

Residues of Antibiotics Anabolic Steroids Pesticides in Assorted Broiler Chicken Meat and Meat Products Available in Omani Market

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Abstract

Analyses of 90 fresh, frozen, processed broiler chicken meat and meat products available in Omani markets revealed no violative residues of Antibiotics (Streptomycin, Tetracycline, Sulfamethazine, Sulfamethoxazole), antibacterial (Triclosan), pesticides (2,4 D, DDE/DDT, Alachlor, Organophosphate), melamine, macrolide, β -Lactam, Chloramphenicol, Sulphur drugs, and Gentamicin residues. There were three violative residues of anabolic steroids (Progesterone, Testosterone, and Estrogen) in broiler meat products. The residues of anabolic steroids were above the maximum residual limits (MRLs). However, the trace of their chemical compounds in broiler chicken meat products may have adverse effects on human health.

Keywords: Meat and Meat Products; Chemical Contaminants; Pesticides; Antibiotic; Hormones; Melamine

Introduction

Broiler chicken meat production represents one of the major food industries in Oman, which led to an increase in broiler meat consumption and made it very popular among the Omani population. It is difficult to predict how the industry in Oman will develop, but, no doubt, attention will be focused on meat safety. Broiler chickens are reared intensively to provide a high-quality protein to meet the Omani protein demand. Intensive rearing of broiler chickens is one way of rapidly increasing animal protein supplies for rapidly increasing populations, with intensive rearing conditions with high stock densities in broiler chicken houses that have provided ideal conditions for manifestation and transmission of parasitic and viral diseases (Piatkowska *et al.* 2012). These diseases are not only influencing broiler growth [1,2], with increase disease loads, the use of veterinary medicines, antiparasitic, or antibiotic drugs and feed additives has become vital to prevent or eliminate diseases and enhance production [1,3-6]. However, one of the risks of using of drugs is that they might be accumulated in the tissues and organs of treated broiler as residues and eventually become part of the food pyramid [3-5,7]. Antibiotics are administered parenterally or intravenously, topically, and orally [8-10].

Broiler chicken meat is a rich and continuous source of valuable nutrients and plays a vital role in human life diets therefore; emphasis should be given to its safety for human consumption. Humans around the world are exposed to many chemical contaminants throughout their lifetime. Over past 50 years, more than 80K chemicals have been synthesized, consumed, protruded and renounced in the environment, which livestock may have accessed through feed and water, and then accumulated in their bodies and products [11]. Less frequent sources of livestock's exposure to chemicals include accidental spills, improper waste disposal and areas contaminated from past use of products that are persistent in the environment [12].

The awareness that chemical and biological contaminants may occur along the food chain has led to the establishment of a maximum residue limits in meat products. The persistence nature of some of the chemical contaminants led to their accumulation in broiler tissues and subsequently causes human dietary exposure to these contaminants through consumption of broiler meat products [13]. Though violative level of contaminants is relatively uncommon, a low violation rate even remains an important public health consideration [14].

Scientific evidence suggests that even such low dose, but long-term exposure can cause serious health hazards to human health such as cancer, immune system disturbances and disruption of hormonal functions [15-18]. Residues of various chemicals and biological compounds including pesticides have been found in meat and meat products [19-23] at different levels. Children and developing fetuses are generally at greatest risk from exposure to different chemicals. A great number of these cases can be attributed to contaminants found in food. More than 90% of human exposure to harmful materials is because of use of food items including meat products which are contaminated. Studies suggest an increase in risk in brain cancer, and germ cell tumors associated with paternal occupational exposure during pregnancy [14].

The overwhelming majority of all food poisoning in the world originates from contaminated livestock products. Oman is an open market for various types of food items originally from all around the world. Therefore, there is a growing awareness and concern about the safety and consumption of meat products on human health. Many contaminants such as antibiotics or anabolic agents and antimicrobial drugs, which are used as growth-promoting substances, are banned in many countries. However, they are still found in meat and products in various parts of the world. This is mainly due to its role as promoting substances to increase muscle development or increased water retention and thus obtaining economic benefits. The routine administration of veterinary drugs to farm animals has contributed to the emergence of called "superbugs". Residues of these substances may remain in animal products and may pose a considerable threat to consumers inducing antibiotic-resistance, allergies, risk of cancer and gastrointestinal ailments.

Objective of the Study

The objective of this study was to evaluate three methods to determine the residues of antibiotic (streptomycin, tetracycline, Sulfamethazine and sulfamethoxazole), antibacterial (triclosan), hormone (Testosterone, trenbolone and Estrogen), pesticide (2,4 D, DDE/DDT, Alachlor and Organophosphate) and melamine in broiler chicken meat and meat products in the Omani market.

Materials and Methods

Ninety broiler chicken meat samples (three replicate each) represented fresh, frozen, mince, sausages, burgers and mortadella of local, regional and international brands were collected from major supermarkets in Muscat region. The samples were kept in plastic bags, labeled and transferred in cool box to the laboratory for analysis. The broiler meat product samples were tested using Enzyme Linked Immunosorbent Assay (ELISA), High Performance Liquid Chromatography (HPLC), CHARM II apparatus and Gas Chromatography apparatus (GC) techniques following the extraction methods and procedures as described by manufacturers. ELISA kits have shown good performance for the analysis of contaminants residues in meat [22,24,25]. The ELISA kits were purchased from abraxia, Pennsylvania and kept at 4°C until the time to use. The CHARM II technology is a new technique that is a rapid, comprehensive and semi-quantitative testing system capable of detecting residual compounds. The use of Charm II along with HPLC separation provides an excellent method for the

detection and identification of individual contaminant residues in animal tissue. HPTC allows the qualitative and quantitative detection of multi-residues in meat and meat products [26,27]. Gas Chromatography apparatus was also used to determine contaminant residues in broiler meat samples.

Results and Discussion

Although the levels of streptomycin, tetracycline, sulfamethazine, and sulfamethoxazole in broiler meat and meat products were below the Maximum Residue Limits (MRLs) (Table 1-6), their hazards for human health still exist. The risks of antibiotic residues in broiler products include toxic and allergic reactions, disturbances of consumer’s intestinal flora, and also the development of drug-tolerant bacteria [28]. Residues of streptomycin levels (ppb) of broiler meat samples ranged between 0.95 to 1.01, 1.06 to 1.07, 1.04 to 1.70, 1.10 to 1.14, 1.09 to 1.23 and 1.08 to 1.11 (ppb) for frozen, fresh, minced, sausage, burger and mortadella meat samples, respectively. The overall mean of the antibiotics residues in the current study were well below the MRLs (European Union standards for meat products) but indicated that chickens have been treated with the veterinary drug and probably were not allowed adequate withdrawal period. Similarly, Nigitung [29] found that meat broiler sold on the traditional markets, itinerant traders, and supermarkets in the city of Makassar safely consumed by people because there are no residues of antibiotics in meat broiler. The mean residual sulfamethazine levels were 0.74, 0.64, 0.71, 0.69, 0.63 and 0.57 ppb. The maximum residual level detected in chicken sausage from brand 1 was 0.85 ppb, which is below the MRLs (100 ppb). The sulfamethoxazole concentrations in the present study were also below the MRLs for meat. In contrast to the present study, the ELIA technique was used by [25] to detect residues in 128 broiler chicken breast muscles and found broiler meat products contained various levels of residues of several antibiotic and anabolic agents with some of the residues were above MRLs.

		Brand								
		1	2	3	4	5	6	7	8	9
Streptomycin	HPLC ¹	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA ²	0.96	0.96	0.98	0.96	0.96	1.01	0.95	0.99	0.98
Tetracycline	HPLC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.66	0.59	0.12	0.73	0.49	0.74	0.54	0.49	0.48
Sulfamethazine	HPLC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.79	0.80	0.79	0.72	0.78	0.62	0.77	0.67	0.71
Sulfamethoxazole	HPLC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.05	0.05	0.07	0.05	0.05	0.05	0.07	0.07	0.07
Macrolide	CHARM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
β-Lactam	CHARM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chloramphenicol	CHARM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sulphur Drugs	CHARM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gentamicin	CHARM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Testosterone	HPLC	1.02	1.27	1.11	1.56	1.09	0.71	1.36	1.18	1.31
	ELISA	0.66	0.75	0.64	0.93	0.95	0.79	0.85	0.65	0.75
Trenbolone	HPLC	0.22	0.43	0.28	0.59	0.60	0.41	0.49	0.14	0.26
	ELISA	0.38	0.35	0.35	0.22	0.18	0.34	0.25	0.36	0.34
Estrogen	HPLC	0.98	1.55	0.79	2.11	1.59	1.59	1.87	0.96	1.23
	ELISA	0.57	0.52	0.59	0.44	0.41	0.52	0.43	0.54	0.54

Triclosan	HPLC	0.36	0.39	0.33	0.45	0.60	0.56	0.54	0.44	0.55
	ELISA	0.30	0.38	0.19	0.54	0.64	0.34	0.22	0.14	0.30
Melamine	HPLC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.38	0.34	0.32	0.32	0.38	0.33	0.35	0.26	0.26
2,4, D	GC ³	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.63	0.31	0.48	0.43	0.33	0.13	0.36	0.55	0.34
DDE/DDT	GC	0.005	0.00	0.002	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.80	0.89	0.89	0.85	0.88	0.78	0.91	0.89	0.87
Alachlor	GC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.61	0.34	0.43	0.41	0.67	0.43	0.15	0.35	0.46
Organophosphate	GC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 1: Levels of accumulated Antibiotics (streptomycin, tetracycline, sulfamethazine, sulfamethoxazole), antibacterial (triclosan), hormones (testosterone, trenbolone, estrogen), pesticides (2,4 D, DDE/DDT, Alachlor, Organophosphate), melamine, macrolide, β -Lactam, chloramphenicol, Sulphur drugs, and gentamicin residues (ppb) in frozen broiler chicken meat from nine brands available in Omani market using four different techniques.

¹HPLC: High Performance Liquid Chromatography. ²ELISA: ELISA: Enzyme Linked Immuno Sorbent Assay.

³GC: Gas Chromatograph-Mass Spectrometer.

The veterinary drug residues in broiler products can potentially be transmitted to humans via consumption of contaminated edible tissues and may lead to several pathological implications that are considered as major health issues. Broiler chicken meat products contaminated with drug residues may pose serious public health hazards in the form of antibiotic-resistant bacteria formation, allergic manifestations, or alteration of useful microflora of digestive tract to no microflora and/or harmful or non-healthy microflora [30]. For instance, residual β -lactams, including cephalosporin and penicillin, have been reported to cause dermatitis, cutaneous eruptions, anaphylaxis, and gastrointestinal symptoms in humans via ingestion of contaminated poultry products [31].

The residual levels of antibiotics in meat products intended for human consumption have been reported to cause poor development of fetuses, staining of teeth in young children, gastrointestinal disorders, pro-inflammatory and autoimmunity effects and carcinogenicity [9,32-34]. Moreover, consume broiler meat contaminated with nitrofurans metabolites as residues may produce toxic, mutagenic, or carcinogenic side effects and may transmit antibiotic resistance among human microflora [35,36].

Triclosan, found in products, is one of the most prevalent antibacterial compounds. It has adverse health effects, including an impact on the relative thyroid hormone. The triclosan residues (ppb) detected in the current study in frozen, fresh, minced, sausages, burgers and mortadella of broiler meat chicken were below the MRLs. Low concentration of triclosan in broiler meat and meat products may be because of washing hands and utensils with antibacterial soap before handling the products.

Pesticides have been used for decades for disease vector control. However, recently there has been a rapid increase in the number of pesticides used in agriculture in many countries. The 2,4-D is an herbicide and a plant regulator in pasture and crop plants. The residues of 2,4-D detected in various products of broiler meat samples in the present study were also below the MRLs (70 ppb). Even in small residues, the 2,4-D may lead to reduce sperm motility and viability following occupational exposure [37]. Organochlorine pesticides are widely used by farmers because of their effectiveness and their broad-spectrum activity. Organochlorine pesticides such as DDT are also

	Brand 1			Brand 2		
	Method			Method		
	HPLC ¹	CHARM	ELISA ²	HPLC	CHARM	ELISA
Streptomycin	0.00	0.00	1.06	0.00	0.00	1.07
Tetracycline	0.00	0.00	0.50	0.00	0.00	0.48
Sulfamethazine	0.00	-----	0.64	0.00	-----	0.63
Sulfamethoxazole	0.00	-----	0.07	0.00	-----	0.07
Triclosan	0.45	-----	0.52	0.38	-----	0.58
Testosterone	1.62	-----	0.85	0.51	-----	0.55
Trenbolone	0.37	-----	0.27	0.28	-----	0.47
Estrogen	1.84	-----	0.50	1.58	-----	0.57
2,4 D	0.00	-----	0.47	0.00	-----	0.53
DDE/DDT	0.00	-----	0.93	0.00	-----	0.92
Alachlor	0.00	-----	0.12	0.00	-----	0.24
Organophosphate	0.00	-----	0.00	-----	-----	0.00
Melamine	0.00	-----	0.30	0.00	-----	0.28
Macrolide	-----	0.00	-----	-----	0.00	-----
β-Lactam	-----	0.00	-----	-----	0.00	-----
Chloramphenicol	-----	0.00	-----	-----	0.00	-----
Sulpha Drugs	-----	0.00	-----	-----	0.00	-----
Gentamicin	-----	0.00	-----	-----	0.00	-----

Table 2: Levels of accumulated Antibiotics (streptomycin, tetracycline, sulfamethazine, sulfamethoxazole), antibacterial (triclosan), hormones (testosterone, trenbolone, estrogen), pesticides (2,4 D, DDE/DDT, Alachlor, Organophosphate), melamine, macrolide, β-Lactam, chloramphenicol, Sulphur drugs, and gentamicin residues (ppb) in broiler chicken fresh broiler chicken meat using four different techniques.

¹HPLC: High Performance Liquid Chromatography, ²ELISA: Enzyme Linked Immuno Sorbent Assay.

employed to control ectoparasites of farm animals [38]. The residues of DDE/DDT levels detected in frozen, fresh, minced meat, sausages, burgers, and mortadella were below the MRLs. Potential mechanisms of small residues of DDT action on human are genotoxicity and endocrine disruption and pre-puberty exposure increases the risk of breast cancer [39]. Alachlor is a herbicide from the chloroacetanilide family [40]. Use of alachlor as herbicide has been banned in the European Union, but still it has been used as illegal herbicides in many countries. The Environmental Protection Agency in USA has set the MRLs Goal for Alachlor at zero to prevent long-term effects. The MRLs for food has been set at two parts per billion (2 ppb). The Environmental Protection Agency has described the following long-term effects of consuming contaminated meat with alachlor, slight skin and eye irritation, at lifetime exposure to levels above the MRLs, potential damage to liver, kidney, spleen, the lining of nose and eyelids, and cancer. Levels of alachlor in broiler meat products were low compared to the 2ppb specified as maximum level. Analytical results of organophosphate obtained in the current study were zero, which means the poultry products were free from this compound.

The present results revealed that broiler meat and meat products slightly contaminated with melamine in frozen, fresh, minced meat, sausages, burgers and mortadella samples. Although, the small detected values (below the MRLs) of melamine in broiler meat products, it can lead to the formation of insoluble compounds that can precipitate in the kidneys leading to renal failure. Toxicity in mammals occurs

	Brand 1			Brand 2			Brand 3		
	Method			Method			Method		
	HPLC ¹	CHARM ²	ELISA ³	HPLC	CHARM	ELISA	HPLC	CHARM	ELISA
Streptomycin	0.00	0.00	1.04	0.00	0.00	1.72	0.00	0.00	1.70
Tetracycline	0.00	0.00	1.07	0.00	0.00	1.02	0.00	0.00	1.14
Sulfamethazine	0.00	-----	0.70	0.00	-----	0.91	0.00	-----	0.81
Sulfamethoxazole	0.00	-----	0.01	0.00	-----	0.01	0.00	-----	0.01
Triclosan	0.45	-----	0.72	0.36	-----	0.69	0.51	-----	0.70
Testosterone	0.48	-----	0.22	0.81	-----	0.23	0.95	-----	0.23
Trenbolone	0.45	-----	0.41	0.86	-----	0.35	0.71	-----	0.19
Estrogen	1.49	-----	0.55	2.59	-----	0.45	2.44	-----	0.38
2,4 D	0.00	-----	0.28	0.00	-----	0.29	0.00	-----	0.35
DDE/DDT	0.00	-----	0.88	0.00	-----	0.79	0.00	-----	0.87
Alachlor	0.00	-----	0.15	0.00	-----	0.16	0.00	-----	0.17
Organophosphate	0.00	-----	0.00	0.00	-----	-----	0.00	-----	0.00
Melamine	0.00	-----	0.29	0.00	-----	0.22	0.00	-----	0.23
Macrolide	-----	0.00	-----	-----	0.00	-----	-----	0.00	-----
β-Lactam	-----	0.00	-----	-----	0.00	-----	-----	0.00	-----
Chloramphenicol	-----	0.00	-----	-----	0.00	-----	-----	0.00	-----
Sulphur Drugs	-----	0.00	-----	-----	0.00	-----	-----	0.00	-----
Gentamicin	-----	0.00	-----	-----	0.00	-----	-----	0.00	-----

Table 3: Levels of accumulated Antibiotics (streptomycin, tetracycline, sulfamethazine, sulfamethoxazole), antibacterial (triclosan), hormones (testosterone, trenbolone, estrogen), pesticides (2,4 D, DDE/DDT, Alachlor, Organophosphate), melamine, macrolide, β-Lactam, chloramphenicol, Sulphur drugs, and gentamicin residues (ppb) in broiler chicken minced meat using four different techniques.

¹HPLC: High Performance Liquid Chromatography. ²CHARM, CHARM II, ³ELISA: Enzyme Linked Immuno Sorbent Assay.

because of the ability of melamine and Cyanuric acid to self-assemble and form a hydrogen-bonded bimolecular network. Melamine is 66% nitrogen by mass and if added to any matrix will artificially increase the protein content of the matrix [41].

Therefore, melamine has been added to poultry feed ingredients to increase their apparent crude protein value and subsequently their monetary value [42]. Cyanuric acid along with ammeline and ammelide are structural analogs of melamine [43] and all belong to the class of chemicals known as s-triazines [44].

The residues of testosterone and estrogen agents in broiler meat and meat products were above the MRLs (Table 1-6). The residues of the estrogen and some of the testosterone agents in the current study were above the MRLs in broiler chicken meat products. Anabolic compounds are frequently used for boosting growth and to increase deposition of proteins in the muscle mass of broiler chickens. The residual trenbolone levels (ppb) detected in the present study were much below the detectable limits (10 ppb) set by the Germany Residue Control Plan. Anabolic steroid residues and their metabolites in broiler meat may cause adverse toxic effects on consumers' health. The residual testosterone levels (ppb) detected in poultry meat products (0.71 - 1.56 HPLC; 0.66 - 0.95 ELISA) in brands of frozen broiler meat chicken samples, which were above the detectable limit (0.10) set by the European Union. The range of residual estrogen (ppb) detected

		Brand ¹					
		1	2	3	4	5	6
Streptomycin	HPLC ³	0.00	0.00	0.00	0.00	0.00	0.00
	CHARM	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA ⁴	1.10	1.11	1.13	1.14	1.13	1.13
Tetracycline	HPLC	0.00	0.00	0.00	0.00	0.00	0.00
	CHARM	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	1.15	1.15	1.00	1.14	0.90	1.11
Sulfamethazine	HPLC	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.85	0.00	0.00	0.68	0.52	0.69
Sulfamethoxazole	HPLC	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.03	0.01	0.03	0.04	0.04	0.02
Triclosan	HPLC	0.55	0.63	0.57	0.60	0.51	0.63
	ELISA	0.67	0.73	0.69	0.66	0.61	0.67
Melamine	HPLC	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.34	0.38	0.28	0.31	0.26	0.25
Testosterone	HPLC	0.69	1.68	1.89	1.91	1.90	1.79
	ELISA	0.34	0.38	0.28	0.31	0.27	0.25
Trenbolone	HPLC	0.443	0.66	0.87	0.75	0.87	0.45
	ELISA	0.31	0.24	0.25	0.10	0.35	0.43
Estrogen	HPLC	1.97	1.94	2.54	2.52	2.43	1.75
	ELISA	0.55	0.40	0.34	0.32	0.38	0.60
Pesticides 2,4 D	GC	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.31	0.29	0.32	0.27	0.19	0.24
DDE/DDT	GC	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.88	0.87	0.87	0.89	0.88	0.87
Alachlor	GC	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.40	0.42	0.37	0.43	0.46	0.40
Organophosphate	GC	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.00	0.00	0.00	0.00	0.00	0.00
Macrolide	CHARM	0.00	Posit.	0.00	0.00	0.00	0.00
β-Lactam	CHARM	0.00	0.00	0.00	0.00	0.00	0.00
Chloramphenicol	CHARM	0.00	0.00	0.00	0.00	0.00	0.00
Sulpha Drugs	CHARM	0.00	0.00	0.00	0.00	0.00	0.00
Gentamicin	CHARM	0.00	0.00	0.00	0.00	0.00	0.00

Table 4: Levels of accumulated Antibiotics (streptomycin, tetracycline, sulfamethazine, sulfamethoxazole), antibacterial (triclosan), hormones (testosterone, trenbolone, estrogen), pesticides (2,4 D, DDE/DDT, Alachlor, Organophosphate), melamine, macrolide, β-Lactam, chloramphenicol, sulpha drugs, and gentamicin residues (ppb) in broiler chicken sausages using four different techniques.

¹HPLC: High Performance Liquid Chromatography. ²ELISA: ELISA: Enzyme Linked Immuno Sorbent Assay,

³GC: gas chromatograph-mass spectrometer. CHGARM; CHARM II.

in the present study was 0.79 to 2.11 ppb for HPLC method and 0.41 to 059 ppb for ELISA method, which above the MRL (0.10) set by the European Union. In agreement with current study, [25] reported that the anabolic steroids in broiler meat products were above the MRLs. According to Salem [45], estrogen treatment result in its accumulation in different tissues and organs of livestock, even if the treatment was stopped for 4 weeks and its meat boiled for 1.5h. Among the three anabolic agents (testosterone, trenbolone and estrogen) studied in the present study, testosterone and estrogen residues in fresh of broiler chicken meat samples of two major brands (Brand 1 and Brand 2) in the country (Table 2) were above the acceptable level by both techniques (HPLC and ELISA). The fresh meat samples form brand 1 contained 0.85 ppb (HPLC) and 1.62 ppb (ELISA) while the residues of the same contaminants were 0.55 ppb (HPLC) and 0.51 ppb (ELISA) for samples from Brand 2. In both brands and using two different techniques indicated that the presented data should be considered as an indicator of the seriousness of the usage of the testosterone and estrogen agents in broiler production system. Estrogen regulates most metabolic, behavioral and morphological requirements essential in reproduction of the female sex of livestock [46,47] while testosterone increase growth rate of broilers. Residues of anabolic steroids are the most common in broiler meat products as are directly exposed to the birds [14]. But this could be avoided, if used properly with sufficient withdrawal period of times. Approximately, 42% of all veterinary drugs used world-wide are used as feed additives, 19% are used as anti-infective, 13% as parasiticides, 11% are used as biological and 15% represent other pharmaceuticals [14]. Several other factors have contributed to the problem of the residue such as poor treatment records or failure to identify the animals and result from the use of a drug in same manner that is consistent with the labeling. This occurs primarily through not observing label of withdrawal times as well as extra-label use of drugs. Residues of anabolic steroids in brands 1, 3 and 3 minced meat samples available in local market were above MRLs (Table 3). The results of the current study revealed that broiler chicken gave anabolic agents within a short period before being slaughtered without appropriate withdrawal period from the drug. High level of estrogen or testosterone hormone in the population diets is may be carcinogenic. The results indicated that small variation in anabolic agents in minced meat between the three brands available in local market.

Broiler chicken sausage formulations from six brands available in local market differed slightly in testosterone, trenbolone and estrogen residues (Table 4). Similar to other chicken products, the concentrations of testosterone and estrogen hormones were higher than MRLs.

		Burger Brand							
		1	2	3	4	5	6	7	8
Streptomycin	HPLC ¹	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CHARM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA ²	1.23	1.11	1.19	1.17	1.14	1.18	1.20	1.09
Tetracycline	HPLC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CHARM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.44	0.10	0.16	0.55	0.18	0.12	0.61	0.41
Sulfamethazine	HPLC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.63	0.59	0.65	0.68	0.64	0.63	0.65	0.57
Sulfamethoxazole	HPLC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.06
Triclosan	HPLC	0.44	0.51	0.50	0.48	0.35	0.22	0.41	0.35
	ELISA	0.32	0.60	0.61	0.58	0.49	0.14	0.57	0.42
Melamine	HPLC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.19	0.23	0.20	0.10	0.22	0.18	0.17	0.25
Testosterone	HPLC	0.96	0.99	1.75	1.18	1.42	1.26	1.09	1.65
	ELISA	0.90	0.69	0.77	0.82	0.80	0.79	0.67	0.87
Trenbolone	HPLC	0.13	ND	0.11	0.28	0.42	0.53	0.39	0.63
	ELISA	0.19	0.55	0.52	0.40	0.20	0.13	0.15	0.08
Estrogen	HPLC	1.54	1.66	2.22	1.97	1.73	1.90	1.79	2.34
	ELISA	0.43	0.45	0.47	0.45	0.43	0.42	0.41	0.33

Pesticide 2,4 D	GC ³	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.42	0.48	0.63	0.50	0.45	0.30	0.19	0.42
DDE/DDT	GC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.92	0.90	0.88	0.91	0.86	0.87	0.90	0.93
Alachlor	GC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.23	0.19	0.65	0.58	0.39	0.38	0.22	0.40
Organophosphate/ Carbonate	GC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ELISA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Macrolide	CHARM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
β-Lactam	CHARM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chloramphenicol	CHARM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sulpha Drugs	CHARM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gentamicin	CHARM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 5: Levels of accumulated antibiotics (streptomycin, tetracycline, sulfamethazine, sulfamethoxazole), antibacterial (triclosan), hormones (testosterone, trenbolone, estrogen), pesticides (2,4 D, DDE/DDT, alachlor, organophosphate), melamine, macrolide, β-Lactam, chloramphenicol, sulpha drugs, and gentamicin residues (ppb) in broiler chicken burger used 4 different methods.

¹HPLC: High Performance Liquid Chromatography. ²ELISA: ELISA: Enzyme Linked Immuno Sorbent Assay, ³GC: Gas Chromatograph-Mass Spectrometer. CHGARM; CHARM II.

	Brand 1			Brand 2		
	Method			Method		
	HPLC ¹	CHARM	ELISA ²	HPLC	CHARM	ELISA
Streptomycin	0.00	0.00	1.11	0.00	0.00	1.08
Tetracycline	0.00	0.00	0.47	0.00	0.00	0.42
Sulfamethazine	0.00	0.00	0.55	0.00	0.00	0.59
Sulfamethoxazole	0.00	0.00	0.06	0.00	0.00	0.07
Triclosan	0.16	-----	0.00	0.25	-----	0.32
Testosterone	1.55	-----	0.83	1.65	-----	0.84
Trenbolone	0.39	-----	0.22	0.46	-----	0.20
Estrogen	1.75	-----	0.45	1.97	-----	0.41
2,4 D	0.00	-----	0.51	0.00	-----	0.41
DDE/DDT	0.00	-----	0.85	0.00	-----	0.90
Alachlor	0.00	-----	0.42	0.00	-----	0.46
Organophosphate	0.00	-----	0.00	0.00	-----	0.00
Melamine	0.00	-----	0.29	0.00	-----	0.33
Macrolide	-----	0.00	-----	-----	0.00	-----
β-Lactam	-----	0.00	-----	-----	0.00	-----
Chloramphenicol	-----	0.00	-----	-----	0.00	-----
Sulpha Drugs	-----	0.00	-----	-----	0.00	-----
Gentamicin	-----	0.00	-----	-----	0.00	-----

Table 6: Levels of accumulated Antibiotics (streptomycin, tetracycline, sulfamethazine, sulfamethoxazole), antibacterial (triclosan), hormones (testosterone, trenbolone, estrogen), pesticides (2,4 D, DDE/DDT, Alachlor, Organophosphate), melamine, macrolide, β-Lactam, chloramphenicol, Sulphur drugs, and gentamicin residues (ppb) in broiler chicken mortadella samples used three different techniques.

¹HPLC: High Performance Liquid Chromatography. ²ELISA: Enzyme Linked Immuno Sorbent Assay.

Residues of anabolic steroids in eight broiler chicken burger brands sold in local market are presented in table 5. The range of testosterone (0.96 to 1.75 ppb: HPLC) and estrogen (0.00 to 0.63 ppb: ELISA) were above MRLs. Consumers may be exposed to high level of anabolic agents by consumption of commercial broiler chicken burgers available in local market. Health effects of concern for human exposure to anabolic agents are cancer, reproductive effects and other endocrine disruption outcomes.

Residues of three anabolic steroids in brand 1 and brand 2 mortadella broiler chicken available in local market are presented in table 6. The brand 1 and 2 mortadella samples contained 0.83 and 0.84 ppb (ELISA) and 1.55 and 1.64 ppb (HPLC), respectively. For estrogen residues, the levels were 0.45 and 0.41 ppb (ELISA) and 1.75 and 1.97 ppb (HPLC), respectively. Both anabolic agents (testosterone and estrogen) residues were above MRLs. The anabolic effect exerted by certain veterinary and chemical drugs, having hormonal or thyrostatic action as well as β -agonists, promotes an improvement in the feed conversion efficiency and an increase in the lean-to fat ratio.

Conclusion

This study showed that several antibiotics residues in broiler meat products were small and below the MRLs. Widespread use of antibiotics in livestock without allowing withdrawal periods may lead to accumulation of small amounts in the broiler meat products. Residues of anabolic steroids in broiler meat products were found and their levels were above the MRLs. Anabolic steroid residues and their metabolites in broiler meat products may cause adverse toxic or carcinogenic effects on consumers' health. The levels of antibacterial, melamine and pesticides in broiler meat products were below the MRLs, but the trace of their chemical compounds may have adverse effects on human health. Therefore, local people consumers are exposed to variety dietary levels of these contaminants. The available information regarding chemical and biological residues in broiler meat products in Oman is very little. Therefore, the design and implementation of appropriate epidemiological studies and their integration with monitoring of samples from broiler meat products would be a major step in assessing the risk of chemical residues in broiler meat products and controlling and eliminating them. Moreover, it was found that High Performance Liquid Chromatography (HPL) Immunosorbent and CHARMII are adequate instruments for the detection and screening of various contaminants in broiler meat and meat products because they are automated and computer-controlled.

Bibliography

1. Chapman HD and Jeffers TK. "Vaccination of Chickens Against Coccidiosis Ameliorates Drug Resistance in Commercial Poultry Production". *International Journal of Parasitology: Drugs and Drug Resistance* 4 (2014): 214-217.
2. Bera AK, *et al.* "Evaluation of Economic Losses Due to Coccidiosis in Poultry Industry in India". *Agriculture Economic Research* 23 (2010): 91-96.
3. Goetting V, *et al.* "Pharmacokinetics of Veterinary Drugs in Laying Hens and Residues in Eggs: A Review of the Literature". *Journal of Veterinary Pharmacology and Therapeutics* 34 (2011): 521-556.
4. Rokka M., *et al.* "Trace Level Determination of Polyether Ionophores In Feed". *BioMed Research International* (2013): 1-12.
5. Seri HI. "Introduction to Veterinary Drug Residues: Hazards and Risks". The National Medicinal and Poisons Board (2013).
6. Ezenduka EV, *et al.* "Rapid Detection of Antimicrobial Residues in Poultry: A Consequence of Non-Prudent Use of Antimicrobials". *Health* 6 (2014): 149-152.
7. Kempe M and Verachttert B. "Cartridges with Molecularly Imprinted Recognition Elements for Antibiotic Residues Monitoring in Milk Cream. Pure and Applied Biochemistry". *Lunds Universitét Centre for Chemistry and Chemical Engineering* (2000): 1-10.
8. Marshall BM and Levy SB. "Food Animals and Antimicrobials: Impacts on Human Health". *Clinical Microbiology Reviews* 24 (2011): 718-733.

9. Lawal JR., *et al.* "Antibiotic Residues in Edible Poultry Tissues and Products in Nigeria: A Potential Public Health Hazard". *International Journal of Animal and Veterinary Advances* 7 (2015): 55-61.
10. Adel M., *et al.* "Antibiotics and Malachite Green Residues in Farmed Rainbow Trout (*Oncorhynchus Mykiss*) from the Iranian Markets: A Risk Assessment". *International Journal of Food Properties* 4 (2016): 837-846.
11. MacLachlan DJ and Bhula R. "Estimating the residue transfer of pesticides in animal feedstuffs to livestock tissues, milk and eggs: a review". *Australian Journal of Experimental Agriculture* 48 (2008): 589-598.
12. Robertson ID., *et al.* "The sources of pesticide contamination in Queensland livestock". *Australian Veterinary journal* 67 (1990): 152-153.
13. Jadhav VJ and Waskar VS. "Public Health Implications of Pesticide Residues in Meat". *Veterinary World* 4 (2011):178-182.
14. Biswas AK., *et al.* "Food safety concerns of pesticides, veterinary drug residues and mycotoxins and meat products". *Asian Journal of Animal Science* 4 (2010): 46-55.
15. Horrigan L. "How sustainable agriculture can address the environmental and human health harms of industrial agriculture". *Environmental Health Perspectives* 110 (2002): 445-456.
16. Vincenzo Russo M., *et al.*, "Determination of organophosphorus pesticide residues in human tissues by capillary gas chromatography-negative chemical ionization mass spectrometry analysis". *Journal of Chromatography B* 780 (2002): 431-441.
17. Castillom M., *et al.* "Pesticide residue analysis in animal origin food: Procedure proposal and evaluation for lipophilic pesticides". Open access book "Pesticides - Resent trends in pesticides residues assay", publisher in Tech, Chapter 3 (2012).
18. Dimitrova RT., *et al.* "Development of analytical method for determination of organochlorine pesticides residues in meat by GC-ECD". *Revue de Médecine Vétérinaire* 169 (2018): 77-86.
19. Hernandez LM., *et al.* "Organochlorine pollutants in meats and cow's milk from Madrid (Spain)". *Bulletin of Environmental Contamination Toxicology* 52 (1994): 246-253.
20. Herrera A., *et al.* "Organochlorine pesticide residues in Spanish meat products and meat of different species". *Journal of Food Protection* 57 (1994): 441-444.
21. Gallo P., *et al.* "Monitoraggio dei residui di pesticidi organoclorurati ed organofosforati in alimenti di origine animale: risultati e considerazioni relative agli anni 1990-1994". *Alimentaria* 35 (1996): 253-257.
22. Lee MH., *et al.* "Public health risks: chemical and antibiotic residues: Review". *Asian Australasian Journal of Animal Science* 14 (2001): 402-413.
23. Chung SWC and Chen BLS. "Determination of organochlorine pesticide residues in fatty foods: A critical review on the analytical methods and their testing capabilities". *Journal of Chromatographic A* 1218 (2011): 5555- 5567.
24. Wang S., *et al.* "Analysis of sulphonamides residues in edible animal products: A review". *Food Additives Contaminants* 23 (2006): 362-384.
25. Kadim IT., *et al.* "Enzyme-Linked Immunosorbent Assay for screening antibiotic and hormones residues in broiler chicken meat in the Sultanate of Oman". *Journal of Muscle Foods* 21 (2010) 243-254.

26. De Brabender HF, *et al.* "Determination of thyrostatic drugs by HPTLC with confirmation by GC-MS". *Journal of Planar Chromatography* 5 (1992): 124-130.
27. Gaugain M and Abjean JP. "High-performance thin-layer chromatographic method for the fluorescence detection of three nitroimidazole residues in pork and poultry tissue". *Journal of Chromatographic A* 737 (1996): 343-346.
28. Berends BR, *et al.* "Human health hazards associated with the administration of antimicrobial to slaughter animals. Part 1: An assessment of the risks of residues of tetracycline in pork". *Veterinary Quarter* 23 (2001): 2-10.
29. Nigitung R. "Residual Antibiotic and Number of Bacteria Meat Broiler in Makassar" (2018): 29-30.
30. Mumtaz A, *et al.* "Rational Use of Drugs in Broiler Meat Production". *International Journal of Agriculture and Biology* 2 (2000): 269-272.
31. Paige JC, *et al.* "Public Health Impact on Drug Residues in Animal Tissues". *Veterinary and Human Toxicology* 9 (1997): 1-27.
32. Nisha AR. "Antibiotic Residues-A Global Health Hazard". *Veterinary World* 1 (2008): 375-377.
33. Idowu F, *et al.* "Antimicrobial Screening of Commercial Eggs and Determination of Tetracycline Residue Using Two Microbiological Methods". *International Journal of Poultry Sciences* 9 (2010): 959-962.
34. Palmieri MD, *et al.* "Antibiotic Treatments in Zootechnology and Effects Induced on the Food Chain of Domestic Species and Comparatively, the Human Species". *Nutrition Hospitalaria* 29.
35. Amiri HM, *et al.* "The Occurrence of Residues of Furazolidone Metabolite, 3-Amino-2-Oxazolidone, in Eggs Distributed in Mazandaran Province". *Scimetr* 2 (2014): e19353.
36. Khan GJ, *et al.* "The Frequent Use in Poultry and Its Consequences on Human Health". *The Professional Medical Journal* 22 (2015): 1-5.
37. Munro IC, *et al.* "Comprehensive, integrated Review and Evaluation of the scientific evidence relating to the safety of the herbicide 2,4-D". *Journal of the American College of Toxicology* 11 (1992): 559-664.
38. Ntow WJ, *et al.* "Farmer perceptions and pesticides use practices in vegetable production in Ghana". *Pest Management Science* 62 (2006): 356-365.
39. Cohn BA, *et al.* "DDT and breast cancer in young women: new data on the significance of age at exposure". *Environmental Health Perspectives* 115 (2007): 1406-1414.
40. Arnold P, *et al.* "Weed Control in Ullmann's Encyclopedia of Industrial Chemistry". *Wiley-VCH* 28 (2002): 165.
41. Yang HH, *et al.* "Molecularly imprinted polymer as SPE sorbent for selective extraction of melamine in dairy products". *Talanta* 80 (2009): 821-825.
42. WHO. Report from Expert Meeting to Review Toxicological Aspects of Melamine and Cyanuric Acid. (2008): 1-4.
43. Tyan YC, *et al.* "Melamine contamination". *Analytical and Bioanalytical Chemistry* 395 (2009): 729-735.
44. Wackett LP, *et al.* "Biodegradation of atrazine and related s-triazine compounds: from enzymes to field studies". *Applied Microbiology and Biotechnology* 58 (2002): 39-45.
45. Salem EG, *et al.* "Antibiotic and Hormone Residues in Chicken". Animal Production Research Institute (2003).

46. Meyer HHD. "Comparative aspects of estrogen biosynthesis and metabolism and in the endocrinological consequences in different animal species". *Handbook of Experimental Pharmacology* 135.11 (1999).
47. Reig M and Toldra F. "Veterinary drug residues in meat: Concerns and rapid methods for detection". *Science* 78 (2008): 60-67.

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