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Effect of litter treatment on growth performance, intestinal development, and selected cecum microbiota in broiler chickens

Gilaneh Taherparvar[1], Alireza Seidavi[1], Leila Asadpour[2], Rita Payan-Carreira[3,4], Vito Laudadio[5], Vincenzo Tufarelli[5]

[1] *Rasht Branch, Islamic Azad University, Department of Animal Science, Rasht, Iran.*
[2] *Rasht Branch, Islamic Azad University, Department of Veterinary Science, Rasht, Iran.*
[3] *Universidade de Trás-os-Montes e Alto Douro, Department of Zootechnics, Vila Real, Portugal.*
[4] *Universidade de Trás-os-Montes e Alto Douro, Animal and Veterinary Research Centre, Vila Real, Portugal.*
[5] *University of Bari "Aldo Moro", Section of Veterinary Science and Animal Production, Department of Emergency and Organ Transplantation, Valenzano, Bari, Italy.*

ABSTRACT - The objective of this study was to determine whether the type of bedding materials (sand, wood shavings, and paper) and of two chemical amendments (lime and bentonite) could interfere with litter quality (moisture, pH, and total bacterial counts), thereby influencing also the growth performance and the development of intestinal traits and cecum microbiota of chickens. Two hundred and seventy male Ross 308 broiler chickens were randomly assigned into nine treatment groups with three replicates per treatment. Broiler productive parameters, relative weight of different intestinal segments, content of cecal total bacterial counts (total aerobic bacteria, *Lactobacilli*, and coliforms), as well as litter moisture, pH, and total aerobic bacteria and coliforms counts, were assessed. Litter material, *per se*, did not significantly affect the productivity parameters at the end of the experimental period (42 days) with the exception of protein efficiency. A significant trend was found among treatments with regard to weight gain and feed intake, with lower performance in birds on sand beddings. Litter pH was relatively homogenous between bedding types and amendments, but the moisture was significantly lower when sand was used. Litter type did not influence the relative weight of the different intestinal segments; however, the type of amendment affected the relative jejunum weight, which was increased in bentonite-treated litter. The use of lime and bentonite treatments may be helpful to decrease the differences in litter moisture associated with particular bedding materials. The tested amendments do not interfere with the productive performance of birds.

Key Words: bedding material, broilers, growth, litter quality, microbiota

Introduction

In intensive commercial broiler production, birds are reared in floors using different types of litter material. As poultry production increases, the amount of litter required by the system is also increased. Consequently, both litter management and disposal raise important challenges to the poultry industry, and a major parameter is its economy. Litter source material usually varies according to regions (Monira et al., 2003; Škrbić et al., 2012); sawdust, rice or oat hulls, sugarcane pulp or bagasse, chopped straw, paper mill by-products, sand, wood shavings, corn cobs, and dried leaves are often used as litter source (Swain and Sundaram, 2000). Litter is composed of the bedding material plus the excreta, feed, feathers, and water. Its moisture and quality have been associated with health and performance as well as with broiler welfare, as it should reduce the floor humidity while giving the sense of comfort and allowing natural scratching behaviour (Karamanlis et al., 2008; Škrbić et al., 2012). It also helps the thermal insulation, moisture absorption, and reduction of ammonia emissions and serves as a protective barrier from the ground (Bjedov et al., 2013). Litter quality may be the origin of environmental and management problems in the commercial poultry industry (Karamanlis et al., 2008; Garcia et al., 2010) if not properly selected or managed. In broilers, 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 as partially due to litter condition (Bilgili et al., 1999; Garcia et al., 2010). Ideally, the bedding material has to be absorbent, have a reasonable drying time, and be innocuous to poultry and farmers (Grimes et al., 2007), but it also needs to meet hygienic requirements and ensure controlled ammonia concentrations throughout the productive cycle (Villagrá et al., 2011).

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Corresponding author: vincenzo.tufarelli@uniba.it
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The litter quality is a major concern in chicken production not only because it affects the flock health and productivity (Bilgili et al., 2006; Bjedov et al., 2013; Garcês et al., 2013), but it may act as potential reservoir and transmission vehicle for pathogens and potential pathogens. Besides seeking increased performance, the industry is also concerned with consumer confidence in the food supply chain, thereby endorsing several studies on the benefits of different bedding materials and litter chemical treatments/amendments to reduce the presence of pathogenic bacteria and to improve broiler productive traits. Manipulation of pH and water activity of the litter will modulate its microbiota content and may also contribute to improving the competence of the immune system of birds (Lee et al., 2011).

Because the litter type can significantly affect the efficiency of the broiler immune system and therefore its growth, this study aimed to analyse the effects of two alternative amendments (lime and bentonite) on the quality of litter using three different bedding materials (sand, wood shavings, and paper) and their effects on growth performance, intestinal traits, and cecum microbiota (total aerobic bacteria count, lactic acid, and coliforms) in broilers.

Material and Methods

This study was conduct at a commercial poultry farm in Abkenar (37°27' N, 49°19' E, 26 m below sea level), Rasht Branch, Iran. The study was approved by the Scientific Board of the Islamic Azad University, and was conducted in compliance with the International Guidelines for Research Involving Animals (Directive 2010/63/EU).

A total of 270 one-day-old male Ross 308 broiler chicks were randomly distributed into nine treatments, with three replicates per treatment, in a total of 30 birds per treatment. Chicks were purchased from a local hatchery and randomly assigned into groups with similar mean body weight. Chicks were reared until the age of 42 days, thus covering three periods: starter (1-14 days), grower (15-35 days), and finisher (36-42 days).

The animals were housed in a unique floored land compartment, split by metal divisions into smaller experimental units (1.5 × 1.5 m). All broilers had a common environment except for the litter materials. Thermo-neutral ambient temperature, cross-ventilation, and relative humidity were maintained in accordance with standard brooding practices (Laudadio et al., 2012) and adapted to the bird rearing stages (Aviagen, 2009). Lighting was provided for 24 h on day 1, and thereafter for 23 h/day, with one hour of darkness, from 19.00 h to 20.00 h. Broiler chickens received *ad libitum* diets (Tables 1 and 2) and water. Routine vaccination and deworming complied with regional veterinary authority.

A completely randomized design was used, with animals logged in experimental pens containing different bedding materials/subtracts (sand, wood shavings, and paper), according to the treatment group, in a total of 27 units. Bedding sources were treated as follows: Treatments 1 to 3 used beddings of sand comprising a control group with no litter treatment (Group 1), a group treated with bentonite (Group 2), and a group treated with lime (Group 3), respectively. Treatments 4 to 6 used wood shavings beddings comprising a control group without treatment (Group 4), a group treated with bentonite (Group 5), and another treated with lime (Group 6), respectively. Treatments 7 to 9 used paper as bedding material and comprised a control group without treatment (Group 7), a group treated with bentonite (Group 8), and a group treated with lime (Group 9), respectively. Bentonite was used at 3 kg/m^3, while the lime was used at 1.5 kg/m^3.

Feed intake and weight gain were recorded weekly and used in the calculation of productivity. The efficiency parameters were estimated as follows:

Feed conversion ratio (FCR) = feed intake (kg)/weight gain (kg);

Table 1 - Ingredient composition of the experimental basal diet fed to broiler chickens

Ingredient (g/kg)	Age (days)				
	1-7	8-15	16-23	24-35	36-42
Corn	454.0	510.0	500.0	460.0	436.0
Wheat	90.0	100.0	140.0	190.0	255.0
Soybean meal	385.0	330.0	307.0	298.0	264.0
Soybean oil	20.0	20.0	20.0	20.0	20.0
Sodium bicarbonate	1.2	1.4	1.4	2.0	1.5
Dicalcium phosphate	23.0	10.0	10.0	6.0	6.0
Oyster powder	12.0	-	-	-	-
NaCl	2.3	2.0	1.8	2.0	1.7
Mineral mixture[1]	2.5	-	-	2.5	2.0
Vitamin mixture[2]	2.5	2.9	2.5	2.5	2.0
DL-methionine	2.6	3.1	2.0	2.2	1.0
L-lysine	2.0	2.0	2.0	0.5	0.5
Threonine	0.9	0.5	0.5	-	-
$CaCO_3$	-	15.0	12.0	12.0	10.0
Coccidiostat	0.5	0.5	-	-	-
Multi-enzyme	0.5	-	-	-	-
Avizyme® enzyme	-	0.5	0.5	0.5	-
Phyzyme® enzyme	-	0.1	0.1	0.1	0.1
Turmeric (*Curcuma longa*)	-	1.5	-	1.5	-
Probiotics	0.5	-	-	-	-
Toxin binder	0.5	0.5	0.2	0.2	0.2
Price (Rial/kg)	18,000	16,000	15,800	15,500	15,000
Price (Euro/kg)	0.43	0.38	0.37	0.37	0.35

[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 - 5,000 IU/g; vitamin D3 - 500 IU/g; vitamin E - 3 mg/g; vitamin K3 - 1.5 mg/g; vitamin B2 - 1 mg/g.

Protein conversion ratio (PCR) = protein intake (kg)/ weight gain (kg);

Energy conversion ratio (ECR) = metabolizable energy intake/weight gain (kg); and

Production index = chick weight at 42 days of age ×10)/(FCR × 42).

Additionally, feed cost per kg live weight was calculated in local currency (Rial).

At 42 d, three birds per group (one bird from each replicate) were randomly selected, weighted, subjected to a 6-h feed withdrawal, and stunned; after bleeding and debeaking, the most representative male birds were carefully selected with respect to body weight compared with the average body weight of the replicate. Intestines were aseptically removed and separated into anatomic portions (duodenum, jejunum, ileum, colon, and cecum) as described in Quinteiro-Filho et al. (2010), and then weighed. For the study of the gross anatomy of the small intestine, the total weight, length, and diameter of the duodenum, jejunum, ileum, right and left cecum, and rectum were determined.

For the microbiology studies, cecal segments were taken and transported to the laboratory on ice. At the laboratory, a representative sample of the cecal contents was collected into new plates, freshly prepared, poured, and weighted a day before use; after determination of the amount collected, the diluted cecal contents were incorporated into selective media (Dibaji et al., 2014). Collected cecum samples were homogenized in buffer phosphate saline (PBS) and serial 10-fold serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}, respectively) in PBS were prepared. From the 10^{-4}, 10^{-5}, and 10^{-6} dilutions, 100 µL were removed and smeared onto the Petri dish containing the medium. The growth media used in the study were MRS agar (Man Rogosa Sharpe agar) to enumeration of Lactobacilli, Macconkey agar for coliforms, and nutrient agar for total aerobic bacteria counts. All the plates were incubated at 37 °C. Lactobacilli bacteria were incubated in anaerobic conditions within an anaerobic jar for 72 h; Coliforms were incubated in aerobic conditions for 24 h; total aerobic bacteria were incubated in aerobic conditions for 48 h. At the end of the incubation periods, the number of colony forming units (cfu) was counted. Bacterial counts were reported as logarithm number of colony-forming units per gram of sample.

Table 2 - Nutritional composition of diets fed to broiler chickens

Item	Age (days)				
	1-7	8-15	16-23	24-35	36-42
Dry matter (%)	85.47	86.39	86.76	87.04	87.25
Metabolizable energy (kcal/kg)	2924	3058	3096	3100	3145
Crude protein (%)	22.09	19.57	18.94	18.73	17.79
Crude fiber (%)	2.71	2.64	2.63	2.63	2.60
Ether extract (%)	4.27	4.45	4.47	4.40	4.40
Choline (g/kg)	1.65	1.58	1.52	1.52	1.44
Linoleic acid (%)	2.22	2.33	2.32	2.26	2.23
Folic acid (mg/kg)	2.15	2.07	1.91	1.88	1.66
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
Isoleucine	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
Glycine + Serine	2.567	2.317	2.237	2.226	2.109
Phenylalanine + tyrosine	2.062	1.877	1.817	1.808	1.720
Methionine + cysteine	0.995	0.991	0.866	0.910	0.737
Minerals (%)					
Calcium	1.064	0.888	0.769	0.684	0.601
Available phosphorus	0.301	0.309	0.300	0.300	0.301
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

Simultaneously, after depopulation, litter samples were collected for physical and microbiological analysis. For the analysis of litter microbiota, one litter sample per replicate was collected with a spatula into tubes/plates for refrigerated transportation to the lab; thereafter, these samples were subjected to the same procedures described for the cecum content. Another sample was collected for measurement of physical properties (pH and moisture) of litter, based on conventional protocols. At the laboratory, the percentage moisture content in the litter was determined as the difference in the weight of the litter sample before and after drying up for 48 h at 65 ºC. Litter pH was determined using an electronic meter after macerating a litter sample in deionized water, at a ratio of 1:5.

Results are presented as means ± standard error of the mean (SEM). Shapiro-Wilks test confirmed the normal distribution of data, which was then analysed using a 3 × 3 factorial arrangement with three litter treatments (sand, wood shavings, and paper) and three chemical reagent treatments (no reagent/control, lime and bentonite). The significance of differences between means was analysed using the ANOVA procedure followed by a Tukey's HSD *post-hoc* test to separate means using IBM SPSS Statistics 19 software for Windows®. A P-value of 0.05 was used to assess significance among means.

Data regarding the gross intestinal measurements (length, width, and diameter) were extracted into a unique variable for each segment, using the principal components method of the factor analysis procedure and a correlation matrix. The resulting variable was used to assess the effects of treatment on the development of the intestinal segments. Pearson's correlation was used to test a putative association amongst equivalent microbiota counts in the cecal content and the litter, the microbiota content in the litter and pH or moisture; to establish the relationship between microbiota content, moisture, or pH with the development of the intestinal segments; and to test the relation between the latter and the productive parameters. P-values ≤0.05 were considered statistically significant.

Results

Overall, neither bedding materials nor treatments individually affected (P>0.2 and P>0.5, respectively) the productivity parameters at the end of the experiment, with the exception of protein efficiency (P<0.01). Bedding material influenced protein efficiency during the entire rearing period, whereas the wood shavings and paper materials showed similar performances. Moreover, when taken together, the litter material used showed to affect the weight gain and the energy efficiency in the starter period (P = 0.002 and P = 0.004, respectively), as well as the feed and the energy intakes (P = 0.002 and P = 0.022, respectively) in the grower period (data not shown).

Overall, although no statistical differences were found, feed intake was lowest in treatment groups 1 to 3, reared in sand litters. Treatments did not change feed efficiency values compared with controls, but when wood shavings litter was applied, there was a slight increase in feed intake. Similarly, non-significant lower weight gains were obtained at the end of the experiment in the sand litter compared with all the other groups, which is related to the slightly increased weight gain recorded for treatment groups 4 to 6 (wood shavings litters) and groups 8 and 9 (treated paper litters) during the rearing period (Table 3). Similarly, the mean final BW by the end of the trial was slightly, non-statically higher in wood shavings groups and in lime-treated paper bedding group, contrasting the lowest value in sand litter groups (Table 4). Despite the non-significant effects, in general, lime treatments seemed to be more effective than bentonite treatments in final weight gain.

In general, neither feed, energy, nor protein efficiency was significantly affected by treatments (Table 3). Lime-treated groups (Groups 3, 6, and 9, respectively) showed lower feed, energy and protein efficiencies than bentonite-treated groups (Groups 2, 4, and 8, respectively), except for groups with wood shavings bedding (Groups 4 to 6). Despite the lack of significance, sand and paper bentonite-treated litters (Groups 2 and 8) presented similar feed and energy efficiencies to their control counterparts (Groups 1 and 7), while both treatments in wood shavings litters (Groups 5 and 6) showed better feed and energy indices than their control (Group 4). This effect recorded for the wood shavings litters also extended to the energy and protein efficiency.

Table 3 - Effects of bedding materials and chemical treatments on performance parameters of Ross 308 broilers (1-42 days)

Treatment		Feed intake (g/day)	Weight gain (g/day)	Feed efficiency (g/g)	Energy intake (kcal/day)	Energy efficiency (kcal/g)	Protein intake (g/day)	Protein efficiency (g/g)
Litter	Sand	113.6	65.42	1.64	352.74	5.04	21.14	0.31b
	Wood shavings	118.5	68.74	1.60	368.01	4.93	22.07	0.30a
	Paper roll	115.6	67.74	1.59	359.15	4.90	21.53	0.29a
	SEM	1.80	1.32	0.03	5.21	0.07	0.32	0.02
	P-value	0.205	0.217	0.298	0.211	0.296	0.189	0.003
Chemical reagent	No reagent	116.9	68.06	1.61	363.07	4.95	21.76	0.30
	Bentonite	115.2	68.25	1.63	357.94	5.06	21.45	0.30
	Lime	115.6	67.59	1.59	358.89	4.90	21.53	0.30
	SEM	1.68	1.55	0.04	4.99	0.06	0.29	0.01
	P-value	0.829	0.642	0.417	0.830	0.422	0.827	0.801
Sand litter / No reagent		118.3	67.90	1.66	367.58	5.10	22.01	0.31
Sand litter / Reagent - bentonite		110.6	63.03	1.65	343.52	5.08	20.59	0.32
Sand litter / Reagent - lime		111.8	65.32	1.60	347.13	4.94	20.82	0.31
Shavings litter / No reagent		116.6	68.57	1.58	362.08	4.86	21.71	0.29
Shavings litter / Reagent - bentonite		120.5	68.67	1.62	374.29	4.99	22.44	0.30
Shavings litter / Reagent - lime		118.4	68.98	1.60	367.66	4.93	22.06	0.29
Paper litter / No reagent		115.8	67.70	1.59	359.56	4.90	21.56	0.30
Paper litter / Reagent - bentonite		114.6	67.05	1.62	356.02	4.99	21.32	0.29
Paper litter / Reagent - lime		116.5	68.47	1.57	361.88	4.82	21.70	0.29
	SEM	3.79	2.89	0.05	5.01	0.12	0.73	0.02
	P-value	0.579	0.772	0.753	0.588	0.757	0.552	0.102

SEM - standard error of the mean.
Means (±SEM) within each column of treatments with no common letter differ significantly at P<0.05.

The bentonite-treated groups presented non-significant higher costs per kg of live weight, particularly the treated wood shavings bedding groups (Table 4). With respect to the production index, it was slightly higher in wood shavings and paper-treated bedding groups (Groups 5, 6, 8, and 9) compared with those in sand (Groups 2 and 3), particularly for the lime-treated groups (Groups 3, 6, and 9), though the differences were not significant (Table 4).

Table 4 - Effects of bedding materials and chemical treatments on productive parameters of broilers

Treatment		Final body weight (g)	Production index
Litter	Sand	2797.4	408.6
	Wood shavings	2936.3	437.9
	Paper roll	2894.3	433.7
	SEM	54.14	12.65
	P-value	0.219	0.229
Chemical reagent	No reagent	2907.6	432.2
	Bentonite	2832.2	414.9
	Lime	2888.3	433.0
	SEM	59.33	12.15
	P-value	0.645	0.556
Sand litter / No reagent		2901.0	420.9
Sand litter / Reagent - bentonite		2689.3	389.5
Sand litter / Reagent - lime		2793.0	415.3
Shavings litter / No reagent		2928.7	442.1
Shavings litter / Reagent - bentonite		2933.7	432.8
Shavings litter / Reagent - lime		2946.7	438.7
Paper litter / No reagent		2893.0	433.4
Paper litter / Reagent - bentonite		2864.7	422.7
Paper litter / Reagent - lime		2925.3	445.0
	SEM	74.02	22.11
	P-value	0.775	0.841

SEM - standard error of the mean.

The bedding material or treatments did not affect the litter pH (Table 5). Also, treatments seemed not to alter the litter moisture (P = 0.833) despite the small variations among the treated litters and the controls (Table 5). Contrasting the treatments, the basic bedding material *per se* showed to affect the litter moisture (P≤0.0001), which was considerably lower in sand litter groups (Table 5). Individually, treatments significantly affected the litter moisture (P = 0.008).

The overall effects of the bedding materials or treatments on the total aerobic bacteria in both cecal content and in the litter were not significant (Table 5). Similarly, neither bedding material nor chemical treatment affected the cecal content in lactic acid-producing bacteria. No relationship was found between pH or moisture and the measurements collected for the different intestinal segments. The microbiota content in the cecal content and in the litter was not correlated. Moreover, no association was found between litter pH or moisture and the microbiota content in the litter (P>0.33). Likewise, the total aerobic bacteria, the lactic-acid producing bacteria and coliforms in the cecal content were independent of the litter pH or moisture. Also,

Table 5 - Effects of bedding materials and chemical treatments on cecal and litter microbiota (log of cfu/g) and on the litter pH and moisture at the end of the experiment (at day 42)

Treatment		Bacterial counts in cecal content			Bacterial counts in litter		Litter	
		Total aerobic bacteria	Lactic acid-producing bacteria	Coliforms	Total aerobic bacteria	Coliforms	pH	Moisture (%)
Litter	Sand	11.00	10.33	10.20	10.02	6.20	7.7	26.57a
	Wood shavings	10.94	10.49	10.79	10.18	5.99	7.9	47.27b
	Paper roll	11.24	10.84	10.52	10.25	6.08	7.4	41.49b
	SEM	0.22	0.19	0.23	0.11	0.14	0.03	2.15
	P-value	0.693	0.209	0.348	0.426	0.973	0.130	<0.001
Chemical reagent	No reagent	11.30	10.56	10.59	10.34	9.82	7.9	36.41
	Bentonite	10.88	10.47	10.05	10.05	9.56	7.6	39.41
	Lime	11.06	10.69	10.79	10.16	9.80	7.6	39.50
	SEM	0.09	0.21	0.19	0.13	0.10	0.02	3.85
	P-value	0.367	0.789	0.121	0.326	0.123	0.311	0.833
Sand litter / No reagent		11.33	10.51	10.00	9.77	9.79	7.7	26.10a
Sand litter / Reagent - bentonite		10.77	10.24	9.98	9.96	9.61	7.7	22.50a
Sand litter / Reagent - lime		11.02	10.17	10.84	10.15	9.85	7.7	31.11ab
Shavings litter / No reagent		11.17	10.00	11.46	10.44	9.88	8.3	44.43ab
Shavings litter / Reagent - bentonite		10.76	10.49	10.13	10.09	9.32	7.5	52.63b
Shavings litter / Reagent - lime		11.09	11.00	10.78	10.19	9.82	7.9	44.73ab
Paper litter / No reagent		11.36	11.23	10.45	10.50	9.80	7.7	38.70ab
Paper litter / Reagent - bentonite		11.18	10.70	10.13	10.08	9.66	7.6	43.10ab
Paper litter / Reagent - lime		11.04	10.80	10.79	10.15	9.71	7.0	42.66ab
	SEM	0.39	0.25	0.32	0.23	0.20	0.02	5.01
	P-value	0.926	0.674	0.540	0.726	0.655	0.194	0.008

SEM - standard error of the mean.
Means (±SEM) within each column of treatments with no common letter differ significantly at P<0.05.

no relationship was found between the bacterial content in the cecum or in the litter and the efficiency parameters tested in the present study.

Regarding the gross anatomy of the intestine, despite the small numerical variations among the results, the bedding material *per se* did not affect the relative weight of the different intestinal segments (Table 6). The type of chemical molecule used affected only the jejunum relative weight (P = 0.050). Moreover, the relative weight of all other intestinal segments was independent of the main chemical treatment used. Considering the relative jejunum weight, similar differences were observed between treated groups and their counterparts, but, in this respect, lime treatments showed higher relative weights, except for the paper bedding group (Group 9). Concerning the right or the left cecum relative weights, treatments slightly reduced the values of the groups (Table 6), but changes were not significant (P>0.05).

The principal component analysis was used to extract the information regarding additional anatomic parameters for the intestinal segments (length, width, and diameter). The bedding material *per se* did not influence the gross morphology of intestinal segments. However, the type of chemical used for litter treatment influenced the jejunum and the right cecum measurements (P = 0.005 and P = 0.030) respectively), with lime treatments showing lower values than controls and bentonite treatments regarding the jejunum measurements, or with bentonite presenting lower results than lime and control with regard to the right cecum measurements. Nevertheless, these differences lost significance when the effects of each treatment over the extracted variables were analysed individually. Despite the tendencies found concerning the intestinal segments relative weight, only a tendency was observed (P = 0.071) for treatment effects on the jejunum measurements, which seemed to be associated with lower measurements presented in lime-treated groups (Groups 3, 6, and 9) compared with controls (Groups 1, 4, and 7) and bentonite-treated groups (Groups 2, 5, and 8). Positive correlations were established between the ileum and left cecum measurements (P = 0.005) and the right and left cecum measurements (P = 0.003), but only a positive tendency was found for the association between the ileum and right cecum measurements (P = 0.056). No additional correlations, either positive or negative, were detected between the different intestinal segments. Duodenum measurements were negatively correlated with the total aerobic content in the litter (P = 0.016); no additional correlations were found between the measurements of the other intestinal segments and the microbiota content in the cecum or the litter. With regard to the relationship between the measurements collected at the different intestinal segments and the production parameters, positive correlations were noticed between the left cecum and feed efficiency (P = 0.007), the left cecum and energy efficiency (P = 0.007), and the duodenum and the production index (P = 0.047). The following negative associations were found between the intestinal segments extracted measurements and the production parameters: the duodenum and feed

Table 6 - Effects of bedding materials and chemical treatments on the relative weight of the intestinal segments of broilers (at day 42)

Treatment		Duodenum (%)	Jejunum (%)	Ileum (%)	Colon (%)	Right cecum (%)	Left cecum (%)
	Sand	1.01	3.72	0.75	0.12	0.37	0.40
	Wood shavings	1.01	3.01	0.76	0.10	0.36	0.40
Litter	Paper roll	0.98	3.31	0.78	0.10	0.33	0.38
	SEM	0.05	0.22	0.04	0.03	0.02	0.03
	P-value	0.908	0.237	0.935	0.223	0.859	0.889
	No reagent	1.05	3.13a	0.73	0.10	0.39	0.43
	Bentonite	1.03	3.52ab	0.82	0.11	0.33	0.35
Chemical reagent	Lime	0.93	3.40b	0.73	0.12	0.35	0.40
	SEM	0.06	0.25	0.05	0.04	0.03	0.04
	P-value	0.407	0.049	0.471	0.330	0.389	0.249
Sand litter / No reagent		1.07	3.29	0.81	0.11	0.42	0.45
Sand litter / Reagent - bentonite		1.04	3.72	0.83	0.10	0.29	0.30
Sand litter / Reagent - lime		0.93	4.13	0.60	0.16	0.41	0.46
Shavings litter / No reagent		1.07	2.73	0.71	0.08	0.40	0.43
Shavings litter / Reagent - bentonite		1.07	3.00	0.87	0.10	0.36	0.36
Shavings litter / Reagent - lime		0.89	3.31	0.70	0.11	0.32	0.40
Paper litter / No reagent		0.99	3.37	0.68	0.11	0.11	0.41
Paper litter / Reagent - bentonite		0.96	3.83	0.76	0.12	0.34	0.39
Paper litter / Reagent - lime		0.97	2.74	0.89	0.10	0.32	0.34
	SEM	0.15	0.29	0.14	0.02	0.04	0.05
	P-value	0.959	0.545	0.539	0.187	0.590	0.666

SEM - standard error of the mean.
Means (±SEM) within each column of treatments with no common letter differ significantly at P<0.05.

efficiency (P = 0.017) or the energy efficiency (P = 0.017); the ileum and weight gain (P = 0.050) or the final weight (P = 0.049); the colon and feed efficiency (P = 0.009), energy efficiency (P = 0.009) or the protein efficiency (P = 0.000); the left cecum and feed intake (P = 0.042), weight gain (P = 0.010), final weight (P = 0.010) and the production index (P = 0.004). A tendency was also found for a positive correlation between the extracted measurements for colon and the production index (P = 0.069), and for negative associations between the measurements extracted for the ileum and feed intake (P = 0.052) or the production index (P = 0.060).

Discussion

In poultry systems, the litter often integrates various dry and absorptive materials as bedding; throughout the rearing period, the quality of bedding material changes due to addition of excreta, feed, and feathers, and accumulation of wasted feed and water, which are further decomposed by moisture and local microbiota. One main goal for litter use is to provide a dry environment to birds, insulating chicks from cooling effects of the ground, reducing the contact with manure while allowing them to explore their natural scratching behaviour (Karamanlis et al., 2008; Škrbić et al., 2012; Bjedov et al., 2013).

Litter is of upmost importance to ensure a good in-house environment, since moisture and growth of bacteria or mould in the litter can largely influence poultry production either by reducing the incidence of lesions (such as breast blisters, skin burns, scabby areas, or bruising), leading to condemnations and downgrades of carcasses, or by reducing the side-effects of accumulation of gas emissions (in particular ammonia) that can negatively influence the growth of birds and increase feed conversion as well as increase the incidence of respiratory and ocular diseases (Liang et al., 2005).

Many products have been used as bedding. The type of bedding material determines the litter capacity for moisture absorption and control of the environmental quality. The present study, using three different bedding materials (sand, wood shavings, and paper) for a 42-day period, confirmed that different materials showed different drying properties. Dryness was higher when sand was used as bedding material, in comparison with paper rolls or wood shavings. Similar findings were reported in a previous study; Garcês et al. (2013), comparing the physical properties of different sources of litter bedding materials (including sand, wood shavings, and paper), refer to sand as the material showing the best absorptive properties, despite producing increased amount of litter. Contrastingly, wood shavings were the material with highest moisture, as was found in the present study. Those authors also report that by the end of the rearing period, there were no differences in litter pH amongst the bedding materials, further supporting the findings of the present work. Chemical amendments to the bedding material reduced in a non-significant manner the differences among groups concerning moisture or pH value. Additional studies in a larger number of cases are envisaged to highlight the existence of beneficial effects of either treatment on litter physical properties

The present study showed that despite the differences in moisture, the litter types and treatments did not influence the litter bacterial counts, or the productive traits analysed. Although the indices used to assess the poultry productive performance were slightly worse in sand beddings than for the other bedding materials (a reduced weight gain at the end of the experiments and final weight despite the increased feed intake, contrasting increased feed and energy efficiencies), those differences were not statistically significant when the entire rearing period was considered. Similar results were also described by Asaniyan et al. (2007), who compared the performance of broilers raised on sand and wood shavings litters.

Litter type did not significantly affect the absolute or relative weight of the different intestinal segments, whilst treatment only affected the relative jejunum weight. However, litter treatments influenced the extracted gross anatomy of the jejunum and the right cecum. Birds raised on sand litter or treated with bentonite showed the highest jejunum weights. Under normal circumstances, the major absorption of nutrients occurs in the duodenum and proximal jejunum (Noy and Sklan, 1995). Whether this finding would relate to a physical adaptation to the litter type, to the need to expand the digestive absorption capacity, or to an irritative reaction to the chemical needs to be addressed in the future. Considering that the extracted duodenum traits were negatively associated and feed efficiency, the hypothesis of a possible need to expand the digestive capacity cannot be discarded on the bases of the data presented here. Results from a study in turkeys showed that wood shavings can increase the intestinal development, particularly the jejunum, which was considered as a positive influence in gut health (Santos, 2006). This was also found in the present experiment.

Litter bedding materials or treatments did not determine the bacterial counts in the cecum at the end of the rearing period. Moreover, no association was found between the bacterial counts in the litter at the end of the experiments and the bacterial counts in the cecal content. This could be related to the fact that treatments normalised the moisture in the different bedding materials, allowing the litter microbiota to be kept under control. These findings also agree with the non-significant differences found regarding the intestinal traits and the productive parameters at the end of the rearing period, which are suggestive of inexistence of an environmental bacterial challenge (O'Reilly et al., 2013).

Conclusions

The use of lime and bentonite treatments allows a reduction of differences in moisture associated with diverse bedding materials throughout the experimental period. Moreover, the tested amendments do not interfere with the production traits or the bacterial contamination of the litter at the end of the rearing period and do not cause changes in the normal intestinal microbiota, thereby contributing to gut health and production traits.

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