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DRUG AND HORMONAL RESIDUES IN BROILERS CHICKEN IN KINGDOM OF SAUDI ARABIA

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ABSTRACT :

During the period of 2001- 2005 the Laboratory of Toxicology, Drug and Hormonal Residues, Ministry of Agriculture in Saudi Arabia has analyzed 2366 chicken tissues and serum samples for drug residues included tetracycline, β-lactams, sulfonamides, macrolides, fluoroquinolones, aminoglicosides, nitrofurans and chloramphenicol and also for natural and synthetic hormones. Also, growth promoters included 17ß-estradiol, testesterone, progesterone, nortestesterone, diethylstilbestrol, zeranol, trenblone and β-agonist using CHARM Π, ELISA, HPLC, LC-MS and GC-MS were estimated. Chicken samples were collected periodically allover the year from broiler farms just before marketing from different regions in the kingdom.

Results showed that all analyzed samples were free from drug residues mentioned before except 293 samples were contains residues for tetracycline, β-lactams and sulfamethazine with a total ratio of 12.38%. All recorded drug residues in 2004 and 2005 were below the European maximum residue limit (MRL) in comparison with the results obtained in previous years (2001 to 2003). In 2002, residues of 17ß-estradiol have only been detected in 6 serum samples (5.36%) collected from one farm with average 137.33±91.4 ng/l and 12 chicken meat samples collected from two sources with average 2.15±1.43 and 13.75±3.26 ng/kg (11.1%).

INTRODUCTION:

Antibiotics mainly used for therapeutic or prophylactic in poultry industry especially broiler production. The antimicrobial drugs are also used as growth promotants; in lieu of growth hormones for at least two reasons, first, no growth hormones are approved for use in poultry production, second, hormones are ineffective in young birds because natural levels of hormones remain high for most of their relatively short production cycle (Kenneth, 2001). This use of drugs to food-producing animals requires not only consideration of effects on the animal but also for the consumers. Over use, misuse or failure to observe the withdrawal times of these drugs leave residues in edible tissues. These residues consist of the parent compound and/or compounds derived from the parent drug including metabolites which can lead to health problems for the consumers (Weber, 1979). This potential problems or hazard effects associated with antibiotic and hormonal residues can be classified in two broad categories; first, aesthetices consumers don't like the idea of foreign substances being present in food. The second problem is that of potential health risks

that includes:- birth defects, heart attacks and the ability to cause cancer either direct or indirect as furazolidone, chloramphenicol or some hormones (Young, 2001), increased microbial drug resistance, allergic reactions and sensitization to antimicrobials and drug toxicity (Kalid and Rehman, 2002), immunotoxicity, arterial fibrillation and other tachycardia and hormonal reproductive disrupting activity (Yamada *et al*, 2006). Drug toxicity may result in an immediate clinical reaction (allergy to penicillin's and sulfonamides), gradual development of an aplastic anaemia (in people sensitive to chloramphenicol), acquisition of an allergy or sensitivity to the drug or acquired resistance by human bacteria (Black, 1984 and Anjum, 2006).

To prevent unwanted drug residues from entering the human food chain, both the government authorities and the industries have established extensive control measures (Sternesjo and Johnsson 1998). In Sudia Arabia, Zaki and Al-Ghamdi (2002) reported that the misuse of antibiotics in the local poultry industry poses a serious health risk to the public and may complicate the treatment of human infections. The veterinary use of antimicrobial agents, especially those with dual animal and human applications, should be restricted with establishment of a government department concerned with food and drug safety. To avoid all these serious effects for drug and hormonal residues the Ministry of Agriculture implements a system to prohibit the distribution of animal foods origin that contain these residues specially if it is above MRL. In this paper a comp-rehensive survey was undertaken to determine the levels of drugs and hormones residues in broilers in the Kingdom of Saudi Arabia during the period from 2001-2005.

MATERIALS AND METHODS:

Sampling: Chicken samples (1958 breast muscle and serum samples) were collected periodically allover the year (2001-2005) from broiler farms just before marketing from different regions in the kingdom. Other samples (408 frozen and chilled chicken samples from local production) were collected from Riyadh markets. All these samples were send to the Laboratory of Toxicology, Drug and Hormonal Residues, Ministry of Agriculture in Riyadh-Saudi Arabia. Breast muscle and serum samples were stored at -20 ºC until examination.

Analysis: Meat samples were analyzed for detection of drug residues which includes tetracycline's, β-lactams, sulfonamides, macrolides, fluoroquinolones, aminoglycosides, nitrofurans, and chloramphenicol by using CHARM II (Charm LSC 7600, SN:LSC 593 Instrument), ELISA (Model ELx800^{TM},BIO-TEK Instrument), HPLC (Waters 1525 Binary HPLC pump & Waters 2487 Dual λ Absorbance Detector) and LC-MS (Waters micromass ZQ serial # LAA1375 (Juhel-Gaugain, *et a.l*, 1999 and Dreassi, *et al*, 2000).

For CHARM II: samples were extracted and measured according to manual with kits (using MSU extraction buffer, incubate at 80±2ºC) CHARM II kits (TIIHT-100 for tetracycline's, PIIT-100 for β-lactams, SMIIHT-100 for sulfa; EIIT-100 for macrolides, GIIHT-100 for gentamicin and neomycin, STIIHT-100 for streptomycin, AIITHT-100 for chloramphenicol and LF-QUIN-100K for enrofloxacin).

For ELISA: samples were extracted and measured quantitatively using ELISA kits in duplicate according to manual enclosed in kits. ELISA kits (r-biopharm Art. No.: R3501 for tetracycline, R3001 for sulfamethazine, R3101 for streptomycin, R1501 for chloramphenicol,

R3111 for enrofloxacin, R3701 for nitrofuran AOZ and R3711 for nitrofuran AMOZ) and (RANDOX, BL3448&BL1371 for β-lactams).

For HPLC: drugs were extracted from muscle samples with acetonitrile, fat was removed by liquid-liquid extraction with isooctane, the extract was cleaned on C18 cartridges then injected in HPLC with UV detector using C18 column (150 × 4mm) (Juhel-Gaugain, *et al*, 1999).

For LC-MS: drugs were extracted from muscle samples with acetonitrile-methanol (95:5,v/v) and the extracts were delipidated with n-hexane saturated with acetonitrile. The extracts were evaporated, dissolved with methanol, analyzed by LC-MS with gradient elution on C18 column (Dreassi, *et al*., 2000).

Reference materials used for HPLC and LC-MS (Standard) were C17322500 for tetracycline, C16996500 for sulfamethazine, C16974900 for streptomycin and C14000200 for gentamycine. These standareds were obtained from Dr. Ehrenstorfer GmbH bD-86199 Augsburg, Germany.

ELISA tests for natural hormones (17ß-estradiol, testesterone, progesterone) to each serum samples were undertaken by using ELISA kits (r-biopharm Art. No.: R2301, R2401 and BRID 0402 kits respectively) in duplicate after extraction of 17ß-estradiol and testesterone by using tert-butylmethyl ether (TBME)/petroleum ether (Rattenberger and Matzke 1989). Samples for, progesterone detection were used directly. Samples with absorbance higher than that of the internal standard were considered to be negative and those with absorbance lower than that of the internal standard were considered as positive. The reading of microtitre plates was performed by Automated Microplate Reader Model ELx800^{TM} (BIO-TEK Instrument). Positive samples for17ß-estradiol was confirmed by using GC-MS after extraction according to the method reported by Rossum, *et al*., (2000). Detection of natural hormones in meat samples were performed by using HPLC-MS after extraction by using TBME/petroleum ether.

Synthetic hormones and growth promoters in serum and meat samples were extracted by TBME for 19-nortestesterone/trenblone, diethylstilbestrol, zeranol and trishydroxymethyl-aminomethane for β-agonist using zeranol immunoaffinity columns (cat. No. ZR 2420) and RIDA C18 (Art. No. R2002) column for purification. Detection of the synthetic hormones and growth promoters were performed using ELISA kits (RANDOX cat. No. NT 2105-TB2106; r-biopharm Art. No.: R2701; RANDOX Cat. No. ZR 2421 and r-biopharm Art. No.: R 1701 respectively). Positive samples were confirmed using GC-MS. The methods used had adequate sensitivity to measure residues comfortably below the levels of concern.

Statistical analysis was carried out according Snedecor and Cochran (1967).

RESULTES:

Results of the present survey (2001-2005) revealed that, all analyzed samples were free from drug residues except 293 samples (12.38%) which contain drug residues and 18 samples (0.76%) were above European MRL (table 1). Percentages of samples contain drug residues above European MRL were 6.25% in 2001, 5.36% in 2002, and 1.72% in 2003 and no residues were detected in samples of the years 2004 and 2005. On the other hand, the most prevalent drug residues above MRL (24 samples) were 15 sulfamethazine, 8 tetracycline and 1 streptomycin as shown in tables 1&3. In 2001, 5 samples of chilled chicken represented 3

local farms (6.25%) contain drug residues above European MRL for tetracycline (2 samples contained 160±2.83 ng/g), streptomycin (1 sample contained 1200ng/g) and sulfamethazine (2 samples contained 148.25±10.25 ng/g). While in 2002, 6 samples from one farm (5.36%) contained tetracycline with sulfamethazine above European MRL (121.08±9.17 and 115.13± 9.13 ng/g respectively). In 2003, there were 7 samples from 2 farms (1.72%) contained sulfamethazine above European MRL (125.13± 11.79 and 105.17±5.86 ng/g in 4 and 3 samples respectively) as shown in Tables 1 & 3 and Figures 1 & 2.

Table (1): Positive samples contain drug residues under/above European MRL during period 2001-2005

Years	Type of samples	Source of samples	Total No.	Positive samples		Positive samples under MRL		Positive samples above MRL	
				No.	%	No.	%	No.	%
2001	Tissues& serum	Different regions in K.S.A.	200	26	13.00%	26	13.00%	00	00%
	Chilled chicken	Riyadh markets	80	11	13.75%	6	7.50%	5	6.25%
2002	Tissues& serum	Different regions in K.S.A.	112	42	37.50%	36	32.14%	6	5.36%
	Chilled chicken	Riyadh markets	108	30	27.78%	30	27.78%	00	00%
2003	Tissues& serum	Different regions in K.S.A.	406	29	7.14%	22	5.42%	7	1.72%
	Frozen chicken	Riyadh markets	120	40	33.33%	40	33.33%	00	00%
2004	Tissues& serum	Different regions in K.S.A.	447	19	4.25%	19	4.25%	00	00%
	Chilled chicken	Riyadh markets	100	35	35.00%	35	35.00%	00	00%
2005	Tissues& serum	Different regions in K.S.A.	793	61	7.69%	61	7.69%	00	00%
Total			2366	293	12.38%	275	11.62%	18	0.76%

Table (2): Positive samples contain hormonal residues in chicken samples analyzed in the lab. During period 2001-2005

Years	Type of samples	Source of samples	Total No.	Positive samples (detectable)		Positive in limit		Positive above MRL	
				No.	%	No.	%	No.	%
2001	Tissues& serum	Different regions in K.S.A.	200	00	00%	00	00%	00	00%
	Chilled chicken	Riyadh markets	80	00	00%	00	00%	00	00%
2002	Tissues& serum	Different regions in K.S.A.	112	6	5.36%	6	5.36%	00	00%
	Chilled chicken	Riyadh markets	108	12	11.11%	12	11.11%	00	00%
2003	Tissues& serum	Different regions in K.S.A.	406	00	00%	00	00%	00	00%
	Frozen chicken	Riyadh markets	120	00	00%	00	00%	00	00%
2004	Tissues& serum	Different regions in K.S.A.	447	00	00%	00	00%	00	00%
	Chilled chicken	Riyadh markets	100	00	00%	00	00%	00	00%
2005	Tissues& serum	Different regions in	793	00	00%	00	00%	00	00%

		K.S.A.							
Total			2366	18	0.76%	18	0.76%	00	00%

Table (3): Amounts recorded for drug residues(ng/g) in poultry samples during period 2001-2005

Years	Total No.	Tetracycline		Betalactam		Sulfamethazine		Streptomycin		Gentamycin	
		No	mean ± SD	No	mean ± SD	No	mean±SD	No	mean±SD	No	mean±SD
2001	26 Tissues & serum	3 6 6 3*	65.33±2.02 64.83±4.71 80.17±10.8 40.33±4.04	2	45±4.24	6 3*	50.25±9.09 20.16±4.25	-	-	-	-
	11 Chilled poultry	2 2	80.25±6.01 160±2.83#	4	44.9±8.11	2	148.25±10.25#	1	1200 #	-	-
2002	42 Tissues & serum	6 6* 6* 6* 6* 6*	70.08±9.74 75.2±9.5 60.25±8.12 80.08±7 70.14±9.07 121.08±9.17#	6 6* 6* 6* 6* 6*	44.1±8.07 35.05±6.56 40.02±8.29 30.05±6.47 35.15±7.9 40±8.25	 6* 6*	 83±6.93 115.13±9.13#	-	-	-	-
	30 Chilled poultry	6 6 6	80.05±9.69 50.04±7.4 76.07±6.1	6 6	30±9.82 44.08±6.02	-	-	-	-	-	-
2003	29 Tissues & serum	3 4	35.33±6.66 40.13±10.56	3 2 2	40.17±8.58 50±8.49 25.25±5.3	8 4 3	50.06±10.13 125.13±11.79# 105.17 ±5.86#	--	-	-	-
	40 Frozen poultry	5 5 5 5 5	45.1±12.33 60.1±9.63 45±4.85 30.05±9.44 35.25±5.91	5 5 5	28.1±6.56 45.22±8.49 20±2	-	-	-	-	-	-
2004	19 Tissues & serum	9	45.6±8.87	4	35±4.83	6	70±8.72	-	-	-	-
	35 Chilled poultry	5 5 5	50.16±7.87 55.1±6.09 60.16±10.53	5 5	35.14±6.26 30.18±8.47	5	30.05±9.12	-	-	5	80±11
2005	61 Tissues & serum	6 6 6 6	30.03±6.5 45.05±7.65 35.15±4.16 45.08±11.77	2 3 6	25±2.12 35±7.94 25.05±7.77	2 2 8 6 8	85±8.49 65.25±5.3 55.6±8.24 65±6.2 85.08±7.84	-	-	-	-

N.B. European MRL in poultry meat for tetracycline and sulfamethazine are 100, betalactam, 50 and streptomycin 500 ng/gm .
*** The samples were contains more than one drug residues.**
 # recorded amount for drug residues were above European MRL .

Results of hormones and growth promoters revealed that neither synthetic hormones nor growth promoters tested have been detected during the period of 2001-2005 in 2366 analyzed samples. For the natural hormones analyses (17ß-estradiol, testesterone, progesterone) results do not detect any of the three hormones analyzed during 2001-2005 except 17ß-estradiol. In 2002, residues of 17ß-estradiol have only been detected in 6 serum samples (5.36%) collected from one farm with average 137.33±91.4 ng/I and 12 chicken meat samples collected from two sources with average 2.15±1.43 and 13.75±3.26 ng/kg (11.1% of samples analyzed in this year).

DISCUSSION:

Immunoassay method (CHARM II or ELISA) were used as a regulatory monitoring for drug residues for the large numbers of samples that reach to the laboratory then positive samples were confirmed by HPLC, GC-MS or LC-MS. Using these screening and confirmatory tests was supported by De Wasch *et al*, (1998) and Schneider and Donoghue, (2004). They mentioned that bioassay methods as microbiological inhibition and immunological method can be used as a screening method for examining large numbers of samples and positive samples should then confermed by a more extensive and accurate method as liquid chromatography-fluorescence-mass spectrometry.

Results of the present survey (2001-2005) revealed that, all analyzed samples were free from drug residues except 293 samples (12.38%) which contains drug residues and 18 samples (0.76%) were above European MRL (table 1). (N.B. European MRL for tetracycline, streptomycin and sulfamethazine in poultry meat are 100 ng/g (European Community, 1999).

Report of Food and Drug Administration (FDA) in Feb 1989, as a casual check, found 143 drugs and pesticide residues in meat and poultry. Forty-two are known to cause cancer or are suspected of causing cancer, whereas twenty cause birth defects and six cause mutations. Penicillin (including ampicillin), tetracycline (including chlortetracycline and oxytetracycline), sulfonamides (including sulfadimethoxine and sulfamethazine and sulfamethoxazole), neomycin, gentamicin, flunixin, streptomycin, arsenicals were considered as the most likely to be detected drugs in meat (Maneka Gandhi, 2006). Also Zaki and Al-Ghamdi (2002) mentioned that twenty-nine antimicrobial agents were identified as being available for poultry use in Sudia Arabia, of which 22 (75.9%) were important for the treatment of human infections. Enrofloxacin, oxytetracycline, ampicillin, neomycin, sulfamethoxazole, colistin, doxycycline and erythromycin were the most frequently used drugs. They added that the food-borne hypersensitivity reactions and the emergence of microbial resistance, as well as cross-resistance to the various groups of antibiotics in animals and its transfer to human pathogens, are well documented.

These results are lower than that obtained by Al-Ghamdi *et al*.,(2000) in Saudi Arabia when they undertaken a survey in the eastern province over a period of two years starting from January 1996, chicken muscle, liver and egg samples from 33 broiler and 5 layer farms. They can identified antibiotic-residue positive samples in the products of 23 broiler (69.7%) and 3 layer (60%) poultry farms. Out of them 87% and 100% of the antibiotic-residue positive broiler farms were positive for at least one tetracycline compound in raw muscle and liver respectively, while 73.9% and 95.5% were positive for 2 or more tetracyclines in these two tissues, respectively. Furthermore, 82.6% and 95.5% of the antibiotic-residue-positive farms had mean concentrations of at least one tetracycline compound in excess of the permissible MRL in raw muscle and liver, respectively. They confirmed the widespread misuse of tetracycline agents including multiple use of drugs belonging to the same pharmacological group and lack of implementation of recommended withdrawal times. This may be contributing to the high resistance rates to tetracyclines in both chicken and human microbial isolates observed in the region. They stresses the need for stricter regulations for the use of antimicrobial drugs in

the poultry industry as well as the inspection of chicken for drug residues prior to marketing. Also results of the present study revealed lower level than results reported by Zaki and Al-Ghamdi, (2000). They detected norfloxacin in 35.0% and 56.7% of raw antibiotic-residue-positive muscles and livers respectively. The norfloxacin-positive muscles and livers were respectively obtained from 11 (50.0%) and 14 (63.6%) of the 22 antibiotic-residue-positive farms. Toxicological studies indicated that tetracycline's was not mutagenic, carcinogenic, or teratogenic, but some toxic effects were observed at high doses with a no-effect of level 18 ng/g body weight/day (Ellin Doyle, 2006).

Estrogens govern reproductive functions in vertebrates, and are present in all animal tissues. Poultry probably contain similar amounts of estrogens as untreated cattle (Daxenberger *et al*., 2001). The use of growth-enhancing hormones (including steroids) in poultry has been illegal in the U.S., Canada, and Europe for decades. Though the practice apparently still exists in other parts of the world, this type of substance would be administered to poultry via feed additives (David, 2004).

Interpretation of our results for 17ß-estradiol hormone seems to be difficult because it is not generally possible to distinguish administered 17ß-estradiol from those produced naturally by the animal. However, the presence of 17ß-estradiol in poultry serum and meat detected in the samples considered to be a safe level. Hartmann *et al*., (1998) considered that 0.03-0.02 mg/kg 17ß-estradiol concentrations in chicken meat as a significant hormone concentration. The possible biological significance of very low levels of estradiol is neglected (Andersson and Skakkebaek 1999). In the USA, the FDA has established an acceptable level of exposure for estradiol in muscle as 120 ng/kg. These values represent parent hormone residue levels in uncooked meat that are considered unlikely to produce any physiological effects in individuals chronically ingesting animal tissues (Electronic Code of Federal Regulations, 2006).

An analysis of residues from anabolic agents found in commercially available meat and poultry was undertaken in Alexandria, Egypt by Sadek *et al*., 1998. The study showed that estradiol levels were much higher in meat of chicken obtained from private growers (0.521-1.3 ug/kg muscle) than samples purchased from government cooperative supermarkets (0.206-0.721 ug/kg muscle). It appears that some poultry growers in Egypt use hormones or hormone-like agents to improve the rate of growth. It is worth mentioning that feeding chickens with oral contraceptive steroids leads to the formation of high estrogen residues in muscle and liver in comparison to controls (Sadek *et al*., 1998). A broiler poultry chemical residue monitoring programme operated during 1999 by Keast, (1999). A total of 6330 analyses were performed on samples from 355 birds randomly selected throughout the year from poultry processing plants. The results revealed that no positive samples for stilbine, zeranol like compounds and 19 nortestesterone tested.

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متبقيات الأدوية والهرمونات في الدجاج اللاحم بالمملكة العربية السعودية

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يقوم مختبر السموم وبقايا الأدوية والهرمونات بالرياض التابع لإدارة المختبرات البيطرية بوزارة الزراعة بعمل تحاليل للعينات التي ترد للمختبر بصفة دورية، وذلك للتأكد من خلوها من المتبقيات الدوائية والهرمونية حيث تجمع هذه العينات من مشاريع الدجاج اللاحم في جميع أنحاء المملكة قبل التسويق مباشرة بمعرفة لجان متخصصة بمديرات الزراعة في هذه المناطق. خلال الفترة من 2001–2005 وصل للمختبر عدد 2366 عينة من أنسجة وأمصال الدجاج، وقام المختصون بالمختبر بالكشف عن المتبقيات الدوائية (التتراسيكلين– البيتالكتام– السلفوناميد– الميكروليد– الفلوركينولون– الأمينوجليكوسيد– النيتروفيوران– الكلورامفينيكول) ، وكذلك الكشف عن متبقيات الهرمونات الطبيعية والمصنعة ومنشطات النمو (17–ب–استراديول – التستستيرون– البروجستيرون– النور تستيستيرون– الداي إيثيل استلبوستيرول– الزيرانول– الترنبلون– البيتاأجونست) باستخدام أجهزة شارم والإليزا وكروماتوجرافيا الضغط العالي السائل وكروماتوجرافيا السائل مع مطياف الكتلة وكروماتوجرافيا الغاز مع مطياف الكتلة. وأظهرت نتائج هذه التحاليل خلو جميع العينات من متبقيات الأدوية والهرمونات سالفة الذكر عدا عدد 293 عينة وجد بها بقايا لكل من التتراسيكلين – البيتالكتام – السلفاميثازين – ستربتومايسين –جنتاميسين بنسبة إجمالية 12.38٪. جدير بالذكر نتائج فحص العينات خلال أعوام 2004 و 2005 أفضل من نتائج السنوات السابقة (2001 إلى 2003) لعدم وجود عينات تحوي بقايا أعلى من النسب المسموح بها دولياً .

وبالنسبة لنتائج تحليل العينات لمتبقيات الهرمونات الطبيعية والمصنعة ومنشطات النمو سالفة الذكر فقد أظهرت النتائج خلو العينات المذكورة من هذه المتبقيات عدا وجود هرمون 17–ب–استراديول في بعض عينات عام 2002م فقط. حيث وجد هرمون 17–ب–استراديول في عدد 6 عينات من المصل لإحدى المزارع بنسبة 5.36٪، ومعدل 137.33± 91.4 نانوجرام/لتر وعدد 12 عينة من أنسجة الدجاج بنسبة 11.1٪ من عدد 2 مزرعة ومعدل 5.15±1.43 و 13.75±3.26 نانوجرام/كجم واسترشاداً بالتقارير العلمية الصادرة من المنظمات العالمية لا تمثل هذه المستويات أي خطورة على صحة المستهلك.