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Zonation in closed house affecting ammonia emission, immune system and broiler performance in the dry season

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Abstract. The aim of the study was to evaluate effect of zonation in closed house on ammonia emission, immune system and broiler chicken performance in the dry season. A total of 720 unsexed broilers were subjected into 4 treatment : zone 1, zone 2, zone 3, and zone 4 (at 0, $\frac{1}{4}$, $\frac{1}{2}$, and $\frac{3}{4}$ the length of closed house from inlet). Ammonia emissions, relative weights of thymus, spleen and bursa fabricius, neutrophil, lymphocyte levels, and N/L ratio broilers were measured. Performance index parameters were also observed to illustrate the impact of zoning on broiler performance. Obtained data were statistically tested by analysis of variance. Results showed that the farther zone of broiler placement from inlet significantly increase ammonia emissions start from zone 3, decrease lymphocyte levels started form zone 4 and performance index started from zone 2, meanwhile relative weight of thymus, spleen, bursa fabricius, and N/L ratio were not significantly affected. This research concluded that the farther zone from inlet increase the ammonia emissions, reducing broilers immune system and performance in dry season.

1. Introduction

Broiler farmers in the tropical areas such as Indonesia facing a lot of problems. During dry season, this area has a low rainfall of 50 – 100 mm [1], high temperature and low humidity. As a result temperature and humidity changes that enter the inlet contribute to microclimate changes. Microclimate changes would be followed by ammonia volatility. High temperature conditions would be increased ammonia volatility [2,3]. Increased ammonia volatility caused an increased ammonia emissions in the broiler house, which ammonia emissions could increase the broiler's susceptibility of diseases. Ammonia emissions are not constant throughout the year, it changes with the season, geographic area, level of precipitation, relative humidity and poultry housing management [4]. Therefore, the dry season might contributes to increase ammonia emissions in the broiler house.

Broiler maintained in closed housing system in order to resolve temperature and humidity fluctuations problems. In a closed house, the temperature and relative humidity can be controlled [5] moreover excess heat, water vapor, and other harming gasses like CO, CO₂, and NH₃ normally can be reduced [6]. However, there were some problems in the closed house such as differences in temperature and humidity distribution between zones. The closer zone from inlet has a lower air and fresher temperature, but increases continuously until it reaches the outlet [7]. Higher temperature and relative humidity at the farther zone from inlet was the result of temperature and humidity accumulation from closer zone [8]. As a result, we predict that ammonia emissions in the farther zone from inlet were



different from the closer one, because the amount of ammonia volatility rate might increased due to effective temperature and humidity changes inside the closed house.

Ammonia is a colorless gas from decomposition of nitrogen in excreta by microbial processes [9]. When exposed to an excessive amount, ammonia may irritates the broiler respiratory tract. As a result of the respiratory tract damage, there are reduces in body's immune system lead to susceptibility of respiratory diseases [10]. Irritated respiratory tract could affect neutrophils to work and increase it levels. In other study [11], reported that 70 ppm of ammonia level can reduce lymphoid organ weight by 9,73%. The decrease in lymphoid weight such as thymus, bursa fabricius, and spleen in broilers can reduce lymphocyte levels [12], and increasing disease susceptibility. Decreased lymphocyte levels may cause a high value of the neutrophil/lymphocyte (N/L) ratio, which indicates broiler stress state. The bad litter quality such as wheat straw produce 19% higher ammonia emission and has an impact on reducing the final weight gain of broiler [13]. Based on the description, the increase of ammonia emissions in different closed house zones could affect broiler immune system and performance.

This research was conducted in a commercial closed house, wet tropical areas, during dry season. The objectives of this research were to evaluate the effect of zonation in a closed house on ammonia emission, immune system, and performance of broilers in the dry season.

2. Materials and methods

Seven hundred and twenty unsex broilers with initial body weight $49,25 \pm 1,13$ g were used and maintained for 30 days in a closed house at Faculty of Animal and Agriculture Science, Diponegoro University, Semarang with 60 m length \times 12 m wide, and 11.000 broilers capacity. This research used a complete randomized block design with 4 treatments and 6 groups. The treatments were zone 1, zone 2, zone 3, and zone 4 respectively at 0 (0 m), $\frac{1}{4}$ (15 m), $\frac{1}{2}$ (30 m), and $\frac{3}{4}$ (45 m) from inlet. This research was conducted in a commercial closed house during dry season with 4 pens divisions that adjusted in standard density based on chicken weight every week. A complete feed (table 1) with code: S10, S11, and S12 were given during maintenance as a starter, grower and finisher diets. Each trial units was consist of 30 broilers. From each unit, 2 broilers were taken which presented the unit's average standard body weight for observing lymphoid relative weight, neutrophil levels, lymphocyte levels, and N/L ratio samples, while broiler performance was assessed from all experimental units.

Table 1. Nutrient Content of Experimental Diets

Nutrient Content	S10	S11	S12
	------(%)-----		
Dry Matter*	10.59	10.79	12.20
Crude Fat*	5.56	6.04	5.60
Crude Fiber*	4.94	6.32	5.57
Crude Protein*	20.22	19.31	18.27
Ash*	5.44	5.39	5.58
Ca*	1.08	1.16	0.91
NFE**	53.24	52.15	52.79
ME**	3155	3122	3072

*Chemical analysis at the Laboratory of Nutrition and Feed Science, Faculty of Animal and Agriculture Science, Diponegoro University (2017); **Calculated value based on Bolton Formula [14]

Parameters measured were ammonia emissions, relative weight of thymus, spleen and bursa fabricius, neutrophil level, lymphocyte level and N/L ratio broilers. Ammonia emissions was measured in each unit with an ammonia detector at 5.00; 13.00; and 21.00 with 3 days interval observation. Ammonia detector was placed between 10 – 15 cm above litter to read ammonia emissions in the unit. Lymphoid relative weight such as thymus, bursa fabricius, and spleen expressed in g/kg of body weight. In the measurement of neutrophil and lymphocyte levels, blood serum were taken through the brachial vein on wing, analyzed using a hematology analyzer with electrical impedance methods. Every blood

cells that passed through a small narrow between two electrodes on the device were counted. The N/L ratio obtained from comparison between neutrophil and lymphocyte blood levels. In this study, performance index changes were also observed to measure the impact of ammonia emission by the following formula [15]:

$$\text{Performance Index} = \frac{\text{Body weight (kg)} \times \text{livability (\%)} \times 100}{\text{Age (days)} \times \text{feed conversion ratio}}$$

To find out some factors that might have an impact on ammonia volatilization, microclimate and macroclimate conditions were also observed in this study (table 2). Obtained data were statistically tested by analysis of variance and followed by Duncan test at 5% probability when the treatment indicated significant effect.

Table 2. Averages of Macroclimates and Microclimates during Research.

Macroclimates	Value			
Temperature (°C)	29,53 ± 4,89			
Humidity (%)	68,67 ± 22,19			
Air Velocity (m/s)	2,40 ± 1,43			
Average of Monthly Rainfall* (mm)	50 – 100			
Microclimates	Zone 1	Zone 2	Zone 3	Zone 4
Temperature (°C)	26,23	26,78	27,78	27,86
Relative Humidity (%)	76,99	76,24	74,84	73,37
Air velocity (m/s)	1,90	1,39	1,11	0,72

* Data collected from the Indonesian Meteorological, Climatological, and Geophysical Agency (2017).

3. Results and discussion

The anova results are provided in table 3 showed that ammonia emissions was significantly ($P \leq 0,05$) increased start from zone 3, and decreased lymphocyte levels start from zone 4. However, relative weight of lymphoid organs, neutrophil levels and N/L ratio were not significantly affected ($P > 0,05$). There were significant decrease on broiler performance index started from zone 2 ($P \leq 0,05$).

3.1. Ammonia emissions

In our research, ammonia emissions at the highest point were still classified as low and below the tolerant limit which started to affect broiler performance at more than 6 ppm [16]. We presume at least there were 3 things that caused the ammonia emission to increase with the farther distance from inlet, first the accumulation effect due to negative pressure air velocity that brings the ammonia toward outlet direction, second the increase of ammonia volatility probability due to microclimate condition that are more conducive to perform bacterial fermentation, and third the increase of broiler oxidative stress will lead to excessive nitrogen excretion due to digestion process inefficiency.

Table 3. Ammonia emissions, relative weight of lymphoid organs, neutrophil levels, lymphocyte levels, N/L ratio, and Performance Index

Parameter	Zone 1	Zone 2	Zone 3	Zone 4	P	SE
Ammonia (ppm)	1.75 ^c	2.82 ^c	4.37 ^b	6.56 ^a	0.01	0.40
Thymus (g/kg)	2.75	3.16	2.78	2.30	0.24	0.03
Spleen (g/kg)	1.25	1.15	0.98	0.92	0.25	0.01
Bursa Fabricius (g/kg)	0.45	0.45	0.40	0.38	0.68	0.00
Neutrophil ($\times 10^3$ /uL)	0.97	1.15	1.20	0.73	0.58	0.26
Lymphocyte ($\times 10^3$ /uL)	21.89 ^a	21.02 ^a	25.57 ^a	13.30 ^b	0.02	0.33
N/L Ratio	0.04	0.05	0.04	0.05	0.72	0.01
Performance Index	388.53 ^a	345.89 ^b	336.45 ^{bc}	308.42 ^c	0.00	10.09

Significantly higher ammonia emission in zone 4 was due to ammonia accumulation effect. The negative pressure created by exhaust fan bring the ammonia emissions produced at closer zone from inlet to be carried by wind toward farther zone from inlet and then pass through the outlet. Ammonia emissions at zone 4 was ammonia accumulations from zone 1, 2, and 3. The lower air velocity at zone 4 (see table 2.) might also contribute to escalate higher ammonia emission and elimination in this particular zone. Ammonia emissions in a broiler house correlate with the air velocity [17]. The average of air velocity in this study was 0.24 – 0.99 m/s, higher than the other study [18], air velocity ranges from 0.12 – 0.21 m/s.

Higher temperature in farther zone from inlet was also predicted to increase ammonia emissions, because the chance of ammonia volatility was greater than the other zones. Hotter air temperature that pulled toward outlet direction would increase the effective temperature in particular zone. Increased of temperature would lead to poor litter quality such as warmer and damp litter which are ideal for microbial fermentation process which eventually increase the ammonia volatility and emission. Relatively high temperature in the dry season may also contribute to the high ammonia emissions inside closed house due to higher input of air temperature for the closed house. High ammonia emissions was induced by higher housing temperature [19].

We presume that the increase of ammonia emission in different zone might lead to oxidative stress. Oxidative stress is a condition which presence of reactive species in excess of the body's capacity [20] and predicted to have an impact on inefficiency of nutrient digestibility, particularly the protein. These conditions increase the nitrogen excretion as results the more available material for ammonia emission. Ammonia exposure known to changes the pulmonary structure, which affecting the heat loss efficiency and body temperature [21]. As explained increase of pulmonary tension and inefficiency of heat loss increase the body temperature which may lead to triiodothyronine (T3) and thyroxine (T4) activity reduction [21,22]. This thyroid hormone involved in controlling metabolic rate [23] and protein turnover [22].

3.2. *Lymphoid organs relative weight*

Relative weight of thymus, spleen, and bursa fabricius as provided in table 3 were not significantly different ($P>0.05$). The relative weight of thymus was 2.30 – 3.16 g/kg, spleen was 0.92 – 1.25 g/kg, and bursa fabricius was 0.38 – 0.45 g/kg of body weight. These results are lower than weight of lymphoid organs in other study [24] that relative weight of thymus was 4.79 g/kg, spleen was 1.16 g/kg and bursa fabricius was 2.67 g/kg. Meanwhile in another research thymus, spleen, and bursa fabricius relative weight were 4.8 g/kg, 1.8 g/kg and 0.98 g/kg of the body weight [25].

Ammonia emissions (table 2) was below the broiler tolerant limit of ammonia, so they had not affected the lymphoid organs relative weight. Ammonia emissions with 30 ppm had not significantly reduced the lymphoid organs weight [11]. Ammonia levels at least 20 ppm have not affected the immune system such as relative weight of thymus and bursa fabricius [26].

3.3. *Neutrophil, lymphocyte levels, and N/L Ratio*

The neutrophil value in the table. 3 ranged 0.73 - $1.20 \times 10^3/\mu\text{L}$ and it was lower than normal value that the neutrophil value in broiler chickens is around $6.12 \times 10^3/\mu\text{L}$ [27]. Neutrophil levels in broilers was not significantly affected by ammonia emissions. Neutrophils have the function of destroying foreign material through the process of phagocytosis [28]. Generally ammonia emissions will increase the risk of infection, especially in the respiratory tract and followed by the increase of neutrophil levels. Neutrophils belong to a non-specific immune response whereby this immune system will limit the spread of pathogens before reaching the specific immune system (lymphocytes) [29]. Thus, we presume that neutrophil levels are not affected since neutrophils acts on short-term stress responses. We expect that neutrophils will work first before lymphocytes, to resolve foreign objects due to short-term irritation, hence in the long-term, neutrophils will be adjust and return to normal levels. In our research the different zone inside broiler closed house start to have significant different ammonia emissions since the broiler is 2 weeks old (unpublished data) while neutrophils observed at fifth week.

The increase of ammonia emissions significantly decrease the lymphocyte levels in zone 4 ($P \leq 0.05$). The farther zone from inlet reduced the lymphocyte levels in broilers. The higher ammonia emissions in zone 4 is potentially irritate the respiratory tract. We predict that the lowest lymphocyte levels found in zone 4 were due to significant long-term exposure of higher ammonia emissions despite it was in low levels, hence causing lymphocytes to have an extra work forming antibodies. In our research we categorize the ammonia emissions as a long-term stressor for broilers since the exposure happens for more than three-quarter of their life cycle. Long-term stress or chronic stress affects lymphocyte levels [30], otherwise short-term stress or acute stress affects neutrophil levels. We predict that long-term ammonia exposure, affecting body's immunity that indicated by a significant decrease on lymphocyte levels.

Table 3 above informed that N/L ratio of broilers was not significantly ($P > 0.05$) affected by the zone. Ammonia emissions in the closed house has not affected the N/L ratio yet since it still below tolerant limit for broilers. The higher N/L ratio, the higher stress level on broilers [12]. The value of N/L ratio 4 – 5 in table 2 was on normal range [31]. Broiler standard value of N/L ratio in broilers is 0.4, the bigger levels refer to the state of stress condition.

3.4. Performance Index

The highest performance index was obtained in zone 1, and the lowest performance index was obtained in zone 4. In this research performance index can be used as an indicator of successful housing management. The slower air velocity the lesser ammonia elimination lead to higher ammonia emissions in farther zone from inlet. Respiratory tract irritation lead to the increase of pulmonary tension and heat loss inefficiency followed by the increase body temperature [32,21,33]. This condition potentially suppressed broiler feed intake which eventually affecting broiler performance. This is according to another research [34] that ammonia emissions reducing broilers feed intake and performance. Performance index between 351 – 400 indicates that livestock has very good performance, while the value that lower than 300 means that broiler has bad performance [35]. The performance index has begun to decrease from its standard starting from zone 2. The continuous slight exposure to ammonia emissions at 2.82 ppm (zone 2) has already start to decrease the broiler performance. We provide more sensitive results compared to other researcher which reported that ammonia emission started to affect broiler performance within the range of 6 to 10 ppm [16]. While another researcher [34] reported that atmospheric ammonia concentrations at 50 and 75 ppm depressed the commercial broilers final body weight at 7 weeks 6 and 9 % respectively.

4. Conclusion

The farther zone from inlet increase the ammonia emissions, reducing broilers immune system and performance in dry season.

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