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# INVESTIGATION OF ZERANOL IN BEEF OF UKRAINIAN PRODUCTION AND ITS REDUCTION WITH VARIOUS TECHNOLOGICAL PROCESSING

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#### ABSTRACT

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Synthetic growth stimulants are widely used to get high productivity of animals. These preparations can accumulate in the meat and their residual quantities will adversely affect the health of consumers. The purpose of the work was to monitor the content of zeranol, growth stimulant of ruminants in beef which goes to meat processing enterprises of the Western region of Ukraine and to determine the effect of heat treatment on its quantity. It was found out that 29.8% of beef samples taken at meat processing enterprises contained a stimulant for the growth of ruminant zeranol. It was found that during the storage of beef samples frozen at a temperature of -18 °C with different content of zeranol there is a decrease in its number. The most intense process of destruction of zeranol occurred during the first month of storage, during this period of time the amount of zeranol is reduced by an average of 20%, regardless of the initial content. Within two months of storage of frozen beef, the content of zeranol decreases by  $28.2 \pm 0.17\%$ , and at the end of the sixth month its quantity decreases to  $33.2 \pm 0.58\%$ . It was also found that the dynamics of zeranol reducing in beef samples with large quantities (22.5  $\mu$ g.kg<sup>-1</sup>) and small (2.3 µg.kg<sup>-1</sup>) were the same. It was set up that during 30 min of meat cooking there was a decrease in the content of zeranol 24.7  $\pm 0.23\%$  and 32.0  $\pm 0.35\%$  for 60 min, compared to its content in fresh meat. At the same time, when stored in the frozen state and subsequent cooking, the reduction of zeranol content in meat was  $39.3 \pm 0.3\%$ . Therefore, it is proposed to revise and amendments into the regulatory documents of Ukraine regarding the control and supervision of the presence of hormone (zeranol) residues in meat and meat products in order to prevent their sale and consumption by humans.

Keywords: beef; zeranol; frozen meat; synthetic growth promoters; meat temperature processing

#### INTRODUCTION

The meat industry is one of the main branches of agriculture, which provides the population with food rich in high protein. Currently, beef is an important part of the human diet, since meat has good taste, high nutritional levels and is also considered a dietary product (Tonu, 2013; Salata et al., 2017). However, various synthetic hormonal growth promoters have become widely used for short periods of time in animal husbandry, in particular: zeranol, trenbolone acetate, diethylstilbestrol and others (Galbraith, 2002; Brynes, 2005; Azza, Sania and Weam, 2015; Lykholat, Grigoryuk and Lykholat, **2016**). Meat obtained from animals on the use of anabolics is characterized by a gentle consistency and lower fat content. However, according to many researches of many scientists (Larrea and Chirinos, 2007; Jeong et al., 2010; Wang et al., 2013), excessive amounts of residues of hormonal preparation in meat and meat products adversely affect the health of consumers, causing various

metabolic disorders and causing cancer. Therefore, in the countries of the European Union it is forbidden to use hormonal preparations – stimulants of growth of live weight of ruminants and it is regulated by directives (EC, 1996a; EC, 1996b). At the same time in countries of Latin America and the USA the use of synthetic preparations in animal husbandry is allowed by national legislation (CFR, 1999).

Our attention was drawn to the synthetic stimulator anabolic zeranol, known as  $\alpha$ -zearalanol, it is a nonsteroidal estrogen of the lactone group of resorbic acid and is used as an implant in the ear of cattle to increase muscle mass (Leffers et al., 2001). Ukrainian legislation does not oblige the definition of this anabolic in beef at slaughterhouses and when implemented within the state. Therefore, researches on the presence of zeranol in the bovine muscle grown in Ukraine in domestic scientific publications are virtually none. Therefore, conducting experimental researches on the monitoring of zeranol in beef at meat processing enterprises in Ukraine will allow to determine the real status of its circulation. Besides, based on the obtained information, it will be possible to propose appropriate amendments to the legislation of Ukraine on the safety of meat and meat products.

The purpose of the work was to monitor the content of zeranol, growth stimulant of ruminants in beef which goes to meat processing enterprises of the Western region of Ukraine and to determine the effect of heat treatment on its quantity.

# Scientific hypothesis

The main hypothesis of the investigation is in the detected beef containing the synthetic anabolic stimulant zeranol, which goes to meat processing enterprises of the Western region of Ukraine and the possible use of meat processing that would reduce the amount of meat.

# MATERIAL AND METHODOLOGY

Research on beef meat was conducted during years 2016 – 2018. Samples of beef meat were selected at the meat processing enterprises of the Western region of Ukraine to determine the amount of zeranol. Determination of zeranol in meat was performed using the test system for enzyme immunoassay RIDASCRIN® Zeranol (Art. No.:R3301) (manufactured by firm Art-Biopharm/R-Biopharm, Darmstadt, Germany) at the Stepan Gzhytskyj Lviv National University of Veterinary Medicine and Biotechnologies.

# Statistical analysis

Statistical processing of the results was carried out using methods of variation statistics using the program Statistica 9.0 (StatSoft Inc., USA). Non-parametric methods of research were used (Wilcoxon-Mann-Whitney test). The arithmetic mean (x) and the standard error of mean (SE) were determined. The difference between the comparable values was considered to be significant for p < 0.05.

# **RESULTS AND DISCUSSION**

The results of the searches of the content of zeranol in fresh beef are shown in Figure 1. From Figure 1 it can be seen that, on average, 30% of beef samples taken from meat at processing enterprises in the Western region of Ukraine contained a stimulant for the growth of ruminant zeranols. The number of negative samples without zeranol content was 70.2  $\pm$ 2.1%. The detected amount of zeranol in meat was different (Figure 2). The highest number of samples – 35.7% with the content of zeranol was found in the smallest range from 1 to 5 µg.kg<sup>-1</sup>. The number of samples with zeranol content from 5.1 to 10.0 µg.kg<sup>-1</sup> was found in 28.6%. A significant number of samples – 21.5% contained zeranol at high concentrations from 10.1 to 15.0 µg.kg<sup>-1</sup> and 14.2% of the investigated samples had its content greater than 15.1 µg.kg<sup>-1</sup>.

Thus, the conducted searches have established the fact of receipts at processing plants of the Western region of Ukraine of beef containing the prohibited in the European Union countries the stimulant of growth of live mass of ruminants – zeranol.

Considering that when consuming food products containing residues of hormonal preparation, there is a violation of metabolism in humans we conducted research of the influence of different technological processes of temperature meat processing on reducing of zeranol content. In Figure 3 the results of the research are presented of changes in the content of zeranol in beef during its storage in the frozen state at a temperature of -18 °C.

The results of the research indicate that there is no complete reduction in the content of zeranol in meat during the six-month refrigerated storage. However, we note the same dynamics of zeranol reducing in beef samples, which contained both large and small amounts. The most intense process of destruction of zeranol occurred during the first month of storage. During this period of time, the amount of zeranol is decreased by an average of 20% in all samples, regardless of initial content. During the two-month term of storage of frozen beef, the content of zeranol was decreased by  $28.2 \pm 0.17\%$ , compared to the initial amount in fresh meat. Next storage of beef for three months did not cause a significant decrease in the content of zeranol and at the end of the sixth month their number was decreased by  $33.2 \pm 0.58\%$ .

Analogical results of the research on the dynamics of changes in zeranol in beef were obtained using the freezing temperature of meat -25 °C and -30 °C. However, when storing meat containing zeranol in the cooled state at +2 to +4 °C and frozen for -2 to -3 °C for 20 days, no decrease in the amount of zeranol was observed.

Thus, obtained scientific data indicate that the storage of meat in the frozen state has a positive effect on the dynamics of reducing the content of zeranol. This process is particularly active within two months of the start of storage.

In Figure 4 results of researches are given of the influence of the cooking process on the dynamics of changes in zeranol in beef. Installed that the process of cooking also affects to reduce the content of zeranol in beef. After 30 minutes of meat cooking, the amount of zeranol in all samples was decreased by  $24.7 \pm 0.23\%$  and over the next 30 minutes to  $32.0 \pm 0.35\%$ , compared to its content in fresh meat. Further heat processing up to 120 minutes did not cause a significant decrease in the zeranol content compared to the 60 minutes of cooking process.

Consequently, during cooking, about 30% of the zeranol from the meat to the broth is destroyed or transferred, which is almost the same amount as in the frozen storage process.

Changes in the content of zeranol in meat were also examined after 6 months of storage in the frozen state and subsequent cooking for 60 min (Figure 5). After freezing, the decrease in the amount of zeranol was found to be  $33.2 \pm 0.58\%$ , and the subsequent meat cooking process led to a slight reduction of the zeranol content to  $6.1 \pm 0.2\%$ . Therefore, in general, after the freezing process and further meat cooking process, the amount of zeranol is reduced by  $39.3 \pm 0.3\%$ .

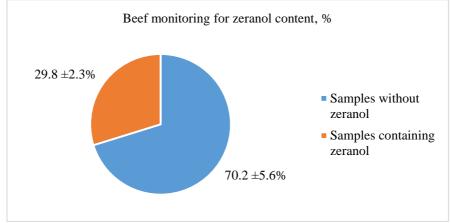


Figure 1 Searches of beef for the presence of zeranol.

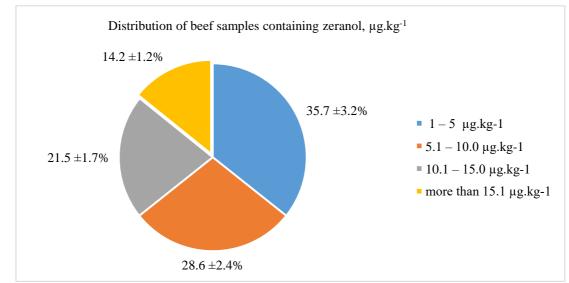
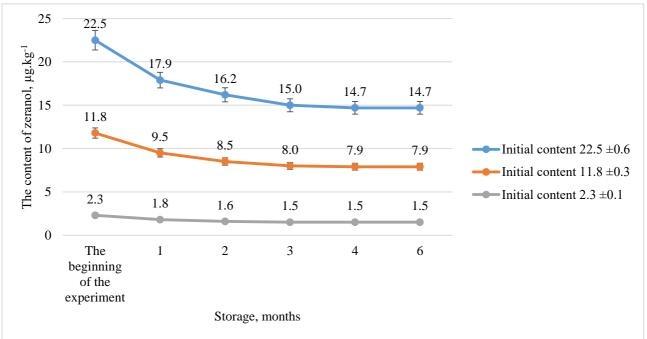


Figure 2 Characteristic of beef samples by zeranol content.



**Figure 3** The intensity of the process of reducing zeranol in frozen beef for storage at a temperature of  $-18 \pm 1$  °C for 6 months.

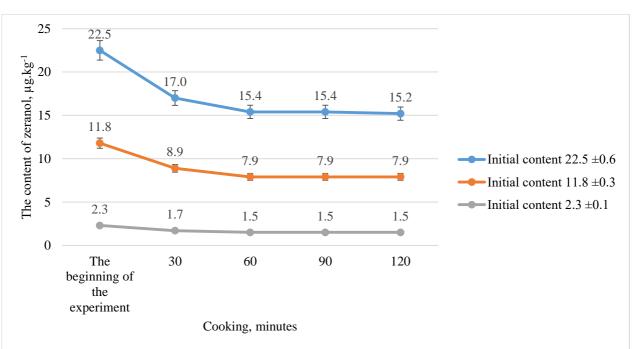


Figure 4 The intensity of the process of reducing zeranol in beef after cooking.

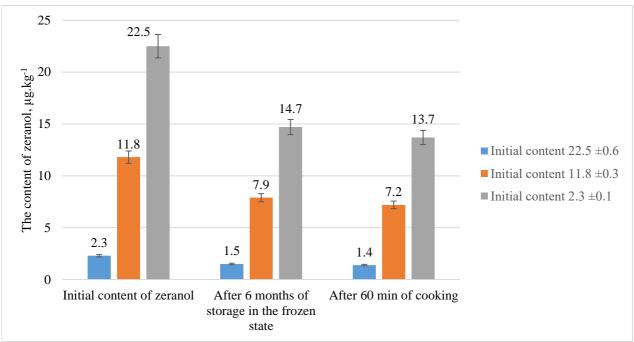


Figure 5 The intensity of the process of reducing zeranol in beef after 6 months of storage and 60 min of cooking

Meat and meat products are essential components of the human diet, so first of all they must be safe in biological, chemical and physical terms. It is currently widely used in the world to increase the growth of live weight of animals and improve the use of feed anabolic hormonal preparations of synthetic production (Galbraith, 2002; Brynes, 2005; Azza, Sania and Weam, 2015; Lykholat, Grigoryuk and Lykholat, 2016). However, in violation of the fattening technology, these preparations can accumulate in the products of slaughter, and their residual quantities adversely affect the health of consumers (Larrea and Chirinos, 2007; Jeong et al., 2010; Wang et al., 2013). Synthetic anabolic stimulator – zeranol is banned in the European Union for use in veterinary medicine because of its adverse influence on humans. At the same time in the USA this preparation is allowed and its amount in ruminant muscles is allowed up to 2  $\mu$ g.kg<sup>-1</sup> and 10  $\mu$ g.kg<sup>-1</sup> in liver (CFR, 1999). In Ukraine, regulatory and legal documents do not regulate the definition of this anabolic in domestic beef and imported abroad.

Our research has found that 29.8% of beef samples taken at meat processing enterprises in the Western region of Ukraine contained a stimulant for the growth of ruminant zeranol. In addition, a significant number of samples -35.7% contained zeranol at high concentrations greater than 10 µg.kg<sup>-1</sup>. Our researches are consistent with the results of scientists from other European countries (Borazan et al., 2007; Salata, et al., 2017), who reported the detection of zeranol in 100% of the investigated meat samples and meat products. Consequently, conducted research have found that there is an income for the processing of beef, which contains the hormone to stimulate the growth of live weight - zeranol. Therefore, we consider it necessary to revise and making changes in the regulatory and legal documents of Ukraine on the control and supervision of the presence of residues of hormone preparations (zeranol) in meat and meat products in order to prevent their sale and consumption by humans. In addition, at the present time, due to the large number of food supplies from different countries, it is impossible to guarantee the safety of meat in terms of residues of animal growth stimulants (Gladij and Sychevs'kyj, 2018). Therefore, when importing beef in Ukraine, it is necessary to control it for the presence of residual quantities of zeranol.

Considering the fact of receipts for beef processing with high content of zeranol, we conducted a research to determine the influence of different types of heat treatment and storage of beef on the quantitative content of zeranol. It has been established that storage of beef in chilled and frozen state for 20 days does not affect the content of zeranol. At the same time, it was found that the storage of beef samples in the frozen state at a temperature of -18 °C with different content of zeranol decreases its amount. Thus, the most intense process of destruction of zeranol occurred during the first month of storage, during this period of time the amount of zeranol was decreased by an average of 20%, regardless of the initial content. During the two-month term of storage of frozen beef, the content of zeranol was decreased by  $28.2 \pm 0.17\%$ , and at the end of the sixth month its amount was decreased to 33.2  $\pm 0.58\%$ . It was also found out that the dynamics of zeranol reducing in beef samples with large quantities  $(22.5 \ \mu g.kg^{-1})$  and small  $(2.3 \ \mu g.kg^{-1})$  were the same. Therefore, conducted researches have given us reason to affirm that the storage of meat in the frozen state has a positive influence on changes in the content of zeranol, that is, it significantly reduces its amount. With the small content of zeranol in meat, it can be reduced to the limit of 2  $\mu$ g.kg<sup>-1</sup> in the US. In addition, it was found out that during 30 min of cooking meat there is a decrease in the content of zeranol by 24.7 ±0.23%, and for 60 min by  $32.0 \pm 0.35\%$ , compared with its content in fresh meat. Further heat processing did not cause a significant decrease in the content of zeranol. At the same time, when stored in the frozen state and subsequent cooking, the reduction of zeranol content in meat was 39.3 ±0.3%. In researches (Braekevelt et al., 2011) it was also found a decrease in the amount of estrogen hormones in beef by 25 - 30% after its two-hour cooking. Moreover, the researchers found that in the non-fat beef, the process of disintegration of hormonal preparations was less and ranged from 5 to 20%. Thus, cooking reduces the content of anabiolics in meat, but in the presence of high concentrations, this process is not effective enough to produce a safe product.

#### CONCLUSION

In summary, it can be noted that the revealed fact of earnings to the processing of beef containing zeran in high concentrations, therefore, we consider it necessary to carry out a selective control and selection of beef at meat processing enterprises in order to establish safety indicators, namely the content of zeranol. To reduce the number of detected samples, the meat must be frozen at -18 °C and stored for at least two months and then subjected to heat processing (cooking). However, if significant concentrations of zeranol (greater than 2  $\mu$ g.kg<sup>-1</sup>) are found, such meat is prohibited. Consequently, the planned monitoring will allow the monitoring and analysis of the situation of beef zeranol in Ukraine.

# REFERENCES

Azza, E. A., Sania, T. E., Weam, M. B. 2015. Study on Hormonal and Heavy metals residues in fresh beef meat. *Egyptian Journal of Chemistry and Environmental Health*, vol. 1, no. 1, p. 552-562. Available at:

Borazan, G. O., Karagul, H., Çelik, S., Ünal, N., Pekcan, M., Sel, T. 2007. Determination of zeranol residues and the serum testosterone oestrogene and progesterone levels in lambs around Ankara region. *Veteriner Fakültesi dergisi*, vol. 54, no. 1, p. 7-10. https://doi.org/10.1501/Vetfak\_0000000247

Braekevelt, E., Lau, B. P. Y., Tague, B., Popovic, S., Tittlemier, S. A. 2011. Effect of cooking on concentrations of  $\beta$ -estradiol and metabolites in model matrices and beef. *Journal of Agricultural and Food Chemistry*, vol. 59, no 3, p. 915-920. <u>https://doi.org/10.1021/jf103064q</u>

Brynes, S. D. 2005. Demystifying 21 CFR Part 556tolerances for residues of new animal drugs in food. *Regulatory Toxicology and Pharmacology*, vol. 42, no. 3, p. 324-327. <u>https://doi.org/10.1016/j.yrtph.2005.05.009</u>

CFR. 1999. Food and drugs, 21, ch. I, part 556.240. Government Printing Office, Washington DC, USA. Available at:

http://www.access.gpo.gov/nara/cfr/waisidx\_99/21cfrv6\_99.html

EC. 1996a. 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  $\beta$ -agonists, and repealing Directives 81/602/EEC, 88/146/EEC and 88/299/EEC OJ L 125, 23.5.1996, p. 3-9.

EC. 1996b. 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 and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC OJ L 125, 23.5.1996, p. 10-32.

Galbraith, H. 2002. Hormones in international meat production: biological, sociological and consumer issues. *Nutrition Research Reviews*, vol. 15, no. 2, p. 293-314. https://doi.org/10.1079/NRR200246

Gladij, M. V., Sychevs'kyj, M. P. 2018. Meat-processing industry of Ukraine in the global food system. *Bulletin of Agricultural Science*, vol. 5, p. 5-11. https://doi.org/10.31073/agrovisnyk201805-01

Jeong, S. H., Kang, D. J., Lim, M. W., Kang, C. S., Sung, H. J. 2010. Risk assessment of growth hormones and antimicrobial residues in meat. *Toxicological research*, vol. 26, no. 4, p. 301-313. https://doi.org/10.5487/TR.2010.26.4.301 Larrea, F., Chirinos, M. 2007. Impact on human health of hormonal additives used in animal production. *Revista de Investigacion Clinica*, vol. 59, no. 3, p. 206-211.

Leffers, H., Naesby, M., Vendelbo, B., Skakkebaek, N. E., Jørgensen, M. 2001. Oestrogenic potencies of Zeranol, oestradiol, diethylstilboestrol, Bisphenol-A and genistein: implications for exposure assessment of potential endocrine disrupters. *Human Reproduction*, vol. 16, no. 5, p. 1037-1045. https://doi.org/10.1093/humrep/16.5.1037

Lykholat, O. A., Grigoryuk, I. P., Lykholat, T. Y. 2016. Metabolic effects of alimentary estrogen in different age animals. *Annals of Agrarian Science*, vol. 14, no. 4, p. 335-339. <u>https://doi.org/10.1016/j.aasci.2016.09.012</u>

Salata, V., Kuhtyn, M., Semanjuk, V., Perkij, Y. 2017. Dynamics of microflora of chilled and frosted beef during storage. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies*, vol. 19, no. 73, p. 178-182. https://doi.org/10.15421/nvlvet7337

Tonu, P. 2013. Toxicological issues associated with production and processing of meat. *Meat Science*, vol. 95, no. 4, p. 844-853. <u>https://doi.org/10.1016/j.meatsci.2013.04.032</u>

Wang, Y., Li, L., Wang, C. C., Leung, L. K. 2013. Effect of zeranol on expression of apoptotic and cell cycle proteins in murine placentae. *Toxicology*, vol. 314, no. 1, p. 148-154. https://doi.org/10.1016/j.tox.2013.09.011

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