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CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF BROILER LITTER KEPT AT DIFFERENT ALTITUDES

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ABSTRACT: The research objective was to assess the chemical and microbiological properties of broiler litter raised in broiler closed house at different altitudes. The design used was a completely randomized design with 3 treatments and 5 replications. The treatments consisted of T1 (broiler closed house at an altitude of ≤100 meters above sea level (masl), T2 (broiler closed house at an altitude of 100 - 300 masl) and T3 (broiler closed house at an altitude of 300 - 500 masl). The results showed that the difference in the altitude where broilers were raised had no significant effect on moisture, ash, extract ether (EE), crude fibre (CF), nitrogen free extract (NFE), total digestible nutrients (TDN), cuprum (Cu), lead (Pb), mercury (Ag), lactic acid bacteria (LAB), *Salmonella, E. coli, Clostridium sp.,* antibiotic contamination (below the threshold) and predominance of gram-positive bacteria. The results of water content 22.71-24.65%, crude protein 13.13-13.47%, Pb 35.15-47.80 ppm, Cu 102.46-136.96 ppm, LAB 3.48 - 7.00 × 10^6 cfu/g. In conclusion, the differences in the altitude did not affect the chemical and microbiological properties of broiler litter.

Keywords: Altitude, Bacteria, Chemical properties, Litter, Poultry.

INTRODUCTION

The increase in demand for broiler chicken meat is in line with the increase in livestock business. The increase in demand for broiler chicken meat in Central Java, Indonesia in 2019 was around 13,000 tons (Statistics of Central Java, 2019). Broiler chicken farming industry produces waste in the form of litter and can be a cause of environmental pollution (Dunlop et al., 2016; Seidavi et al., 2019). Efforts to reduce poultry waste can be done by processing the waste into materials with higher economic values (Sahoo et al., 2017).

The macroclimate and microclimate conditions of broiler house are one of the determining factors of success in the world of animal husbandry (Kic, 2016). The macroclimate and microclimate conditions of broiler house are affected by the altitude of the region where the broiler farm is built (Nazareno et al., 2016; Dróżdż et al., 2020). Extreme temperatures can be stressful for livestock and therefore affecting livestock productivity (Henry et al., 2018).

In general, the optimum temperature for the growth of broiler chickens ranges from 18-21 °C, and those highland areas have lower temperatures than lowland areas (Vilchis et al., 2012). Farm management is the key to successful production of broiler chickens; one of the efforts to improve management is the use of pedestals and chicken warmers in the form of litter (Wang et al., 2016). Traditionally, the litter can be prepared from rice straw, husks, or sawdust (Garcia, 2007). The constituent composition of litter is manure, chaff and limestone. Litter contains 9-11% protein, 91-94% dry ingredients, 11-50% crude fiber and 1-3% crude fat (Rahimi et al., 2018). Good quality litter will not be the growth medium for parasite development (Najibulloh et al., 2020). Good litter has the characteristic that the water content is 20-25 percent, capable of well absorbing water, does not contain harmful materials and is dust-free (Petek et al., 2014).

The study aimed to examine the chemical and microbiological qualities of broiler litter raised in broiler closed house at different altitudes. It is expected that the study results will provide preliminary information on the quality of broiler litter obtained at different altitudes so that it can be used as the basis for the processing of litter into alternative feed materials.

MATERIALS AND METHODS

Materials

The material used was litter of 15 broiler closed house of PT. Citra Unggas Lestari located in Demak Regency (altitude ≤ 100 meters above sea), Semarang city (altitude 100-300 meters above sea) and Kendal Regency (altitude 300-500 meters above sea level).

Methods

The research began with litter sampling of broiler chickens from 15 partnership cages PT Citra Unggas Lestari. Sampling is done purposive random sampling that can represent the percentage of area and capacity of the cage. The

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research design used is complete randomized design (CRD) with 3 treatments and 5 repeats. The treatment given is T1 (closed house cage at an altitude of ≤100 meters above sea level), T2 (closed house cage at an altitude of 100-300 meters above sea level) and T3 (closed house cage at an altitude of 300-500 meters above sea level).

Research parameter testing

Proximate analysis

Proximate analysis includes testing water content, ash, crude protein, crude fat, crude fiber, calculation of extract materials without nitrogen and TDN (AOAC, 2005). Water content analysis is done using drying method with oven. Analysis of ash content is done by the method of smoking using *muffle furnace* (AOAC, 2005). Analysis of crude protein levels using the Kjeldahl method (AOAC, 2005). Crude Fat level analysis is done by ether extraction method (AOAC, 2005). Analysis of crude fiber levels using a method of boiling with a solution of weak acids and weak bases (AOAC, 2005). NFE levels are calculated using a formula (Alagbe et al., 2020) namely:

NFE = 100 - (Ash + Crude Fat + Crude Fiber + Crude Protein)

The calculation of total digestible nutrients is calculated by formula (AOAC, 2005) namely:

TDN = % Crude Fiber digestible + % NFE + % Crude Protein + 2.25% Crude Fat

Metal content testing Pb

Testing of pb metal content in the sample was done using Atomic Absorption Spectophotometer (AAS) method with different wavelength principle for each type of metal (Oliviera et al., 2017). Absorbance measured at a wavelength of 283 nm using AAS variant AA240 Australia and calculated the concentration of lead metal (Pb) using lead metal concentration equation (Pb) as follows (AOAC, 2005).

(µg/g) = "C x VC x V" / "W W"

Description:

C = concentration of mg/L (ppm) measurement results converted into units of µg/L (ppb)

V = total sample volume (mL) converted to liter unit (L)

W = sample weight (g)

Cu content testing

Testing of Cu content in litter samples is done by AAS method (Gu et al., 2020). The pre-prepared sample was measured with an AAS at a wavelength (λ) of 324.8 nm to determine its concentration.

Mercury testing

Mercury test was conducted using qualitative and quantitative analysis of mercury using atomic absorption spectrophotometer method (Pasinszki et al., 2020). The tools used are mercury analyzer.

Antibiotic contamination analysis

Antibiotic contamination test is done using the method of screening tester bioassay (Jonkers et al., 2020). Testing using special tools such as HPLC *(*high pressure liquid chromatography*),* TLC (thin layer chromatography*)* and GC *(Gas Chromatography)* to find out the type of antibiotic compounds quantitatively (Mahmood et al., 2019).

Total lactic acid bacteria analysis

Determination of the number of total lactic acid bacteria (LAB) colonies of each litter sample is measured using the total plate count (TPC) method (Kwak et al., 2008). Colonies that grow stem-shaped, have positive grams, not form spores, are non-motile, are anaerobic, catalase negative and oxidation positive. Then the LAB population is calculated as follows:

LAB Population (cfu/g) = Number of Colonies x Dilution

Gram positive and negative bacterial analysis

Testing gram positive and gram negative is done by pour method (pour plate) followed by gram staining method (gram staining method) (Rajivgandhi et al., 2018). Observations are carried out under a microscope with a magnification of 1000x, observed the shape of the cell and its color. If the bacteria is pink belongs to the gram negative group.

Salmonella content testing

Salmonella content testing using carter method (Andoh et al., 2017).

Escherichia Coli content testing

Identification of *E. Coli content* is done by Most Probable Number (MPN) method (Ahmed et al., 2015).

Clostridium sp content testing

Clostridium sp content testing is done by Total Aerobic Count method (Angelotti et al., 1961).

Statistical analysis

The data obtained was analyzed using analysis of variance (ANOVA) test to test data diversity and if there is any real influence, it will be continued with Duncan's multiple range test (DMRT) at a 5% confidence level.

Ethical approval

The *in vitro* experiment did not need ethical approval from the animal ethics committee.

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RESULTS AND DISCUSSION

Proximate composition and TDN of litter from broiler chickens raised at different altitudes

The results of the analysis indicate that the altitude of the various cage locations, namely T1 (altitude of 0-100 meters above sea level), T2 (altitude of 100-300 meters above sea level) and T3 (altitude of 300-500 meters above sea level)) did not have a real impact on the content of litter water (P>0.05).In the broiler litter, the total water content was 9.2 %. Owen al. (2008) noted that in broiler house with a height of 120 meters above sea level, litter in the broiler chicken had a water content of 12-25%. Qian et al. (2018) documented that broiler house at low altitudes had high temperatures that cause the chickens to consume more water so that the excreta released was moist. As the litter in all broiler houses came from the same substance, namely husks, the moisture content of the litter was not largely different. Ritz et al. (2017) stated that a suitable litter has a characteristic that the water content ranges from 20-25%, able to absorb water well, does not contain harmful materials and is free from dust. The effect of altitude also does not affect the water content of litter because the type of litter source cage is the same, namely closed house cages. *Closed house* has a mechanism to maintain litter quality in order to remove excess moisture in the cage. This is thought to result in the litter water content produced in this study, which is not influenced by the altitude of the location. Kaukonen et al. (2016) stated that a good litter condition can be measured through the water content parameter; the optimal litter water content indicates that the litter condition is good.

The findings of the present study revealed that different altitudes of broiler house had no significant (P>0.05) effects on the ash content of litter. In broiler chicken litter, the total ash content was 24.11%. Barnes et al. (1997) reported that in broiler litter the average ash content value is 20-25 percent. In general, there are several factors affecting the ash content in broiler litter, which are the type of litter material as well *as* the altitudes of broiler house located. Pappas et al. (2010) stated that litters prepared from the materials such as wood shavings have higher absorption and have more organic content. The altitude of the location did not affect the litter ash content, presumably because there was no difference in maintenance management from each study cage. The ash content in the litter is influenced by the mineral composition of the excreta and husks, so that no differences in the mineral content have an impact on the ash content of the litter which is not different either. Owen et al. (2008) stated that the mineral content in raw materials is influenced by mineral content, because the ash content describes the amount of mineral substances that do not burn into steam.

It was shown in this study that the differences in the altitudes of location of broiler houses showed no substantial effect on the content of crude protein litter (P>0.05). Crude protein content in litter has an average value of 13.31%. Cross (1995) stated that the crude protein content in poultry chicken litter is 11-15%. The high protein content in litter is caused by ammonia. Ferguson et al., (1998) states that the protein content in litter is still relatively high due to the presence of ammonia formed from the decomposition of nitrogen by microbes and the amount of nitrogen that is not absorbed by chickens so that it is released along with substances that are not absorbed by the body, namely excreta. This is also the reason why the elevation of the location does not affect the protein litter levels. The relatively same cage management makes no difference in ammonia in litter, so that the height of the location does not affect the litter protein content.

In this study, the altitudes of location of broiler houses had no effect on the content of Crude fiber of broiler litter (P>0.05). The average crude fiber content in broiler litter was 23.34%. Stephenson et al. (1990) stated that the fiber content in poultry manure is 18-69%. The factor that causes high crude fibers in litter is the constituent component of fibers, namely lignin. Knudsen (2014) states that high fiber content is caused by lignin content as the main component of plant tissue formation. The altitude of the location does not affect the litter crude fiber content produced. The main fiber content of litter comes from rice husks, so that the fiber measured in litter is a large contribution from rice husk fiber. The cages for the source of research litter used the same base, namely rice husks, so that there was no effect of height differences on litter fiber content. The altitudes of location of broiler houses had no substantial effect on crude fat content of broiler litter. The altitude of the location does not affect the litter fat content. This can happen presumably because the litter water content is not significantly different from the location height treatment. Pappas et al. (2010) stated that the high and low levels of fat are influenced by the moisture content of the material, the amount of fat is calculated to be greater in the proximate analysis, it can occur if the water content that comes out of the material is higher. The average content of Crude fat in chicken litter was 1.72%.

The results of the analysis showed that the altitude of different cage locations, namely T1 (cage altitude 0-100 meters above sea), T2 (cage altitude 100-300 meters above sea level) and T3 (altitude 300-500 meter above sea) had no real effect on the content of NFE Digestibility litter (P>0.05). The absence of the influence of the location height treatment on the crude protein and crude fiber, the cause of the NFE litter value is also not affected by the location height treatment. The average content of NFE Digestibility litter chicken is 37.54. Caswell et al. (1978) stated that NFE digestibility content in chicken litter is 26.1%. The value of nitrogen free extract ingredients is determined by other nutrient levels. Alam et al. (2008) stated that NFE digestibility value is influenced by the ups and downs of other levels such as crude fiber, crude protein on the material.

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The results of the analysis showed that the different altitudes of broiler house locations, namely T1 (cage altitude 0-100 meters above sea), T2 (cage altitude 100-300 meters above sea) and T3 (altitude 300-500 meters above sea level) showed no real effect on the content of crude fat litter (P>0.05). TDN was not different because other proximate contents such as moisture content, ash content, crude fiber, crude protein, crude fat and NFE were not affected by the altitude of the location. This results in the altitude of the location not giving different results to the TDN litter. Alam et al. (2008) stated that TDN is digestible energy derived from crude protein, crude fiber, crude fat, and NFE. The average content of chicken litter TDN was 44.86%. Bagley et al. (1996) stated that the content of chicken litter TDN is 60%. The TDN value of chicken litter is influenced by the fiber content of the feed. Utama et al., (2019) states that the higher the crude fiber feed, the smaller the digestibility of the feed.

Contamination of Pb, Cu, Mercury and antibiotics of litter from broiler chickens raised at different altitudes

Table 2 points to the results that the average Pb content in chicken litter was 42.14 ppm. Uchimiya et al. (2012) states that the average Pb content in the chicken litter was 38 ppm, which indicated that the level was still in normal condition. T1, T2, and T3 treatments showed no real different results. The factor that affects Pb levels in the cage is the altitudes of the cage area. Uchimiya et al. (2010) states that the use of litter in cages is determined by moisture an area, if the area has a high moisture then it can be ascertained the addition of litter so that the soil element in litter more and more.

Table 2 also shows that the average content of Cu in broiler chicken litter was 118.66 ppm. Codling et al. (2008) stated that in broiler chicken manure has an average content of Cu of 332 ppm which is still classified as normal and when consumed ruminant livestock will not dissolve in the bloodstream and do not settle on the organs of livestock. The T1, T2 and T3 treatments showed no significant different results. The factor that affects the value of Cu is the humidity of the excreta. According to Hoeven (2014) that moisture in excreta can affect the content of Cu in broiler litter. High humidity in the high altitude can cause excreta to get wet. According to Santos et al. (2020), the height of the altitudes, which is a part of the macro environment, is related to the conditions of the broiler house microclimate. When the land level of the cage is low, the humidity in the cage is high. Environmental variables are another aspect that influences levels of Cu. In contrast to regions with higher altitudes caused by soil elements in high areas, dissolved and settled in low areas.

Based on Table 2, that mercury content in chicken litter is low. The value of mercury in the litter of broiler chickens used ranges from 0-0.3 ppm. Malone and Chaloupka (1983) argues that mercury levels range at least 0.03-0.79 ppm, while at 0.3-0.5 ppm mercury content begins to be high. According to SNI: 7387 (2009), mercury content threshold is 0.005 ppm. Mercury levels can be overcome by some microorganisms such as *pseudomonas fluorescens* bacteria (Gupta and Kelly, 1992). Table 2 shows that no antibiotic residues were found in the sample chicken litter. This shows that farmers already understand the importance of discontinuing antibiotic administration and administering antibiotics according to dosage of Sun et al. (2014). According to SNI No: 01 – 6366 (2000), tetracycline antibiotic content should not exceed 0.1 Mg/Kg.

Table 1 - Proximate composition and TDN litter broiler chickens kept at different altitudes.

Parameters	Treatment (%)		
	T1	T2	T3
Water content	22.71±0.64	24.65±0.80	22.85±0.22
Ash content	25.55±3.51	22.79±1.36	23.98±1.23
Crude protein	13.13±1.69	13.33±2.47	13.47±1.16
Crude fibers	24.31±5.28	24.94±8.03	20.77±1.81
Crude fat	2.47±1.95	1.28±0.81	1.41±0.74
Nitrogen free extract (NFE)	34.54±3.92	37.65±6.29	40.37±3.39
Total digestible nutrients (TDN)	43.25±6.75	43.76±10.02	47.57±2.81

Table 2 - Content of Pb, Cu, Mercury and antibiotics of litter from broiler chickens raised at different altitudes.

Parameters	Treatment (ppm)		
	T1	T2	T3
Pb	43.49±16.67	35.15±9.62	47.80±19.61
Cu	102.46±21.03	136.96±38.06	116.58±24.35
Mercury	0.001	0.001	0.001
Antibiotics	0.001	0.001	0.001

Non-significant mean (P<0.05) on all treatments.

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Table 3 - Microbiological quality of broiler chicken litter maintained at different altitudes.

Parameters	Treatment (cfu/g)		
	T1	T2	T3
Total LAB	7.00×10^6	3.48×10^6	5.46×10^6
Gram +	Stem, Solitary	Stem, Solitary	Stem, Solitary
Gram -	Not Found	Not Found	Not Found
Salmonella	0	0	0
E. Coli	0	0	0
Clostridium sp	Negative	Negative	Negative

Microbiological quality of broiler chicken litter maintained at different altitudes

The population of lactic acid bacteria in litter is an indicator of the microbiological quality of the material. The results of the variety analysis showed that the height of different places had no real effect on the total LAB litter. Total LAB observations ranged from 3.48×106 – 7.00×106 cfu/g. The environment suitable for LAB life includes temperature, potential hydrogen (pH) and nutrient content. Too high temperatures will damage the proteins that support bacterial life and cause bacteria to die (Kwak et al., 2008). Too low temperature will result in LAB dormant and not growing. Lactic acid bacteria have an optimal temperature range of 40– 45°C and can live at pH 4 - 6.5 (Adamberg et al., 2003).

The results of identification of the presence of gram-positive bacteria are rod-shaped and solitary. One type of bacteria that grows in litter is a type of gram-positive bacteria that is *Lactobacillus Sp* which is LAB. Lactic Acid bacteria is a bacterium that produces lactic acid that is able to inhibit the growth of gram-negative bacteria. Kwak et al., (2008) states that gram-negative bacterial permeability can be weakened by lactic acid by damaging the outer membrane of gram-negative bacteria.

Test results on litter samples showed no *salmonella* found results on litter. *Salmonella* is a type of bacteria that is pathogenic (Wiedemann et al., 2015), and according to SNI 7388 (2009) the safe limit of *Salmonella* content is less than 25 g. Litter may contain *Salmonella* derived from chicken excreta infected with *Salmonella* then mixed with litter. The impact of *Salmonella* bacterial infection on livestock can lead to Salmonellosis disease. One way to reduce *salmonella* content in an ingredient is by heating at a temperature of about 80^0C (Kim et al., 2012). Investigation on the litter sample obtained no results of *Escherichia coli* found on the sample. *E. coli* are bacteria that include pathogens for humans and livestock. According to SNI 7388 (2009) the threshold of *Escherichia coli* infection is 10 g. *E. coli* in litter comes from excreta infected with *E. coli*. The presence of *E. coli* bacteria in livestock that are often found in the gastrointestinal tract that cause colibacillosis disease will have an impact on the inhibition of nutrient absorption until the death of livestock (Hinton et al., 2000). The provision of antibacterial ingredients can inhibit the growth of *E. coli*. Khan et al. (2018) states that antibacterial compounds such as essential oils, triterpenoids, saponins, flavonoids, and tannins can inhibit the development of bacteria *E. coli*.

Testing the presence of *Clostridium sp* on litter samples showed negative results in all treatments. *Clostridium sp* includes bacteria that are often found in livestock products and belong to the category of pathogenic bacteria (Lepp et al., 2021). According to SNI 7388 (2009) the content limit of *Clostridium sp* is 1×10^2 colonies / g. Infection from *Clostridium sp* can cause Clostridial Necrotizing Enteritis (CNE) disease in poultry, both diseases can result in death (Mwangi et al., 2019). Efforts can be made to inhibit the growth of *Clostridium sp,* namely by heating the indicated material *Clostridium sp* and administering vaccines to livestock.

CONCLUSION

In conclusion, the difference in the altitudes of the region does not affect the chemical quality, contamination and microbiology of litter broiler chickens that are kept at different altitudes. The chemical quality of litter that is not affected by the height of the area is moisture, ash, crude protein, crude fibre, crude fat, NFE and TDN. The Pb content in broiler chicken litter was 35.15-43.49 ppm, Cu content was 102.46-116.58 ppm, mercury content was 0.001 ppm and no antibiotic content was found. Total litter bacteria ranged from 3.48 - 5.46×10^6, gram-positive bacteria that grew on solitary and rod-shaped litter, gram-negative bacteria, bacteria, *Salmonella*, *E. coli*, *Clostridium sp* in broiler litter were not found.

DECLARATION

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Conflict of interests

The authors declare that they have no competing interest.

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