

DETERMINATION OF ANTIBIOTIC GROWTH PROMOTER (POLYMYXIN E) RESIDUES IN LAYERS

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DOI: <https://doi.org/10.5281/zenodo.22829612>

Keywords

Polymyxin E; Antibiotic Residues; Layer Chicken; Poultry Meat; Liver; Eggs; Maximum Residue Limit; HPLC; Withdrawal Period; Veterinary Antibiotics.

Article History

Received: 10 June 2026

Accepted: 01 September 2026

Published: 17 September 2026

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Abstract

Background: Antibiotics are agents that inhibit or kill microorganisms and are widely used in veterinary practice. Polymyxin E is commonly used to treat bacterial infections in poultry. However, excessive or improper use may result in drug residues in poultry meat, organs, and eggs, which may pose health risks to consumers, particularly children. Therefore, determining appropriate withdrawal periods is important to minimize antibiotic residues.

Methodology: A total of 225 samples of layer meat, liver, and eggs were collected from different poultry farms. Polymyxin E residues were determined using high-performance liquid chromatography (HPLC) with a UV detector at 215 nm. Ethyl acetate and phosphate-buffered saline were used for extraction, followed by dissolution in the mobile phase and n-hexane. The data were statistically analyzed using ANOVA.

Results: Overall, 67% of the samples had Polymyxin E concentrations above the maximum residue limit (MRL), while 33% were below the MRL. Residue concentrations in muscle samples ranged from 101.6–704.8 µg/kg, liver samples from 911.6–3573.8 µg/kg, and egg samples from 14–202.6 µg/kg. ANOVA showed statistically significant differences among the obtained results.

Conclusion: The findings indicate a high occurrence of Polymyxin E residues in layer poultry products, possibly due to improper antibiotic use and failure to observe recommended withdrawal periods. Proper antibiotic administration and adherence to withdrawal periods are necessary to reduce drug residues and protect public health.

Introduction:

Livestock production plays an important role in food security, nutrition, employment, and the agricultural economy. Among the livestock sectors, the poultry industry is one of the important components of agriculture in Pakistan. The demand for poultry meat has increased considerably because chicken meat is relatively

less expensive than meat from other animals and is therefore accessible to a large proportion of the population. Poultry production also provides employment opportunities and contributes substantially to the national meat supply. In Pakistan, poultry meat has become an important source of animal protein and contributes significantly to total meat production (Ghafoor et

al., 2010; Anonymous, 2018). The increasing demand for poultry products has consequently encouraged intensive production systems aimed at achieving rapid growth, efficient feed utilization, and disease control. A healthy and adequate diet is essential for maintaining physical strength, good health, growth, and productive capacity. Proteins are an essential component of a balanced human diet, and poultry meat and eggs are important sources of high-quality protein (Abedullah et al., 2007). Poultry meat also provides fats, vitamins, minerals, selenium, heme iron, zinc, and copper, making it an important nutritional component of the human diet (Marangoni et al., 2015). Increasing consumption of poultry meat has been observed over time in Pakistan and other countries, reflecting its nutritional value, affordability, and availability. Poultry production can therefore contribute to improving protein availability while maintaining relatively affordable retail prices (Maqbool et al., 2005). To increase productivity and maintain animal health, various feed additives are used in poultry production. These include probiotics, prebiotics, synbiotics, organic acids, vitamins, minerals, herbs, carnitine, exogenous enzymes, antioxidants, and antibiotics. Such additives may improve growth, feed efficiency, intestinal health, and overall production performance. Antibiotic growth promoters (AGPs) have historically been used in poultry production at low concentrations to improve growth performance and feed utilization (Mahima et al., 2012, 2013). Growth-promoting substances may increase growth, improve feed consumption or feed efficiency, or reduce the amount of feed required to achieve a particular level of growth. However, products that only increase feed consumption without improving production are considered commercially less useful, while additives that negatively affect meat quality or taste are generally undesirable (Castanon, 2007). Antibiotics have been administered to food-producing animals, including broilers and layers, for the prevention and treatment of infectious diseases as well as for growth promotion. Their beneficial effects have been

associated with the suppression of harmful microorganisms, maintenance of intestinal microbial balance, improved nutrient absorption, and reduction in microbial competition for nutrients. Under stressful conditions or during digestive disturbances, pathogenic organisms, particularly *Escherichia coli* and other Gram-negative bacteria, may increase and disturb the normal intestinal microbiota. These organisms can adhere to the intestinal epithelium, interfere with nutrient absorption, and consequently reduce poultry growth and performance (Dhama et al., 2014). Antibiotic growth promoters may reduce the occurrence and severity of intestinal infections, decrease microbial consumption of nutrients, enhance nutrient absorption, and reduce the production of harmful microbial metabolites. However, their growth-promoting effects are not observed in germ-free animals, suggesting that interactions with the intestinal microbiota play an important role in their action (Niewold, 2007). The use of antibiotic growth promoters has raised increasing concerns because prolonged or inappropriate antimicrobial exposure can contribute to antimicrobial resistance and the accumulation of antimicrobial residues in animal-derived foods. Continuous antibiotic use may select resistant strains of microorganisms, including *Staphylococcus aureus* and *Streptococcus* spp. (Upadhyay et al., 2014). Antimicrobial resistance can develop through genetic mutations or biological mechanisms that reduce the effectiveness of antibiotics. These mechanisms may include enzymatic destruction or modification of antimicrobial agents, changes in bacterial drug targets, reduced uptake, and active efflux of antibiotics from bacterial cells. Some bacteria may also prevent antibiotics from reaching or binding effectively to their target sites, thereby reducing antimicrobial activity (Adewuyi et al., 2011). These developments represent an important public-health concern because resistant bacteria can potentially spread between animals, humans, food, and the environment. Long-term use of antimicrobial agents may also result in drug residues in edible animal tissues. Failure to follow appropriate

withdrawal periods may lead to the persistence of antimicrobial compounds in meat, milk, and eggs. Such residues can create food-safety concerns and may contribute to unnecessary antimicrobial exposure among consumers. Some antibiotics may also produce allergic reactions or disturb the normal gastrointestinal microbiota, potentially affecting the production or availability of vitamins such as vitamins B and K. Because of these concerns, the use of several antibiotics as growth promoters has been restricted or banned, while alternative feed additives such as probiotics, prebiotics, synbiotics, enzymes, toxin binders, organic acids, organic minerals, and oligosaccharides have received increasing attention (Niewold, 2007). Among the antimicrobial agents used in food-producing animals, colistin has received particular attention because of its activity against Gram-negative bacteria and its importance in the treatment of multidrug-resistant infections. Colistin, also known as polymyxin E, belongs to the polymyxin class of antibiotics and is a cyclic decapeptide linked to a fatty-acid chain. It is active against several Gram-negative organisms and has been used for the treatment and prevention of enteric infections caused by organisms belonging to the family Enterobacteriaceae (Kempf et al., 2016). Colistin and polymyxin B have historically been considered important therapeutic options against problematic multidrug-resistant Gram-negative bacteria. Colistin has also been used in poultry production in countries including China, India, Vietnam, and Japan (Liu et al., 2016; Vishnuraj et al., 2016; Kim et al., 2013; Makita et al., 2016). Colistin acts primarily by altering bacterial cell membrane permeability, ultimately causing bacterial cell damage and death. Its activity includes organisms such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella* spp. (Zhao et al., 2016). The increasing prevalence of Gram-negative bacteria resistant to multiple antibiotics has resulted in the reintroduction and increased importance of polymyxins (Yiun et al., 2018). However, colistin use has raised concerns because resistance to this antimicrobial can compromise an important therapeutic option for multidrug-

resistant infections. Colistin resistance and its increasing use in animal production have therefore become important issues in antimicrobial-resistance surveillance (Kempf et al., 2016). Colistin differs from polymyxin B by the substitution of D-leucine for D-phenylalanine, and its clinical use has also been associated with nephrotoxicity and neurotoxicity (Lim et al., 2010).

The concern regarding antimicrobial use in food-producing animals has led to stricter regulations and monitoring. In European countries, the use of antibiotics as growth promoters was restricted because of concerns regarding antimicrobial residues and public health, and growth-promoting use of antibiotics was banned in the European Union in 2006 (Maron et al., 2016). Nevertheless, antimicrobial use in poultry production remains a concern in several developing countries. Therefore, reliable methods for detecting antimicrobial residues are necessary to monitor banned substances and determine whether residues of permitted products remain within maximum residue limits. Several analytical methods are available for detecting antimicrobial residues in animal-derived foods. Screening approaches include swab tests, agar plate methods, and four-plate tests, while more advanced analytical techniques include high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), thin-layer chromatography (TLC), radioimmunoassay (RIA), and enzyme-linked immunosorbent assay (ELISA) (Hakeemzadegan et al., 2014). These methods can support the identification and quantification of antimicrobial residues and help assess compliance with food-safety standards. Therefore, monitoring antimicrobial residues, particularly those associated with critically important antibiotics such as colistin, is essential for evaluating food safety and understanding the potential contribution of poultry production to antimicrobial resistance. Responsible antimicrobial use, appropriate withdrawal periods, regulatory surveillance, and reliable

residue-detection methods are consequently important for protecting animal health, maintaining food quality, and reducing potential risks to public health

Materials and Methods

The study was conducted to determine the presence and concentration of Polymyxin E (Colistin) residues in layer chicken muscle, liver, and eggs collected from poultry farms around Faisalabad, Pakistan. Samples were randomly collected from 15 poultry farms located in different areas around Faisalabad, including Toba Tek Singh, Kamalia, Samundari, Jhumra, Gojra, and Thikriwala. From each farm, five layer birds were randomly selected, and muscle, liver, and egg samples were obtained from each bird. Thus, 15 samples were collected from each farm, consisting of five muscle, five liver, and five egg samples, giving a total of 225 samples. After collection, samples were placed in polythene bags, transported under refrigerated conditions, homogenized, and stored at -18°C until analysis. Analytical-grade and HPLC-grade reagents were used throughout the study. Acetonitrile, ethyl acetate, n-hexane, disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, potassium chloride, hydrochloric acid, and HPLC-grade water were used for sample extraction and preparation of the mobile phase and phosphate-buffered saline (PBS). Colistin reference standard was used for preparation of calibration solutions. The major equipment included an analytical weighing balance, homogenizer, refrigerated centrifuge, vortex mixer, micropipettes, pH meter, ultrasonic sonicator, membrane filters, and HPLC system. Colistin residues were analyzed using a high-performance liquid chromatograph equipped with a C18 column, solvent delivery system (SYKAM S1122), autosampler, column oven, and UV/VIS detector (SYKAM S3210).

The mobile phase was prepared by mixing acetonitrile and HPLC-grade water in a ratio of 27:73 (v/v). The solution was filtered and sonicated for 30 minutes to remove dissolved air and was freshly prepared before analysis.

Phosphate-buffered saline was prepared by dissolving 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 , and 0.24 g KH_2PO_4 in HPLC-grade water and making the final volume up to 1 L. The pH was adjusted to 7.4 using hydrochloric acid. A Colistin stock solution of 1000 $\mu\text{g}/\text{mL}$ was prepared by accurately weighing 1 mg of the reference standard and dissolving it in 1 mL of HPLC-grade acetonitrile. Working standard solutions of 100, 80, 60, and 10 $\mu\text{g}/\text{mL}$ were prepared by serial dilution using acetonitrile according to the equation $C_1V_1 = C_2V_2$. The prepared standards were filtered through a 0.22 μm membrane filter and used for HPLC calibration. For sample extraction, frozen muscle, liver, and egg samples were thawed and homogenized. Five grams of each homogenized sample were transferred into centrifuge tubes, followed by the addition of 5 mL phosphate-buffered saline and 20 mL ethyl acetate. The samples were homogenized for 3–5 minutes and centrifuged at 1500 rpm for 20 minutes. The supernatant was collected, and the extraction procedure was repeated twice to improve recovery. The combined extracts were evaporated to dryness under a nitrogen stream at 60°C . The residue was reconstituted with 3 mL of mobile phase and mixed with 2 mL n-hexane, followed by centrifugation at 4000 rpm for 10 minutes. The resulting extract was filtered through a 0.22 μm membrane filter and transferred into HPLC vials. A volume of 20 μL was injected into the HPLC system for analysis. The extraction procedure was performed according to the method described by Zhao et al. (2016).

HPLC analysis was performed using a C18 column (4.6×250 mm) with an isocratic mobile phase consisting of acetonitrile and water (27:73, v/v). The flow rate was maintained at 1.0 mL/min, and the injection volume was 20 μL . The UV detector was set at 215 nm, and the system pressure was approximately 100 bar. Peak height and peak area were recorded using Peak Sample software version 3.29. Colistin concentrations in the samples were determined from the calibration curve prepared using the standard solutions. The calibration curve was

obtained by plotting the peak areas of the Colistin standards against their corresponding concentrations. The concentration of Colistin in each sample was calculated from the regression equation of the calibration curve while considering the sample weight and extraction volume. Method validation was performed by evaluating linearity, limit of detection (LOD), and limit of quantification (LOQ). Linearity was assessed using a matrix-matched calibration curve prepared from four Colistin concentrations over the selected analytical range. The LOD was defined as the lowest concentration producing a signal-to-noise ratio of approximately 3:1, whereas

the LOQ was defined as the lowest concentration providing acceptable analytical performance at a signal-to-noise ratio of approximately 10:1. The reported LOQ for the method was 0.1 µg/mL. Statistical analysis was performed by calculating the mean and standard deviation of Colistin concentrations in the analyzed samples. Differences among sample groups were evaluated using analysis of variance (ANOVA), followed by Duncan's Multiple Range (DMR) test. A statistical approach similar to that reported by Markou et al. (2008) was used for comparison of the groups.

Result

Table 4.1: No. of Polymyxin E positive and negative samples of layer's muscle, liver and eggs obtained from different poultry farms of Faisalabad.

Type of sample	Antibiotic positive	Antibiotic negative
Muscle (n=75)	58	17
Liver (n =75)	60	15
Egg (n=75)	54	21

Table 4.2: overall percentage of antibiotic residues present in muscle, liver and egg samples obtained from layers by HPLC analysis.

Type of sample	Percentage of positive Antibiotic	Percentage of negative Antibiotic
Muscle (n=75)	77%	23%
Liver (n =75)	80%	20%
Egg (n=75)	72%	28%

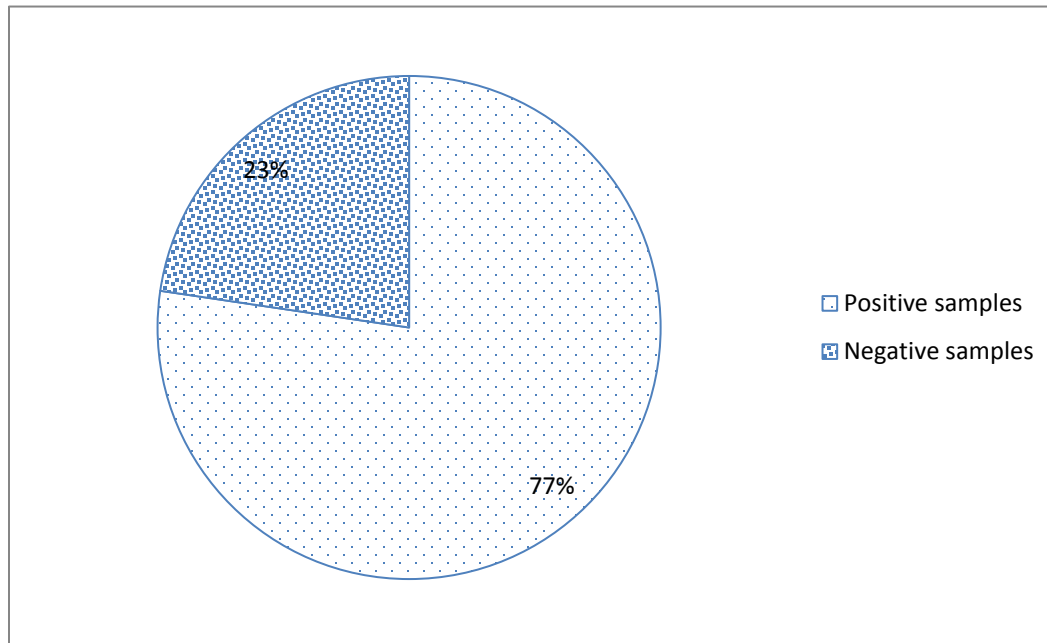


Fig. 4.1: Pie chart representing positive and negative muscle samples of layers containing Polymyxin E

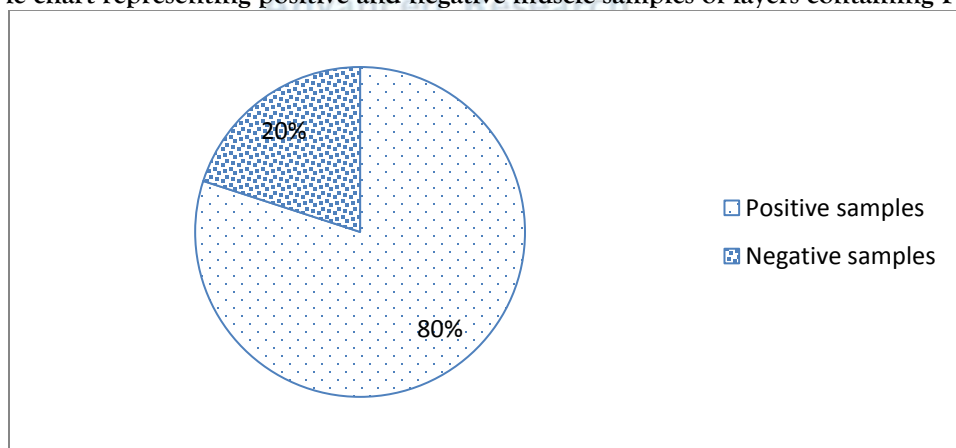


Fig. 4.2: Pie chart representing positive and negative liver samples of layers containing Polymyxin E

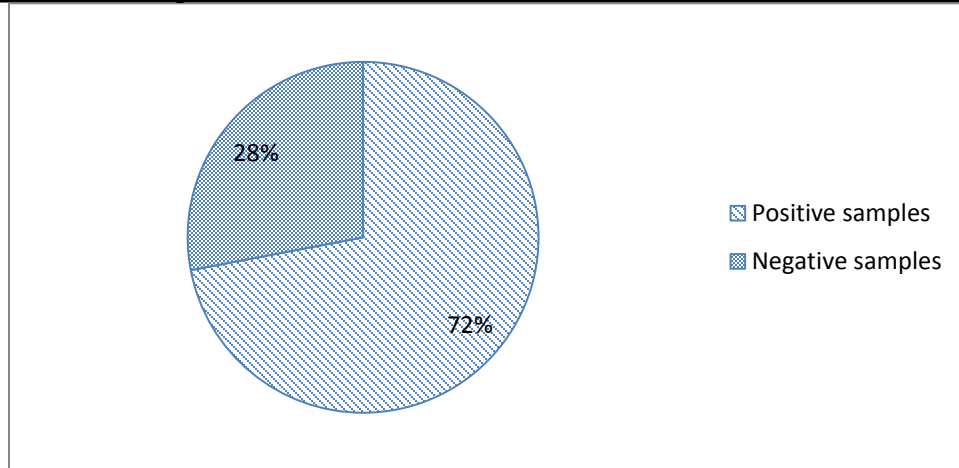


Fig. 4.3: Pie chart representing positive and negative egg samples of layers containing Polymyxin E

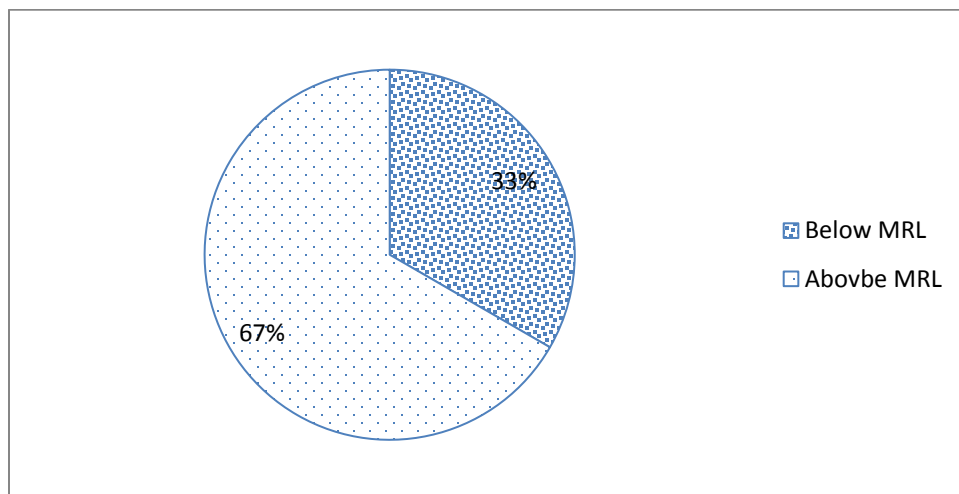


Fig. 4.4: Pie chart indicating the percentage of overall samples above and below maximum residual limits (MRL).

Table 4.3: Mean $\pm$ S.D concentration of polymyxin E in Layer's muscle samples from different poultry farms around Faisalabad

No. of farms	Mean $\pm$ S.D of polymyxin E in Muscles ($\mu\text{g/kg}$)
Farm 1	101.8 $\pm$ 2.387
Farm 2	250.2 $\pm$ 7.328
Farm 3	704.8 $\pm$ 3.271
Farm 4	123.8 $\pm$ 3.033
Farm 5	164.4 $\pm$ 3.361
Farm 6	193.4 $\pm$ 3.435
Farm 7	263.8 $\pm$ 3.346

Farm 8	342.6 ± 2.408
Farm 9	220.6 ± 2.408
Farm 10	151.8 ± 2.387
Farm 11	437.4 ± 2.073
Farm 12	453.6 ± 2.302
Farm 13	280.2 ± 3.492
Farm 14	363 ± 2.236
Farm 15	425.2 ± 4.147

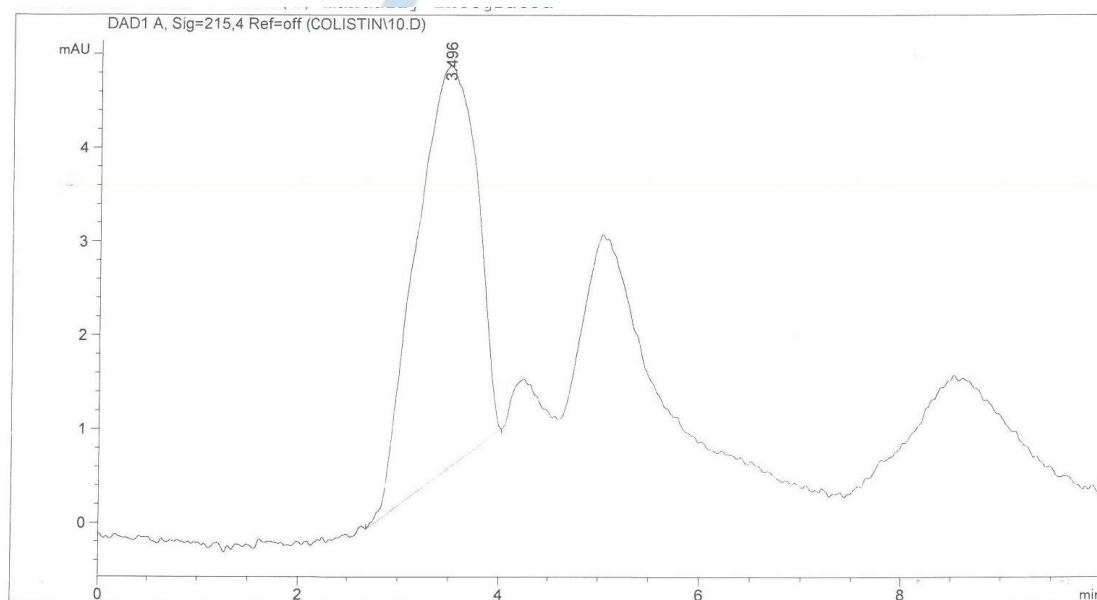


Fig 4.5: Chromatogram indicating polymyxin E residues in muscle samples of layers

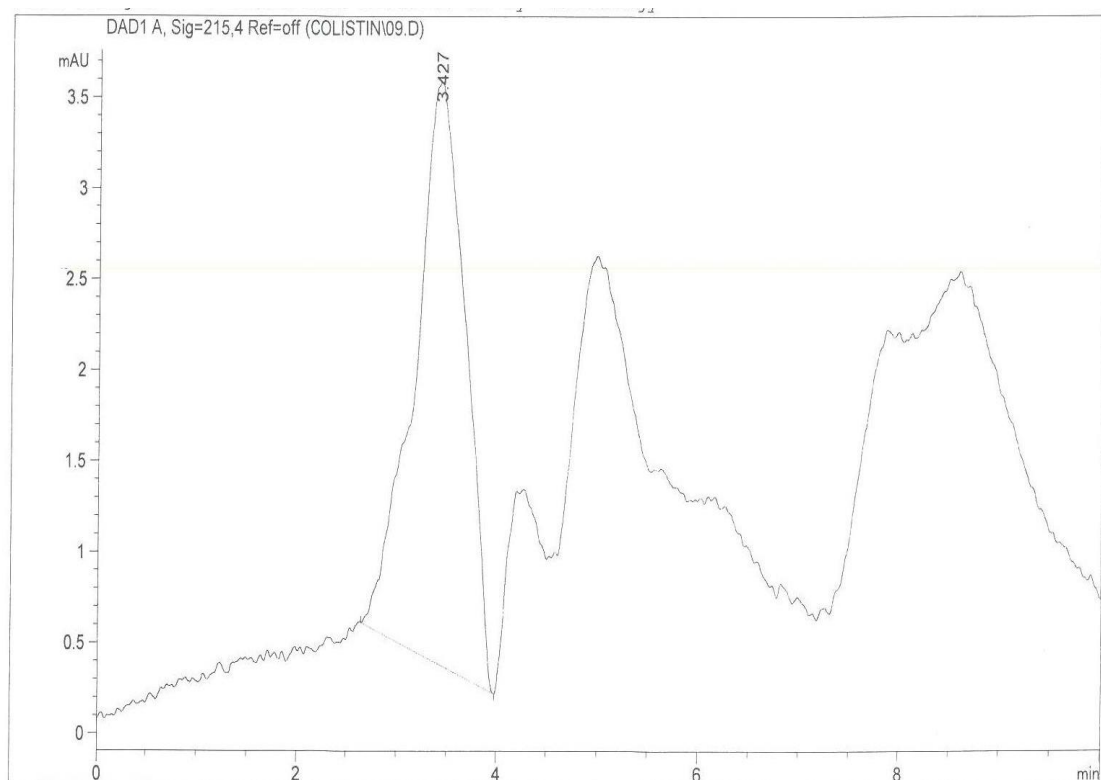


Fig 4.6: Chromatogram indicating polmyxin E residues in muscle samples of layers

Table 4.4: Mean $\pm$ S.D concentration of polmyxin E in Layer's liver samples collected from different poultry farms around Faisalabad

No. of farms	Mean $\pm$ S.D of polmyxin E in Liver ($\mu\text{g}/\text{kg}$)
Farm 1	1074.8 $\pm$ 1.923
Farm 2	1747.4 $\pm$ 2.073
Farm 3	1563 $\pm$ 2.236
Farm 4	1827 $\pm$ 4.527
Farm 5	1045.8 $\pm$ 15.417
Farm 6	6727.6 $\pm$ 7.436
Farm 7	5644.2 $\pm$ 3.193
Farm 8	911.6 $\pm$ 2.701

Farm 9	1416.6 ± 4.669
Farm 10	1504 ± 1.581
Farm 11	3351 ± 6.557
Farm 12	3037.4 ± 2.408
Farm 13	2759.8 ± 3.492
Farm 14	3573.8 ± 2.588
Farm 15	2373.8 ± 2.863

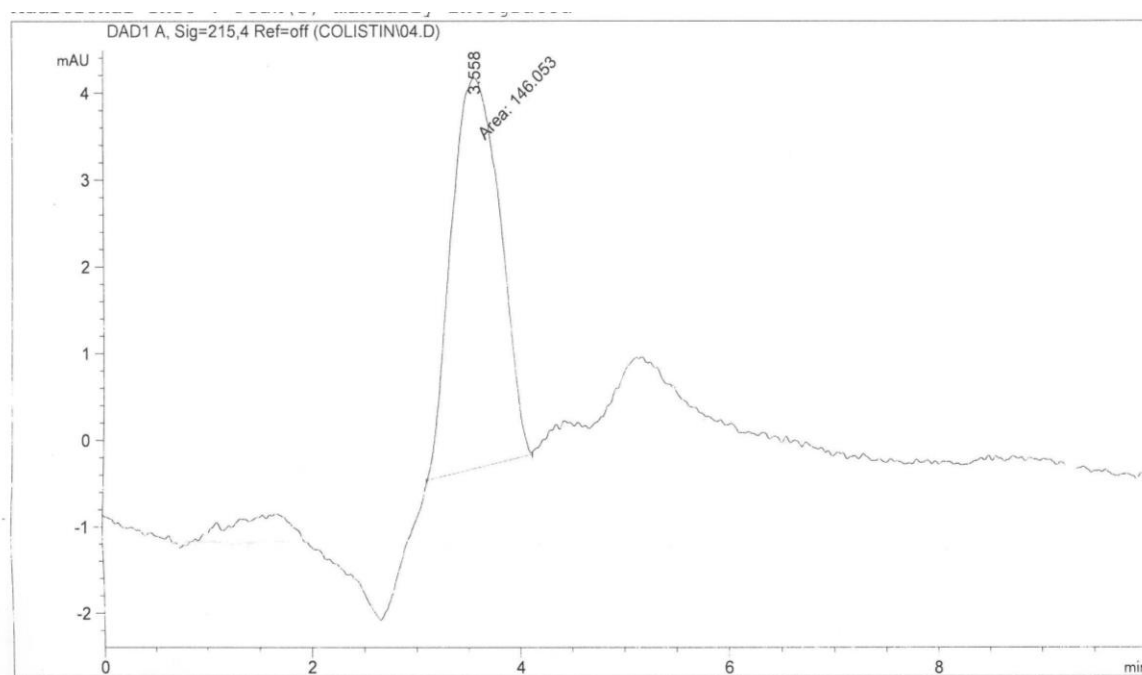


Fig 4.7: Chromatogram indicating polmyxin E residues in liver samples of layers

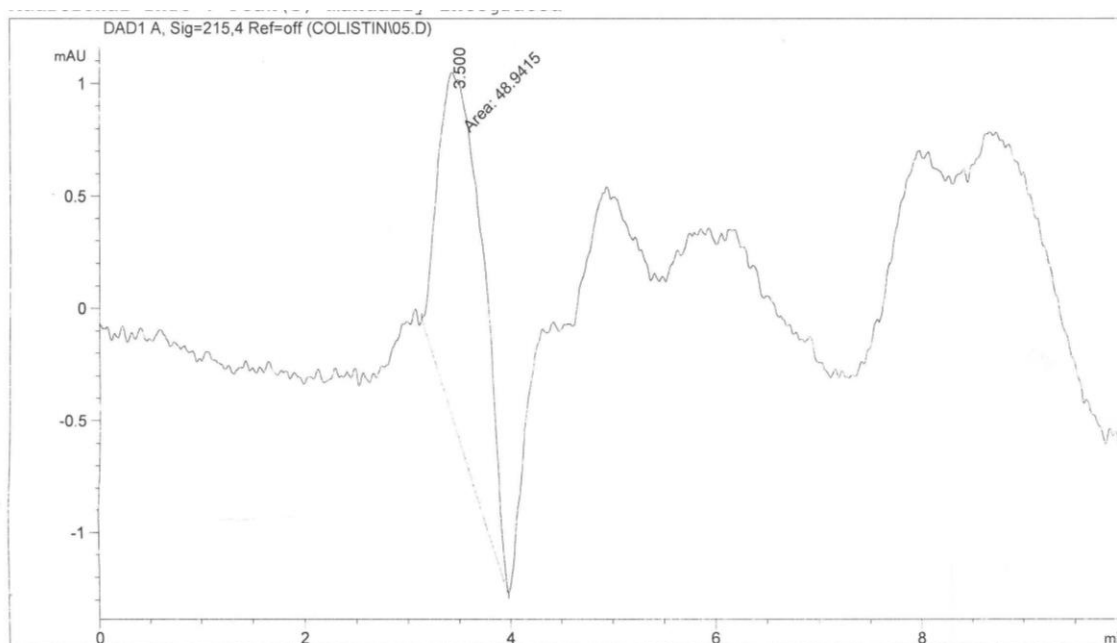


Fig 4.8: Chromatogram indicating polmyxin E residues in liver samples of layers

Table 4.5: Mean $\pm$ S.D concentration of polmyxin E in Layer's Egg samples collected from different poultry farms around Faisalabad

No. of farms	Mean $\pm$ S.D of polmyxin E in Eggs ($\mu\text{g}/\text{kg}$)
Farm 1	47.8 $\pm$ 1.923
Farm 2	173.4 $\pm$ 2.408
Farm 3	14 $\pm$ 3.937
Farm 4	53.4 $\pm$ 2.408
Farm 5	33.2 $\pm$ 2.588
Farm 6	45 $\pm$ 1.581
Farm 7	93.6 $\pm$ 2.302
Farm 8	53.4 $\pm$ 2.408
Farm 9	51 $\pm$ 3.391
Farm 10	54.2 $\pm$ 2.588
Farm 11	45 $\pm$ 2.738

Farm 12	26 ± 3.162
Farm 13	202.6 ± 2.408
Farm 14	24 ± 1.581
Farm 15	34 ± 3.162

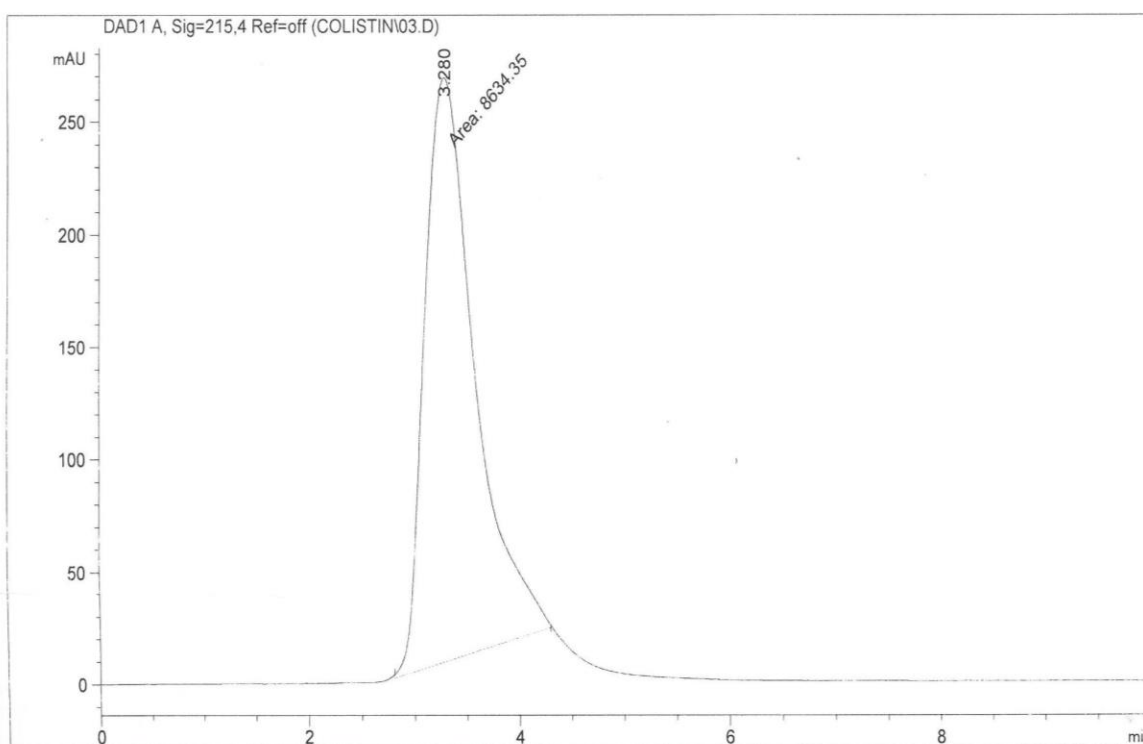


Fig 4.9: Chromatogram indicating polymyxin E residues in egg samples of layers

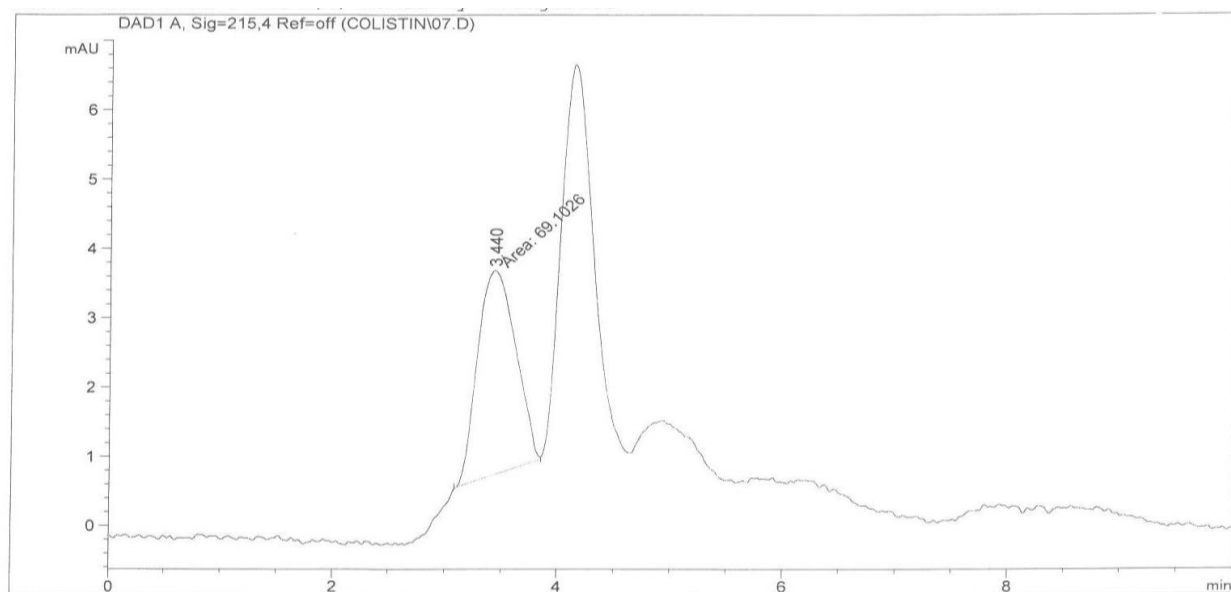


Fig 4.10: Chromatogram indicating polymyxin E residues in egg samples of layers

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Table 4.6: Comparison of Mean $\pm$ S.D concentration ($\mu\text{g/kg}$) of polymyxin E in Layer's muscle, liver and egg samples collected from different poultry farm of Faisalabad

No. of farms	Mean $\pm$ S.D of polymyxin E in muscles ($\mu\text{g/kg}$)	Mean $\pm$ S.D of polymyxin E in Liver ($\mu\text{g/kg}$)	Mean $\pm$ S.D of polymyxin E in Eggs ($\mu\text{g/kg}$)
Farm 1	101.8 $\pm$ 2.387A	1074.8 $\pm$ 1.923C	47.8 $\pm$ 1.923D
Farm 2	250.2 $\pm$ 7.328G	1747.4 $\pm$ 2.073G	173.4 $\pm$ 2.408H
Farm 3	704.8 $\pm$ 3.271O	1563 $\pm$ 2.236F	14 $\pm$ 3.937A
Farm 4	123.8 $\pm$ 3.03B	1827 $\pm$ 4.527H	53.4 $\pm$ 2.408F
Farm 5	164.4 $\pm$ 3.361D	1045.8 $\pm$ 15.417B	33.2 $\pm$ 2.588C
Farm 6	193.4 $\pm$ 3.435E	6727.6 $\pm$ 7.436O	45 $\pm$ 1.581D
Farm 7	263.8 $\pm$ 3.346H	5644.2 $\pm$ 3.193N	93.6 $\pm$ 2.302G
Farm 8	342.6 $\pm$ 2.408J	911.6 $\pm$ 2.701A	53.4 $\pm$ 2.408F
Farm 9	220.6 $\pm$ 2.408F	1416.6 $\pm$ 4.66D	51 $\pm$ 3.391E
Farm 10	151.8 $\pm$ 2.387C	1504 $\pm$ 1.581E	54.2 $\pm$ 2.588F
Farm 11	437.4 $\pm$ 2.073M	3351 $\pm$ 6.557L	45 $\pm$ 2.738D
Farm 12	453.6 $\pm$ 2.302N	3037.4 $\pm$ 2.408K	26 $\pm$ 3.162B
Farm 13	280.2 $\pm$ 3.492I	2759.8 $\pm$ 3.492J	202.6 $\pm$ 2.408I
Farm 14	363 $\pm$ 2.236K	3573.8 $\pm$ 2.588M	24 $\pm$ 1.581B
Farm 15	425.2 $\pm$ 4.147L	2373.8 $\pm$ 2.863I	34 $\pm$ 3.162C

Different alphabets for all of the groups show that overall mean differ significantly.

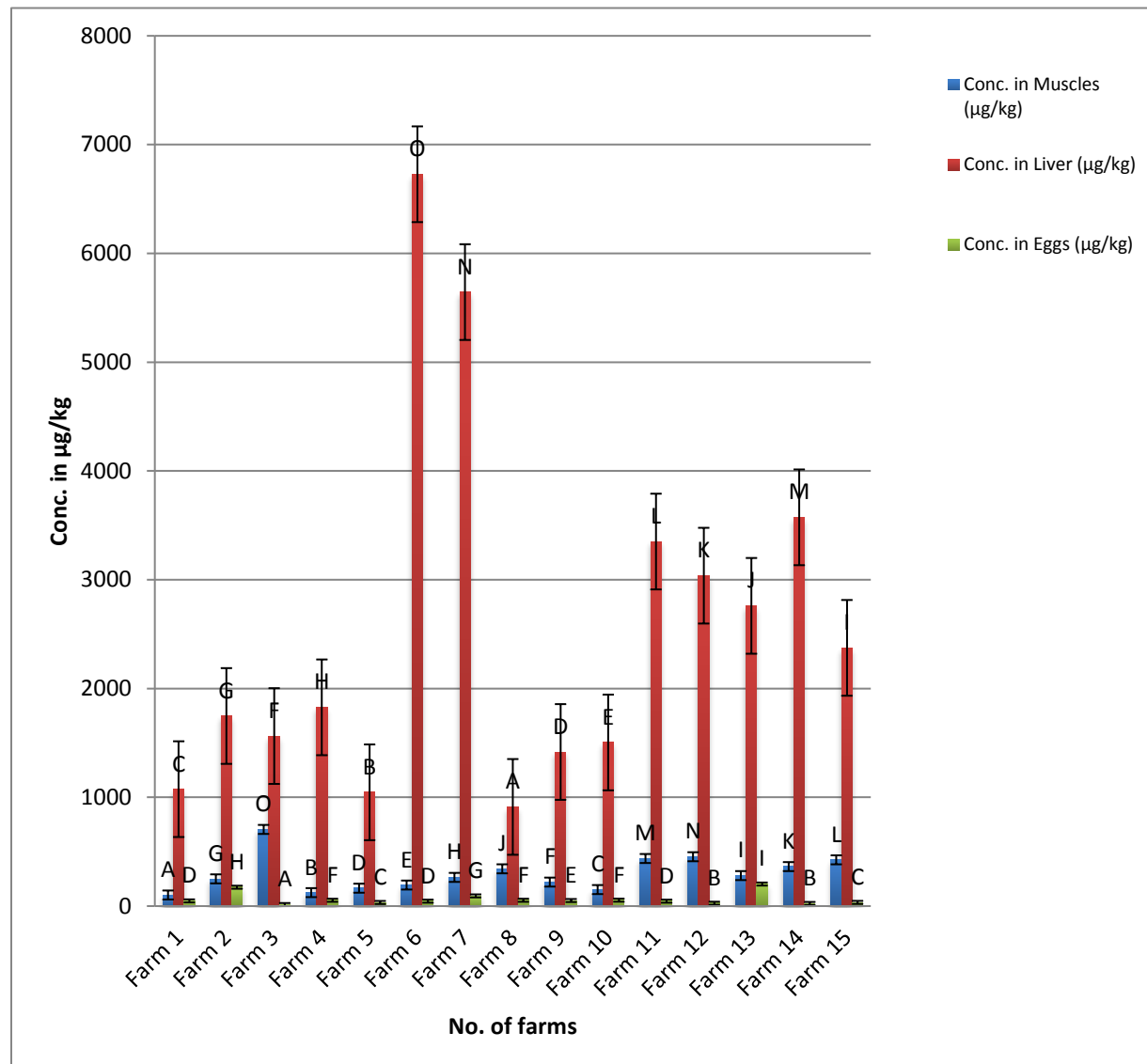


Fig 4.10: Graph indicating the concentration of residues of ploymyxin E in muscle, liver and egg samples of layers taken from 15 poultry farms

Table 4.7: ANOVA of muscle samples of layers

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1801438.880	14	128674.206	11035.524	.000
Within Groups	699.600	60	11.660		
Total	1802138.480	74			

Table 4.8: ANOVA of liver samples of layers

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	203327257.920	14	14523375.566	490433.664	.000

Within Groups	1776.800	60	29.613		
Total	203329034.720	74			

Table 4.9: ANOVA of egg samples of layers

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	204577.147	14	14612.653	2085.536	.000
Within Groups	420.400	60	7.007		
Total	204997.547	74			

Discussion:

Layer's meat is one of the essential needs of human diet as it source of proteins, fats, carbohydrates and minerals. As it is low in cost and is easily available it is widely used throughout the world to fulfill the basic needs of humans in developed countries as well as in developing countries (Ghafoor *et al.*, 2010). But there are also chances of some infectious diseases caused by multi drug resistant bacteria among poultry animals which may cause harmful effects to humans who use infected meat (Kemmet *et al.*, 2013). To avoid such problems as well as to increase the growth of poultry animals many kind of antibiotics are used widely by poultry industry. Several types of antibiotics are used for this purpose but long term use of such antibiotics may also cause risks in consumers because of less information about the drug withdrawal time and misuse of such drugs as a result of which residual deposition may occur in muscle and tissues of meat which is main cause of side effects in persons taking meat. That's why it is essential to properly know about the drug withdrawal time and the maximum residual limits of the drugs that are used either as growth promoters or to treat the infectious diseases that would be helpful to inhibit the formation of residues in concerning animal tissues (Lee *et al.*, 2012).

Polymyxin E belongs to class of polypeptides having broad spectrum activity against gram negative bacteria used to treat the diseases caused by multi drug resistant bacteria but its long term use may also cause the resistance and residue formation in layers meat so MRL should be

determined to ensure the safety of chicken meat after drug use. For this purpose different techniques like microbiological methods and chromatographic techniques are used to determine MRL (Hakeemzadegan *et al.*, 2014). This study was done in Faisalabad, Pakistan as it is agricultural city and Polymyxin E is used in different poultry farm. Purpose of this study is to determine the polymyxin E residues in layer's liver, muscle and egg samples by using HPLC combined with UV detector. 15 poultry farms were selected for this purpose and liver, muscle and egg samples were collected from each farm. 15 samples were collected from each farm, stored properly at suitable temperature and analyzed by HPLC to check the presence of residues in respective samples and the results obtained were subjected to ANOVA. Here out of 225 samples of liver, muscle and eggs, 172 samples had polymyxin E residues called as antibiotic positive samples and 53 samples had no residues called as antibiotic negative samples. Table 4.1 presents the data about positive and negative samples. Withdrawal times had also been determined and the data obtained by this study is also in line by the study conducted by Choosakoonkriang *et al.*, 2013 that showed the presence of polymyxin E residues in muscle, liver and egg samples of chicken where it had been used in feed promote the growth of chicken. Furthermore it has also been accomplished from the fig 4.1, 4.2 and 4.3 that 77%, 80% and 72% layer's muscle, liver and egg samples were positive respectively whereas 23%, 20% and 28% were negative respectively. Similarly fig 4.4 depicted that 67% of overall

samples showed results above maximum residual limits whereas 33% were below MRL. Maximum residual limit given by FDA and European commission for polymyxin E is 150µg/kg for liver and muscle samples of layers and 300µg/kg for egg samples of layers (Seri *et al.*, 2013). Mean concentrations of polymyxin E residues in muscle samples have been depicted in table 4.3 that ranged from 101.6 to 704.8µg/kg. Similarly table 4.4 showed that mean concentration in liver samples ranging from 911.6 to 3573.8µg/kg and table 4.5 depicted the concentrations in egg samples ranging from 14 to 202.6µg /kg. Final data showed that liver had higher concentrations as compared to muscle and egg samples. The reason for high concentrations in liver is that it is metabolizing organ and mostly drugs are metabolized by liver. Moreover liver is also highly perfused organ than muscles and eggs so drug is present in more concentrations in it. The concentrations in egg samples compared with the study conducted by Rouduat, 2011 in which residual depletion of polymyxin E was observed in laying hens. That study showed that orally no residual concentrations were observed in eggs as compared to IV administration and in that study it was concluded that concentrations were present in yolk rather than albumin. Lipid soluble drugs have ability to cross the tissues of the body like liver, lungs, skin, kidney and muscles and because of more half-lives these may result in the formation of residues which are ultimate harmful responses in humans. Other reason of residue formation is improper information about drug withdrawal times (Seri *et al.*, 2013). For the Extraction of polymyxin E, ethyl acetate and buffer saline has been used this was also confirmed through previous studies. N-hexane was also used (Choosakoonkriang *et al.*, 2013). High performance liquid chromatography was used for the purpose of analysis associated with C18 column, flow rate was maintained at 1.0ml/min at the wavelength of 215nm. 20µL of samples was taken into HPLC vials and samples were subjected to analysis to determine residues (Zhao *et al.*, 2016). Polymyxin E has been used as growth promoter as well to treat diseases, its use

is safe but misuse or prolonged use may lead to the adverse effects so for all type of veterinary medicines it should be important to know about the maximum residual limits given by FDA and European commission to confirm that residue present in edible products should not exceed that limit to avoid any kind of harmful effects in consumers. It should also be noted that drug has to be eliminated before slaughtering of animals. Final data of this study showed that residues of polymyxin E were more than MRLs concluding that this meat is not suitable to consume so it is essential to slaughter the animals at least after five to seven days of drug administration to provoke drug elimination from the body of animals.

Conclusion

Concentration of polymyxin E residues in liver and muscle samples are above the maximum residual limits than those given in literature. Mean concentrations of polymyxin E in muscle samples ranged from 101.6 to 704.8µg/kg, in liver samples ranging from 911.6 to 3573.8µg/kg and in egg samples ranging from 14 to 202.6µg /kg. The concentration presented in literature for polymyxin E in muscle and liver is 150µg/kg and that in egg is 300µg/kg. Results of this study also depicted that liver samples have more concentrations than other egg and muscle samples concluding that liver is a metabolizing organ.

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