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# DETERMINATION OF PESTICIDE AND ANTIBIOTIC RESIDUES IN MUSCLES OF SUDANESE CAMEL (*Camelus dromedarius*)

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## ABSTRACT

An effective analytical procedure was used for determination of organochlorine pesticides (Endrin, Aldrin, DDT, Endosulfan sulfate and heptachlor) and antibiotics (Tetracycline, Sulfonamides, Gentamycin and Cephalexin) in 12 Sudanese dromedary camel muscles. Forty-eight muscle samples from two age groups: group 1 (3-4 year old) and group 2 (6-7 year old) were collected. Meat samples from four muscles, i.e. longissimus thoracis (LT), semitendinosus (ST), semimembranosus (SM) and biceps femoris (BF) were extracted with acetonitrile and purified with acetonitrile-saturated n-hexane for removing impurities. After evaporation to dryness, the residue was passed through a Sep-Pak C18 cartridge for sample cleanup prior to Gas Chromatography coupled with various detectors such as Mass Spectrometer or electron capture detector (GC-ECD). Liquid Chromatography Mass Spectrometry (LC-MS) was also used to quantify of chemical concentrations in camel muscles. Pesticides residues in all camel muscles were below the Maximum Residual Limit (MRL). A Thin Layer Chromatographic (TLC) method was used to determine the residual of veterinary drugs and the results were confirmed by Liquid Chromatography-Mass Spectrometry (LCMSMS). With the exception of tetracycline, no antibiotic residues were detected in camel muscles. Tetracycline residues in some muscles was significantly higher than the MRL. Meat sample from group 1 had significantly ($P<0.05$) lower tetracycline residues than group 2 in LT (32.13 vs. 36.75 ppm), BF (34.35 vs. 36.94), ST (29.07 vs. 35.83) and SM (28.42 vs. 35.92). This study confirmed that Sudanese camel meat is free from organochlorine residues but tetracycline residues were accumulated in both age groups. Following medication treatment, a withdrawal period of two weeks should be practiced to avoid any hazard for human health.

**Key words:** Antibiotics, muscles, organochlorine pesticides, residues, sudanese camel

Livestock may have access to different chemical contaminates through feed and water and it may be accumulated in their products (MacLachlan and Bhula, 2008; Hamamoto *et al,* 2017). Synthetic pesticides are fat-soluble and rapidly absorbed to accumulate in the tissue of animals continuously exposed to them through spraying of the environment or feed contamination (Hansen and Lambert, 1987). Pesticides including hexachlorocyclohexane (HCH), chlorocyclodienes (aldrin,dieldrin, endrin, heptachlor and heptachlor epoxide), DDTs and the fungicide hexachlorobenzene (HCB) are widespread used in developing countries and extensive leading to serious public health cancer, immune system disturbances and disruption of hormonal functions and environmental problems (Blair and Zahm, 1993; Hamamoto *et al,* 2017; Vincenzo *et al,* 2002; Garcia and Gotah, 2017). Residues of pesticides have been found in meat and meat products at different levels (Hamamoto *et al,* 2017; Hernandez *et al,* 1991; Herrera *et al,* 1994; Gallo *et al,* 1996). More than 90% of human exposure to harmful materials is due to consumption of contaminated meat products (Bantobal and Jodral, 1995). Antibiotics are also widely prescribed as antimicrobial drugs for human and animals. It is estimated that 100–200 thousand tons of antibiotic substances are annually produced in the world (Kümmerer, 2003; Wise, 2002). The improper use of veterinary drugs could lead to drug residue in animal products. The residues of these substances or its metabolites in animal products may cause adverse effects on consumers' health (Shankar *et al,*

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2010). Consumer demand for safe food has resulted in the introduction of numerous laws and regulations designed to control environmental distribution of these potential food contaminants. Therefore, many countries strictly regulated the use of chemical in agriculture. Despite the longer life span of camel compared to cattle and sheep, lower levels of some of chemical contaminates were found in camel meat and meat products compared to cattle and sheep products (Sallam and Morshedy, 2008). However, effect of environmental chemical contaminants in different muscles residues at different age have not been investigated. The present study was aimed to determine the residues of Endrin, Aldrin, DDT, Endosulfan sulfate, Tetracycline, Sulfonamides, Gentamycin, Cephalexin and heptachlor in musdes, i.e. longissimus thoraces (LT), semitendinosus (ST), semimembranosus (SM) and biceps femoris (BF) muscles of two age groups (3-4 and 6-7 year-old) of Sudanese camels.

## Materials and Methods

### *Meat samples*

Forty-eight muscle samples from two age group, i.e. group 1 (3-4-year old) and group 2 (6-7 year old) were collected from 12 Sudanese camels slaughtered at Tambol slaughterhouse (yard) at Al-Butana State, Sudan. Animals were slaughtered after having been held in a lairage for 12 hrs and dressed following routine commercial slaughterhouse procedures. The LT, ST, SM and BF muscles were dissected from the left side of each carcass within 60 min of postmortem. Each individual muscle was trimmed of external fat, kept in zipped plastic bags and transported in insulated ice box and kept at -18$^{o}$C for 7 days at the Meat Science Lab, Faculty of Animal production, University of Khartoum, then transported to Meat Lab at the Department of Animal and Veterinary Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University. The samples were kept in a freezer at -18$^{o}$C until analysis.

### *Reagents*

Individual stock solutions of Organochlorine pesticides standards of Carbadox (CDX) 50 mg and penicillin, cephalexin, aminoglycoside, tetracycline and sulfanilamide (100 mg of each) purities of greater than 99% were prepared in acetonitrile. Whereas (CLP 100mg) was prepared in acetonitrile/ water (1/1, v/v) solution. Working standard solution for each antibiotic were diluted with acetonitrile (0.05 M sodium hydrogen phosphate (3/7, v/v)) to a series of concentrations ranging from 0.2 to 2.0 µg/mL.

### *Sample extraction and cleanup*

The methods used for extraction of Multi-residue (pesticides and antibiotic) carried out according to (Minkao *et al,* 2001). Concentration of pesticides in camel meat samples were determined used Gas Chromatography/ Mass spectrometer GC/ MS (Shimadzu GC-MS system QP2010 Ultra with GC-2010 Plus Advanced flow Controller (AFC). Concentration of antibiotics were determined using a thin Layer Chromatography and conformed by a Liquid Chromatography-Mass Spectrometry (LCMS/ MS) following the procedure described by Tajick and Shohreh (2006).

### *Pesticides*

Approximately 5 g meat sample from each muscle was homogenised with 20 ml acetonitrile in a 50 ml centrifugation tube using Ultra Turrax T25 homogeniser. Twenty ml of acetonitrile was added and vigorously shaken for 3 min. The mixture was filtered and the residues were mixed with another 50 ml of acetonitrile. The mixing and filtration steps were repeated more than once. The filtrated materials were combined and transferred into a separation funnel containing 30 ml of acetonitrile-saturated n-hexane and shaken vigorously for 5 min. The acetonitrile layer was collected into a concentration flask and evaporated to dryness at 40$^{o}$C using a rotary evaporator. The dry residue was reconstituted with 20 mL of (0.05 M sodium dihydrogen phosphate) and was introduced in a Sep-Pak C18 cartridge, which was pre-conditioned with 10 mL of methanol and 10 mL of 0.05 M sodium dihydrogen phosphate. The concentration flask was washed twice with 5 mL of sodium dihydrogen phosphate and then applied into the cartridge. The eluate was discarded and the flask was washed twice with 5 mL of methanol and the resulting solution was passed through the cartridge. The eluate was collected and evaporated to dryness at 40°C using a rotary evaporator.

The dry matter was reconstituted with 1 mL of acetonitrile /water (3/7, v/v) solution. After spiking 0.5 mL of acetonitrile-saturated n-hexane, the resulting solution was thoroughly mixed and then centrifuged at 3000 rpm for 5 min. The acetonitrile layer was collected and filtered through a membrane filter .45µl into vial and stored in a freezer at -20$^{o}$C until analysis.

### *Analytical determination*

GC-MS analysis of pesticides was performed on a Shimadzu GC-MS system QP2010 Ultra with

GC-2010 Plus Advanced Flow Controller (AFC). The GC/MS temperature set at 275°C in spilt less mode with a 10.6 psi pressure constant flow. The flow of He through a GC column is set at 1m l/min. The oven programme at 100°C for one min, ramp at 20°C column was set at 140°C, then ramp at 5°C/min until reached 280°C, then held for 8 minutes. Interface temperature of the GC to the MS was set at 250°C and the MS ion source was set at 200°C. The MS was operated in electron –ionization (EI) mode scan range 60-500 m/z. The GC/MS was calibrated with each new sample batch. Three calibration standards were run. The calibration range for GC/MS is 200 to 1000g/L.

### *Antibiotics*

Thin Layer Chromatography (TLC) was used to detect tetracycline, Sulfonamides, Gentamycin and Cephalexin residues following the procedure of Tajick and Shohreh (2006). Two g of meat from each muscle was homogenised with 5 ml phosphate buffer (pH 6.5). The protein was precipitated by adding 1 ml of trichloroacetic acid (30%). The solvent transferred to a 15 ml centrifuge tubes and centrifuged at 7000 rpm for 15 min. The supernatant was collected, then extracted by an equal volume of diethyl ether. The mixture was kept at room temperature for 10 min for separating layers; the mixture was separated from each other by using separating funnel. The upper oily layer was discarded and the bottom layer was collected. The steps were repeated from 5-8 times with diethyl ether and evaporated until dryness. The evaporated sample were reconstituted with 2 ml of mobile phase was done (methanol and acetone 1:1) and transferred into in a screw cap vial and kept in a refrigerator for antibiotic analysis.

Glass plates washed in acetone bath had 10 × 20 cm dimensions. For each plate 2 gm of Silica gel F256 (Merck, Germany) mixed in 5 ml DW and shacked thoroughly to produce fine paste. Clean glass plates coated with silica paste by TLC gel spreader system (Shandon, England) in 0.25 mm thickness. Plates activated in 120 C for two hours o (Boyer, 1993). Raw antibiotics (sulfaamidine, tetracycline, amoxicillin, ciprofloxacin and gentamicin) were prepared by dissolving of 0.1g of each material in 4ml methanol (Thangadu *et al,* 2002). Approximately, 50 µl of methanol dissolved antibiotic were applied at certain point on the line of the silica plates. The treated sample was transferred to TLC tank containing acetone-methanol (1:1) as mobile phase. After the solvent front retching to end of plates, chromatograms observed on UV light at 256 nm (Thangadu *et al,* 2002).

### *Statistical analysis*

The data were analysed by Statistical Analysis System Software (SAS, 1993), using General Linear Model procedure. The procedure was used to evaluate the concentrations of pesticides and antibiotics at two age groups across four muscle types. Least Significant Difference (LSD) test was used for mean separation.

## Results and Discussion

### *Pesticides*

The organochlorine residues (α-HCH, β-HCH, Heptachlor, hexachlorobenzene (HCB), Endosulfan, Endosulfansulfate, 1,1,1-Trichloro-2,2-bis (chlorophenyl) ethane (DDT), Aldrin, Endrin Dieldrin in camel meat and the influence of muscle types (*longissimus thoracic, Semitendinosus, semimembranosus* and *biceps femoris*) and the age of camel (3-4 and 6-7 year-old) are presented in Table 1. With the exception of Endrin residues, the analysis procedure used did not detect any pesticides residues in camel meat samples. Although the level of Endrine residues was below MRL value established by FAO/WHO (2006), camel meat samples from group 2 (6-7 year-old) had 0.034 ppb in *semitendinosus* muscle. This may probably be due to that the camel has the ability to evacuate organochlorine compounds from their body tissues more efficiently than other livestock. Similarly, Khalid *et al* (2007) found that Endrin residues in meat samples from camel, cattle and sheep were below the MRL. The same authors reported the mean values of the residual concentrations (ng/g wet weight) of DDTs in camel, cattle and sheep muscles were 13.9, 17.9 and 20.3, respectively, were below MRL. However, residues of pesticides have been found in meat and meat products in different species and at different levels (Hamamoto *et al,* 2017; Hernandez *et al,* 1991; Herrera *et al,* 1994; Gallo *et al,* 1996). An LC-MS/MS multiresidue method coupled with modified QuEChERS ( (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction method was used by Hamamoto *et al* (2017) for the investigation of eight pesticide residues: prallethrin (PR), resmethrin (RMT), imidacloprid (IMC), diflubenzuron (DFB), cyromazine (CYR), etofenprox (EFP), dinotefuran (DNT) and phthalthrin (PTLT). The mean concentration of the pesticides ranged between 74.7% and 113.5%, for bovine, swine and chicken muscle and liver tissue samples. Organochlorine pesticides

**Table 1.** Concentrations of pesticide residues (ppb) in s *longissimus thoraces* (LT), *biceps femoris* (BF), *semitendinosus* (ST) and *semimembranosus* (SM) muscles of Sudanese dromedary camels.

| Parameters | Muscle | | | | | | | | MRL[2] (mg/kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| | LD | | BF | | ST | | SM | | |
| | Age (years) | | | | | | | | |
| | 3-4 | 6-7 | 3-4 | 6-7 | 3-4 | 6-7 | 3-4 | 6-7 | |
| Organochlorine (ppb) | | | | | | | | | |
| α-HCH | ND | ND | ND | ND | ND | ND | ND | ND | 0.01 |
| β-HCH | ND | ND | ND | ND | ND | ND | ND | ND | 0.20 |
| Heptachlor | ND | ND | ND | ND | ND | ND | ND | ND | 0.01 |
| HCB | ND | ND | ND | ND | ND | ND | ND | ND | 0.10 |
| Endosulfan | ND | ND | ND | ND | ND | ND | ND | ND | 0.10 |
| Endosulfan sulfate | ND | ND | ND | ND | ND | ND | ND | ND | 0.10 |
| DDT | ND | ND | ND | ND | ND | ND | ND | ND | 0.05 |
| Aldrin | ND | ND | ND | ND | ND | ND | ND | ND | 0.01 |
| Endrin | ND | ND | ND | ND | ND | 0.034 | ND | ND | 0.10 |
| Dieldrin | ND | ND | ND | ND | ND | ND | ND | ND | 0.01 |

MRL*s of EU regulation guidelines (CE 396/2005) = Maximum Reside Limits. HCB =hexachlorobenzene. ND: Not detected

and in particular Aldrin, DDT, Endrin and Dieldrin are widely used by farmers as insecticides that act against a wide range of agricultural pests. In addition to organochlorine pesticides, polychlorinated biphenyls (PCBs) are other type of persistent organic pollutants that may contaminate meat (Garcia and Gotah, 2017). Although, PCBs were banned many years ago, their residues were still present in meat products because of their stability and lipid-soluble properties; these compounds have harmful effects on consumer health including carcinogenicity, neurotoxicity and developmental disorders in children. Moreover, potential mechanisms of Endrin action on humans at a toxic dose may lead within few hours to signs and symptoms of intoxication excitability and convulsions. Death may follow within 2–12 hr after exposure if appropriate treatment is not applied immediately. Public concern about the adverse environmental and human health impacts of organochlorine contaminated led to strict regulations on their use in developed country. Nonetheless, DDT and several other organochlorine pesticides are still being illegally used for agriculture and animal production programs in many developing countries and led to the contamination food stuffs, especially those having a high fat content such as meat and meat products which contributed to the higher dietary intakes of most of the organochlorine (Kannan *et al,* 1994). In Nigeria, 96% of bovine meat and organs contaminated with organochlorine chemicals (Osibanjo and Adeyeye, 1997), while 88% of meat and meat products contaminated within organochlorine in Spain (Herrera *et al,* 1996). In Canada, DDT was detected in 21% of analysed fat samples from different animals (Frank *et al,* 1990). The higher detection frequencies in DDT in developing countries could be due to illegal use of DDT for agriculture purposes.

## Antibiotic

With the exception of the tetracycline, there were no antibiotics residuals in the longissimus thoraces, semitendinosus, semimembranosus and biceps femoris muscle from the two age groups of Sudanese camel (Table 2). The results showed that tetracycline residues increased with increasing age of the camel from 3-4 to 6-7 year (Table 3). Although, meat samples from group 2 (6-7 year) had higher level of tetracycline residue than those from group 1 (3-4 year), the amount below the MRL values established by the EU law of drugs (European Commission 2004). The biceps femoris muscle had the highest tetracycline residues among all muscles (Fig 1). The acceptable maximum residues level (MRLs) for tetracycline as recommended by the joint FAO and WHO expert Committee on food Additives is 200,600 and 1200 mg/kg for muscles, liver and kidney, respectively. Antibiotics can act as growth promoters even though these substances can contribute to an increase in the human exposure to antibiotics, development of antibiotic-resistant pathogens and increased allergies due to its presence in foods (Reig and Toldrá, 2008; Mungroo and Neethirajan, 2014; Jalal *et al,* 2015; Garcia and Goitah, 2017).

**Table 2.** Levels of antibiotic residues (ppb) in longissimus thoraces (LT), semitendinosus (ST), semimembranosus (SM) and biceps femoris (BF) muscles of two different age groups of dromedary camel

| Parameters | Muscle[1] | | | | | | | | STM[1] | MRL |
|---|---|---|---|---|---|---|---|---|---|---|
| | LD | | BF | | ST | | SM | | | |
| | Age (year) | | | | | | | | | |
| | 3-4 | 6-7 | 3-4 | 6-7 | 3-4 | 6-7 | 3-4 | 6-7 | | |
| Tetracycline | | | | | | | | | | |
| TLC | 32.13$^{b}$ | 36.75$^{c}$ | 34.35$^{bc}$ | 36.94$^{c}$ | 29.07$^{a}$ | 35.83$^{c}$ | 28.42$^{a}$ | 35.92$^{c}$ | 4.78 | 100 |
| LCMS/MS | 8.34$^{a}$ | 46.33$^{b}$ | 15.42$^{ac}$ | 50.62$^{b}$ | 9.97$^{a}$ | 36.72$^{bc}$ | 9.67$^{a}$ | 32.18$^{abc}$ | 4.78 | |
| Sulfonamides | | | | | | | | | | |
| TLC | ND | ND | ND | ND | ND | ND | ND | ND | | 50 |
| LCMS/MS | ND | ND | ND | ND | ND | ND | ND | ND | | |
| Gentamycin | | | | | | | | | | |
| TLC | ND | ND | ND | ND | ND | ND | ND | ND | | 50 |
| LCMS/MS | ND | ND | ND | ND | ND | ND | ND | ND | | |
| Cephalexin | | | | | | | | | | |
| TLC | ND | ND | ND | ND | ND | ND | ND | ND | | 50 |
| LCMS/MS | ND | ND | ND | ND | ND | ND | ND | ND | | |

[1]STM: standard error of means. MRL (maximum residues limit: Tetracycline (ppb) part per billion. (TLC): thin layer chromatography and tetracyscline by (LCMS/MS): liquid chromatography-mass spectrometry. ND: not detected

**Table 3.** Effect of Sudanese camel age on tetracycline residue (ppb) by Thin Layer Chromatography (TLC) and Liquid Chromatography-Mass Spectrometry (LCMS/MS).

| Antibiotic (ppb) | Age (year) | | SEM[1] |
|---|---|---|---|
| | 3-4 | 6-7 | |
| TLC | 11.296a | 41.466b | 3.34 |
| LCMS/MS | 30.995b | 36.365a | 0.48 |

[1]SEM: Standard Error of Mean

Widespread use of antibiotics in livestock without withdrawal periods may probably led to accumulate drugs in animal products (Jalal *et al,* 2015; Garcia and Gotah, 2017). The present results indicated that antibiotics have been used at least once during the animal's lifetime for treatment of bacterial infections. However, the improper use of veterinary drugs may result in the presence of their residues in edible animal tissues, which can be toxic and dangerous for human health and potentially cause allergic reactions. Small residues of antibiotic in products consumed for long periods can lead to the spread of drug-resistant microorganisms (Shalaby *et al,* 2001; Masawat and Slater, 2007; Yu *et al,* 2011; Beyene, 2016). Health hazard concerns are raised on the antibacterial resistance in zoonotic enteropathogens (*Salmonella* spp., *Campylobacter* spp.), commensal bacteria (*Escherichia coli, Enterococci*) and bacterial pathogens of animals (*Pasteurella, Actinobacillus* spp.) (Di Stefano and Avellone, 2014). Monitoring of antibiotic residues is very important in controlling the safety of products for human consumption (Koesukwiwat *et al,* 2007). Antibiotic residues in animal products may be the cause of numerous health concerns in humans. These problems include toxic effects, transfer of antibiotic resistant bacteria to humans, immunopathological effects, carcinogenicity (sulphamethazine, oxytetracycline and furazolidone), mutagenicity, nephropathy (gentamicin), hepatotoxicity, reproductive disorders, bone marrow toxicity (chloramphenicol) and allergy (penicillin) (Paige *et al* 1997). Failure to observe the instructions for antibiotic use can lead to antibiotic residues entering animal-derived foods (Darwish *et al,* 2013). The significance of this contamination depends on the pharmacodynamics of the compound and the species (McEvoy, 2002). In Sudan, the most commonly used antibiotics by farmers are quinolones and tetracycline. The majority of farmers use antibiotics for prevention and control of disease; only 5% of farmers use antibiotics for livestock health maintenance (Eltayb *et al,* 2012). The risk of tetracycline residues in meat include toxic and allergic reaction and development of resistant strains of bacteria following the ingestion of sub-therapeutic doses of antimicrobials (Botsoglou and Fletuvris, 2001).

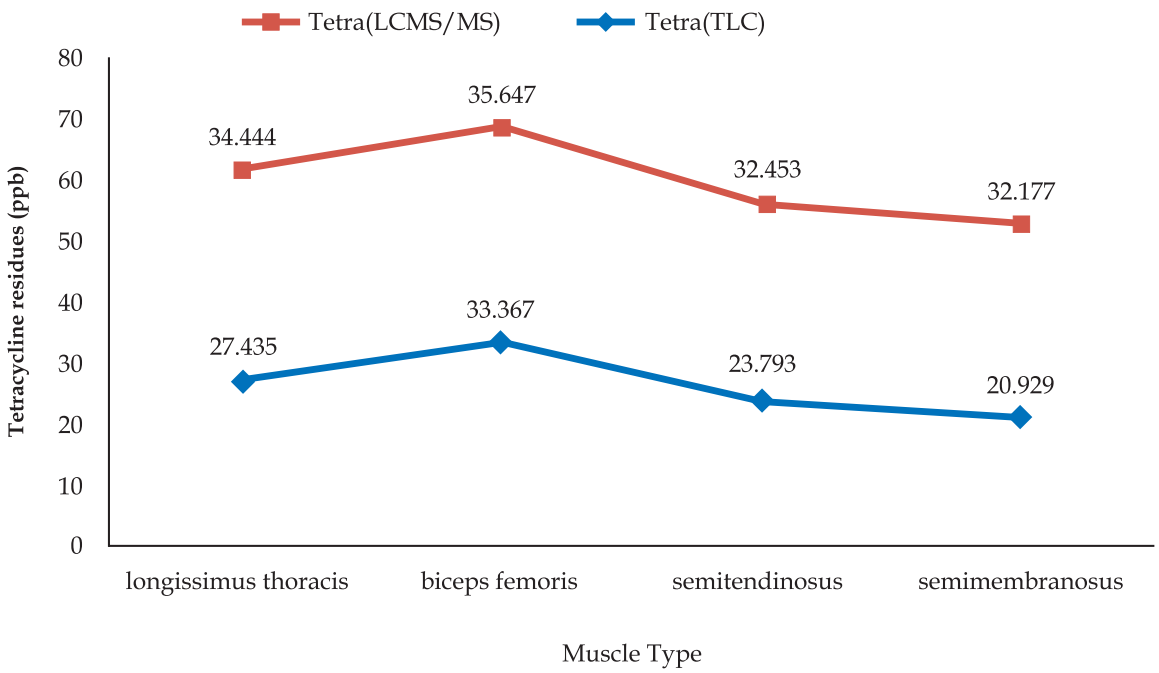


**Fig 1.** Tetracycline residues (ppb) in four muscles of Sudanese dromedary camel using (TLC) Thin Layer Chromatography (TLC) and Liquid Chromatography-Mass spectrometry (LCMS/MS).

## Conclusion

The Organochlorine pesticides including DDT, Endosulfan sulfate and heptachlor were not detected in Sudanese camel muscle samples but trace residue of Endrin found in semitendinosus muscle from group 2. With the exception of tetracycline, Sudanese camel muscles were free from residue of sulfonamides, gentamycin and cephalexin. Tetracycline residue increased significantly with increase camel age. This study conclude that Sudanese camel meat is free from most pesticides and antibiotics, therefore it can marketed as a safe meat for human consumption.

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