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HEALTHY FOOD FOR TEENAGERS PART 2: MEAT ANALYSIS FOR HORMONAL DRUGS

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Abstract: *Considering the widespread use of hormonal drugs in animal husbandry, the problem of their possible content in adolescents' food becomes urgent. The remains of synthetic steroid hormones that enter the human body along with meat can have a negative effect on the body that develops. The main goal of our work was to study meat from urban markets for the content of synthetic hormone residues in it. The article presents the results of the study for 2019-2020. From three organized markets in the city of Chernivtsi, 23 samples were tested for the content of hormonal drug residues. For the study of each sample, 6 standard qualitative reactions were carried out. A total of 138 tests were carried out. The results showed that, on average, over the period studied, only 22% of the meats tested were not contaminated with hormone residues. Most of the meat samples tested contained residues of synthetic hormonal drugs: 75% in 2019 and 82% in 2020. The most popular meat varieties among Bukovina adolescents (pork and chicken) contained residues of synthetic hormonal preparations in 100% and 71% of the samples, respectively. This may indicate an increase in the likelihood of eating unhealthy meat with all the negative consequences for the teenager's body.*
Given the small number of samples, the data obtained are preliminary and require further study.

Keywords: *meat, hormonal drugs, steroid hormones, quality response, sensitivity threshold.*

1.Introduction

The main structural elements of all body tissues are proteins. Almost half of a person's protein needs is from meat. Meat is a valuable food product, so nowadays the consumption of animal products has increased significantly. To meet consumer demand in agriculture, synthetic steroid hormones are widely used to stimulate the growth and muscle mass of animals. This creates the risk of concomitant hormones entering the human body through meat, milk, fish, eggs. The developing organism has the greatest risk from exposure to various biologically active substances [1-3].

Hormones – biologically active, chemically diverse substances produced by the endocrine glands in small quantities. The term "hormone" was coined by Bayliss and Starling in 1905 when they studied the effects of secretin. Currently, more than one and a half hundred hormones from various multicellular organisms have been described and isolated. There are many classifications of hormones. Given the topic of our study, we give a classification adopted in pharmaceutical chemistry. According to this classification, all hormones are chemically divided into two groups. The first group includes hormones – amino alcohols, amino acids, polypeptides, proteins and compounds

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related to them in chemical structure (hormones of the cerebral layer of the adrenal glands, pituitary gland, thyroid and parathyroid glands, pancreas). The second group of hormones has a steroidal structure, they include hormones of the cortical layer of the adrenal glands (corticosteroids), sex hormones (androgens, estrogens, progestogens) [4].

Hormones are carried with the blood and regulate all vital processes in the body: metabolism; morphogenesis; sexual development and reproductive function; adaptation to the conditions of existence (reactions of adaptation of the organism). This regulation is due to a biological feedback mechanism. There is also a close relationship between hormones and the nervous system, which is also bilateral. All hormones have common properties: high biological activity (hormones show their effect in very low concentrations); specificity of action (each hormone causes strictly specific corresponding reactions of organs and tissues); distance of action (hormones have their effect on the metabolism of organs and tissues located at a distance from the place of their formation); high selectivity of action (hormones show their effect only on the target organs sensitive to them, whose cells have specific protein receptors for this hormone). Excess or deficiency of hormones is equally dangerous. Hormonal disorders are the cause of failure of the physiological state of the body and the development of relevant diseases [5-7].

The unique properties of hormones have prompted the creation of a large number of hormonal drugs – drugs that contain hormones or hormonoids are similar in pharmacological effects to hormones. For medical purposes, hormones are secreted from the endocrine glands of animals, it can be both individual substances and total biopreparations. Synthetic and semi-synthetic analogues of hormones are also used. There are hormone – like substances of plants (phytohormones). These drugs are widely used in medical practice, veterinary medicine, various fields of agriculture. For example, sex hormones, their synthetic analogues and anabolic steroids: estradiol, testosterone, progesterone, trenoboloneacetate are used to stimulate meat and dairy productivity of poultry, laying birds. Hormonal drugs, in addition to the positive effects, have a very dangerous negative effect. For example, due to the great economic effect in animal husbandry, thyrostatics, which block thyroid hormones, are often used illegally. These substances disturb the heat balance of the animal's body, which provides high weight gain, the animals become hydrated (such meat is advantageous to sell raw). At the same time, they have a very negative effect on the human thyroid gland, which can lead to mental retardation, reproductive dysfunction. Sexual dysfunction and even cancer can be caused by steroid hormones. Therefore, the use of hormonal drugs, which by a similar effect outweigh natural hormones more than 100 times, requires strict control, and their impact on the development of the body – further careful study [2, 4, 8].

One of the most difficult tasks is to provide the population with food. Nutrition from birth to the last day of human life affects our body. Nutrient ingredients enter the human body with food and are converted in the process of metabolism as a result of complex biochemical transformations into structural elements of cells. A balanced diet provides our body with plastic material and energy, creates the necessary physiological and mental performance, and determines the health, activity and

life expectancy, the ability to perceive. Nutrition is an important factor in determining the health of a nation.

Hormonal drugs are widely used in modern animal husbandry for various purposes. As a result, meat sold or processed contains substances which, if constantly supplied, present a serious danger to human health [9].

Hormones, especially steroids, which are stored in meat for a long time, can withstand deep freezing and high temperatures. Together with food, they are easily absorbed in the intestines, enter the bloodstream and interfere with the work of organs and systems. Pregnant women, children, and adolescents are most prone to this effect. The adolescent's body is actively growing, and this happens under the influence of the endocrine system. It is her delicate work that ensures the harmonious development of both the physical and the mental. Even small hormonal changes at this age can have consequences. Residual amounts of hormonal drugs in meat significantly disrupt hormonal processes, causing serious illness. For example, eating meat that contains male and female sex hormones can cause premature puberty in adolescents or lead to malformation. Steroids can act as a factor in the development of obesity in children. Synthetic estrogens have been shown to be the most dangerous steroids for human health. These substances are carcinogenic and can stimulate the development of hormonally active tumors of the breast [10].

In violation of the terms of use of drugs, as well as the shutter speed of animals to excrete a stimulant from the body, harmful compounds remain in the meat. To prevent the negative effects of the use of bioactive substances, there are mandatory conditions for keeping animals before slaughter until the complete elimination of hormonal drugs from their body, but these conditions are not always met[6].

2. Materials and methods

Meat is one of the most popular foods. For a healthy diet, information about the presence of hormones in the meat we eat is very relevant. The purpose of our work was to investigate the content of hormone residues in meat from the city markets; justify the need to monitor meat for steroid hormone residues as a guarantee of safe and healthy nutrition for adolescents. 23 samples of meat from the markets of Chernivtsi were tested for the presence of residues of hormonal drugs using standard qualitative reactions in 2019-2020 years. To study each sample, 6 qualitative reactions for the presence of hormones were performed. A total of 138 qualitative tests for hormonal drugs were performed. For qualitative reactions, a concentrate extract from the test meat sample was used, which was prepared for each sample separately. Weighing the prepared minced meat according to state standard, weighing 5 g was transferred to a conical flask with 20 ml of twice boiled distilled water; with triple stirring, boiled for 15 minutes The resulting extract was filtered.

Hormones are chemical compounds that have certain chemical functional groups, each of which can be determined by an appropriate qualitative reaction. Due to the fact that the amino acids that are part of the animal protein have identical functional groups with hormones, the protein was denatured by boiling the filtrate for ten minutes.

Natural hormones undergo chemical transformations in living organisms and in their usual form can not be manifested by qualitative reactions. Also, for each of the

qualitative reactions there is such a thing as a "threshold of sensitivity", which directly depends on the concentration of the substance being determined. It is known that in the studied samples the initial concentration of synthetic hormones is much lower than the concentration of these substances in animals [8]. That is why when studying the filtrate of one sample in the experimental series of qualitative reactions in some cases, we observed a positive analytical effect of "+" (positive reaction) – the formation of sediment, gas evolution, turbidity, etc .; and in others these signs were absent, which was interpreted as a negative analytical effect "-" (negative reaction).*The lowest concentration of hormone that can be detected is the open minimum, which is less than one microgram in the minimum volume of the extremely dilute solution* [11].

Qualitative analysis of steroid hormones in the studied samples was carried out taking into account the chemical classification according to standard methods.

The structural basis of steroid hormones is the skeleton of the hydrocarbon cyclopentaneperhydrophenanthrene (Fig. 1.).A common reaction for all steroid hormones and their synthetic analogues is the reaction with concentrated sulfuricacid.

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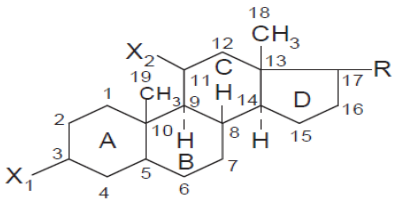

Fig. 1. General formula of steroid hormones

Reaction I. When dissolved in concentrated sulfuric acid and heated, the substance gives a specific color, sometimes fluorescence, with the subsequent addition of water, chloroform, iron (III) solution of ammonium sulfate color changes, there is a specific fluorescence.

Steroid hormones that have a keto group in position 3(Fig. 2.), enter a substitution reaction with hydroxylamine hydrochloride, phenylhydrazine,2,4-dinitrophenylhydrazine, isoniazid – precipitation with a characteristic melting point is observed or a characteristic color appears(*yellow,orange-red*).

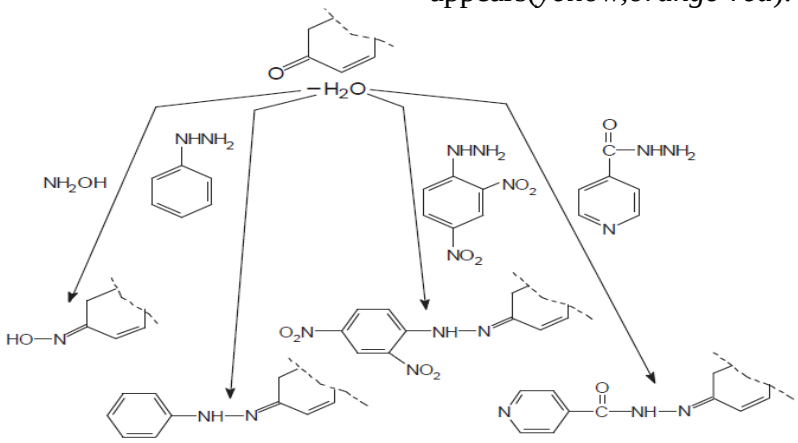

Fig. 2. Reactions of steroid hormones that have a keto group

Reaction II. For the identification of hormones having hydroxy groups in positions 3 or 17, the formation of esters is often used: acetates, benzoates. The reaction is carried out in 80% acetic acid, and gently concentrated sulfuric acid is added – an orange color appears at the phases boundary; the upper phase is characterized by yellow-green fluorescence. The esterification reaction (Fig. 3.), resulting in the formation of an ester group, which can be detected using a hydroxamic test (Fig. 4.).

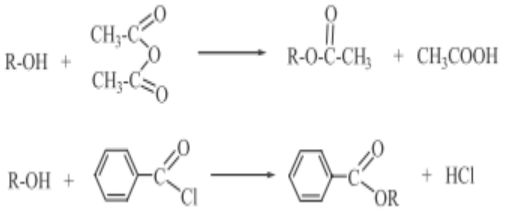

Fig. 3. Esterification reactions

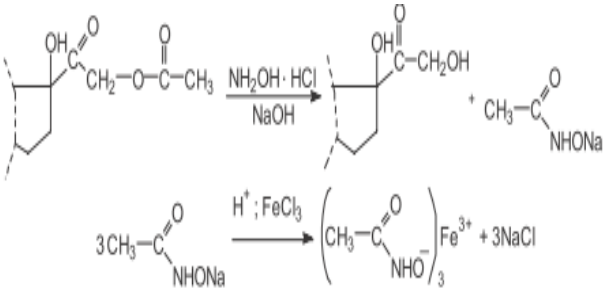

Fig. 4. Hydroxamic test

Reaction III. Hydrolysis of hormones with potassium hydroxide in ethanol produces estradiol, which precipitates when diluted with water and acidified with hydrochloric acid.

Reaction IV. Steroid hormones are dissolved in sulfuric acid and added to ammonium sulfate solution (III). The precipitate is reddish-brown; after dilution with water, a pink-red precipitate precipitates.

Reaction V. The extract concentrate of hormones is dissolved in chloroform, a solution of $FeCl_3$ and pyridine are added. There is a blue color that turns green from the excess pyridine: the phenolic groupAlso, iron trichloride can act as an oxidant for alcohol hydroxyl (Fig. 5.).

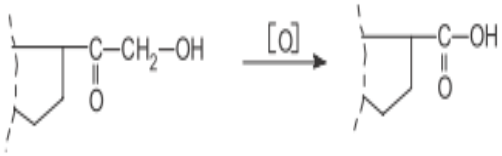

Fig. 5. Oxidation reaction of alcohol hydroxyl

Reaction VI. When added to the test sample isoniazid in the presence of hydrochloric acid, a yellow color appears [11-12].

3. Results and discussion

To study the presence of hormonal drugs in meat at random in the markets of Chernivtsi, 12 samples of different types of meat were selected (2019). We performed 72 qualitative reactions to the content of hormonal drugs in the studied samples, 13 of which showed a positive effect, ie 18% of specific reactions had a sufficient threshold of sensitivity to detect hormonal drugs in the studied meat samples (Table 1). Tests for synthetic

hormones showed that only in three samples (№1 rabbit; №5 veal; №8 chicken) all qualitative reactions were negative, ie it can be assumed that 25% of the studied meat did not contain synthetic hormones. Qualitative reactions gave a positive analytical effect in 9 samples, which indicates the presence of hormonal drugs in 75% of the studied meat.

Table 1

The results of the study of the presence of hormonal drugs in meat products from the markets of Chernivtsi for 2019 year

№ meat sample	Kind of meat	Sampling market	Reaction number					
			I	II	III	IV	V	VI
1	Rabbit	Lower	-	-	-	-	-	-
2	Chicken	Main	-	-	-	+	-	-
3	Pork	Main	-	-	+	+	-	-
4	Chicken	Main	+	-	-	-	-	-
5	Veal	Central	-	-	-	-	-	-
6	Pork	Central	+	-	-	-	-	-
7	Chicken	Central	-	-	-	+	-	-
8	Chicken	Lower	-	-	-	-	-	-
9	Veal	Main	+	-	+	-	-	-
10	Veal	Central	+	-	+	-	-	-
11	Pork	Central	+	-	-	-	-	-
12	Pork	Lower	+	-	+	-	-	-

Notes:*I; II; III; IV; V; VI – see Materials and methods; «+» – positive reaction; «-» –negative reaction.*

In order to determine the trend of contamination of meat with hormonal drugs, similar studies were conducted in 2020 (Table 2). 66 qualitative reactions to the content of hormonal drugs in the test samples were performed, 14 of which showed a positive effect, which is 21% of the total. Compared to 2019, we see an increase in this indicator by 3%.

In 2020, only in two samples (№1 chicken; №2 rabbit) all qualitative reactions were negative. It can be assumed that 18% of the studied meat did not contain synthetic hormones, and 82% (9 samples) were contaminated with hormonal drugs.

Table 2

The results of the study of the presence of hormonal drugs in meat products from the markets of Chernivtsi for 2020 year

№ meat sample	Kind of meat	Sampling market	Reaction number					
			I	II	III	IV	V	VI
1	Chicken	Central	-	-	-	-	-	-
2	Rabbit	Main	-	-	-	-	-	-
3	Chicken	Lower	-	-	+	+	-	-
4	Veal	Central	+	-	-	-	-	-
5	Pork	Central	-	-	+	-	-	-
6	Veal	Central	+	-	+	-	-	-
7	Chicken	Main	-	-	-	+	-	-
8	Pork	Lower	-	-	+	-	-	-
9	Pork	Main	+	-	+	-	-	-
10	Veal	Central	+	-	+	-	-	-
11	Pork	Central	+	-	+	-	-	-

Notes: *see table 1.*

Compared to 2019, we see an increase in this indicator by 7%. This may indicate an increase in the use of hormonal drugs in livestock and an increase in the likelihood of purchasing unsafe meat.

Analyzing the results for the entire period of research (Fig. 6), we can conclude that about 78% (18 of 23 samples) of the studied meat – contaminated with residues of hormonal drugs. Steroid hormones are more likely to enter the body of a teenager along with meat. Synthetic steroid hormones can adversely affect the sexual development of adolescents, be a cause of obesity in children.During the experiment, it was found that only 22% of meat (5 of 23 samples: rabbit, chicken and veal) were uncontaminated by hormonal drugs. The most commonly used type of pork in 100% (8 samples) contained residues of hormonal drugs. Hormone contamination of chicken was detected in 71% (5 samples out of 7). Veal samples contained hormones in 83% (5 samples out of 6). 100% (2 samples) of rabbit had no residues of hormonal drugs.

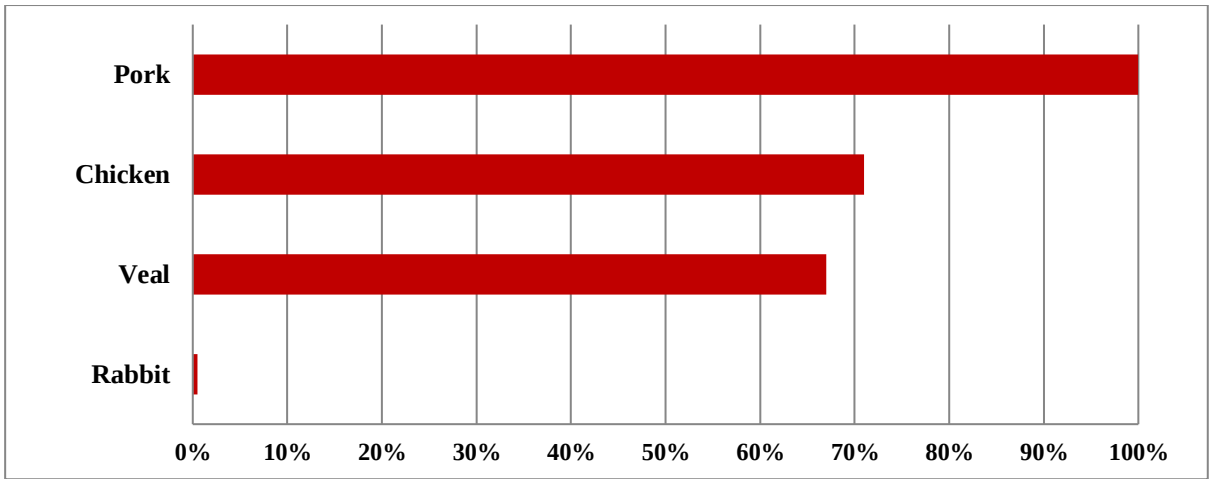

Fig. 6.The content of residues of synthetic hormones in samples of popular meat varieties

Given the small number of samples, the data obtained are pilot in nature and require further study.

4.Conclusion

78% of the meat tested is contaminated with hormonal residues. There is a tendency to increase the content of synthetic hormones in meat from the markets of Chernivtsi. 22% of the studied meat (rabbit, chicken and veal) does not contain residues of hormonal drugs. The most common types of meat: 100% of the tested pork samples contain residues of hormonal drugs; 71% of chicken contains residues of synthetic hormones. Hormone steroids can get into the body of a teenager along with meat. The content of harmful substances in meat does not depend on its type. The obtained data are of a pilot nature and require further research.

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