

The Effect of Colistin Administration as Medicated Feed on Alanine Aminotransferase and Creatinine Level in Broiler Infected with *Escherichia coli*

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Abstract. Colistin is a decapeptide antibiotic with narrow spectrum activity, mainly used as treatment for Gram negative bacteria. This study aims to scientifically determine the effect of colistin administration as medicated feed on *alanine aminotransferase* (ALT) and creatinine level in broiler infected with *Escherichia coli*. KTOP group as positive control, KTON group as negative control, while I, II, and III groups were infected with *Escherichia coli* 1 x 10⁸ CFU/ml 0.1 ml via intratracheal route. Group I, II, and III were given colistin treatment dosage of 0.3 g/kg food, 0.6 g/kg food, and 1.2 g/kg food. Blood samples were taken through brachial veins for ALT and creatinine examination with a Caretium NB-201 semi-auto chemistry analyzer. Data were examined statistically using IBM SPSS Statistics 24 software and graphically using Microsoft Excel 365. Conclusion of the research by statistical analysis with Kruskal-Wallis test obtained ALT test results P = 0.147 and creatinine test results P = 0.815. Based on the results of this study, the administration of colistin medicated feed did not cause a significant effect on ALT and creatinine level in broiler infected with *Escherichia coli*, indicating that colistin has low potential toxicity while given as medicated feed.

1 Introduction

Antibiotics are substance, produced by one microorganism, or of biological origin which at low concentrations can inhibit the growth of, or are lethal to other microorganisms. Antibiotics will express similar pattern of effectiveness, toxicity, and allergic potential side effects within the same structural class. Beta-lactams, Macrolides, Tetracyclines, Quinolones, Aminoglycosides, Sulfonamides, Glycopeptides and Oxazolidinones are some common classes of antibiotics based on chemical or molecular structures [3]. The use of Antibiotics Growth Promotor or AGP has been regulated in UU No. 41 Year 2014 about Animal Health and Husbandry stating that animal feed mixed with certain hormones and/or

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feed additive antibiotics are prohibited ^[16]. Regarding to Technical Guidelines for the Use of Veterinary Drugs in Animal Feed for Therapeutic Purposes in Decree of Directorate General of Animal Health and Husbandry No. 09111/KPTS/PK.350/F/09/2018, antibiotics can be mixed in animal feed and may only be used for therapeutic purposes either through feed or drinking water in right therapeutic dose and maximum 7 days duration of use ^[6].

Colistin or often known as colistin sulfate or polymyxin E, one of the antibiotics listed in Critically Important Antimicrobials for Human Medicine, is a narrow spectrum antibiotic and is effective against Gram negative bacteria ^[18]. It is categorized as the last drug choice for the treatment of infectious diseases caused by multiresistant Gram-negative bacteria such as *Acinetobacter* species, *Pseudomonas aeruginosa*, and *Enterobacteriaceae* with multiresistance to carbapenemase ^[9]. The mechanism of colistin, apart from being bactericidal, is lipopolysaccharides and phospholipids binding to the outer membrane of bacterial cell. It is also able for calcium and magnesium ions binding to neutralize lipopolysaccharides (LPS) ^[15]. Studies of colistin pharmacokinetics in poultry are very limited despite their frequent use for decades although colistin has been extensively used in veterinary medicine for prophylaxis, metaphylaxis, treatment of bacterial infections and growth promoters ^[2].

Escherichia coli (*E. coli*) is a Gram-negative, rod shaped, facultative anaerobic bacterium that belongs to the *Enterobacteriaceae* family ^[1]. Basically, this disease is not a new one, yet it has been attracting concern in veterinary field due to the occurrence of more frequent cases of pathogenic *E. coli* in poultry industry. Numerous studies show antibiotics resistance to tetracycline, nalidixic acid, ampicillin, amoxicillin, streptomycin, trimethoprim, and cotrimoxazole appeared to be the most common among the *E. coli* isolates circulating in poultry farms ^[8]. The aim of this study was to determine the effect of colistin as medicated feed on Alanine Transaminase (ALT) and creatinine level in broiler infected with *E. coli*. Changes in ALT and creatinine profiles were measured after oral administration of colistin medicated feed for 5 days duration in broiler infected with *E. coli*.

2 Material and Methods

2.1 Animal models

Approval by the Ethical Clearance Commission of Faculty Veterinary Medicine Universitas Gadjah Mada No. 0027/EC-FKH/Int./2018 and protocol of PT. NovindoAgritech Utama, forty broiler chickens strain Cobb CP 707 bought from PT. Charoen Pokphand Jaya Farm were included in this study. The broilers were housed in UP2KH animal facilities from Day Old Chick (DOC) to 30-day old broilers. KTOP group as positive control, KTON group as negative control, while I, II, III groups were infected with *E. coli* 1×10^8 CFU/ml 0.1 ml via intratracheal route. Group I, II, III were given colistin treatment dosage of 0.3 g/kg food, 0.6 g/kg food, 1.2 g/kg food. All broilers were judged based on results of serum biochemical analysis prior to study. Blood samples were taken through brachial veins for ALT and creatinine examination with a Caretium NB-201 semi-auto chemistry analyzer.

2.2 Study design

The broilers were housed in UP2KH animal facilities from Day Old Chick (DOC) to 30-day old broiler. When broilers arrived, the first adaptation was carried out in a 3 x 1.5 m cage with rice husks litters to absorb the smell of Ammonia. During adaptation, broilers were fed with commercial feed Japfa Comfeed BR I Crumble (BR1) and 7 litres of drinking water/day (ratio 15 litres of water : 500 g of mashed brown sugar) that was replaced every

morning for 3 (three) days. Feeding with Japfa Comfeed BR I Crumble (BR1) was continued during starter period and Japfa Comfeed BR II Crumble (BR2) was given during finisher period. Weighing is done once a week. On the 3rd day, the Newcastle Disease (ND) and Infectious Bursal Disease (IBD / Gumboro) vaccination was carried out with the live MedivacND-IBD vaccine via eyedrops. On the 13th day, the IBD Vaccination via drinking water was carried out with the Medivac Gumboro A live vaccine, continued with the administration of vitamin B complex at a dose of 5 g dissolved in 7 litres of drinking water. This vitamin is given until 15th day. On the 17th day, ND vaccination was carried out via drinking water with the Medivac ND clone 45 live vaccine.

Escherichia coli infection was given on the 17th day by dividing the broilers into 5 groups. Groups I, II, and III each contained 10 broilers were infected with *E. coli* and were given medicated feed treatment, the KTOP group as a positive control contained 4 broilers infected with *E. coli* without medicated feed treatment, and the KTON group as a negative control contained 6 broilers without medicated feed treatment and without being infected with *E. coli*. *Escherichia coli* infection to KTOP, I, II, and III groups was carried out by injection of *E. coli* strain O78 as much as 0.1 ml/broiler with total colony count of 1×10^8 CFU/ml via intratracheal. On day 20 to day 24 groups I, II, and III were each treated with colistin at 0.3 g/kg of feed, 0.6 g/kg of feed, and 1.2 g/kg of feed, while the KTON and KTOP groups were not given colistin medicated feed. At 3rd week of maintenance, chickens in KTOP group died one by one before blood samples were taken for data analysis. On the 29th day, blood samples were taken via brachial veins for ALT and creatinine examinations.

2.3 Serum preparation

Blood samples were taken via brachial veins and collected in a non-EDTA conical tube for ALT and creatinine examination. Centrifugation at 3000 rpm for 10 minutes was carried out at the Laboratory of Pharmacology, Faculty of Veterinary Medicine, Universitas Gadjah Mada. After centrifugation process was complete, the serum was seen on the top layer. Serum was then collected using a micropipette into the eppendorf tube to be stored in 4°C temperature until further examination.

2.4 Serum biochemical analysis

Serum alanine transaminase (ALT) and creatinine were measured using standard reagent kits (Stanbio ALT/GPT Liqui-UV®, Stanbio Liqui-UV®, Stanbio Direct Creatinine LiquiColor®, Stanbio LiquiColor®) and were examined using semi-autochemistry analyzer Caretium NB-201. Based on the results of the examination, the ALT value appeared on the instrument is expressed in units/litre (U/L) and the creatinine value is expressed in units of milligrams/decilitre (mg/dL).

2.5 Statistical analysis

All results are expressed as mean \pm SEM. Data were compared using Kruskal-Wallis test with IBM SPSS Statistics 24 for measurements to investigate both the differences between treatments compared with control values. Significance was set at $P < 0.05$. Data were illustrated graphically using Microsoft Excel 365.

3 Results

The graphic in **Fig 1.** shows ALT mean values of five treatments analysed using Microsoft

Excel 365 and IBM SPSS 24. The KTOP group as a positive control could not be compared to the results of the data analysis because all the chicken in KTOP group died before blood sampling for data analysis. Based on the results, group II had the highest ALT mean (33.47 ± 8.54 U/L) and the KTON group as a negative control had the lowest mean (9.53 ± 0.86 U/L). The results of group I, II, and III had means and standard deviations of 24.87 ± 15.08 U/L, 33.47 ± 8.54 U/L, 23.23 ± 0.15 U/L. Group II had the highest ALT mean compared to the control group and other treatment groups. Normal ALT concentrations in chicken blood are 9.50-37.2 U/L [11]. From the results of the data analysis showed that all groups are still in the normal range.

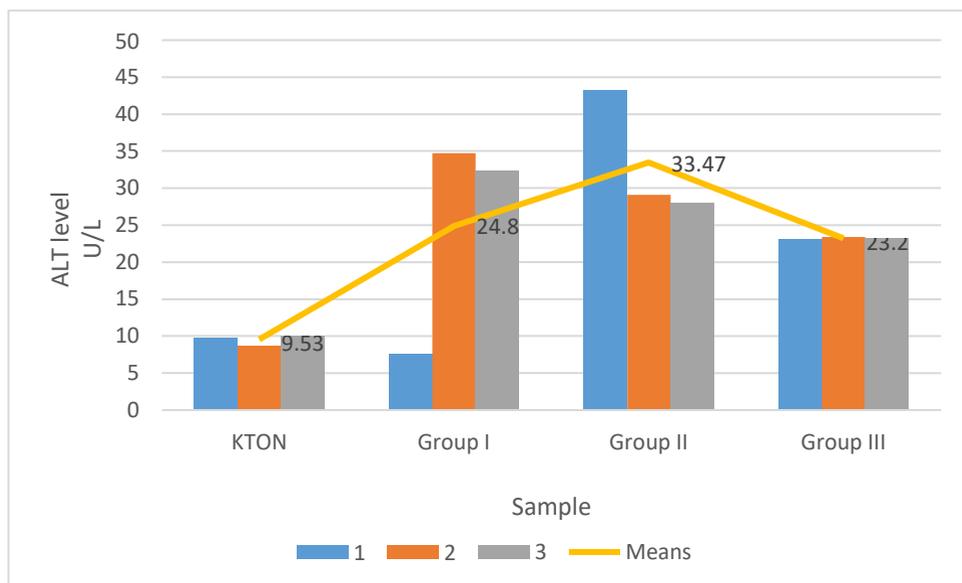


Fig. 1. Comparison of ALT level and ALT means between treatments. KTON = Not given colistin medicated feed treatment and not infected with *E. coli*. Group I = given colistin medicated feed at dose 0.3 g/kg feed. Group II = given colistin medicated feed at dose 0.6 g/kg feed. Group III = given colistin medicated feed at dose 1.2 g/kg feed.

The graphic in **Fig 2.** shows creatinine mean values of five treatments analyzed using Microsoft Excel 365 and IBM SPSS 24. The KTOP group as a positive control could not be compared to the results of the data analysis for the same reason shown in **Fig. 1.** Based on the results of examination and data analysis, KTON group has 0.33 ± 0.058 mg/dL mean value, group I has 0.3 mg/dL mean value, group II has 0.30 ± 0.10 mg/dL mean value, and group III has 0.33 ± 0.058 mg/dL mean value. The KTON group and group III had the same mean value (0.33 ± 0.058 mg/dL), while group I and group II also had the same mean value (0.30 mg/dL). Normal creatinine levels in chicken are 0.10 - 0.40 mg/dL [5]. From the results of data analysis, it showed that the creatinine levels of each group I, II, and III treated with colistin medicated feed were still classified as normal.

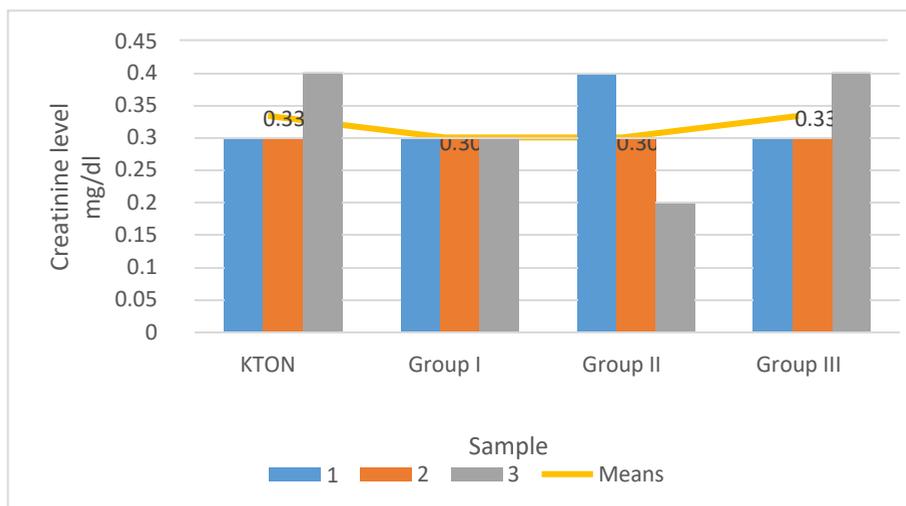


Fig. 2. Comparison of creatinine level and creatinine means between treatments. KTON = Not given colistin medicated feed treatment and not infected with *E. coli*. Group I = given colistin medicated feed at dose 0.3 g/kg feed. Group II = given colistin medicated feed at dose 0.6 g/kg feed. Group III = given colistin medicated feed at dose 1.2 g/kg feed.

4 Discussion

The result of ALT means comparison using Kruskal-Wallis test was $P = 0.147$ ($P > 0.05$), it showed no significant difference of ALT means between groups. Colistin medicated feed at a dose of 0.3 g/kg of feed, 0.6 g/kg of feed, and 1.2 g/kg of feed effect in blood serum broilers infected with *E. coli* has no significant result compared to broilers that were not given medicated feed and were not infected with *E. coli*. It means colistin considered safe and may not be associated with hepatotoxicity in broilers infected with *E. coli*.

Table 1. Descriptive ALT and creatinine level of each group.

Parameter	Group	Mean	Std. Error
ALT (U/L)	KTON	9.533	0.4978
	I	24.867	8.7087
	II	33.467	4.9269
	III	23.233	0.0882
Creatinine (mg/dL)	KTON	0.33330	0.03333
	I	0.30000	0.30000
	II	0.30000	0.05774
	III	0.33333	0.03333

Descriptively, group II had the highest mean due to one of the samples had ALT levels increased significantly for up to 43 U/L. The body detoxification process is carried out by the liver enzymes such as AST and ALT through oxidation, reduction, hydrolysis, or conjugation of potentially harmful substances and converting them into physiologically inactive substances. The liver is very open to gut microorganisms and microbial products called pathogen-associated molecular patterns (PAMPs) through the portal vein. In a healthy condition, the liver acts as a gatekeeper to prevent things that trigger infection, such as bacteria and endotoxin from entering the circulatory system. Kupffer cells, macrophages present in the liver, efficiently phagocyte pathogens and PAMPs that enter the liver via arterial or portal circulation or translocation (migration of active bacteria or bacterial products

from the intestinal lumen to normal sterile tissue) has been known to induce and guard against systemic resistance. In animals infected with *E. coli*, the elimination of *E. coli* is slow, while the release of endotoxin is high so that it influences the hepatic and systemic circulation^[4]. An increase in ALT can be caused by the direct effect of bacterial toxins, drugs, chemicals on liver cells, especially those that are closed to the central vein. These cells receive the least amount of nutrients from the blood and are sensitive to hepatotoxins. Inflammation products will directly affect the permeability of the liver cell membranes^[12].

From the results, it showed that the creatinine levels of each group I, II, and III treated by colistin medicated feed were still classified as normal. The result of creatinine means comparison using Kruskal-Wallis test showed $P = 0.815$ ($P > 0.05$), which showed there was no significant difference between the creatinine groups. Based on the results of statistical analysis, colistin medicated feed with a dose of 0.3 g/kg of feed, 0.6 g/kg of feed, and 1.2 g/kg of feed in broiler groups infected with *E. coli* did not have a significant effect on creatinine levels. Creatinine is a muscle protein product produced by muscle metabolism which is released from muscles at an almost constant rate and is excreted in the urine at the same rate. Creatinine is a more reliable indicator of renal function than BUN because it is less influenced by other factors such as diet and hydration^[17], a level greater than the normal value indicates impaired kidney function. The mechanism action of colistin involves interactions with lipopolysaccharide molecules in the outer membrane of bacteria, which causes displacement of calcium and magnesium ions and destabilizes the outer membrane causing the leakage of cell content and leading to cell senescence^[7]. The normal dose of colistin administration ranges from 5-80 mg/kg body weight^[13]. In this study, the doses used as variables were 0.3 g/kg of feed, 0.6 g/kg of feed, and 1.2 g/kg of feed. There is no further information regarding the toxicity of colistin in poultry via parenteral or enteral route such as medicated feed. Colistin has been used as an antimicrobial agent for broiler maintenance in Belgium^[10]. No observations of colistin effect in hematobiochemical serum on broilers were found^[13]. Further information regarding the toxicity of colistin to broiler creatinine parameters was also not found.

5 Conclusion

Group II had the highest ALT levels with an average of 33.47 ± 8.54 U/L. The KTON group and group III had the same creatinine level (0.33 ± 0.058 mg/dL), while group I and group II also had the same average creatinine level (0.30 mg/dL). In the Kruskal- Wallis test ($P > 0.05$), the results were no significant difference of ALT and creatinine level between treatments and control groups. Based on these results, the administration of colistin did not have a significant effect on the ALT and creatinine levels between colistin medicated feed treatment groups and control group. The summary of this study is colistin considered safe and may not be associated with hepatotoxicity and nephrotoxicity regardless of the prohibition of colistin use in Indonesia^[16] due to the Decree of Directorate General of Animal Health and Husbandry No. 09160/PK.350/F/12/2019, colistin is the last drug of choice for gastrointestinal infections and bacteremia caused by multidrug resistance in humans, which in its widespread use has the potential to cause bacterial resistance.

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