Journal of Animal and Veterinary Advances 7 (5): 630-632, 2008
ISSN: 1680-5593

Occurrence of Zeranol in Ground Beef Produced in Kars, Turkey

[1]Asim Kart, [2]Mehmet Elmali, [3]Kursat Yapar and [4]Hilmi Yaman
[1]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine,
[2]Department of Food Hygiene and Technology, University of Kafkas, 36100, Kars, Turkey
[3]Department of Pharmacology, School of Health, Giresun University, 28100, Giresun, Turkey
[4]Department of Food Hygiene and Technology, Faculty of Veterinary Medicine,
University of Afyon Kocatepe, 03200, Afyonkarahisar, Turkey

Abstract: Anabolic agents are used to increase live body weight in food animals. Zeranol is such an agent applied to cattle as subcutaneous implants in some countries. There is the potential risks that zeranol could affect hormonal and mineral metabolisms and have carcinogenic and teratogenic effects. Due to potential risks on animal and public health, use of zeranol as an anabolic agent was banned in Europian Union and in Turkey. In this study, it was aimed to investigate the presence of zeranol in ground beef samples marketed in Kars city which is one of the important ports in food animal production in Turkey. Seventy ground beef samples were collected from the retail shops to monitor occurrence of zeranol. The quantitative analysis of zeranol in beef samples were carried out by Competitive Enzyme Immune Assay (ELISA) test procedure as described by R-Biofarm GmBH, Germany ((Ridascreen® Zeranol, Tissue Samples, muscle, liver, kidney etc. Art. No: R3301). Of the 70 ground beef samples analyzed, 66 samples (94.28%) had level of zeranol under the detection limit (62 ppt), while 4 samples out of 70 (5.72%) contained zeranol ranging from 100-110 ppt.

Key words: Zeranol, beef, ELISA, harmonal and mineral metabolism

INTRODUCTION

Increased productivity has been the main objective in agriculture and animal husbandry to feed the growing world population. Application of anabolic agents in animals is one method to increase beef production. Among the anabolic agents, zeranol has found an application in some countries (Demet *et al*., 1992). Zeranol is a semi-synthetic estrogenic agent derived by chemical modification of zearelenone, a mycotoxin produced by *Fusarium* sp. (*Fusarium roseum*, *F. tricinctum*) (Sanli *et al*., 1995). The compound binds with cytoplasmic estrogenic reseptors in the target cells leading to increased DNA polymerase I and II synthesis with ensuing increased protein synthesis. The anabolic effects of zeranol are reported to be observed on the growth (Rhind *et al*., 1984; Sawyer *et al*., 1987), fat deposition (Prichard *et al*., 1989), mineral (Chanetsa *et al*., 2000) and endocrine metabolisms (Staigmiller *et al*., 1985; Gulyuz *et al*., 1996).

In addition to the beneficial effects on these systems, there are also some serious side effects that are of importance for animal and public health. Most anabolic agents used in food animals are hormones in nature, or at least they show hormone-like effects. Therefore, it is not surprising that these agents could also affect human health following consumption of contaminated beef. It is reported that zeranol interferes with hormonal and mineral metabolisms as well as shows carcinogenic and teratogenic effects (Chanetsa *et al*., 2000; Sundlof and Strickland, 1985; Lindsay, 1985; WHO, 1988; Anon Ymous, 1989; Moran *et al*., 1990). Following the intake of zeranol, children show early sexual maturation and abnormal fat distribution similar to adults. In adult males, zeranol causes alterations including feminine characters (such as female-like fat distribution and voice), atrophy in testes and prostate, decreased spermatogenesis and testosterone production. The use of zeranol for anabolic effect in food animals was banned in the Turkey (TFC, 2003) and EU. In addition, monitoring zeranol in animals in member States of EU has been required by law for possible abuse of this banned substance.

In this study, it is aimed to investigate and monitor presence of zeranol in beef samples marketed in retail shops of Kars province which is the leading city in Turkey for cattle population. The study will also provide information about the potential risks posed by zeranol in beef samples.

Corresponding Author: Dr. Asim Kart, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Kafkas, 36100, Kars, Turkey

MATERIALS AND METHODS

Seventy beef samples obtained from the retail stores of Kars city were analyzed for the presence of zeranol. Ground beef samples each weighing approximately 200 g. were stored at-18°C until further analysis following the collection of each sample. The quantitative analysis of zeranol in ground beef samples were carried out by competitive enzyme immunoassay (ELISA) test procedure according to the method described by Biopharm (2008) (Ridascreen® Zeranol, Art. No: R3301, GmBH, Germany).

Preparation of Samples: Fat residues from the samples were removed and 1 g of ground sample is homogenized with 2 mL of distilled water. Ten mililiter of diethyl ether were added to the samples. The samples were centrifuged at 3000 g at 15°C for 10 min and then frozen at-25°C for 60 min. The ether layer was decanted to a centrifuge tube. The aqueous phase was thawed and 5 mL of diethyl ether was added to the aqueous phase. The ether extract was pooled and evaporated to dryness. The dried residue was dissolved in 1 mL of chloroform. Ten mililiter of 1 M NaOH was added and shaken for 30 sec. The mixture was then centrifuged again at 2000 g at 15°C for 10 min. The extract was pipetted into a vial containing 250 μL of 90% acetic acid. Another 5 mL of diethyl ether was added and shaken again. The mixture was then centrifuged again at 2000 g at 15°C for 10 min. The samples were frozen again and ether phase was decanted into a vial. The supernatant was evaporated to dryness then dissolved in 0.5 mL of sample dilution buffer and used for the assay.

Test procedure: A sufficient number of microtiter wells were inserted into the microwell holder for all standards and samples. Fifty microLiter of each standard and prepared sample was added to separate wells and 50 μL of diluted enzyme conjugate was added. Fifty microLiter of the diluted anti-zeranol antibody solution was added and incubated at room temperature for 2 h. The liquid was removed from the wells by tapping the microwell holders against absorbent paper. All wells were filled with 250 μL of distilled water and the liquod part was poured out again. Fifty microLiter of substrate and 50 μL of chromogen were added to each well and incubated at room temperature in the dark for 30 min. A hundred microiter of the stop solution was added and shaken gently. The samples were measured at 450 nm against blank in ELISA reader (Spectra Max 384 Plus).

Evaluation: The samples were evaluated according to the Rida® Soft Win computer program developed by R-Biopharm.

RESULTS AND DISCUSSION

Of the 70 beef samples analyzed, the level of zeranol in 66 beef samples (94.28%) was under the detection limit (62 ppt), whilst zeranol was detected in 4 (5.72%) samples in concentrations ranged from 100-110 ppt. The data obtained from the study were presented in Table 1.

Although, use of anabolic agent is banned for food animals in many countries including Turkey, previous studies indicate that use of zeranol as an anabolic agent in food animals is still an important issue in Turkey. Presence of zeranol in beef samples was reported in several studies. Liman *et al.* (2005) reported that 5 samples out of 84 beef kidneys contained zeranol ranging from 300-500 ppt. Nazli *et al.* (2005) studied the presence of zeranol in visceral organs from 30 beef samples (10 liver, 10 kidney 10 spleen). Zeranol was detected at concentrations of 0.01-1.00 ppb in 56.7%, 1.01-2.00 ppb in 16.7%, 2.01-4.00 ppb in 6.6% and 4.01-8.00 ppb in 20% of the total analyzed samples. Demet *et al.* (1992) reported that 28 samples out of 63 from different type of beef tissues were found to contain zeranol. Similarly, Akilli (1995) reported that while no zeranol was detected in Turkish sausage and pastrami samples in a total of 200 samples, 5 samples (1.66%) out of 300 samples from the unprocessed meat contained zeranol. In another study, Akilli (1996) also reported that 7 beef samples out of 1317 contained zeranol. In comparison to the previously reported studies, rate of zeranol contamination in beef samples reported here is similar to that of Liman *et al.* (2005), lower than those of Nazli *et al.* (2005) and Demet *et al.* (1992) but higher than those of Akilli (1995, 1996).

The great portion of zeranol (about 80%) is eliminated by 65 days in cattle following subcutaneous implantation (Kaya *et al.*, 2007). As growth promotant, zeranol is applied to cattle at 36 mg via subcutaneous ear implant. Withdrawal times for zeranol from the tissues of cattle were reported to be 65 days. Consumption of the meats from the treated animals before the withdrawal times entails a special risk to consumers (Demet *et al.*, 1992).

Therefore, WHO/FAO (1988) and Cordle (1988) recommended that cattle should be slaughtered following 65 days of zeranol treatment. One important result from the current study is that beef samples containing zeranol are presented to the markets before the withdrawal times. In addition, the results from the present study also

Table 1: Occurrence of Zeranol in ground beef samples marketed in Kars, Turkey

Under detection Limit (<62 ppt)	66	94.28
100-110 ppt	4	5.72
Total no. of samples	70	100

indicate that meat market in Kars city is supplied with beef containing zeranol although the use of this substance in food animals is banned. The possible sources of zeranol in this region could be due to 2 different ways. First, it appears that some cattle growers in Turkey use anabolic agents for growth promotion. Secondly, there is a possibility that meats of cattle imported or smuggled from neighboring countries enter illegally to retail markets.

CONCLUSION

Since, the use of zeranol in food animals is banned in Turkey, it is important to take more efficient legal and preventive measures. In addition, considering the possible adverse effects of anabolic agents, use of zeranol could pose a special risk to public health.

REFERENCES

Akilli, A., 1995. Türkiyede üretilen et ve mamüllerinde (pastırma, sucuk) dietilstilbestrol ve zeranol kalıntıları. Etlik Vet. Mikrobi. Derg., 8: 127-146.

Akilli, A., 1996. Gıdalarda veteriner ilaç ve anabolizan maddelerin kalıntı düzeylerinin tespiti. Gıdalarda katkı, kalıntı ve bulaşanların izlenmesi T.C. Tarım ve Köy işleri Bakanlığı Koruma Ve Kontrol Genel Müdürlüğü Yayınları. Ankara, Türkiye.

Anonymous, 1989. Hormon ve benzeri maddelerin hayvancılıkta verim arttırıcı olarak kullanılması. Türkiye Bilimsel ve Teknik Araştırma Kurumu. 17 Mayıs Ihtisas Komisyonu Toplantısı. Ankara, Turkey.

Chanetsa, F., L.S. Hillman, M.G. Thomas and D.H. Keisler, 2000. Estrogen agonist (zeranol) treatment in castrated male lamb model: effects on growth and bone mineral accretion. J. Bone Mineral Res., 15: 1361-1367.

Cordle, M.K, 1988. USDA regulation of residues in meat and poultry products. J. Anim. Sci., 66: 413-433.

Demet, O., B. Tras, A.L. Bas and Y. Dogruer, 1992. Konya'da et ve balık kurumunda kesilen sığırlardan alınan kas, karaciğer ve böbrek dokularında zeranol kalıntılarının araştırılması. Selçuk Üniversitesi Araştırma Fonu, Konya, Türkiye.

Gulyuz, F., A. Aksoy, S. Ugras, I. Turel and G. Dağoglu, 1996. Zeranol ve 19-nortestosteron'un (nandrolon) akkaraman ırkı erkek kuzuların genital organlarına etkisi uzerine histopatolojik incelemeler. Y.Y.U. Saglik Bil. Derg., 2: 6-11.

Kaya, S., I. Pirincci, A. Unsal, Z. Karaer, B. Tras, A. Bilgili and F. Akar, 2007. Veteriner Farmakoloji. Cilt 2, 4. Baskı, Sayfa 287, Medisan Yayıncılık, Dışkapı, Ankara, Türkiye.

Lindsay, D.G., 1985. Zeranol a nature identical oestrogen. Food. Chem. Toxicol., 23: 767-774.

Liman, B.C., G. Eraslan, M. Kanbur, S.E. Hismiogulları, N. Seybek and A. Alan, 2005. Zearalenone and ochratoxin residues in the kidney of bulls. Indian Vet. J., 82: 615-617.

Moran, C., D.J. Prendivile, J.F. Quirke and J.F. Roche, 1990. Effects of oestradiol, zeranol or trenboane acetate implants on puberty, reproduction and fertility in heifers. J. Reprod. Fertil., 89: 527-536.

Nazli, B., H. Colak and H. Hampikyan, 2005. A study on the presence of some anabolic residues in offals marketed in Istanbul. Turk. J. Vet. Anim. Sci., 29: 691-699.

Prichard, D.L., T.T. Marshall, D.D. Hargove and T.A. Olson, 1989. Effects of creep feeding, zeranol, Implants and breed type on beef production: II. Reproductive development and fat deposition in heifers. J. Anim. Sci., 67: 617-623.

R-Biopharm, 2008. (Ridascreen® Zeranol, Art. No: R3301, Gmbh, Germany).

Rhind, S.M., D. Zygoyiannis, J.M. Doney, L.D. Leslie and I.C. Hart, 1984. Effects of zeranol implante and dietary supplemet on growth rate, endocrine status and blood metabolic levels of growing lambs at pasture. Anim. Prod., 39: 269-276.

Sanlı, Y., S. Kaya, I. Pirincci, H. Yavuz, E. Baydan, O. Demet and A. Bilgili, 1995. Editor: Sezai Kaya, Veteriner Klinik Toksikoloji, Medisan Yayınevi, Dıskapı, Ankara, Türkiye, pp: 324-328.

Sawyer, G.J., R.H. Casey and D.J. Barker, 1987. Growth response of steer calves treated with zeranol oestradiol 17β or progestone-oestrodiol benzoate implants before and after weaning. Aust. Vet. J., 64: 371-374.

Sundlof, S.F. and C. Strickland, 1985. Zeralenon And Zeranol: Potential residue problem in livestock. Vet. Hum. Toxicol., 28: 242-250.

Staigmiller, R.B., R.M. Brownson, R.J. Kartchner and J.H. Williams, 1985. Sexual development in beef bulls following zeranol implants. J. Anim. Sci., 60: 342-351.

Turkish Food Codex (TFC), 2003. Gıda değeri olan hayvanlara uygulanması yasaklanan ve belli şartlara bağlanan hormon ve benzeri maddeler hakkında tebliğ (Tebliğ No: 2003/18, Madde 5), Bakanlıklar, Ankara, Turkey.

World Health Organisation (WHO), 1988. Evaluation of certain veterinary drug residues in food. Thirty second report of the joint FAO/WHO expert committe on food additives. Technical Report Series, 763, Genova.