The effect of zonation in the closed house broiler on the litter *Escherichia coli* count and ammonia concentration

The effect of zonation in the closed house broiler on the litter *Escherichia coli* count and ammonia concentration

N Badrah, R Malaka, Daryatmo, K I Prahesti and Hajrawati

Faculty of Animal of Science, Universitas Hasanuddin Makassar, Indonesia

Email: malaka_ag39@yahoo.co.id

Abstract. The closed house is designed in length model form to provide differences in temperature, humidity and wind speed between the zone close to the inlet and the zone far from the inlet. This difference in environmental conditions has not been widely studied, especially for the growth of microorganisms present in the litter, especially *Escherichia coli* from chicken faeces contamination. *Escherichia coli* is a Gram-negative bacteria that normally occurs in the digestive tract and will be pathogenic if the numbers are excessive and outside the digestive tract. The purpose of this study was to determine the total *E. coli* bacteria in litter zoning in closed house facility. This is very important to be used as a reference in considering the proper litter handling. In this study, the closed house enclosure is formed 4 zones (zone 1 is 0-30 m from the inlet, zone 1 is 30-60 m from the inlet, zone 3 is 60-90 m from the inlet, and zone 4 is 90-120 m from the inlet. inlet). Each zone is 20×30 meters in size. The research was conducted using a sample of litter by taking 10 gr from each zone. The samples were then planted on EMBA (Eosin Methylene Blue Agar) media based on SNI 19-2897: 1993 work guidelines. The results showed that the total average of *E. coli* bacteria in zone 1 was 5.3×10^7 CFU/g, 2.5×10^7 CFU/dg in zone 2, 3.8×10^7 CFU/g in zone 3, and 5.4×10^7 CFU/g in zone 4. These results indicated that zoning did not affect the total *E. coli* bacteria on the litter so that it can be concluded that environmental conditions in the form of temperature, humidity, and wind speed in the zoning in the closed house were quite even. During the maintenance period, litter scraping was performed on the 7th day in zones 1 and 2, the 14th day in zones 3 and 4, and the 21st day in zones 1 and 2. On the 21st day, the litter bed is reversed in zone 3 and 4. Then add new husks on the 28th day. The conclusion showed that the treatment was effective in reducing the total *E. coli* bacteria in litter.

1. Introduction

Broiler chickens are one of the poultry commodities that play an important role in meeting people's protein needs [1]. The need for animal protein per capita per year continues to increase, especially those from broiler chickens [2]. Based on the 2018 statistical data, it was reported that the consumption of purebred chicken meat in Indonesia in 2017 reached 5.68 kg per capita or an increase of 11.2% from the consumption in 2016 of 5.11 kg per capita. The increase in consumption is due to the open mindset of the community regarding the advantages of applying closed system cages so that the application of closed system cages is increasingly developing.

The advantages of a closed house are that the capacity or population is much more, the chickens are more protected from external disturbances, both physical, weather, and disease, are protected from pollution, the uniformity of chickens is better [3]. This advantage can be achieved because the closed house system applies the latest technology in controlling environmental conditions and maintenance management in the cage.

A closed house used a cooling pad/inlet (where the air enters) and an exhaust/outlet (where the air comes out) to regulate air circulation in the cage. Closed houses have zoning in them, where zone 1 near the cooling pad has a lower temperature than zone 4 which is close to the exhaust fan, which gets heat accumulation from zone 1 to zone 4 [4]. This condition causes differences in temperature and humidity which may affect the condition of the litter as a potential medium for the development of bacteria.

Litter is generally used in postal cages to avoid injury to chicken feet, reduce wetness, and breaking down feces. Materials that are often used as litter are rice husks, rice straw, and sawdust. Litter in chicken coops is mixed with feces, leftover feed, and chicken feathers shed [5]. Feces that have been mixed with litter will break down and form ammonia gas (NH_3) by the metabolism of Gram-negative bacteria.

The most common Gram-negative bacteria found in the litter are *E. coli*. *Escherichia coli* has opportunistic properties [6], which is normally found in the digestive tract of chickens in a controlled amount, but in poor conditions due to stress, can develop into pathogens. As a result, *E. coli* excreted together with feces can pollute the environment, especially in the litter. Cages containing 10^5-10^6 CFU/g *E. coli* and ammonia (NH_3) that exceed 20 ppm are potential sources of disease transmission, especially in wet and dirty environmental conditions [7,8]. Colibacillosis is an infectious disease in poultry caused by pathogenic *E. coli* as a primary or secondary agent [9,10].

The inlet to exhaust distance may result in differences in microenvironmental conditions in the cage such as temperature, humidity, and wind speed. This is thought to affect the litter condition which will affect the total *E. coli* which is likely to affect the production of ammonia gas in the closed house cage.

This study aimed to determine the total *E. coli* bacteria in litter zoning in closed house cages, as a reference in considering proper litter handling. The usefulness of this research is expected to be a source of information for the community, especially breeders, regarding the appropriate litter management system to control *E. coli* bacterial contamination on litter in closed house cages.

2. Materials and Method

2.1. Closed house management

The cage used is a closed house with 4 plots (size 20×30 meters). The litter material used is rice husk with a thickness of ±10 cm. Each plot was provided with a place for feeding and drinking. Before chick-in, sanitation is carried out first such as cleaning the cage, spraying the cage using ACT formalin (dose: 1 liter in 50 liters of water) and disinfectant (VirkonTMS dose: 300 mL/m^2), washing the feed and drinking containers using a disinfectant (VirkonTMS dose: 300 mL/m^2), then calcifying the floor and walls and installing litter. Furthermore, fumigation is carried out one day before the chickens are put into the cage. After that, install the brooding and plastic on top of the litter. Brooding is heated 2-3 hours before DOC (Day Old Chick) is put into the house.

2.2. Research design

The research implementation stage includes the maintenance of 22,000 unsexed Cobb strains DOC for 28 days. The research illustration can be seen in figure 1.

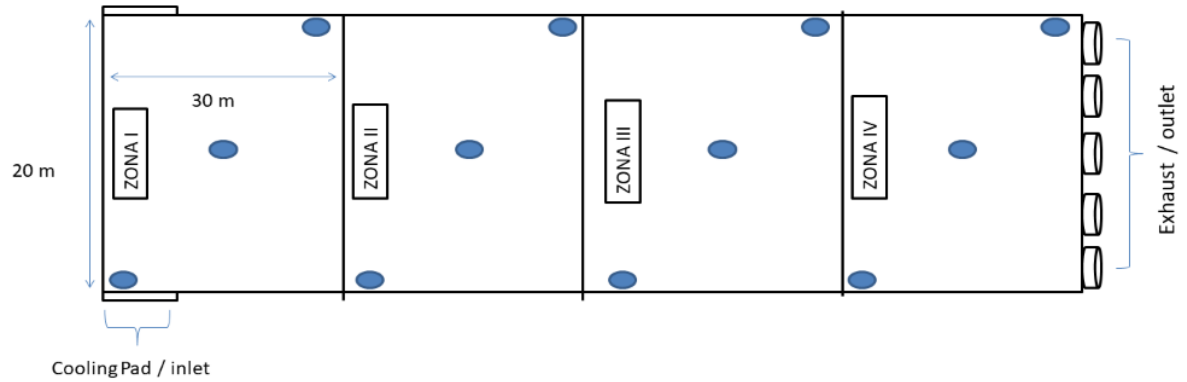

Figure 1. Illustration of the zonation in the closed house, showing sampling points.

In the closed house, there are 4 zones (zone I: 0-30 meters; zone II: 30-60 meters; zone III: 60-90 meters; and zone IV: 90-120 meters). The maintenance phase is divided into 2 phases, the starter phase from the age of 1-14 days and the finisher phase from the age of 15-28 days. The plastic that is attached to the litter will be removed after 2 days. During the maintenance period, litter scrapping was performed on the 7th day in zones 1 and 2, the 14th day in zones 3 and 4, and the 21st day in zones 1 and 2. On the 21st day, litter reversal was performed in zones 3 and 4. Then new husks were added on the 28th day.

Table 1. Microenvironmental conditions in the closed house.

Parameter	Long maintenance time (day)	Zone				Average
		Zone 1	Zone 2	Zone 3	Zone 4	
Temperature (°C)	7	30.73	30.47	31.03	31.97	31.05
	14	30.47	30.70	31.03	31.23	30.86
	21	29.53	29.97	30.30	30.70	30.13
	28	29.37	2967	29.97	30.17	29.79
Average		30.03	30.20	30.58	31.02	30.46
Temperature (%)	7	77.80	81.00	76.33	74.90	77.51
	14	78.57	78.23	75.47	74.33	76.65
	21	78.90	77.60	77.77	77.27	77.88
	28	80.15	77.80	76.33	74.90	77.30
Average		78.86	78.66	76.48	75.35	77.33
wind velocity (m/s)	7	1.82	1.70	1.60	1.43	1.72
	14	1.83	1.53	1.30	1.10	1.44
	21	3.60	2.83	2.53	2.27	2.81
	28	3.53	2.87	2.67	2.10	2.79
Average		2.78	2.23	2.03	1.73	2.19

Microenvironmental conditions in the cage were done by measuring temperature, humidity, and wind speed. Temperature (°C) was measured using a laser thermometer, humidity (%) was measured using the GM816 anemometer, wind speed (m/s) was measured using the GM816 anemometer. Sampling, measurement of temperature, humidity, wind speed, and ammonia measurements were carried out on the 7th, 14th, 21st and 28th days at 08.00 am. The temperature, humidity, and wind speed in each zone in the closed house cage can be seen in table 1.

The total *E. coli* bacteria are expressed in colony-forming units (CFU) per gram of litter. The number of colonies found is then entered in the following formula:

$$\text{Total bacteria} = \frac{1}{\text{dilution}} \sum \text{bacterial colonies}$$

Production of ammonia gas (NH_3) was determined as ammonia levels (ppm). The ammonia levels were measured using a digital ammonia test (Instrument H1700) placed in the middle of the zone for 5 minutes. This measurement was done every day at 8.00 am, 1.00 pm and 6.00 pm.

2.3. Sample preparation

Sample testing was carried out at the Laboratory of Animal Microbiology, Faculty of Animal Science, Universitas Hasanuddin based on the SNI 19-2897: 1993. The sample was weighed as much as 10 grams aseptically and then put in a sterile container filled with distilled water until the total weight reached 100 grams. The sample was then homogenized using a stomacher for 1-2 minutes. This is a solution with a 10^{-1} dilution [11]. The dilution was carried out by transferring 1 mL of the 10^{-1} dilution suspension with a sterile pipette into a solution of 9 mL of distilled water to get a 10^{-2} dilution, continue in series until the dilution is 10^{-10}. Furthermore, 1 mL of suspension from 10^{-8}, $10^{-9,}$ and 10^{-10} dilutions was put into a petri dish in duplo and then incubated at 35°C±2°C for 18-24 hours. Colonies thought to be *E. coli* have a diameter of 2-3 mm, have a black or dark center, and a greenish metallic color.

2.4. Data analysis

The data obtained were analyzed using descriptive quantitative data that describe or explain an event with significant numbers [12].

3. Results and Discussion

3.1. Total Escherichia coli in Litter

Escherichia coli have opportunistic properties, which are normally found in the digestive tract but can become pathogenic if the amount is excessive both in the intestine and outside the intestine [6,13] and [14]. In litter, the *E. coli* bacteria develop and can cause colibacillosis. the total average of *E. coli* bacteria on litter can be seen in table 2.

Table 2. Average *Escherichia coli* (CFU/g) in litter in closed house cages at different zones and ages.

Day	Total of *Escherichia coli* (CFU/g)				Average
	Zone I	Zone II	Zone III	Zone IV	
0	2.0×10^3	9.4×10^4	0	3.7×10^1	2.4×10^4
7	1.8×10^6	4.1×10^6	2.3×10^7	4.2×10^7	1.8×10^7
14	4.2×10^7	1.7×10^7	6.6×10^6	6.1×10^6	1.8×10^7
21	9.2×10^6	2.5×10^6	8.8×10^6	6.2×10^6	6.7×10^6
28	5.3×10^4	1.6×10^6	42×10^5	2.0×10^5	5.7×10^5
Average	5.3×10^7	2.5×10^7	3.8×10^7	5.4×10^7	4.3×10^7

Based on table 2, the results obtained on day 0 show the total *E. coli* bacteria in litter in the zone I (2.0×10^3 CFU/g), zone II (9.4×10^4 CFU/g), and zone IV (3.7×10^1 CFU/g) where the expected condition is that there are no *E. coli* bacteria as in zone III (0) because the cage has been sanitized and fumigated. This condition shows that fumigation in a closed house does not reach every corner of the litter in the cage. Litter sterilization using fumigation techniques is not able to kill all microorganisms in the litter [15]. However, this fumigation method is quite effective in reducing the risk of *E. coli* infection at the beginning of chicken rearing where the tolerance limit for *E. coli* bacteria in litter according to Barnes et al [6] is 10^5-10^6 CFU/g.

In table 2 can be seen that the total average *E. coli* bacteria in each zone is 10^7 CFU/g. These results indicate that the difference in temperature, humidity, and wind speed in zoning in closed house cages is

not enough to have a significant effect on the total *E. coli* bacteria on litter. This is following the statement of Metasari et al. [16] and Yusrizal [17] stated that the use of modern tools in closed house cages causes the ventilation and circulation systems in the cage to be well controlled so that ammonia emissions from bacterial metabolism can be minimized.

Table 2 showed that zones 3 and 4 on day 7 and zones 1 and 2 on day 14 exceed the tolerance limit for *E. coli* on litter, was 10^5-10^6 CFU/g [6]. This is probably because on the 7th day the litter mat is scraped off in zones 1 and 2 so that it can prevent a significant increase in total *E. coli* on the litter, while zones 3 and 4 are not scraped. Likewise, on the 14th day, the litter bed was scraped off in zones 3 and 4. On the 21st day, zones 1 and 2 showed a decrease in the total *E. coli* bacteria, which was probably due to the removal of the litter bed on that day. On the 21st day zone 3 and 4, there was no significant increase in total *E. coli* because on that day the litter bed was reversed. On the 28th day, there was a significant decrease in the total *E. coli* because on that day a new husk addition treatment was carried out.

3.2. Ammonia (NH_3) gas production

Ammonia (NH3) is a gas produced from the process of overhauling nitrogen residues by decomposing bacteria (ureolytic bacteria) from chicken feces [18]. Air polluted with ammonia gas will affect livestock health and can have an impact on livestock production. Ammonia gas production in the cage at different zones and maintenance times can be seen in table 3.

Table 3. Ammonia gas production (ppm) in closed house cages in different zones and maintenance.

Maintenance time (day)	Zone I	Zone II	Zone III	Zone IV	average
0	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00
21	2.20	3.67	2.97	12.77	5.40
28	19.53	16.43	14.63	11.53	15.53
average	5.3	5.03	4.40	6.08	5.23

Each zone of placement of broilers has different microenvironmental conditions. These different environmental conditions are presented in table 3. The data shows that the more distant zone from the inlet, the temperature will increase and humidity and wind speed will decrease. In table 6, it can be seen that on the 28th day the zone is farther from the inlet, the production of ammonia gas will decrease. This is because zone 1 has higher humidity and lower temperature so that it can break down nitrogen-containing urea into ammonia gas. An increase in temperature could lead to more optimum microbial growth so that more ammonia production [19]. High temperatures and normal or higher humidity result in increased microbial activity, which results in more optimal volatilization of ammonia. According to Pereira [18], the ammonia gas produced in the cage comes from fermentation between excreta and cage litter. High humidity and relatively low temperatures will cause urea-containing nitrogen to break down into ammonia and CO_2 gas. On the 21st day, the production of ammonia gas varied, probably due to differences in the amount of chicken feces production, the diversity of chickens, the number of culled chickens, and the mortality rate. The degradation process of metabolic waste into ammonia is influenced by humidity, temperature, pH, litter material, feed composition, livestock density, and ventilation circulation in the pen [20].

The wind speed in the closed house is increased as the chickens age. This is because in the starter phase the production of ammonia gas is still low so that the wind speed used is only 1-2 m/s, while in the finisher phase the wind speed is increased to 3 m / s because ammonia production has increased. Table 3 showed that the highest average production of ammonia gas levels detected by the ammonia detector was 19.53 ppm. This level of ammonia gas is still considered safe for breeders and livestock. The tolerance limit for ammonia for human safety and animal welfare is 25 ppm [21]. The compromise limit for NH_3 (ammonia) levels is 20 ppm, if it exceeds this limit it can affect the immune system of

chickens. This table also showed that the production of ammonia gas has increased significantly on the 28th day. Table 4 shows the total number of *E. coli* bacteria decreased significantly on day 28. This condition may occur due to the presence of other Gram-negative bacteria that affect ammonia production in the cage. The activity of this pathogenic microorganism (*E. coli*) which is metabolized effectively can produce NH_3 emissions [17]. This means that the more the number of *E. coli* was the cause of increasing ammonia. Ammonia (NH_3) in chicken coops is formed from a chemical reaction between uric acid ($C_5H_4N_4O_3$) and water (H_2O) as well as uricase enzymes from Gram-negative bacteria. The same thing was stated by Riza [22] that ammonia gas (NH_3) is formed as a result of the metabolism by microbes in excreta.

4. Conclusion

Environmental conditions in the form of temperature, humidity, and wind speed in the zoning in a closed house cage are quite evenly distributed so that they do not have a significant effect on the total *E. coli* on the litter. The zone close to the inlet has higher humidity and wind speed and lower temperature, which causes the production of ammonia gas in zone 1 to be higher than the zone far from the inlet. Turning, scraping, and adding litter is very effective in reducing the total *E. coli* in the litter but less effective in reducing the production of ammonia gas in the cage.

References

[1] Hidayat M N, R Malaka, W Pakiding and L Agustina 2016 Abdominal fat percentage and carcass quality of broiler given probiotic *Bacillus* spp *Scientific Research Journal (SCIRJ)* **4** 33-7
[2] Thaha A H, R Malaka, W Hatta, D Marmansari, E Purwanto and K Kiramang 2020 Sanitary hygiene implementation at *Salmonella* sp. critical control points in layer farms *IOP Conf. Ser.: Earth Environ. Sci.* **492** 012098
[3] Ferry T 2012 *Ayam Broiler, 22 Hari Panen Lebih Untung* (Jakarta: Penebar Swadaya)
[4] Renata T, A Sarjana dan S Kismiati 2018 Pengaruh zonasi dalam kandang *closed house* terhadap kadar amonia dan dampaknya pada kualitas daging boiler di musim penghujan *Jurnal Ilmu-Ilmu Peternakan* **28** 183-91
[5] Chen Z and X Jiang 2014 Microbiology safety of chicken litter or chicken litter based organic fertilizers: a Review *Agriculture Journal* **4** 1-29
[6] Hidayat M N, Malaka R, Agustina L and Pakiding W 2018 Effect of *Lactobacillus* sp. probiotics on intestinal histology, *Escherichia coli* in excreta and broiler performance *J. Indonesian Trop. Anim. Agric*. **43** 445-52
[7] Barnes H J, Vaillancourt J P and Gross W B 2003 *Colibacillosis In Diseases of Poultry. 11th ed.* (Ames: Lowa State University Press)
[8] Moore Jr P A, Burns R and Miles D M 2008 Reducing Ammonia Emissions from Poultry Litter with Alum *Proceedings of Mitigating Air Emissions from Animal Feeding Operations Conference* May 19-21, 2008 (Des Moines: Iowa) p 90-4Tarmudji 2003 Kolibasilosis pada ayam: etiologi, patologi dan pengendaliannya *Wartazoa* **13** 65-73
[9] Bauzad M, F N Yuliati, K I Prahesti and R Malaka 2019 Total plate count and *Escherichia coli* in raw buffalo milk in Curio District Enrekang Regency *IOP Conf. Ser.: Earth Environ. Sci.* **247** 012027
[10] Islam M M, M N Islam, Sharifuzzaman and M Fakhruzzaman 2014 Isolation and identification of *Escherichia coli* and *Salmonella* from poultry litter and feed *Int. J.Nat. Soc. Sci.* **1** 1-7
[11] Sudjana N dan Ibrahim *2004 Penelitian dan Penilaian Pendidikan Ed. III* (Bandung: Sinar Baru Algesindo)
[12] Hatta W, Sudarwanto M, Sudirman I and R Malaka 2013 Prevalence and sources of contamination of *Escherichia coli*, and *Salmonella* spp. in cow milk dangke, Indonesian fresh soft cheese *Global Veterinaria* **11** 352-56
[13] Jawetz E, Manick J L and Adelberg E A 1980 *Review of Medical Microbiology14th Ed.* (Singapore: Huntsman Offset Printing Pte Ltd)

[14] Fontenot J P, Webb K E, Harmon B W, Trucker R E and Moore W E C 1971 Studies of processing nutritional value and palatability of broiler litter for ruminants *In Proceeding of the International Symposium of Livestock Wastes, Colombus* 19-22
[15] Metasari T, Dian S and Veronica W 2015 Pengaruh jenis bahan litter terhadap kualitas litter broiler fase finisher di *closed house Jurnal Ilmiah Peternakan Terpadu* **3** 23-9
[16] Yusrizal, Manin F, Yatno and Noverdiman 2012 The use of probiotic and prebiotic (symbiotic) derived from palm kernel cake in reducing ammonia emission in the broiler house *Proceeding the 1st Poultry International Seminar* 334-43
[17] Pereira J L S 2017 Assessment of ammonia and greenhouse gas emissions from broiler houses in Portugal *Atmospheric Pollution Research* **8** 949–55
[18] Sarjana T A, Mahfudz L D, Ramadhan M, Sugiharto, Wahyono F and Sumarsih S 2017 Kadar amonia dan kondisi litter pada kendang ayam broiler sistem terbuka yang mendapatkanadditif berbeda dan kombinasinya dalam ransum *Prosiding seminar Nasional Pengembangan Peternakan Berkelanjutan*
[19] Estevez I 2002 NH_3 and poultry welfare *Poultry Perspectives* **4** 1-3
[20] Kristensen H H and Wathes C M 2000 Ammonia and poultry welfare: A review *World's Poultry Science Journal* **56** 235-45
[21] Riza H, Wizna, Rizal Y and Yusrizal 2018 Pengaruh level energi dan protein dengan bakteri *Bacillus amyloliquefaciens* sebagai probiotik untuk mengurangi pencemaran amonia pada kandang ayam broiler *Jurnal Peternakan Indonesia* **20** 99-107