

The effect of using different litter materials in broiler rearing on performance, carcass yield, antioxidant status, some litter parameters, and coccidiosis oocysts

Gökhan ŞEN^{1,*}, Mehmet Naci OKTAY², Şevket EVCI³, Sami GÖKPINAR⁴, Ali ŞENOL⁵

¹Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Kırıkkale University, Yahşihan, Kırıkkale, Türkiye

²Department of Animal Breeding and Husbandry, Faculty of Veterinary Medicine, Kırıkkale University, Yahşihan, Kırıkkale, Türkiye

³Department of Plant and Animal Production, Kırıkkale University, Delice VHS, Delice, Kırıkkale, Türkiye

⁴Department of Parasitology, Faculty of Veterinary Medicine, Kırıkkale University, Yahşihan, Kırıkkale, Türkiye

⁵Department of Biochemistry, Faculty of Veterinary Medicine, Kırıkkale University, Yahşihan, Kırıkkale, Türkiye

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Abstract: The aim of this study was to determine the effects of various mixtures of different litter materials containing wood shaving and barley straw on body weight gain, feed consumption, feed efficiency, carcass yield, antioxidant state, litter quality, and coccidiosis oocysts in broilers rearing. For this purpose, a total of 120, 0-day-old Ross 308 broilers were assigned to 3 groups with 4 replicates. Wood shaving, barley straw, and wood shaving + barley straw group had 10 chicks in each replicate, a total of 40 chicks in each group. When the overall period of the study was examined, it was determined that the feed conversion ratio (FCR) of the barley straw group was higher than other groups in the second half of the study ($p < 0.05$). It was observed that while nitric oxide (NO) level was higher in the wood shaving + barley straw group, enzymatic antioxidants could be more functional in the wood shaving group ($p < 0.05$). As a result, it has been concluded that the use of wood shaving and barley straw separately or mixed in equal proportions as litter will have a similar effect on performance and litter quality, whereas wood shaving will positively influence on the antioxidant status.

Keywords: Litter, broiler, performance, antioxidant, coccidiosis

1. Introduction

For economically viable broiler breeding, it is important to reduce feed and maintenance costs, reduce chick mortality rates and increase the quality of the produced carcass. One of the important factors for profitability in farms is litter material and management. Litter material and quality affect broiler welfare, behavioral traits, some oxidative stress parameters, fattening performance, and carcass quality [1–6].

A litter is a bedding material that is a mixture of feces, feathers, waste feed, and water [7]. In broiler breeding, coarse wood shavings are generally used as litter material. Apart from this, rice husk and straw of some grains, by-products obtained from some plants, and wastepaper are also tried as litter material [8–11]. The litter material should provide a comfortable environment for the chicks by preventing direct contact of the chick with the ground and providing insulation and should improve the maintenance conditions by absorbing the moisture in the feces [12]. The litter material to be used for these purposes should be soft and have a high-water holding capacity, should not contain

toxic substances, and be easy and cheap to supply [13]. Excessive water retention in the litter material causes various disorders such as bruising and burns in the breast skin and muscle. It also prepares the conditions for important health problems such as the growth of pathogenic bacteria and molds and the release of ammonia. Ammonia emission is one of the most important environmental factors affecting broiler performance. Controlling litter moisture is very important in preventing these problems [7].

Ammonia emission from the litter increases as a result of the moisture level in the litter material increases and the rise of pH level, especially above 9 [14–15]. Increasing ammonia emission deteriorates the air quality of the poultry house and causes health problems in the chicks, and decreased performance, low quality of the feces at the end of production due to nitrogen loss [16–19].

The aim of this study was to investigate the effects of using wood shaving and barley straw as separate or mixed litter on broiler performance, antioxidant status, and litter quality.

* Correspondence: gokhansen@kku.edu.tr

2. Materials and methods

The study was carried out at Kırıkkale University, Faculty of Veterinary Medicine, Poultry Research Unit. The study was approved by the decision of the Kırıkkale University Animal Experiments Local Ethics Committee (49, 2019/10). A total of one hundred twenty 0-day-old Ross 308 broiler chicks from a commercial hatchery were brought to the research unit and their body weights were weighed and divided into groups. The study was composed of 3 groups with 4 subgroups, and it was distributed equally so that there were 10 chicks in each subgroup. The study groups; in which i-wood shaving, ii-barley straw, and iii-wood shaving and barley straw are mixed in equal amounts as litter material. The study was carried out for 42 days on ground in 1 m² compartment. Chicks were fed with chick feeders and water was given with chick waterers as ad libitum for the first 10 days. In the remaining time, water and feed were provided with nipple water and stalactite feeders. Ingredients and nutrient compositions of diets used in the experiment are presented in Table 1. The house environment was heated to 33 °C on the first day with electric radiant heaters, and the temperature was gradually reduced to 22 °C on the following days. Lighting is provided with fluorescent lamps for 24 h. Chicks were weighed and body weight gains calculated individually every week. Feed consumptions were determined by calculating the difference between the amount of feed put in the feeders

for a week and the feed left in the feeders at the beginning of the next week. Feed conversion rates were calculated by dividing the average feed consumption of the groups by the average body weight gains.

At the end of the experiment, blood samples of 12 animals from each group (total of 36 animals) were centrifuged at 1600 × g for 10 min to obtain plasma. Plasmas were stored at -80 °C until biochemical analysis. Plasma total oxidant status (TOS) analysis was carried out by reading at 530 nm in a spectrophotometer (Shimadzu UV-1700) device according to Erel [20]. Plasma total antioxidant status (TAS) analysis was determined by reading at 660 nm in a spectrophotometer (Shimadzu UV - 1700) device according to the method of Erel [21]. Oxidative stress index (OSI) was calculated according to Kösecik et al. [22]. Plasma malondialdehyde (MDA) level was determined by the method of Buege and Aust [23] as a result of thiobarbituric acid reaction (TBARs) in spectrophotometer. Plasma vitamin A and β carotene levels were determined by using the spectrophotometric (Shimadzu UV-1700, Japan) method of Suzuki and Katoh [24] based on the highest light absorption at 325 and 453 nm, respectively. Plasma vitamin C analysis was performed by reading at 520 nm in a spectrophotometer (Shimadzu UV- 1700) device according to Haag [25]. Plasma NO levels were calculated spectrophotometrically by using the method of Miranda et al. [26].

Table 1. Ingredients and nutrient compositions of diets used in the experiment.

	Starter	Grower	Finisher
Corn, %	48.75	52.61	57.66
Soybean meal, %	43.40	38.46	33.18
Vegetable oil, %	4.20	5.35	5.43
Marble dust, %	1.15	1.08	1.13
Premix ¹ , %	2.5	2.5	2.5
Lysine sulfate, %	-	-	0.1
Crude Protein, %	24.31	21.84	20.34
Ash, %	7.00	6.00	6.35
Crude cellulose, %	5.00	5.05	4.16
ME ² , Kcal/kg	3081	3196	3248

¹Content of premix: Vitamin A 500,000 IU, Vitamin D3 200,000 IU, Vitamin E 2000 mg, Vitamin K3 150 mg, Vitamin B1 100 mg, Vitamin B2 128 mg, Vitamin B6 160 mg, Vitamin B12 1 mg, Niacin 1200 mg, Pantothenic acid 400 mg, Folic acid 40 mg, Biotin 4 mg, Vitamin C 2000 mg, Choline 12,000 mg, Calcium 17.78%, Available phosphorus 12.43%, Total phosphorus 5.46 %, Potassium 0.05%, Chlorine 5.12%, Sodium 5.37%, Manganese 3200 mg, Iron 2575 mg, Zinc 4025 mg, Copper 400 mg, Iodine 62 mg, Selenium 13.50, Lysine 8.94%, Methionine 8.89%, Methionine + Cystine 9.15%, Arginine 0.51%, Threonine 1.53%, Leucine 0.50%, Isoleucine 1.36%, Valine 0.51%, Tryptophan 0.91%, Linoleic acid 0.55%, Sugar, 0.19%, Starch 1.92%, Antioxidant 4000 mg, Phytase 40,000 FTU, Xylanase 40,000 TXT, Glucanase 10,000 TGU, Mannanase 4000 U, Protease 4000 U, Alpha amylase 6000 U, Lipase 4000 U, Pectinase, 3200 U, Toxin binder, 40,000 mg, Salinomycin sodium 24,000 ppm, Organic acid 12,000 mg, Herbal extract 10,000 mg.

² Calculated metabolizable energy

To determine ammonia nitrogen and moisture levels as litter parameters, samples taken from five different points, four corners, and the middle of each subgroup compartment, were mixed homogeneously. The nitrogen level of the litter mixture obtained in this way was examined by the Kjeldahl method according to The Association of Official Analytical Chemists (AOAC) [27]. The moisture levels of the same samples were calculated on the dry matter obtained by drying at 105 °C for 24 h in the drying cabinet according to AOAC [27].

To determine the presence of coccidiosis in chicks, fresh stool samples were taken daily from all subgroups from day 10 to 42 days old. Stool samples taken were delivered to Kırıkkale University Faculty of Veterinary Medicine Routine and Epidemiology laboratory as soon as possible. These stool samples were examined for the presence of *Eimeria* spp. oocysts using the Fülleborn flotation technique.

Statistical analysis of the data was performed using one-way analysis of variance (ANOVA). Statistical analysis results were written as mean and standard errors of the data. The differences between the groups were determined

Duncan's multiple range test. Differences between groups with $p < 0.05$ were considered statistically significant.

3. Results

The average body weights of the experimental groups are weekly given in Table 2. Statistically, there was a difference between the groups only in the second and third weeks, and there was no difference between the groups in the other weeks ($p > 0.05$). At the end of the second week, the statistical differences between the wood shaving and the barley straw groups were similar and significantly higher than the wood shaving + barley straw group ($p < 0.01$). In the third week, the live weight average of the barley straw group was significantly higher than the wood shaving + barley straw group ($p < 0.05$) and showed similarity with the wood shaving group. In the same week, there was no difference between the wood shaving group and the wood shaving + barley straw group.

Weekly average body weight gains of the experimental groups in three-week periods and the overall study period are given in Table 3. There was no difference between the groups at the third, fifth, and sixth weeks ($p > 0.05$). In the

Table 2. Average body weights of experimental groups, g.

Week	Wood shaving	Barley straw	Wood shaving + Barley straw	p
0	40.83 ± 0.46	40.48 ± 0.50	41.63 ± 0.52	0.245
1	159.46 ± 2.39	166.38 ± 2.68	159.63 ± 2.45	0.089
2	392.49 ± 5.42 ^a	405.58 ± 5.89 ^a	370.88 ± 5.69 ^b	0.000
3	736.64 ± 12.57 ^{ab}	757.51 ± 12.55 ^a	698.60 ± 14.00 ^b	0.019
4	1091.06 ± 22.06	1094.15 ± 18.72	1066.35 ± 16.24	0.662
5	1589.66 ± 33.54	1569.46 ± 36.84	1553.90 ± 30.86	0.808
6	2101.50 ± 48.76	2091.79 ± 47.20	2101.63 ± 69.10	0.988

^{a,b}: Different letters in the same row indicate significant differences ($p < 0.05$)

Table 3. Average body weight gains of experimental groups, g.

Week	Wood shaving	Barley straw	Wood shaving + Barley straw	p
1	118.64 ± 2.26 ^b	125.90 ± 2.69 ^a	118.00 ± 2.51 ^b	0.049
2	233.03 ± 5.81 ^a	239.20 ± 6.53 ^a	211.25 ± 6.24 ^b	0.005
3	343.94 ± 14.47	352.23 ± 12.30	318.10 ± 16.15	0.298
4	354.42 ± 24.81 ^b	336.64 ± 21.51 ^b	499.00 ± 73.28 ^a	0.009
5	507.43 ± 36.47	475.31 ± 42.85	487.55 ± 34.97	0.833
6	508.76 ± 55.44	522.33 ± 57.01	549.53 ± 81.39	0.918
0-3	702.08 ± 20.53	715.78 ± 18.18	644.77 ± 24.23	0.108
3-6	1372.75 ± 17.58	1335.50 ± 49.01	1399.55 ± 48.85	0.590
0-6	2074.83 ± 33.90	2051.28 ± 62.12	2059.40 ± 16.00	0.937

^{a,b}: Different letters in the same row indicate significant differences ($p < 0.05$).

first week, the wood shaving and the wood shaving + barley straw groups' results were similar, but barley straw group was higher than the others ($p < 0.05$). At the end of the second week, wood shaving and barley straw groups were similar and significantly higher than the wood shaving + barley straw group ($p < 0.01$). In the fourth week, the wood shaving and barley straw groups were similar again, but this time, significantly lower than the wood shaving + barley straw group ($p < 0.01$). It was observed that there was no significant difference between the groups in the three-week periods and overall period ($p > 0.05$).

Feed consumption of the experimental groups as weekly, three-week periods, and the whole study period are given in Table 4. It was observed that the average feed consumptions of the groups were similar weekly, in three-week periods, and overall period ($p < 0.05$).

Feed conversion rates of the experimental groups as weekly, three-week periods and the whole study period are given in Table 5. Accordingly, weekly feed conversion rates were similar ($p > 0.05$). When the three-week periods were examined, wood shaving and wood shaving + barley straw

groups were similarly determined in the second three-week period and were also found at a lower level than the barley straw group ($p < 0.05$). There was no statistically significant difference between the feed conversion rates of the groups in the overall period ($p > 0.05$).

The carcass yields of the experimental groups are given in Table 6 and there is no significant difference between the groups ($p > 0.05$).

Plasma antioxidant statuses obtained from the groups are shown in Table 7. Plasma NO level increased significantly in the wood shaving + barley straw group ($p < 0.05$), and TAS, TOS, OSI, and MDA parameters were similar between the groups ($p > 0.05$). While the nonenzymatic antioxidant vitamin C level was similar between the groups, the β -carotene level was lower in the barley straw group than in other groups ($p < 0.05$) and the vitamin A level was higher in the wood shaving group compared to other groups ($p < 0.05$).

The moisture levels and changes determined from the weekly litter samples of the groups are shown in Figure. The litter moisture ratios of the groups were determined

Table 4. Feed consumptions of experimental groups, g.

Week	Wood shaving	Barley straw	Wood shaving + Barley straw	p
1	126.23 ± 4.89	128.03 ± 3.97	131.98 ± 1.43	0.558
2	292.39 ± 9.63	269.33 ± 13.64	279.58 ± 18.60	0.549
3	380.00 ± 17.38	410.25 ± 17.00	342.67 ± 36.79	0.190
4	703.73 ± 37.28	666.09 ± 9.85	684.60 ± 18.70	0.608
5	782.40 ± 18.71	805.00 ± 33.72	834.05 ± 23.45	0.542
6	899.81 ± 17.32	889.66 ± 40.01	911.22 ± 66.28	0.932
0-3	798.61 ± 23.97	807.60 ± 19.60	756.73 ± 62.22	0.593
3-6	2385.93 ± 38.40	2360.75 ± 81.29	2429.87 ± 108.43	0.835
0-6	3184.55 ± 57.14	3168.35 ± 92.15	3248.67 ± 100.83	0.829

Table 5. Feed conversion rates of experimental groups, g/g.

Week	Wood shaving	Barley straw	Wood shaving + Barley straw	p
1	0.95 ± 0.03	0.98 ± 0.02	0.90 ± 0.03	0.165
2	1.25 ± 0.03	1.13 ± 0.06	1.32 ± 0.05	0.053
3	1.10 ± 0.03	1.17 ± 0.06	1.26 ± 0.18	0.511
4	1.95 ± 0.02	1.99 ± 0.04	1.88 ± 0.15	0.501
5	1.53 ± 0.07	1.72 ± 0.11	1.71 ± 0.01	0.316
6	1.78 ± 0.04	1.71 ± 0.07	1.66 ± 0.14	0.555
0 - 3	1.14 ± 0.01	1.13 ± 0.01	1.17 ± 0.08	0.709
3 - 6	1.74 ± 0.01 ^b	1.77 ± 0.01 ^a	1.74 ± 0.02 ^b	0.049
0 - 6	1.53 ± 0.01	1.54 ± 0.00	1.58 ± 0.04	0.195

^{a,b}: Different letters in the same row indicate significant differences ($p < 0.05$).

Table 6. Carcass yields of experimental groups.

	Wood shaving	Barley straw	Wood shaving + Barley straw	p
Preslaughter body weight, g	2265.00 ± 86.57	2173.00 ± 57.97	2262.38 ± 46.70	0.485
Hot carcass weight, g	1687.14 ± 66.75	1619.17 ± 45.90	1656.25 ± 31.68	0.619
Carcass yield, %	74.47 ± 0.33	74.50 ± 0.53	73.25 ± 0.64	0.223

Table 7. Plasma antioxidant statuses of experimental groups.

	Wood shaving	Barley straw	Wood shaving + Barley straw	P
TOS (µmol/L)	8.47 ± 0.79	7.69 ± 0.45	9.56 ± 1.16	0.313
TAS (mmol/L)	1.01 ± 0.02	0.96 ± 0.04	1.02 ± 0.02	0.272
OSI (arbitrary unit)	0.84 ± 0.08	0.81 ± 0.06	0.94 ± 0.12	0.583
MDA (µmol/L)	1.00 ± 0.16	0.69 ± 0.10	0.81 ± 0.09	0.199
NO (µmol/L)	27.96 ± 2.79 ^b	24.66 ± 2.11 ^b	32.70 ± 1.82 ^a	0.041
Vitamin C (µg/dL)	27.15 ± 1.13	28.79 ± 1.50	26.99 ± 1.36	0.581
β-carotene (µg/dL)	183.53 ± 8.22 ^a	159.38 ± 5.30 ^b	188.37 ± 7.24 ^a	0.008
Vitamin A (µg/dL)	71.73 ± 4.05 ^a	57.37 ± 2.22 ^b	57.48 ± 1.59 ^b	0.001

^{a,b}: Different letters in the same row indicate significant differences (p < 0.05).

TOS: Total oxidant status, TAS: Total antioxidant status, OSI: Oxidative stress index, MDA: Malondialdehyde, NO: Nitric oxide,

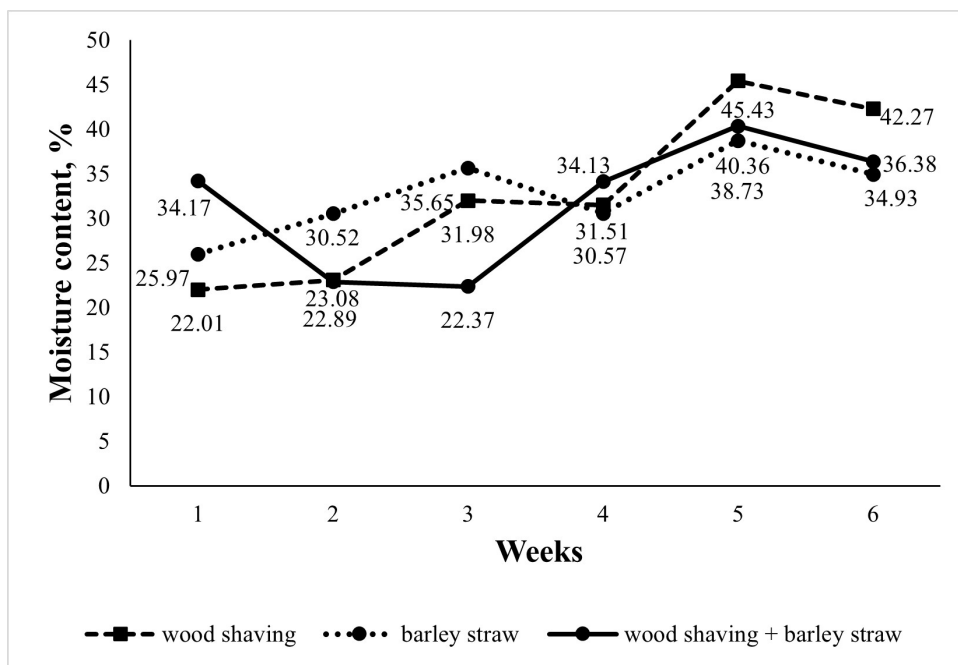


Figure. Moisture levels measured from weekly litter samples of experimental groups, %.

as 22.01%, 25.97%, and 34.17% in the wood shaving, barley straw, and wood shaving + barley straw groups, respectively, and there was no statistical difference between the groups ($p > 0.05$). In the following weeks, in the moisture levels of the wood shaving and the barley straw groups an increase was determined, while in the litter moisture rate of the wood shaving + barley straw group, a fluctuation was shown. However, when each week is examined individually, it is seen that there is no statistical difference between the groups.

The ammonia levels analyzed from the samples taken from the litter of the groups onwards the third week of the experiment are given in Table 8. There was no statistically significant difference between the groups ($p > 0.05$). In the third week, the ammonia level in the litter sample taken from the wood shaving + barley straw group was numerically lower than the wood shaving and barley straw groups.

Eimeria spp. oocysts were not found in any of the stool samples examined daily for 32 days. All subgroups were determined as negative in terms of coccidiosis. Therefore, the effect of litter type on the prevalence and development of *Eimeria* spp. oocysts could not be evaluated.

4. Discussion

Poor environmental conditions are a reason why broilers cannot perform their genetic potential. Litter quality highly affects the quality of the in-house environment [7]. In the results of our study, although there are differences in some weeks, the body weight gains of the groups were similar at the overall period. This result is similar to those of Hafeez et al. [28] and Sigroha et al. [29]. At the end of study conducted with different litter types, Toghyani et al. [4] reported that the group using rice hulls had lower body weight than the others. They stated that this result may be due to feed consumption depression of the rice hull group. In this study, there was no significant difference between the feed consumptions of the groups. Accordingly, it can be said that it is an expected situation that there is no difference in the body weight gains of the groups. In the three-week period results of our study, it is seen that there is no difference between the body weight gains of the groups. In the present study, it was determined that the results at the overall period were similar to Onbaşlar

et al. [30]. However, they have reported that there is a difference between the groups in the first three weeks of the same study and it was stated that this difference may be due to the color of the litter used. Since the colors of wood shaving and straw used in our study are similar, the results may be similar in both separate groups and mixed groups.

The feed consumption and feed conversion ratios of the groups in the present study were also not significant found at the overall period. El-Deek et al. [31] found significant differences in a similar study, emphasizing that this may depend on the type of litter. In the second three-week period, although it caused a numerical decrease on feed consumption and body weight gain values in the straw group, the feed conversion ratio of the straw group increased significantly. Straw has less water holding capacity and shows less caking. Considering that the particles in the noncaking substrate will cause more discomfort to the soles of the feet, it can be said that this decrease may be due to this. This result is also consistent with the results of Youseff et al. [32]. Even so, there was no significant difference between feed conversion rates of the groups at the overall period, and the result was consistent with results of Toghyani et al. [4].

Similarly, there was no difference between the body weights of the groups at the end of present study, the preslaughter weights of the randomly selected animals were similar. While the results of preslaughter weights of Škrbić et al. [33] were significant between different type genotypes in the straw groups, they were determined that it was similar between the same genotypes and different litter material groups as our results. This shows that different genotype can cause different results with the same material. As the hot carcass weights of the groups were also similar, there was no significant difference between the carcass yields. This result was consistent with results of other studies [4,31,33].

Reactive oxygen species, also called free radicals, are formed as a result of cell metabolism. These metabolites, which increase rapidly under environmental stress conditions, damage the cell structure. The negative effects of these metabolites are balanced by the effect of antioxidants. Free radicals and their elimination are in balance in the organism. MDA is also an important indicator of the presence of stress and is revealed as the

Table 8. The ammonia levels were analyzed from the litter of experimental groups (g/kg of litter).

Week	Wood shaving	Barley straw	Wood shaving + Barley straw	p
3	12.01 ± 0.83	11.34 ± 0.90	7.08 ± 2.24	0.065
4	15.89 ± 1.32	18.08 ± 1.11	18.62 ± 2.65	0.423
5	19.72 ± 1.33	22.37 ± 1.82	19.61 ± 2.06	0.459
6	18.13 ± 0.93	23.32 ± 1.11	21.86 ± 5.18	0.154

end product of lipid peroxidation [34–36]. In the present study, TAC, TOC, OSI, and MDA values were found to be similar between the groups, indicating that chicks were raised in similar environmental conditions and different litters protect the oxidative balance. Different litter materials can also cause statistically significant results in TAC and MDA levels. In the study conducted by Nawar et al. [37], wood shavings and crushed corncob materials were used as litter, both MDA levels increased, and total antioxidant levels decreased in the group using corncob material. In the current study, while it was determined that the NO level increased in the wood shaving + barley straw group compared to other groups, β – carotene level was the same with the wood shaving group. But vitamin A levels of these two groups were not the same and in the wood shaving + barley straw group, it was decreased. This was interpreted as increasing NO negatively affecting the conversion of existing β – carotene to vitamin A.

It is desirable that the litter used in broiler farming has a high-water holding capacity [38]. The ideal moisture level expected to be found in the litter has been reported as 15% in summer and 30% in winter by Gençođlan and Gençođlan [39]. If the litter is higher than the recommended rates, it will promote to increase in the numbers of pathogenic bacteria and molds, while too dry will cause dehydration and respiratory problems [7,39]. In the results of our study, although the litter moisture level was not significantly different between the groups, it increased from the beginning to the last week in all groups. In the results of the study conducted by Ogan [40], it was stated that the litter moisture increased during the experiment. Similar results were seen by Hafez et al. [28], and they stated that this was due to accumulating feces and increased respiration due to growth.

In litter with high moisture change rapidly pH and ammonia levels. Litter pH plays an important role in ammonia release because ammonia production tends to increase with pH. A litter pH of 9 and above creates a suitable environment for ureolytic bacteria (for example, *Bacillus Pasteuri*), causes bacteria to multiply, and creates a suitable environment for ammonia production [41–42]. In the present study, litter ammonia levels were found to be similar between the groups. The results obtained are consistent with the study conducted by Onbařilar

et al. [43] with wood shavings and rice hull in terms of both litter moisture and ammonia-N status between groups. There is a close relationship between moisture and ammonia production. In the presence of sufficient ambient temperature, moisture also facilitates the growth of ammonia-producing bacteria by breaking down the organic material in the environment [17].

Eimeria spp. oocysts were not found in any of the samples in the stool examination in the study. The presence of anticoccidial salinomycin in the feeds given to the chicks may be effective in the emergence of these results. However, in many studies, it has been reported that *Eimeria* species that cause infection in chickens are resistant to salinomycin [44,45]. It has been determined that the mortality rate due to coccidiosis in chickens using salinomycin as an anticoccidial is higher than in chickens using other anticoccidials [44]. Arabkhazaeli et al. [46] reported that the use of anticoccidial did not prevent the formation of subclinical or clinical coccidiosis, and some *Eimeria* species developed partial resistance to salinomycin in broiler chickens. Sarı and akmak [47] reported that feeds containing anticoccidial additives may be insufficient to protect against coccidiosis in chickens. Conway et al. [48] reported that samduramicin was more effective than salinomycin in *E. tenella* and *E. acervulina* infections. Kaewthamasorn et al. [49] reported in their study that the use of salinomycin as an anticoccidial reduced the number of lesions caused by coccidiosis by 69.58% but could not completely eliminate it. No study conducted to date has found that the use of salinomycin as an anticoccidial completely eliminates *Eimeria* spp. oocyst excretion. For this reason, we think that the inability to detect *Eimeria* spp. oocysts in chickens in our study may not be due to the use of salinomycin as an anticoccidial in the feed, and the litter materials used also affected the results. As a result, it has been determined that the use of wood shaving and barley straw separately or mixed in equal proportions as litter will have a similar effect on performance and litter quality, but wood shaving will positively affect the antioxidant status.

Conflict of interest

The authors declare no conflict of interest.

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