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# Validation of the method for determining lincomycin levels and calculating lincomycin levels in broiler chicken plasma using high-performance liquid chromatography

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**Abstract**

**Background:** Antibiotic residues that come from food of animal origin, such as broiler chicken, have a variety of consequences on human health and increase the likelihood of antibiotic resistance. Lincomycin residue investigations in broiler chicken especially in plasma broiler chicken should be undertaken utilizing the validation method analysis.

**Aim:** The purpose of this study is to determine the high-performance liquid chromatography (HPLC) as a validation method for calculating the residual concentration of lincomycin in broiler chicken blood plasma and compare it with the minimum Inhibitor Concentration (MIC) and Maximum Residue Limits (MRLs) standards for lincomycin.

**Methods**: Thirty-five-day-old broiler chickens cobb 700 were weighed and randomly allocated to and separated into control (placebo) and six treatment groups of varying doses and duration. The treatment group's suggested dosage of lincomycin was 50, 100, or 150 mg/kg/day given to 18-day-old chicken, along with drinking water for a week (A group) and 2 weeks (P group). Lincomycin levels in blood plasma were validated using HPLC. The residual lincomycin concentrations 24 hours and 1 week after injection were compared to the lincomycin MIC and the Indonesian National Standard of MRL.

**Result:** The validation of linscomycin reveals a linear value in blood plasma with an $R^2$ of 0.9983. Precision and accuracy levels indicate promising results for detecting lincomycin. The retention duration for 100 µg/ml lincomycin was 10.0–10.5 minutes. Lincomycin had LOD and LOQ values of 13.98 and 4.86 µg/ml, respectively. After 1 week of dosing at 50 and 100 mg/kg dosages, lincomycin residue detection was 0.00, which was below the MRL criterion of <0.1 ppm. The study found that the residual concentration of 150 mg/kg dosages for a week and 100/150 mg/kg doses for 2 weeks above the lincomycin MIC limits against *Mycoplasma synoviae, Staphylococcus aureus, and Salmonella enteritidis*.

**Conclusion:** Lincomycin detection by HPLC in chicken blood plasma showed promising results in terms of linearity, accuracy, precision, specificity, and sensitivity. Lincomycin administration for 1 week at doses of 50 and 100 mg/kg resulted in the lowest residual concentration below the lincomycin MIC and MRL standards.

**Keywords:** Broiler chicken, High-performance liquid chromatography, Lincomycin, Plasma, Residue.

## Introduction

Antibiotics are medicines produced by microbes that can hinder cell wall production, increase cytoplasmic membrane permeability, and interfere with protein synthesis, hence suppressing or killing microorganisms (Kanchugal and Selmer, 2020). Antibiotics are mostly utilized for therapeutic purposes and disease prevention in hens. In addition, it functions as a feed supplement (Goren *et al*., 2011; Wang *et al*., 2020). Antibiotics in feed supplements can increase growth performances, increase microorganisms in the digestive tract, and improve nutrient uptake, digestion, and absorption (Foroutankhah *et al*. 2019). Antibiotics, based on their working power, are split into two groups: broad spectrum and narrow spectrum (Yuningsih, 2009). Lincomycin is a broad-spectrum antibiotic that is widely

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used in broiler chicken farms to treat diseases caused by Gram-positive and Gram-negative bacterial poultry infections. Lincomycin is typically added to drinking water in chickens (Goren *et al*., 2011). Lincomycin, like the macrolide group, inhibits protein synthesis in microbial cells by attaching to the 50S ribosomal subunit. Increased broiler chicken consumption is followed by antibiotic use in farm-raised chickens (Muzaki *et al*., 2023). Continuous and irregular use of antibiotics can cause cross-resistance and increased resistance of pathogenic bacteria such as *Salmonella enteritidis*, *Mycoplasma synoviae*, *Staphylococcus aureus*, and *Pasteurella multocida* (Landy *et al*., 2020). Antibiotic use is becoming increasingly limited in Indonesia, as evidenced by the issuance of Indonesia Minister of Agriculture Regulation number 14 of 2017 prohibiting the use of antibiotics as Antibiotic Growth Promoters and Indonesia Minister of Agriculture Regulation number 22 of 2017 prohibiting the use of antibiotic-laced feed. Antibiotics are increasingly being used for purposes other than treating microbial illnesses. To avoid antibiotic residues and antibiotic resistance, which risk human health, the consequences of residues include effects on human health, such as hypersensitivity, gastrointestinal disorders, tissue damage, and neurological diseases (Ministry of Agriculture, 2017; Hakimah *et al*., 2019; Nadzifah *et al*., 2019). The primary issue with incorrect doses is that they can leave antibiotic residues. Antibiotic residues are analytes or metabolites that remain after antibiotic metabolism in tissue, liver, and plasma. The presence of antibiotic residues in animal food, especially in chickens, is associated with several effects on human health, including hypersensitivity, gastrointestinal disorders, tissue damage, and neurological disorders, leading to the risk of resistance to several pathogenic microorganisms, causing significant problems in the field of human and animal health (Hakimah *et al*., 2019; Choirunnisa *et al*., 2019). According to Mehdi *et al*. (2018), delivering a greater dose than normal can result in plasma residue. Several drug content analysis technologies are developed alongside the rising use of antibiotics in the poultry business, particularly in broiler chicken raising. Chromatography is one technology used to analyze drug levels. Chromatography is a physical separation technique of a mixture of chemical substances (analytes) based on the differences in migration/distribution of each individual component of the mix in the stationary phase under the influence of the mobile phase. The mobile phase might be gas or liquid, while the stationary phase can be liquid or solid (Susanti and Dachriyanus, 2014). High-performance liquid chromatography (HPLC) is a tool used for residue analysis. HPLC is a chemical and physicochemical process that separates nonvolatile organic chemical components quickly and efficiently using column technology, a high-pressure pump system, and a sensitive detector (Anastasia, 2011). This study aims to validate the analytical method for measuring lincomycin levels by spiking lincomycin in plasma by examining the peak/area, linearity, accuracy, precision, Limit of Detection (LOD), and Limit of Quantification (LOQ), as well as the potential effectiveness and lincomycin levels in broiler chicken plasma. It was compared to the Minimum Inhibitor Concentration (MIC) value for administration through drinking water and the antibiotic dose of lincomycin in the combination formulation of ½, 1, and 2 times the dose given in the drug usage guide.

## Materials and Methods

Lincomycin levels are measured by treating chickens as experimental animals, collecting samples, and preparing plasma samples. Plasma extraction employs a modified approach from Sholihah *et al*. (2021). The sample preparation technique employed is protein precipitation, which is a method of separating the drug from the bonds in the protein such that it does not interfere with the drug's quantification. Protein precipitation procedures often use solvents that may be combined or dissolved in water, such as acetonitrile, acetone, ethanol, methanol, acids, metal ions, or salts. The process of preparing the sample involves first centrifuging the separated plasma sample in a tube for 20 minutes at a speed of 4,500 rpm. Following this, 100 µl of the sample is taken and the solvent, acetonitrile, is added. Following the addition of 100 µl of acetonitrile and a 1:1 ratio of plasma, the sample was homogenized for 30 seconds in a vortex mixer. After centrifuging the homogenized solution for 20 minutes at 4,500 rpm, the precipitate was first filtered using a 0.45 µm syringe filter, and the filtered material was then placed in an Eppendorf tube for storage. An injection of 20 µl of the sample was made into the HPLC. The organ samples, techniques validation for the analysis of the antibiotic drug levels of lincomycin, and the levels of antibiotic drug in the plasma of broiler chickens. Plasma samples from broiler chickens, examination of method validation outcomes, and findings from lincomycin tests on plasma samples. HPLC method established by Abualhasan *et al*. (2012) and Muzaki *et al*. (2023) for the detection measurement of lincomycin levels in plasma. Finding the values for accuracy, precision, specificity, and sensitivity is the first step in method validation. Lincomycin levels in broiler chicken bloodstream were measured using an HPLC instrument. The results were then analyzed by comparing the levels of the antibiotic drug with the maximum residue limit (MRL) specified by SNI 01-6366-2000, which is 0.1 ppm, or equivalent to 0.1 µg/ml, and by evaluating the possible efficacy of the levels of the antibiotic drug by comparing the MIC values of various bacteria. Additionally, an SPSS *T*-test was used to compare and analyze the lincomycin levels in each group.

***Ethical approval***

The experimental animal treatment approach has obtained ethical approval from the Faculty of Veterinary Medicine Ethics Commission of Universitas Gadjah Mada, Yogyakarta, Indonesia, with number 00100/EC-FKH/Int./2021.

## Results

The results reveal that lincomycin medication levels were accurately detected. Linearity, precision, accuracy, specificity, and sensitivity were all taken into account when validating the method. Figure 1 depicts the chromatogram results for detecting lincomycin drug levels, which reveal the appearance of a peak area around the 10th minute.

Table 1 shows the results of calculating lincomycin levels after 1–2 weeks of treatment. Lincomycin levels are markedly significant ($p < 0.05$), as indicated by the same row symbol. Treatment: A1 receives ½ dosage, A2 receives one dose, and A3 receives two doses. P1 receives ½ dose, P2 receives one dose, and P3 receives two doses.

## Discussion

The results revealed that UV HPLC can detect lincomycin levels in broiler chicken plasma. Lincomycin levels in plasma can be determined using HPLC, which has been verified in this investigation. The lincomycin standard curve's linearity was determined using the calibration findings of the drug concentration ratio to the peak area visible on the chromatogram. A standard curve linearity test was performed on the yield of spiking lincomycin at plasma drug concentrations of 50, 25, and 10 µg/ml. The linear calibration equation for spiking lincomycin in broiler chicken plasma is $y = 388.24x + 1367.1$, with $R^2 = 0.9999$. Figure 1 depicts the calibration curve for lincomycin medication levels. The calibration curve's deviation value ($R^2 > 0.99$) indicates a linear association between drug concentration and peak area in the method utilized (Razi *et al*., 2020). The precision value of the method for detecting lincomycin levels in broiler chicken plasma is determined using the %CV (coefficient of variation) value in a test procedure. A suitable test procedure will yield findings with generally low variance between repetitions of the same concentration. The precision number (%CV) is derived from the standard deviation of test findings divided by the average and multiplied by 100%. (Jellife *et al*., 2015). The precision value (% CV) is calculated by repeating at least six times with a 100% analyte concentration, or by repeating at least nine times, with three concentrations and three repetitions (Hakimah *et al*., 2019). The precision value (% CV) is bad if it exceeds 15%. (Jellife *et al*., 2015). This study used nine trials to determine the precision value (%CV) at doses of 50 (µg/ml), 10 (µg/ml), and 1 (µg/ml). Table 2 shows the results of measuring precision values (%CV). The method for detecting lincomycin levels in broiler chicken plasma has average precision values (%CV) of 6.72%, 10%, and 7%. The findings of measuring this study's accuracy value (% CV) are categorized as good according to the literature, which is less than 15%. The method for detecting lincomycin levels in plasma was calculated by computing the %recovery value from the results of peak area measurements on three known concentration standards, namely 1, 10, and 50 µg/ml. According to Abualhasan *et al*. (2012), an excellent approach for measuring lincomycin levels in broiler

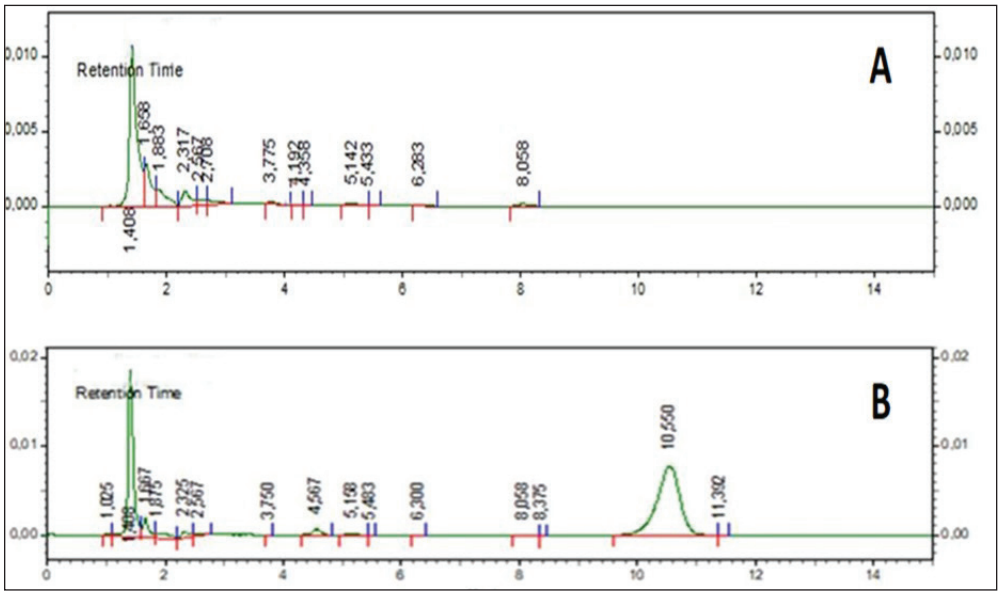


**Fig. 1.** Chromatogram results for both blank and spiking samples. (A): A plasma blank chromatogram. (B): Spiking chromatogram of lincomycin concentration 50 µg/ml in plasma.

**Table 1.** Results from the measurement of lincomycin levels after 1–2 weeks of treatment

| Group | Treatment 1 week | | Group | Treatment 2 Weeks | |
|---|---|---|---|---|---|
| | 1-Day post-treatment | 1 Week post-treatment | | 1-Day post-treatment | 1 Week post-treatment |
| - | Mean ± Standard Deviation (ppm/ (µg/ml)) | Mean ± Standard Deviation (ppm/ (µg/ml)) | | Mean ± Standard Deviation (ppm/ (µg/ml)) | Mean± Standard Deviation (ppm/ (µg/ml)) |
| A1 | 1,75 ± 0,02* | 0 ± 0,0 | P1 | 3,77 ± 1,27 | 1,46 ± 0,83 |
| A2 | 2,22 ± 0,28 | 0 ± 0,0 | P2 | 6,59 ± 1,06* | 2,28 ± 0,63 |
| A3 | 5,37 ± 0,99 | 1,41 ± 0,62 | P3 | 13,99 ± 1,40 | 4,23 ± 0,68 |

The row's symbols represent the importance of the lincomycin levels ($p < 0.05$). Information: A1 receives a ½ dosage, A2 receives one dose, A3 receives two doses, P1 receives a ½ dose, P2 receives one dose, and P3 receives two doses.

**Table 2.** Results of assessing the accuracy value (% recovery) of lincomycin medication levels in plasma broiler chickens.

| Sample | Concentration (µg/ml) | peak area wide | Average Area | %Recovery |
|---|---|---|---|---|
| Plasma | 50 | 20,737 | 20643.3 | 99.30% |
| | | 20,545 | | |
| | | 20,648 | | |
| | 25 | 12,560 | 11049.67 | 103.47% |
| | | 10,324 | | |
| | | 11,345 | | |
| | 10 | 4,874 | 5208.33 | 98.94% |
| | | 5,164 | | |
| | | 5,587 | | |

chicken plasma using HPLC has a recovery value of 80% to 110%. The percentage recovery calculated for the three benchmarks in this investigation ranged from 90.39% to 108.43%. These findings suggest that the approach for detecting lincomycin levels is reliable. Table 2 displays data from the derivation of the percentage recovery value.

The sensitivity of the analytical method is assessed by calculating the linearity of the Limit of Quantitation (LOQ) and LOD. The LOQ, also known as the LOQ, is the least amount of analyte in a sample that may fully meet the requirements. At the same time, LOD is the lowest analyte in a sample that can still be detected despite not always being quantifiable, and it offers a more meaningful response than LOQ. The LOD value of lincomycin in broiler chicken plasma was 1.12. The LOQ for lincomycin in broiler chicken plasma is 4.86 µg/ml. The technique validation results from this study are shown in Table 3.

Based on the results of measuring lincomycin levels in plasma, the results obtained after one day of treatment for 1 week in group A1 were 1.75 ± 0.02 µg/ml, A2 was 2.22 ± 0.28 µg/ml, and A3 was 2.22 ± 0.28 µg/ml. One week after the first treatment, group A1 was 3.77 ± 1.27 µg/ml, A2 was 0 ± 0.0 µg/ml, A3 was 0 ± 0.0 µg/ml, and Measurement of lincomycin levels in plasma showed results after one day of treatment for 2 weeks in group P1 of 0.03 ± 0.05 µg/ml, P2 of 6.59 ± 1.06 µg/ml, and P3 of 13.99 ± 1, 40 µg/ml, then after 1 week after the first treatment, group P1 was 1.46 ± 0.83 µg/ml, P2 was 2.28 ± 0.63 µg/ml, and P3 was 4.23 ± 0.68 µg/ml. The majority of the groups had elevated lincomycin levels the day following treatments 1 and 2. The measurement's levels surpass the 0.1 ppm or 0.1 µg/ml Indonesia MRL for lincomycin allowed in SNI 01-6366–2000, with the exception of groups A1 and A2, which showed figures above and below MRL 1 week following treatment. Park *et al*. (2019) reported that the downtime or withdrawal period for oral administration of lincomycin is 3 days. On the other hand, chicken products manufactured 1 day after treatments one and two are harmful to eat, according to the European Medicines Agency (2008), which specifies that the lincomycin withdrawal period is 5–7 days. However, P1 and P2 revealed that drug levels dropped and were no longer detectable 1 week following treatment 2. The normality test compares the levels of lincomycin in groups A1, A2, and A3 1 day

**Table 3.** Results of the validation process for the HPLC method of measuring the levels of lincomycin in the plasma of broiler chickens.

| Method validation parameters | Method validation results | Information |
|---|---|---|
| Linearity | The $r^2$ values resulting from spiking lincomycin with concentrations of 50 µg/ml, 25 µg/ml, and 10 µg/ml in broiler chicken plasma are: $r^2 = 0.9983$ | Good |
| Accuracy | The % recovery value is between 90% and 110% | Good |
| Precision | % coefficient of variation (CV) value ≤ 15% | Good |
| Specificity | No peak area appears in the chromatogram results of the blank sample. The lincomycin peak area appeared in the chromatogram results of the spiking sample at the 10th minute. | Good |
| LOD | Limit of quantification = 1.12 µg/ml | Good |
| LOQ | Limit of Quantification= 4,86 µg/ml | Good |

and 1 week after treatment 1. The data are normally distributed because the significance level is greater than 0.05, indicating a difference between samples 1 day after treatment and 1 week later. Homogeneity tests were performed on groups A1, A2, and A3 1 day after treatment and 1 week later. The sample failed the homogeneity test due to a significance value of less than 0.05. The Kruskall-Wallis test was used to continue the analysis, and the findings revealed no significant difference because the significance value was greater than 0.05. Groups P1, P2, and P3 1 day after treatment, 1 week after undergoing the same test as groups A1, A2, and A3. The significance value is greater than 0.05, indicating that the normality test is broadly distributed. Homogeneity tests were conducted on groups P1, P2, and P3 1 day after treatment and 1 week later. Based on these findings, it was determined that the sample passed the homogeneity test, as the significance value was >0.05. The one-way ANOVA test revealed a significant difference ($p$-value < 0.05). Treatment group 2 performed a Posh Hoc test or additional test. The test revealed a significant difference between groups A1 1 day after treatment and P2 1 week after treatment ($p$-value < 0.05). Another group used the same test and found no change since the significance level was greater than 0.05. Thus, it is possible to conclude that providing half a dose of lincomycin and one dose considerably impacted lincomycin levels in the plasma.

The lincomycin levels in this study were compared to the MIC value against a variety of microorganisms, particularly harmful bacteria. The MIC values utilized in this investigation were derived from numerous sources. Catania *et al*. (2019) found that the therapeutic level of lincomycin for *M. synoviae* infection is 0.5 to 32 µg/ml. Yang *et al.* (2017) explained that the MIC value of lincomycin for *S. aureus* infections was 1 µg/ml, for *Escherichia coli* and *Pasteurella multocida* infections, the MIC value showed 512 µg/ml, while for *S. enteritidis* infections according to Aranda *et al.* (2019) shows a figure of 1.97 µg/ml. Based on the research results, it was found that groups A1 1 day after, A2 1 day after treatment, and A3 1 day after treatment found lincomycin levels of 1.75 ± 0.02; 2.22 ± 0.28; 5.37 ± 0.99, this figure is still above the MIC value for *M. synoviae* infection of 0.5 to 32 µg/ml. These findings indicate that group A1 1 day after treatment has the potential for successful therapy and effectiveness against *M. synoviae* infection since it exceeds the MIC value. One week after treatment, groups A1, A2, and A3 had therapeutic level values of 0 ± 0.0, 0 ± 0.0, and 1.41 ± 0.62, respectively. Groups A1 and A2 had lincomycin levels below the MIC value for infection, while group A3 still met the MIC value. As a result, the potential effectiveness in groups A1 and A2 decreased due to the lower lincomycin levels.

Groups P1, P2, and P3 1 day after treatment obtained a therapeutic level of 3.77 ± 1.27; 6.59 ± 1.06; 13.99 ± 1.40. Groups P1, P2, and P3 1 week after treatment, received a therapeutic level value of 1.46 ± 0.83, 2.28 ± 0.63, and 4.23 ± 0.68; this figure still has an MIC value for *M. synoviae* infection of 0.5 to 32 µg/ml. These data reveal that groups P1, P2, and P3 are above the MIC value 1 day and 1 week after treatment, indicating therapeutic efficacy and effectiveness against *M. synoviae* infection. Groups A1, A2, and A3 1 day after treatment obtained therapeutic levels of 1.75 ± 0.02, 2.22 ± 0.28, 5.37 ± 0.99, and groups P1, P2, and P3 1 day after treatment obtained a therapeutic level value of 3.77 ± 1.27; 6.59 ± 1.06; 13.99 ± 1.40. Groups P1, P2, and P3 1, a week after treatment, obtained a therapeutic level value of 1.46 ± 0.83, 2.28 ± 0.63, and 4.23 ± 0.68; this figure still has a therapeutic value above the MIC for *S. aureus* infections, namely one ppm, which has the potential for therapeutic success and therapeutic effectiveness against *S. aureus* infections because it is above the MIC value. Lincomycin levels were below the MIC values in all groups 1 day and 1 week

after treatment for *Escherichia coli* and *Pasteurella multocida* infections. Lincomycin levels in groups A1, A2, and A3 were below the MIC for *S. enteritidis* 1 day and 1 week after treatment, reducing their potential effectiveness. Groups A1, A2, and A3 had lincomycin levels below the MIC for *S. enteritidis* 1 day and 1 week after treatment, reducing their potential effectiveness.

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***Conflict of interest***

The authors declare that they have no conflict of interest.

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***Author contributions***

CW, ADW, DAW, AYM, AN. Designed the study. CW, ADW, and AYM. Field research and sample analysis in the laboratory. All authors wrote, edited, read, and approved the final manuscript.

***Data availability***

All data are provided in the manuscript.

**References**

Abualhasan, M.N., Batrawi, N., Sutcliffe, O.B. and Zaid, A.N. 2012. A validated stability-indicating HPLC method for routine analysis of an injectable lincomycin and spectinomycin formulation. Sci. Pharm. 80(4), 978–986.

Aranda, M.I.R., Gomez, G.A.T., Barros, M., Santos, M.H.S., Oliviera, L.L., Pena, J.L. and Moreira, M.A.S. 2019. Antimicrobial and synergistic activity of 2,2',4- tryhydroxybenzophenone against bacterial pathogens of poultry. Front. Microbiol. 10, 490.

Anastasia, Y. 2011. Teknik analisis residu golongan tetrasiklin dalam daging ayam secara kromatografi cair kinerja tinggi. Buletin Teknik Pertanian. 16, 68–69.

Catania, S., Botinelli, M., Fincato, A., Gastaldelli, M., Barberio, A., Gobbo, F. and Vincenzoni, G. 2019. Evaluation of minimum inhibitory concentration for 154 *Mycoplasma synoviae* isolates from Italy collected during 2012- 2017. PLoS One 14, 11.

Choirunnisa, S., Wuryanto, M.A., Kusariana, N. And Saraswati, L.D. 2019. Survei kandungan residu oksitetrasiklin pada hati ayam yang dijual di pasar tradisional kecamatan banyumanik Kota Semarang. J. Kesehat. Masy. 7, 447–453.

European Medicines Agency. 2008. Target animal safety for veterinary pharmaceutical products. Veterinary Medicines and Inspections, London, UK.

Foroutankhah, M., Toghyani, M. and Landy, N. 2019. Evaluation of *Calendula officinalis* L. (marigold) flower as a natural growth promoter in comparison with an antibiotic growth promoter on growth performance, carcass traits and humoral immune responses of broilers. Anim. Nutr. 5, 314–319.

Goren, E., De Jong, W.A. and Doornenbal, P. 2011. Therapeutic efficiency of medicating drinking water with spectinomycin and lincomycin spectinomycin in experimental *Escherichia coli* infection in poultry. Vet. Ext. Q. 10, 191–197.

Hakimah, N., Donny, R. Gagak, S., Pawestri, W. and Indarjulianto, S. 2019. Validasi metode analisis tetrasiklin pada Ikan Nila (*Oreochromis sp.*) menggunakan Alat Kromatografi Cair Kinerja Tinggi (KCKT). Jurnal Sains Veteriner. 37, 213–218.

Jellife, R.W., Fu, X., Schumitzky, A., Bayard, D. and Neely, M. 2015. Describing assay precision—reciprocal of variance is correct, not CV percent: its use should significantly improve laboratory performance. Therap. Drug Monit. 37(3), 389–394.

Kanchugal, S. and Selmer, M. 2020. Structural recognition of spectinomycin by resistance enzyme ANT (9) from *Enterococcus faecalis*. Int. J. Antimicrob. Agents. 64, 10–1128.

Landy, N., Farshid, K. and Mostafa, F. 2020. Evaluation of cottonseed bioactive peptides on growth performance, carcase traits, immunity, total antioxidant activity of serum and intestinal morphology in broiler chickens. Ital. J. Anim. Sci. 19(1), 1375–1386,

Mehdi, Y., Létourneau, M., Marie-Pierre, G., Marie-Lou, C., Younes, S., Gayatri, R., Tarek, B., Satinder K., Côté, C., Ramirez, A.A. and Godbout, S. 2018. Use of antibiotics in broiler production, global impacts and alternatives. Anim. Nutr. 4, 170–178.

Muzaki, A.M., Wibisono, C., Irianti, N.I., Wijayanti, A.D. and Widiasih, D.A. 2023. Validasi metode analisis penetapan kadar linkomisin menggunakan high performance liquid chromatography (HPLC). J. Sains Vet. 41, 207–214.

Park, N., Pervin, R., Hossain, Md.A. and Park, S. 2019. Establishment of withdrawal period after oral administration of lincomycin and colistin combination in broiler chicken. Pak. Vet. J. 40, 267–270.

Sholihah, S., Putriana, N.A. and Pratiwi, R. 2021. Review metode analisis warfarin dalam plasma dengan berbagai instrumen. J. Sains. Farm. Klin. 8, 128–144.

Standard Nasional Indonesia (SNI). 2000. Batas Maksimum Cemaran Mikroba dan Batas Maksimum Residu Dalam Bahan Makanan Asal Hewan. SNI 01- 6366–2000. National Standardization Council.

Susanti, M. and Dachriyanus, A. 2014. Kromatografi cair kinerja tinggi. Padang, Indonesia: LPTIK Universitas Andalas.

Wang, B.Y., Wang, X., Xie, Z., Diao, K., Xie, G. Zhiang., T. and Zhang. G.D. 2020. Quantitative analysis in poultry eggs by accelerated solvent extraction coupled with gas chromatography tandem mass spectrometry. Foods 9(5), 651.

Yang, B., Lei, Z., Zhao, Y., Ahmeed, S., Wang, C., Zhang, S., Fu, S., Cao, J. and Qiu, Y. 2017. Combination susceptibility testing of common antimicrobials *in Vitro* and effects of Sub-MIC of antimicrobials on *Staphylococcus aureus* biofilm formation. Front. Microbiol. 8, 270495.

Yuningsih, A. 2009. Keberadaan residu antibiotika dalam produk peternakan (Susu dan Daging). Lokakarya Nasional Keamanan Pangan Produk Peternakan, pp: 48–54.