Kafkas Univ Vet Fak Derg
20 (1): 129-134, 2014
DOI: 10.9775/kvfd.2013.9639

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

The Effect of Vermiculite as Litter Material on Some Health and Stress Parameters in Broilers [1]

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[1] *This study was supported by Atatürk University, Scientific Research Foundation (Project Number:2011/288)*

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Makale Kodu (Article Code): KVFD-2013-9639

Summary

This study was conducted to determinine the effect of vermiculite as the litter material on some health and stress parameters such as alanin aminotransferaz (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), glucose (GLU), cholesterol (CHOL), superoxide dismutases (SOD), malondialdehyde (MDA), catalase (CAT), footpad burn (FPB), gait score (GS), tonic immobility duration (TI) and feathering score (FS) in broilers. In present study, 195 day-old chickens were divided into three treatment groups placed on litter materials such as woodshavings (WS), mixture of woodshavings-vermiculite (WSV) and vermiculite (V), respectively. The experiment lasted for 42 days. At the end of the experiment, it was ascertained that the litter material did not have significant effect on ALT, AST, TP, ALB, GLU, CHOL, CAT and feathering score, but had significant effect on FPB, GS, TI, SOD and MDA values ($P<0.01$). FPB and GS were lower in the pens littered with V and WSV than in the pen littered with WS. The foot health was positively affectef by the use of V and WSV as litter material. TI of WS group was longer than those of V and WSV groups. SOD, MDA values from oxidative stress parameters were found lower ($P<0.01$) in the V and WSV groups than that of WS group. Consequently, vermiculite may be used as litter material in the rearing of broiler without adverse health problem.

Keywords: *Broiler, Litter material, Vermiculite, Health, Stress*

Vermikülitin Broilerlerde Altlık Olarak Kullanımının Bazı Sağlık ve Stres Parametreleri Üzerine Etkisi

Özet

Bu çalışmada, farklı altlık materyallerinin ALT, AST; TP, ALB, GLU, CHOL, SOD, MDA, CAT, ayak taban yanığı, yürüme skoru, tonik immobilite ve tüylenme skoru gibi bazı sağlık ve stres parametreleri üzerine etkisini belirlemek amaçlanmıştır. Araştırmada 195 adet günlük civciv kullanıldı. Civcivler üç farklı altlık materyali (talaş, talaş-vermikülit karışımı ve vermikülit) kullanılan bölmelere yerleştirildi. Deneme 42 günlük yaşa kadar sürdürüldü. Denemenin sonunda ALT, AST, TP, ALB, GLU, CHOL, CAT ve tüylenme skoru bakımından altlık materyalleri arasında fark bulunamadı ($P>0.05$), ayak taban yanığı, yürüme skoru, tonik immobilite, SOD ve MDA değerleri bakımından ise altlık grupları arası fark önemlidir ($P<0.01$). V ve WSV gruplarında WS grubuna göre FPB ve GS daha düşüktür. V ve WSV altık olarak kullanımı ayak sağlığının olumlu etkilemiştir. TI süresi WS grubunda V ve WSV gruplarından daha yüksek bulunmuştur. Oksidatif stres parametrelerinden SOD, MDA değerleri V ve WSV gruplarında WS grubundan daha yüksektir. Sonuç olarak, broylerlerde sağlık üzerine zararlı bir etkisi olamadığından, vermikülit atlık olarak kullanılabilir.

Anahtar sözcükler: *Broyler, Altlık, Vermikülit, Sağlık, Stres*

INTRODUCTION

All of the broiler raising is carried out as flocks on floor with litter. In view of emerging demands related to the new animal welfare rules implemented in European Union Member States, some modifications have been made in floor-based growth systems (i.e. free range systems) [1]. Especially broiler production is performed intensive and

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widely in the world, considering the importance of the litter is better understood. Litter material should also have low thermal conductivity, to retain warmth and act as insulation. It should therefore be capable of drying quickly and be soft and compressible, absorb moisture and buoyant [2].

The researchs for alternative poultry litter sources continue because availability of softwood shavings is limited. In many areas, decreased supplies and increased demand for wood shavings has increased the cost of these products and has resulted in a search for supplemental or alternative litter sources [3]. Several alternative litter materials have been tested with favourable results [4].

Wood shavings, commercial litter, rice hulls, sand and vermiculite were used as litter material by Miles et al.[5], they reported that vermiculite was a new litter material with high moisture absorption capacity but, it caused more ammonia production than wood shavings, commercial litter, rice hulls and sand.

Arunlertaree et al.[6] reported that when vermiculite and hydrated sodium calcium aluminosilicate were added to feed, they bind aflatoxin B1 on account of toxin-binding characteristics of the vermiculite.

Vermiculite is a siliat mineral that is obtained from volcanic magma resources. High heat treatment creates an expantion in volume, an increase in permeability and a descrease in weight. The obtained product is very light and sterile. With thermal insulated and fire-resistant features, vermiculite is used as the land regulator in agriculture. The chemical composition of vermiculite is: SiO_2 38-46%, AL_2O_3 10-17%, MgO 16-35%, CaO 1-5%, K_2O 1-6%, Fe_2O_3 6-13% TiO_2 1-3% and H_2O 8-16%. Material has a relatively high water-holding capacity (200-325% of weight and 20-50% by volume) and thermal conductivity (0.065-0.062 watt) and it has a gold colored, accordion shaped physical appearance. Although it has the same function as perlite, vermiculite has better thermal properties and lesser dust ratio than perlite [7].

Either consumer or broiler health can be negatively affected by unsuitable use of litter material [8]. Also litter materials should be free of other substances as chemicals, disease organisms and moulds that may damage the birds' health [2]. Litter materials compose about 4% of total consumption of broilers during growing period [9]. Litter material must be free of any contaminants, that can be absorbed by edible parts of the chicken.

According to Knowles et al.[10], modern management techniques associated to the genetic characteristics of rapidly-growing broilers have compromised their welfare, as well as their walking ability. A major welfare concern for the poultry industry is leg deformaties and lameness. It is critical to understand that lame birds experience pain. To assess lameness in a flock, use the gait scoring [11]. Foot sores and hock burns are related to leg disorders. Birds with leg problems spend more time sitting and, if the litter is wet and dirty with faeces, this results in burns and sores. Foot and hock burns in turn reduce walking activity because they make walking painful. Litter substrate significantly affected some of the indices of leg weakness measured [12]. Contact dermatitis is a 'relatively widespread' problem which can affect many of the birds in some flocks, and that it is associated with crowding, restricted movement, leg weakness and poor litter quality [13]. The material used as litters should protect birds from the impact and the friction on the poultry house floor, and this is particularly important when footpad lesions are considered, as their incidence is closely related to the quality and quantity of litter material. High litter moisture may lead to cycles of wetting and drying that compact the material and causes burns and footpad dermatitis in broilers. However, the incidence of dermatitis was significantly different ($P<0.05$) among litter materials, possibly due to differences in moisture content [14]. Footpad dermatitis, lesions on the back of the legs and feet, respectively, which may be superficial or progress into deep ulcers may also develop indirectly by deteriorating litter quality [15]. When birds lie in wet litter, ammonia produced by the decomposing organic material may irritate the skin [16].

Duration of TI has been used as a measurement for evaluating fearful behaviour and may be used as a criteria for measuring well-being and levels of stress of chickens [17].

Several factors may influence the feathering of broilers, particularly feed nutritional levels and environmental temperatures. Edens [18] found that chickens reared in cold environments presented higher feathering index, and the authors consider it an important characteristic for the maintenance of thermal homeostasis.

Formation and transformation of metabolic events takes place in liver. Measurement of ALT and AST activities is important to determine hepatic disorders. Serum total protein, albumin, total protein, glucose, cholesterol level is important for liver functions. Oxidative stress may occur as a result of increase of free radical production and decrease of antioxidant defense. Oxidative stress can be determined with SOD, MDA and catalase (CAT) measurements [19].

The objective of this study was to evaluate the effects of vermiculite as a litter material on some health and stress parameters of broiler chickens.

MATERIAL and METHODS

The experiment was carried out at Ataturk University in Research and Application Unit of School of Veterinary Faculty in accordance with approval by Ataturk Universitesi Local Etics Committee for Animal Experiments (Number: 2011/84). One hundred and ninety five (n=195) 1-d-old

(Ross 308) male commercial broiler chicks were used in the experiment. The chicks were weighed and assigned at random to 15 floor pens (13 chicks per pen) at a density of 12 broiler/m^2, in a naturally-ventilated. Chicks were brooded with one suspended electric brooder in each pen during the first 2 weeks. Birds were fed (mash form) with a starter diet from 1 to 21 d of age (24% of crude protein and 3075 kcal/ME/kg) and grower diet from 22 to 42 d of age (20% of crude protein and 3200 kcal/ME/kg). During the experiment feed and water were provided *ad libitum*. Feeder and drinker spaces were identical in each pen, and lighting was continuous (24L:0D).

The experiment consisted of 3 treatments with 5 replicates, and animals were allocated in a complete randomised design into the treatments. The 3 treatments were woodshavings (WS), a mixture of 50% woodshavings - 50% vermiculite (WSV) and vermiculite (V).

All the birds were assessed for tonic immobility (TI) at 40 d of age, and assessed for walking ability or gait score (GS) and feathering scores (FS) at 41 d of age. After slaughter at 42 d of age, carcasses were also assessed for prevalence of footpad burn (FPB). Bird feet were also assessed for prevalence of footpad burn. Feet were gently washed with a wet cloth before scoring. A scale from 1 (no lesions) to 4 (very severe lesions) was used to evaluate degree of burn, inflammation, wounds and scratches. With an aim to determine the gait score**,** each chicken was taken out of its cage and allowed to walk alone along the passageway for observation. Those reluctant to move were gently prodded. Scoring was made from "0" to "5" (GS from 0, for a perfectly normal bird, to 5, for a bird that could not walk at all) as described by Kestin et al.[20]. Chickens were caught avoiding any harm, and were transferred to a silent room, where they were restrained on their back in a cradle like U-shaped apparatus to determine tonic immobility periods as described by Jones and Faure [21]. All the birds were given feathering scores average of back, breast, wing and tail using scores of 1 to 4 for feather coverage with 1 representing maximal uncovered and 4 covered.

The blood that taken from V. subcutenea ulnaris of 10 chicks from each group, transferred to anticoagulant vacuum tubes, centrifuged at 3.000 rpm, +4°C for 10 min and stored at -20°C until the biochemical analysis. Serum SOD and MDA levels were measured according to methods reported by Sun at al.[22], Yoshioka at al.[23], CAT levels was measured according to Goth [24] with Biotek ELISA Reader. ALT, AST, TP, ALB, GLU and CHOL levels in serums were determined with Mindray perfect Plus 400 auto analyzer by ready kits.

The difference among the in terms of parameters related to footpad burn, gait score, tonic immobility and feathering score were determined with Kruskal Wallis variance analysis, the significance of difference was tested with Mann-Whitney U test. Blood biochemistry data were subjected to analysis of variance for a complete randomised design using the General Linear Models procedure of SPSS software [25]. A probability of $P<0.05$ was used for statements of significance using a Duncan multiple range comparison test.

RESULTS

The effect of use of vermiculite, wood shavings and vermiculite-wood shavings mixture as litter material in broilers on footpad burn score, gait score, tonic immobility and feathering score are presented in *Table 1* and *Fig. 1*, and the effect of litter materials on some biochemical parameters are given in *Table 2*.

DISCUSSION

Litter material significantly affected some of the indices of leg weakness measured. Footpad burn and gait scores were lower in the pens littered with V (1.06±0.05, 0.2±0.08) and WSV mix (1.02±0.01, 0.06±0.03) than in the pens littered with WS (1.98±0.12, 1.34±0.15). The use of V and WSV mixtures as litter material effected foot health positively. Although difference between V and VSW groups was not significant, observation of FPB frequency and GS in WS group was increased. So, the broilers in WS group were negatively affected by the kind of litter material.

Table 1. *Footpad burn score, gait score, tonic immobility and feathering scores of broilers reared on different litter materials*
Tablo 1. *Farklı altlık materyallarinde yetiştirilen broylerlerde ayak taban yanığı, yürüme skoru, tonik immobilite ve tüylenme skorları*

Parameters[1]	Litter Treatments[2]						P
	V		WS		WSV		
	X±Sx	Median	X±Sx	Median	X±Sx	Median	
FPB	1.06±0.05^B	1.00	1.98±0.12^A	2.00	1.02±0.01^B	1.00	0.000
GS	0.20±0.08^B	0.00	1.34±0.15^A	1.00	0.06±0.03^B	0.00	0.000
TI	172.25±31.86^B	128.00	310.60±35.29^A	360.00	153.75±29.33^B	118.50	0.005
FS	2.92±0.03	3.00	2.93±0.03	3.00	2.89±0.03	3.00	0.503

*[1] **FPB** = footpad burn score; **GS** = gait score; **TI** = tonic immobility; **FS** = feathering score; [2] **V** = vermiculite; **WS** = woodshavings (control); **WSV** = a mixture of 50% woodshavings - 50% vermiculite, [AB]: Means within rows with different superscripts are significantly different*

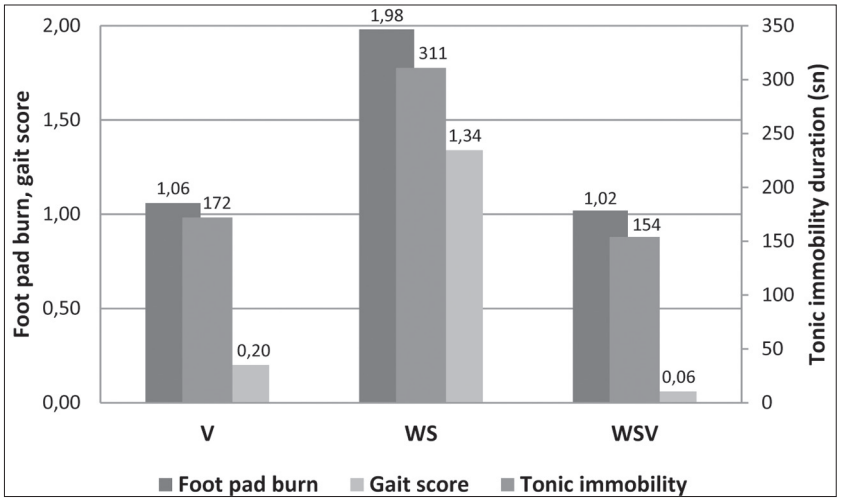

Fig 1. Footpad burn, gait score and tonic immobility duration of broilers reared on different litter materials

Şekil 1. Farklı altlık materyallerinde yetiştirilen broylerlerde ayak taban yanığı, yürüme skoru ve tonik immobilite skorları

Table 2. *Some blood biochemical parameters of broilers reared on different litter materials.*
Tablo 2. *Farklı altlık materyallerinde yetiştirilen broylerlerde bazı biyokimyasal kan parametreleri*

Biochemical Parameters	Litter Treatments			SEM	P
	V	WS	WSV		
ALT (U/l)	6.28	6.10	6.02	1.96	0.636
AST (U/l)	364.20	363.64	361.62	5.90	0.949
TP (g/dl)	3.06	3.05	3.02	0.03	0.747
ALB (g/dl)	1.39	1.42	1.40	0.02	0.797
GLU (mg/dl)	252.20	253.16	252.72	2.94	0.974
CHOL (mg/dl)	131.56	130.62	131.68	1.89	0.911
SOD (U/ml)	4.34^{B}	6.52^{A}	4.21^{B}	0.21	0.000
MDA (mol/l)	4.45^{B}	5.04^{A}	3.97^{B}	0.17	0.001
KATALAZ	54.43	59.89	53.35	2.01	0.065

^{AB} Means within rows with different superscripts are significantly different

Miles et al.[5] reported that V has not only higher water holding capacity than WS, but also it has higher NH_3 production capacity than WS. While the number of broiler with FPB was expected to increase related to ammonia production with use of V as litter, present study pointed out a decrease in the number of broiler with FPB and a positive affect on GS *(Fig. 1)*.

Sorbara et al.[26], comparing citrus pulp with wood shavings as litter material for broilers, did not find any significant difference in the incidence of footpad lesions between treatments. Santos et al.[27], in a study on the incidence of footpad lesions in broilers, concluded that the most probable cause of these lesions was excessive litter moisture. Su et al.[12], noticed that wood shavings is better litter material than chopped straw on walking ability and the incidence of FPB, comparing chopped straw with wood shavings as litter material for broilers.

As to findings obtained from present study, the effect of litter type on the tonic immobility duration was found significant (P<0.05). Tonic immobility of WS group (310.6±35.29) was longer than V (172.25±31.86) and WSV groups (153.75±29.33). WS group chickens were more fearful than the V and WSV group chickens. Campo et al.[28] confirmed that the development of footpad dermatitis had an increasing effect on the fearfulness of cocks kept in cages. Parallel to findings of present study, TI duration was found lower in groups, that has lower FPB levels.

The effect of litter type was not significant (P>0.05) on the feathering score of broilers in this study. Several factors may influence the feathering of broilers, particularly feed nutritional levels and environmental temperatures. Edens [18] found that chickens reared in cold environments presented higher feathering index, and researcher considered it an important characteristic for the maintenance of thermal homeostasis. Differences among the V, WSV and WS groups in terms of feathering index were not significant. That may be caused insignificant difference among the kind of litter materials in terms of termal insulation.

ALT, AST, TP, ALB, GLU, CHOL and CAT were not significant in V, WSV and WS groups (P>0.05). The most common used litter material in the world is wood shavings. Indifference between wood shavings and vermiculite pointed out

that vermiculite may be used as litter material in broilers without adverse health effects. Davis [29] reported that vermiculite did not have not negative effect on health as a result of study in rotends.

While TP and ALB levels in groups raised on wood shavings were compatible with the study findings performed by Yalçınkaya et al.[30], serum AST and CHOL levels were found higher than those of Yalçınkaya et al.[30]. Seven et al.[31] found lower GLU, TP, CHOL levels and higher ALB levels than those of present study. Serum TP and ALB values obtained from our study were parallel with the results of some other researchers [32,33].

SOD and MDA parameters varied significantly due to the kind of litter material in the present experiment *(Table 2)*. SOD, MDA values of oxidative stress parameters were found lower (P<0.01) in the V (4.34±0.21, 4.45±0.17) and WSV (4.21±0.21, 3.97±0.17) groups than that of WS group (6.52±0.21, 5.04±0.17). The study result showed that a lower value of MDA in V group in comparison to WS group because of decreasing effect of V on oxidative stress. Arivuchelvan et al.[34] found lower serum MDA level in the group reared on WS as litter material than that of other materials used as litter.

Consequently, the use of vermiculite and vermiculite-wood shavings mixture as litter material in broiler did not have any adverse effect on health and stress parameters, and V and WSV provided better conditions than WS in terms of some health and stress parameters. But, furter studies on the effects of different ratios of wood shavings and vermiculite mixture on performance of broilers should be researched.

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