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Hormone residues in milk and meat products and their public health significance

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Abstract
India after the post-independence era has developed many strategies to overcome the long-standing food crisis problem to become a leading country of food grains production in world market. In recent years, increasing attention has been paid to chemical and hormonal residues like chlorinated pesticides, organophosphates, herbicides, exogenous and endogenous hormones that contaminate milk and meat. Seven hormone drugs (testosterone propionate, trenbolone acetate, estradiol, zeranol, progesterone, melengestrol acetate, and bovine somatotropin) are approved by the U.S. Food and Drug Administration for use in food animals. There is concern that these drugs or their biologically active metabolites may accumulate in edible tissues, potentially increasing the risk of exposure for consumers. The occurrence of hormonal residues in the milk of lactating cows is a matter of public health concern, since dairy products are widely consumed by infants, children and many adults throughout the world. Governments have responsibility for making regulations to protect consumers against harm arising from chemical in food.

Keywords: hormonal residues, milk, meat, public health

Introduction

India, after independence has to meet with most difficult challenge of overcoming the problem of long-standing food crisis owing to rapidly growing population. For attaining self-sufficiency in production Indian government played a crucial role on the agricultural as well as animal husbandry sector as both are interdependent with each other [1]. Animal husbandry constitutes backbone of Indian farming, where animals are used as a source of draft power as well as food in the form of milk, meat and eggs. India accommodates a huge livestock as 108.7 million buffaloes, 190.9 million cattle, 135.2 million goats, 65.1 million sheep, 10.3 million pigs and 729.2 million chickens [2]. India ranks first in milk production, accounting for 18.5 Per Cent of World Production [3] with a status of 155.5 million tonnes in the year 2015-16 with per capita availability of 337 gm/day [4]. India ranked 5th in the world with 2.08% contribution in global meat production [5]. Meat production from all sources in the beginning of twelve plan (2012-13) was 5.95 million tonnes which has been further increased to 7.0 million tonnes in 2015-16 [6].

With these impressive figures of India in worlds picturesque, India has evolved as a powerhouse of milk and its derived products like cheese, curd, yoghurt as well as meat and its products like nuggets and sausages. For this, Indian government had taken several bold steps like Operation Flood, the world's largest integrated dairy development program, attempted to establish linkages between rural milk producers and urban consumers. But during this period, certain banned substances like veterinary drugs, pesticides and hormonal active growth promoters were used effectively for promotion of muscle growth in farm animals and milk production. So, the presence of residues of these banned substances in milk and meat products is a matter of concern for public health. So, our main aim is to investigate the exogenous hormonal effect on milk and meat products and the impact of its residual effect on public health.

Milk

Cow's milk naturally contains a number of steroid and protein (peptide) hormones in minute amounts. Steroid hormones include the glucocorticoids (cortisol and corticosterone) and the sex hormones – progesterone, 17 ß-estradiol (E2), estrone (E1), and estradiol (E3) [7-10]. Protein hormones in milk include prolactin, gonadotropin-releasing hormone, insulin-like growth factor-I (IGF-I) and bovine somatotropin [11].

Steroid Hormones

Glucocorticoids: Glucocorticoids are the group of hormones in milk which act synergistically with other hormones of lactogenesis. Endogenous glucocorticoids are mainly synthesised in the adrenal gland and is controlled by the adreno-corticotrophic hormone (ACTH). Cortisol is the predominant glucocorticoid (GC) in goats and cow's milk [12, 13]. The normal concentration of total GCs (0.46-0.65 ng/ml) has been determined by means of protein binding method in raw cow's milk [14].

Various research studies suggested that widespread utilisation of dexamethasone, a synthetic glucocorticoid which is used for the treatment of ketosis and several inflammatory diseases has its maximum residual limits in milk and other tissue [15]. Another study revealed that, use of adrenocorticotropic hormone (ACTH) in lactating Holstein cows leads to four-fold increase of milk cortisol level [16]. Quantification of GC can be done through comparative protein binding (CPB) [17] and radioimmunoassay (RIA) method [18]. But recently some advanced techniques like ELISA[19], gas chromatography – mass spectrophotometry (GC-MS) [20] and liquid chromatography-mass spectrophotometery (LC-MS) [21] have been developed in bovine's milk. Despite of all the progress in terms of detection and quantification, biological effects of milk GCs in animals and humans are negligible.

Sex hormones

Progesterone

Progesterone and 20-dihydroprogestrones are largely produced in ovaries and placenta and is regarded as one of the essential hormones in the whole processes of animals and humans reproduction starting from ovulation to the maintenance of the pregnancy, development of mammary glands and neurobehavioral roles associated with it [22-23]. Earlier it was detected in milk of different species of animals but for the first time in 1975 scientists detected in cow's milk due to its higher non-polar property.

Melengestrol acetate (MGA) is progesterone that is used as an animal feed additive to improve feed efficiency, increase the rate of weight gain, and suppress oestrus in beef heifers [24]. MGA is fed at daily doses of 0.25 – 0.50 mg per heifer for 90 to 150 days prior to slaughter [25] but it has several carcinogenic effects. MGA possess reproductive toxicity as several research findings revealed mammary and endometrial hyperplasia in rats fed MGA at doses of 0.15mg/kg/day or higher for 90 days [26]. Scientists found mammary carcinogenicity in small observational studies of captive wild felids in which MGA was used as a contraceptive [27]. There is also evidence that prepubertal exposure to MGA accelerates the onset of puberty in the beef heifer [28]. In spite of some carcinogenic effects, WHO in 2000 declared a maximum acceptable daily intake of 0.03µg/kg/day in human females as it doesn't affect the fertility, pregnancy and lactation in treated women.

Estrogens

The concentration of naturally occurring estrogens in food varies from species to species alongwith its age, gender & physiological status [29]. Milk is considered to be one of the potent source of steroids including oestrogens [30]. Milk-borne steroid hormones either originate from active transport or from passive diffusion through blood-brain-barrier [31]. Estrogens are utilised in the body in the form of estradiol (E2) because it is more potent than other two physiologic estrogens, estrone (E1) and estriol. Among the estrogenic steroids, mammary biosynthesis of β-E2 (17β estradiol) has been observed in cattle, sheep and goat as compared to E1(estrone) or 17α-estradiol (αE2) [32, 33]. In high yielding lactating cows, the concentration of 17β estradiol is found to be higher in mammary drainage than in peripheral circulation [33]. In cattle, estrogen benzoate is converted to estradiol [34]. Following the administration of radiolabelled E2, significant amount of 17α estradiol was detected in urine. It is used as hormone-replacement therapy in menopause but results of large-scale epidemiological investigations evidenced it as a mammary carcinogen and act both as an initiator and promoter of breast carcinogenesis.

Zeranol is an ER agonist with potency similar to diethylstilbestrol and estradiol-17β. Repeated in-vitro zeranol treatments were shown to reduce cell doubling time, stimulate colony formation, and, most notably, induce expression of ER-β mRNA in the proliferation of human breast epithelial cell line (MCF-10A) and downregulates the tumour suppressor genes (P53) in tissues of rats and beef heifers [35]. Since estrogens pass the blood-brain barrier easily, so, main estrogen in cow's milk is the biologically inactive 17β-estradiol, which followed by estrone and 17α-oestradiol.

Protein hormones

Insulin-Like Growth Factor 1 (IGF-1)

IGF-1 is a 70 amino acid-linked polypeptide produced mainly by mammary gland and liver in response to somatotropin [36]. Bovine IGF-1 in milk is identical to human IGF-1. As recommended by FDA, use of bST (Bovine somatotropin) increases IGF-1 levels in the milk. It is rapidly degraded in gut rather than being absorbed as it is structurally similar to insulin. The beneficial effects of colostrums IGF-1 in neonates indicates higher absorption and less degradation of milk IGF-1 in milk-consumer neonates as compared to neonates who receives milk replacer with lack of IGF-1.

Numerous epidemiological studies revealed a healthy relationship between plasma and serum IGF-1 levels and breast cancer risks. Meta-analysis of various case-controlled studies during different times revealed a significant higher level IGF-1 associated breast cancer in premenopausal women than in post-menopausal women. Heightened IGF-1 levels have also been associated with increased risk of colorectal and prostate cancers [14].

Recombinant bovine growth hormone

Recombinant bovine growth hormone (rBGH) or bovine somatotropin is a synthetic (man-made), genetically engineered protein which is identical to naturally synthesised pituitary product and is marketed to dairy farmers to increase milk production and efficiency of milk synthesis in cows [37]. Some rBGH products on the market differ chemically from a cow's natural somatotropin by one amino acid [38]. Therefore, BST is a protein hormone that increases milk production in cows between 10 and 15% [39]. Both the natural and recombinant forms of the hormone stimulate the activity and longevity of mammary secretory cells by increasing levels of another hormone known as insulin-like growth factor (IGF-1) produced by either liver or mammary gland. It has been used in the United States since it was approved by the Food and Drug Administration (FDA) in 1993, but its use is not permitted in the European Union, Canada, and some other countries.

Of greater concern is the fact that milk from rBGH-treated cows has higher levels of IGF-1, a hormone that normally helps some types of cells to grow. Bovine Growth Hormone increases activity and/or longevity of mammary secretary cells, probably via Insulin-like Growth Factor (IGF) I produced by the liver and/or the mammary gland [40]. Several studies have found that IGF-1 levels at the high end of the normal range may influence the development of certain prostate, colorectal and breast cancers. Second, cows treated with rBGH tend to develop more udder infections (clinical mastitis) due to increased milk yield and an increased incidence of lameness in cows and subclinical mastitis in ewes [41]. Reproductive problems in dairy cows have become very common as consequence of using rBST's, resulting with large numbers of cows being culled. These cows are given more antibiotics than cows not given rBGH. BST treated cows consume sufficient quantities of nutrients to meet the energy needs for more milk production. But, as a result, body lipid mobilisation doesn't get increased; rather lipid synthesis is reduced instantly [42].

Oxytocin

Oxytocin is a cyclic nonapeptide hormone released by the posterior pituitary and showing uterotonic and galactogenic activity. In the brain oxytocin is classically viewed as primarily involved in the milk let down reflex and in the stimulation of uterine smooth muscle during parturition [43]. In veterinary practice, xytocin is used universally to induce letdown of milk and expulsion of retained placenta after delivery. It is also helpful in the management of post-parturient uterine prolapse and also used in antibiotic therapy for treatment of mastitis [44].

If at all oxytocin is secreted in the milk and is ingested alongwith milk then it will produce no harmful effects because oxytocin is degraded by the gut enzymes and can't able to reach the blood circulation. Research in 1993 suggested that oxytocin injections before and after milking significantly increases the milk production in bovines by 3% and a non-significant effect on milk plasma activity, fat, protein and lactose [45]. But excess oxytocin in milk leads to hazardous effects in humans by causing headache, nausea, abdominal pain, drowsiness, deafness and blindness in children. It also increases the risk of post-partum haemorrhage. Sometimes, it reduces the growth hormone production leading to attainment of precocious puberty [46].

Meat

Growth promoters, including hormonal substances and antibiotics, are used legally and illegally worldwide in meat-producing animals for accelerating their growth. However, the use of hormones in such animals has provoked many concerns on human health impacts. The hormonal substances used for growth promotion in cattle are the naturally occurring steroids such as estradiol-17β, progesterone, and testosterone, as well as synthetic compounds such as zeranol, trenbolone acetate and melengestrol acetate which are generally classified as anabolic agents [47]. These hormones are administered to cattle mainly as an ear implant, to increase rates of weight gain, feed efficiency, storing of protein and decreasing fatness [48, 49, 50].

From research findings, it is clear that the intake amount of hormones via the meat of treated animals is approximately 40 times to thousands of times lower than the amount of human daily production of the hormone [51]. JECFA (Joint FAO/WHO Expert Committee on Food Additives) [52] concluded that the amount of exogenous hormones ingested via meat from treated cattle would be incapable of exerting any hormonal effects in human beings since bioavailability is very low in the case of orally administered hormones, and even when absorbed into the circulatory system, circulating hormones are predominantly in their inactive form. JECFA also recommended that establishing MRLs (maximum residue limits) for the natural hormones are unnecessary because exogenous estradiol, progesterone and testosterones are structurally identical to that produced endogenously in human beings.

Zeranol (α-zearalanol) is a nonsteroidal, oestrogenic mycotoxin produced by several Fusarium species which is used in livestock to increase the rate of weight gain, feed efficiency and high quality carcass [53, 54]. In cattle, it is implanted through ear implantation and is discharged after 65 days with a residual effect of ≤ 2ppb (µg/kg) in all organs and tissues [55]. Keeping in view the public health significance, the level of zeranol residues must not exceed 0.05 ppb in routine human diet, 2 ppb in cattle muscle and 10 ppb in cattle liver [56, 57].

Trenbolone acetate (TBA), a 19-nortestesteron, is a synthetic steroid with anabolic properties which simultaneously decreases the protein synthesis and degradation [58-59]. But when the rate of synthesis exceeds the rate of degradation, then muscle protein synthesis initiates in cattle [60]. The maximum permissible limits for trenbolone are 2 ppb in muscle and 10 ppb in liver [56, 57].

Diethylstilbestrol (DES) is a synthetic non-steroidal estrogen, which produces the same pharamacological and therapeutic effects in livestock as that of estradiol. In animals, DES is widely used as growth promoter to improve feed conversion ability, growth rates, increased protein metabolism leading to daily gain and reduced fat. Besides this, it is used to treat estrogen hypothyroidism and hormonal imbalance [61]. However, various reports suggested that it has mutagenic, carcinogenic and teratogenic properties which have raised a widespread public health concern [62, 63, 64]. Its use in veterinary food products as growth stimulant for food producing animals has been banned in several countries (since 1979 in USA & 1981 in EU). Therefore EU has proposed a minimum required performance limit (MRPL) of 0.5–2.0 lg/kg to control its abuse in meat producing animals [65].

Besides this, several feed additives such as beta-adrenergic agonists (ß-agonist), have been used to increase the carcasses lean content which substantially increases muscle to fat ratios. Clenbuterol, Paylean and Optaflex have been the most popular ß-agonists used for this purpose. Since Clenbuterol increases muscle mass, increases lipolysis, decreases lipogenesis, decreases protein degradation, increases protein synthesis and lowers the amount feed needed for finishing animals it makes a substantially larger profit for livestock farmers per animal [66]. Clenbuterol is easily and quickly eliminated from the body and not easily detectable after a small withdrawal period making it a profitable black market product [67]. The anabolic effect can be observed when clenbuterol is administered at dosages in excess of 5-10 fold the recommended therapeutic dose in meat-producing animals (> 1 µg/kg of body weight per day) [68]. The maximum residual level is 0.5 ppb in all edible tissues [69]. According to various research findings, Clenbuterol have a higher receptor affinity among all ß-agonists [70] which causes tachycardia and depressed appetite during initial days of administration [71].

Conclusion

The use of hormonal growth promoters has raised many concerns on their human health impacts. Risk assessments play a key role in the security of food safety. By following through with hazard identifications, hazard characterizations, exposure assessments, and risk characterizations, we attain more scientific background for decisions on risk management options in the protection of public health.

References

1. Jadhav VJ, Waskar VS. Public Health Implications of Pesticide Residues in Meat. Veterinary World. 2011; 1;4(4):178-182.
2. NDDB, 2012. @http://www.nddb.org/information/stats/pop
3. Ministry of Finance, Govt. of India, 2016 @http://mof.gov.in/
4. NDDB, 2015-16. @http://www.nddb.org/information/stats/pop
5. FAO, 2014. Vision 2050 NRCM @http://www.fao.org/docrep/014/i2280e/i2280e.pdf.
6. DAHD, Annual report – 2015-16@ http://dahd.nic.in/sites/default/files/ Livestock % 20%205.pdf.
7. Canapana W, Baunarucker CI. Hormones and Growth Factors in Bovine Milk. Handbook of milk composition. 1995; 23:476.
8. Jouan PN, Pouliot Y, Gauthier SF, Laforest JP. Hormones in bovine milk and milk products: a survey. International Dairy Journal. 2006; 16(11):1408-14.
9. Pape-Zambito DA, Roberts RF, Kensinger RS. Estrone and 17β-estradiol concentrations in pasteurized-homogenized milk and commercial dairy products. Journal of dairy science. 2010; 93(6):2533-40.
10. Macrina AL, Ott TL, Roberts RF, Kensinger RS. Estrone and estrone sulfate concentrations in milk and milk fractions. Journal of the Academy of Nutrition and Dietetics. 2012; 112(7):1088-93.
11. Vicini J, Etherton T, Kris-Etherton P, Ballam J, Denham S, Staub R *et al*. Survey of retail milk composition as affected by label claims regarding farm-management practices. Journal of the American Dietetic Association. 2008; 108(7):1198-203.
12. Schwalm JW, Tucker A. Glucocorticoids in Mammary Secretions and Blood Serum during Reproduction and Lactation and Distributions of Glucocorticoids, Progesterone, and Estrogens in Fractions of Milk1. Journal of dairy science. 1978; 61(5):550-60.
13. Gorewit RC, Tucker HA. Comparison of Binding Proteins of Glucocorticoids in Mammary Tissue and in Blood Sera from Lactating Cows1. Journal of dairy science. 1976; 59(7):1247-53.
14. Malekinejad H, Rezabakhsh A. Hormones in Dairy Foods and Their Impact on Public Health-A Narrative Review Article. Iranian journal of public health. 2015; 44(6):742.
15. Cherlet M, De Baere S, De Backer P. Quantitative determination of dexamethasone in bovine milk by liquid chromatography–atmospheric pressure chemical ionization–tandem mass spectrometry. Journal of Chromatography. 2004; 80(1):57-65.
16. Fox L, Butler WR, Everett RW, Natzke RP. Effect of adrenocorticotropin on milk and plasma cortisol and prolactin concentrations. Journal of dairy science. 1981; 64(9):1794-803.
17. Schwalm JW, Kirk J, Secrest S, Tucker HA. Effects of Processing Milk on Concentrations of Glucocorticoids in Milk1. Journal of dairy science. 1978; 61(10):1517-8.
18. Butler WR, Des Bordes CK. Radioimmunoassay technique for measuring cortisol in milk. Journal of dairy science. 1980; 63(3):474-7.
19. Groër M, Davis M, Casey K, Short B, Smith K, Groer S. Neuroendocrine and immune relationships in postpartum fatigue. MCN: The American Journal of Maternal/Child Nursing. 2005; 30(2):133-8.
20. Delahaut P, Jacquemin P, Colemonts Y, Dubois M, De Graeve J, Deluyker H. Quantitative determination of several synthetic corticosteriods by gas chromatography-mass spectrometry after purification by immunoaffinity chromatography. Journal of Chromatography B: Biomedical Sciences and Applications. 1997; 696(2):203-15.
21. Caloni F, Belloli C, Crescenzo G, Ormas P, Archimbault P. Determination of dexamethasone in milk of dairy cows by immuno-enzymatic assay. Veterinary and human toxicology. 2000; 42(6):345-8.
22. Zalanyi S. Progesterone and ovulation. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2001; 98(2):152-9.
23. Conneely OM, Lydon JP, De Mayo F, O'Malley BW. Reproductive functions of the progesterone receptor. Journal of the Society for Gynecologic Investigation. 2000; 7(1):S25-S32.
24. United States Food and Drug Administration. Animal Drugs @ FDA 2013 Available at: http://www.accessdata.fda.gov/scripts/animaldrugsatfda.
25. Canadian food inspection agency 2008. Available at: http://www. inspection. gc.ca/food/chemical-residues-microbiology/chemical-residues/foods-of-plant-and animal origin/eng/1389127041198/1389127042104.
26. Nachman KE, Smith TJ. Hormone use in food animal production: assessing potential dietary exposures and breast cancer risk. Current environmental health reports. 2015; 2(1):1-4.
27. Harrenstien LA, Munson L, Seal US. Mammary cancer in captive wild felids and risk factors for its development: a retrospective study of the clinical behavior of 31 cases. Journal of Zoo and Wildlife Medicine. 1996; 1:468-76.
28. Imwalle DB, Patterson DJ, Schillo KK. Effects of melengestrol acetate on onset of puberty, follicular growth, and patterns of luteinizing hormone secretion in beef heifers. Biology of reproduction. 1998; 58(6):1432-6.
29. Daxenberger A, Ibarreta D, Meyer HH. Possible health impact of animal oestrogens in food. Apmis. 2001; 109:S103-S112.
30. Hartmann S, Lacorn M, Steinhart H. Natural occurrence of steroid hormones in food. Food Chemistry. 1998; 62(1):7-20.
31. Schams D, Karg H. Hormones in milk. Annals of the New York Academy of Sciences. 1986; 464:75-86.
32. Janowski T, Zduńczyk S, Małecki-Tepicht J, Barański W, Raś A. Mammary secretion of oestrogens in the cow. Domestic Animal Endocrinology. 2002; 23(1):125-37.
33. Malekinejad H, Scherpenisse P, Bergwerff AA. Naturally occurring estrogens in processed milk and in raw milk (from gestated cows). Journal of agricultural and food chemistry. 2006; 54(26):9785-9791.
34. Kuiper GG, Carlsson BO, Grandien KA, Enmark E,

Häggblad J, Nilsson S *et al*. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology. 1997; 138(3):863-70.
35. Liu S, Lin YC. Transformation of MCF-10A Human Breast Epithelial Cells by Zeranol and Estradiol-17β. The breast journal. 2004; 10(6):514-21.
36. Jones LP, Stefansson S, Kim MS, Ahn SN. Comparison of radioimmuno and carbon nanotube field-effect transistor assays for measuring insulin-like growth factor-1 in a preclinical model of human breast cancer. Journal of nano biotechnology. 2011; 9(1):36.
37. Girma K, Tilahun Z, Haimanot D. Review on Milk Safety with Emphasis on Its Public Health. World Journal of Dairy and Food Science. 2014; 9(2):166-83.
38. Aytenfsu S, Mamo G, Kebede B. Review on Chemical Residues in Milk and Their Public Health Concern in Ethiopia. Journal of Nutrition and Food Science. 2016; 6(5):2-13.
39. Hoagland TA, Zinn SA, Tripp MW, Schreiber D, Cowan WA. Effect of Bovine Somatotropin (BST) on milk production in Angus and Hereford cows. Journal of Animal Science. 1997; 75:131-139.
40. Hilliard Y, Lerondelle C, Disenhaus C, Mouchet C, Paris A. Recombinant growth hormone: potential interest and risks of its use in bovine milk production. In Biotechnology in animal husbandry Springer Netherlands, 2001, 65-97.
41. Jahed KGR. Chemical Contaminants in Milk and Public Health Concerns: A Review. International Journal of Dairy Science. 2007; 2:104-115.
42. Serratosa J, Blass A, Rigau B, Mongrell B, Rigau T, Tortades M *et al*. Residues from veterinary medicinal products, growth promoters and performance enhancers in food-producing animals: a European Union perspective. Rev Sci Tech. 2006; 1;25(2):637-53.
43. https://www.fssai.gov.in/dam/jcr:af2704ba-9e73-4fd2-82b9e59e27b24e52/ Manual_ Antibiotics _Hormones_Residues_09_01_2017.
44. Ijaz A, Aleem M. Exogenous administration of oxytocin and its residual effects. Pakistan Veterinary Journal. 2006; 26(2):99-100.
45. Ballou LU, Bleck JL, Bleck GT, Bremel RD. The effects of daily oxytocin injections before and after milking on milk production, milk plasmin, and milk composition. Journal of dairy science. 1993; 76(6):1544-9.
46. Nostrand SD, Galton DM, Erb HN, Bauman DE. Effects of daily exogenous oxytocin on lactation milk yield and composition. Journal of Dairy Science. 1991; 74(7):2119-27.
47. Nazli B, Çolak H, Aydin A, Hampikyan H. The presence of some anabolic residues in meat and meat products sold in Istanbul. Turkish Journal of Veterinary and Animal Sciences. 2005; 29(3):691-9.
48. Moran C, Quirke JF, Prendiville DJ, Bourke S, Roche JF. The effect of estradiol, trenbolone acetate, or zeranol on growth rate, mammary development, carcass traits, and plasma estradiol concentrations of beef heifers. Journal of animal science. 1991; 69(11):4249-58.
49. Cecava MJ, Hancock DL. Effects of anabolic steroids on nitrogen metabolism and growth of steers fed corn silage and corn-based diets supplemented with urea or combinations of soybean meal and feathermeal. Journal of animal science. 1994; 72(2):515-22.
50. Sawaya WN, Lone KP, Husain A, Dashti B, Al-Zenki S. Screening for estrogenic steroids in sheep and chicken by the application of enzyme-linked immunosorbent assay and a comparison with analysis by gas chromatography–mass spectrometry. Food Chemistry. 1998; 63(4):563-9.
51. Jeong SH, Kang D, Lim MW, Kang CS, Sung HJ. Risk assessment of growth hormones and antimicrobial residues in meat. Toxicological research. 2010; 26(4):301.
52. Joint FAO/WHO Expert Committee on Food Additives Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additive 2000b Series, no. 45. Geneva: WHO.
53. Dağoğlu G, Aksoy A. Hayvansal üretimde zeranol. YY Ü. Sağ. Bil. Derg. 1995; 1:83-88.
54. Xiong YL, Moody WG, Blanchard SP, Liu G, Burris WR. Postmortem proteolytic and organoleptic changes in hot-boned muscle from grass-and grain-fed and zeranol-implanted cattle. Food research international. 1996; 29(1):27-34.
55. Egan CL, Wilson LL, Drake TR, Henning WR, Mills EW, Meyer SD *et al*. Effects of different doses of zeranol on growth, hemoglobin, and carcass traits in veal calves. Journal of animal science. 1993; 71(5):1081-107.
56. Codex Alimentarius. Food and Nutrition Division. FAO/WHO Food Standards. Viale delle Terme di Caracalla, Rome, Italy, 1997.
57. European Commission: Unit B3-management of scientific committees II: Opinion of the scientific committee on veterinary measures relating to public health. Assessment of potential risks to human health from hormone residues in bovine meat and meat products. 1999.
58. Laitem L, Gaspar P, Bello I. Detection of trenbolone residues in meat and organs of slaughtered animals by thin-layer chromatography. Journal of Chromatography A. 1978; 147:538-9.
59. Hsu SH, Eckerlin RH, Henion JD. Identification and quantitation of trenbolone in bovine tissue by gas chromatography-mass spectrometry. Journal of Chromatography B: Biomedical Sciences and Applications. 1988; 424:219-29.
60. Apple JK, Dikeman ME, Simms DD, Kuhl G. Effects of synthetic hormone implants, singularly or in combinations, on performance, carcass traits, and longissimus muscle palatability of Holstein steers. Journal of animal science. 1991; 69(11):4437-48.
61. Yang JY, Zhang Y, Lei HT, Wang H, Xu ZL, Shen YD *et al*. Development of an ultra-sensitive chemiluminescence enzyme immunoassay for the determination of diethylstilbestrol in seafood. Analytical Letters. 2013; 46:2189-2202.
62. Andersson AM, Skakkebaek NE. Exposure to exogenous estrogens in food: possible impact on human development and health. European Journal of Endocrinology. 1999; 140(6):477-85.
63. Chuanlai X, Huting W, Chifang P, Zhengyu J, Liqiang L. Colloidal gold-based immumochromatographic assay for detection of diethylstilbestrol residues. Biomedical Chromatography. 2006; 20(12):1390-4.
64. De Brabander HF, Le Bizec B, Pinel G, Antignac JP, Verheyden K, Mortier V *et al*. Past, present and future of mass spectrometry in the analysis of residues of banned substances in meat-producing animals. Journal of mass

spectrometry. 2007; 42(8):983-98.
65. Taşçi F. Determination of Diethylstilbestrol Residue in Raw Meat Sold in Burdur. Journal of Applied Biological Sciences. 2014; 8(3):32-4.
66. Strydom PE, Frylinck L, Montgomery JL, Smith MF. The comparison of three β-agonists for growth performance, carcass characteristics and meat quality of feedlot cattle. Meat Science. 2009; 81(3):557-64.
67. Wood A, Foo TK, Ahmad M, Fagan JM. Clenbuterol: Effects and Usage in Livestock and Show Animals.
68. Degand G, Bernes-Duyckaerts A, Maghuin-Rogister G. Determination of clenbuterol in bovine tissues and urine by enzyme immunoassay. Journal of Agricultural and Food Chemistry. 1992; 40(1):70-5.
69. Rose MD, Shearer G, Farrington WHH. The effect of cooking on veterinary drug residues in food: Food Additives and Contam. 1995; 12:67-76.
70. Spurlock ME, Cusumano JC, Mills SE. The affinity of ractopamine, clenbuterol, and L-644,969 for the beta-adrenergic receptor population in porcine adipose tissue and skeletal muscle membrane. Journal of Animal Science. 1993; 71(8):2061-5.
71. Ricks CA, Dalrymple RH, Baker PK, Ingle DL. Use of a-agonist to alter fat and muscle deposition in steers. Journal of Animal Science. 1984; 59(5):1247-55.