Research Article

Carbonized Rice Husk as an Effective Bedding Material for Broiler to Reduce Burden of *E. coli* through Minimization of Ammonia Production from Litter

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Abstract | Broilers do not perform to their genetic potential in a stressful and diseased condition as concerned with poor bedding materials, high ammonia production from litter, and so on. The maximum ammonia production from litter depends on factors like moisture (%), pH, total nitrogen (%) of litter, and temperature of broiler houses. Carbonized Rice Husk (CRH) is a good poultry bedding material that consists of carbon and SiO_2 which interestingly enhance ammonia adsorption. Keeping the above fact, this study was conducted to reduce ammonia reducing immunosuppression that manages susceptibility of *E. coli*. When the maximum level of ammonia was produced i.e., on day 34, intra-nasal administration of *E. coli* of strain χ^{7122} (serotype 078: K80:H9) at pathogenicity amount (9.4 x 10^5 cfu *E. coli* per bird) was done and morbidity and mortality rate was also recorded. The significantly minimum ammonia (ppm) was recorded in CRH (30.53±0.77); however, the maximum ammonia (ppm) was recorded in RH (49.78±0.42) with that compared to SD (33.42±0.25) and RS (43.22±0.56). Similarly, the mortality rate due to colisepticemia was recorded minimum in CRH (68.75%), however, the maximum was recorded in RH (86.25%) with that of compared to SD (70.00%) and RS (77.50%). It showed that CRH adsorbs ammonia produced in poultry houses reducing the immunosuppression managing susceptibility of *E. coli*. This proves CRH as the best poultry bedding material for the rearing of broiler chickens for better production.

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Introduction

Poultry is a term used for domestic fowls such as chickens, ducks, geese, which are raised for the production of meat (also called flesh), and eggs that can be used as food. Poultry is a growing enterprise and is a major source of income generation in urban and peri-urban areas (Bhattarai, 2008). The protein of high quality are obtained from poultry meat and eggs which are nutritionally beneficial food for humans. Besides this, poultry meat and eggs consist of low fat and contained a favorable mix of fatty acids (FAO, 2013). The genetic performance of birds is greatly reduced due to high levels of ammonia in broiler houses (Sa *et al*., 2018) which ultimately causes high mortality due to the ultimate increase in the susceptibility to diseases (Kristensen and Wathes, 2000; Leinonen and Kyriazakis, 2016). The enzymes urease and uricase break down the urea and uric acid present in the litter which ultimately generates ammonia. Prolonged exposure of NH_3 to about 20 ppm concentration shows a variety of disorders, including respiratory tract damage-causing coli-septicemia and risk towards Newcastle disease (Yi *et al*., 2016). Increments of mucus-secreting cells and loss of cilia leading to changes in the respiratory epithelium are due to the exposure of birds to NH_3 of concentration levels from 75 ppm to 100 ppm (Al-Mashhadani and Beck, 1985; Cressman, 2014). *E. coli* are gram-negative, non-spore-forming, non-capsulated facultative anaerobic bacilli measuring 1-3 µm x 0.4 - 0.7 µm which are normally found inside the intestine of poultry. Some of the strains such as the Avian Pathogenic Escherichia coli (APEC) strain χ^{7122} serotype O78:K80:H9 cause infection at ages between 4 -6 weeks only and when they are establishing themselves in the respiratory tract of poultry causing diseases of the respiratory system (Blaak *et al*., 2015; Manges, 2016).

Due to lack of proper bedding material, poultry industries have to face a lot of problems such as the susceptibility towards various diseases that generally come from litter. So, this acts as a barrier towards the success of rearing broiler chickens. Amongst the various factors to control the reduction of ammonia and finally mortality, one of the most important factors was the presence of good quality bedding materials (Munir *et al*., 2019; Soliman and Hassan, 2020). Lacy (2002) accounts for different poultry bedding materials (hardwood shavings and sawdust, pine shavings and sawdust, rice hulls, hay or maize stay-over, pine or hardwood chips, sugarcane pomace, peanut hulls, chopped straw, processed paper, crushed maize cobs, sand) which have been tried somehow with success. Different bedding materials such as RH, CRH, SD, and RS are used which have the properties of adsorption and help in reducing ammonia which further decreased the risk of coli-septicemia and finally the reduction in the mortality rate. Although a variety of products have been used as poultry bedding materials, due to their shortage, the most common interest of poultry producers in alternative bedding materials with broad advantages (Hester *et al*., 1997). CRH has a good ammonia gas adsorption capacity (Kumagai *et al*., 2006) and thus is the best bedding material for broilers. This study was conducted to determine whether non-conventional bedding materials- Carbonized rice husk (CRH) - could be the best bedding materials as poultry litters, which can adsorb ammonia and reduce the risk of infectious disease as coli-septicemia.

Materials and Methods

Preparation for research

The study was conducted at the livestock farm Institute of Agriculture and Animal Science (IAAS), located at Rampur, Chitwan, Nepal from September to November 2013. A research trial was conducted using a Complete randomized design (CRD) with four treatments i.e., T_1 (rice husk as control), T_2 (carbonized rice husk), T_3 (sawdust) and T_4 (finely chopped rice straw) replicated four times and altogether there were 16 treatments with 20 Venn Cobb 500 broiler birds in each treatment, so there was 320 total number of broiler birds. Ethical clearance for conducting the research work in the field was provided by the IAAS livestock farm of Rampur, Chitwan.

CRH was prepared by the number of serial processes, 100 kg of rice husk produces 45 kg of charred or carbonized rice husk (CRH) using an open-type carbonizer. 55 kg of charred rice husk was utilized in 2 plots for the use of poultry bedding materials for my research. The total number of plots for the trials for CRH in my research was 4. So, 110 kg of CRH was used in all 4 plots.

Research design

The research was conducted in eight steps:

Step 1: Broiler birds throughout the trial period (42 days) were raised. Commercial broiler diet (pellet feed) was fed ad libitum with regular vaccination against viral diseases, along with supporting drugs.

Step 2: Nutrient analysis as moisture content (%), pH, NH_3 gas production (ppm) and total nitrogen content (%) of poultry litter along with broiler shed temperature (°C) parameters was analyzed.

Step 3: On day 30, aerosol samples from the level of height of the broilers and litter samples with the help of cotton swabs for *E. coli* culture were taken from every treatment in Petri dishes containing media MacConkey's agar and Eosin methylene blue (EMB) agar following standard guidelines. For the minimization of existing *E. coli* present in air and litter of poultry house chlorine @ 1ppm was sprayed in the shed on day 31, for the minimization of error during research for obtaining better data for better results. Again, on day 33, samples of aerosol and litter of poultry house as previously described were taken and cultured for 24 hr. in an incubator at 37 °C which were analyzed on day 34 and confirmed *E. coli* due to production of pink colonies on MacConkey's agar and black colonies on Eosin methylene blue (EMB) agar. These were in lower amounts as compared to those of previous culture results in aerosol samples as well as in litter samples of broiler houses.

Step 4: The samples collected from aerosol and litter from broiler houses were cultured for about 24 hr. in an incubator at 37 °C and were analyzed and confirmed as *E. coli* due to the production of pink colonies on MacConkey's agar and black colonies on Eosin methylene blue (EMB) agar. The samples collected from aerosol and litter from the broiler house were cultured for about 24 hr. in an incubator at 37 °C and were analyzed as *E. coli* due to the production of pink colonies on MacConkey's agar and black colonies on Eosin methylene blue (EMB) agar which was in excessive amount, confirming or otherwise the presence of *E. coli* in air and as well as in litter of broiler house.

Step 5: For the minimization of existing *E. coli* present in air and litter of poultry house chlorine @ 1ppm was sprayed in the shed on day 31 and then after re-culture lower amounts of *E. coli* compared to those of previous culture results was as confirmed pre-control of aerosol and litter *E. coli* for better results.

Step 6: On day 34, APEC of strain χ^{7122} serotype O78:K80:H9 at amount 9.4 x 10^5 cfu *E. coli* per bird (LD = 9.4 x 10^5 cfu *E. coli*) was administered intra-nasally as suggested by Brown and Curtiss, 1996.

Step 7: The number of sick and dead broiler chickens for the morbidity and mortality rate up to the rearing period from each treatment was recorded and finally, morbidity and mortality rate was calculated.

Step 8: For the diagnosis of the cause of morbidity and mortality of broiler chickens' various diagnostic procedures as clinical signs/symptoms, post-mortem (PM) examinations, culture examinations, and serological test (ELISA) for the confirmatory diagnosis were performed.

Dead birds and sacrificed sick birds from each replication of every treatment were taken for post-mortem (PM) and were examined. The PM examination showed lesions of milky fluid in the pericardium (i.e., due to pericarditis), thicker and cloudy air sac with dark liver covering of fibrinous exudates. Thus, examined PM lesions confirm coli septicemia.

For cultural identification of *E. coli*, Cotton swabs were dipped in various organs like the liver, duodenal contents and also dipped in heart blood, then sprayed with a slight touch over media MacConkey's agar and Eosin methylene blue (EMB) agar present in Petri dishes which was then incubated for 24 hr. in an incubator at 37 °C. After 24 hr., the Petri dishes with an excessive number of pink colonies in MacConkey's agar and black colonies in Eosin methylene blue (EMB) agar shows *E. coli* and thus confirm as coli septicemia. In addition, serological test was conducted. Samples of liver, heart blood, and duodenal portion with its contents were taken in samples collecting plastic bags and were dispatched to MiRON Laboratory and Research Center (MLRC), Subidanagar, Tinkune, Kathmandu for Enzyme-Linked Immuno-Sorbent Assay (ELISA) as confirmatory diagnosis of colisepticemia which finally shows Avian Pathogenic *E. coli* of strain χ^{7122} serotype 078: K80:H9 which confirmed diagnosis as colisepticemia.

Data analysis

Processing and analysis of samples were done at the Veterinary Teaching Hospital (VTH), IAAS, Rampur, Chitwan, and the MiRON Laboratory and Research Center (MLRC), Subidanagar, Tinkune, Kathmandu. The data, thus, obtained were analyzed statistically by MSTAT (Microsoft Excel), ANOVA, LSD, and DMRT at the 0.05 level of significance. The significance of difference was determined by analysis of variance (ANOVA) at $p<0.05$ level of probability. The mean comparison of treatment groups was determined by Duncan's Multiple Range Test (DMRT) at the 0.05 level of significance.

Table 1: *Main effect of different bedding materials on moisture, temperature, pH, ammonia, total nitrogen of broiler litter.*

	Moisture	Temperature	pH	Ammonia	Total nitrogen
Rice husk (RH)	52.79 a	28.51 ab	8.30 a	49.78 a	0.86 bc
Carbonized rice husk (CRH)	52.66 a	28.55 ab	7.77 d	30.53 d	0.85 c
Sawdust (SD)	53.19 a	28.49 b	7.87 c	33.42 c	0.89 ab
Rice straw (RS)	50.44 b	28.60 a	8.15 b	43.22 b	0.89 a
F- value	27.29	2.66	85.76	1113.56	4.12
Probability	<0.01	>0.05	<0.01	<0.01	<0.05
CV %	1.13	0.55	1.22	3.21	1.75

Means column with different super script differ significantly by LSD (P<0.05).

Results and Discussion

Moisture (%), pH, ammonia (ppm), total nitrogen (%) with different bedding materials also differed significantly (P<0.05) (Tables 1,2). Overall, maximum ammonia (ppm) was recorded in RH, and minimum ammonia (ppm) was recorded in CRH. Overall, the results revealed that the significantly maximum total nitrogen (%) was recorded in RS, and the minimum total nitrogen (%) was recorded in CRH. The maximum level of ammonia in treatments RH, RS, SD, and CRH was recorded as 60.25 ppm, 52 ppm, 40.25 ppm, and 38.50 ppm respectively on day 34 (Table 2).

Table 2: *Main effect of different bedding materials on moisture, temperature, pH, ammonia, total nitrogen of broiler litter during different time interval.*

	Moisture	Temperature	pH	Ammonia	Total nitrogen
20 Days	35.58 e	27.24 f	7.42 f	14.88 f	0.84 g
25 Days	42.03 d	28.01 e	7.77 e	24.44 e	0.85 f
30 Days	51.02 c	28.31 d	8.04 d	36.81 d	0.87 e
32 Days	54.16 b	28.80 c	8.13 c	43.00 c	0.88 d
34 Days	57.76 a	29.03 a	8.33 a	47.75 a	0.89 a
36 Days	57.62 a	28.88 bc	8.24 b	46.94 ab	0.88 c
38 Days	57.41 a	28.81 c	8.08 cd	46.50 b	0.88 d
40 Days	57.44 a	28.83 c	8.07 cd	46.19 b	0.88 c
42 Days	57.43 a	28.95 ab	8.13 c	46.63 b	0.88 b
F- value	3008.94	226.40	124.30	1415.75	22.99
Probability	<0.01	<0.01	<0.01	<0.01	<0.01
CV %	1.13	0.55	1.22	3.21	1.75

Means column with different super script differ significantly by LSD (P<0.05).

After the intranasal administration of *E. coli* of strain χ^{7122} (serotype 078: K80:H9), the lowest mortality rate due to colisepticemia was recorded in CRH (68.75%), and the maximum was recorded in RH (86.25%) with that of SD (70.00%) and RS (77.50%) (Table 3).

CRH showed the properties of adsorption and reduction of ammonia which further decreased the risk of coli septicemia and finally reduction in the mortality rate. It is clear from the results that overall, the lowest ammonia (ppm) was recorded in CRH (30.53±0.77), and the maximum ammonia (ppm) was recorded in RH (49.78±0.42) with that of SD (33.42±0.25) and RS (43.22±0.56) (Table 2). The maximum level of ammonia in treatments RH, RS, SD, and CRH was recorded as 60.25 ppm, 52 ppm, 40.25 ppm, and 38.50 ppm respectively on day 34 (Table 2). Similarly, maximum mortality, after the intranasal administration of *E. coli* of strain χ^{7122} (serotype 078: K80:H9), was recorded in RH which was 86.25% followed by RS (77.50%), SD (77.50%), and finally the lowest with CRH (68.75%).

Table 3: *Morbidity and mortality rate of broiler chickens during experimental period.*

Treatments	Morbidity		Mortality	
	No.	%	No.	%
RH	80	100	69	86.25
CRH	80	100	55	68.75
SD	80	100	56	70.00
RS	80	100	62	77.50

The morbidity and mortality observed in this research is confirmed to be a result of colisepticemia by applying different types of confirmatory diagnostic procedures. The rate of morbidity was the same in all treatments but the mortality varied in each treatment. This difference was due to the difference in each of the parameters i.e., moisture (%), temperature (°C), pH, ammonia (ppm), and TN (%) (Carlile, 1984; Fairchild *et al*., 2006; Woorley *et al*., 2002), which are co-related with each other. This finding also shows that higher ammonia in broiler houses might have caused increased immunosuppression causing colisepticemia which led to an increased mortality rate. Earlier studies by (Dietert *et al*., 1994; Koller, 1979; Chauhan and Chandra, 1997) also found that environmental contaminants such as ammonia cause immunosuppression that allows opportunistic pathogens to overwhelm and cause mortality. The current result also shows that variation in mortality rate was directly proportional to the production of ammonia between different treatments, which was likely due to the damage of the bird's respiratory system. As the level of ammonia increases, the damage of the bird's respiratory also increases which allows increment in the risk of infectious disease-causing increment in mortality as reported by (Brown and Curtiss, 1996; Sojka, 1965). The production of ammonia in the current research was less in CRH as compared to that of other treatments i.e., RH, SD, and RS, which could be due to the adsorption properties of CRH as mentioned by Kumagai *et al.* (2006). The adsorption properties of CRH in this research are due to the presence of carbon and SiO_2 which enhance the ammonia adsorption which is similar to the finding of Kumagai and Sasaki (2009).

Conclusions and Recommendations

It is clear from the research that the litter with CRH has the lowest ammonia (30.53±0.77 ppm), while the highest in RH (49.78±0.42 ppm). Similarly, ammonia in the litter with SD and RS is 33.42±0.25ppm and43.22±0.56, respectively. The mortality rate was the lowest in CRH (68.75%), and the highest was in RH (86.25%). This shows that CRH has ammonia adsorptive properties, as a result of which it adsorbs excess amounts of ammonia produced in poultry houses, which reduces the death due to colisepticemia, which indicates the CRH as the best poultry bedding materials for poultry producers for the rearing of broiler chickens for better production.

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Author's Contribution

All authors contributed equally.

Conflict of interest

The authors have declared no conflict of interest.

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