ENZYME-LINKED IMMUNOSORBENT ASSAY FOR SCREENING ANTBIOTIC RESIDUES IN CHICKEN AND GOAT MEATS

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Abstract - This study aimed to detect residue levels of tetracycline, streptomycin and sulfamethazine in chicken and goat meat samples. The Enzyme-Linked Immunosorbent Assay (ELISA) was used to detect residues in 128 meat samples. In chicken meat samples, tetracycline, streptomycin and sulfamethazine levels were 52.75 ppb kg^{-1}; 105.00ppb kg^{-1}, and 7.89ppb kg^{-1}, respectively. In goat meat samples, tetracycline, streptomycin and sulfamethazine levels were 53.62 ppb kg^{-1}; 17.81 ppb kg^{-1} and 3.19ppb kg^{-1}, respectively. In general, levels of these residues were below the international allowable levels set by the German Residue Control Plan (GRCP) and the EU. However, this study indicated that meat and meat products available in the local markets contained various levels of residues of antibiotics and hormones. Although levels of these residues did not exceed the maximum allowable limits, they still poise a human health hazard.

I. INTRODUCTION

Veterinary drugs and growth promotants are used extensively in food-producing animal for the treatment and prevention of diseases improving feed efficiency and promoting growth (1, 2). These agents are reported to increase protein deposition and decrease fat content in meat (3, 4). Meat and meat products therefore, could become a health hazard if they contain chemical contaminants. Due to the demand for increasing meat production, various types of antibiotics and synthetic as well as natural hormones were used. Residues of these substances may increase human risk from antibiotic-resistant bacteria (5,6,7). Resurreccion and Galvez (8) reported that 77% of consumers considered veterinary drug residues in meats to be an extreme health concern. These perceptions are supported by popular media (9). Some anabolic agents possess estrogenic properties, while others are androgens, which interfere with natural human physiological functions. There has been an increasing awareness about widespread drug therapy and the possibility of deposition of residues in meat and consequent potential human health hazards. Many growth promoters are now classified as carcinogenic, toxic and allergenic. Some of which may interfere with the natural physiological functions of humans. Therefore, detection of these residues in various meat products intended for human consumption is very important for consumer's safety. This study was aimed to use ELISA to detect the concentration levels of residues of the tetracycline, streptomycin and sulfamethazine in meat.

II. MATERIALS AND METHODS

One hundred and twenty-eight meat samples representing broiler chicken and goat were randomly collected from local markets. Five samples (each 10–20 g) were dissected from each sample using scalpel blades and placed in individual, labeled plastic bags. Samples were then stored at -80°C until analysis. The samples were randomly allocated to one of three residual assays, namely tetracycline, streptomycin and sulfamethazine. The ELISA is based on an antigen-antibody interaction and is highly specific for particular residues (7). ELISA kits were obtained from R-Biopharm AG, Darmstadt, Germany and stored at 4ºC.

Meat sample (5-10 g) was mixed with 10–25 mL of special buffer and shakes for 30 min, then homogenized and centrifuged at 3,000–4,000 rpm for 10 min at 15°C ad then dried using rotary evaporator, then re-dissolved with solvent. Five milliliters of the filtrate was purified with RIDA C18 column and the supernatant stored at -20°C. Four hundred and

fifty microliters of sample dilution buffer were added to 50 mL of eluate or supernatant of each of the meat samples in glass vials. Fifty microliters of each diluted standard solution and each diluted sample of antibody solution was added to each of the 96 wells. Fifty microliters of each diluted standard solution were added to separate duplicate wells and 50 mL of the antibody solution were added to each well. The plates were then incubated for 2 h at room temperature after mixed by rocking the plate. Fifty microliters of each substrate and chromogen were added to each well (after washed and dried) and then carefully mixed by manually rocking the plate, and were incubated for 30 min at room temperature in the dark. Finally, 100 mL of the stop reagent were added to each well and cautiously mixed by manually rocking the plate. The absorbance was measured at 405 nm or 450 nm against an air blank. The absorbance of the color was read within 60 min after the addition of the stop solution in a Multiskan Spectrophotometer using Ascent Version 2.6 for Multiskan software (Model MultiSkan 355, Thermo Lab systems, Helsinki, Finland). The concentration of the antibiotic in ppb kg^{-1} that corresponds to the absorbance of each sample was read from the calibration curve. The concentration from the calibration graph was multiplied by the dilution factor divided by mass of meat. Analyses were carried out in duplicates. The data were subjected to analysis of variance using the General Linear Model (SAS). Significant differences between mean residual levels of antibiotics agents were assessed using the least significant difference procedure at $P < 0.05$.

II. RESULTS AND DISCUSSION

Radioimmunoassay is considered as one of the most sensitive and accurate method to detect antibiotic residues in meat (10). The quantity of antibiotic and hormonal residues in the broiler meat samples are presented in Table 1. Broiler chicken meat contained various concentrates of various agents. Although, the residual levels of antibiotics were within the acceptable limits of German Residue Control Plan (GRCP), it may pose human health hazards as allergic reactions or by the development of drug resistance bacteria. Shao *et al*. (11) indicated that exposure to certain chemical agents, even at low level, may lead to a potential risk to human. The residual tetracycline levels (ppb kg^{-1}) detected (37.55–67.15: mean 52.75) in the present study is within allowable limits (100) set by the EU's law of drugs. This indicates that the tetracycline was used at least once during the chicken's lifetime for treatment or as a growth promoter.

Table 1 Mean, standard deviation (SD), minimum and maximum residual levels (ppb kg^{-1}) of antibiotic residues detected in 128 meat samples of broiler chickens.

Agent	Mean	SD	Min.	Max	AL[1]
Tetracycline	52.75	4.95	37.55	67.15	100
Streptomycin	105.00	32.73	33.12	176.88	500
Sulfamethazine	7.89	3.23	0.18	15.60	100

[1] AL: Acceptable Limit; Maximum international acceptable level (European Union and National Residue Control Plan, Germany). The minimum detection limit for tetracycline (<0.10 ppb), streptomycin (<0.25 ppb) and sulfamethazine (<0.05 ppb).

The risks of tetracycline residues in poultry products include toxic and allergic reactions and also disturbances of consumer's intestinal flora (12). The range of residual streptomycin levels (33.12-176.88; average 105.00 ppb kg^{-1}) in poultry meat samples indicated that birds have been treated with the drug and probably was not allowed adequate withdrawal period. The low level of sulfamethazine in broiler meat may probably be because it is virtually used in all food animal species, not only to treat and prevent the disease but also to improve the weight gain at sub-therapeutic levels (1). However, their use requires extreme care and good husbandry to prevent illegal residues. Only granulated formulations of sulfamethazine should be used in feed medication. The electrostatic properties of powdered sulfamethazine, which is not approved for feed use, increase the risk of residues. In the U.S.A., the limit for residues of sulfamethazine in uncooked tissues is 0.1 ppm, and the withdrawal period is 15 days (13). Consequently, its use has been prohibited for the treatment of animals destined for food production.

Goat meat samples in the present study contained some levels of residues of all antibiotic agents tested (Table 2). The exception was the sulphamethazine, which

was minimal (<1 ppb). In most cases, levels of residues were less than the international levels set by the EU. The range of tetracycline concentrations detected in the current study (48.34-58.89 ppb kg^{-1}) is below the allowable international levels. Bogialli et al. (14) stated the concentrations of tetracycline in bovine, swine and poultry muscles ranged between 1 and 9 ng/g. However, the current study indicated that most of the animals have been treated with the drug and probably not allowed an adequate withdrawal period. Streptomycin levels in the current study ranged between 0-35.88 ppb kg^{-1} with a mean of 17.81 ppb kg^{-1} which is very low according to EU MRL. Sulfonamides are widely used as feed additives for calves, pigs and poultry. They are also used for treating intestinal infections and other systemic diseases. Levels of sulphonamides in goat meat were negligible because the range between 0-35.88 ppb kg^{-1}.

Table 2 Mean, standard deviation (SD), minimum and maximum residual levels (ppb kg^{-1}) of antibiotic residues detected in 40 meat samples of imported and local sheep and goats.

Agent	Mean	SD	Min.	Max	AL[1]
Tetracycline	53.62	3.567	48.34	58.89	100
Streptomycin	17.81	5.76	00.00	35.88	500
Sulphamethazine	3.19	1.08	00.00	6.37	100

[1] AL: Acceptable Limit, Maximum international acceptable level (European Union and German Residue Control Plan). The minimum detection limit for tetracycline (<0.10 ppb), streptomycin (<0.25 ppb) and sulfamethazine (<0.05 ppb).

Occurrence of antibiotics agent residues in meats of various species is of great alarm and concern to consumers. There are numerous reports that the use of antibiotics agents may lead to a presence of their residues in meat and it is for this reason that statutory authorities commonly require “withdrawal periods” prior to slaughter (15). Antibiotic agent residues may cause hypersensitivity reactions, or contribute to the development of bacterial resistance in humans (16). According to Bass (17), the use of antibiotics as feed additives do not constitute an appreciable risk for the consumers of meat because of their low absorption rate and extensive biotransformation to microbiologically inactive and less toxic metabolites. However, the development of bacterial resistance is a major problem resulting from antimicrobial feed additives. In the current study, generally levels of the antibiotics residues were below the international levels set by the Germany Residue Control Plan and EU.

IV. CONCLUSION

This study indicated that chicken, sheep and goats meats sold in Omani market contain residues of antibiotics and anabolic agents. Although antibiotic levels were generally within allowable limits, hormone levels are above the allowable limits. Both may pose a human health hazard as they may cause allergic reactions or produce drug-tolerant bacteria

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