

Influence of sepiolite additions to different litter materials on performance and some welfare parameters of broilers and litter characteristics

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ABSTRACT The litter quality is an important factor affecting the performance, welfare and carcass quality of the broilers. Depending on the progress of the fattening duration, some materials may be added to the litter in order to keep the pH, moisture and ammonia levels in the litter under control. Sepiolite is a natural material and it has strong absorbing ability to the water. Therefore, the aim of this study was to determine the effects of sepiolite additions to different litter materials on performance and some welfare parameters of broilers and litter characteristics. A total of 288 1-d-old male broiler chicks (ROSS-308) were used. Birds were randomly allocated to 2 litters (wood shavings and rice

hull) and 3 sepiolite groups (0, 25, 50%) with 6 replication, and each pen contains 8 chicks. Fattening performance, IgG, tonic immobility period, feather score, skin injures, breast burns, and footpad burns of broilers were not affected significantly by sepiolite additions to the litter. However, sepiolite addition to the litter can improve litter quality. There was no significant difference between 2 litter materials because both have same color. There were no significant interactions in examined parameters. In conclusion, addition of sepiolite at 25 and 50% levels to litter materials may be used as a litter material in the broiler production without adverse welfare and performance problem.

Key words: broiler, litter, sepiolite, performance, welfare

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INTRODUCTION

Litter is an important factor of broiler production because broilers spend their lifetime in contact with it (Onbaşlar et al., 2014a). Different types of litter materials such as wood shavings, rice hull, pine shavings, and corncob are used in the poultry houses. Litter conditions influence broilers performance and welfare, which in turn affect profit of producers (Sahoo et al., 2017). Litter moisture content ranges from 15 to 57% depending on litter type, excreta, water spillage from the drinker, and ventilation in the broiler house and also production season (Groot Koerkamp, 1994; Hayes et al., 2000; Miles et al., 2011; Van der Hoeven-Hangoor, 2014). High litter moisture increases the ammonia level in the litter. High level of ammonia causes respiratory disease, inefficient feed conversion, and decreased body weight (Anderson et al., 1964; Charles and Payne, 1966; Miles et al., 2004, 2006). And also it causes some hock, footpad and breast dermatitis. This leads to reduced carcass quality of the broilers and increased number of

downgraded carcasses at the slaughterhouse. In addition, broilers suffer from pain due to the burns, and this may be less eager to go to the feeders than broilers have less body weight compared with broilers with no burns (Martland, 1984,1985; De Jong et al., 2014). Some natural and chemical materials such as zeolite, *Yucca schidigera*, sepiolite, vermiculite, aluminum chloride, aluminum sulfate and sodium bisulphate etc may be added to the litter in the poultry house for moisture, ammonia, and pH control (Çabuk et al., 2004; Eleroğlu and Yalçın, 2004; Do et al., 2005; Nagaraj et al., 2007; Choi and Moore, 2008; Okanovic et al., 2014; Onbaşlar et al., 2014a,b; Yıldız et al., 2014).

Sepiolite is a clay mineral having a wide range of industrial applications derived mainly from its sorptive, rheological, and catalytic properties, which are based on the fabric, surface area, porosity, crystal morphology, structure, and composition of these minerals (Galan, 1996). Sepiolite has high porosity and surface area, strong absorptive power and high structural stability (Eser et al., 2012; Yalçın et al., 2016, 2017). It is a feed additive (E-562) used as a binder and anti-caking agent up to 2% in all feeds for all animal species (EFSA, 2013) and it is nontoxic (Galan, 1996). Sepiolite in the broiler diets increased body weight gain and reduced the relative weight of abdominal fat and the levels of

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Table 1. The chemical composition of sepiolite (%).

SiO ₂	Al ₂ O ₃	MgO	CaO	Fe ₂ O ₃	Na ₂ O	K ₂ O	Mn ₂ O ₃	TiO ₂
41.3	1.09	24.44	11.39	0.48	0.23	0.30	0.01	0.08

Sepiolite (Exal T, Tolsa Turkey—Polath—Turkey) consist of 74% sepiolite, 18% dolomite, and 8% dolomite.

serum cholesterol and triglyceride (Quhida et al. 2000; Ayed et al. 2011; Eser et al., 2012; Yalçın et al., 2017). Sepiolite is a reactive mineral exhibiting strong absorbing ability to the water (Zhang et al., 2005). For this reason, sepiolite can be used to control the moisture in the broiler litter. However, there is no study about this directional effect. Therefore, the objective of this trial was to investigate the effects of sepiolite additions to different litter materials on performance and some welfare parameters of broilers and litter characteristics.

MATERIALS AND METHODS

Experimental Design and Animals

This study was approved by Ankara University Animal Care and Use Committee (2015-14-156). A total of 288, 1-d-old male broiler chicks (Ross-308) were obtained from a commercial hatchery. The trial was designed with 2 litter (wood shavings and rice hull) and 3 sepiolite (0, 25 and 50%) groups each containing 6 replicate pens of 8 chicks. Half of the pens contained wood shavings and the other half of the pens contained rice hulls as a litter material. Each litter groups were divided into 3 sepiolite groups; control (only 4.32 kg litter per pen), 75% litter with 25% sepiolite mixture (3.24 kg litter and 1.08 kg sepiolite per pen) and 50% litter with 50% sepiolite mixture (2.16 kg litter and 2.16 kg sepiolite per pen). Sepiolite (Tolsa, Polatlı, Turkey) was used and the composition of sepiolite is shown in Table 1. Chicks of each replicate group were placed in separate floor pens measured as 80 × 90 cm, width × length, respectively. During the first week, each pen was equipped with 1 chick drinker and 1 chick feeder and the other weeks each pen was equipped with 2 nipples and 1 feeder. Feed and water were provided ad libitum. Broilers were fed with a commercial starter diet from 1 to 21 d of age and commercial grower diet from 22 to 42 d of age (Table 2). The trial was carried out during 42 d in a naturally ventilated broiler house in the sum-

mer season. The lighting period was continuous lighting during the whole experiment. Average room temperature was 34 ± 2°C on the first week and then is gradually lowered to average 24 ± 2°C, and this temperature was maintained up to slaughter age.

Performance and Some Welfare Parameters

Body weight gain, feed intake were measured weekly by pen, and feed efficiency was calculated as the feed to gain ratio weekly. At 35 and 42 day of age, all broilers were assessed for feathering score, skin injuries, foot and breast burns. Birds were scored for the feather condition and skin injuries of 5 individual parts of the body, i.e., neck, breast, back, wings, and tail. A score (graduated from 1 = very poor feather to 4 = intact plumage) was assigned for feather condition for each area of the body parts. Feather conditions of all parts were added and calculated as feather condition of broiler (5–20). Skin injuries were scored from 1 to 4 points, where 1 = large defects on skin and 4 = no defects (Tauson, 1984). A 3-point visual ranking system was used for the footpad dermatitis scoring system, where a score of 1 indicated footpads with no lesions present and intact dermal ridges within central plantar footpad surface; a score of 2 indicated footpads with mild lesions, and dermal ridges with oval or round ulcers covered with a crust (<7.5 mm); and a score of 3 indicated footpads with severe lesions, with a dark brown crust (>7.5 mm) adhering to the central plantar footpad (Bilgili et al., 2009). A 2-point visual ranking system was used for the breast burns scoring system, where a score of 1 indicated with no lesions present and a score of 2 indicated with lesions (Allain et al., 2009).

Tonic Immobility and Immunity

At day 40, 2 broilers for each pen were selected according to the group body weight and were assessed for tonic immobility (TI) duration (Jones and Faure, 1980). Each broiler was carefully restrained for 15 s by covering the head with 1 hand while placing the other hand on the sternum. Latency to self-righting was used as the measure of TI. If this had not happened after 10 min, this session was terminated and the individual was assigned a value of 600 s. If the broiler terminated the state of immobility before 10 s the trial was repeated.

At day 42, 1 broiler for each pen was selected according to the group body weight and bled from the brachial vein. Blood samples were centrifuged at 1500 rpm for

Table 2. Composition of the diets.

Ingredients	Starter diet (1 to 21 d)	Grower diet (22 to 42 d)
Metabolizable energy (kcal/kg)	3025	3200
Crude protein (%)	23.0	20.2
Ether extract (g/kg)	62.0	85.0
Crude fiber (g/kg)	37.0	36.0
Crude ash (g/kg)	53.0	48.0
Calcium (g/kg)	9.98	9.92
Total phosphorus (g/kg)	6.83	6.38

Table 3. Effect of sepiolite additions to the different litter materials on body weight gain, feed intake, and feed conversion ratio of broilers.

Litter material	Level of sepiolite (%)	Initial weight (g)	Body weight gain (g)				Feed intake (g)				Feed conversion ratio (g/g)			
			Days 1 to 21		Days 22 to 42		Days 1 to 21		Days 22 to 42		Days 1 to 21		Days 22 to 42	
			Mean	NS	Mean	NS	Mean	NS	Mean	NS	Mean	NS	Mean	NS
Wood shavings		42.1	599	1391	1990	811	2503	3314	1.36	1.80	1.67			
Rice hull		42.3	596	1398	1995	814	2508	3322	1.37	1.80	1.67			
	0	42.4	599	1389	1988	818	2492	3310	1.37	1.80	1.67			
	25	42.1	599	1389	1987	810	2519	3328	1.35	1.82	1.68			
	50	42.1	596	1405	2001	811	2506	3317	1.36	1.79	1.66			
SEM		0.102	3.037	9.858	10.239	3.995	8.787	8.986	0.007	0.012	0.008			
Litter		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS			
Level of Sepiolite		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS			
Litter X Level of Sepiolite		0.619	NS	NS	NS	NS	NS	NS	NS	NS	NS			

NS: Non-significant.

5 min. After separating the serum, IgG levels were determined with ELISA (Gao et al., 2008).

Litter Analysis

A litter sample was collected from pens to determine pH, moisture, and ammonia analyses at days 28, 35 and 42 of ages. Litter samples were collected from 5 sites (4 corner and 1 central samples), then mixed and 100 g sub sample from mixed sample was taken in a plastic bag. To determine litter pH, 20 g of litter sample was mixed with 30 mL of sterile distilled water. Then pH was measured (Pope and Cherry, 2000) with a pH meter (Selecta, pH-2004, Barcelona, Spain). Litter samples (2 g) from each pen were dried in an oven at 105°C for 8 h to determine moisture content of samples (AOAC, 2000). Litter ammonia-N of each pen was determined using a spectrophotometer (Chaney and Marbach, 1962).

Statistical Analysis

Statistical analysis was conducted using SPSS version 23.0 for Windows software (SPSS Inc., Chicago, IL). Data were tested for normal distribution and homogeneity of variance. Data set showed normality, and a 2-way ANOVA was used to determine the differences between litter and sepiolite groups as well as their interactions with respect to the initial weight, body weight gain, feed intake, feed efficiency, IgG, and TI duration. Three-way ANOVA was used for litter characteristics, feather score, and skin injures. Comparisons among means were made by Tukey test. Breast and footpad burn values were expressed as the number of observations and frequencies. Then, differences in frequencies of breast and footpad burn were assessed by chi-square or Fisher exact tests where appropriate (Dawson and Trapp, 2000). A value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Many materials are linked to specific regions and harvesting seasons, and the choice of material for a particular location ultimately depends on its availability and economic advantage, which have been used as a broiler litter (Garcês et al., 2017). The broilers' performance during the rearing periods was shown in Table 3. No significant differences were observed in the performance parameters of broilers reared on the examined litter materials. Several studies have reported that litter type would affect bird performance and welfare (Huang et al., 2009; Youssef et al., 2010; El-Deek et al., 2011; Onbaşilar et al., 2014a,b). However, other studies reported that litter type had no effect on performance and welfare (Monira et al., 2003; Hafeez et al., 2009). Onbaşilar et al. (2014b) indicated that color of litter materials were an important factor for performance parameters in certain periods. In our study, there may be

Table 4. Effect of sepiolite additions to the different litter materials on litter characteristics.

Fattening duration (day)	Litter material	Level of sepiolite (%)	pH	Moisture (%)	Ammonia (g/kg)
28			7.9 ^a	17.4 ^a	2.70 ^a
35			7.9 ^a	19.6 ^b	3.09 ^b
42			8.4 ^b	22.0 ^c	3.40 ^c
	Wood shavings		8.1	19.8	3.03
	Rice hull		8.0	19.6	3.10
		0	8.2 ^y	21.5 ^y	3.32 ^y
		25	8.1 ^{x,y}	19.1 ^x	3.00 ^x
		50	7.9 ^x	18.5 ^x	2.88 ^x
SEM			0.034	NS	0.046
Fattening duration			0.000	0.000	0.000
Litter			0.018	NS	NS
Level of sepiolite			0.014	0.000	0.001
Fattening duration X Litter			NS	NS	NS
Fattening duration X Level of sepiolite			NS	0.030	NS
Litter X Level of sepiolite			NS	NS	NS
Fattening duration X Litter X Level of Sepiolite			NS	NS	NS

^{a,b,c} and ^{x,y} means within columns with different letters are significantly different ($P < 0.05$). NS: Non-significant.

a similarity in the colors of the litter materials used as a reason for not having a difference in performance values in our study. Total body weight gain, feed intake, and feed conversion ratio values of broilers reared on the wood shavings and rice hull were 1,990 and 1,995 g, 3,314 and 3,322 g, 1.67 and 1.67, respectively. There were not any negative effects of sepiolite addition to the litter on performance parameters. There was no significant interaction between litter materials and level of sepiolite for body weight gain, feed intake, and feed efficiency.

Litter quality is an important management factor during the production. The quality of the litter is determined by the moisture, pH, and ammonia content, level of caking and water holding capacity of the litter (Meluzzi et al., 2008; Bilgili et al., 2009; Shepherd and Fairchild, 2010; Xavier et al., 2010). High litter moisture causes to change the ammonia and pH levels in a short time. Litter pH plays a key role in NH_3 evaporation in that NH_3 generation tends to increase with pH. Caked litter increases the ammonia level and this negatively affecting broiler's health, welfare, growth performance, and carcass quality (Reece et al. 1981; Kristensen and Wathes 2000; Miles et al. 2004). In our study, at the end of the fourth week, the pH, moisture, and ammonia levels of litter were 7.9, 17.4%, 2.70 g/kg, respectively. At the end of the sixth week, the pH, moisture, and ammonia levels of litter were significantly ($P < 0.001$) increased to 8.4, 22.0%, 3.40 g/kg, respectively. Researchers reported that the pH of the litter used in the production is between 8 and 10, and the pH in the dry litter is about 7 (Lavergne et al., 2006; Gholap et al., 2012). The pH level of wood shavings was higher than that of rice hulls ($P < 0.05$). However, Onbaşlar et al. (2014a,b) reported that pH levels of wood shavings and rice hulls were not different. This result also indicated that the type of rice hull used is important for litter characteristics. However, moisture and ammonia levels in the different litter materials were not different. The ideal moisture content in the litter should be 15 and

Table 5. Effect of sepiolite additions to the different litter materials on Ig-G level and tonic immobility duration of broilers.

Litter material	Level of sepiolite (%)	Ig-G (mg/dL)	TI duration (s)
Wood shavings		6.1	308
Rice hull		6.8	243
	0	6.5	211
	25	6.4	323
	50	6.4	292
SEM		0.233	26.037
Litter		NS	NS
Level of sepiolite		NS	NS
Litter X Level of sepiolite		NS	NS

NS: Non-significant.

30% in the summer and in the winter seasons, respectively (Gençoğlan and Gençoğlan, 2017). Hafeez et al. (2009) reported that the moisture content of sawdust during the trial increased from 13.07 to 46.55%. Sepiolite addition to the litter affected the examined litter characteristics ($P < 0.05$). Increase in the sepiolite ratio from 0 to 50% in the litter reduced the pH, moisture, and ammonia levels of the litter (Table 4). Only fattening duration and sepiolite interaction for moisture level was found statistically significant ($P < 0.05$). As the fattening duration increased, the ability of sepiolite to suppress the moisture increased.

There are many kinds of stress factors (environment, diseases, psychological factors, and feeding management) in broiler breeding, which can directly or indirectly change the immune system (Mashaly et al. 2004; Shini and Kaiser 2009; Wilkinson et al. 2011; Li et al., 2017). The type of litter has a significant effect on immune system efficiency of broiler chickens (Taherparvar et al., 2016). However, litter type or sepiolite addition to the litter in our study was not affected by the IgG level of broilers (Table 5).

TI is an index to assess fearfulness and stress and an innate response of animals (Liu et al., 2016). Broilers show a TI response when they are facing danger

Table 6. Effect of sepiolite additions to the different litter materials on feather score and skin injures of broilers.

Fattening duration (day)	Litter material	Level of sepiolite (%)	Feather score	Skin injures
35			16.7	19.97
42			16.0	19.95
	Wood shavings		16.3	19.95
	Rice hull		16.5	19.97
		0	16.4	19.98
		25	16.3	19.95
		50	16.5	19.95
SEM			0.064	0.012
Fattening period			0.000	NS
Litter			NS	NS
Level of sepiolite			NS	NS
Fattening period X Litter			0.012	NS
Fattening period X Level of sepiolite			NS	NS
Litter X Level of sepiolite			NS	NS
Fattening period X Litter X Level of sepiolit			NS	NS

NS: Non-significant. Birds were scored for the feather condition and skin injures of 5 individual parts of the body, i.e., neck, breast, back, wings, and tail. A score (graduated from 1 = very poor feather to 4 = intact plumage) was assigned for feather condition for each area of the body parts. Feather conditions of all parts were added and calculated as feather condition of broiler (5–20). Skin injuries were scored from 1 to 4 points, where 1 = large defects on skin and 4 = no defects (Tauson, 1984).

or fear (Duan et al., 2014). Broilers stay in a state of nervousness and fear when in danger and they exhibit long TI duration than the others (Hazard et al., 2008).

In our study, there were no significant differences in the litter and sepiolite treatments for the TI duration of broilers. The improvement in the litter characteristics due to the sepiolite did not affect the duration of TI in broilers. TI duration was 308 and 243 s for broilers reared on the wood shavings and rice hull, respectively, and it was 211, 323, and 292 s in the broilers reared on the 0, 25, and 50% sepiolite addition to the litters, respectively. The feather scores were similar in litter and sepiolite groups (Table 6).

Over time, the feathering score decreased due to the deterioration of the litter ($P < 0.001$). Only fattening period and litter interaction was found statistically significant ($P < 0.05$). The feather score of broilers reared on the wood shavings litter decreased faster from 35 to 42 d of age than that on the rice hull litter. Different litter usage and sepiolite addition to the litter did not affect skin injures of broilers. Footpad and breast burns are an audit criterion in welfare assessments of broiler production systems (Haslam et al., 2007). These lesions are a direct source of pain and reflect many aspects of rearing conditions, being considered valid welfare indicators (Haslam et al., 2007; Meluzzi et al., 2008). Litter improvement is a critical step we should take to control breast and footpad burns. In this study, breast and footpad burns were not affected by treatment. The difference in litter quality due to the treatment did not affect the incidence of burn and footpad lesions. Breast burn incidences at 35 d of age 0 and 0.8% and at 42 d of age 0.9 and 0.9% of broiler breed on the wood shavings and rice hull, respectively. 1.2 and 2.7% of the broilers reared on the no sepiolite addition to the litter had

mild breast lesion at 35 and 42 d of age, respectively. However, broilers reared on the sepiolite addition to the litter had no lesion both of the ages. Footpad burn incidences at 35 d of age 3.1 and 3.0% and at 42 d of age 6.2 and 5.2% of broiler breed on the wood shavings and rice hull, respectively. At 35 d of age, 4.7, 2.2, and 2.3% and at 42 d of age, 9.5, 3.8, and 4.1% of the broilers had mild footpad lesions in groups reared with 0, 25, and 50% additions of sepiolite, respectively.

In conclusion, this study provides the knowledge about the applicability of sepiolite addition to the different commonly used litter materials in the summer season. And it shows that there are no negative effects of sepiolite on the broilers. Differences among examined groups in the fattening performance, some welfare and immunity parameters such as IgG, TI duration, feather score, skin injures, breast burns and footpad burns of broilers were not found statistically significant. However, sepiolite addition to the litters improved the litter quality compared to untreated litter materials. This may be due to the experiment being carried out in the summer. No significant difference was observed in the use of different litter materials due to the same color. But, not only the type and size of the litter, but also the working of the litter color will be important in interpreting the differences. Litter material and sepiolite interactions were not found statistically significant in the examined parameters. It can be said that the addition of sepiolite at 25% and 50% levels in the broiler litter may use to improve the litter quality. In large-scale poultry houses, the disruption in control of environmental factors such as ventilation can affect the litter material and therefore the effect of the litter material on the broiler performance in different ways. For this reason, field studies will be done in the different season and different dose of sepiolite in the litters.

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