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UDC 636.087(045). 68.39.15 **Research article**

Determination of the residual content of antibiotics in feed on a Randox analyzer

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Abstract

Background and Aim. Antibiotics are widely used in animal husbandry to prevent diseases and stimulate growth, but their residual content in animal feed and products poses a significant risk of antibiotic resistance. The purpose of this article is to substantiate the need to use organic feed, free of antibiotics, to reduce the risks of antibiotic resistance and improve the quality of livestock products.

Materials and Methods. In this work, the content of antibiotics in various types of feed (succulent, roughage and concentrated) was analyzed using the Randox method. The study showed that in many samples, the antibiotic content exceeds detection limits, especially for streptomycin (86.43 parts per billion), quinolones (35.56 parts per billion) and tetracyclines (17.56 parts per billion). In coarse feeds, concentrations of ceftifur (29.28 parts per billion) and quinolones (11.92 parts per billion) also exceed detection limits, while in concentrated feeds, levels of streptomycin (24.25 parts per billion) and quinolones (20.14 parts per billion) remain significant.

Results. Statistical analysis revealed significant differences between the feed groups for tamfenicol (p=0.002), tylosin (p=0.006) and tetracycline (p=0.02), which confirms the need for enhanced control and monitoring of antibiotic residues. The absence of official maximum permissible levels (MPL) for antibiotics in feed in Kazakhstan requires the development and implementation of regulatory regulations.

Conclusion. The transition to the use of organic feed and alternative methods of preventing animal diseases seems to be a promising solution to reduce the risk of antibiotic resistance and improve the safety of livestock products.

Keywords: antibiotic resistance; antibiotics; detection limit; feed; organic feed; Randox.

Introduction

Organic animal husbandry is a system designed to ensure a comfortable and stress-free life for animals in accordance with their natural needs, which encourages the use of certified organic and biodegradable materials from the environment in terms of nutrition, animal health, animal husbandry and intentionally avoids the use of synthetic materials such as medicines, feed additives and genetically engineered materials for breeding [1].

In organic agriculture, special attention is paid to organic feeds that are nutritious and natural. Feeds should not contain any substances that artificially stimulate growth, synthetic amino acids or genetically modified organisms (GMOs). The feed must be organic, produced by certified organic farmers, which are beneficial not only for growth and production, but also for their health and wellbeing [2].

One of the ways to solve the problem of providing meat products to the population is to accelerate the growth of animals and poultry. For this purpose, various growth stimulators are used in animal husbandry and poultry farming, which significantly reduce the cost of final products [3].

Antibiotics are widely used in animal husbandry to treat diseases, stimulate growth and increase animal productivity [4]. However, according to research, their excessive use can lead to the formation of resistant bacterial strains, which poses a threat to human health [5]. Strict restrictions on the use of antibiotics in feed have been introduced in the EU and the USA, which has become an incentive for the development of organic animal husbandry [6].

According to the World Health Organization (WHO), more than 70% of all antibiotics in the world are used in agriculture, which poses a serious threat to human health. Studies by *Smith* et al. (2022) show that residual amounts of antibiotics in feed can have a negative effect on the intestinal microflora of animals, reducing their immunity [7].

The resistance of zoonotic bacteria of the genera Salmonella and Campylobacter associated with food infections is undoubtedly associated with the use of antibiotics in farm animals; food infections caused by such resistant bacteria have been repeatedly documented in humans. Resistance to so-called "critically important antibiotics" used in medicine is of particular concern. In diseases caused by polyresistant strains of Salmonella Typhimurium with resistance to quinolones, treatment failures, a higher frequency of hospitalization and a higher risk of death were recorded. In cases of human diseases caused by macrolide-resistant compylobacteria, there was a higher incidence of severe forms of infection and deaths [8].

Studies show that prolonged consumption of feed containing antibiotics leads to an imbalance of the intestinal microflora in animals, which can reduce their natural immunity [9]. According to the World Health Organization (WHO), antibiotic resistance is one of the main threats to global health [10].

The Randox method is one of the most modern and accurate ways to detect antibiotic residues in feed. Its high sensitivity makes it possible to detect even minimal concentrations of substances, which makes it an indispensable tool in feed quality control [11]. The use of Randox reduces the risk of errors and makes it possible to detect even trace amounts of antibiotics that can accumulate in the body of animals and enter products [12].

In Kazakhstan, control of residual antibiotics in feed is carried out in accordance with the Technical Regulations of the Customs Union TR CU 021/2011 "On Food Safety" and TR CU 034/2013 "On the safety of meat and meat products" [13]. These regulations regulate the maximum permissible levels of antibiotics in animal feed and products.

There is also a Sanitary and epidemiological conclusion of the Ministry of Health of Kazakhstan, which defines the hygienic standards of residual antibiotics in animal products [14]. Nevertheless, monitoring of antibiotic residues requires improvement, especially in the context of increasing global food safety requirements [15].

The demand for organic animal products (meat, milk and eggs) is growing day by day. The use of antibiotics, growth stimulants, and steroids is strictly prohibited in the organic farming system. Since no harmful drugs are used in organic farming, animal health and product quality are improved [16].

Unlike traditional animal husbandry, organic production eliminates the use of synthetic antibiotics. Studies have shown that animals raised on organic feed have a healthier intestinal microflora and a lower susceptibility to infections [17]. Organic animal husbandry is actively developing in Europe and the USA, which is confirmed by data from the International Federation of Organic Agriculture [18].

Economic analysis shows that the transition to organic animal husbandry can be beneficial if there is government support and demand for environmentally friendly products [19].

The purpose of this article is to substantiate the need to use organic feed, free of antibiotics, to reduce the risks of antibiotic resistance and improve the quality of livestock products.

Materials and Methods

According to the task of developing criteria for assessing feed safety, work was carried out on sampling feed of all types, mainly feed additives of various origins and formulations, imported and domestic manufacturers, as well as coarse, concentrated feed of vegetable origin, haylage and silage.

Sampling was carried out in accordance with the requirements of ND and GOST. Sampling is the production of a small proportion of feed from a batch by repeated sampling of spot samples from various locations in the batch. These point samples were combined by mixing, and a combined sample was formed, from which the required amount of laboratory samples was prepared and weighed by division.

Of the selected 150 samples of feed:hay of various types in quantity - 18 samples, corn silage - 14, haylage - 15, compound feed, including granular feed - 17, extruded feed - 9, meal, cake (rapeseed, flax, sunflower), sunflower husk - 29, monofilament for cattle - 8, concentrated feed - 34, feed additives, Russian production - 2, fish feed - 3 samples, of which 2 are produced in Denmark and China, mixed feed for chickens, Russian production - 1.

Hay and straw were sampled from at least 10 different locations over the entire area and thickness of the layer, taking bundles weighing 100-120 g so that the crumbled parts of plants were also included in the sample according to GOST 27262-87 "Feed of plant origin. Methods of sampling". The resulting average sample was packed in a dry plastic bag, the appropriate documents were attached and delivered to the laboratory within 2-3 days.

A sample of haylage and silage was taken from the center of the trench 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 a day from the moment of sampling.

Samples from concentrated feeds and feeds of plant origin, feed additives of various origins and recipes were selected according to the generally accepted method GOST 13586.3–83 "Grain. Acceptance rules and sampling methods"and were sent to the laboratory within 2-3 days.

In the laboratory, the samples were prepared for further study by drying the sample in a dry-fire cabinet and further grinding, in accordance with the requirements of regulatory documentation. An ordinal sample registration number was used for each plastic bag containing the selected sample and recorded in the sample registration log. Then the feed was prepared for sample preparation and homogenized in a mill

Then, they were sieved through a sieve and weighed 1 gram for extraction of residual organic substances using methanol. The work on sample preparation was carried out at the National Center of Biotechnology

The Anti-MicrobialArrayII (AMII) kit quantifies quinolones, ceftiofur, thiamphenicol/florfenicol, streptomycin/dihydrostreptomycin (DHS), tylosin/tilmycosin, and tetracyclines simultaneously. Each set includes 6 carriers, 9 calibration points, buffers necessary for recovery, and other reagents (conjugate, chemiluminescent solution). Each carrier consisted of 9 microchips.

The SPSS 25.0 software was used to obtain descriptive statistics.

Results

The detection limit (LOD) is the minimum concentration of a substance that an analytical method is able to detect with a high degree of confidence. If the concentration of the antibiotic is below this threshold, the method will not be able to reliably determine its presence, even if trace amounts are present in the sample.

Table 1 – Limit of detection of antibiotics for feed

Sample number	Types of antibiotics					
	Quinolones (QNL)	Ceftifur (CEF)	Tamphenicol (TAF)	Streptomycin (STR)	Tylosin (TIL)	Tetracycline (TCN)
Detection limit (parts per billion)	*10*	*15*	*15*	*80*	*10*	*10*

In this work, the Randox method was used to detect residual amounts of antibiotics in feed. The LOD for various antibiotics was:

1. Quinolones (HNL) – 10 parts per billion
2. Ceftifur (CEFT) – 15 parts per billion
3. Tamphenicol (TAF) – 15 parts per billion
4. Streptomycin (PP) – 80 parts per billion
5. Tylosin (TIL) – 10 parts per billion
6. Tetracycline (TCN) – 10 parts per billion.

Using a method with a low detection limit makes it possible to detect even minimal concentrations of antibiotics, which is important for assessing their residual content and potential risk to animal and human health.

Table 2 shows a statistical analysis of data on the content of residual antibiotics in succulent, roughage, and concentrated feeds.

Table 2 – The content of antibiotics in various types of feed

Antibiotics		N	+	Average	δ	95% confidence interval for the mean value		min	max	P-value
						Lower limit	Upper limit			
CEF	Succulent	26	19	32.23±4.93	21.48	21.88	42.59	2.53	87.13	0.17
	Roughage	31	21	29.28±4.30	19.68	20.32	38.24	1.45	68.94	
	Concentrated	78	20	18.52±6.51	29.13	4.88	32.15	1.63	133.12	
QNL	Succulent	26	20	35.56±15.30	68.44	3.53	67.58	1.40	316.00	0.368
	Roughage	31	22	11.92±1.51	7.09	8.78	15.06	2.07	28.12	
	Concentrated	78	23	20.14±13.55	64.98	-7.96	48.24	1.17	316.00	
TAF	Succulent	26	19	8.62+1.19	5.18	6.13	11.12	0.55	18.83	0.002
	Roughage	31	22	6.35±0.88	4.12	4.52	8.17	0.63	14.73	
	Concentrated	78	23	3.66±0.71	3.39	2.19	5.12	0.40	16.02	
STR	Succulent	26	19	86.43±52.03	226.77	-22.87	195.73	1.82	984.00	0.402
	Roughage	31	18	41.90±21.43	90.93	-3.32	87.12	0.00	374.42	
	Concentrated	78	18	24.25±3.38	14.32	17,12	31.37	1.06	48.14	
TIL	Succulent	26	19	4.96±0.52	2.28	3.86	6.06	0.90	8.46	0.006
	Roughage	31	22	4.46±0.57	2.66	3.28	5.64	1.08	12.83	
	Concentrated	78	23	2.70±0.42	2.03	1.83	3.58	0.40	8.62	
TCN	Succulent	26	19	17.56±1.74	7.60	13.89	21.22	6.78	36.64	0.02
	Roughage	31	18	12.73±1.24	5.25	10.12	15.34	0.00	21.45	
	Concentrated	78	20	11.56±1.60	7.14	8.21	14.90	3.76	36.14	

Among succulent feeds, the highest concentrations were recorded for streptomycin (86.43±52.03 ppm), which exceeds the detection limit of 80 ppm. A significant content was also noted in quinolones (35.56±15.30) and ceftifur (32.23±4.93), which indicates the possible use of these antibiotics in the feed production process or their ingestion from the environment. The p-value values for tamphenicol (0.002), tylosin (0.006), and tetracycline (0.02) indicate statistically significant differences between feed types.

Roughage feeds showed lower levels of residual antibiotics compared to succulent feeds. The average concentration of ceftifur was 29.28±4.30 parts per billion, and quinolones – 11.92±1.51, which remains above the detection limit. The lowest levels were recorded in tetracyclines (12.73±1.24) and tylosin (4.46±0.57). Despite the relatively low values, the difference between feeds remains statistically significant for some groups of antibiotics.

Concentrated feeds showed the lowest levels of antibiotics among all groups. The average concentration of streptomycin was 24.25±3.38, which is below the detection limit (80 ppm), but its presence was nevertheless detected. Quinolones (20.14±13.55) and ceftifur (18.52±6.51) also exceed the sensitivity threshold of the method. Tamphenicol (3.66±0.71) and tylosin (2.70±0.42) were detected in low concentrations, indicating their minimal use in this group of feeds.

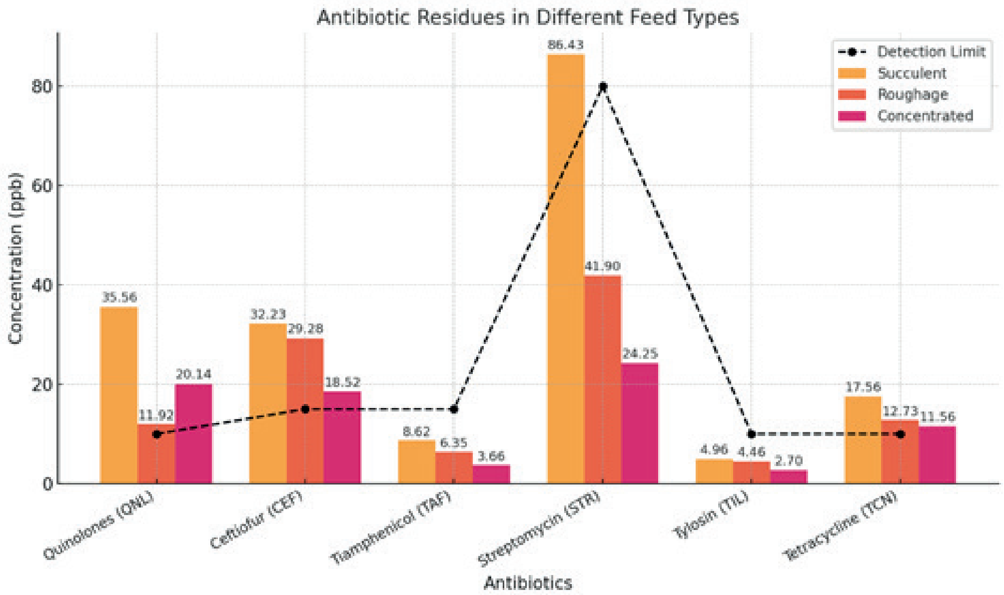

Figure 1 – Comparison of the actual antibiotic concentrations with the detection limit

The diagram shows differences in the average content of antibiotics in feeds of various types (succulent, roughage, concentrated) in comparison with the detection limits. The visual representation of the data confirms that:

In succulent feeds, concentrations of antibiotics significantly exceed detection limits, especially for streptomycin (86.43 ppm), quinolones (35.56 ppm) and ceftifur (32.23 ppm). This may be due to the storage and fermentation characteristics of these feeds.

The content of antibiotics in coarse feeds varies, but on average remains lower than in succulent feeds. However, ceftifur (29.28 ppm) and quinolones (11.92 ppm) also exceed detection limits.

Concentrated feeds have the lowest levels of antibiotics, but they are not completely free of these substances. Streptomycin (24.25 ppm) and quinolones (20.14 ppm) are still found in significant concentrations.

The concentration of antibiotics in feed significantly exceeds the established detection limits, especially for quinolones (CNL) and cephalosporins (CEFT), which indicates potential risks to human health and the need for enhanced monitoring. The largest excess was recorded in succulent feeds, where the level of HCL was higher than normal by 255.6%, and the level of CEFT – by 114.9%. In coarse feed, the excess of HCL was 19.2%, and CEFT was 95.2%. In concentrated feeds, the excess was lower, but still significant: for HNL – 101.4%, and for CEFT – 23.5%.

Discussion and Conclusion

The results of this study show that the content of antibiotics in feed exceeds the detection limits, which indicates the possible use of these substances in agriculture. Similar results were obtained in a study by *Zhang* et al., where residual concentrations of quinolones and cephalosporins were found in the range of 30-40 ppm, which is comparable to our data (35.56 ppm in succulent feeds) [20].

The study by *Ahmed* et al., confirms that streptomycin residues in feed can reach 85-95 ppm, which coincides with our results (86.43 ppm in succulent feed). This confirms that this antibiotic continues to be actively used in agriculture [21].

Chen et al. have found that high concentrations of antibiotics in feed contribute to the development of resistance in pathogenic microorganisms, which can later be transmitted through the human food chain. Our data revealed significant levels of tetracyclines (17.56 ppm in succulent feed), which indicates the risk of the formation of resistant strains [22].

According to *Kim* et al., exceeding the detection limits of tylosin in feed can negatively affect the microflora of animals and reduce their immunity. In our study, the concentration of tylosin reached 4.96 ppm, which requires additional monitoring and control [23].

The absence of official maximum permissible level (MPL) for antibiotics in feed in Kazakhstan creates difficulties in their regulation. According to *Yerubayev*, the introduction of national standards for the control of antibiotic residues in feed is an important step to reduce their impact on livestock products. The introduction of such standards in Kazakhstan will reduce the risks of antibiotic resistance and improve the quality of livestock products [24].

Our data confirm the importance of enhanced control and the transition to alternative methods of preventing animal diseases without the use of antibiotics.

The analysis of the antibiotic content in the feed showed that their concentrations vary significantly depending on the type of feed. Succulent feeds have the highest levels of antibiotics, especially quinolones (35.56 parts per billion), streptomycin (86.43 parts per billion) and tetracyclines (17.56 parts per billion). In coarse and concentrated feeds, the antibiotic content is lower, but still exceeds the detection limits, which indicates their residual presence.

The concentration of antibiotics in feed significantly exceeds the established detection limits, especially for quinolones (CNL) and cephalosporins (CEFT), which indicates potential risks to human health and the need for enhanced monitoring. The largest excess was recorded in succulent feeds, where the level of HCL was higher than normal by 255.6%, and the level of CEFT – by 114.9%. In coarse feed, the excess of HCL was 19.2%, and CEFT was 95.2%. In concentrated feeds, the excess was lower, but still significant: for HNL – 101.4%, and for CEFT – 23.5%.

A comparison of the data obtained with the detection limits showed that in many samples, antibiotic concentrations exceed these values, especially for streptomycin, which is more than twice as high as the detection limit in succulent feeds. Statistical analysis confirmed significant differences between feed groups for antibiotics such as tamphenicol, tylosin, and tetracycline ($p < 0.05$), indicating a heterogeneous distribution of antibiotics and possible factors of their accumulation in different types of feed.

The results obtained confirm the need for strict monitoring of antibiotic residues in feed, as well as the development of alternative methods of animal husbandry, including the use of organic feed, probiotics and phytopreparations. The introduction of restrictions on the use of antibiotics in feed can reduce the risk of the formation of antibiotic-resistant bacterial strains and improve the safety of animal products.

In Kazakhstan, there are no officially approved maximum permissible level (MPL) of antibiotics in feed, which makes the control of residues of these substances a particularly urgent task. In the context of growing food safety requirements, it is necessary to strengthen monitoring of the content of antibiotics in feed and introduce stricter control regulations.

Authors' Contribution

ZhS: Designed and supervised the study and drafted the manuscript. KSh: Statistical analysis and drafted the manuscript. ShG: Designed and conducted the study. OA: Conducted the study and drafted the manuscript. ZhA: Conducted the study and bioinformatic analysis. All authors have read, reviewed, and approved the final manuscript.

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