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Effect of Extracts and Flour of Batak Onions on the Number of Escherichia Coli Colonies in Broiler

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Abstract. This study aims to find out the effect of giving extracts and onion flour batak as a controller Escherichia coli also as an antibacterial in broiler chickens as well as to know the relative weight of the digestive organs of broiler chickens. The research method used is a complete randomized design (RAL) with 7 treatments and 3 replays. Treatment consists of Non-infectious Control (POA); Control+E.coli infection (POB); POA+Batak Onion Extract (A.chinense) concentration 20% dose 1 ml/tail (P1); POA+Batak Onion Flour (A.chinense) with a dose of 0.05% of the amount of feed given (P2); POB+Batak Onion Extract (A.chinense) concentration of 20% at a dose of 1ml/tail (P3); POB+Batak Onion Flour (A.chinense) with a dose of 0.05% of the amount of feed given (P4); POB+Tetracycli Antibiotic (P5). The result of this studies have an significant effect (P<0,05) on the decrease in the number of *E.coli* bacterial colonies in chicken digesta, batak onion (*A.chinense*) extract and flour were able to control *E.coli* bacteria. The treatment also had no significant effect (P>0,05) on the relative weight of the digestive. Based on this research, it can be concluded the giving of batak onion (*A.chinense*) extract and flour has an effect on E.coli bacteria and can be used as an alternative to antibiotics, but it does not effect the relative weight of the digestive organs of the chicken.

Key words: Batak onion, extract, flour, *Escherichia coli*, broiler chicken

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1. Introduction

Broiler chicken is a chicken that is much in demand by the public, because of the tender meat and relatively cheap prices. Broiler chickens have a kunggulan that is rapid weight growth and has a weakness easily exposed to diseases derived from bacteria. Bacteria that commonly attack farmers are *Escherichia coli* bacteria that can cause diarrhea. One of the diseases commonly found on farms in Indonesia is Kolibasilosis. This disease is often encountered even as if it has become a mandatory disease in chicken farms. The incidence of this disease is generally directly related to the selection of the location and environment of the farm, especially cleanliness. Antibiotics are believed to suppress the growth of pathogenic bacteria that result in soaring populations of beneficial bacteria in digestive tract. The high beneficial microflora can stimulate the formation

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of antimicrobial compounds, free fatty acids and acidic substances so as to create an uncomfortable environment for the growth of pathogenic bacteria.

Breeders usually take precautions with the use of drugs or antibiotics derived from the plant. These antibiotics can result in antibiotic residues in farm products and can cause resistant microbes to develop in the bodies of livestock and the human body that consumes such products. The administration of natural antibiotics can help the immune system.

One of the most nal plant genus and widely used by the community is *Allium*. The genus Allium consists of more than 280 species scattered throughout the world [1]. Various antimicrobial compounds of ge nus have long been known such as allicin, dial lyl disulfide, ajoene, and 3-(Allyltrisulfanyl)-2-amino-propanoic acid. Allium plants can inhibit the growth of microorganisms such as bacteria, fungi, viruses and parasites [2]. Batak onions (A. chinense) are rich in biological compounds such as sulfur, steroidal, saponins, nitrogen, flavonoids, amino acids and others [3]. Batak onions can be used as a natural antibacterial for livestock according to research [4], which states that methanol extract of batak onion (Allium chinense) has a high antimicrobial effect on Escherichia coli bacteria. Escherichia coli attacks the digestive tract, therefore researchers want to see the effect of giving extracts and onion flour to escherichia coli bacteria used as natural antibiotics and see the effect on the increase and decrease in the weight of digestive organs in broiler chickens.

So far there have been no studies that use extracts and onion flour as a prevention to reduce the number of Escherichia coli bacteria in broiler chickens as well as to find out its influence on the weight of chicken digestive organs including cache, proventrikulus, gizzard, small intestine (duodenum, jejenum and ileum), broiler chicken sekum and colon infected with Escherichia coli bacteria.

2. Research Methods

2.1. Time and Place of Research

The research was conducted in August-September 2020, located in the poultry cage, Jl. Limau Manis Gg. Harapan Desa Sinnembah, Tanjung Morawa District, Deli Serdang Regency and Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara.

2.2. Research Materials and Tools

2.2.1. Materials

The ingredients used in this study were DOC (day old chick) as many as 168 heads, ethanol solution 70%, formalin, KMnO4, rodalon, vitachick, ND vaccine, ration feed, drinking water, aquades, antibiotic tetracycline onions, Escherichia coli bacteria, solid media EMB (Eosin Methylene Blue).

2.2.2. Tools

The tools used are oven, rotary vaccum evaporator, water heater, mortal and pastle, stirrer, sieve, scales, petri dish, autoclave, drip pipette, incubator, test tube, ose wire, gloves, mask, incandescent lamp trial cage, feed place, drinking place and syringe, incandescent lamp cage, tarpaulin, thermometer, feed place, drinking place, syringe, erlenmeyer and label paper.

2.3. Research Methods

The research method used is a complete randomized design (RAL) consisting of 7 treatments and 3 replays of each replay consisting of 8 broiler chickens. The treatment is given by infecting E. coli bacteria at a dose of ± 10^6 CFU/tail administered perorally. The treatment given is:

P0A = Control without infection
P0B = *E.coli infection*
P1 = P0A + Batak Onion Extract (A.chinense) (Concentration 20%, 1ml/tail)
P2 = P0A + Batak Onion Flour (A.chinense) (0.05% of rations)
P3 = P0B + Batak Onion Extract (A.chinense) (Concentration 20%, 1ml/tail)
P4 = P0B + Batak Onion Flour (A.chinense) (0.05% of rations)
P5 = P0B + Tetracycline Antibiotics (0.05%)

The data obtained will be analyzed using fingerprint analysis using *Analisis of Varian* (ANOVA) and if obtained very real or tangible results then continued by using Duncan's Multiple Range Test with a significant rate of 5% to be able to know the best treatment.

2.3.1. *Escherichia coli* bacterial isolate

Isolates of Escherichia coli bacteria given to broiler chickens were obtained from the Microbiology Laboratory of the Faculty of Mathematics and Sciences of the Universitas Sumatera Utara.

2.3.2. Batak onion extraction

Extraction of batak onions by maceration method

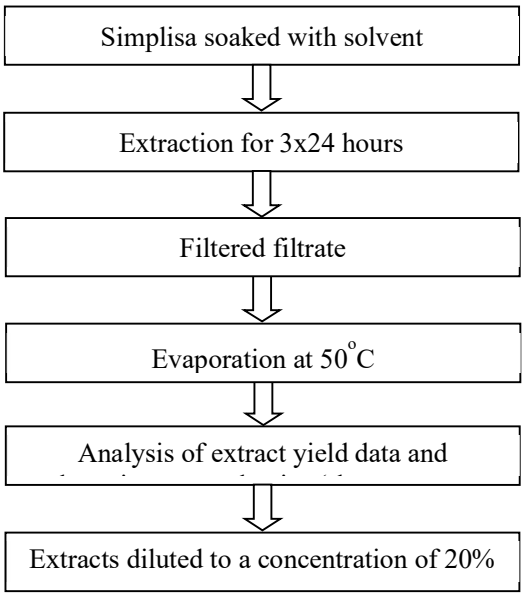

Figure 1. Maceration Method Extraction Flow Chart

Extract making is done with the first step of onions cleaned and peeled skin, cut 1-2 mm thick then dried using the oven at a temperature of 40o Celsius. Dried onions are pounded until smooth to obtain powder (simplisia). Batak onion powder is soaked using ethanol solvent 70% for 3×24 hours at room temperature. Then the filtrat is taken by filtering. Stirring on the maceration method is carried out 12 times for 15 minutes. Then a filter is performed to separate the fitrat from the pulp.

The filtering results are then evaporated using rotary vaccum evaporator at a temperature of 50o Celcius. The remaining solvent is evaporated over the water bath to obtain ethanol viscous extract, then the extract is analyzed for yield data used to determine the presentation produced by simplisa

$$\%\text{Rendemen} = \frac{Extract\ Weight\ (End)}{Simplisa\ Weight(Early)} x100\% \quad \text{.....(1)}$$

After that observe organoleptic extract consists of shape, aroma, color and taste. Batak onion extract is then diluted into extracts with a concentration of 20% using 2 grams of extract dissolved with sterile aquades to mix the volume on a measuring glass of 10 ml.

2.3.3. Batak onion flour

For the manufacture of onion flour batak using fresh onions, after which it is washed thoroughly. Then the clean onion is ovened for 12 hours with a temperature of 40ºC, then done grinding to the onion batak until it becomes flour.

2.3.4. Test challenge using broiler chicken

The challenge test in this study is based on [5] research, by injecting Escherichia coli in chicken livestock and given treatment so that it can be known the growth and development of E. coli in its host. Sampling in chickens aged 13 days before infection and conducted isolate of E. coli with a concentration of ± 10^6 in chickens aged 14 days.

The procedure used in this method is based on [6] research, the first intake of digesta is done in chickens aged 13 days before treatment and then checked for E.coli isolates at the age of 14 days. After the incubation period of chicken age 16 days conducted digesta retrieval that has been infected and calculated CFU, then re-taken digesta in chickens aged 18 days by taking each 1 tail / unit treatment. Samples of chickens are slaughtered and then the chest is split to make it easier to take the digestive tract. Parts of the small intestine (duodenum, jejenum and ileum) are separated to be sampled digesta, then digesta is removed and accommodated.

Digesta is put in a vial bottle and tightly closed, then put in an ice bag that has been given ice cubes to keep the sample from dying bacteria. Samples are taken to the laboratory for further microbiological testing. The total calculation of E. coli by poure plate method, in dilution taken samples as much as 1 g then put in a petri dish. The media is poured in a petri dish as much as 15 ml, then closed and placed on the table by shaking forming the number eight and silenced until solidified. Solidified samples were incubated in an inverted incubator for 48 hours at 37^{o}C. After the incubation period is complete, the fortified colonies are counted. Each colony can be thought to be derived from one cell splitting into many cells, although it also comes from another adjacent cell.

2.3.5. Giving extracts and flour of batak onions to broiler chickens

Batak onion extract is given to broiler chickens aged 15 days orally for 5 days and the dose according to the treatment of each batak onion flour is given from DOC up to the age of 35 days.

2.3.6. Broiler chicken maintenance

Randomization of the cage is done before the broiler chicken enters the cage by arranging the treatment number and replay first on each unit.

Chickens are infected with E. coli bacteria on day 14 at a 1 ml dose containing a population of 10^6CFU/ml, except for P0A, P1 and P2 treatments. Administration of antibiotics in the form of onion extract batak done 5 days with a dose of 1 ml / tail and the administration of onion flour batak starting from the beginning of maintenance. While the administration of tetracycline at a dose of 5 g/ 10 liters of drinking water or 150 mg / kg per day for 5 consecutive days, treatment is given 1 day after infection, administered in drinking water [7]. Feed and drinking water are given in ad-libitum. Cages, feed places, and drinking water places are cleaned in the morning at 08.00 WIB and in the afternoon at 17.00 WIB.

2.3.7. Observed modifiers

1. **Antimicrobial Bacteria test**: obtained from by looking at the presence of clear zones around the extract, this shows that the extract has the potential as an antimicrobial ingredient. The diameter of the zone that has been formed can be seen with the sash term.
2. **Calculation of Escherichia coli**: Bacterial Colony obtained from the number of colonies multiplied by 1 per dilution
3. **Crop**: obtained from the crop weight divided by the live weight
4. **Proventricular**: obtained from proventricular weight divided by live weight
5. **Gizzard**: obtained from gizzard weight divided by live weight
6. **Duodenal**: obtained from duodenal weight divided by live weight
7. **Jejenum**: obtained from the weight of the jejenum divided by the live weight
8. **Ileum**: obtained from the weight of the ileum divided by the live weight
9. **Cecum**: obtained from the weight of the cecum divided by the live weight
10. **Colon**: obtained from the weight of the colon divided by the live weight

3. Results and Discussion

3.1. Antimicrobial Test

Based on the results of antimicrobial tests of onion extract against E.coli bacteria that batak onion extract can inhibit E.coli bacteria. As the test results of clear zone diameter (mm) can be seen in Table 1.

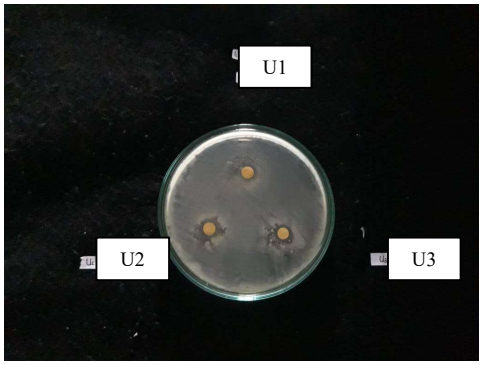

Figure 3. Antimicrobial Test Results of Onion Batak Extract

Table 1. Antimicrobial Test Results on Onion Extract Against *Eschericia coli* Bacteria

Bacteria	Sample Name	Clear Zone Diameter (mm)			Average	Antimicrobial Index
		U1	U2	U3		
Eschericia coli	Extract	12	10,1	11,2	11,1	0,85

This antibacterial test by looking at the coolness of bacteria, where the extract will form a clear zone (obstacle zone). Based on Table 1. antimicrobial extract of onion batak has a clear zone diameter of U1 12 mm, U2 10.1 mm and U3 11.2 mm with an average of 11.1 mm and an antimicrobial index of 0.85 this indicates that onions are effective in inhibiting eschericia coli bacteria. According to [8] the blandness (clear zone) is divided into 4 parts, namely very strong in the clear zone >20 mm, strong in the clear zone of 10-20 mm, while in the clear zone 5-10 mm and weak in the clear zone <5 mm.

Based on this, batak onion extract is strong in inhibiting E.coli bacteria so that onion extract has the potential as an antibiotic. This is reinforced based on the results of [9] research, antibacterial test results antibiotic treatment (tetrachlor) resulted in a tasteless zone of 10.97±0.03 mm from the result of the result of the obstacle zone of onion extract batak does not differ much from the antibiotic obstacle zone (tetrachlor) so that the extract of onion batak can potentially be a natural antibiotic.

Onion batak has antibacterial compounds in the form of flavonoids, steroida / terpenoida and saponins so that the formation of a tasteless zone in bacteria. [10], said that flavonoid compounds act as antibacterial by disrupting the function of microorganisms. This compound can inhibit bacterial growth by causing permeability damage to bacterial cell walls, microsomes and lysosomes. As well as saponins according to [11] states saponins have a mechanism of action that can cause damage to cell membranes so that the exit of various important components from the bacterial cells in the form of proteins, nucleic acids and nucleotides.

3.2. Number of E. coli Bacteria in Broiler Chicken Digesta

Based on the results of research that has been done it is known that the administration of extracts and flour batak onions affect the amount of E. coli bacteria in the digesta chicken boiler. The administration of extracts and flour of batak onions can reduce the amount of E.coli bacteria in chicken digesta and can be seen in Table 2. with CFU (Colony Forming Units) log units.

Table 2. Effect of Extracts and Flour on the Average Number of Colonies of E. Coli bacteria (CFU/g logs) in Broiler Chicken Digesta

Treatment	Before Infection (Age 13 Days)	Days After Infection	
		After Infection (2 Days, Age 16 Days)	After Treatment (4 days, Age 18 Days)
POA	4,26^{tn}	5,20^{d}	5,19^{ab}
POB	4,20^{tn}	5,36^{cd}	5,28^{a}
P1	4,68^{tn}	5,20^{d}	3,91^{c}
P2	4,64^{tn}	5,43^{bcd}	3,95^{c}
P3	4,50^{tn}	6,10^{a}	4,27^{bc}
P4	4,75^{tn}	5,81^{abc}	4,35^{bc}
P5	4,49^{tn}	5,95^{ab}	4,03^{c}

Note: Superscript with different letters towards the column, showing a real difference (P<0.05%); tn: unreal (P>0.05)

3.2.1. Before infection (age 13 days)

Table 2 Result. indicates that the amount of E.coli bacteria in chicken digesta before infection is 4.20-4.75 CFU logs are still relatively normal. [12], said the normal condition of E.coli is present in the chicken's digestive tract. About 10-15% of all E.coli found in healthy chicken intestines are classified as pathogenic serotypes. Dust in the chicken coop can contain 10^5-10^6 E.coli/grams and these bacteria are durable against dryness.

3.2.2. After infection (2 days, age 16 days)

Table 2 Result. shows that the number of E.coli colonies in broiler chickens in the infection phase has a noticeable effect (P<0.05) on non-infectious treatment (POA) with E.coli Infection (POB) treatment. In this study, infected healthy boiler chickens using 10^6 E. coli bacteria isolates. After infection with E.coli bacteria in digesta increased where the highest in P3 treatment was 6.10 CFU Logs.

Treatment without infection also occurs an increase in E.coli bacteria is caused because E.coli does live in the gastrointestinal tract and also fecal contamination, as well as the taking of different chickens and it could be that the chicken is already infected with E.coli bacteria. This is in accordance with the opinion of [13], which states that most E. coli bacteria live in poultry cage environments through fecal contamination. The onset of pathogenic occurrence of E. coli may occur in the hatchery of contaminated infections or eggs, but systemic infections usually require a predisposition environment or the causes of those infections.

[12], stated that transmission of kolibasilosis usually occurs orally through feed, drinking water or dust/dirt contaminated with E. coli. [14], said there is a mutual connection between chickens and the environment (as a source of transmission). Litter, as the main source of transmission of E.coli, in addition to feed and drinking water contaminated by faeces.

3.2.3. After Treatment (4 days, Age 18 Days)

Table 2 Result. the average number of E.coli colonies infected after treatment decreased the amount of E.coli seen in the P3 treatment decreased by 4.27 CFU logs treated by onion extract, P4 treatment decreased by 4.35 CFU logs given the treatment of onion flour, variety analysis showed a real influence ($P<0.05$).

Batak onions can inhibit E.coli karane bacteria have saponin compounds that can inhibit bacteria. This is in accordance with the statement of [15] which states saponin compounds have antibacterial, antifungal, antipyretic effects, increase the synthesis of DNA and proteins, as well as increase immunity. A study mentions that steroid compounds Onion batak can prevent the occurrence of cardiac injury due to oxidative substances as well as have anti-inflammatory effects because the tubers contain succinic acid.

Batak onions are rich in biological compounds such as sulfur, steroidal, saponins, nitrogen, flavonoids, amino acids and others [3]. Flavanoids are the largest group of phenol compounds, phenol compounds themselves have effective properties inhibiting the growth of viruses, bacteria and fungi. [16] added that flavanoid compounds are generally antioxidant and are usually widely used as one of the raw material components of medicines. Flavanoid compounds and derivatives have two specific physiological functions, namely as chemicals to be able to overcome disease attacks (as antibacterial) and anti-viral for plants. This is supported also by [17] states that flavonoids can also inhibit bacterial motility.

P5 treatment dropped to 4.03 CFU logs given tetracycline antibiotics. Tetracycline is an antibiotic that belongs to the group of antibiotics that react by inhibiting the synthesis of bacterial proteins.

3.3. Relative Weight of Broiler Chicken Digestive Organs

The relative weight of the organ is obtained by weighing the digestive organs (crop, proventricular, gizzard, duodenal, jejenum, ileum, cecum and colon) then the acquired number is divided by the live weight. From the results of the study obtained the average relative weight of the digestive organs of broiler chickens in Table 3.

Table 3. Average Relative Weight of Digestive Organs (g/kg) Broiler Chickens Aged 35 Days Given Various Treatments

Digestive Organs (g/kg)	Treatment						
	POA	**POB**	**P1**	**P2**	**P3**	**P4**	**P5**
Crop$^{(tn)}$	3,31	3,02	3,68	3,87	3,20	3,15	3,50
Proventricular $^{(tn)}$	4,63	4,82	3,99	4,73	4,77	4,66	4,39
Gizzard$^{(tn)}$	13,75	14,24	14,92	15,56	13,78	15,31	14,58
Duodenal$^{(tn)}$	8,44	8,90	8,37	8,77	8,29	8,94	8,59
Jejenum$^{(tn)}$	14,12	12,47	14,30	13,06	12,31	12,56	12,43
Ileum$^{(tn)}$	8,61	8,34	7,16	7,77	7,03	7,84	8,30
Cecum$^{(tn)}$	4,96	5,78	4,68	5,16	5,11	5,64	5,33
Colon$^{(tn)}$	1,08	1,21	1,10	1,11	1,25	1,36	1,31

Note: tn = unreal differences (P>0,05)

Based on Table 3 above, the results of diversity analysis on the administration of extracts and onion flour to chickens infected with E.coli bacteria or not infected with E.coli give a different influence is not real (P>0.05) to the relative weight of chicken digestive organs (crop, proventricular, gizzard, duodenal, jejenum, ileum, cecum and colon). This indicates the treatment gives the same response to the weight of the digestive organs of broiler chickens. This can also be caused by the consumption of animal feed, activities, palatability and health of livestock [18], saying that the consumption of rations influenced by the nation and large chickens, environmental temperature, livestock health, warehousing and balance of feed substances.

The size of the thick length as well as the weight of the digestive tract of poultry is not a static magnitude. Changes may occur during the development process because it is influenced by the type of ration given. Rations containing a lot of fiber in it will cause changes in the size of the digestive tract so that it can become heavier, longer, and thicker [19].

4. Conclusion

Based on this research, it can be concluded that the administration of onion extract and onion flour in rations can decrease the number of E.coli colonies. The administration of extracts and onion flour has no effect on the relative weight of the digestive organs of broiler chickens in the form of crop, proventricular, gizzard, duodenal, jejenun, ileum, cecum, and colon.

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