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DEVELOPMENT OF METHOD FOR DETERMINATION OF BETA-AGONISTS CONTENT IN MEAT AND DAIRY PRODUCTS BY ENZYME IMMUNOASSAY

The article presents the results of the development of methods for determining the content of beta-agonists in livestock products. The paper analyzes the issue of the use of hormones in the feed for farm animals, existing threats and risks to human health, determines the maximum allowable levels of clenbuterol residues and ractopamine in the animal products, describes the existing methods for determining the content of beta-agonists physical-chemical and chemical-biological methods. The objects of investigation were frozen beef slabs, beef liver, milk. The content of ractopamine and clenbuterol in the samples was determined by ELISA method. Based on the data basic the metrological characteristics of the determination of clenbuterol in animal products were established – the detectable concentration range from 0.04 to 2000 ng/kg, the relative standard deviation of repeatability 0.8% (meat), 0.7% (milk), the relative standard deviation of intermediate precision of 1.0% (meat), 0.9% (milk), and ractopamine – detectable concentration range from 0.02 to 8,100 ng/kg, the relative standard deviation of repeatability 0.3% (meat), 0.4% (beef liver), the relative standard deviation of the intermediate precision of 0.8% (meat) and 0.9% (beef liver). Extended standard uncertainty measurements of clenbuterol and ractopamine did not exceed 1%. The results will be used to develop the methods for determining the content of beta-agonists by ELISA quality control and safety of animal products for human consumption.

Key words: ractopamine, clenbuterol, beta-agonists, enzyme immunoassay method, repeatability, precision.

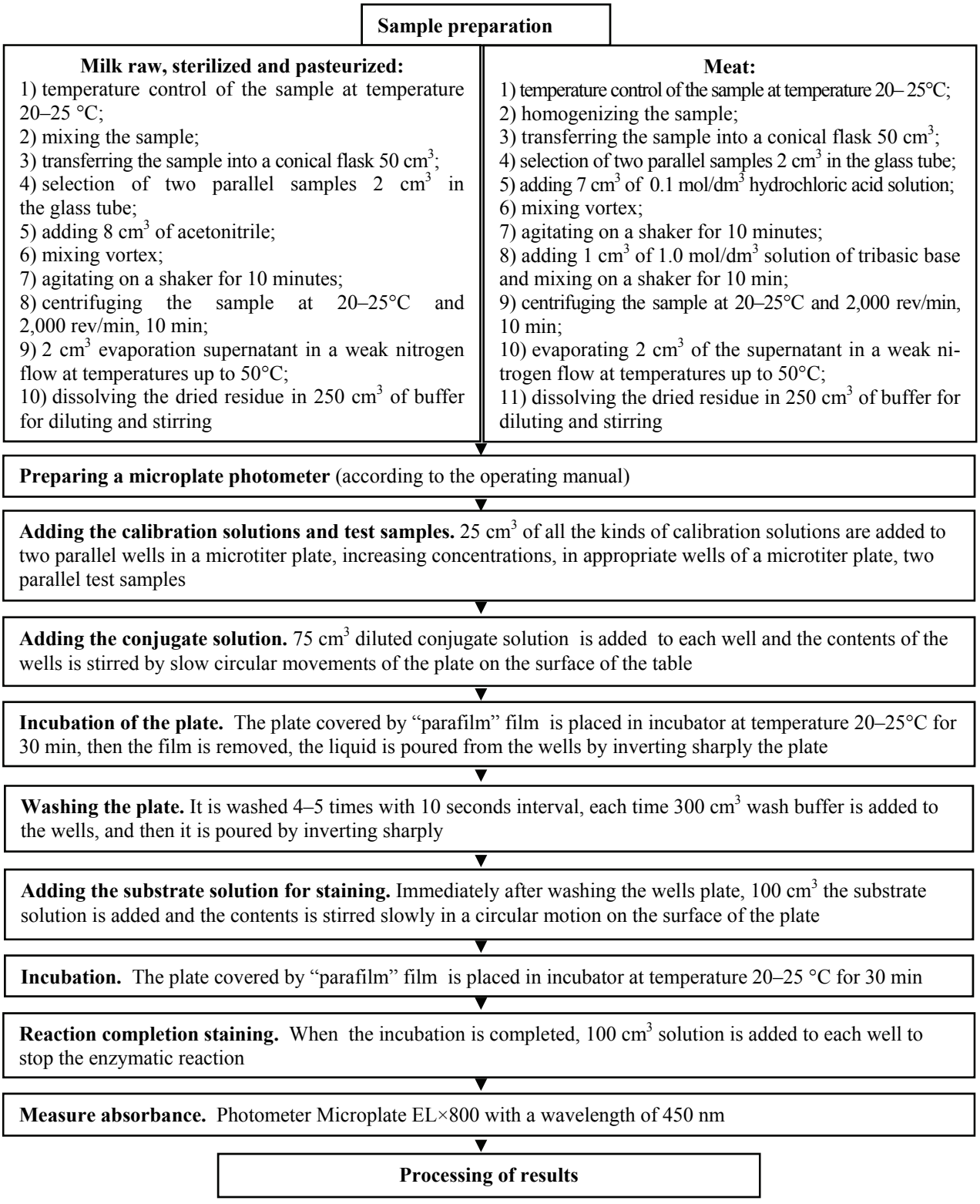
Introduction. The use of animal hormones, including beta-agonists is one of the widely discussed international issues of food raw materials and food products security. This group of drugs stimulates growth, causes reduced-mass of adipose tissue and hypertrophy of antiplaque-fibers; that supply eventually leads to the production of lean meat raw materials. The attention is paid to the use of raktopamine and clenbuterol that have beta-adrenoceptor selective action [1, 2].

Ractopamine has been approved by the Office of FDA for the US Food since 1999, by the Agency of Canada for the inspection of food products ractopamine has been approved as a feed additive for livestock cattle industry since 2003, and since 2005 ractopamine has been approved for the industrial pig farming as it increases body weight by 10% and reduces the need for feed by 6%. The use of growth promoters is permitted in 24 countries, including the United States, Canada, Austria, New Zealand, Brazil, but it is banned in 160 countries, including the EU, China, and Russia. EU Directive 96/22/EC contains a general prohibition on the use of beta-agonists [3]. 17 cases of food poisoning, containing beta-agonists have been registered in China for the past 10 years, which affected more than 1,000 people [4]. On December 7, 2012 the import of meat products containing residual amounts of beta-agonist was banned in Russia. Medicinal products based on beta-agonists are not included in the "Register of medicinal products and feed additives registered for use in the Russian Federation" [1]. According to the decree of the Ministry of Agriculture and Food of the Republic of Belarus, by December, 16, 2005 No. 78 products supplied to other countries, must be completely free from beta-agonists residues [5].

Tachycardia, muscle tremor, hypokalemia, tachyphylaxis, anxiety, headaches, and high blood pressure are side effects of beta-agonists to humans. It makes particularly dangerous consumption of meat containing residues of beta-agonists by people suffering from cardiovascular diseases [6].

FAO/WHO co-programme has set the maximum allowable level of ractopamine 0.1 mg/kg for pig and cattle meat; 0.04 mg/kg – for pig and cattle liver, and 0.09 mg/kg – for the pig and cattle kidneys [7].

Analysis of the published data shows that ion exchange, gas, immune affinity, high-performance liquid chromatography have been used for the determination of beta-agonists in products [5, 8–9]. However, the high cost of research and maintenance of the equipment, labor intensity and duration of sample preparation limit the widespread use of these methods. In addition, a well-known radio immune assay method for the determination of hormones has drawbacks; i.e. limited lifetime radioactive label, a high probability of pollution [8]. According to the information available in the scientific literature, enzyme immune assay method has a high speed of analysis setting and ease of registration data [9], so the use of this method is the most promising one.



Basic measurement of beta-agonists residues

Main part. The purpose of this work is the development of methods of measurement of beta-agonists (clenbuterol and ractopamine) in meat and dairy products by immune enzymatic assay method. The objects of research are beef frozen lump, beef liver, milk.

The microplate photometer EL×800 was used for tests. The measurement parameters of beta-agonists is shown in Figure.

The repeatability and intermediate precision were defined to evaluate the characteristics of the methodology.

Table 1

The content of beta-agonists

Hormone	Products	Content, ng/kg	MRLs, mg/kg
Clenbuterol	Meat	0.5	0.1
	Milk	0.2	–
Ractopamine	Meat	0.4	0.1
	Liver	0.5	0.04

Table 2

Characteristics of methods for determining the content of beta-agonists

Hormone	Products	Measurement range, ng/kg	The relative standard repeatability deviation S_r, %	The relative standard deviation of the intermediate precision S_R, %	Standard uncertainty u, ng/kg	Extended standard uncertainty U, ng/kg, $K = 2$, $P = 95\%$
Clenbuterol	meat	from 0.02 to 2,000.00 incl.	0.8	1.0	0.5	0.1
	milk	from 0.02 to 2,000.00 incl.	0.7	0.9	0.5	0.1
Ractopamine	meat	from 0.02 to 8,100.00 incl.	0.3	0.8	0.2	0.6
	liver	from 0.02 to 8,100.00 incl.	0.4	0.9	0.4	1.6

The proximity between research results obtained under repeatability conditions (same laboratory, one test, one and the same equipment and reagents, test for a short time) was evaluated by the ISO 5725-2 p. 7, ISO 5725-3 p. 8.2, ISO 5725-4 p. 5 [10–12]. Estimation of the intermediate precision was carried out according to ISO 5725-6-2002 [13].

The results of performance evaluation methods and research on the content of ractopamine and clenbuterol by ELISA are shown in Table 1 and 2.

As it can be seen from the data presented in Table 1, the content of hormones in the products does not exceed the permissible levels.

According to the data given Table 2, determination of the content of clenbuterol standard deviation of repeatability beef samples was 0.8%, milk – 0.7% and intermediate precision standard deviation beef S_R – 1.0%, milk – 0.9%. Limit intermediate precision determination of clenbuterol for beef was 2.9%, milk – 2.6%.

The extended standard uncertainty of Clenbuterol measurement was calculated according to the alternative method for both beef and milk; it was 0.1 ng/kg at a confidence level of $P = 0.95$.

The results of the determination of ractopamine showed that the standard repeatability deviation *Sr* of beef samples – 0.3%, liver – 0.4% and intermediate precision standard deviation beef S_R – 0.8%, milk – 0.9%. Extended standard uncertainty of ractopamine at a confidence level $P = 0.95$ for the beef sample – 0.6 ng/kg, milk – 1.6 ng/kg.

Conclusion. Experimental tests have allowed us to establish basic metrological characteristics (repeatability and intermediate precision, extended standard uncertainty) measurement of beta-agonists ELISA. Data statistical processing of the results formed the basis for the development of methods for determining the content of beta-agonists in meat and dairy products by immune enzymatic assay using microplate photometer EL×800. This technique can be widely used to control the quality and safety of animal products in agriculture, food and medicine, as well as for research purposes.

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