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Original article

Effect of using biochar as litter on broiler welfare and health

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ABSTRACT

This study was undertaken to assess the effect of biochar used as litter on the welfare and health of broilers. 201 day-old broiler chicks of the Cobb 500 strain were divided into three experimental groups of 67 chicks each: the Biochar group (litter made up of 100% biochar from corn cobs), Mixed group (litter made up of 50% biochar and 50 % corn cobs) and the Corn cobs group (litter made up of 100% corn cobs). After 52 days of rearing, a blood sample was taken from the chickens to determine haematological, biochemical parameters and the concentration of the T_3 hormone. On the 56th day, 15 chickens from each batch were slaughtered. The presence of contact dermatitis was assessed by visual examination. The weights of the carcass and various organs were determined. The type of litter induced significant differences ($p<0.05$) for contact dermatitis The incidence of foot pad dermatitis was 0%, 46.67%, 26.67% and that of kock burns was 0%, 6.67% and 0% respectively for Biochar group, Mixed group and Corn cobs group. Significant variations ($p<0.05$) were recorded from the relative weights of the lungs. The number of white blood cells was higher ($p<0.05$) in the mixed group while no difference was recorded for the neutrophil/lymphocyte ratio ($p>0.05$). The

biochemical and hormonal parameters were similar whatever the type of litter ($p>0.05$). Biochar therefore improves the health status of broilers and does not harm their welfare. The evaluation of the physico-chemical and microbiological quality of the litters would be interesting for the understanding of the observed effects.

1. Introduction

The management of litter in the poultry sector is a crucial aspect of environmental protection since this litter, being dry at the beginning of breeding, becomes moistened as the bird droppings, which are rich in water, are added and drinking water leaking from water troughs (Martrenchar et al., 2002; Eichner et al., 2007). There is then a lowering of dry matter in the litter, a release of NH3 and a proliferation of the anaerobic bacterial flora consisting of pathogenic germs for poultry (Carlile, 1984). Ammonia releases can cause keratoconjunctivitis, respiratory damage and immunosuppression (Rylander and Carvalheiro, 2006). In addition, the contact of the legs and the bodies of the chickens with the litter is also a source of lesions, all factors which can lead to negative influence on the performance of the chickens (Zhang et al., 2011; Linhoss et al., 2019).

Several strategies are used to control humidity and ammonia (NH3) levels in poultry houses. These include ventilation and litter amendments (Sanni, 2014; Linhoss et al., 2019). Therefore, few studies have focused on the use of biochar as a litter amendment in poultry farming. The few studies that have been done in this area focused on chars produced from peanut hulls, pine chips, and coconut husks (Ritz et al., 2011) and pine chips biochar (Linhoss et al., 2019). The work carried out by Major et al. (2009) showed that biochar has a good water absorption capacity and its incorporation into the litter does not affect broiler performance (Ritz et al., 2011; Linhoss et al., 2019). However, the characteristics of biochar such as carbon content, energy and porosity vary depending on the type of biomass used (Weber and Quicker, 2018).

Several criteria are used to assess the welfare of chickens. These include the presence or absence of contact dermatitis and the variation in the proportions of blood elements. Bignon et al. (2015) reported that the type and presentation of litter can influence broiler welfare. The objective of this study is to evaluate the effects of the use of biochar from corn cobs as litter on the welfare and health of broiler chickens.

2. Materials and methods

2.1. Study area

The study took place in the municipality of Parakou, located in the department of Borgou in northern Benin. The municipality of Parakou is located between 9°15' and 9°27' of latitude North and 2°30' and 2°46' of longitude East (Anonymous, 2019). The climate of Parakou is of sudanese type with an alternation of a rainy season (May to October) and a dry season (November to April). The annual rainfall is between 858 and 1,400 mm and the average annual temperature varies between 26 and 27°C (Kassa et al., 2016).

2.2. Experimental design and animal management

The trial was carried out for 8 weeks from April 19 to June 14, 2022 on 201 day-old chicks of the Cobb 500 strain. These chicks were raised and distributed on three different poultry houses which differ in the type of litter: a group named “Corn cob” with a litter of 100% corncobs; a second group named “Mixed” with a litter consisting of a mixture of corncobs and biochar (50%-50%) and a third group named “Biochar” with a 100% biochar litter made only from biochar of corn cobs.

An identical quantity of 5 kg/m² of litter was put in place for each group at the start of the trial. The chicks were raised with a density of 5.5 chickens/m² in newly built barns and which have never housed poultry. They were cleaned and disinfected with VIRUNETND (potassium sulphate and persulphate) at a dose of 1% by spraying two weeks and two days before the installation of the chicks after the installation of the litter. The breeding

equipment has been thoroughly cleaned and disinfected with bleach. During rearing, the birds received adequate prophylactic care. They were fed commercial broiler starter feed from the Veto Services Group (GVS) for the first twelve days and from the thirteenth day to the end of the experiment, they were fed commercial growth feed GVS. Feeding and watering of the birds was provided *ad libitum*. Food was served twice a day, at 7 a.m. in the morning and 5 p.m. in the evening.

2.3. Data collection

2.3.1. Determination of ambient parameters

The ambient temperature and hygrometry of each barn were recorded using thermo-hygrometers every day at 7 a.m. under static ventilation. During the start-up period, the thermo-hygrometers were suspended in the middle of the buildings by strings at 30 cm from the level of the litter and during the rest of the time of the experiment at 50 cm.

2.3.2. Assessment of body injuries

The foot pad dermatitis scoring grid used by Olivere (2010). The lesion score is between 1 and 5. Thus score 1 corresponds to no lesion or to simple hyperkeratosis of the epidermis, scores 2 and 3 correspond to the presence of elongated brown scales on less than 50% of the skin surface of the footpad (score 2) or over more than 50% (score 3), scores 4 and 5, in the same way, correspond to ulcerative lesions covering less than 50% of the footpad (score 4) and more than 50% (score 5). Hock burns were scored using this same grid, replacing the plantar surface with the surface of the tarsal joint. The wishbone, possible signs of diarrhea and scratches were also observed.

2.3.3. Blood sample collection and analysis

The blood sample is taken between 8 a.m. and 10 a.m. on the 52nd day of the experiment. A venous blood sample is taken from the brachial vein in dry tubes and in tubes containing Ethyl Diamine Tetra Acetic Acid (EDTA) from six chickens per group. These blood samples were placed in a cooler with ice and transported to the laboratory for the determination of hematological and biochemical parameters. All these parameters were determined using automata.

The hematological parameters measured are white blood cell WBC count and its differentials (heterophils, lymphocytes, monocytes, eosinophils, immunoglobulin G and basophils), red blood cells (RBC), Hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelets (PLT).

The biochemical parameters measured are blood sugar, urea, serum creatinine, serum protein, transaminases (TGO and TGP). The dosage of the thyroid hormone triiodothyronine or T_3 was also carried out.

2.3.4. Determination of treatment effects on chicken weights and organs

At the end of the experiment, the chickens were weighed and then slaughtered using cervical dislocation method. After plucking with hot water, organs such as the liver, lungs, heart, and spleen were removed and examined macroscopically. The carcass and organ weights were then recorded. Relative organ weights were calculated. Relative organ weight = organ weight (g) / live body weight (LBW) (g).

2.4. Statistical analyzes

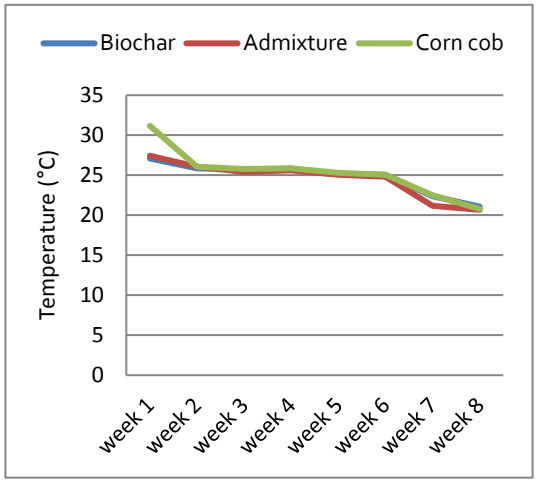
For assessing the effect of treatment on biochemical and haematological parameters, blood hormone levels, organ weights, an analysis of variance was performed in cases of normality. When normality was not acquired, the Kruskal-Wallis non-parametric test was performed. When the probability was significant ($p<0.05$), means were structured using the SNK function of the "agricolae" package (de Mendiburu, 2021). Then, the generalized linear model of the Poisson family enabled to evaluate the effect of the treatment on the PLT, the RDW-CV, the hock burn and foot pad dermatitis scores. All analyzes were performed with R 3.5.1 software (R Core Team, 2018).

3. Results

3.1. Ambient parameters

The ambient temperatures recorded in the barns were little different, except during the first and seventh weeks (Figure 1). A gradual decrease in temperature was noted in all batches as a function of time. Figure 2 shows

the evolution of relative humidity (RH) in livestock buildings as a function of time. HR increased gradually over time and was not highly variable with treatment.



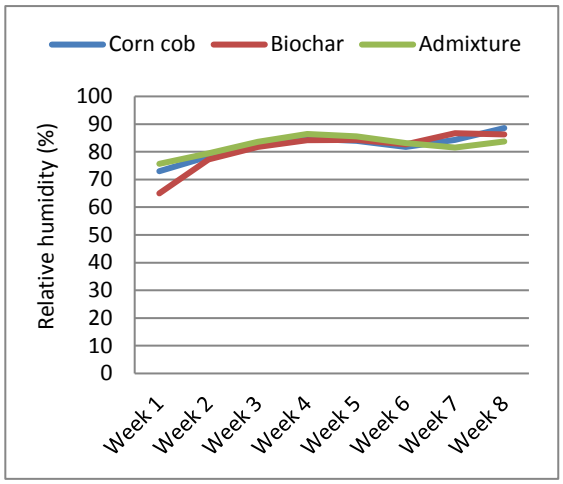
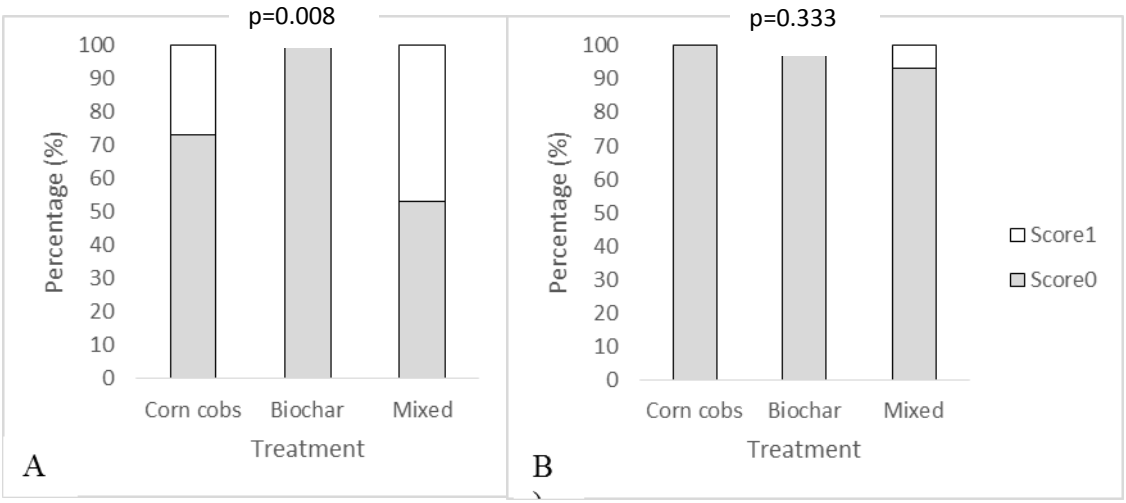


Fig. 1. Evolution of the temperature in the paddocks. **Fig. 2.** Evolution of relative humidity in the paddocks.

3.2. Effects of different litters on contact dermatitis in chickens

The type of litter significantly influenced ($p < 0.05$) the presence of contact dermatitis (Figure 3). Biochar did not cause contact dermatitis in chickens. The Mixed batch recorded the highest rates of contact dermatitis. The incidence of foot pad dermatitis is 0%, 46.67%, 26.67% respectively for the Biochar, Mixed, Corn cobs batches. Only the Mixed batch recorded cases of hock burns (6.67% of chickens).



Foot pad dermatitis (A); Hock burn (B)

Fig. 3. Distribution of chicken contact dermatitis according to litter type

3.3. Effects of different litters on live body weight and organs

Macroscopically and by direct visual examination, the organs showed no difference depending on the treatment. Table 1 shows the variation of organ weight in relation with live body weight. The type of litter had no effect on most organs except the lungs for which significant differences were recorded ($p < 0.05$). The relative weight of this organ was lower in the Mixed group compared to Biochar and Corn cobs groups.

Table 1
Variations in relative organ weight at 8 weeks of age according to type of litter.

	Treatement			
Parameters	**Biochar**	**Mixed**	**Corn cobs**	**P**
Carcass weight	1653.40[a]±77.00	1589.50[a]±61.80	1457.60[a]±76.60	0.167
Live weight (LBW)	2227.00[a]±104.00	2249.70[a]±87.40	2136.00[a]±112.00	0.706
Heart weight/LBW	0.006[a]±0.000	0.005[a]±0.000	0.005[a]±0.000	0.136
Liver weight/LBW	0.02[a]±0.00	0.02[a]±0.00	0.02[a]±0.00	0.394
Lung weight/LBW	0.007[b]±0.000	0.006[a]±0.000	0.007[b]±0.000	0.031
Spleen weight/LBW	0.0008[a]±0.0001	0.0009[a]±0.0001	0.0010[a]±0.0001	0.607

LBW: Live body weight. Letters on the same row compare the results of different treatments. Different letters show significant difference ($p < 0.05$).

3.4. Effects of different litters on biochemical and hematological parameters

Table 2 shows the variations in biochemical parameters in chickens according to the type of litter. No significant difference was noted between the batches for the parameters considered ($p > 0.05$). The variations of the haematological parameters according to the type of litter are presented in Table 3. The number of white blood cells and the number of lymphocytes of the Mixed batch are significantly higher ($p < 0.05$) than those of the Biochar and Rafle batches. No significant difference was noted between the batches concerning the Neutrophils / Lymphocytes ratio.

Table 2
Variations in blood biochemical parameters depending on the type of litter (mean ± standard deviation).

	Treatements			
Parameters	**Biochar**	**Mixed**	**Corn cobs**	**P**
Blood sugar (g/l)	1.89[a]±0.18	2.19[a]±0.13	1.88[a]±0.13	0.262
Urea (g/l)	0.08[a]±0.005	0.09[a]±0.005	0.09[a]±0.004	0.273
Creatinemia (mg/l)	13.28[a]±1.23	15.15[a]±1.83	16.32[a]±1.57	0.449
Protidemia (g/l)	33.89[a]±4.99	48.37[a]±4.23	38.46[a]±3.76	0.084
Transaminase TGP (UI/l)	10.71[a]±1.30	13.08[a]±2.25	7.07[a]±1.27	0.079
Transaminase TGO (UI/l)	49.90[a]±11.40	31.39[a]±6.63	49.10[a]±10.50	0.304

Letters on the same row compare the results of different treatments. Different letters show significant difference ($p < 0.05$).

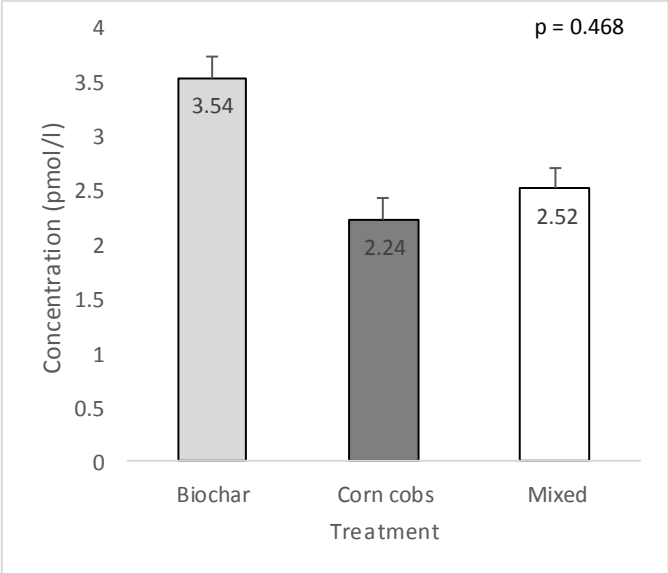


Fig. 4. Variation of the plasma concentration of the hormone Triiodothyronine (T_3) according to the type of litter.

Table 3
Variations in hematological parameters depending on the type of litter (mean ± standard deviation).

Parameters	Treatment			P
	Biochar	Mixed	Corn cobs	
WBC (10^3/µl)	6,54^b±11,80	10,73^a±4,35	6,79^b±12,40	0,016
RBC (10^6/µl)	1,87^a±0,18	5,60^a±3,46	2,21^a±0,07	0,410
HGB (g/dl)	5,80^a±0,52	6,38^a±0,33	6,68^a±0,09	0,221
HCT (%)	23,66^a±2,17	27,05^a±1,52	27,38^a±0,92	0,225
MCV (fl)	126,66^a±2,17	125,00^a±2,74	123,88^a±1,95	0,711
MCH (pg)	31,18^a±0,96	29,52^a±0,39	30,33^a±0,79	0,313
MCHC (g/dl)	24,62^a±0,71	23,65^a±0,28	24,52^a±0,66	0,426
PLT (10^3/µl)	3,40^a±0,51	3,67^a±0,49	3,50^a±0,76	0,972
PCT (%)	0,00±0,00	0,002±0,002	0,00±0,00	-
Heterophils (10^3/µl)	3,38^a±5,26	4,60^a±5,38	2,99^a±6,19	0,139
Lymphocytes (10^3/µl)	2,91^b±0,79	5,62^a±0,52	3,48^b±0,78	0,038
H/L	1,43^a±0,26	0,88^a±0,16	0,91^a±0,13	0,105
Monocytes (10^3/µl)	0,071^a±0,021	0,148^a±0,037	0,063^a±0,02	0,072
Eosinophils (10^3/µl)	0,002^a±0,002	0,002^a±0,001	0,001^a±0,00	0,896
Basophils (10^3/µl)	0,174^a±0,053	0,363^a±0,12	0,255^a±0,061	0,344
Immunoglobulins G (10^3/µl)	0,017^a±0,017	0,156^a±0,156	0,217^a±0,138	0,561

WBC: white blood cell, RBC: red blood cells, HGB: Hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, H/L: heterophils/lymphocytes, PLT: platelets
Letters on the same row compare the results of different treatments. Different letters show significant difference ($p < 0.05$).

The type of litter did not induce any significant variation in the blood concentration of the T_3 hormone in chickens (Figure 4). The highest average concentration (3.54 pmol/l) is obtained in the Biochar batch and the lowest (2.24 pmol/l) in the Corn cobs.

4. Discussion

The ambient temperatures measured during the test period fluctuated between 20.7°C and 31.2°C and are close to those (22.9°C and 23.9°C) reported by (Sourokou Sabi, 2014) in Senegal. These temperatures, which are supposed to be among the lowest of the day because they are recorded early in the morning, are higher than those recommended by Anonymous (2008) for Cobb broilers. The same applies to the relative humidity which is higher in this study. In warm conditions, increased relative humidity may result from evaporative water loss due to panting, with compensatory consumption leading to wetter droppings and litter (Anonymous, 2000). However, the positioning of thermo-hygrometers in poultry houses could be partly responsible for these reported differences.

The temperature difference observed during the first week could be linked to the heating of buildings with charcoal. In addition, the evolution of the relative humidity of the air was negatively correlated with that of the temperature. This is in agreement with the results of Koukpemedji et al. (2015) who found that temperature and relative humidity are two quantities that vary in opposite directions: when the temperature increases the relative humidity increases and vice versa.

The type of litter significantly influenced the occurrence of contact dermatitis in chickens. None of the chickens reared on the biochar showed any physical lesions unlike the chickens reared on the corncob and on the mixed litter. There are many causes of contact dermatitis. According to Webster (1995) and Kjaer et al. (2006), the development of foot pad dermatitis is linked to the growth rate of broiler chickens and the physiological changes it causes (bone and muscle disorders). They are therefore mainly lying down and consequently a non-negligible surface of their bodies remains continuously in contact with litter products such as ammonia. Kjaer et al. (2006) by evaluating the susceptibility to foot pad dermatitis of two strains of chickens, one of which was fast growing and the other slow growing, did not observe any signs in the chickens of the slow growing strain unlike the fast growing one.

According to Bignon et al. (2015), the frequency and severity of contact dermatitis vary with the type of litter used, taking into account characteristics such as water absorption capacity, strand size, abrasiveness and density. For Eichner et al. (2007), the friability of the litter also plays a role in its quality. Thus, the quality of the litter would be improved the more friable it is. Martland (1985) suggested that humidity leads to bacterial proliferation responsible for the degradation of faeces and therefore an increase in the content of ammonia and other irritating substances leading to an increase in the severity of lesions. However, Olivere (2010) reported an appearance of the first foot pad dermatitis from the 9th day of age of the chicks while the litter was still dry. Foot pad dermatitis therefore turns out to be lesions that develop rapidly and early. According to Greene et al. (1985), these tissue deformations would generally be due to irritations and would be caused by irritating substances contained in the litter such as treatment residues or other pollutants.

The season can also have indirect consequences on the frequency of foot pad dermatitis (McIlroy et al., 1987; Bruce et al., 1990; Martrenchar et al., 2002). In fact, in winter, in order to save heating, breeders tend to ventilate less in order to keep the heat. Physiological indices are widely used to provide information about welfare. The most commonly used indices are blood concentrations of hormones and enzymes and the composition of leukocytes (Anonymous, 2000). However, changes in these characteristics may vary depending on the nature of the stressor. Measuring short-term responses can also be complicated by method effects such as taking a blood sample (Anonymous, 2000).

Among all the haematological parameters considered in this study, only the number of white blood cells and the number of lymphocytes were influenced by the type of litter. The Mixed litter recorded the highest values for these two parameters compared to the Biochar and Corn cobs litters. The values obtained in this study for these parameters are higher than those reported by Kokore et al. (2021) and reference values reported by Jain (1993) in broiler chickens. But for the other parameters, the values found in the present study are lower than those reported by these same authors.

The main haematological response to stress in chickens is a change in the heterophil/lymphocyte (H/L) ratio (Anonymous, 2000). According to this author, a normal ratio is around 0.4, but it can reach 8 in birds subjected to severe stress. The values found for the H/L ratio are not very high and are respectively 1.43±0.26, 0.88±0.16 and 0.91±0.13 for the Biochar, Mixed and Corn cobs batches. An increase in H/L has also been reported as an initial response to restricted feeding but the change is not necessarily maintained over a prolonged period of restricted feeding (Maxwell et al., 1990; Maxwell et al., 1991). The number of basophils can also be increased during stress, but the increase is associated with acute stress of a potentially life-threatening magnitude, which is not the case in this study (Anonymous, 2000).

The type of litter did not cause any variation in biochemical parameters. Aspartate transaminase (AST) or TGO transaminase, is a plasma enzyme which is widely used as an indicator of stress, the increase of which indicates muscle damage (Anonymous, 2000). Mixed litter resulted in a significant decrease in relative lung weight compared to Biochar and Corn cobs litter. This could be a sign of a respiratory disease when considering the significant increase in the number of white blood cells and lymphocytes in this batch. In fact, Ritz et al. (2011) observed after necropsies a darker coloring of the nostrils and frontal sinuses in broilers reared on litter amended with biochar. This coloring would be caused according to these authors to the inhalation of particles of biochar in suspension in the air. It can also be non-infectious respiratory problems that are mainly caused by poor air quality (air composition, dust and ammonia).

No difference was recorded between batches for the T_3 hormone, indicating that the type of litter does not influence its production. Considering the studies conducted by Beckford et al. (2020), the concentration of T_3 decreases in the presence of heat stress. Abhay et al. (2015) reported a negative correlation between thyroid hormone T_3 secretion and ambient temperature. Thus, at high ambient temperatures, the activity of this hormone is reduced, which decreases the performance of birds. In fact, according to Decuypere et al. (2005), thyroid hormones have various effects on energy metabolism and development in vertebrates. They would also be the basis of the increase in respiratory rate (Souza et al., 2016).

5. Conclusion

This study shows that the type of litter influence the welfare and health of broilers. Biochar did not cause contact dermatitis in chickens. The incidence of foot pad dermatitis was higher for mixed litter compared to corn cobs. Only the mixed litter caused hock burns in some chickens. The type of litter did not influence the relative

weight of the organs, except for the lungs where significant differences were noted. An increase in the white blood cell count was noted in the Mixed batch with, however, an absence of difference for the neutrophil/lymphocyte ratio. The biochemical and hormonal parameters are similar regardless of the type of litter. The use of biochar as litter therefore improves the health status of broilers and does not harm their welfare. However, it would be interesting in future studies to evaluate the physicochemical and microbiological quality of the different litters in order to identify the possible causes of the observed effects.

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