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Impact of litter and litter amendments on blood variables and immunity of broiler chickens

G Taherparvar[1], AR Seidavi[2*], L Asadpour[3] and R Payan-Carreira[4]

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[1]MSc Graduated, Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran
[2]Professor, Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran
[3]Associate Professor, Department of Biology, Rasht Branch, Islamic Azad University, Rasht, Iran
[4]Professor, ICAAM & Department of Veterinary Medicine, Universidade de Évora [Pole at Mitra], Évora, Portugal
[*]Corresponding author E-mail: alirezaseidavi@iaurasht.ac.ir

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Abstract

Introduction: Poultry litter is composed of bedding material, excreta, feed, feathers and water. It is currently accepted that litter quality can add to environmental and management problems in the commercial poultry industry. Several studies are available on the impact of litter material or quality on the intestinal health immunity of poultry, but often they focus on particular pathogens, potentially detrimental to humans or other livestock, or on imposed limitations to broilers performance. **Aim:** This study aimed to assess whether the type of bedding materials (sand, wood shaving, and paper) or chemical amendments (lime and bentonite *vs*. controls) affect blood parameters and immunity of broiler chickens. **Materials and methods:** Two hundred and seventy male Ross broiler chickens were randomly assigned into nine treatment groups with three replicates per each treatment (a total of 30 birds per each treatment). A completely randomized 3 × 3 design was used, with the main effects of bedding materials/subtracts (sand, wood shaving, or paper) and amendments (no amendment (control), bentonite, or lime), in three replicate pens of 10 chicks each, in a total of 27 experimental units. Starting at day one, groups were created according to the combination of beddings and amendments, as follows: Group 1 (Grp 1) - control sand bedding; Group 2 (Grp 2) - sand bedding treated with Bentonite; Group 3 (Grp 3) - sand bedding treated with lime; Group 4 (Grp 4) - control wood shaving bedding; Group 5 (Grp 5) - wood shaving bedding treated with Bentonite; Group 6 (Grp 6) - wood shaving treated with lime; Group 7 (Grp 7) - control paper bedding; Group 8 (Grp 8) - paper bedding treated with Bentonite; and Group 9 (Grp 9) - paper treated with lime. **Results:** The results showed that different bedding materials (sand, wood shavings, and paper) had not effect on most analyzed traits; though, we detected a small significant increase in the influenza antibody titres at day 36 ($P < 0.05$) in sand reared groups, and an increase in the total immunoglobulins (Ig) titres (due to increased IgM) on 14 days after challenged with sheep red blood cells ($P < 0.05$). Treatment of the bedding material with Bentonite or lime mainly affected the humoral immunity traits assessed herein. Lime treatments slightly increased the antibody titres for Influenza at the first challenge, but did not affect them on the

second challenge. In contrast, no treatments increased total Ig titres (due to an increase in IgM) at day 38. The comparison of the nine groups (bedding type × amendment) indicated small differences in particular blood parameters and the humoral immunity traits. **Conclusion:** Although, no deleterious effects were found on broilers, the results suggested that different litter materials with distinct amendments may affect the final quality of carcasses.

Keywords: Bedding material; Broilers; Chemical supplementation/treatment; Immune system; Litter quality; Microbiota

Introduction

The broiler industry uses genetically improved birds with rapid juvenile growth, breast-meat yield and increased efficiency of feed utilization (Klasing 2007; Dawkins and Layton 2012). However, this improvement in performance is accompanied with an increased susceptibility of birds to environmental stressors (Klasing 2007; Dawkins and Layton 2012).

For birds reared in confinement, pen-litter may become an important environmental stressor because high moisture and poor sanitary conditions, may interfere with bird health and productivity. Ideally, bedding material should be very absorbent, have a reasonable drying time and must be innocuous to poultry or farmers (Bilgili et al. 2006; Grimes et al. 2007; Bjedov et al. 2013; Garcês et al. 2013), but it also needs to meet hygienic requirements and control ammonia concentrations throughout the productive cycle (Karamanlis et al. 2008; Villagrá et al. 2011; Skrbic et al. 2012; Bjedov et al. 2013).

litter is composed of bedding material mixed with excreta, feed, feathers and water. It is currently accepted that poor litter quality may cause environmental and management problems in the commercial poultry industry (Karamanlis et al. 2008; Garcia et al. 2012, Sohirat Torfy et al. 2017) if not correctly managed. Poor growth performance, compromised immune system and increased incidence of breast burns and blisters, leg abnormalities, and footpad dermatitis have been reported in the literature partially due to poor litter conditions (Bilgili et al. 1999; Garcia et al. 2012). Several studies are available on the impact of litter material or quality on the intestinal health and immunity in poultry (Garrido et al. 2004; Torok et al. 2009), but often they focus on particular pathogens, potentially detrimental effects to humans or other livestock (Monira et al. 2002; Macklin and Krehling, 2010), or on imposed limitations to broilers performance (Huang et al. 2009; Bjedov et al. 2013).

Many different materials, varying according to regional availability (Swain and Sundaram 2000; Monira et al. 2003; Torok et al. 2009; Skrbic et al. 2012), are used for poultry bedding in intensive commercial broiler production, resulting in differences in its physical and microbiological characteristics. Thus, the source of bedding can determine the need for specific amendments to improve its quality. Poultry litter maybe a potential reservoir and transmission vehicle for pathogenic bacteria. Diverse treatments or amendments have been proposed to minimize the risk of pathogens in letter during the productive cycle (Ivanov 2001; Line 2002; Garrido et al. 2004; Choi et al. 2008, Taherparvar et al. 2016). However, limited information is available on the effect of litter amendments on blood variables or immunity in broliers. Therefore, the present study aimed to compare the effects of two alternative amendments (Lime and Bentonite) applied over three different bedding materials (sand, wood shaving and paper) on the haematology parameters and immunity of broilers reared in an intensive 42 daycycle.

Materials and methods

This study was conduct in August-September 2013, at a commercial poultry farm at Abkenar

(37° 27' North, 49° 19' East, -26 m below sea level) and at the Laboratory of Nutrition and Dairy Industry from the Agriculture Faculty of Islamic Azad University, Rasht Branch, Iran.

The study was approved by the Ethic Committee of the Islamic Azad University, and was conducted in respect to the International Guidelines for research involving animals (Directive 2010/63/EU); care was taken to minimize the number of animals used.

Animals and housing

In this study, a total of 270 male Ross 308 broiler chicks were randomly distributed into nine treatments, with three replicates per treatment, in a total of 30 birds per treatment. The one-day-old chicks were purchased from a local hatchery and randomly assigned into groups with similar mean body weights. Chicks were reared until the age of 42 days. The animals were housed in 1.5 x 1.5 m cages.

All broilers had a common environment except for the litter beddings. Thermo-neutral ambient temperature was maintained in accordance to standard brooding practices and adapted to the birds rearing stages (Aviagen 2009). Lighting was provided 24 h on the first day and thereafter, 23 h/day with one hour of darkness from 19 to 20 pm.

Broiler chickens received feed and water *ad libitum* throughout the trial. Broilers were unable to feed from adjoining cages. Formula and chemical composition of experimental diets are present in Tables 1 and 2. Routine vaccination and deworming was designed by the farm veterinarian and coped with regional veterinary authority.

Vaccination was made against infectious bronchitis (Infectious Bronchitis Virus (IBV, H120); Razi Co, Iran) at days 1 and 14, and a Gamboro vaccination (Gamboro IBD071IR; Razi Co, Iran) was administered at days 8, 16 and 23.

Experimental design

A completely randomized design with a 3 × 3 factorial arrangement of treatments was used, with three types of bedding materials/subtracts (sand, wood shaving, and paper) and three amendment treatments (no amendment or control, Bentonite and Lime), in three replicate pens of 10 chicks each, resulting in a total of 27 experimental units.

Commencing from day one, the following groups were created by combination of bedding and amendments, as follows:

Group 1 (Grp 1)-Control sand bedding;

Group 2 (Grp 2)-Sand bedding treated with Bentonite;

Group 3 (Grp 3)-Sand bedding treated with lime;

Group 4 (Grp 4)-Control wood shaving bedding;

Group 5 (Grp 5)-Wood shaving bedding treated with Bentonite;

Group 6 (Grp 6)-Wood shaving treated with lime;

Group 7 (Grp 7)-Control paper bedding;

Group 8 (Grp 8)-Paper bedding treated with Bentonite;

Group 9 (Grp 9)-Paper treated with lime.

Bentonite was used at three kg/m^3 and lime was used at 1.5 kg/m^3 based on Taherparvar et al. (2016).

Measurements of broiler blood metabolites and hepatic enzymes

At the end of the experiment (42 days), one bird from each replicate pen, to total of three birds for each experimental group, was randomly selected for blood sampling.

Prior to blood collection and slaughter, feed was removed from all the birds for a period of four hours in order to stabilize the plasma constituents. Further, all blood sampling was done in the morning to avoid the diurnal variability of the blood parameters to be measured. Care was taken to choose the most representative male birds with respect to body weight compared to the group mean body weight.

Blood samples (~5 mL/bird) were collected from the wing vein (*Vena cutanea ulnaris*) into tubes coated with 10 mg of the anticoagulant ethylenediamine tetra acetic acid (EDTA) for plasma separation, and transferred to the

laboratory for analysis within two hours of collection. Plasma was harvested after centrifugation (3000 g, for 10 min at room temperature) and stored at -20°C until analyzing. Blood parameters analyzed in this study included: cholesterol (Chol), triglycerides (TG), very low-density lipoprotein (VLDL), high density lipoprotein (HDL), low density lipoprotein (LDL), HDL/LDL ratios, total protein, uric acid (UAc), albumin (Alb), Globulin (Glob), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Plasma blood parameters were analyzed using a Roche Cobas Integra autoanalyzer (Roche Diagnostics, GmbH, Mannheim, Germany), based in standard protocols using commercial kits from Pars Azmoon (Pars Azmoon Co., Tehran, Iran), according to the manufacturer's instructions, as described elsewhere (Nahavandinejad et al. 2014; Shabani et al. 2015).

Table 1- Diet ingredients fed to broilers

	Age periods (days)				
Ingredients (g/kg)	**1-7**	**8-15**	**16-23**	**24-35**	**36-42**
Corn	454.9	510.5	500.5	460	436
Wheat	90	100	140	190	255
Soybean meal	385	330	307	298	264
Soybean oil	20	20	20	20	20
Sodium bicarbonate (NaHCO3)	1.2	1.4	1.4	2	1.5
Ca%22P%18	23	10	10	6	6
Oyster powder	12	-	-	-	-
NaCl	2.3	2	1.8	2	1.7
Mineral Mixture[1]	2.5	-	-	2.5	2
Vitamin Mixture[2]	2.5	2.9	2.5	2.5	2
DL-Methionine	2.6	3.1	2	2.2	1
L-Lysine-Hydro-Chloride	2	2	2	0.5	0.5
Threonine	0.9	0.5	0.5	-	-
CaCO3	-	15	12	12	10
Coccidiostat Salinomycin	0.5	0.5	-	-	-
Multi-enzyme	0.5	-	-	-	-
Avizyme enzyme	-	0.5	0.5	0.5	-
Physasyme enzyme	-	0.1	0.1	0.1	0.1
Turmeric (*Curcuma longa*)	-	1.5	-	1.5	-
Probiotics (Technomos)	0.5	-	-	-	-
Anti fungus toxin binder	0.5	0.5	0.2	0.2	0.2
Total	1000	1000	1000	1000	1000

[1] Calcium Pantothenate: 4 mg/g; Niacin: 15 mg/g; Vitamin B6: 13 mg/g; Cu: 3 mg/g; Zn: 15 mg/g; Mn: 20 mg/g; Fe: 10 mg/g; K: 0.3 mg/g

[2] Vitamin A: 5000 IU/g; Vitamin D3: 500 IU/g; Vitamin E: 3 mg/g; Vitamin K3: 1.5 mg/g; Vitamin B2: 1 mg/g

Table 2- Calculated concentrations of nutrient in diets fed to broilers for the different rearing periods

	Age periods (days)				
	1-7	**8-15**	**16-23**	**24-35**	**36-42**
Dry Matter (%)	85.470	86.390	86.760	87.040	87.249
Energy (ME) (kcal/kg)	2.924	3.058	3.096	3.100	3.145
Crude Protein (%)	22.091	19.573	18.939	18.727	17.794
Crude Fiber (%)	2.712	2.649	2.633	2.630	2.601
Ether Extract (%)	4.274	4.458	4.473	4.407	4.405
Choline (g/kg)	1.650	1.582	1.521	1.526	1.445
Linoleic Acid (%)	2.222	2.333	2.325	2.263	2.235
Folic acid (mg/kg)	2.153	2.070	1.911	1.883	1.667
	Amino acids (%)				
Leucine	1.977	1.838	1.780	1.753	1.663
Phenylalanine	1.137	1.037	1.008	1.007	0.964
Arginine	1.564	1.400	1.340	1.322	1.232
Lysine	1.442	1.298	1.244	1.115	1.034
Valine	1.092	1.000	0.970	0.965	0.921
Iso-Leucine	0.999	0.906	0.877	0.875	0.834
Tyrosine	0.925	0.840	0.809	0.801	0.755
Threonine	0.884	0.802	0.771	0.761	0.714
Methionine	0.613	0.636	0.518	0.564	0.402
Tryptophan	0.328	0.293	0.282	0.282	0.267
Gly + Ser	2.567	2.317	2.237	2.226	2.109
Phen + Tyr	2.062	1.877	1.817	1.808	1.720
Met+Cys	0.995	0.991	0.866	0.910	0.737
	Ions (%)				
Calcium	1.064	0.888	0.769	0.684	0.601
Available Phosphorus	0.148	0.141	0.139	0.138	0.135
Sodium	0.118	0.103	0.096	0.104	0.094
Potassium	0.957	0.867	0.835	0.827	0.780
Chloride	0.219	0.201	0.189	0.173	0.155

Measurements of broiler immune competency

Immunization program and challenge

To study the humoral immune competence in treated groups, the following challenge tests were performed on three birds/pen:

a) Response to the Newcastle lentogenic vaccine was assessed in blood sampled twice, at days 15 and 26; commercial lyophilized vaccines (Razi Co, Iran), prepared with the strains Hitchner B1, La Sota and Clon 30, were administered on days 1, 8, and 19, respectively.

b) Response to the Influenza vaccine (Avian Influenza- H9N2- Razi Co, Iran) administered at day 8 was assessed in blood sampled at 21 and 28 after the first administration (i.e., 29th and 36th days of age).

c) Response to sheep red blood cell (SRBC) inoculation - The antigenic challenge with SRBC was performed twice, at days 13 and

24, and blood sampling was performed at days 22 and 38 for assessment of total antibody, IgG and IgM production. One half millilitre of a 10% suspension of SRBC in sterile PBS (phosphate buffered saline solution; v/v) was inoculated under skin of the breast. In each replicate, only two birds were inoculated and tested. In these birds a pre-immune blood sample was collected based on Pourhossein et al. (2015).

For the assessment of the immune parameters, blood samples (two ml) were collected from the wing vein on the pre-scheduled days. The samples were centrifuged at 1,500 rpm for 10 min and the serum harvested and stored at -20 C until analysis.

Haemagglutination inhibition (HI) assays were used to determine the vaccine titres of Newcastle disease (ND) and avian influenza (AI), following the procedure described in previous work (Seidavi et al. 2014; Ebrahimi et al. 2015). The total antibodies or the immunoglobulin titers were expressed as log 2. Total antibody titers to SRBC were determined by hemagglutination assay in serum from birds. In U-bottom microtiter plates, two-fold serial dilutions of heat-inactivated (at 56°C) serum were made with PBS (0.01 mol/L; pH 7.4) for total antibody, or PBS with 1.4% 2-mercaptoethanol for immunoglobulin G (IgG) antibody. All antibody titers were recorded as log2 of the highest dilution of serum that agglutinated an equal volume of a 0.5% SRBC suspension in PBS. The IgM titer was determined by the difference between total and IgG titer (Pourhossein et al. 2015).

Lymphoid organs and liver weight

After four hours of fasting, one bird per each replicate, aged of 42 days, for a total of three broilers per group, was chosen and slaughtered to collect the main lymphoid organs (thymus, spleen and bursa of Fabricius) and liver. Care was taken to choose the most representative male birds, presenting a live body weight similar to the mean live body weight of their cohorts. Birds were fully plucked by dry pecking method; the post-slaughter weight was recorded and used to estimate the relative organ weight. The thymus (all the lobes), liver, spleen and bursa of Fabricius were immediately removed, stripped of adherent connective tissue, and individually weighed in an electric balance. Relative organ weights were calculated as percentage of live body weight.

Statistical analysis

Results are presented as means ± standard error of the mean. Shapiro-Wilks test confirmed the normal distribution of data, which was then analyzed using a 3×3 factorial arrangement with three litter treatments (sand, wood shaving and paper) and three chemical reagent treatments (no reagent/control, Lime and Bentonite). The significance of the differences among group means was analyzed using the ANOVA procedure, followed by a Tukey's post hoc test to separate means, using IBM SPSS Statistics 21 software for Windows®. An α-value of 0.05 was used to assess significance among means. *P* values $\leq$ 0.05 were regarded as statistically significant.

Results

The results obtained in this study are reported in Tables 3 to 5. Overall, we found that the type of bedding material used had no effect on plasma metabolites (Table 3), except for the HDL/LDL ratio (P=0.023), which were higher in broilers reared on paper bedding. Similarly, the chemical amendments had little effect on the measured metabolites or enzymes (Table 3).

Concentrations of hepatic enzymes were slightly decreased in the groups with bedding treated with Lime (P=0.068 for AST and P=0.068 for ALT), in line with a tendency for a decrease in the absolute liver weight (P=0.055, Table 5) in the same groups.

We also found that litter treatments influenced the concentration of several blood metabolites, namely the total cholesterol, the triglycerides and VLDL (P=0.040, P=0.036 and P=0.036, respectively) or the uric acid (P=0.008), as well as the hepatic enzymes AST ($p \leq 0.001$) and ALT

(P=0.035). The major differences found for uric acid blood concentration were represented by Grp 2, which showed the highest values, and groups 3 and 6 that showed the lowest (Table 3). On respect to the total cholesterol, the extreme values represented the groups 8 and 9 (122.33±9.76 mg/mL and 96.33±5.82 mg/mL, respectively; Table 3).

The type of material used for bedding influenced the Influenza titres at day 36 (P=0.020), and there was a tendency was observed regarding the Newcastle titres at day 26 (P=0.068). In addition, the bedding material also affected the total antibody production at day 38 after the SRBC-stimulation (P=0.033) mainly due to differences in IgM (P=0.010), which were increased in wood shaving bedding groups compared to those on sand or paper beddings (Table 4). In contrast, chemical amendments influenced a larger number of immune parameters in the current study, namely the Influenza and Newcastle vaccine titres on day 15 (P=0.007 and P=0.001, respectively) although the differences were attenuated at the time of the second testing, at day 26 (Table 4). The differences were due to higher titres in the Lime treated litters. Similarly, differences were found for the SRBC-stimulated total antibody and IgM production (P=0.012 and P=0.004, respectively), which were increased seven days after the second challenge in groups whose litter has been treated with Bentonite. In contrast, at day 14 after the second challenge, the differences observed in total immunoglobulins (P=0.005) and IgM (P=0.012) were associated to control treatments (Table 4).

Differences among treatments were also detected in the immune parameters analyzed. The Influenza and Newcastle vaccine titres differed among groups either at day 29 and 36 (P=0.040 and P=0.051 *vs.* P=0.001 and P=0.002, respectively for Influenza and Newcastle tested on the first and second times; Table 4). A group effect was found in the amount of IgM on both tested times (P=0.006 and P=0.003, respectively at days 22 and 38; Table 4).

The bedding materials used in this experiment did not influence the absolute or relative weight of the main immune organs. However, the chemical amendment affected only the absolute and relative spleen weight (P=0.004 and P=0.001, respectively), with a decrease in the weight of the spleen in birds reared in litters treated with Lime, despite the tendency found for a slight increase in the absolute liver weight litters non-treated (P=0.055; Table 5). Treatments affected the absolute liver weight (P=0.028) and both the absolute and relative spleen weight (P=0.009 and P=0.001, respectively), (Table 5). The liver weight was the highest in birds from Grp 4, but they were the lowest in Grp 2 and 3 (Table 5). The absolute spleen weight was the highest in Grp 8 broilers, compared to birds in Grp 3 that had the lowest spleen weight (Table 5). Bursa of Fabricius weight, the highest values were found in Grp 2 and the lowest value was in Grp 4 (Table 5).

Table 3- Blood metabolite mean (±SEM) of Ross 308 broilers reared under different types of bedding (sand, wood shavings and paper) and bedding amendments (no amendment - controls, Bentonite and lime).

Parameters		Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	VLDL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Ratio HDL/LDL	Total Protein (g/dL)	Albumin (g/dL)	Uric Acid (mg/dL)	Globulin(g/dL)	AST (U/L)	ALT (U/L)
Bedding	sand	111.44±3.03	55.11±5.73	11.11±1.16	66.89±1.67	33.44±1.96	0.50±0.03^{ab}	3.54±0.68	1.58±0.03	4.98±0.47	1.96±0.03	360.22±23.27	9.89±0.68
	wood shavings	112.78±2.50	74.11±8.49	14.89±1.65	69.22±2.29	29.11±2.34	0.43±0.046^{b}	3.61±0.09	1.60±0.04	4.89±0.34	2.01±0.04	333.11±17.10	9.77±0.69
	paper	110.667±4.85	69.22±6.98	13.89±1.39	62.33±3.22	37.44±2.34	0.63±0.07^{a}	3.61±0.09	1.58±0.04	4.72±0.29	2.06±0.04	362.33±25.28	9.89±0.80
P-value		0.916	0.160	0.159	0.146	0.100	0.023	0.806	0.886	0.891	0.392	0.585	0.585
Amendments	Controls	109.889±3.15	77.56±6.65	15.56±1.67	64.78±1.75	30.44±2.14	0.47±0.03	3.49±0.06	1.56±0.036	4.93±0.29	1.967±0.036	392.44±25.03	11.33±0.76
	Bentonite	118.111±4.07	57.33±6.17	11.56±1.31	67.56±2.74	38.56±3.57	0.60±0.07	3.63±0.11	1.61±0.05	5.40±0.40	2.022±0.05	338.44±23.54	9.11±0.62
	Lime	106.889±2.94	63.56±8.39	12.78±1.21	66.11±3.01	31.00±1.98	0.49±0.05	3.64±0.07	1.59±0.02	4.26±0.37	2.056±0.02	324.78±12.12	9.11±0.65
P-value		0.065	0.132	0.132	0.746	0.064	0.150	0.328	0.083	0.574	0.473	0.068	0.068
Treatments	Grp 1	103.333±4.36 ^{ab}	57.33±10.92 ^{b}	11.67±2.23 ^{b}	61.67±2.32	30.00±4.43	0.48±0.06	3.57±0.42	1.57±0.04	4.27±0.53 ^{ab}	2.00±0.04	340±67±19.10 ^{ab}	10.33±0.92 ^{ab}
	Grp 2	112.333±3.47 ^{ab}	48.67±10.36 ^{b}	9.67±2.01 ^{b}	66.00±2.85	36.67±2.57	0.56±0.04	3.47±0.17	1.60±0.06	6.73±0.80 ^{a}	1.867±0.06	447.33±43.05 ^{ab}	12.00±0.97 ^{a}
	Grp 3	118.667±6.24 ^{ab}	59.33±9.77 ^{b}	12.00±2.03 ^{b}	73.00±1.32	33.67±2.93	0.46±0.05	3.6±0.12	1.57±0.02	3.93±0.61 ^{b}	2.033±0.02	292.67±28.25 ^{ab}	7.33±0.84 ^{b}
	Grp 4	113.000±5.54 ^{ab}	98.33±12.66 ^{a}	19.67±2.43 ^{a}	70.67±0.92	25.33±0.92	0.36±0.11	3.37±0.11	1.50±0.10	5.60±0.19 ^{ab}	1.86±0.10	380.67±43.81 ^{ab}	11.33±1.52 ^{a}
	Grp 5	119.667±3.39 ^{ab}	45.67±9.48 ^{b}	9.33±1.87 ^{b}	72.67±5.35	36.33±5.95	0.54±0.13	3.90±0.17	1.70±0.06	5.37±0.22 ^{ab}	2.20±0.06	283.67±8.57 ^{b}	7.67±0.56 ^{b}
	Grp 6	105.667±1.46 ^{ab}	78.33±14.24 ^{ab}	15.67±2.74 ^{ab}	64.33±4.07	25.67±2.01	0.40±0.02	3.57±0.08	1.60±0.04	3.70±0.79 ^{b}	1.96±0.04	335.00±6.76 ^{ab}	10.33±0.92 ^{ab}
	Grp 7	113.333±6.39 ^{ab}	77.00±16.42 ^{ab}	15.33±3.31 ^{b}	62.00±3.81	36.00±3.85	0.57±0.03	3.53±0.11	1.60±0.04	4.93±0.62 ^{ab}	2.03±0.04	456.00±52.31 ^{a}	12.33±1.52 ^{a}
	Grp 8	122.333±9.76 ^{a}	77.67±11.35 ^{ab}	15.67±2.23 ^{ab}	64.00±5.62	42.67±9.11	0.71±0.16	3.53±0.20	1.53±0.11	4.10±0.44 ^{ab}	2.00±0.11	284.33±13.05 ^{b}	7.67±0.76 ^{b}
	Grp 9	96.333±5.82 ^{b}	53.00±4.49 ^{b}	10.67±0.84 ^{b}	61.00±7.67	33.67±4.36	0.61±0.13	3.77±0.17	1.60±0.06	5.13±0.43 ^{ab}	2.16±0.06	346.67±24.27 ^{ab}	9.67±1.17 ^{ab}
P-value		0.040	0.036	0.036	0.304	0.200	0.186	0.261	0.674	0.008	0.687	<0.001	0.035

VLDL- very low-density lipoprotein; HDL - high density lipoprotein; LDL - low density lipoprotein; aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Table 4- Effect of different bedding sources (sand, wood shavings and paper) and bedding treatments (no treatment - controls, Bentonite and lime) on broilers' immune system (log2).

Parameters		Vaccinal antibody titres				TSRBC_d22			TSRBC_d38		
		Influenza_d29	Influenza_d36	Newcastle_d15	Newcastle_d26	Total_AB	IgG	IgM	Total_AB	IgG	IgM
Bedding	Sand	3.33±0.16	2.44±0.12^{a}	1.44±0.16	0.44±0.16	1.11±0.29	0.33±0.11	0.78±0.25	2.33±0.23^{ab}	0.89±0.14	1.44±0.23^{ab}
	Wood shavings	2.89±0.31	2.11±0.08^{b}	1.44±0.22	0.11±0.08	0.89±0.16	0.56±0.12	0.44±0.12	3.44±0.58^{a}	0.78±0.10	2.67±0.55^{a}
	Paper	2.89±0.27	2.11±0.08^{b}	1.44±0.20	0.11±0.08	0.78±0.19	0.33±0.11	0.44±0.12	2.00±0.28^{ab}	0.89±0.14	1.11±0.21^{b}
P-value		0.370	0.020	0.396	0.068	0.559	0.305	0.305	0.033	0.774	0.010
Amendements	Controls	2.67±0.28^{b}	2.11±0.08	1.67±0.11^{a}	0.11±0.08	0.78±0.21^{ab}	0.44±0.12	0.44±0.10^{b}	3.67±0.52^{a}	1.00±0.11	2.67±0.40^{a}
	Bentonite	2.78±0.28^{b}	2.22±0.10	1.00±0.16^{b}	0.44±0.17	1.44±0.26^{a}	0.44±0.12	1.00±0.23^{a}	2.11±0.37^{b}	0.78±0.10	1.33±0.36^{b}
	Lime	3.67±0.11^{a}	2.33±0.11	1.66±0.23^{a}	0.11±0.08	0.56±0.12^{b}	0.33±0.11	0.22±0.10^{b}	2.00±0.16^{b}	0.78±0.15	1.22±0.15^{b}
P-value		0.007	0.228	0.001	0.068	0.012	0.747	0.004	0.005	0.353	0.012
Treatments	Grp 1	3.33±0.21^{ab}	2.33±0.21	2.00±0.00^{a}	1.00±0.37^{a}	0.67±0.42	0.33±0.21	0.33±0.21^{b}	3.00±0.37^{ab}	1.00±0.00	2.00±0.37^{ab}
	Grp 2	3.33±0.21^{ab}	2.33±0.21	1.00±0.00^{b}	0.00±0.00^{b}	2.00±0.63	0.33±0.21	1.67±0.56^{a}	2.00±0.37^{aa}	0.67±0.21	1.33±0.42^{ab}
	Grp 3	3.33±0.42^{ab}	2.67±0.21	1.33±0.42^{ab}	0.33±0.21^{ab}	0.67±0.21	0.33±0.21	0.33±0.21^{b}	2.00±0.37^{ab}	1.00±0.37	1.00±0.37^{b}
	Grp 4	2.67±0.21^{ab}	2.00±0.00	1.67±0.21^{ab}	0.00±0.00^{b}	1.00±0.37	0.67±0.21	0.67±0.21^{ab}	5.33±1.12^{a}	1.00±0.00	4.33±1.12^{a}
	Grp 5	2.00±0.73^{b}	2.33±0.21	1.00±0.37^{b}	0.33±0.21^{ab}	1.00±0.37	0.67±0.21	0.67±0.21^{ab}	2.67±1.05^{ab}	0.67±0.21	2.00±0.97^{ab}
	Grp 6	4.00±0.00^{a}	2.00±0.00	1.67±0.21^{ab}	0.00±0.00^{b}	0.67±0.21	0.33±0.21	0.00±0.00^{b}	2.33±0.21^{ab}	0.67±0.21	1.67±0.21^{ab}
	Grp 7	2.00±0.63^{b}	2.00±0.00	1.33±0.21^{ab}	0.33±0.21^{ab}	0.67±0.42	0.33±0.21	0.33±0.21^{b}	2.67±0.76^{ab}	1.00±0.37	1.67±0.56^{ab}
	Grp 8	3.00±0.00^{ab}	2.00±0.00	1.00±0.37^{b}	0.00±0.00^{b}	1.33±0.21	0.67±0.21	0.67±0.21^{ab}	1.67±0.21^{b}	1.00±0.00	0.67±0.21^{b}
	Grp 9	3.67±0.21^{ab}	2.33±0.21	2.00±0.37^{a}	0.00±0.00^{b}	0.33±0.21	0.00±0.00	0.33±0.21^{b}	1.67±0.21^{b}	0.67±0.21	1.00±0.00^{b}
P-value		0.040	0.051	0.001	0.002	0.053	0.293	0.006	0.005	0.752	0.003

Table 5- Effect of different bedding sources (sand, wood shavings and paper) and bedding treatments (no treatment - controls, Bentonite and lime) on the weight (absolute and relative) of broilers' main immune organs.

Parameters		Thymus weight		Liver weight		Spleen weight		Bursa of Fabricius weight	
		Absolute (g)	Relative (%)	Absolute (g)	Relative (%)	Absolute (g)	Relative (%)	Absolute (g)	Relative (%)
Bedding	Sand	7.00±0.51	0.28±0.02	61.13±1.65	2.50±0.06	3.201±0.21	0.13±0.01	2.01±0.25	0.08±0.01
	Wood shavings	8.37±0.70	0.32±0.03	67.78±4.00	2.62±0.15	3.21±0.15	0.12±0.01	1.55±0.11	0.06±0.01
	Paper	8.28±0.55	0.31±0.02	66.71±3.12	2.49±0.12	3.26±0.22	0.12±0.01	1.99±0.22	0.07±0.01
P-value		0.193	0.435	0.271	0.676	0.976	0.731	0.196	0.150
Amendements	Controls	8.33±0.61	0.30±0.02	71.07±3.80	2.61±0.16	3.23±0.20^{ab}	0.12±0.01^{b}	1.83±0.28	0.07±0.01
	Bentonite	7.85±0.73	0.31±0.03	63.54±3.03	2.52±0.11	3.65±0.20^{a}	0.15±0.01^{a}	1.98±0.14	0.08±0.01
	Lime	7.47±0.44	0.30±0.02	61.01±1.80	2.48±0.50	2.79±0.11^{b}	0.11±0.00^{b}	1.74±0.17	0.07±0.01
P-value		0.606	0.965	0.055	0.732	0.004	0.001	0.701	0.429
Treatments	Grp 1	7.56±1.19	0.29±0.04	66.45±1.41^{ab}	2.57±0.06	3.32±0.36^{ab}	0.13±0.01^{ab}	2.18±0.71	0.08±0.02
	Grp 2	6.91±1.00	0.28±0.03	58.40±2.10^{b}	2.38±0.36	3.80±0.36^{a}	0.15±0.01^{a}	2.41±0.00	0.10±0.00
	Grp 3	6.52±0.42	0.29±0.02	58.54±3.61^{b}	2.54±0.12	2.49±0.06^{b}	0.11±0.00^{ab}	1.46±0.11	0.06±0.01
	Grp 4	8.03±1.39	0.29±0.05	81.69±9.84^{a}	3.01±0.42	3.63±0.29^{ab}	0.13±0.01^{ab}	1.40±0.04	0.05±0.00
	Grp 5	8.82±1.69	0.35±0.06	59.83±2.46^{b}	2.39±0.13	3.24±0.09^{ab}	0.13±0.01^{ab}	1.63±0.31	0.07±0.01
	Grp 6	8.26±0.29	0.33±0.00	61.83±1.66^{ab}	2.46±0.08	2.76±0.25^{ab}	0.11±0.01^{ab}	1.63±0.12	0.07±0.01
	Grp 7	9.39±2.0	0.33±0.03	65.07±3.73^{ab}	2.25±0.17	2.73±0.31^{ab}	0.09±0.01^{b}	1.92±0.49	0.07±0.02
	Grp 8	7.82±1.12	0.30±0.04	72.39±7.72^{ab}	2.79±0.27	3.93±0.45^{a}	0.15±0.02^{a}	1.92±0.17	0.07±0.01
	Grp 9	7.63±1.18	0.29±0.04	62.66±3.91^{ab}	2.43±0.09	3.11±0.17^{ab}	0.12±0.01^{ab}	2.13±0.45	0.08±0.01
P-value		0.688	0.927	0.028	0.214	0.009	0.001	0.467	0.350

Discussion

In poultry systems, various dry and absorptive materials are often used as bedding. The composition of litter quality changes throughout the rearing period due to addition of excreta, feed and feathers, and accumulation of wasted feed and water, which are further decomposed by moisture and local microbiota. These changes may affect the productivity of broilers (Huang et al. 2009; Uno et al. 2011; Bjedov et al. 2013) by indirectly interfering with gut health and immunity (Garrido et al. 2004; Torok et al. 2009). The search for alternative bedding materials of economical and regional interest led us to test sand, wood shavings, and paper as suitable substitute bedding materials. We found that the type of bedding material did not affect the blood biochemistry parameters of broilers with exception of alanine aminotransferase. We also found some differences in the bird's immunity, as measured by the antibody production against Influenza and after SRBC stimulation. To maintain the hygienic quality of litters, chemical treatments can be implemented (Ivanov 2001; Line 2002; Garrido et al. 2004; Choi et al. 2008), but it is of upmost importance that the product used should not interfere with the health and productivity of broilers. In this study, we found some evidence that amendments used to treat litter beddings interfered with the bird's parameters analyzed in the current study. Generally, Lime amendments increased the values for the hepatic enzymes, as well as the liver weight. Moreover, litter amendments, particularly the Bentonite, interfered with the bird humoral immune competency, but these effects were beneficial as the broilers as resulted in better humoral immunity traits. From the major immune organs, differences among chemical amendments were only found for the spleen weight. However, none of the additives compromised the final weight or productivity of the birds (Seidavi et al. 2015).

Overall different types of bedding and various amendments caused, minor changes in lipids (total cholesterol and triglycerides) and uric acid concentrations in blood. Total cholesterol and triglycerides were slightly increased in the group of Bentonite-treated paper litters, but decreased when sand and wood shavings were treated with Bentonite. This result suggests possible hypocholesterolaemic and hypolipidemic actions that would also limit fat deposition in tissues (Piotrowska et al. 2011). In contrast, it would be expected that in paper beddings treated with Bentonite, fat accumulation in tissues would be increased compared with the other groups.

Conclusion

In conclusion, we found that despite the differences in the blood parameters and the humoral immunity traits, the type of litter and amendments did not have negative effects on broilers. The fact that Bentonite treatment showed different effects depending on the type of used bedding materials also suggests that different litter materials may need distinct amendments to maximize the final quality of carcasses.

Conflict of interest statement

The authors have no conflict of interest to declare.

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References

Bilgili SF, Alley MA, Hess JB and Nagaraj M, 2006. Influence of age and sex on footpad quality and yield in broiler chickens reared on low and high density diets. Journal of Appllied Poultry Research 15: 433-441

Bjedov S, Žikić D, Perić L, ĐukićStojčić M and Milošević N, 2013. Effect of different litter treatments on production performance of broiler chickens. Biotechnology in Animal Husbandry 29: 625-630

Choi IH, Kim JN and Kwon YM, 2008. Effects of chemical treatments on pH and bacterial population in poultry litter: a laboratory experiment. British Poultry Science 49: 497-501.

Dawkins MS and Layton R, 2012. Breeding for better welfare: genetic goals for broiler chickens and their parents. Animal Welfare-The UFAW Journal 21: 147-155.

Ebrahimi A, Santini A, Alise M, Pourhossein P, Miraalami N and Seidavi A, 2015. Effect of dried citrus sinensis peel on gastrointestinal microbiota and immune system traits of broiler chickens. Italian Journal of Animal Science 14: 712-717.

Garcês A, Afonso SMS, Chilundo A and Jairoce CTS, 2013. Evaluation of different litter materials for broiler production in a hot and humid environment: 1. Litter characteristics and quality. Journal of Appllied Poultry Research 22: 168-176.

Garcia R, Almeida PI, Caldara FR, Nääs IA, Pereira DF, Freitas LW and Graciano J, 2010. Effect of the litter material on drinking water quality in broiler production. Revista Brasileira de Ciência Avícola 12: 165-169.

Garrido MN, Skjervheim M, Oppegaard H and Sørum H, 2004. Acidified litter benefits the intestinal flora balance of broiler chickens. Applied and Environmental Microbiology 70: 5208-5213.

Grimes JL, Carter TA, Gernat AE, Godwin JL, 2007. A novel bedding material made from cotton waste, gypsum, and old newsprint for rearing turkeys. Journal of Applied Poultry Research 16: 598-604.

Huang Y, Yoo JS, Kim HJ, Wang Y, Chen YJ, Cho JH and Kim IH, 2009. Effect of bedding types and different nutrient densities on growth performance, visceral organ weight, and blood characteristics in broiler chickens. Journal of Applied Poultry Research 18: 1-7.

Ivanov IE, 2001. Treatment of broiler litter with organic acids. Research in Veterinary Science 70: 169-173.

Karamanlis X, Fortomaris P, Arsenos G, Dosis I, Papaioannou D, Batzios C and Kamarianos A, 2008. The effect of a natural zeolite (clinoptilolite) on the performance of broiler chickens and the quality of their litter. Asian-Australian Journal of Animal Science 21: 1642-1650.

Klasing KC, 2007. Nutrition and the immune system. British Poultry Science 48: 525-537.

Line JE, 2002. Campylobacter and Salmonella populations associated with chickens raised on acidified litter. Poultry Science 81: 1473-1477.

Macklin KS and Krehling JT, 2010. The use of metam-sodium to reduce bacteria in poultry litter. Journal of Applied Poultry Research 19: 274-278.

Monira KN, Islam MA, Alam MJ and Wahid MA, 2003. Effect of litter material on broiler performance and evaluation of manurial value of used litter in late autumn. Asian Australian Journal of Animal Science 16: 555-557.

Nahavandinejad M, Seidavi A, Asadpour L and Payan-Carreira R, 2014. Blood biochemical parameters of broilers fed differently thermal processed soybean meal. Revista MVZ Córdoba 19: 4301-4315.

Piotrowska A, Burlikowska K and Szymeczko R, 2011. Changes in blood chemistry in broiler chickens during the fattening period. Folia Biology of Krakow 59:183-187.

Pourhossein Z, Qotbi AAA, Seidavi AR, Laudadio V, Centoducati G and Tufarelli V, 2015. Effect of different levels of dietary sweet orange (*Citrus sinensis*) peel extract on humoral immune system responses in broiler chickens. Animal Science Journal 86: 105-110.

Seidavi A, Asadpour L, Dadashbeiki M and Payan-Carreira R, 2014. Effects of dietary fish oil and green tea powder supplementation on broiler chickens immunity. Acta Scientiae Veterinariae 42: 1-13.

Shabani S, Seidavi A, Asadpour L and Corazzin M, 2015. Effects of physical form of diet and intensity and duration of feed restriction on the growth performance, blood variables, microbial flora, immunity, and carcass and organ characteristics of broiler chickens. Livestock Science 180: 150-157.

Škrbić Z, Pavlovski Z, Lukić M, Petričević V and Milić D, 2012. The effect of lighting program and type of litter on production and carcass performance of two broiler genotypes. Biotechnology in Animal Husbandry 28: 807-816.

Sohirat Torfy M, Mirzadeh KH, Tabatabaei Vakili S and Chaji M. 2017. Effects of different levels of nano-selenium solution in water on performance, ileum microbial populations, tibia bone parameters, litter quality and some blood parameters of broiler chickens. Journal of Animal Science Researches 26(4): 189-211.

Swain BK and Sundaram RNS, 2000. Effect of different types of litter material for rearing broilers. British Poultry Science 41: 261-262.

Taherparvar G, Seidavi AR, Asadpour L, Payan-Carreira R, Laudadio V and Tufarelli V, 2016. Effect of litter treatment on growth performance, intestinal development, and selected cecum microbiota in broiler chickens. Revista Brasileira de Zootecnia (Brazilian Journal of Animal Science) 45: 257-264.

Torok VA, Hughes RJ, Ophel-Keller K, Ali M and MacAlpine R, 2009. Influence of different litter materials on cecal microbiota colonization in broiler chickens. Poultry Science 88: 2474-2481.

Villagra A, Olivas I, Benitez V and Lainez M, 2011. Evaluation of sludge from paper recycling as bedding material for broilers. Poultry Science 90: 953-957.

تأثیر بستر و مواد شیمیایی اضافه شده به آن بر فراسنجه‌های خونی و ایمنی جوجه‌های گوشتی

گیلانه طاهرپرور[1]، علیرضا صیداوی[2]*، لیلا اسدپور[3]، ریتا پایان-کاریرا[4]

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[1] دانش‌آموخته کارشناسی ارشد گروه علوم دامی، واحد رشت، دانشگاه آزاد اسلامی، رشت، ایران
[2] استاد گروه علوم دامی، واحد رشت، دانشگاه آزاد اسلامی، رشت، ایران
[3] دانشیار گروه زیست‌شناسی، واحد رشت، دانشگاه آزاد اسلامی، رشت، ایران
[4] استاد بخش ICAAM و گروه دامپزشکی، دانشگاه Évora، [Pole at Mitra]، Évora، پرتغال
* مسئول مکاتبه:Email: alirezaseidavi@iaurasht.ac.ir

چکیده

زمینه مطالعاتی: کف سالن پرورش جوجه‌ها شامل بستر و مواد اضافه شده ناشی از دفع مدفوع، خوراک، پرها و آب است. در حال حاضر پذیرفته شده است که کیفیت بستر ممکن است منشأ مشکلات زیست محیطی و مدیریتی در صنعت طیور تجاری باشد. مطالعات متعددی در مورد تأثیر جنس یا کیفیت مواد بستر بر سلامت روده و ایمنی طیور در دسترس است، اما اغلب آنها بر روی عوامل بیماری‌زای خاصی که به طور بالقوه برای انسان یا دام های دیگر مضر هستند و یا سبب محدودیت بر عملکرد جوجه‌های گوشتی می‌شوند متمرکز شده‌اند. **هدف**: این مطالعه با هدف ارزیابی اینکه آیا نوع مواد بستر (ماسه، تراشه چوب و رول کاغذی) یا افزودن دو ماده شیمیایی (آهک و بنتونیت در مقابل گروه کنترل) ممکن است در پارامترهای خونی و مصونیت جوجه‌های گوشتی اختلال ایجاد کند انجام شد. **روش کار**: دویست و هفتاد جوجه گوشتی نر سویه راس به طور تصادفی به نه تیمار با سه تکرار در هر تیمار (در مجموع ۳۰ پرنده در هر تیمار) تخصیص داده شدند. از آزمایش فاکتوریل ۳×۳ بر پایه طرح کاملاً تصادفی استفاده شد. حیوانات وارد قفس‌های زمینی شدند که حاوی بسترهای مختلف (ماسه ، تراشه چوب و رول کاغذی) و مواد شیمیایی (بدون تغییر یا شاهد، بنتونیت و آهک) بودند. هر تکرار شامل ۱۰ جوجه بود و در مجموع ۲۷ واحد آزمایشی استفاده شد. از روز اول، نه گروه آزمایشی با توجه به جنس بستر و مواد شیمیایی افزوده شده، به شرح زیر ایجاد شد: گروه ۱: – بستر ماسه بدون افزودنی؛ گروه ۲: بستر ماسه همراه با بنتونیت؛ گروه ۳: بستر ماسه همراه با آهک؛ گروه ٤: بستر تراشه چوب بدون افزودنی؛ گروه ٥: بستر تراشه چوب همراه با بنتونیت؛ گروه ٦:بستر تراشه چوب همراه با آهک؛ گروه ۷: بستر رول کاغذی بدون افزودنی؛ گروه ۸: بستر رول کاغذی همراه با بنتونیت؛ و گروه ۹:بستر رول کاغذی همراه با آهک. **نتایج**: یافته‌ها نشان داد که سه نوع بستر استفاده شده (ماسه، تراشه چوب و رول کاغذی) اکثر صفات مورد بررسی را تحت تأثیر قرار نمی‌دهند. اگرچه افزایش معنی‌داری در تیترهای آنتی‌بادی آنفلوانزا در روز ۳٦ ($P<0.05$) در گروه پرورش یافته روی ماسه و نیز افزایش معنی‌داری در تیترهای ایمونوگلوبولین کل (Ig) به دلیل افزایش IgM در ١٤ روز پس از چالش با گلبول‌های قرمز خون گوسفند($P<0.05$) مشاهده شد. فرآوری بستر با بنتونیت یا آهک تاثیر عمده‌ای بر صفات ایمنی هومورال داشت. افزودن آهک، تیترهای آنتی بادی برای آنفلوانزا را در اولین چالش افزایش داد، اما بر روی چالش دوم آنها تأثیری نداشت. در مقابل، تیمارهای آهک باعث کاهش تیتر Igبه دلیل افزایش IgM در روز ۳۸ شدند. مقایسه این نه گروه (نوع بستر x مواد شیمیایی اضافه شده) حاکی از تفاوت‌های اندکی در برخی

پارامترهای خون و صفات ایمنی هومورال بود. **نتیجه‌گیری نهایی**: اگرچه هیچ‌گونه اثرات مضری بر جوجه‌های گوشتی مشاهده نشد، اما نتایج حاکی از آن است که افزودن مواد مختلف به بستر نیاز به بررسی و حداکثرسازی کیفیت لاشه هم دارد.

واژگان کلیدی: جوجه‌های گوشتی، سیستم ایمنی، کیفیت بستر، ماده بستر، مکمل‌سازی/ تیمار شیمیایی، میکروبیوتا