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Hanaa A. Zaid[1], Hanaa T. El-Bahnasy [1], Magda A. Elkomy[1], Mona M. Elsayed [2], Abeer E. Abdrabouh [1]

[1]Zoology Department, Faculty of Science, Mansoura University, Egypt

[2] Hygiene and Zoonoses Department, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt

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Hanaa A. Zaid[1], Hanaa T. El-Bahnasy [1], Magda A. Elkomy[1], Mona M. Elsayed [2], Abeer E. Abdrabouh [1]

[1]Zoology Department, Faculty of Science, Mansoura University, Egypt
[2] Hygiene and Zoonoses Department, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt

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Abstract

This study investigated the air quality in poultry house for ammonia concentration along with N%, pH, and moisture in litter material and the related poultry health. The poultry litter was used for biochar production for mitigating ammonia emissions. Accordingly, 20 chicks of one day age were reared without any treatment as a control group for 35 days. After this period the poultry litter was used in biochar production at 450 ° C. Two broiler groups (each of 20 chicks) were constructed as 10% biochar (10%BC group) and 20% biochar (20%BC group) added to the litter. The concentration of ammonia in air, as well as litter moisture in control group were significantly high comparing with biochar groups. Although feed consumption by the control group was more than biochar groups, results showed significant decrease in the body weight gain in control group compared to the biochar groups, especially 10%BC. Feed conversion ratio was highly recorded in control group compared to biochar groups. Moreover, serum total protein and albumin contents were significantly decreased in control group compared to biochar groups, especially 10%BC. However, levels of liver nitric oxide and interlukin-6 (IL-6) was significantly increased in control group compared to the two biochar groups. On contrast, interlukin-10 (IL-10) recorded significant decrease in control group compared to both biochar groups. Consequently, addition of biochar 10% and 20% to the bedding material resulted in decreasing ammonia emission in the air environment of poultry house which in turn lead to improvement in the chicken health, especially with 10%BC group.

1. Introduction

Poultry farms are one of the main suppliers for meat in Egypt. A chicken produces 80-100 g of manure daily, corresponding to 3-4% of its body weight (Tańczuk et al. 2019). The poultry litter bedding material covers the floor either for protection and insulation, as well as to receive animal waste and absorb moisture (Oliveira et al., 2021). Poultry litter residue is traditionally used as a fertilizer due to rich nutrients, including nitrogen (N) and phosphorus (P). However, the excess use can cause soil and groundwater contamination due to the presence of organic matter and heavy metals, as well as pathogenic microorganisms (Kyakuwaire et al, 2019). In poultry facility, chicken themselves excrete unused nitrogen as ammonia and urea. The environment of poultry sheds is always provided with stress causing agents, including carbon dioxide, hydrogen sulphide, methane, and ammonia. However, ammonia (NH3) is considered as the most deleterious due to its irritant pungent smell which is primarily released through litter degradation in poultry houses (Asif et al., 2021). The main source of NH3 in poultry houses is the chicken fecal material that contain uric acid, urea and NH3 which result from high amino acids and proteins diet that added to accelerate growth (Maliselo and Mwaanga 2016). Despite this, volatilization of ammonia that result from the breakdown of uric acid and urea has a major impact on the environment and the poultry industry (Zhou et al.,2020). On the other hand, increased NH3 levels in the air can contribute to the formation of nitrogen oxides that are considered as greenhouse gases (Oliveira et al., 2021). Moreover, raised NH3 levels in the environment has detrimental effects on different bird organs such as the lung, spleen, brain and liver which in turn may lead to reduced growth rate, high mortality and low feed efficiency (Wang et al.,2019).

Cytokines are playing an important role in the inflammatory response. Among these cytokines, IL-

6 is a potent proinflammatory and immunomodulator (Mucksova et al.,2018), while IL-10 is known as an anti-inflammatory cytokine which is participating in blocking production of inflammatory cytokines (Sabat et al., 2010). Zhou et al. (2020) referred that the disturbance in both types could induce harmful effects that may affect the growth rate of birds and feed efficiency.

The reuse of litter several times during chicken rearing to reduce the production costs and decrease the amount of generated wastes has widely been adopted, however, this may affect human and poultry health (Asif et al., 2021). Previous studies reported that the permissible limit of NH3 in air is 25 ppm that is suitable for human and animal health (Anderson et al. 1964 and United Egg Producers, 2016). However, Cobb (2018) established the maximum limit of ammonia in poultry facilities as 10 ppm.

Various practices have been used to minimize NH3 emissions in poultry farms, including acidification of litter (Kavanagh et al., 2019), and biochar addition (Hung et al., 2022; Baral et al., 2023). Biochar is low-cost, sustainable biomaterial with many environmental remediation applications. Biochar is produced by thermochemical transformation of biomass in the absence of oxygen (pyrolysis) (Baral et al., 2023). Its physicochemical properties have been proven to provide environmental benefits via the adsorption of organic and inorganic contaminants, promote plant growth, improve soil quality, and provide a form of carbon sequestration (Rawat et al. 2019). Regarding biochar production, application methods to biological systems have a significant influence on the moisture content, pH, microbial communities, and carbon & nitrogen retention (Oni et al. 2019).

Several studies have been concerning the use of biochar as a bio-cover or bio-mix for reducing NH3 emissions with showing variable effectiveness in reducing emissions showing that the efficiency was highest in the first week and gradually reduced over the period (Miirkhanuly et al., 2020; Covali et al., 2021). However, McGuiggan et al. (2022) did not show any reduction in NH3 emissions with applying biochar.

The environmental stress resulting from poultry practice is an issue that needs to be studied and solved. Therefore, the objectives of the present study are: 1-recycling the poultry wastes for producing the biochar to control the release of greenhouse gases inside the poultry house. 2-evaluation of air quality inside the poultry house before and after using the biochar. 3-evaluation of chicken body weight changes and liver cytokines before and after using biochar.

2. Materials and Methods

2.1. Broiler birds and experimental protocol

2.1.1. Control group

In an experimental room of (3.5m length x1.5m width x 3m height), equipped for housing the broiler chicks, where sawdust was used as a bedding material. The experiment started with 20 broiler chicks of Cobb breed, one day age forming the control group (CN) in January 2023 for 35 days. During this period, sawdust bedding material was added daily. However, at the end of experimental period, all the litter of the control bird group was collected to reuse in biochar production used with the following two experimental broiler groups.

2.1.2. Biochar groups

For the same period of investigation, the second group included 20 Cobb broiler breed chicks of one-day age, where biochar was added to the bedding sawdust regularly as 10% of the total amount of the bedding sawdust (10%BC group). Moreover, the third group consisted of the same number and age of broiler chicks, but bedding material was frequently mixed with biochar, which constitute 20% of the bedding sawdust (20%BC group).

The initial weight of the broiler chicks in the three studied groups was 60±3g. The room temperature was averaged 20±3°C, relative air humidity was 60±5%, and airflow was controlled to ensure adequate ventilation, minimizing buildup of contaminants generated by animals and avoid thermal stress. All investigations of the experiment were approved by Animal Care Committee of Mansoura University, Egypt, MU-ACUC (SC. PhD.23.02.4).

2.2. Biochar production

Poultry litter (PL) of the control group was collected after 35 days, avoiding feathers as much as possible. Collected PL was sun dried for 3 days to remove extra moisture. The dried PL material was preserved in plastic pot and was transferred to the laboratory for further use. Poultry litter biochar was produced through pyrolysis process using muffle furnace under limited oxygen condition. For pyrolysis, the crucibles were completely filled with poultry litter residue, to ensure maximum oxygen removal. They were subsequently covered and the treatments for biochar production were performed in a JUNG muffle furnace at 450 ° C for 0.5 h (Pereira, et al. 2019).

2.3. Environmental hygiene investigations

2.3.1 Ammonia concentration in air

The concentration of ammonia (NH3) in air environment of the poultry house was evaluated using Aeroqual S200L, 0202181-3304 portable apparatus, where the current study concerned with NH3 concentration (ppm) at the end of the experimental period.

2.3.2. Litter measurements

Total nitrogen % (N%) was estimated through the official Kjeldahl method described in AOAC (2019), where a 0.5g of air-dried litter was digested with 8ml of concentrated sulphuric acid in Kjeldahl flask in the presence of 2.14g digestion mixture (1kg potassium sulphate and 60g of mercuric oxide). After digestion, the solution was treated with 10ml of 40% sodium hydroxide solution, where the liberated NH3 was

received into 10ml of 1% boric acid in the presence of 2 drops of Tachero indicator (1.25g methyl red+0.32g methylene blue in one liter of 90% ethanol). The received ammonia was titrated against 0.01N sulphuric acid. The percentage of total nitrogen was estimated by subtracting the NH4 +-N from the Kjeldahl N. Moreover, the pH-value in the poultry litter was measured by using an electrical-pH meter (Model Corning, NY 14831 USA) digital analyzer after dissolving the chicken litter into the water in a ratio of 1:10. Then the pH of the mixture was measured. The litter moisture was detected according to the method of AOAC (2019), where litter was aerated and then dried in an oven at 60 ºC for 48 hours and then weighed. After that, the same sample was continued to dry using an oven at 105 ºC for 3 hours. The total moisture% was calculated from subtraction the reading at 105 ºC from that at 60 ºC.

2.4. Estimation of body weight change and feed conversion ratio

After the experimental period of 35 days, the final body weight for all the bird groups was recorded, and the body weight gain (g) was calculated from: final body weight – initial body weight. Additionally, the mean of total liver weight (g) was recorded for each investigated bird group. Moreover, the amount of total feed consumption (Kg) for each group was recorded along with the feed conversion ratio (FCR) which measure how a flock of birds can convert feed intake into live weight forming the amount of meat chicken produces. FCR was measured by dividing the weight of consumed feed (Kg)/ body weight gain (Kg). The greater weight of chicken, the lower FCR. So, lower FCR indicates higher efficiency (Fry et al, 2018).

2.5. Samples collection

Chicken of each group were slaughtered, and their blood samples were collected and centrifuged at 1500 rpm for 15 min. to separate serum. After that, chickens were dissected to obtain 0.5 g from the right lobe of liver that was washed with normal saline and homogenized in 5 ml phosphate buffer solution (PBS), centrifuged at 1500 rpm for 15 min. All the collected liver supernatants and serum samples were frozen at -20°C till analysis.

2.6. Estimation of biochemical parameters

In the serum of investigated chicken groups, total protein (TP) and total albumin (Alb) contents were detected through kits from Biodiagnostic Company, Giza, Egypt. Moreover, in the liver of investigated groups, nitric oxide (NO) as an oxidative stress marker was estimated according to the method enclosed in the kits of Biodiagnostic Company, Giza, Egypt. However, cytokines (IL-6 and IL-10) concentrations were measured also in the liver by commercial ELIZA kits specialized for chicken produced by My biosource company, San Diego, USA according to the manufacturer instructions.

2.7. Statistical analyses

One way analysis of variance (ANOVA) followed by Tukey's test were performed for all measurements through using GraphPad Prism program (v 5.04, GraphPad Software Inc., La Jolla, CA, USA). Results were expressed as mean± standard deviation (SD), where significance was recorded at $p< 0.05$.

3. Results

3.1. Environmental hygiene investigations

As seen in Fig.(1), levels of NH3 in the air environment of the CN group recorded 6.61±0.52 ppm, while in biochar groups were 5.37±0.65 and 4.95±0.35 ppm for 10%BC and 20% BC groups, respectively. Statistically, the two biochar groups showed significant decrease in NH3 concentration compared to CN group. However, N% in the litter of CN group was nearly the same as in 10%BC and 20%BC groups, represented as 2.99%±0.57, 2.81%±0.78 and 2.88%±0.64, respectively. Similarly, pH-value of the three investigated groups showed non-significant difference and tended to be alkaline, where CN, 10%BC and 20%BC recorded 7.64±0.63, 7.59±0.24, and 7.80±0.35, respectively. Furthermore, the percentage of litter moisture of 10% and 20% biochar groups decreased (38.46±4.90 and 42.04±11.00)% compared with CN group (53.35±8.72%), however this decrease with only significant with 10%BC group.

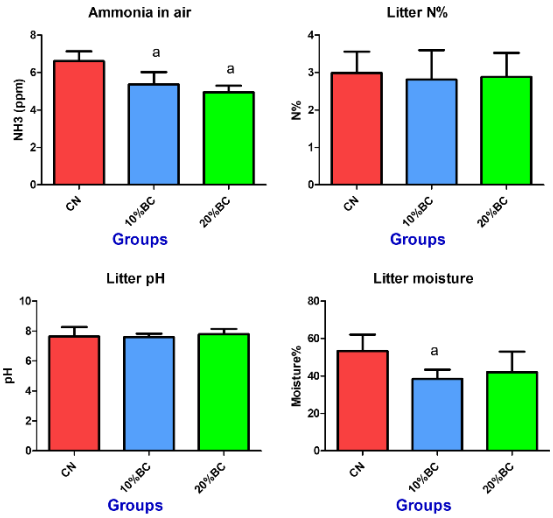

Figure. 1 Concentration of ammonia in air, and litter N%, pH and moisture in different investigated groups. Values are represented as mean±SD. CN: control,10%BC:10%biochar, 20%BC: 20%biochar. a: significance between BC groups and CN, b:significance between the two BC groups ($p<0.05$).

3.2. Changes in growth performance

Table (1) illustrates the changes in the body weight gain at the end of the experimental period, where 10%BC group (2787±292g) showed significant increase in the body weight gain compared to CN group (2268±489.6g). However, 20%BC group (2401±303.3g) showed non-significant increase in the body weight gain compared to CN. In parallel, the liver weight of 10%BC group (65.33±7.967 g) significantly increased compared to CN group (50.57±4.826 g), while 20%BC group (57.00±6.455 g) showed non-significant increase in liver weight compared to CN group. On the other hand, feed consumption in the three groups was not greatly different, however, CN group

consumed more feed than biochar groups. In this concern, the present study recorded higher feed conversion ratio in CN group (24.69) compared to the 10% and 20% BC groups (18.30 and 22.50), respectively.

3.3. Biochemical analyses

As shown in Table (2), serum biochemical analyses for the total protein and albumin contents showed remarkable reduction in the control group (3.31±0.20 g/dl and 1.49±0.11 mg/dl) compared to biochar groups which was significant, especially with 10%BC group (4.28±0.59 g/dl and 2.69±0.46 mg/dl), respectively.

Table.1 Changes in the body, liver weights and feed consumption in different investigated groups.

Weights	CN	10%BC	20%BC
Body weight gain (g)	2268 ±489.6	2787[a] ±292.0	2401 ±303.3
Liver weight (g)	50.57 ±4.826	65.33 [a] ±7.967	57.00 ±6.455
Feed consumption (kg)	56	51	54
Feed conversion ratio (FCR)	24.69	18.30	22.50

Values are represented as mean±SD. CN: control,10%BC:10%biochar, 20%BC: 20% biochar.a: significance between BC groups and CN, b:significance between the two BC groups ($p<0.05$).

However, 20%BC group showed non-significant increase compared to the CN group (3.78±0.40 g/dl and 1.85±0.35 mg/dl), respectively. On the other hand, the obtained results showed high level of the oxidative stress marker, NO in the liver of CN group (55.4±4.15 mmol/g) which was significantly decreased in both biochar groups, where 10% BC group recorded 25.3±2.00 mmol/g and 20% BC group showed 33.2±3.37 mmol/g.

Table.2 Changes in biochemical parameters in serum and liver of different investigated groups.

Parameters	CN	10%BC	20%BC
Serum TP (g/dl)	3.31 ±0.20	4.28[a] ±0.59	3.78 ±0.40
Serum Alb (mg/dl)	1.49 ±0.11	2.69[a] ±0.46	1.85 ±0.35
Liver NO (mmol/g)	55.4 ±4.15	25.3[a] ±2.00	33.2[a] ±3.37
Liver IL-6 (ng/g)	134.0 ±5.108	106.7[a] ±5.412	117.6[a] ±2.107
Liver IL-10 (ng/g)	61.53 ±3.623	95.37[a] ±5.823	83.40[a] ±7.562

Values are represented as mean±SD. CN: control,10%BC:10%biochar, 20%BC: 20%biochar.a: significance between BC groups and CN, b:significance between the two BC groups ($p<0.05$)

Furthermore, the concentration of proinflammatory cytokine, IL-6 in the liver was highly observed in the control group (134.0±5.11 ng/g). However, in biochar groups, concentrations of IL-6 were significantly decreased compared to the CN group, where in 10%BC group it was 106.7±5.41 ng/g and in 20%BC it was 117.6±2.11 ng/g. On contrast, the liver anti-inflammatory marker, IL-10 concentration, exhibited significant decrease in the control group (61.53±3.623 ng/g) compared to 10%BC and 20%BC groups (95.37±5.823 ng/g and 83.40±7.562 ng/g), respectively.

4. Discussion

The present study showed high ammonia concentration in the air environment of the control group reared on ordinary poultry litter. He et al.(2016) attributed the ammonia release in poultry production to two main sources, the first source is the hydrolysis of urea in the urine of animals through the urease enzyme in a process known as ureolysis. However, urea degradation is considered a lower source than the second source which results from degradation of nitrogen excreted by birds in the form of uric acid that represented about 50% of the undigested proteins by birds (Vilela et al. 2020). In addition, Miles et al. (2011) attributed the increase of ammonia in the air to the presence of microbial enzymes, high pH and moisture in the litter that helps in converting uric acid and urea into NH3 by the action of uricase and urease enzymes, respectively. In the present study, especially moisture% was highly recorded in the litter of CN group, which may play a fundamental role in elevation of NH3 concentration in the air environment of CN group.

However, the significant decrease in concentration of ammonia in biochar groups (10%BC and 20%BC) was explained by Sajjadi et al. (2019) who described biochar by porous nature that gives a large surface area to volume ratio and have polar oxygenated functional groups help in adsorbing NH3 released from animal manure. Accordingly, the biochar can reduce NH3 emissions through its surface functional groups that provide a favorable site to adsorb NH3 while its porosity and surface area are effective for ammonium ion (NH4+) adsorption which consequently reducing NH3 emissions. Furthermore, the decreased moisture% in biochar groups could explain the role of biochar in adsorbing moisture and in turn reducing ammonia emission in air.

Although NH3 concentration in air environment of CN group (6.61 ppm) was lower than limits 10 ppm and 25ppm suggested by Cobb (2018) and United Egg Producers (2016), respectively that related to human and chicken health impacts, the present study showed pronounced alterations in chicken health in CN group compared to biochar groups. These hazards may be resulted from NH3 emission in a small area compared to other studies, so it was effective in inducing health hazards. In this concern, the body weight always gives general indication for the body health. The current data showed significant decrease in the body weight gain in CN group, especially when compared to 10%BC group. This was in accordance with the study of Mohammed (2022) who explained that as poultry exposure to high levels of ammonia for 20 days possibly affecting the nutrient absorption in birds along with decreasing the bird capability to resist oxidative stress. This may lead to alteration in the ability of the intestinal tract to break down nutrients and affecting the immune organs. At the same time, the recorded body weight gain in the CN group was not in consistent with its feed consumption

that was elevated compared to biochar groups. Ferket and Gernat (2006) attributed the decrease in the body weight gain with increasing feed consumption to inefficiency of nutrient absorption and utilization. Feed conversion ratio (FCR) supported these results, where the obtained data showed an increase in FCR in control group which gives an indication of lower efficiency of birds to convert feed intake into live weight (Fry et al.,2018).

Taken together the obtained data showed significant decrease in serum total protein and albumin contents in CN group compared to biochar groups. This may be resulted from oxidative protein damage as a result of NH3 emissions (Mohammed 2022). In this concern, the obtained results showed significant elevation in the liver NO levels in CN group compared to biochar groups. NO is normally produced in the liver, however, increased production can result in formation of peroxynitrite which is responsible for oxidative protein damage that can lead to decrease in serum total protein and albumin contents (Rehman et al.,2018).

Furthermore, Ferket and Gernat (2006) reported that the decrease in body weight gain may result from the increase in proinflammatory cytokines which could decrease anabolic hormones, such as growth hormone and increase catabolic hormones, such as glucocorticoids that may lead to decrease in the body weight even the feed consumption increase. The authors added that once the proinflammatory cytokines decline, feed intake increases, and the body health alleviated. This was achieved in the current results where CN group showed elevation in inflammatory IL-6 and reduction in anti-inflammatory cytokine, IL-10, referring to incidence of inflammation. However, biochar groups recorded amelioration in the inflammatory status represented by significant decrease in IL-6 along with significant increase in IL-10 compared to CN group. This may be considered as a marker of improvement of the bird's immune system due to depletion of NH3 emissions in air. This was agreed with the results of Zhou et al. (2020), where birds exposed to 15 ppm of NH3 for three days showed elevated concentrations of the inflammatory cytokine, IL-6, indicating that short term exposure to low NH3 concentration result in inflammatory response in bird's trachea. In support, Wu et al. (2017) indicated that a longer period of exposure to a low concentration of NH3 is believed to cause long-term health problems. Another explanation was provided by Asif et al., (2021) who attributed these changes to stress conditions that may increase corticosterone level in plasma which in turn it can deplete the immune function and lead to inflammatory conditions.

Above all these considerations, the current study showed more better results with 10%BC group than 20%BC group. This may be explained by Czekala et al.,(2015) who reported that the addition of more biochar increased C-CO2 emission and the total C-CO2 emission, where carbon was reported to constitute more than 40% of total elements of biochar (Xue et al., 2022). Excessive level of CO2 in poultry farms lead to respiratory distress, gasping and decreased appetite for feed and so, it causes changes in the activity, behaviour and production (Gerritzen et al. 2008). Consequently, the increased percentage of biochar may lead to elevation of CO2 which may participate in lowering the efficiency of 20% BC group compared to 10%BC group (Al-Kerwi et al.,2022).

Conclusion

The reuse of poultry litter in producing biochar material showed effective role in mitigating the litter emissions for ammonia in air may be through moisture adsorption. In turn, poultry health hazards, including body weight gain and inflammatory status were ameliorated. The addition of biochar to poultry litter either 10%BC or 20%BC are effective, however, 10%BC showed better results especially with poultry health.

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