter treatments might have a positive (decreasing) effect on FPD occurrence (Pursswell *et al.*, 2013). Alum-treated litter had no detrimental effect on the development of footpad and hock burns (Madrid *et al.*, 2012) while other authors (Nagaraj *et al.*, 2007) stated there was a trend of decreasing incidence and FPD severity when NaHSO_4 was used.

Histological analyses of footpad lesions were performed in order to establish connection between macroscopic and microscopic features of the four grades of footpad lesions. The samples of each lesion type corresponded to a typical macroscopic appearance and shared the same histological features. Together with the increase of the surface of footpad which is affected with lesions, there is an increase of the depth of the affected skin layers. The described microscopic lesions in this study were very close to those described by other authors (Martland, 1984; Greene *et al.*, 1985; Martland, 1985; Michel *et al.*, 2012). The results showed that applied scoring method (scoring from 0-3) could be applied in monitoring of FPD in welfare-monitoring programs. Histological analyses indicated the presence of the severe damage of the deeper layers of the skin which could cause a strong pain in birds with higher FPD score. From that reason the behavior of the broilers was observed at 3 and 6 weeks of age.

In both weeks, for all observed groups, walking was below 5% of the total daily time budget, which coincides with the results of other authors (Bizeray *et al.*, 2000; Weeks *et al.*, 2000; Cornetto & Estevez, 2001; Reiter & Kutritz, 2001; Bokkers, 2004; Djukic, 2006; Jordan *et al.*, 2011). The appearance of the lesions did not affect the decrease in the activity of broilers. Other observed parameters, such as resting, feeding, drinking, dust bathing, and scratching are in accordance with results from other similar studies (Reiter & Kutritz, 2001; Bokkers, 2004; Djukic, 2006; Jordan *et al.*, 2011).

For feeding, dust bathing and scratching, differences between chopped and un-chopped straw were statistically significant. The drinking, feeding, dust bathing and scratching are significantly different between the different materials (sawdust, sand, rice husks and recycled paper) (Toghyani *et al.*, 2010; Villagr a *et al.*, 2014). If given a choice, chickens prefer to spend more time on the loosest litter - sand and dust bathe (Arnould *et al.*, 2004; Toghyani *et al.*, 2010; Villagr a *et al.*, 2014). On the other hand, other researchers showed that broilers behavior did not differ between chickens reared on different types of



litter (El-Laithy, 2003; Karousa *et al.*, 2012). The choice of material for the litter can also be an effective way to increase activity of broiler chickens (Shields *et al.*, 2005). Chickens reared on chopped straw, without the addition of MP showed significantly more dust bathe and scratch. The reason was loose litter. Addition of MP led to the formation of crust on the surface of the litter. Therefore, surface was not friable and chickens showed less scratching and dust bathing.

CONCLUSIONS

The present study has produced evidence that the size of the straw has a significant effect on occurrence of FPD. The shorter fibers of chopped straw significantly lowered the incidence of FPD. Chopped straw in combination with MP showed the lowest score of FPD in broilers. Histological procedures validated and confirmed the macroscopic evaluation of the FPD severity. A strong positive correlation was found between macroscopic and microscopic evaluation of FPD. The chopped straw had a significant effect on some behavior patterns of broiler chickens. Differences were found for dust bathing and scratching. Correlation between footpad dermatitis and locomotor activity of the birds was not confirmed. Chopped straw is more suitable material for a litter compared to un-chopped, because it facilitates birds to express a more comfort behavior and thereby improve their welfare state.

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