Hormonally active substances in the food chain from farm animals to consumers

*Jelka Pleadin and Marko Samardžija**

Abstract

The application of sex hormones and other hormonally active substances in farm animals have an anabolic effect that result in increased protein synthesis and fat degradation, and also a better sensory-profile of final meat products. However, when applied to animals, these substances are transmitted to edible tissues and body fluids, and can be found in final products of animal origin intended for consumer consumption, such as milk, offal, meat and eggs. Given that hormonally active substances can cause intoxications in humans, their use as an animal growth promoter is prohibited in the European Union. As sex hormones are present in the tissues and body fluids of farm animals at physiological levels, and that their occurrence may also be the consequence of therapeutic application, the correct interpretation of established levels in different materials sampled from farm animals is of great significance in proving their illegal use. In recent decades, numerous studies have been carried out in this area, since many substances with hormonal activity have not yet been investigated for their possible toxic effects in humans. At the same time, the development and occurrence of new synthetic hormones and their mixtures continue on the black market. As abuse of hormonally active substances in the livestock industry poses a potential risk to consumers, monitoring their occurrence in all critical points of food production is necessary. In order to produce safe foodstuffs and to ensure the protection of consumer health, there is need for continuous development of analytical methods that are proven to detect a high number of synthetic substances with hormonal activity that might be present in the food chain from farm animals to consumers.

Key words: *hormones; anabolic effect; residues; intoxication; food safety*

Introduction

In recent decades, cases of the appearance of sex hormones and other hormonally active substances in foods of animal origin have been increasing worldwide. Hormones are the collective name for chemical signalling substances synthesized and excreted from the endocrine glands in small quantities in humans and animals, which are important for maintaining homeostasis

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and avoiding metabolic imbalance and the resulting disorders. All steroid hormones are synthesized from cholesterol as the common precursor (Noppe et al., 2008), and differ structurally only in the pattern of chemical bonds within the rings and modifications on the side chain (Norman, 2003). The biochemical pathway for steroid hormone synthesis is shown in Figure 1. Among the steroid hormones, natural sex hormones are substances primarily excreted from male and female sex glands, i.e. testicles and ovaries, though they can also be excreted from other tissues (Samardžija et al., 2016; Gamulin et al., 2018).

Hormones are naturally occurring in farm animals or are synthetically produced xenobiotics with oestrogenic (17β-estradiol and its esters; zeranol), androgenic (testosterone and esters; trenbolone acetate) or progestogenic (progesterone; melengestrol acetate) activity (Galbraith, 2002). Additionally, there are also stilbenes, thyrostats, corticosteroids and beta-adrenergic compounds, which also can be used singly or in effective combinations (Courtheyn et al., 2002; Reig and Toldrá, 2009; Stephany, 2010). They have been used extensively as growth promoters in farm animals in international production to improve the efficiency of conversion of food into live-weight gain, to reduce fat and increase N retention and lean tissue deposition (Lone, 1997; Galbraith, 2002). Their implementation can result in increased yields and higher profits in the livestock industry (Armstrong et al., 2004; Pleadin et al., 2011; Pleadin and Bogdanović, 2016). The achieved yields can range up to 40%, though this is dependent on the animal species, breed, age, reproductive status and hormone delivery (Meyer, 2001; Courtheyn et al., 2002).

Possible adverse effects of hormone residues and their metabolites on consumers has become a major concern in recent decades, as when applied to farm

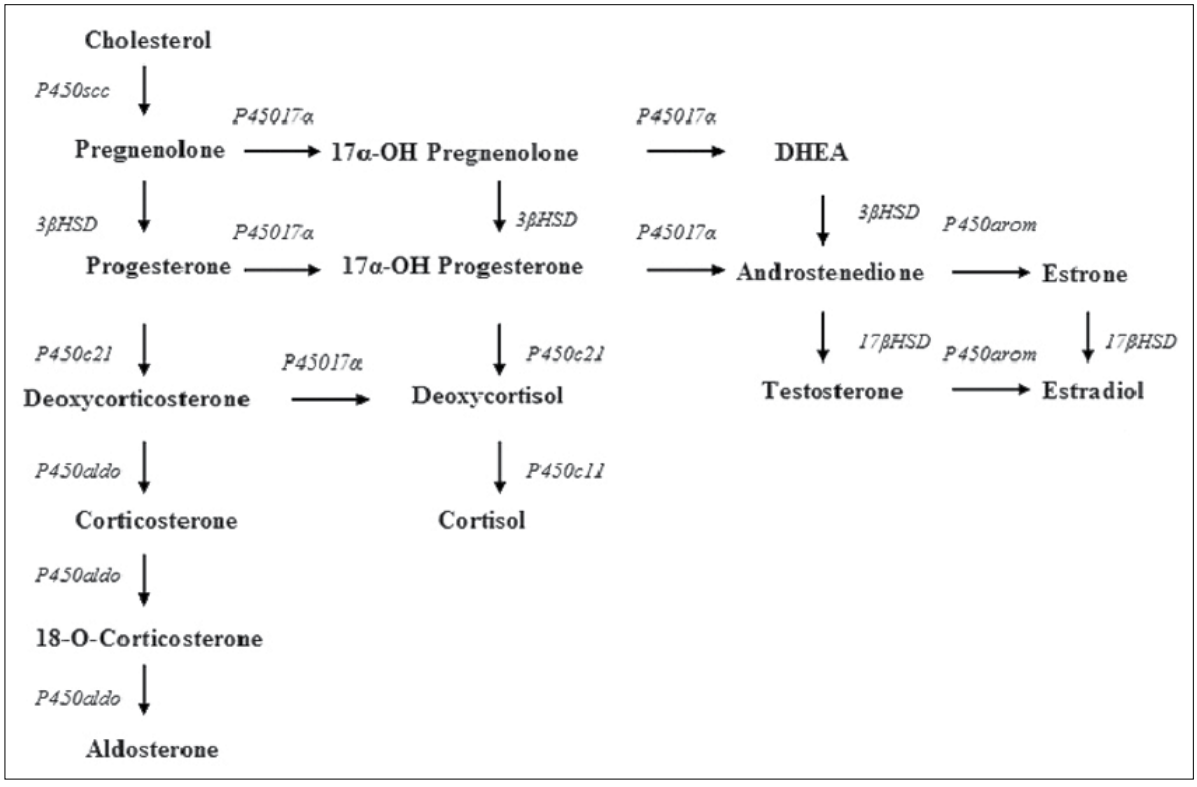

Figure 1. Biochemical pathway for steroid hormone synthesis (Manca, 2008)

animals, these substances are transferred to edible tissue and body fluids, and thus also to finished products of animal origin (Hartmann et al., 1998; Samardžija et al., 2018). Since hormonal intake through food has the same effect as the animal's endogenous hormones, in consuming foods of animal origin, consumers may be exposed to additional hormonal action that can affect many physiological processes in the body and ultimately affect human health. The use of certain hormones as growth promoters in farm animals is permitted in some countries, though it is completely prohibited in the European Union (Galbraith, 2002). However, data obtained under national monitoring programs in European countries has shown a 5-15% frequency of illegal use of substances with a hormonal effect (Stephany, 2010).

This paper provides an overview of the application of natural and synthetic substances with hormonal activity in farm animals, the occurrence of their residues in foods of animal origin, and measures that have to be taken to control or suppress abuses with a view to ensuring food safety and consumer health protection.

Role and anabolic effects in the body

In the reproductive sense, hormone synthesis control plays a primary role in the pituitary, epiphyses, gonads (ovaries and testis) and placenta during pregnancy (Arthur et al., 1997; Samardžija et al., 2015). There are also other endocrine glands that have a particular influence on endocrinology, such as the adrenal glands, the thyroid gland, the uterus and the hypothalamus. In the female, there are two physiological functions of different types of hormones: oestrogen (follicular hormones) and progestogen (yellow body hormones), whereby the ovaries have two basic functions: germinative and endocrine. Sexual hormones, their physiological secretions and their actions are the basis for reproduction. Because of their action, sexual steroid hormones can be used in therapeutic purposes for the treatment of disorders in the sex or reproductive system of farm animals (Samardžija et al., 2015; Gamulin et al., 2018).

Substances having an anabolic effect represent organic chemical substances that stimulate tissue growth by virtue of their impact on metabolic processes, where the latter stimulation is particularly pronounced when it comes to skeletal muscle cells (Pleadin and Bogdanović, 2016). In animals, their action mechanisms can be direct or indirect, ultimately resulting in enhanced nitrogen retention and protein synthesis, i.e. in enhanced growth (Lone, 1997). This is why livestock production started to make use of anabolic substances as early as the 1950s. In addition to natural sex hormones (17β-estradiol, progesterone and testosterone), three synthetic chemical substances with oestrogen (zeranol), progestogen (melengestrol acetate) and androgen (trenbolone acetate) properties were used in the past to stimulate cattle growth by applying earplug implants and by administration via water and animal feed (Galbraith, 2002; Jeong et al., 2010). Since natural hormones are found to be physiologically present in the body, and given that their amounts vary depending on a range of factors, their presence in tissues or fluids does not automatically indicate illegal use in farm animals (Pleadin et al., 2011; Pleadin et al., 2013).

The application of these substances has been a point of international contention on the safety of meat and other types of food of animal origin. However, proper therapeutic use of natural hormones does not bear adverse human health consequences (FAO/WHO 2000). As opposed to the total ban of

all hormone-based growth promoters in livestock production imposed by the EU, the controlled use of certain hormones as an anabolic is legalized, for example in the United States, Canada, Australia, New Zealand and some South American, Asian and African countries. 17β-estradiol, testosterone, progesterone, trenbolone and zeranol are applied in the form of small compact earplug implants. Furthermore, the use of melengestrol acetate and ractopamine in pigs in the form of feed supplements is also permitted. At present, all substances from the beta-agonist group are forbidden for use to stimulate growth; the only exceptions are ractopamine, which is permitted for use in the United States, and zilpaterol, which is permitted for use in South Africa. However, scientists have indicated that despite the ban on the use of these substances, a high percentage of farm animals are grown using hormones in the world (Stephany, 2010).

Most important compounds

Stilbenes and their salts and esters are non-steroidal synthetic estrogenic compounds, the most common of which is diethylstilbestrol (DES), followed by hexestrol and dienestrol (Payne et al., 1999). DES is endocrine disruptor with carcinogenic properties and one of the first growth promoters used in veal production, administered to livestock either orally or via injection. The literature has shown that DES has strong mutagenic, teratogenic and carcinogenic properties, so that the use of this substance may have serious adverse impacts on human health. Due to the known toxic effects of stilbenes, their administration has been banned since 1981 (Council Directive 81/602/EEC), though literature data have indicated that DES has continued to be misused as an anabolic in meat production (Lone, 1997).

Thyreostats or anti-thyroid agents are a complex group of substances that inhibit thyroid function and, as a consequence, reduce the circulation of thyroid hormones (Pleadin and Bogdanović, 2016). Weight gain obtained by thyreostatic treatment should mainly be attributed to the increased filling of the animal gastrointestinal tract and increased water retention (Stolker and Brinkman, 2005). These compounds are carcinogenic and teratogenic. Thiouracil (2-thiouracil), as a particularly most toxic substance of this group, was one of the most frequently abused thyreostats.

Anabolic steroids include oestrogens, progestogens, androgens and corticosteroids as natural compounds and their synthetic derivatives. Among the natural hormones, 17β-estradiol is the most important due to the most pronounced anabolic activity of this hormone (Meyer, 2001; Pleadin et al., 2013). If given to animals in the approved therapeutic dose, its residues in meat are low-levelled and not dangerous for consumers, as in the case of the most important natural androgen, testosterone (Samardžija et al., 2018), which is most commonly given to farm animals in combination with 17β-estradiol. Long-term progesterone use results in toxic effects and can cause ovarian, breast, vaginal and uterine tumours. Of all the synthetic steroids, trenbolone and 19-nortestosteron have received the greatest attention. Trenbolone is a steroid having androgenic and anabolic properties that indirectly affects muscle growth by changing the concentrations and metabolic paths of certain hormones, and it is usually applied in the form of ester derivatives, such as trenbolone acetate (Meyer, 2001). 19-nortestosteron is a steroid having more potent anabolic and androgenic effects than testosterone; in humans, it stimulates muscle growth and stimulates appetite, increases the production of erythrocytes and bone density, and may exhibit hepatotoxic effects, as

well as cause gynecomastia, decreased libido, cardiovascular symptoms and the reduction of luteinising hormone (Noé et al., 1999; Pleadin and Bogdanović, 2016).

Resorcylic acid lactones include taleranol, zearalenol and zeranol, and their effects on the organism have not yet been fully investigated. Literature data have shown oestrogenic effects of zeranol and its variations in affinity to bind to oestrogen receptors (Meyer, 2001), so in the past it was used as an anabolic drug (Kennedy et al., 1998). Zeranol affects animal growth both directly by binding to oestrogen receptors, and indirectly by increasing the concentrations of growth hormone and insulin-like growth factor IGF-I in animal blood (Thomas et al., 2000). Zeranol causes developmental impairments, immune toxicity, genotoxicity, possible carcinogenic effects, and changes in blood concentrations of various hormones (Wilson et al., 2002).

Beta-adrenergic agonists (β-agonists) are substances with hormonal effect that have been used for many decades in human and veterinary medicine for the treatment of chronic bronchitis, chronic obstructive pulmonary disease and asthma, and in animal tocolytics (Anderson et al., 2005). Their effect on the body is achieved by binding to specific β-adrenergic receptors located on cell membranes of the target tissue (Mersmann, 1998). They still represent the most important group of anabolic drugs and major grounds for abuse, since certain substances belonging to this group are permitted for use in some countries (outside the European Union) up to the stipulated Maximum Residue Limits (MRLs). Among them, the long-acting substance clenbuterol has been the one most studied in recent years (Pleadin and Bogdanović, 2016). Many short-acting substances of this class are also present, such as salbutamol, ractopamine, cimaterol, zilpaterol, terbutaline, mabuterol and others (Courtheyn et al., 2002). It is important to point out that there is a difference in the stance of scientists and in the legislation adopted in countries worldwide concerning the use of certain β-agonists, such as clenbuterol, ractopamine, zilpaterol in the livestock industry (Pleadin and Bogdanović, 2016; Van Ginkel et al., 2016).

Residues in food of animal origin

The administration of hormonally active substances in farm animals at doses 10 to 15 times higher than therapeutic doses result in an anabolic effect. However, at the same time, these substances are transmitted to edible tissue and body fluids and are ultimately present in food of animal origin in the form of residues (Anderson et al., 2005; Andrée et al., 2010; Pleadin et al., 2010). Data on hormone presence in food placed on the market are generally very rare and, in many cases, obtained as a result of individual and random action measures carried out by inspection bodies or consumer organizations (Pleadin, 2018). Since sex hormones are naturally present in the tissues and body fluids of farm animals at physiological levels, and their occurrence in the organism may also be a consequence of therapeutic use, it is understandable that these substances in animal food may be present in their physiological levels, but also at higher levels if they have been used for medical treatment and if not completely excreted from the body.

In addition to meat and milk, eggs represent their main source (Stephany, 2010) and consumers of food of animal origin may thus be exposed to increased hormonal activity. At the same time, research has shown that hormone residues in food cannot be inactivated or removed by thermal treatment (Rose et al., 1995). Hartmann et al. (1998) presented results and summarized

much of the previous literature data on hormone levels in foods (Table 1). From these data and information on average food consumption, authors estimated that the average daily intake of estradiol for women, men, prepubescent girls, and prepubescent boys is 0.08, 0.1, 0.07, and 0.08 µg, respectively, with 60–70% supplied by milk products and 15–20% by eggs, meat and fish. According to JECFA, the acceptable daily intake of estradiol is 3.0 µg for a 60-kg adult and 0.5 µg for a 10-kg child (FAO/WHO, 1999). The concentration of oestrogens in milk is associated with the amount of milk fat and the stage of cow pregnancy, as levels are higher in the second half of pregnancy than in earlier stages. Dairy products are also a major dietary source of progesterone and testosterone, though eggs and meat also providing significant amounts.

With regard to consumer exposure, it is not necessary to neglect the intake of hormones through foods of plant origin and the possible synergistic effects resulting from hormone intake in a multi-source organism. A comparison of the intake of natural hormones through food and their relative contribution to consumers is shown in Table 2.

Toxic effects in the body

Many cases of intoxication and varying hormonal effects in humans were previously recognized as a consequence of consuming contaminated food of animal origin (Martinez-Navarro, 1990). Research on the effect of substances with hormonal action in many animal species has shown different sensitivity, and the degree of toxicity was found to be dependent on particular genetic (species and breeds) and physiological (age, sex, nutrition, general state of the organism) factors, causing cytotoxic, hepatotoxic, genotoxic, immunosuppressive, mutagenic, teratogenic and carcinogenic effects (FAO/WHO, 2000). Toxicological parameters and MRLs of certain substances with anabolic effect are shown in Table 3.

The illegal use of clenbuterol, as the main recognized substance abused in the past, lead to numerous cases of poisoning of consumers consuming the meat of treated animals. The poisoning symptoms including rapid heartbeat, tremor, nervousness, general weakness, dizziness and headache (Martinez-Navarro, 1990; Kuiper et al., 1998; Woodward, 2005). The majority of toxicity studies on

Table 1. Hormone concentrations in different types of food of animal origin (modified from Hartmann et al., 1998; Doyle, 2000)

Food	17β-Estradiol	Progesterone	Testosterone
Skim milk (µg/L)	1.4.-2.2	/	/
Whole milk (µg/L)	0.01-0.03	9.5-11.8	0.02-0.05
Butter (µg/kg)	<0.03	141-300	<0.05
Cheese (µg/kg)	0.01-0.03	44.2	0.48-1.41
Eggs (µg/kg)	<0.03-0.22	12.5-43.6	0.04-0.49
Chicken meat (µg/kg)	<0.03-0.02	0.24	<0.02-0.03
Boar muscle (µg/kg)	0.91	3.71	/
Boar fat (µg/kg)	0.43	11.96	/
Boar liver (µg/kg)	9.67	1.2	/
Herring (µg/kg)	<0.03	0.51	0.07

Table 2. Daily intake of natural hormones in the body and their relative contribution through different types of food (Adolf et al., 1994)

	Meat and fish	Milk products	Eggs
17ß-estradiol and estrone			
Men (µg/day)	0.02	0.06	0.02
Women (µg/day)	0.01	0,05	0,02
Boys (µg/day)	0.01	0.06	0.01
Girls (µg/day)	0.01	0.05	0.01
Relative contribution (aprox, %)	15-20	60-70	15-20
Progesterone			
Men (µg/day)	0.63	8.18	0.92
Women (µg/day)	0.45	7.06	0.76
Boys (µg/day)	0.37	7.27	0.62
Girls (µg/day)	0.33	6.57	0.60
Relative contribution (aprox, %)	5	80	10
Testosterone			
Men (µg/day)	0.02	0.02	0.01
Women (µg/day)	0.01	0.02	0.01
Boys (µg/day)	0.01	0.02	0.01
Girls (µg/day)	0.01	0.02	0.01
Relative contribution (aprox, %)	20-30	30-40	15-20

Table 3. Toxicological parameters of certain substances with anabolic effect (Jeong et al., 2010; Pleadin and Bogdanović, 2017)

Substance	NOAEL (µg/kg b.w./ day)	ADI (µg/kg b.w./ day)	MRL (µg/kg) for cattle tissues*
17ß-Estradiol	5	0 – 0.05	-
Testosterone	1700	0 - 2	-
Progesterone	3300 (LOAEL)	0 - 30	-
Zeranol	50	0 – 0.5	2 (muscle), 10 (liver)
Melengestrol acetate	5	0 – 0.03	1 (muscle), 10 (liver), 2 (kidney), 18 (fat)
Trenbolon acetate	2	0 – 0.02	2 (muscle, β-trenbolon), 10 (liver, α-trenbolon)

NOAEL - no-observed adverse effect level; LOAEL - lowest observable adverse effect level; ADI - acceptable daily intake; MRL - maximum residue limit
*MRL values do not apply to the EU (totally prohibited use of substances for anabolic purposes)

natural steroid hormones have focused on 17β-estradiol and its hormonal action, likely because this is the most used and misused of all the natural sex hormones. The mutagenic, carcinogenic and teratogenic effects of this hormone have been proven in many animal species. Oral, particularly parenteral administration of 17β-estradiol, depending on the dose and duration of exposure, may cause an increased incidence of tumours in treated animals in tissues with high concentrations of specific hormone receptors (uterus, vagina, cervix, breast) including the pituitary gland, gut, bone and liver (Zimmerman, 1998). Growth of dairy glands, vaginal epithelial ejaculation and similar toxic effects are clear indicators of morphological changes. Biochemical changes caused by 17β-estradiol (changes in gene expression, signal transduction and cell cycle regulation) are much more subtle and less evident than morphological changes, although they are certainly equally important.

Recent studies have shown the trend of early sexual maturation of girls due to exposure to exogenous oestrogens (Andersson and Skakkebaek, 1999; Maruyama et al., 2010). Increased levels of oestrogen and its metabolites can cause carcinogenic effects of the reproductive system (FAO/WHO, 2000). Examining dietary habits and other risk factors for the development of cancer in menopausal women has established that the risk of ovarian cancer may be related to consumption of dairy products (Asif, 2013). Studies have also shown that in most cases, when these substances are properly used, the meat of treated animals is an insignificant source of hormone compared to physiological endogenous production in humans. However, research on the sex hormone levels in tissues is still incomplete and inadequate for many animal species and breeds and is dependent on many factors. Therefore, further research on the toxic effects of these substances and their metabolites is needed, as their metabolites also have biological activity in the organism (Andersson and Skakkebaek, 1999).

Control of hormonally active substances

It is known that hormonally active substances persist in edible tissues of animals used for food production and that thermal processing is unable to inactivate or remove these substances (Rose et al., 1995; Pleadin et al., 2010). In case of injection of liquid hormone preparations or application of "pour on" preparations, hormone concentrations observed in meat samples of illegally treated animals are typically in the range of a few micrograms to a few tenths of a microgram per kilogram. In case of contaminated product consumption, they can cause alimentary intoxication and serious health consequences in humans. It is important to point out that for European countries, data on natural hormones in meat and other types of food of animal origin are scarce, because of the absence of data about the regular (natural) levels of these hormones that can ultimately be used for comparisons with the levels determined in final products.

Encouraged by a series of scandals concerning hormone residues in food of animal origin, the European Commission has developed a comprehensive strategy for determining their presence in raw materials sampled during animal fattening and at slaughterhouses (Heitzman, 1994). Council Directive 86/469/EEC, which entered into force in 1986, provided a method for screening animals and fresh meat to determine the presence of hormone residues, with a view to ensuring the equivalent application of the prescribed measures

to protect food from their residues. The legislation currently in force in this area are Council Directive 96/22/EC, which prohibits the use of hormones for anabolic purposes in the European Union, Council Directive 96/23/EC, which mandates the monitoring of residues with hormonal activity through annual plans to monitor all animals during fattening and at slaughterhouses, and Commission Regulation 37/2010, which defines the MRLs of pharmacologically active substances in food of animal origin.

The purpose of the inspection programme in countries where the use of certain substances is permitted is to examine the compliance of the established hormone level with the prescribed national legislation. Throughout Europe, these substances do not have the prescribed MRLs, and their occurrence in material derived from animals in terms of natural hormones may be interpreted as purely physiological, while synthetic substances are completely prohibited and their presence can reveal abuse. Since the occurrence of sex hormones may be the consequence of therapeutic application, in order to interpret the results, it is necessary to know the physiological levels and to consider the drug carrier (excretion period from the organism). Inspection programmes are based on the sampling of biological materials more suitable for testing the presence of these substances on farms (urine, blood and animal hair) and at slaughterhouses (liver and muscle tissue). However, according to the findings of National Reference Laboratories conducting research in this area, the number of active compounds is constantly increasing, *i.e*. new substances with a hormonal effect in the body are being continuously identified, either individually or in "smart" combinations (Stephany, 2010).

Analytical methods used in official laboratories to test for the presence of substances with hormonal effects can be divided into screening and confirmation methods. The screening method most widely used is immunoenzymatic method ELISA, since it enables rapid screening or quantification of very low levels of hormones. This method is characterized by its ease of use, acceptable accuracy and availability of reagents, though a shortcoming is its possible cross reactivity with the occurrence of false positive results, and that the application of ELISA kit is limited only for particular substances (Pleadin and Bogdanović, 2016). Since these substances are mainly prohibited, the final identification of these substances is carried out using confirmatory analytical techniques (Stolker and Brinkman, 2005). The most widely used methods are Liquid (LC) and Gas (GC) Chromatography in combination with tandem Mass Spectrometry (MS-MS), which provide unambiguous identification by mass ratio and ion charge characteristic for each particular substance and their precise quantification.

Conclusions

The issue of hormones in food of animal origin needs to be considered, taking into account their overall intake through different sources. Further toxicological research is needed since many substances with hormonal action have not yet been explored, while new synthetic hormones and their mixtures containing a wide variety of low-dose active substances are constantly being developed. Also, continuous development of modern analytical methods is needed to identify new compounds on the black market, and for the analysis of pigmented materials, such as hair, which allows easy sampling and persistence of hormones over a significantly longer period of time. In order to protect consumer health, continuous monitoring of the occurrence of hormonally active

substances in all critical points of food production chain from farm animals to consumers is required.

References

1. ADOLF, T., W. EBERHARDT, H. HESEKER, S. HARTMANN, A. HERWIG, B. MATIASKE, K. J. MOTH, R. SCHNEIDER and W. KIIBLER (1994): Lebensmittel- und Nahrstoffaufnahme in der Bundesrepublik Deutschland. In: VERA-Schrtftenreihe, Band XII, Ktibler, W., Anders, H. J., Heeschen, W. (eds.), Wissenschaftlicher Fachverlag Dr. Fleck, Niederkleen.
2. ANDERSON, D. B., D. E. MOODY and D. L. HANCOCK (2005): Beta Adrenergic Agonists. In: Encyclopedia of Animal Science. Pond, W. G., Bell, A. W. (eds.), Marcel Dekker, USA, pp. 104-107.
3. ANDERSSON, A.-M. and N. E. SKAKKEBAEK (1999): Exposure to exogenous estrogens in food: possible impact on human development and health. Eur. J. Endocrinol. 140, 477-485.
4. ANDRÉE, S., W. JIRA, K.-H. SCHWIND, H. WAGNER and F. SCHWÄGELE (2010): Chemical safety of meat and meat products. Meat Sci. 86, 38-48.
5. ARMSTRONG, T. A., D. J. IVERS, J. R. WAGNER, D. B. ANDERSON, W. C. WELDON and E. P. BERG (2004): The effect of dietary ractopamine concentration and duration of feeding on growth performance, carcass characteristics, and meat quality of finishing pigs. J. Anim. Sci. 82, 3245-3253.
6. ARTHUR, G. H., D. E. NOAKES, H. PEARSON and T. J. PARKINSON (1997): Veterinary reproduction and obstetrics, 7th edition, W. B. Saunders Company, London.
7. ASIF, M. (2013): Effect of estrogen hormones present in animal milk, dairy products and animal proteins in cancer. J. Sci. Innov. Res. 2, 1083-1085.
8. COMMISSION REGULATION (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Official Journal of the European Communities, L 15, p. 1-72.
9. COUNCIL DIRECTIVE 81/602/EEC of 31 July 1981 concerning the prohibition of certain substances having a hormonal action and of any substances having a thyrostatic action. OJ L 222, 1981, 32–33.
10. COUNCIL DIRECTIVE 86/469/EEC of 16 September 1986 concerning the examination of animals and fresh meat for the presence of residues. Official Journal of the European Communities, L 275, p. 36-45.
11. COUNCIL DIRECTIVE 96/22/EC of 29 April 1996 concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of β-agonists, and repealing Directives 81/602/EEC, 88/146/EEC and 88/299/EEC. Official Journal of the European Communities, L 125, p. 3-9.
12. COUNCIL DIRECTIVE 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC, 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC. Official Journal of the European Communities, L 125, p. 10-32.
13. COURTHEYN, D., B. LE BIZEC, G., BRAMBILLA, H. F., DE BRABANDER, COBBAERT, M. VAN DE WIELE, J. VERCAMMEN and K. DE WASCH (2002): Recent developments in the use and abuse of growth promoters. Anal. Chim. Acta. 473, 71-82.
14. DOYLE, E. (2000): Human Safety of Hormone Implants Used to Promote Growth in Cattle. FRI BRIEFINGS. http://fri.wisc.edu/docs/pdf/hormone.pdf
15. Food and Agriculture Organization/World Health Organization (FAO/WHO) (2000): Toxicological evaluation of certain veterinary drug residues in food. Estradiol-17β, progesterone and testosterone. The Fifty-second meeting of the Joint FAO/WHO Expert Committee in Food Additives (JECFA). WHO Food Aditives Series 43.
16. GALBRAITH, H. (2002): Hormones in international meat production: biological, sociological and consumer issues. Nutr. Res. Rev. 15, 293-314.
17. GAMULIN, E., M. SAMARDŽIJA, I. BUTKOVIĆ and J. PLEADIN (2018): Biochemical mechanisms of sex hormones synthesis in domestic mammals. Vet. stn. 49, 425-438.
18. HARTMANN, S., M. LACORN and H. STEINHART (1998): Natural occurrence of steroid hormones in food. Food Chem. 62, 7-20.
19. HEITZMAN, R. J. (1994): Veterinary Drug Residues, Residues in food producing animals and their products: Reference Materials and Methods. Oxford: Blackwell Science.
20. JEONG, S.-H., D. KANG, M.-W. LIM, C. S. KANG and H. J. SUNG (2010): Risk assessment of growth hormones and antimicrobial residues in meat. Toxicol. Res. 26, 301-313.
21. JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES. Summary and Conclusions of the Fifty-second Meeting, Rome, 2–11 February 1999. http://www.fao.org/WAICENT/ FAOINFO/ ECONOMIC/ESN/Jecfa/jecfa52.pdf
22. KENNEDY, D. G., S. A. HEWITT, J. D. MCEVOY, J. W. CURRIE, A. CANNAVAN, W. J. BLANCHFLOWER and C. T. ELLIOT (1998): Zeranol is formed from *Fusarium* spp. toxins in cattle in vivo. Food Addit. Contam. 15, 393-400.
23. KUIPER, H. A., M. Y. NOORDAM, M. M. H. DOOREN-FLIPSEN, R. SCHILT and A. H. ROOS (1998): Illegal use of β-adrenergic agonists: European Community. J. Anim. Sci. 76, 195-207.
24. LONE, K. P. (1997): Natural sex steroids and their xenobiotic analogs in animal production: growth, carcass quality, pharmacokinetics, metabolism mode of action, residues, methods and epidemiology. Crit. Rev. Food Sci. Nutr. 37, 193-209.

25. MANCA, P. (2008): The Relative effectiveness of 2-Methoxyestradiol on undifferentiated and differentiated cells of glial and neuronal origin. Dottorato in Ripruduzione, Produzione e Benessere Animale XX Ciclo 2004-2007. Università Degli Studi Di Sassari. p. 34
26. MARTINEZ-NAVARRO, J. F. (1990): Food poisoning related to consumption of illicit beta-agonist in liver. Lancet 336, 1311.
27. MARUYAMA, K., T. OSHIMA and K. OHYAMA (2010): Exposure to exogenous estrogen through intake of commercial milk produced from pregnant cows. Pediatr. Int. 52, 33-38.
28. MERSMANN, H. J. (1998): Overview of the effects of β-adrenergic receptor agonists on animal growth including mechanisms of action. J. Anim. Sci. 76, 160-172.
29. MEYER, H. H. D. (2001): Biochemistry and physiology of anabolic hormones used for improvement of meat production. Apmis 109, 1-8.
30. NOÉ, G., J. SUVISAARI, C. MARTIN, A. J. MOO-YOUNG, K. SUNDARAM, S. I. SALEH, E. QUINTERO, H. B. CROXATTO and P. LÄHTEENMÄKI (1999): Gonadotrophin and testosterone suppression by 7α-methyl-19-nortestosterone acetate administered by subdermal implant to healthy men. Hum. Reprod. 14, 2200-2206.
31. NOPPE, H., B. LEBIZEC, K. VERHEYDEN and H. F. DE BRABANDER (2008): Novel analytical methods for the determination of steroid hormones in edible matrices. Anal. Chim. Acta 611, 1–16.
32. NORMAN, A. W. (2003): Hormones, Steroid Hormones. In: Encyclopedia of Food Sciences and Nutrition (Second Edition), B. Caballero (ed.), Academic Press, 2003, 3166-3174.
33. PAYNE, M. A., R. E. BAYNES, S. F. SUNDLOF, A. CRAIGMILL, A. I. WEBB and J. E. RIVIERE (1999): Drugs prohibited from extralabel use in food animals. Javma 215, 28-32.
34. PLEADIN, J. (2018): Hormoni u hrani životinjskog podrijetla – prirodna pojavnost ili zlouporaba? Zbornik radova s 10. međunarodne konferencije Hranom do zdravlja, 119-128.
35. PLEADIN, J. i T. BOGDANOVIĆ (2017): Anabolici u proizvodnji mesa - Učinci u farmskih životinja i opasnosti po zdravlje potrošača. Meso 19, 59-67.
36. PLEADIN, J. and T. BOGDANOVIĆ (2016): Chemical hazards in fermented meats. In: Fermented Meat Products: Health Aspects, N. Zdolec (ed.), Boca Raton, CRC Press, Taylor and Francis Group, LLC, pp. 417-449.
37. PLEADIN, J., A. VULIĆ i N. PERŠI (2012): β-adrenergički agonisti: Tvari s anaboličkim učinkom kod životinja za proizvodnju mesa. Meso 14, 51-57.
38. PLEADIN, J., A. VULIĆ, N. PERŠI and N. VAHČIĆ (2010): Clenbuterol residues in pig muscle after repeat administration in a growth-promoting dose. Meat Sci. 86, 733-737.
39. PLEADIN, J., N. PERŠI, A. VULIĆ i N. VAHČIĆ (2013): 17β-estradiol u goveđem mesu, mlijeku i krvi: Fiziološke razine i zlouporaba u stočarskoj proizvodnji. Meso 15, 44-49.
40. PLEADIN, J., N. PERŠI, B. ANTOLOVIĆ, B. ŠIMIĆ and I. KMETIČ (2011): Toksikološki aspekti anabolika u hrani životinjskog podrijetla. Croatian J. Food Sci. Technol. 3, 48-56.
41. REIG, M. and F. TOLDRÁ, (2009): Veterinary Drug Residues. In: Handbook of processed meats and poultry analysis. Nollet, L. M. L., Toldrá, F. (eds.). Boca Raton, CRC Press, Taylor and Francis Group, LLC, pp. 647-664.
42. ROSE, M. D., G. SHEARER and W. H. H. FARRINGTON (1995): The effect of cooking on veterinary drug residues in food: clenbuterol. Food Addit. Contam. 12, 67-76.
43. SAMARDŽIJA, M., B. VUDRAG i J. PLEADIN (2015): Spolni hormoni u farmskih životinja: fiziološke razine, terapeutska i anabolička primjena. Vet. stn. 46, 281-293.
44. SAMARDŽIJA, M., N. KRALJ i J. PLEADIN (2016): Estrogeni u hrani životinjskog podrijetla i utjecaj na ljudsko zdravlje. Vet. stn. 47, 513-521.
45. SAMARDŽIJA, M., T. MAGAŠ, L. RADMANIĆ and J. PLEADIN (2018): The use of testosterone in domestic animals - therapeutic and anabolic activities. Vet. stn. 49, 287-296. (in Croatian).
46. STEPHANY, R. W. (2010): Hormonal growth promoting agents in food producing animals. Handb. Exp. Pharmacol. 195, 355-367.
47. STOLKER, A. A. M. and U. A. Th. BRINKMAN (2005): Analytical strategies for residue analysis of veterinary drugs and growth-promoting agents in food-producing animals - a review. J. Chromatogr. A 1067, 15-53.
48. THOMAS, M. G., J. A. CARROLL, S. R. RAYMOND, R. L. MATTERI and D. H. KEISLER (2000): Transcriptional regulation of pituitary synthesis and secretion of growth hormone in growing wethers and the influence of zeranol on these mechanisms. Domest. Anim. Endocrin. 18, 309-324.
49. VAN GINKEL, L. A., T. BOVEE, M. H. BLOKLAND, S. S. STERK, N. G. E. SMITS, J. PLEADIN and A. VULIĆ (2016): Hormones and beta-agonists, Chemical Analysis of Non-antimicrobial Veterinary Drug Residues in Food, SAD: Wiley, 141-244.
50. WILSON, T. W., D. A. NEUENDORFF, A. W. LEWIS and R. D. RANDEL (2002): Effect of zeranol or melengestrol acetat (MGA) on testicular and antler development and agression in farmed fallow bucks. J. Anim. Sci. 80, 1433-1441.
51. WOODWARD, K. N. (2005): Veterinary pharmacovigilance. Part 2. Veterinary pharmacovigilance in practice - the operation of a spontaneous reporting scheme in a European Union country - the UK, and schemes in other countries. J. Vet. Pharmacol. Therap. 28, 149-170.
52. ZIMMERMAN, H. J. (1998): Hepatic disease. In: Toxicology of the liver. Plaa, G. L., Hewitt, W. R. (eds.), Taylor and Francis, USA, pp. 45-67.

Hormonski aktivne tvari u prehrambenom lancu od domaćih životinja do potrošača

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Primjena spolnih hormona i drugih hormonalno aktivnih tvari u domaćih životinja ima anabolički učinak, rezultirajući povećanom sintezom proteina i razgradnjom masti, kao i boljim senzorskim profilom finalnih mesnih proizvoda. Međutim, ukoliko se primjenjuju na životinjama, ove se tvari prenose u jestiva tkiva i tjelesne tekućine te se mogu naći u konačnim proizvodima životinjskog podrijetla namijenjenim konzumaciji potrošača, poput mlijeka, iznutrica, mesa i jaja. S obzirom da hormonalno aktivne tvari izazivaju intoksikacije u ljudi, njihova uporaba kao promotora rasta životinja u Europskoj uniji je zabranjena. Budući da su spolni hormoni u tkivima i tjelesnim tekućinama domaćih životinja prisutni u fiziološkim razinama, a da njihova pojavnost može biti i posljedica terapijske primjene, kako bi se dokazala ilegalna uporaba od velikog je značenja ispravno tumačenje utvrđenih razina u različitim proizvodima domaćih životinja. U posljednjim desetljećima u ovom području provedene su brojne studije, budući da za mnoge tvari s hormonskim djelovanjem još nisu istraženi toksični učinci u ljudi. Istodobno se nastavlja s razvojem i pojavnošću novih sintetičkih hormona i njihovih mješavina na crnom tržištu. Kako zlouporaba hormonalno aktivnih tvari u stočarskoj proizvodnji predstavlja potencijalni rizik za potrošače, potrebno je pratiti njihovu pojavnost u svim kritičnim točkama proizvodnje hrane. Isto tako, da bi se proizvela zdravstveno ispravna hrana i osigurala zaštita zdravlja potrošača, potreban je kontinuirani razvoj analitičkih metoda za ispitivanje velikog broja sintetičkih tvari s hormonalnom aktivnošću koje mogu biti prisutne u hranidbenom lancu od domaćih životinja do potrošača.

Ključne riječi: *hormoni, anabolički učinak, rezidue, intoksikacije, sigurnost hrane*