



# Study of Residual Antibiotics in Feed and Feed Additives Using Randox Evidence Investigator

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**Abstract.** In Kazakhstan, organic animal husbandry is also developing along with traditional livestock farming. Organic animal husbandry is one of the methods of farming that is as close as possible to natural farming. As part of the scientific program, studies were conducted on samples of feed and feed additives of domestic and imported production. As a result, samples were identified that contained antibiotics in quantities exceeding the permissible limit of the method used using the RANDOX EVIDENCE INVESTIGATOR. However, there is currently no regulatory documentation of the customs union regulating the standards for the content of residual antibiotics in feed and feed additives.

**Keywords:** organic livestock farming, feed, feed additives, antibiotics, analysis, sample.

## 1 Introduction

Organic livestock farming is an important branch of agriculture. Organic livestock farming not only maintains the health and welfare of animals but also plays an important role in providing health benefits to consumers, profits to producers and protection of the environment. While the use of veterinary drugs such as antibiotics and hormones poses a constant threat to human and human health. Organic herds typically spend the maximum amount of time on pastures, also organic, not treated with pesticides or herbicides [1].

Currently, the demand for organic livestock products, including feed and feed additives, is increasing significantly due to the expansion of livestock product markets, the

presence of developing countries such as Brazil and Argentina in the market. Exports of organic livestock products, including feed and feed additives, provide favorable opportunities for other developing countries such as India [2]. Globally, more antibiotics are used in animal husbandry than in medicine, including in countries where livestock accounts for only 20 percent of total food consumption. The widespread use of antibiotics in animal husbandry is primarily associated with disease prevention, as well as the desire to accelerate animal growth by adding such drugs to feed and feed additives [3].

In recent decades, there has been an increase in antibiotic resistance, which is primarily due to the inappropriate use of drugs. The main causes of this phenomenon include the excessive use of antibiotics in therapy, their improper administration, and the use of antibiotics as growth promoters in animal husbandry [4].

It has been found that antibiotic-based drugs can remain in the body of farm animals for a long time. This is why feed and feed additives consumed by animals and poultry, and then the products that people eat, can be contaminated with drugs and, as a result, can negatively affect humans and the environment as a whole. When using antibiotics in feeding, it is possible to stop the development of infections, improve the growth and development of the animal's body, and stabilize metabolism. But despite all the above-mentioned advantages of using antibiotics, there are also negative aspects. Antibiotics can have a bactericidal effect not only on the pathogenic microflora of the intestine, but also on some of the beneficial microflora [5].

Unlimited use of antimicrobial drugs in feeding pets contributes to the emergence of microorganisms that acquire resistance to these antibiotics. This, in turn, is one of the causes of drug resistance [6]. To ensure safety in the countries of the Customs Union, the technical regulation "On the safety of food products" (TR CU 021/2011) was adopted.

The content of antibiotics in livestock products is undesirable, but still, if an antibiotic is present, there is a MAC (maximum permissible concentration).

Unsystematic use of antibiotics leads to the accumulation of residual amounts of antibiotics in livestock products. If the antibiotic is present in quantities higher than the maximum permissible, it can cause side effects: resistance, allergies and various gastrointestinal diseases (dysbacteriosis, candidiasis), and also accumulate toxins in the body [7].

Human health depends on what he eats. When eating foods such as meat and milk, which are valuable sources of nutrition, the body can receive a significant amount of residual antibiotics contained in these products. The use of these products in the feeding diet can lead to dysbacteriosis and allergic reactions. The use of antibiotics can harm beneficial lactic acid bacteria, which makes metabolism more difficult [8].

Residues of antimicrobial drugs in feed, feed additives and animal products pose a serious problem for ensuring the safety of organic food products, since all of them are widely used in traditional animal husbandry [9]. In this regard, studies conducted to determine residual antibiotics in feed and feed additives are very relevant

## 2 Research Materials and Methods

According to the task set to develop criteria for assessing the safety of livestock products, work was carried out to select samples of different types of feed and feed additives of domestic and foreign production in the amount of 150 samples. The studies were conducted from November 2023 to February 2025. Samples of hay, straw, silage, haylage, grain, grain mixture, as well as feed additives of plant and animal origin produced in the Russian Federation were collected. radio, China, Denmark, etc.

To obtain reliable results in assessing the quality of feed and feed additives, primary importance should be given to the correct sampling.

Sampling of feed and feed additives was carried out in warehouses, feed storage areas, pastures of various agricultural formations in Akmola, North Kazakhstan, Karaganda, Pavlodar, East Kazakhstan and other regions of the Republic of Kazakhstan and imported products from Russia, China and other countries.

Sampling of hay and straw was carried out from at least 10 different places over the entire area and layer thickness, selecting bunches weighing 100-120 g so that the fallen parts of plants were also included in the sample according to GOST 27262-87 "Feed of plant origin. Sampling methods". The resulting average sample was packed in a dry plastic bag; the relevant documents were attached and delivered to the laboratory within 2-3 days.

Samples of haylage and silage were taken from the center of the trench and at a distance of 0.5-1.0 m from the wall. The average sample of silage and haylage was placed in a bag and sent to the laboratory within 24 hours from the moment of sampling.

Samples from grain, grain mixture, feed additives of different origins and formulations were selected according to the generally accepted method of GOST 13586.3-83 "Grain. Acceptance rules and sampling methods" and sent to the laboratory within 2-3 days.

In laboratory conditions, samples were prepared for further study by drying the sample in a dry-heat oven and further grinding, in accordance with the requirements of regulatory documentation. For each polyethylene bag with a sample taken, a serial registration number of the sample was used and recorded in the sample registration log. The analysis of feed and feed additive samples was performed on the Evidence Investigator analyzer (Randox, UK) using the Anti Microbial Array II Plus test system according to the manufacturer's instructions and GOST 34285-2017.

The analysis procedure includes the following steps:

1. Calibration of the device using standard calibrators included in the kit.
2. Sample preparation. The feed sample was homogenized. Next, 1 g of the sample was placed in a centrifuge tube (15 ml), 9 ml of the working concentration wash buffer were added and mixed in a vortex for 30 seconds. The sample was centrifuged at room temperature at 4000 rpm for 10 minutes. 200 µl of the supernatant were collected and 200 µl of the working concentration wash buffer was added. Mixed in a vortex before applying to the biochip.
3. Analysis of the sample. The test system was taken out of the refrigerator 30 minutes before the start of the analysis. In accordance with the instructions, solutions of working concentration were prepared. The required amount of analytical buffer and test sample

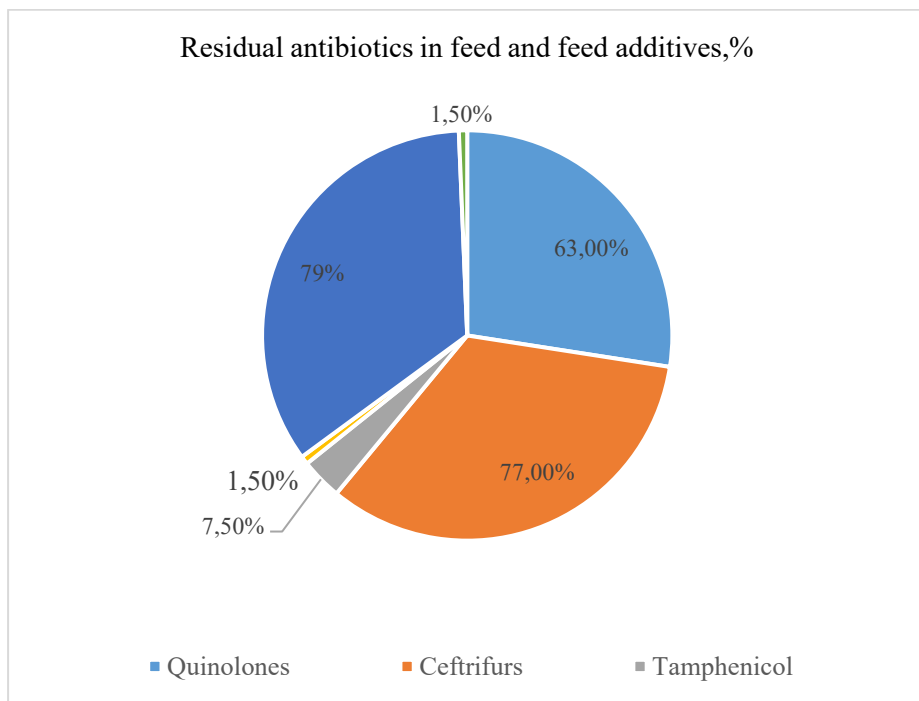
were applied to the biochip. Then the biochips were incubated in a thermosphere for 30 minutes. Then the conjugate solution was applied and incubated for another 60 minutes. The signal reagent of working concentration was prepared. After incubation, the biochips were washed with buffer. Then the signal reagent was applied to the biochip one by one and after 2 minutes it was placed in the device chamber.

4. Obtaining research results using software. Statistical processing of the research results was carried out using variation statistics methods using MS Excel.

### 3 Research Results

During the study, samples of hay, straw, silage, haylage, grain, grain mixture, as well as feed additives of plant and animal origin were taken. These samples were tested for the presence of antibiotics: quinolones, ceftiofur, tamfenicol, streptomycin, tylosin and tetracycline. The results of the analysis are presented in Figure 1.

Figure 1 presents the results of the analysis of the content of six groups of antibiotics in various types of feed: quinolones (CNL), ceftifur (CEFT), tamfenicol (TAF), streptomycin (STR), tylosin (TIL) and tetracycline (TCN). A total of 150 samples of feed and feed additives were analyzed in the studies. Of these, 66 samples were found to contain various types of antibiotics, which amounted to 44%. At the same time, out of 66 samples of feed and feed additives, 42 samples were found to contain quinolones, which amounted to 63%, and 51 samples contained Ceftifur - 77%. The largest number of feeds and feed additives contained tetracycline - 79%, which indicates the use of broad-spectrum antibiotics in the production of feeds and feed additives.



**Fig. 1.** Results of the study of residual antibiotics in feed and feed additives using the Randox Evidence Investigator device

The issue of the maximum permissible amount of antibiotics - a factor of impact on humans, animals or the environment, which is considered safe in the production of feeds and feed additives, remains important.

When analyzing open sources, official standards for the content of MPC antibiotics in feeds and feed additives were not found. The regulatory documents of the countries of the Eurasian Economic Union (EAEU), including Kazakhstan and the Customs Union, establish maximum permissible levels (MRL) for the content of antibiotics in food products of animal origin (meat and meat products, milk and dairy products), but such indicators for feeds and feed additives are absent.

Table 1 presents an analysis of the indicators of residual antibiotics in maximum permissible quantities in feeds and feed additives studied using the Randox Evidence Investigator device

As can be seen from Table 1, the maximum permissible amount (in parts per billion, ppb) is given for each antibiotic according to the determination method using the Randox Evidence Investigator device. Analysis of the table data showed that the greatest number of excesses was recorded for quinolone and ceftifur in most feed samples (hay, straw, haylage, silage). Thus, for the quinolone content in hay, the excess was 2.6, in straw 3.8. At the same time, the greatest excess of the MAC is observed in

haylage and is 33.1 parts per billion. For the content of the antibiotic Ceftifur, the excess of the MAC is observed in five types of feed and feed additives out of seven declared and is from 5.1 to 16.1. Tetracycline also exceeds the MAC in a number of samples (hay, haylage, silage). No MAC exceedances were detected for streptomycin, tylosin and tamfenicol, with the exception of individual values close to the threshold.

**Table 1.** Analysis of antibiotic residues in feed and feed additives using the Radox Evidence Investigator

Name	Types of antibiotics					
	Quinolones (QNL)	Ceftifur (CEFT)	Tamfenicol (TAF)	Streptomycin (STR)	Tylosin (TIL)	Tetracycline (TCN)
MAC (parts per billion)	<b>10,0</b>	<b>15,0</b>	<b>15,0</b>	<b>80,0</b>	<b>10,0</b>	<b>10,0</b>
hay	12,6	29,2	7,28	29,3	5	11,7
straw	13,8	31,1	4,93	3,22	3,94	7,96
haylage	43,1	25,9	7,93	21,3	4,52	15,9
silage	20,6	26,2	7,04	70,5	4,07	15,3
grain	6,20	5,29	2,73	47,6	2,05	5,68
grain mixture	6,91	20,1	4,37	27,3	2,78	9,32
Feed additives of plant and animal origin	7,83	15,0	4,72	32,9	2,81	9,35

## 4 Discussion

Organic production is not only a problem for producers in developing countries, it also offers new export opportunities. Organic agriculture is practiced by 1.8 million producers in 160 countries. The production of organically grown food products continues to increase steadily by 15% per year. Organic livestock farming is still developing, and further research is needed to make it sustainable [10].

To increase the export of organic livestock products and develop strong domestic markets, it is important to keep and feed farm animals without the use of antibiotics, or to develop a system of maximum permissible levels of antibiotics in feed and feed additives. Almost all countries investing in their organic agricultural sector have taken

note of the change in imports of products in industrialized countries, requiring changes in their production standards for antibiotic residues to enable their export to major developing countries in Asia such as China, India, Thailand, Malaysia, Sri Lanka, etc. [11].

According to FAO, livestock accounts for almost 40% of total agricultural production in developed countries and 20% in developing countries, supporting the livelihoods of at least 1.3 billion people worldwide. Thirty-four percent of the world's food protein supply comes from livestock. In 2021, the organic market reached almost €125 billion, an increase of €4 billion or about 3% (FiBL, 2023). The global organic meat products market is expected to grow from USD 16.67 billion in 2022 to USD 17.99 billion in 2023, with a compound annual growth rate (CAGR) of 7.9% (The Business Research Company Report, 2023). Organic dairy products account for 22.3% of the total organic agricultural products in the world. The global organic dairy products market reached USD 23.9 billion in 2022, and the market is expected to reach USD 36.0 billion by 2028, showing a growth rate (CAGR) of 6.5% during 2023-2028 (IMARC Group Report, 2023) [12].

Despite the low level of livestock development, organic livestock production should become a priority for this industry. Since the livestock products obtained today, including feed and feed additives, are essentially organic, but due to the lack of standards and Institutes for certification of organic products, it does not allow registering and selling domestic livestock products as organic [13].

## 5 Conclusion

At the beginning of the 20th century, antibiotics began to be widely used in agriculture. However, after a short period of time, bacterial resistance to the drugs began to appear, which served as an impetus for regulating their use. At the same time, the use of antibiotics is minimized by using pre- and probiotics, plant extracts, organic acids, bacteriophages and vaccines and immunostimulants for prophylaxis.

Our results indicate the use of antibiotics in the production of various types of feed and feed additives for feeding and raising farm animals and poultry in Kazakhstan. At the same time, these products do not correspond to organic production, which in turn initiates the development of standards for the maximum permissible amount of antibiotics in feed and feed additives.

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