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A REVIEW ON BENZANTHRONE LUMINOPHORES FOR RAPID AND HIGH-RESOLUTION IMAGING OF PARASITES BY CONFOCAL LASER SCANNING MICROSCOPY

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Abstract

Nowadays, is growing interest in investigation of parasites and their anatomic structure. Most common tools are linked with usage of luminophores and luminescent microscopy techniques. In recent years, a variety of fluorescent probes have been developed and widely used to realize the visualization of certain structures [1]. The benzanthrone compounds captivated a lot of interest as fluorescent probes for biomedical technologies because of remarkable spectral properties and negligible fluorescence in the aqueous phase [2]. Notably, the spectral characteristics of benzanthrone dyes meet the criteria for an ideal bio-imaging agent, featuring a high extinction coefficient, bright fluorescence, photo-, thermo- and chemical stability, and reduced background signal [3]. Fluorescent molecular dyes, currently used to study cell membranes, make lipid structures visible through optical techniques [4] and act as potential fluorescent probes for biomolecules [5]. There are studies in the literature where the benzanthron phosphors have confirmed their application for visualization of biological objects [6-8]. Despite high activity in this field, still it`s huge request for specific fluorescent probes for biological objects.

The confocal laser scanning microscope (CLSM) is a powerful tool for providing high-resolution optical sections. CLSM can be used to analyse images of morphological structures and to place organ or organ systems of interest in their anatomical context. Luminescence imaging techniques are increasingly utilized for exploring the structure and properties of biological objects. Laser-induced fluorescence stands out as a sensitive method, capable of detecting even a single-molecule under specific conditions [9], which makes it a powerful bioanalytical tool for life sciences [10-12]. Benzanthrone derivates are used for CLSM imaging as fluorescence probe for various biological objects because benzanthrones render the specimen fluorescent to examine the stained samples by optical sectioning [13,14]. This review provides inputs in utilisation of different benzanthrone luminophores for examination of the parasites.

Key words: parasites; luminophores; confocal laser scanning microscopy; staining protocols.

Introduction

Parasites play an important role in healthy and functioning ecosystems. They are part of food chains, biological cycles and even serve as environment indicators showing changes in various habitats [15].

Parasite species identification is crucial for life cycle description, determination of prevalence and intensity, biogeographical distribution and for interaction among species [16,17].

A simple tool used to identify species is a taxonomic key. Taxonomic keys are created based on a specific character present in the group of organisms, these characters are quantitative or qualitative. However, taxonomic keys are created based on group of organisms, which are living in a specific region at a specific time period, therefore, not always an examined organism will comply to all taxonomic keys in the list. There could be minor variations. This is the reason why taxonomic keys are challenging to describe as they would be used worldwide [18].

Modern microscopic methods such as CLSM allows to obtain high-resolution optical images. Thus, increasing our understanding of parasite's biology. The most important advantage of this method is that it allows to visualize and examine both fresh and previously fixed samples. Nowadays, CLSM is widely used for investigation of muscular arrangement [19,20], parasite's internal and external structure [21,22] or to examine general morphology of parasite's specific attachment organs such as oral suckers [14]. All these studies describe more and more species-specific characteristics; therefore, the confocal microscopy method gives more detailed information about species specific characteristics and these differences facilitate to distinguish one species from another [23]. Fluorescence microscopy places great emphasis on luminophores [24]. In past, the fluorescent dyes acridine orange and rhodamine C were widely used for staining the structure of parasites [25,26], but nowadays other synthetic dyes began to appear for the morphological study of biological organisms [27,28]. In recent years, benzanthrone luminophores have gained popularity and their use enables the detection of specific lipids and proteins [29,30]. Previous studies confirmed that benzanthrone luminophores are able to stain biological materials [30-32]. Every time when we think about luminophores, the photobleaching process has to be considered. Photobleaching is an irreversible process in which the fluorophore gradually fades [33]. Photofading occurs as a static process after many cycles of absorption and emission of photons. As a result of photofading, during the investigation of the sample, fading of the sample is observed and the fluorescence intensity drops, which means that the quality of the obtained result begins to gradually disappear [34,35]. Photofading could be considered as the main property of luminophores, as it affects the total number of emitted photons and the quality of the obtained result. As a result, more and more luminophores are continuously being synthesized in which attempts are made to slow down the photofading process [36]. Although staining with benzanthrone luminophores significantly reduces sample preparation time, a specific benzanthrone luminophore must be selected for each group of organisms [37].

Materials and methods

Information on the usage of the benzanthrone luminophores for rapid and high-resolution imaging of parasites by CLSM was gathered by searching in the International online databases Web of Science, ScienceDirect, PubMed (all fields), and Scopus (title, abstract, and keywords) were searched for all published data on the topic. The databases were searched for all published studies in English, from 2000 to 1 November 2023. The search results from the four databases were combined and duplicates were excluded. Eligible studies were selected based on the title and abstract.

Results

In a total, five relevant articles and one monography were identified during the literature search. First articles on utilisation of the benzanthrone luminophores for examination of the parasites are dated by 2018 year and the most recent publication is dated by 2021 year. The following parasite species were studied using the benzanthrone luminophores and confocal laser scanning microscopy: please refer Table 1 below listing articles, which include original research on benzanthrone luminophore utilisation for various parasite examination.

Table 1 – parasite species, luminophore and fixative used in the studies

Study	Parasite species	Luminophore	Fixation
Kirjusina et al., 2018 [7]	Fasciolidae	AZP5	96% ethanol
Kirilova et al., 2018 [21]	*Prosotocus confusus* ad.	P8, AM1, AM2, AM4, AM16	96% ethanol AFA
	Diplostomum spathaceum mtc.	P8, AM1, AM2, AM4, AM16	96% ethanol
	Diplodiscus subclaviatus ad.	P8, AM1, AM2, AM4, AM16	Carnoy's solution
Gavarane et al., 2018 [6]	*Trichinella britovi* larvae	P13	70% ethanol 96,6% ethanol, frozen storage in animal muscle 1-5 years
Gavarāne et al., 2019 [22]	*T. britovi* larvae	AZM	70% ethanol Bouin's solution Carnoy's solution AFA
	T. spiralis larvae	AZM	70% ethanol Bouin's solution Carnoy's solution AFA
Gavarane et al., 2020 [37]	*Diplostomum* sp.mtc.	AM2	96% ethanol
	Diplostomum sp. mtc.	AM2223	70% ethanol
	T. britovi larvae	AZP4	Bouin's solution
	Camallanus lacustris	AZP4	96% ethanol
	Dactylogyrus sp.	AZP4	96% ethanol
	Parafasciolopsis fasciolaemorpha ad.	AZP5	70% ethanol Carnoy's solution
	P. fasciolaemorpha ad.	AZR	70% ethanol
	P. fasciolaemorpha ad.	EAM1	70% ethanol
	P. confusus ad.	P10	AFA
Rubenina et al., 2021 [38]	*P. fasciolaemorpha* ad.	AZPP	70% ethanol 96% ethanol Bouin's solution Carnoy's solution AFA 10% Neutral buffered formalin
	P. fasciolaemorpha ad.	AM323	70% ethanol 96% ethanol Bouin's solution Carnoy's solution AFA 10% Neutral buffered formali

During the studies, mainly the parasite species of the Trematode class were investigated: species from a homothermic host as well as species of parasites from poikilothermic hosts [7, 20, 37-38]. All selected trematode parasite species are endoparasites. They are an important factor influencing the dynamics of wild populations [39]. Even more so, a high intensity of invasion and a wide variety of parasite species can significantly affect the health of the host [40].

Discussion

CLSM is widely used in the study of the morphological and physiological structure of various species, especially for fixed samples of trematodes. Moreover, the various studies and attempts to improve the properties of different luminophores provide opportunities to visualize the morphological features of the parasite's body surface, the organ systems of various trematode species in the adult and larval stages, especially the digestive and reproductive systems. Systematic studies of flatworm parasites focus on the detailed study of structures even on the surface of nutrient openings, reproductive system, excretory system and glands, possible functions of sensory organs and their arrangement on the surface, shape and types of arrangement of trematode spikes [41,42]. The results of the study [21] showed that using 488 nm (with a 500-655 nm filter) laser excitation, it was possible to achieve a 23x smaller autofluorescence signal, compared to 405 nm (with a 425-580 nm filter) wavelength excitation. When autofluorescence was evaluated, different Region of Interests (ROIs) were selected and the selected ROIs were compared to the background ROIs. Based on the obtained data, 488 nm laser with FITC filter (500-550 nm) and 638 nm laser with Cy5 filter (662-737 nm) were the most suitable lasers for suppressing unwanted autofluorescence. On the other hand, in the study with freshwater trematodes [38], a laser with a wavelength of 405 nm was not used, because it induces autofluorescence of the samples. In previous studies, localization of benzanthrone luminophores in model membranes was detected by Förster resonance energy transfer and red-edge absorption shift [29-30]. The hermaphrodite generation of trematodes is characterized by a body wall musculature consisting of three layers: annular, striated and diagonal [43,44]. Kirilova et al. [21] and Rubenina et al. [38] confirmed with their study results that all three characteristic layers of the musculature of the trematode body wall were observed as the developed protocol does not target any specific organ system or skeletal muscle layer. Although, it is possible that by modifying the staining protocol, it would be possible to observe other muscle fiber groups. Relatively complex staining methods are used for studies of muscle layers, for example, Krupenko [19] used a 4% paraformaldehyde solution in PBS as a fixative and stained *D. subclaviatus* with TRITC-conjugated phalloidin. Phalloidin binds to polymeric and oligomeric forms of actin [45]. The standard fluorescent actin staining protocol together with CLSM has been used several times to investigate the muscular system of trematodes [13,14; 46-49]. Preparation of the sample in this case requires one or even more than two days, but as a result, the smallest muscle fibers are highlighted, thus highlighting the most characteristic features of the species. With research-developed staining protocols [7, 21,22, 38], results can be obtained within the first two hours. When the first attempts were made to stain freshwater trematodes, the obtained results did not show the whole-body muscle structure, but the muscular pharynx, mouth and abdominal suckers were marked. On the other hand, in the study with *P. fasciolaemorpha,* a more detailed body muscle structure was observed, visualizing the annular, diagonal and striated musculature layers [7, 38].

The front part of the Trematode body is covered by spines with larger teeth, while in the lower part of the body, spines with smaller teeth or even spines with 2-3 peaks are found [50]. Kirilova et al. [21] and Rubenina et al. [38] in their studies observed that the front part of the parasite’s body is more densely covered with spines than the lower part of the body. Although Krupenko & Dobrovolskij [13] concluded that the shape of the spikes, the number of teeth, etc. not detectable only by the CLSM method, the results of their studies confirmed the opposite. The AZPP luminophore and the CLSM method provided information on the size of the spikes, the number of teeth and their shape [38].

The obtained results showed that due to its high lipophilicity, P8 can enter the hydrophobic regions of the membrane. The luminophore P8 is in the phospholipid head region, although other luminophore binding sites were closer to the membrane surface. Ryzhova and co-authors [30] confirmed that dyes AM2 and AM4 have lower

lipid binding abilities than P8 [21] due to their higher polarity.

The staining protocols developed in the study were not intended for staining and visualization of a specific organ system, therefore, it was checked whether any part of the nervous system would be observed [21, 38]. In the obtained results, when staining trematodes with the synthesized AZPP, AM323, AM1, AM2, AM4, AM16 and P8 benzanthrone luminophores, none of the parts of the nervous system were observed. During the study, the synthesized benzanthrone luminophores in the polar solvents showed fluorescence in the red spectrum region, however, stained samples had a fluorescence shift in the shorter wavelength region [21, 38]. This could be due to more hydrophobic conditions (higher number of lipids, dehydration with ethanol). Adjacent luminescence can be produced by a chemical fixative. For example, using a mixture of formalin-containing chemical fixatives, more intense cellular luminescence in the yellow-green region of the spectrum can be observed [51]. *T. spiralis* and *T. britovi* species of *Trichinella* genus were selected during the study conducted by Gavarane et al. [6] and Gavarane et al. [22]. Both *Trichinella* species are the causative agents of trichinosis, which is dangerous for humans and animals [52,53]. Due to morphological similarities, it has become very challenging to identify isolates of *Trichinella* species to the species level, therefore several biochemical and molecular methods have been developed over the years, thus facilitating the identification of species and genotypes [54]. Almost all cells and tissues are capable of fluorescing near ultraviolet radiation of the visible spectrum. To study the synthesized fluorescent luminophores, the fluorescence signal must be separated from the luminophore and the autofluorescence signal. The easiest way to do this is to choose the wavelength that corresponds to the absorption. Typically, the autofluorescence excitation region is around 400 nm and the 488 nm absorbance was selected in the study [6], suppressing the autofluorescence signal relative to the tracer fluorescence signal [55,56]. Gavarane et al. [6] found that the most suitable chemical fixative for *Trichinella* larvae obtained from animal musculature and fixed, which are then examined by CLSM, is Bouin's fixative. As a result, detailed data on the morphology of the larva and its arrangement were obtained. *Trichinella* larvae studies always analyse larval morphology. One of the features by which the sex of trichinella larvae can be distinguished is the length of the rectum. In male larvae, the average length of the rectum is 40 μm to 50 μm, but in female larvae it is almost half that at 17 μm to 35 μm. During the study, Gavarane et al. [22] developed the staining protocol, which is suitable for determining the sex of the Trichinella larva by measuring the length of the rectum. The results of the study showed that the length of the rectum in male T. britovi larvae is 41.08 ± 4.26 μm SD and in T. spiralis larvae – 46.08 ± 2.95 μm SD; for female larvae 21.19 ± 2.45 μm SD and 20.55 ± 1.48 μm SD. The obtained research data agree with the data of other studies and confirm that the length of the rectum of males is twice that of females [57-59]. By developing new methods for determining the sex of the parasite, the obtained data would be useful in controlling the reproductive strategy of the parasite population [60].

The larval cuticle consists of three or more outer layers that are made of collagen and other components. The epidermis or hypodermis forms the outer cuticle layer and this layer is acellular [61]. The obtained results showed a high fluorescence signal in the cuticle of larvae [6, 22]. The cause of the high fluorescence signal in the cuticle may be the accumulation of lipids in the epicuticle of Trichinella larvae, as lipids are the energy source for the survival of the parasite in the host's muscle cells [62]. In general, the body of Trichinella is covered by a ridged cuticle [63] and during the study [22] it was observed that there are differences between the cuticle of *T. spiralis* and *T. britovi* larvae. In T. spiralis larvae, striation, so-called "pseudo-segmentation", was observed, while no transverse lines or striations were observed in the cuticle of *T. britovi* [22]. When staining the samples with AZM, an esophagus consisting of a single-layered epithelium with a basement membrane on the basal side was also observed [6, 22]. Other studies have confirmed that four types of epithelia are observed in the esophagus of *Trichinella* and that some of the epithelial cells are myoepithelium, which provides esophageal peristalsis [64]. Different epithelial cells were not observed in Gavarane et al. [6, 22] studies, however, there is a possibility that they could be observed using CLSM if the staining protocol is optimized. In total 6 various chemical fixatives have been used for sample preparation. Articles confirmed, chemical fixation

step of the samples has an impact on imaging results as well. At least fifteen new benzanthrone luminophores had been synthesized and several staining protocols had been developed for the study of Nematoda phylum and Trematoda class species. The articles confirmed photobleaching factor, therefore, use of laser wavelengths in descending order is recommended. Interestingly that trematode's thickness has an important role in staining protocol development as thicker samples require dehydration step by 100% xylene.

All articles demonstrated that benzanthrone luminophores are useful for rapid and high-resolution imaging of the parasites. Basically, a new staining protocol should be developed every time when a new benzanthrone is synthetised as high-resolution imaging results are mandatory criteria for rapid object examination. However, results of all studies demonstrated that all benzanthrone luminophores are not equal for all groups of parasites.

Conclusions

Considering the results of literature, benzanthrone luminophores with CLSM can be used to: efficiently and rapid exam various Nematoda and Trematoda species; to study frozen larvae in animal musculature, to study samples stored in 96,6% ethanol or to study larvae that have been isolated from recently collected animal musculature within a day and fixed; to differentiate larvae of the *T. spiralis* and *T. britovi* species, to determine the sex of *T. spiralis* and *T. britovi* larvae based on the length of the larva's rectum length. Developed staining protocols allow to study the anatomical and muscular arrangement within the parasite's body.

Gratitude

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GENERATION OF MONOCLONAL ANTIBODIES AGAINST TRICHINELLA SPIRALIS AND DETERMINATION OF THEIR IMMUNOCHEMICAL PROPERTIES

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Abstract

Diagnosis of trichinellosis in humans and animals is still in the developmental stage of the most effective and sensitive test for early detection. Serological methods are the most sensitive for detecting trichinella invasion. However, there is a high risk of non-specific reactions with other parasitological diseases. To address this issue, monoclonal antibodies (mAbs) against excretory-secretory antigens (ES-Ag) of trichinella muscle larvae are employed. In this study, hybridomas secreting mAbs against ES-Ag of *Trichinella spiralis* muscle larvae were generated. Out of 79 colonies, 5 mAb producer monoclonal clones (1D5, 1E7, 3C10, 4D6, 4F7) were obtained, and further stability testing selected 3 clones (1D5, 1E7, and 4F7) that yielded 14 to 40% active subclones. Western blotting using T. spiralis ES antigens demonstrated that the mAbs recognized a single band at 75 kDa. The productivity of the clones ranged from 2.0 to 4.0 mg/ml of protein. The obtained results indicate that the high activity, specificity of mAbs, and productivity of the clones recommend these mAbs for application in diagnostic purposes.

Key words: diagnostic activity; immunochemical properties; monoclonal antibodies; trichinellosis.

Introduction

Trichinellosis represents a foodborne parasitic zoonosis instigated by the muscle larvae (ML) of *Trichinella spp*. Human incidences of this malady are consistently documented on a global scale, primarily attributed to the ingestion of inadequately cooked or raw pork, along with the consumption of meat from wild animals, including canines [1]. Trichinella infection unfolds in two distinct phases: the initial enteric phase involves the residence of adult worms in the intestines. The clinical manifestations during the first week post-infection manifest as gastroenteritis, diarrhea, and abdominal pain. Subsequently, in the second or parenteral phase, larvae invade muscle tissue, provoking the formation of a nurse cell where they await a new host. Clinically, this stage is characterized by fever, myalgia, and arthralgia. Infections with a low parasite burden may be asymptomatic [2].

Timely detection of trichinellosis during the enteric phase is crucial to mitigate the infection, given the enhanced efficacy of anthelminthic drugs at this juncture. However, early clinical identification of human trichinellosis is challenging due to the absence of pathognomonic signs or symptoms [3]. Diagnosis relies on three principal criteria: patient infection history, clinical assessment, and laboratory investigations, including serological tests [4]. The primary limitation of serological tests, such as ELISA, in detecting antibodies to trichinella lies in the elevated rate of false-negative outcomes during the early stages of infection. Research indicates that the optimal positive dynamics of ELISA for

detecting antibodies to trichinella are not attained until at least 1-3 months post-infection with the parasite [5]. Hence, the acquisition of monoclonal antibodies (mAbs) capable of facilitating the development of serodiagnostic tools for detecting circulating antigens of trichinellosis is imperative.

Various mAbs targeting newborn larvae, muscle larvae, or adults of T. spiralis have been documented in the literature [6,7]. However, immunodiagnostic methods utilizing these mAbs have encountered challenges pertaining to sensitivity, specificity, and reproducibility. Antigens derived from the excretory-secretory (ES) complex of muscle larvae of *T.spiralis* have proven effective in generating mAbs for the development of immunodiagnostic approaches for trichinellosis [8,2].

The aim of this study was to create and characterize mAbs against ES antigens of muscle larvae of *T.spiralis*, providing a foundation for the development of specific serological methods for the early diagnosis of trichinellosis.

Materials and Methods

All stages of the project were conducted at the scientific and technical base of the "S. Seifullin Kazakh Agrotechnical Research University" (KATRU): at the Faculty of Veterinary Medicine and Animal Husbandry in the Professor N.T. Kadyrov Parasitology Laboratory, the "Joint Kazakhstan-China Laboratory for Biosafety," and the Scientific-Production Platform for Agricultural Biotechnology.

Ethics Approval:

All animal-related activities adhered to high biosafety standards and animal welfare. All protocols were in compliance with the International Guiding Principles for Biomedical Research Involving Animals. All animal care and use procedures were approved by the Ethics Committee on Animal Care of the Faculty of Veterinary Medicine and Animal Husbandry, S. Seifullin Kazakh Agrotechnical Research University (KATRU), Astana, Kazakhstan (Protocol No. 2, dated July 20, 2020).

Parasites:

The study utilized samples of muscle tissue from experimentally infected laboratory *Balb/c* mice with *Trichinella spiralis* larvae, generously provided by Dr. Anne Mayer-Scholl, a specialist from the Department of Diagnostics, Genetics, and Characterization of Pathogens at the Reference Center for Risk Assessment (BfR) in Berlin.

Experimental Animals and Cell Line:

Laboratory *Balb/c* mice were used for infection and monoclonal antibody production. The X63Ag8.653 cell line was employed as myeloma cells.

Immunization:

Monoclonal antibodies against trichinella ES antigens were obtained through mouse immunization. The immunization involved administering 100 μL of ES antigens with a concentration of 25 μg/mL on the first day, mixed with Freund's complete adjuvant at a 1:1 ratio. Subsequently, antigen immunization mixed with incomplete Freund's adjuvant continued on the 7 days post immunization (dpi), with additional doses on the 11 dpi, 12 dpi, and 13 dpi, and serum collection was conducted on the 17 dpi.

Hybridization and Monoclonal Antibody Production:

For the experiment, three groups of *Balb/c* mice of approximately the same age and weight were selected. Antigen used for hybridization was trichinella ES antigens. Animals with the highest antibody titers (at least 1:800) determined by ELISA on 4 dpi were sacrificed using cervical dislocation. Spleens were aseptically extracted, and splenocytes were obtained by perfusion. Hybridization of prepared splenocytes with X63Ag8.653 myeloma cells was performed following the method of Oi V. and Herzenberg L. [9].

Production of Monoclonal Antibodies in vitro and in vivo:

Following two rounds of cloning, hybrid cells were propagated to a substantial quantity in plastic culture flasks containing 25-50 mL of complete growth medium. The cells underwent a 3-4 day culture period in a CO2 incubator. Subsequently, detachment from the plastic substrate was achieved by pipetting, followed by centrifugation at 1000 rpm for 7-10 minutes. The resulting cell pellet was reconstituted in an incomplete growth medium, and 2x106 cells were intraperitoneally injected into *Balb/c* mice. These mice had received a prior injection of pristane (Sigma, USA) at a dose of 0.5 mL per mouse 7-10 days before the injection (dbi). The mAbs were then obtained from the culture supernatant of hybridoma cells for subsequent research purposes.

Upon the development of ascitic tumors within 12-15 days, mice were humanely euthanized through cervical dislocation. Ascitic fluid was extracted from the abdominal cavity using a

syringe equipped with a needle. Antibodies from the ascitic fluid were precipitated at 5000 rpm for 30 minutes at 4 °C. Purified monoclonal antibodies in the form of ascitic fluid were either stored at -70 °C without a preservative or at 4 °C with the addition of 0.1% sodium azide. The concentration of antibodies in both ascitic and culture fluids was quantified using the Bradford method [10].

SDS-PAGE and Western Blotting

Electrophoresis of ES antigens was performed on a 10% polyacrylamide gel using the Laemmli method with SDS-PAAG. The electrophoretic transfer of trichinella antigens from the gel to a nitrocellulose membrane and the detection of specific protein bands using sera from infected mice and/or hyperimmunized mice were carried out through standard procedures. The electrophoresis and blotting protocols followed the methods described by U.K. Laemmli et al. (1970) and H. Towbin et al. (1979), respectively [11,12].

Results

Immunization of Balb/c mice with Trichinella spiralis ES-Ag.

The ES antigen used in immunization was obtained by the research group in a previous study [13]. The initial phase of the current study involved generating a sufficient quantity of the ES antigen through mouse immunization. Subsequently, following the immunization protocol, the purified ES antigen was intraperitoneally injected into mice five times at a concentration of 50 μg/mL. Blood serum was collected on the 17 dpi. Serum samples were then tested to determine the maximum antibody titer using the indirect ELISA method (Table 1).

Table 1 – Investigations of Blood Sera from Immunized Balb/c mice

Antigenic preparat	Mouse Serial Numbers	Duration of Immunization	Number of Isolated Splenocytes (million cells)	Antibody Titers
Trichinella Excretory-Secretory Antigen	1	17 days	$7x10^6$	1:6 400
	2		$10x10^6$	1:6 400
	3		$5 x10^6$	1: 6 400

As evident from Table 1, the preparations demonstrated their antigenicity and immunogenicity in *Balb/c* mice. The maximum antibody titer in immunized animals reached 1:6400 for ES-Ag, indicating that the obtained preparations possess significant antigenicity and can be used for mAb production. Based on these results, it was decided to proceed with ES-Ag for further work, as it exhibited higher antigenicity and antibody titers. Several sources also note that ES antigens of *Trichinella spiralis* muscle larvae are valuable antigens for obtaining mAbs for use in developing trichinellosis immunodiagnostic methods [14,15].

Hybridization of Myeloma Cells

The hybridization of myeloma cell line *X63Ag8.653* with immune B-lymphocytes from mice immunized with ES-Ag was performed at a ratio of 1:10. The number of splenocytes isolated from the spleens of immunized mice by perfusion was 80 million cells, while the myeloma cell count was 8×106. Hybridization was conducted in the presence of PEG-4000, and the cell suspension was resuspended in complete growth medium with 10% fetal bovine serum before being seeded on 96-well plates [16].

Twenty-four hours after hybridization, cultivation continued in selective HAT medium (Sigma, USA) containing hypoxanthine, aminopterin, and thymidine, with a switch to selective HT medium (Sigma, USA) on the 7th day, which contains hypoxanthine and thymidine, promoting the growth of hybrid cells exclusively. The formation of hybrid colonies was observed on the 10th day post-hybridization (Figure 1).

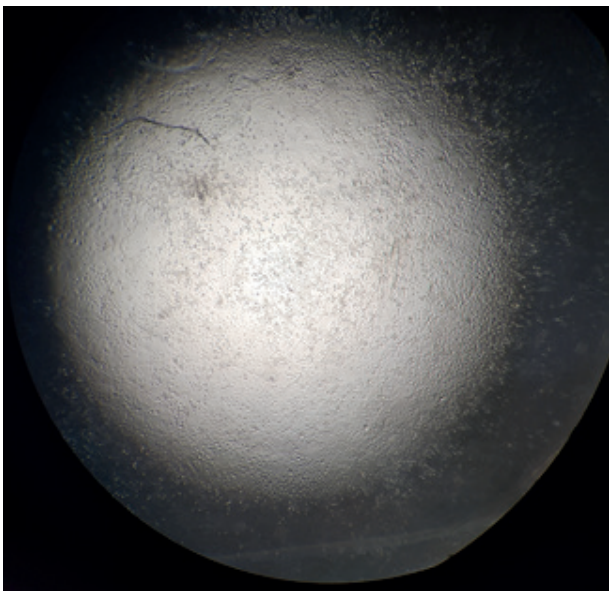

Figure 1 – Growth of hybrid cells

On the 21st day after hybridization, through continuous screening, the growth of hybrid cells was detected in 79 wells out of 384 seeded, or in 20.5% of cases. The ability of the formed hybrids to produce antibodies against trichinella ES preparations was determined by testing the culture fluid using the ELISA method. As a result, out of the 79 colonies, activity was observed in 5 strains: 1D5, 1E7, 3C10, 4D6, 4F7. Subsequent work was conducted with these 5 cell strains. The hybrid cells were then transferred to 24-well plates and subsequently to culture flasks. The hybrid cells exhibited growth as immobile colonies. When introduced into flasks, they exhibited a weak attachment to the substrate, presenting as spherical cells roughly comparable in size to the original myeloma. The hybrid cytoplasm displayed a slender perimeter surrounding the nucleus. When seeded at concentrations of 1-2 million cells/mL, the cells established a monolayer within an average period of 5-7 days, concurrently demonstrating mAb productivity in the range of 30 to 60 μg/mL. To obtain offspring from a single producing cell, the most active clones were further cloned using the limiting dilution method. Culture medium testing was performed on days 10-17 after cloning using the ELISA method. For testing, samples of culture fluid were taken only from wells where single colonies of cells were growing. The cloning results are presented in Table 2.

Table 2 – Activity of subclones of hybrids during the first cloning

Number of bores	Name of clones	Number of formed subclones	
		total	active of them
1	1D5	7	1 (14%)
2	4D6	11	3 (27.2%)
3	1E7	5	2 (40%)

As evident from Table 2, the activity of subclones during cloning was relatively low. For instance, among the 1E7 clone, 40% of subclones exhibited activity, while for the other two hybrids, 1D5 and 4D6, the proportion of active subclones was significantly lower, at 14% and 27.2%, respectively.

Subsequent work was conducted with the hybrid cells 1D5F3, 1E7D4, and 1E7B9, which demonstrated their activity. While obtaining a preparative quantity of mAbs typically requires only one clone, this does not guarantee its productivity and activity.

Monoclonal Antibody Production

Positive active clones were transferred to flasks for the production of preparative quantities of subclones 1D5F3, 1E7D4, and 1E7B9 under in vitro conditions. Based on the results of indirect ELISA, the most active hybrids, 1D5F3 and 1E7B9, were selected for further use in obtaining mAbs under both *in vitro* and *in vivo* conditions. For in vitro mAb production, hybrids were cultured in 50 ml polystyrene flasks. The culture fluid was tested by ELISA, collected in separate

sterile bottles, and preserved using a sodium azide solution. As a result, more than 250 ml of culture fluid containing mAbs from active clones of hybrids 1D5F3 and 1E7B9 was collected. The mAb titers were high, ranging from 1:8 to 1:16, and the overall protein concentration was 30-60 μg/ml. After harvesting the culture fluid, a portion of the hybrids in the logarithmic growth phase underwent cryopreservation.

To generate substantial quantities of mAbs through in vivo means, subclones 1D5F3 and 1E7B9 were introduced into mice. Hybrid cells were intraperitoneally injected into four linear mice (two hybrids per mouse) at a dose of 1 million cells in 0.5 ml, which were pre-treated with pristane at a dosage of 0.5 ml. Upon the formation of ascitic tumors, ascitic fluid was harvested from the peritoneal cavity on the 14th day. The collected ascitic fluid underwent centrifugation, and hybrid cells were cryopreserved in liquid nitrogen. The mAbs were subsequently isolated and purified from the supernatant using ammonium sulfate precipitation and dialysis against phosphate-buffered saline. The outcome yielded 12 ml of ascitic fluid from four mice, with 7 ml attributed to the 1E7B9 hybrid and 5 ml to the 1D5F3 hybrid. The mAb concentration in the ascitic fluid of both hybrids ranged from 2.0 to 4.0 mg/ml.

Despite the abundance of literature on the production of mAbs against trichinellosis for neonatal larvae, muscle larvae, or adult *T.spiralis* individuals, the immunodiagnostic methods developed using these mAbs encountered challenges related to sensitivity, specificity, and reproducibility.

Therefore, through cloning, two hybrid cultured cell clones (1D5F3 and 1E7B9) were selected, possessing all the necessary indicators. Moreover, based on the obtained and tested hybrids, a panel of hybrid cells stably producing mAbs against *Trichinella spiralis* was developed.

Determination of Immunohistochemical Properties of mAbs

The immunohistochemical properties of mAbs were assessed through SDS-PAGE and immunoblotting. The determination of the molecular mass of the obtained mAbs was conducted using vertical electrophoresis in 10% denaturing polyacrylamide gel. It was found that the molecular mass of the investigated mAbs was 65 and 25 kDa, aligning with widely accepted values for heavy and light chains of immunoglobulins.

Immunoblotting results demonstrated that the obtained mAbs reacted with the ES antigen of trichinella, forming a distinct band at approximately 75 kDa (Figure 2).

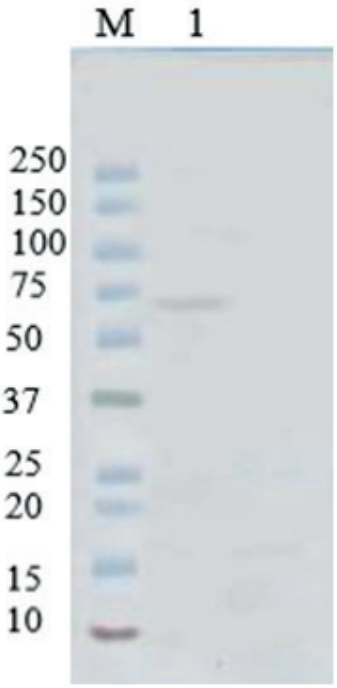

Figure 2 – The result of immunoblotting - binding of mAb to ES-Ag:
M - marker; 1 - monoclonal antibodies

The overall protein concentration in the ascitic fluid was 2.0 mg/ml for the 1D5F3 hybridoma and 4.0 mg/ml for 1E7B9. To determine the titration activity of monoclonal antibodies, an indirect ELISA based on trichinella ES-Ag was employed. The titers of mAbs were found to be high, ranging from 1:8 to 1:16 in the culture medium and 1:3200 to 1:6400 in the ascitic fluid.

The class and subclass of mAbs were identified through immunodiffusion reactions using standard reference sera for determining the class and subclass of immunoglobulins, provided by Sigma, USA. The results revealed that the obtained monoclonal antibodies belong to the IgG class, subclass G1. The immunochemical characteristics of mAbs from hybridoma cells 1D5F3 and 1E7B9 are presented in Table 3.

Table 3 – Characteristics of the mAb of hybrid cells 1E7B9 and 1D5F3

Name of clones	Isotype of mAbs	*In vitro* productivity of mAbs, mg/ml	*In vivo* productivity of mAbs, mg/ml	Titre of mAbs in culture medium	Titre of mAbs in ascitic fluid
1E7B9	IgG1	0.06	4.0	1:16	1:6400
1D5F3	IgG1	0.03	2.0	1:8	1:3200

As evident from Table 3, the hybridoma clones exhibited high productivity both in cell culture and ascitic fluid. Cultivating hybridomas in the abdominal cavity of mice yielded ascitic fluid with antibody titers against trichinella at levels ranging from 1:3200 to 1:6400.

Therefore, the monoclonal antibodies obtained in the course of this study demonstrated high activity and productivity. This suggests their potential application as components for developing serological test systems for diagnosing trichinellosis.

Discussion

The global prevalence of trichinellosis has instigated the development of diverse serological tools aimed at identifying trichinella invasion in both humans and animals. Serological techniques involve the identification of specific antibodies and circulating antigens of parasites present in serum or tissue fluids. In the context of diagnosing human trichinellosis, serological tests designed to detect trichinella-specific antibodies play a crucial role in diagnostic approaches. According to the guidelines set forth by the International Trichinella Commission, serological methods for identifying trichinella infection in animals are not recommended as a substitute for individual carcass meat inspection. Nevertheless, serological approaches for antibody detection are deemed suitable for the surveillance of both domestic and wild animals, contributing significantly to the comprehension of trichinella circulation [17,5].

In this study, the generated IgG mAbs against ES antigens of T.spiralis, as revealed by immunoblotting, exhibited a single band at 75 kDa, indicating specificity and purity of the reaction.

Srimanote et al. (2000) reported a high percentage of growing hybridomas secreting antibodies cross-reactive to many of the 23 tested heterologous parasites. Only six monoclonals (designated 3F2, 5D1, 10F6, 11E4, 13D6, and 14D11) secreted monoclonal antibodies specific to the ES antigen and/or crude extract (CE) of infectious larvae of *T.spiralis* [15].

Data are also available on the use of mAbs to detect circulating antigens and coproantigens using a sandwich-ELISA method without reactivity between mAb and common antigens of other helminths (e.g., *Angiostrongylus cantonensis, Ascaris suum, Echinococcus granulosus, Fasciola hepatica, Strongyloides stercoralis, Taenia solium, Toxocara canis,* and *Trichuris trichiura*). IgM mAbs recognized antigens with masses of 45, 49, and 55 kDa in ES antigens [2].

Trichinella infection triggers a specific antibody response, with the timing of seroconversion influenced by factors such as the infection dose, trichinella species, and host species. The persistence of antibodies varies among different hosts. In humans, seroconversion typically occurs between the second and fifth weeks after primary infection with Trichinella larvae, and specific antibodies may endure for several years. However, antibody levels do not correlate with the severity of the disease or the clinical course during the acute phase of trichinellosis [18,19,20]. In animals infected with trichinella, detectable antibody levels are usually absent for 2-3 weeks or more after infection [21]. Antibodies against trichinella

can persist for at least 6 months after infection without diminishing ES-ELISA results. Yet, in horses, antibody levels decline a few months after infection, despite the presence of infectious larvae in the muscles. Consequently, serological methods are not recommended for detecting trichinella infection in horses [22,23].

In the context of human diagnosis, antibody detection tests serve as a valuable adjunct. Classes of specific antibodies to immunoglobulin G (IgG), immunoglobulin E (IgE), and immunoglobulin A (IgA) become evident only 2-3 weeks after infection with trichinellosis. The literature details diverse methods for detecting antibodies against Trichinella infection in humans and animals, including the indirect fluorescence antibody test (IFAT), Western blot analysis, and indirect ELISA [24,3]. The development of serological tests hinges on the availability of high-quality antigens, with a variety of antigens currently employed for this purpose [25].The mAbs obtained during this study showed a high titer in both culture medium and ascitic fluid, while the 1D5F3 and 1E7B9 subclones showed high productivity in vivo, producing from 2.0 to 4.0 mg/ml of protein.

Thus, obtaining mAbs against trichinella will increase the sensitivity and specificity of the designed serological test systems and will help reduce the level of non-specific reactions with other diseases.

Conclusion

The applied methods of obtaining and operating mAbs have shown their effectiveness in obtaining active and productive hybrid cells. The specific reaction of mAbs with ES-Ag of muscle larvae makes it possible to use these antibodies for the serodiagnosis of trichinellosis.

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TOOLS FOR IMPROVING REPRO ON DAIRY FARMS

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Abstract

The studies presented in this article are part of the PTF of the Kazakhstan Ministry of Agriculture. Most consultants, when dealing with herd fertility problems, advise first setting specific goals (S), measurable (M), achievable (A), relevant (R), and time-bound (T) – SMART goals. Each farm has different assets, goals, and challenges and therefore a consultant must tailor their recommendations to the farm and avoid generalizations or textbook recommendations based on national averages. To create the standards for feeding, reproduction, and maintenance of dairy, during 2021-23 improved diets, assessed animal welfare, and reproduction data was collected monthly, the basis of the collected data in the article shows the dynamics of the fertility of cows in one of the basic farms participating in Execution of the program. By improving the conditions of feeding and maintenance, the fertility of cows had a positive dynamic. One of the important indicators is the number of calves per 100 cows from 77 calves in 2021 increased to 86 in 2023. The number of cows with an elongated dry period from 14.7% decreased to 6.3%. Pregnancy Rate increased by 2 times from 11.9% to 20.8%. The mortality rate of calves also decreased from 23.2% in 2021 to 6.7% in 2023. Introducing the system for heat detection cuts the heat detection rate coefficient, the indicator has grown to 54% from a low 23% in 2021. In general, we can conclude that by improving the level of feeding and maintenance, the fertility of the cows had positive dynamics.

Key words: conception rate; dairy cattle; fertility; pregnancy rate; heat detection rate.

Introduction

In Kazakhstan, many high-yielding herds suffer from the necessity to cull cows for infertility after their first lactation because the body's reserves are insufficient for milk production and reproduction [1]. This reduces the number of replacement calves, for maintaining or growing herd size. Furthermore, few replacements mean that farms must reduce their genetic selection intensity further slowing growth in the genetic value of their animals. Poor fertility means that cows spend more time in the less profitable phase of their lactation curve and compels farms to increase their annual expenses on purchasing replacement heifers, which constitutes a significant portion of their costs. Finally, low fertility necessitates numerous attempts by farmers to inseminate cows (especially first-calf heifers), which ultimately need to be culled, meaning that financial resources are wasted, thereby reducing the profitability of production.

In Kazakhstan, there has been growth in the population of cattle. In 2013, the cattle population numbered 5.7 million head, and by 2022, it had increased to 8.4 million head. As of August 2023, there was a 4% further growth, bringing the total to 8.9 million heads. The production of cow's milk has also been on the rise. In 2013, approximately 2.7 million tons of milk were produced, while in 2022, production reached around 4.0 million tons [2]. It's worth noting that a significant portion of the dairy cattle population is in small-scale family farms, and more than 70% of the total milk production comes from these smaller herds. Despite the noticeable growth in cattle numbers and milk production, the country's increasing population has led to a higher demand for livestock products, including milk with demand now exceeding 6.0 million tons annually. This represents a cost to consumers and a missed economic opportunity for the dairy sector.

The primary goal of a reproductive management program at a dairy farm is to ensure that cows calve once a year to maximize economic efficiency. However, reproductive management goes beyond just breeding cows. A successful reproductive management program encompasses overall animal management, the application of technical skills, and sensible decisions regarding breeding that fit with the economic goals of the farm. The good news is that the lower the fertility, the more the farm can spend on reproductive management and still see a positive return on their investment. Those farms with very high fertility must be careful with their spending decisions as each additional percentage improvement in fertility will return less profit. In these cases of high fertility, reproductive management should focus on approaches to simplify and reduce the number of tasks and labor needed to maintain high fertility.

As part of the BR10764965 program, the scope of which included the preparation of standards for feeding, reproduction and maintenance of dairy cows, large-scale studies were carried out on the base farms cows' reproductive status.

The purpose of this study was to determine the dynamics of reproduction indicators with improved feeding and maintenance conditions during 2021-2023. The consultants for this project were distinguished professors of dairy nutrition Pr. A. Hristov and reproduction Pr. T. Ott at the Pen State.

Materials and Methods

Reproduction data were collected monthly from the beginning of the program and at the end from each base farm. All the data that was accumulated monthly is described below.

Fertility in dairies is conveniently expressed as pregnancy rate (or pregnancy risk to insemination). However, it is important to remember that there are two factors that are involved in the pregnancy rate calculation – heat detection rate (HDR) and conception rate (CR). HDR is calculated by dividing the number of animals detected in heat (typically over a 21-day period) by the number eligible to be detected in heat (e.g., beyond the voluntary waiting period and not inseminated or pregnant). Conception rate is calculated by dividing the number of pregnant cows by the number inseminated (over 21 days). The product of HDR and CR yields the farms' pregnancy rate (PR). When PR is low, in most cases, inadequate heat detection is the primary factor. Cows not cycling at the end of the voluntary waiting period and missed heats are key factors contributing to extended calving intervals. By improving heat detection and ensuring cows are cycling by the end of the voluntary waiting period, calving interval will be reduced. Here again, the focus should be on managing the cows to successfully navigate calving, adjust to lactation, and resume cycling. Each of these is tied closely to nutritional management. The efficiency of heat detection often (but not always) improves with increased milk production. This may seem counterintuitive but the factors (nutrition, management, welfare, health) that contribute to high milk production can also lead to improved fertility. This indicates that producers can achieve higher milk yields along with good reproductive performance. However, it is possible to achieve high milk production, but still struggle with low fertility. The key to understanding this conflict between production and reproduction is understanding that the animal will suppress key reproductive hormones (e.g. Luteinizing hormone that causes ovulation) when it is losing weight (negative energy balance). A negative energy balance suppresses cyclicity, so no matter how diligent the heat checking, few heats will be detected. It is also important to note that high-producing cows in hot conditions, or those that are housed on slippery floors will exhibit less estrous behavior. Therefore, managing for optimal HDR rate requires farmers to feed animals so they return to positive energy balance early after calving (within 4 weeks) and institute management practices that will promote expression and detection of heats. Ovulation synchronization protocols (described below) can be used to induce ovulation in cows that are not cycling, but conception rates to these induced ovulations are low and cows will often not recycle if they do not conceive after insemination.

Goals for optimal heat detection are to observe 70% of all expressed heats and to ensure optimal nutrition so that all cows are cycling by 40-50 days after calving. If conception rates are high (>40%), cow should be inseminated 60-70 days after calving. If conception rates are low (<35%) breeding cows expressing strong heats can occur >50 days after calving. For well-managed dairies using Holstein genetics, the target for days between calving and the next conception are 110-120 (days

open). Therefore, the higher the conception rate, the later the cows can be scheduled to receive their first insemination.

Submitting animals to insemination, whether based on estrus detection or ovulation synchronization, to ovulate a high-quality follicle is only the first step to successful reproductive management. These animals must be inseminated using the appropriate techniques with high quality sperm. If both are accomplished, then the only remaining limit to high fertility is the genetic value of the animals. Measuring success of the reproductive management at this point is done by calculating the conception rate. Various estimates of conception rates include services per pregnancy and the percentage of successful services or pregnancy risk to insemination. These metrics include data from both pregnant and non-pregnant cows. The conception rate is determined by dividing the number of successful services (confirmed pregnancies) by the total number of services rendered in each interval. Confirmed pregnancies are employed to calculate this percentage in herds that routinely conduct pregnancy diagnoses. In cases where actual pregnancy data are unavailable, the 65-day non-return rate is utilized as a substitute. This approach has risks in farms using induced ovulation or where nutrition is not optimal. Cows in these situations will often fail to recycle after a failed insemination. These cows will cause a large economic loss for the producer as the eventual determination of their non-pregnant status places them at high risk for culling.

Conception rate is an essential metric for evaluating the reproductive performance of a dairy farm. It measures the percentage of inseminated cows that become pregnant. Conception rate is determined as follows:

- Define the Time Period: Decide on the specific time frame for which you want to calculate the conception rate. It could be monthly, quarterly, annually, or any other period that suits your analysis. Monthly is recommended and fits into management practices that include pregnancy diagnosis 4-6 weeks after insemination. Longer intervals, again allow problems to accumulate before they are detected (e.g. poor-quality semen, poor insemination technique, problems with equipment etc.),

- Determine the Number of Cows Inseminated: Count the total number of cows that were inseminated during the chosen period.

- Count the Number of Pregnant Cows: Determine how many of the inseminated cows have become pregnant within the same time frame. These are the cows that have successfully conceived.

- Calculate the Conception Rate: Use the following formula to calculate the conception rate as a percentage:

Conception Rate (%) = (Number of Pregnant Cows / Number of Inseminated Cows) x 100

For example, if you inseminated 200 cows in a month and 140 of them became pregnant during that month, your conception rate for that month would be:

Conception Rate = (140 / 200) x 100 = 70%

This means that 70% of the cows you inseminated in that specific month successfully conceived.

Monitoring the conception rate over time is crucial for assessing the effectiveness of your dairy farm's reproductive management program and making necessary adjustments to improve herd fertility. Also, it is recommended that animals are rechecked for pregnancy 2-3 times before dry off. For example, 5-10% of cows identified pregnant between 30-35 days after insemination will lose those pregnancies and be open at a 60-day pregnancy. Lack of follow up pregnancy diagnosis will increase cows detected open at dry off. These cows will be culled at a large economic loss to the producer.

Pregnancy rate is another important metric for assessing the reproductive performance of a dairy farm. It measures the percentage of eligible cows that become pregnant within a specified period. Calculate the Pregnancy Rate: Use the following formula to calculate the pregnancy rate as a percentage:

Pregnancy Rate (%) = (Heat Detection Rate x Conception Rate) x 100

For example, if you have 500 eligible cows in your herd during a specific month, and 350 of them are detected in heat and are inseminated then the HDR = 70% (350/500 = 0.7 x 100 = 70%). Now, if 125 of these cows are diagnosed as pregnant, the CR = 35% (125/350 = .35 x 100 = 35%). Therefore, the pregnancy rate for that month would be:

Pregnancy Rate = (0.7 x .35) x 100 = 24.5%

This means that 24.5% of the eligible cows in your herd were detected in heat, inseminated, and

became pregnant during that specific month.

Average open days between calving and the next conception (days open) serve as an indicator of the reproductive efficiency in a dairy herd. However, because this metric is measured months after insemination. It is not the most current metric for assessing the reproductive performance of a dairy. The Voluntary Waiting Period VWP represents the minimum desired interval from calving to the first service. VWP is set based on the conception rates on a farm. The higher the conception rates, the longer the VWP. In well-managed dairies with high CR, VWP is set between 70-80 days. By delaying insemination, cows have more time to adjust to lactation, recover uterine health, and cycle several times before insemination. All of these will improve CR to first insemination. This is particularly true for first calf heifers who are still growing and take longer to adjust to the metabolic demands of lactation. Because these animals have a more persistent lactation curve, the optimal days open is 10-15 days longer than that for mature lactation cows. The calving interval, measured in months, is the time elapsed between consecutive calvings. To calculate the projected minimum calving interval, one adds 280 days (the average length of pregnancy) to the average days open and divides the result by 30.4 days (the average month length).

The Dry Period is defined as the days between dry off and the subsequent calving. Both excessively long and short dry periods have negative implications for herd profitability. It is advisable to maintain a high proportion of cows within the 40-70-day dry period range. A short dry period deprives cows of adequate rest and recovery time for mammary gland involution and regeneration. Conversely, extended dry periods result in a prolonged period without milk income and increase the risk of over-conditioned (fat) cows. Over-conditioned cows are more susceptible to health and reproductive issues and are particularly susceptible to calving complications. The duration of dry periods, whether too long or too short, may arise from factors such as extended calving intervals, subpar record-keeping practices, or ineffective pregnancy diagnosis methods.

Results

During the program, a lot of work was done to determine the fertility level of all farms involved in the research. Our statistics show that most farms in Kazakhstan have very low reproductive rates of cows. The calving interval is at least 470 days (the benchmark for dairy is 390-420 days), and in some high-producing herds, it is about 500 days. The calving interval is primarily extended due to the number of open days after calving, even when using estrus/ovulation synchronization, the number of open days ranges from 108 (optimal) to 174 (low fertility) days. This can be related to low HDR, which varies between 35-59% in Kazakhstan. The proportion of animals conceiving to the first insemination also varies widely; for cows, it is in the range of 26-56%, and for heifers – 25-82%. Optimal first-service conception rates are 45-50% for lactating cows and 60-75% for dairy heifers. The only indicator that is close to optimal in Kazakhstan is the dry period, on average 64-77 days.

Nutrition is the leading factor driving herd milk production and fertility. Heifer development is a key factor in well-managed dairies. Dairy breeds should achieve puberty between 10-12 months of age, but they must also achieve ~2/3 of their mature body size before breeding should be attempted. Poor nutrition means that farmers cannot inseminate heifers on time because of delayed puberty and low body weight. The first insemination occurs late on average at the age of 16.8-24.6 months, and the first calving occurs at the age of 26.4-35.8 months. Here again, modern dairy breeds should be managed to conceive between 12-15 months of age and calve between 21-22 months. Low fertility (19-27% of culled cows) is second only to low production as the cause of culling in Kazakhstan. In most cases, culling occurs after the first calving due to low fertility. This is particularly devastating to farmers as it takes roughly 2.5 lactations to recover the cost of raising that heifer until she enters the milking herd. Animals culled before this represent a large economic loss to the dairy. It is obvious why in Kazakhstan the dairy business is not profitable for everyone.

The first thing you need to start with is to determine the structure of the herd. In dairy cattle breeding, the main share of the herd will be dairy cows; their share should be at least 55-60%. In dairy farming, AI is mainly used, bulls should not be included in the main herd, and younger bulls are subject to sale, not only because they are an extra cost, but at the same time, males are dangerous.

We carried out an analysis of reproduction over 3 years in an experimental farm in the Akmola region, in which basic research was carried out to improve feeding and maintenance technologies; data on the dynamics of fertility indicators are presented in the following table.

Table 1 – Dynamics of fertility indicators from 2021 to 2023

Indicators	Goal	2021	2022	2023
# Calves per 100 cows	>90	77	80	86
% Culled cows for Repro	<10	6	20	10
% Dry cows: < 40 days > 70 days	 <5 <5	 4,4 14,7	 6,7 8,7	 3,9 6,3
Pregnancy Rate (%)	>22	11,9	13,2	20,8
Calving Interval (months)	12-14	19,3	19,9	17,8
% First Service by 85 DIM*	>90	34,9	54,7	76,1
First Service Conception Rate (cows/heifers)	>45/65	19/33	10/50	28/60
Age of first insemination of heifers, months.	12-15	18	19,2	15,9
% Stillborn calves	≤3	7,8	10,4	8,6
% Calf mortality	≤7	23,2	13,3	6,7
% Calving Difficulty (4-5 on scale of 1-5)	<5	4	3	2

* Day in Milking

The farmer explained the low level of reproductive function by the fact that the level of feeding in previous years was low. Analysis of feed and feeding and housing systems confirmed the farmer's words. As mentioned earlier, cow fertility is influenced by many factors. The productivity of cows, compared to the beginning of 21, decreased from 6360 kg to 5998 kg, by the end of 2022 it had already reached 5708 kg, and at the end of the research it increased again to 6144 kg. At the same time, we see improvements in reproductive function within 3 years, in other words, with proper management of feeding and housing technology, not only productivity increase, but also the cows will not experience stress, which does not affect other functions. In 2021, the calf yield per 100 head was low at 77 per 100 head, which was aggravated by a high proportion of stillborn calves and mortality before 2 weeks of age. If we also consider the fact that this year many young animals left the herd at an earlier age, then the percentage will decrease, the number will drop to 30 calves per head. At the beginning of 2022, many cows continued to retire from the herd due to low productivity of more than 70%, but at the same time 20% of first-calf heifers left due to low fertility. In 2022, the calf yield increased by only 3%, and by 2023 it was close to the norm.

On average, the dry period for cows was within normal limits in all years, but the table shows that in 2021, many cows were started on their own, ceasing to be productive long before calving. The launch of cows is currently being carried out in a timely manner; there are fewer cows that were milked longer, and at the same time the number of self-starting cows has decreased by more than 2 times. The calving interval has also been reduced by 2 months. The period between calving and the first insemination has also shortened to 58.8 days. The age of first insemination of heifers also approached the norm, reaching 15.9 months.

During the study period, 200 Smax Tec [3] boluses were installed on the farm, which doubled the heat detection rate. If at the beginning of the studies this figure was within 23%, then by the end of the studies it increased to 54%. Although this figure increased by more than 30%, this number is also not a reference for dairy farming; we explain this by the fact that boluses were installed only for first-calf heifers, which make up a little more than 1/3 of the entire herd.

Associated with the heat detection rate is the pregnancy rate. So, in 2021, this figure was equal to 11.9%, in 2022 it increased, but here it is worth noting that the fertilization rate in cows became below 10%, and in heifers above 50%, but due to this, the percentage of detection of cows in heat The pregnancy rate increased and increased to 13.2%. Already in 2023, these two related indicators increased, the first to 20.8%, and the second to

28/60 in cows/heifers. Thus, these two indicators make it possible to cover and understand not only the level of fertility of queens, but also the level of management of the fertility of a given herd.

Having analyzed the level of reproductive management in the herd, the next important step is to develop a plan to achieve the goals. The plan should contain clear and detailed objectives, defining who will be responsible for their implementation, what exactly needs to be done, in what time frame, in what place, and how to achieve each goal. These goals should be closely linked to the overall breeding strategy. A suitable person should be assigned to each task. Everyone involved should be trained and then be retrained/ evaluated at yearly intervals. Establishing a reward system for successfully completing tasks and achieving goals can be helpful. The plan also needs to clearly define what will be measured, who will monitor it, what the time frame will be, and how improvements and achievements will be assessed. Be careful in how these rewards are set. For example, rewards based on increasing the percentage of cows detected in heat may encourage workers to "believe" cows are in heat when they are not. Whereas rewards for increasing the pregnancy rate over the year from 20 to 24% will return a real profit to the diary and will justify worker incentives.

Discussion

Milk, or more precisely, its quantity, is the sole goal of the owner since it is the primary source of income for dairy operations. Many authors have reported a negative correlation between milk production and the fertility of cows [4,5]. However, the genetic correlation between these two traits is quite low [6,7]. In fact, it is typical to see some of the best herds in terms of milk production exhibiting the best fertility. What this reveal is that farm/animal management has the greatest impact on both traits.

In practice, it is difficult to increase milk production without the proper number of high-quality replacement heifers. For instance, their review titled "A 100-Year Review: Practical Female Reproductive Management," highlights that in the United States, J. S. Stevenson and J. H. Britt [8] attribute the significant increase in cow productivity from 2005 to 2015, to gains in the genetic value of animals for milk production, improved feeding management, housing, reproductive control, and greater cow welfare and comfort.

As mentioned above, many factors influence the fertility of cows, but feeding [9,10] and health management [11,12] have a greater influence. Once these influences are optimized, the use of strategies to improve cow fertility will begin to have positive effects.

Conclusion

In conclusion, the dairy industry in Kazakhstan faces significant challenges when it comes to the reproductive rates of cows, leading to extended calving intervals, low conception rates, and high culling rates due to fertility issues. These challenges are exacerbated by various factors, with poor nutrition playing a pivotal role. Inadequate heifer development, delayed puberty, and low body weight contribute to delayed breeding, resulting in late first inseminations and calvings. Low fertility, often leading to culling after the first calving, is a major economic setback for dairy farmers, taking multiple lactations to recover the investment made in raising heifers.

In 2021, the economy faced serious problems in practically all indicators of assessing the reproductive function. However, by 2023, significant improvements are noticeable. The number of calves per 100 cows increased to 91, exceeding the target. The percentage of cows culled for reproduction has dropped to 10%, approaching the target. The farm has also been able to reduce the percentage of dry cows falling outside the desired range. Notably, pregnancy and first service rates by day 85 improved significantly, while stillborn calves, calf mortality and calving difficulties decreased. Due to the use of technology for raising heifers, the time of rearing before the first insemination has also been reduced. If in 2021 this figure was 18 months, then in 2023 it became 15.9 months.

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TREMATODA AND CESTODA SPECIES IN CYPRINID FISH FROM SMALL LAKES OF THE KOSTANAY REGION

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Abstract

This study presents data on the epizootiological monitoring of parasitic diseases in fish from Kostanay region. It confirms the presence of previously identified permanent natural foci of opisthorchiasis in the southern districts, specifically in the Uly-Zhilanchik and Torgay rivers. Additionally, the research conducted in Zhangeldy and Amangeldy districts revealed the existence of biohelminths with both epizootiological and epidemiological significance.

This study aimed to investigate the presence of parasites dangerous to humans and carnivores in cyprinid fish species. Specifically, it focused on identifying the second intermediate hosts for opisthorchiasis, metorchiasis, and ligulosis: *ide (Leuciscus idus)* and *bream (Abramis brama)*. From 2021 to 2023 seven reservoirs of the Kostanay region were the monitoring objects of the parasitological situation: Verkhnetobolskoe reservoir, rivers: Torgay, Uly-Zhilanchik, Zhaldama, Tobol, Zhelkuar, Akkol Lake, where shellfish-bitiniids, waterfowl and crustaceans live in large numbers, as the first intermediate host of pathogens *Opisthorchis felineus (Metorchis bilis), Ligula imestinalis.*

Examination of eight ide (Leuciscus idus) specimens caught in the Uly-Zhilanchik River revealed the presence of metacercariae of two parasitic trematodes: O.felineus and M.bilis, while the prevalence was 18.5% and 8.3%, and the infestation intensity was 1-3 and 2, respectively. In the Amangeldy region, metacercariae *O. Felineus* and plerocercoids *Ligula imestinalis* were found in two fish species: one individual of the ide species and two individuals of the bream species caught from the Torgai River. At the same time, the prevalence was 6.6% and 16.6%, and the infestation intensity was 2 and 1, respectively.

Parasitic diseases of fish were not detected in the waters of Denisovsky and Zhitikarinsky districts.

Based on Polymerase Chain Reaction (PCR) analyses conducted using specific primers at the National Center of Biotechnology LLP laboratory in Astana, Kazakhstan, two distinct types of opisthorchid parasites were identified in two *ide (Leuciscus idus)* specimens collected from Kostanay region. The species were confirmed to be Opisthorchis felineus and Methorchis bilis (PCR protocol dated April 24, 2023).

Key words: biohelminths; bitiniid mollusks; fish; invasion; metacercariae; natural focus; opisthorchiasis.

Introduction

Veterinary services play a vital role in safeguarding public health by ensuring the safety and high quality of fish products [1]. While fish is a valuable source of nutrition, it can also harbor dangerous parasites that cause serious helminthiases in humans. Among these, opisthorchiasis ranks as one of the leading concerns. Notably, opisthorchiasis is classified as a natural focal parasitosis, meaning it occurs in specific geographical areas with distinct ecological conditions favorable for parasite transmission [2].

Opisthorchiasis is a type of oral biohelminthiasis.

The pathogen's range extends from the Yenisei River basin to the western borders of Europe, but the spread of the disease in humans is focal [3]. The level of infection of the population with opisthorchis is driven by social and household factors: lifestyle (traditions, habits), the degree of development of fishing, the proportion of fish in the diet, methods of culinary processing of fish, the sanitary condition of the area [4].

Due to the fact that the intermediate and second intermediate hosts live in reservoirs, foci of opisthorchiasis are concentrated near rivers. The world's largest outbreak of this disease was formed in the Ob-Irtysh River basin. In the lower reaches of the Irtysh and the middle reaches of the Ob, the invasiveness of opisthorchiasis among rural populations reaches staggering levels of 90-95%. Even preschool children are frequently infected, highlighting the severity of the public health concern [5].

The highest morbidity rates of the population (up to 1000 per 100 thousand) are registered here. The reason for such an exceptional importance of this territory in the epidemiology of opisthorchiasis is the presence of an extremely developed river floodplain that provides conditions for the circulation of the pathogen of the disease. Territories with the above quantitative indicators of population infestation are hyperendemic. The development of *Opisthorchis felineus* occurs with a triple change of hosts: the first intermediate (mollusks), the second intermediate (fish) and the final (mammals) [6].

The final hosts of the parasite include humans, cats, dogs, pigs and more than 25 species of wild mammals whose diet includes fish (fox, arctic fox, sable, ferret, otter, mink, water vole, muskrat, etc.). As a result, timely diagnosis and monitoring of parasitic diseases of fish such as ligulidosis, diplostomiasis are very much in demand, and liver flukes of the *Opisthorchidae* family are of the greatest epidemiological importance. The life cycle of this trematode is carried out with the participation of gastropods of the family *Bithyniidae*. The chain of the cycle is continued by fish of the *Cyprinidae* family, which play a major role in the spread of opisthorchiasis, as well as domestic and wild fish-eating animals. A person becomes infected with this parasite by eating infected individuals of the carp family. This disease is widespread in the Palearctic and Indochina [7].

Fishing is an efficient industry that generates significant income. The potential reserve of the fishing industry is to limit the spread of parasitic diseases of fish, the organization of rational preventive measures within the framework of epizootological supervision [8].

The Kostanay region boasts numerous rivers and lakes, vital resources for agriculture, industry, and recreational and commercial fishing. In this context, timely diagnosis and monitoring of diseases such as ligulidosis, diplostomiasis is of great importance for fish farming and industry, and liver flukes of the *Opisthorchidae* family are of the greatest epidemiological importance. Ensuring the helminthological safety of fish products for human consumption and addressing the impact of parasitic diseases on fish populations in the region remain pressing concerns [9].

This study investigated the prevalence of specific parasites in different fish species inhabiting water bodies across Kostanay region, aiming to assess the overall parasitic burden and its potential impact.

Materials and Methods

The work was performed at the Department of Veterinary Medicine of the Non-Profit Joint Stock Company "Akhmet Baitursynuly Kostanay Regional University" (Kostanay). For definitive diagnosis, samples were further analyzed at the laboratory of National Center of Biotechnology LLP, Astana.

The study of the epizootological situation for parasitic diseases, including fish opisthorchiasis, was carried out in the Kostanay region from 2022 to 2023. Within the framework of the budget program "To study the epizootological characteristics of the country's territory for especially dangerous diseases and to develop veterinary and sanitary measures to improve their effectiveness" and the concluded agreement between the NLC "Akhmet Baitursynuly Kostanay Regional University" and LLP "Kazakh Scientific Research Veterinary Institute" No. 04/8-21-32 from 07.09.2021, as well as the topics of the master's thesis "Monitoring of the parasitological situation of helminthiasis of fish in the territory of Kostanay region".

The objects of monitoring of the parasitological situation in 7 reservoirs of Kostanay region became: Verkhnetobolskoe reservoir of the Torgai river, the Uly-Zhilanchik river, the Zhaldama river, the

Tobol River, the Zhelkuar river, the Akkol lake, where live in large numbers the bitiniid mollusks (the first intermediate host of *Opisthorchis felineus* metacercariae).

A total of 6 species of fish of the Cyprinidae family (*Cyprinidae*) in the amount of 247 specimens were studied. Namely, crucian carp (*Carassius carassius*), ide (*Leuciscus idus*), bream (*Abramis brama*), tench (*Tinca tinca*), roach (*Rutilus rutilus*), carp (*Cyprinus carpio*).

The species membership of fish was determined by the atlas "Fish Identifier" (Myagkov H.A., 1994) and the textbook "System of commercial fish" (Azizov H.A., Moiseev P.A., 1996).

The study of fish was carried out by a complete helminthological autopsy using the Scriabin method. A compressor method was also used to diagnose fish for infection with *O. Felineus* and *M.bilis* metacercariae.

The fish to be examined was determined up to the species. Then, having freed the middle part of the body from the scales, the skin was cut with scissors along the middle line of the back, and two vertical incisions from the first incision to the lateral line outlined the area of the middle third of the back. The skin was removed from it and a layer of muscles 2-3 mm thick was cut off, which was then examined in the compressor using binoculars (Beer, 1987). For each fish, a thorough external examination assessed skin and fin condition, followed by detailed inspection of the gills. The abdominal cavity was then opened, and internal organs including the heart, liver, gallbladder, spleen, swim bladder, kidneys, genitals, and gastrointestinal tract were systematically examined for any abnormalities. Particular attention was paid to the most likely localities of metacercariae – subcutaneous connective tissue and surface layers of muscle tissue. The diagnosis of diplostomiasis was also made using the compression method of examining the eyes of fish. For this technique, the eyes were removed from the eye cavities, opened with sharp scissors and clamped between two compressor glasses. Then the obtained material was examined under a microscope.

Metacercariae of opisthorchid fish were identified in the laboratory of the Department of Veterinary Medicine, Akhmet Baitursynuly Kostanay Regional University. The identification process involved a compressor method followed by microscopic examination using a Levenguc light microscope. Microscopic analysis of the helminth larvae was performed using an MBS binocular microscope at 16x magnification. Additionally, to confirm the infection of fish with opisthorchiasis, muscle samples of 5 fish affected by opisthorchiasis metacercariae (ide) were sent to the laboratory "National Center of Biotechnology" LLP, Astana (PCR protocol dated 04/24/2023).

Results

The study investigated the epizootiological situation for fish parasitoses in the Kostanay region, focusing on Zhangeldy, Amangeldy, Denisov, and Zhitikara districts due to their close proximity to freshwater resources, primarily smaller rivers. The functional stability of parasite foci in these areas is attributed to the presence of all necessary links in the opisthorchid life cycle: intermediate hosts (bitiniid mollusks as the first host and cyprinid fish as the second) and definitive hosts.

This study aimed to select freshwater fish, specifically those belonging to the *Cyprinidae* and *Salmonidae* families, from rivers, lakes, and reservoirs in the specified regions. Four cyprinid species (*tench, ide, bream*, and *roach*) and one salmonid species (ripus) were chosen for investigation, as detailed in Table 1.

Table 1 – Helminthiasis of fish in the water basins of the Kostanay region

Name of the reservoir	Types of fish	Researched	Affected	Type of parasite	The extent of the invasion%	Infestation intensity, instance
Zhangeldy district						
Uly-Zhilanchik River	*Ide*	45	5	*Opisthorchis felineus*	18,5	1-3
			1	*Metorchis bilis*	8,3	2
	Roach	15				
	Tench	12				
	Bream	11				

Torgai River	*Crucian carp*	17				
	Roach	14				
Akkol Lake	*Sazan*	12				
	Roach	10				
	Ripus	3				
Amangeldy district						
Zhaldama river	*Roach*	13				
	Tench	7				
Torgai River	*Ide*	15	1	*Opisthorchis felineus*	6,6	2
	Roach	13				
	Bream	12	2	*Ligula imestinalis*	16,6	1
Denisov district						
Verkhnetobolskoe reservoir	*Ide*	12				
	Bream	10				
	Roach	15				
Zhitikara district						
Zhelkuar river	*Ide*	9				
	Tench	2				
Total:		247	9			

From the research data reflected in Table 1, it can be seen that most of the fish studied in the reservoirs in Zhangeldy and Amangeldy, Denisov, Zhitikara districts were free of parasites: *ide, roach, tench, bream, crucian carp* (Zhaldama river, Zhelkuar river, Verkhnetobolskoe reservoir and lake.Accol), *bream* (river Uly-Zhilanchik), *carp, ripus* (lake Accol). However, several fish species studied in the reservoirs of Zhangeldy and Amangeldy districts were susceptible to parasitic diseases. Metacercariae were found in eight ide caught in Zhangeldy district from the Uly-Zhilanchik rivers: *O. Felineus* and *M. Bilis,* while the prevalence was 18.5% and 8.3%, and the intensity of 1-3 and 2 specimens, respectively. In Amangeldy district in the Zhaldama River, the fish is completely free of any parasites, and no helminths were found in the roach (Torgai River). And in two species of fish: one individual ide and two individuals bream, caught from the Torgay River, metacercariae *O. Felineus* and plerocercoids *Ligula imestinalis* were found, respectively. At the same time, the prevalence was 6.6% and 16.6%, and the infestation intensity was 2 and 1 specimens, respectively. Parasitic diseases of fish were not diagnosed in the waters of Denisov and Zhitigara districts. The analysis of the infestation of fish from different reservoirs indicates that the ide is the species most susceptible to invasion of *O. Felineus, Metorchis bilis.* In the fish of the species: *bream, pathogens* of ligulosis were found in single specimens. As a result of the conducted studies, a natural focus of opisthorchiasis infestations of the Uly-Zhilanchik and Torgai rivers was established, and a significant accumulation of the intermediate host of the mollusk of the genus *Bithynia* was also found in the coastal waters.

Among the six fish species examined, only the ide harbored opisthorchid metacercariae, as detected by the compressor method. Out of 45 ide infected with opisthorchids with an invasion intensity of 2-3 copies, 8 individuals were found, as shown in Figures 2 and 3. No metacercariae of opisthorchids were found in other fish species (*ripus, crucian carp, carp, roach, bream, tench*).

Analysis by the National Center of Biotechnology LLP, Astana (PCR protocol, April 24, 2023), revealed two opisthorchid species among five fish with metacercariae. Two ides harbored both *Opisthorchis felineus* and *Methorchis bilis* (Figure 1).

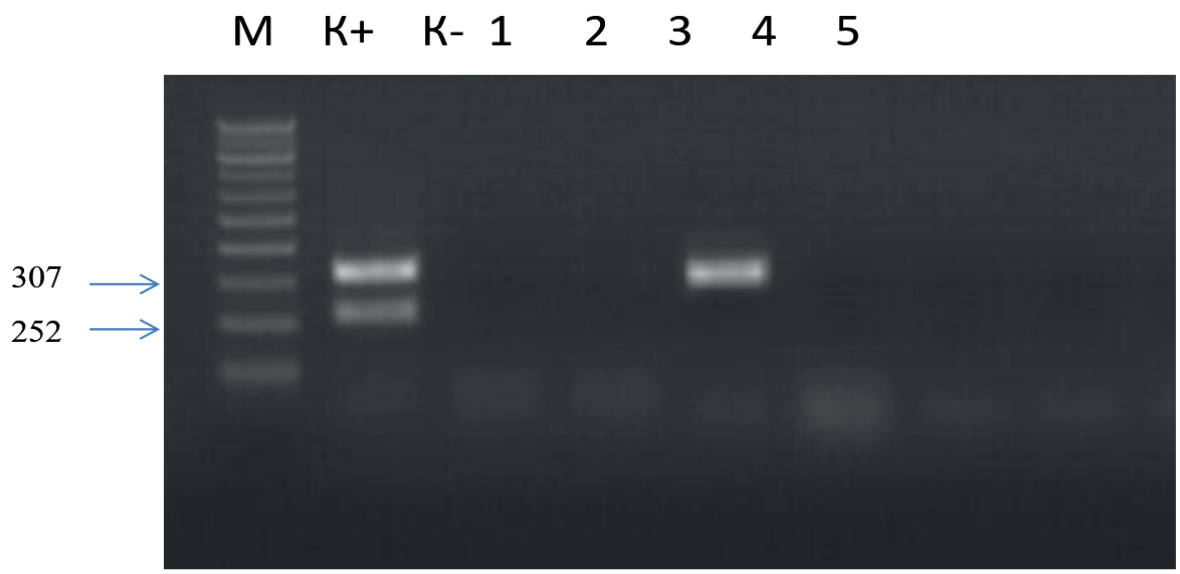

Figure 1 – Electrophoregram of PCR results with specific primers M – marker 1-5 – studied samples, K+ – positive control, K - – negative control

The pathogens *Opisthorchis felineus* and *Methorchis bilis* were confirmed in two of the five provided samples through PCR analysis, as shown in Figure 1.

Discussion

Ligulosis in bream is monoinvasia, the causative agent of which is the cestode *Ligula intestinalis.* Invasive fish are concentrated mainly in shallow water areas. The high rates of infection of fish with ligules are explained by the significant number of fish-eating birds of the *gull* family in reservoirs. Gulls and crustaceans are the main reservoir in which the population of the idler is preserved in nature. The life span of the helminth in the intestines of the bird is limited to 2 – 5 days. However, given the ability of seagulls to travel long distances and make flights from one reservoir to another, the spatial possibilities of the spread of invasion are significant [10].

It is quite difficult to diagnose metorchosis in fish as well as opisthorchiasis by classical parasitological methods of analysis. Identifying the causative agent of metorhoz has been challenging due to the remarkable similarity between existing species of the pathogen [11]. Modern DNA-based diagnostics employing PCR with specific primers enabled the differentiation of two opisthorchid species. PCR successfully amplified fragments (amplicons) with distinct molecular weights: 307 bp for *Opisthorchis felineus* and 252 bp for *Methorchis bilis.* This confirms the presence of mixed metacercariae infections with both parasite species in the isolated specimens [12].

Opisthorchiasis, metorchiasis, and fish ligulosis persist as substantial public health concerns requiring continued focus in medical, biological, and veterinary research and practice.

Conclusion

According to the results of studies conducted in Zhangeldy and Amangeldy districts of Kostanay region, biohelminths of epizootological and epidemiological significance were found.

According to the results of monitoring of parasitic diseases in fish, it was found that the natural focus of opisthorchiasis and metorchiasis was formed within the boundaries of Zhangeldy and Amangeldy districts of the Kostanay region. The Uly-Zhilanchik and Torgai rivers are natural foci of opisthorchiasis with a high level of epidemiological risk. Favorable conditions for shellfish habitat cause the incidence of fish (ide) with opisthorchiasis and metorchiasis. The prevalence by opisthorchiasis and metorchiasis was 18.5% and 8.3%, and the infestation intensity of 1-3 and 2 specimens, respectively. In Amangeldy district in the Torgay River, parasitization of *Ligula intestinalis* plerocercoids was detected in two ticks, the prevalence was 6.6%, and the infestation intensity was 1-2 specimens.

Parasitic diseases of fish were not diagnosed in the waters of Denisov and Zhitikara districts. The findings from the epizootological monitoring of

fish parasitic diseases highlight the crucial role of continued research on parasite system components. This research will provide an objective assessment of the region's epidemiological and epizootological state, facilitating forecasting and monitoring of the parasitological situation.

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EFFECT OF HUMIC FEED ADDITIVE ON METABOLIC PROCESSES AND PRODUCTIVITY OF BEEF CATTLE

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Abstract

The use of made-in-Kazakhstan organic and mineral feed additives to in-crease the meat productivity of cattle is important for the agro-industrial complex of the Republic. During the study, qualitative and quantitative analysis of raw materials was made. Quality control of the finished feed additive was carried out following the requirements of the State Pharmacopoeia of the Republic of Ka-zakhstan. The studies were conducted in accordance with the requirements of the Technical Regulations approved by the Decree of the Government of the Repub-lic of Kazakhstan dated April 23, 2008 No. 380, Regulation No. 7-1/625 dated 11/28/2014 and Regulation (EC) No. 1831/2003 of the European Parliament and of the Council of September 22, 2003, on additives for use in animal feeding. Ka-zakh potassium humate of the Maykubensky deposit is sterile, non-pyrogenic, non-toxic, and harmless, with an average pH of 11.0-1.0, and has a biological ef-fect on laboratory animals (white mice). The effectiveness analysis in the produc-tion experiment allowed us to identify the positive effect of feed additives on metabolic processes and increasing meat productivity in bulls. Regardless of age, on the 7th day of taking the feed additive of potassium humate, the calves' coats acquired a noticeable shine and a bright color, and shedding was completed with-in a month. Clinical signs of metabolic disorders and vitamin deficiency, detected in the farm at the time of the experiment in some animals, pass through 20-30 days from the beginning of the application of the feed additive. The optimal scheme for the use of feed additives was tested on the Kazakh meat breed Akbas, and the concentration of humic substances was selected for the maximum mani-festation of the biologically active effect. Animals became mobile and active, their rumination became more active, they grew noticeably, and the average daily weight gain increased by 7.6-8.9%.

Key words: cattle; feed additive; humic substances; meat productivity; po-tassium humate.

Introduction

The program implemented by the Government of the Republic of Kazakh-stan for the development of beef cattle breeding primarily involves the importa-tion of highly productive livestock from abroad, such as Angus, Aubrac, Here-ford, etc. In most cases, these are animals with productivity from 1,000 g to 2,400 g of daily weight gain and the formation of marbled meat. High meat productivity of livestock and high-quality meat products require a new approach to animal feeding [1].

The use of made-in-Kazakhstan feed additives to increase the meat produc-tivity of cattle is important for the agro-industrial complex of the Republic. This will reduce the cost of beef and also the cost of purchasing and importing similar feed additives from abroad [2].

In recent years, there has been a growing interest in humic substances in an-imal diets. Humic substances are natural organic substances formed in the soil during the humification of dead organic matter. Their main components are hu-mic acids, fulvic acids, and humins. Humic substances are a rich source of easily digestible minerals. They

are considered natural and safe feed additives with many positive effects, including improving animal welfare and the quality of an-imal products [3, 4].

Humic acids are part of the organic mass of peat, coal and brown coal, sludge, some soils, and lignosulphonate, from which they are extracted by treat-ment with weak aqueous alkali solutions [5].

Humic acids are complex high-molecular compounds of an aromatic nature. The main structural unit consists of a flat grid of cyclically polymerized carbon with side branched chains that carry various functional groups: carboxyl, hy-droxyl, phenolic, methoxy, quinoid, and others that are responsible for the reac-tivity of humic acids [6, 7].

In the last decade, there has been a growing interest in the use of humic substances in agriculture, medicine, and biology [8]. Humic substances with a high proportion of humic acids (more than 40%) have been classified by the European Commission as feed materials that can be used in animal nutrition since 2013 [9].

Due to its redox properties, humic acid can reduce iron (III) to iron (II) in aqueous conditions in a wide range of pH values (from 4.0 to 9.0). Humic acids can restore and release iron from ferritin reserves, as well as promote lipid peroxidation. This contributes to the normalization of metabolic processes in animals and humans [10].

According to scientific research, humic acids, improving digestion and as-similation of feed, optimize the condition of the gastrointestinal tract of animals. Replacing antibiotics (added to feed as growth stimulants) with humic acids im-proves the productivity and condition of animals, namely, daily weight gain and feed intake [11].

Humic acids, as natural components of humus, constantly enter the animal body with pasture grasses, feed, or natural or special feed additives, are included in metabolic processes, are completely metabolized in the cell, and are assimilated without a trace in the animal's body, acting as additional sources of biologically active substances, namely, various mineral compounds. They have a positive ef-fect on the general condition and normalize the metabolic processes of the body at the molecular level. Therefore, products obtained from animals that received the preparation can be used without any restrictions [12].

In Russia, humic preparations have been widely used for feeding farm ani-mals and plants since the beginning of the second half of the 20th century. To in-crease the weight gain of animals and strengthen the general nonspecific re-sistance of the organism, in 1987 the Presidium of the Veterinary Pharmacologi-cal Council under the General Directorate of Veterinary Medicine of the USSR, based on the results of state production tests, decided to use ballast-free sodium humate as a feed additive in the cattle and poultry diets [13].

In Kazakhstan, humic substances in the form of sodium humate or potassi-um humate are obtained from brown or stone coal [14]. The use of made-in-Kazakhstan preparations based on humic acids in meat husbandry would allow to activate metabolic processes and increase weight gain in animals.

The purpose of the study is to determine the qualitative indicators of po-tassium humate and the effectiveness for animals of the «Gumka-KZ» feed addi-tive based on Kazakh raw materials.

Research objectives:

1. Determination of organoleptic and physico-chemical properties of potas-sium humate;

2. check of sterility, pyrogenicity, toxicity and harmlessness of potassium humate and finished feed additive;

3. Analysis of the organoleptic properties of the finished feed additive;

4. quantitative analysis of potassium humate content in feed additive;

5. analysis of the biological activity of the feed additive on laboratory ani-mals and in production experiments.

Materials and methods

Potassium humate was obtained and analyzed at the Institute of Coal Chemistry and Technology Limited Liability Partnership (LLP); the development and quality control of the feed additive were carried out at the Laboratory of Mycology and Biotechnology of Fungi at the Kazakh Agrotechnical Research Uni-versity named after S. Seifullin; infrared (IR) spectrometry of the feed additive was carried out at the Provost Office (Collective Use Office of the Nazarbayev University Autonomous Educational Organization (AEO)).

The object of the study:

- Four samples of potassium humate of Kazakh origin obtained from brown coals mined in Kazakhstan.

The control of appearance, pH, sterility, pyrogenicity, toxicity, and harmlessness of raw materials and finished feed additives was carried out following the requirements of the State Pharmacopoeia of the Republic of Kazakhstan [15]. The studies were conducted in accordance

with the requirements of the Technical Regulations approved by the Decree of the Government of the Republic of Kazakhstan dated April 23, 2008 No. 380, Regulation No. 7-1/625 dated 11/28/2014 and Regulation (EC) No. 1831/2003 of the European Parliament and of the Council of September 22, 2003, on additives for use in animal feeding.

IR spectroscopy was performed on the Shimadzu IR Prestige-21 IR Fourier spectrometer with the Miracle attenuated total internal reflection (ATIR) attach-ment produced by the Pike Technologies. IR spectrometry for the qualitative con-tent of humic substances was carried out on a Nicolet iS 10 IR Fourier spectrom-eter, with an average mass of the analyzed sample equaling 2 ml. Quantitative analysis of potassium humate was carried out by ultraviolet (UV) spectrometry on the Evolution 300 UV-VIS Spectrophotometer equipment manufactured by Thermo Scientific. The work on the spectrometers was carried out according to the manufacturer's instructions.

Laboratory animals (white mice) from the S.Seifullin KATU vivarium were used to study harmlessness, acute toxicity and biological activity. Laboratory tests of the harmlessness, toxicity, biological activity of the preparation were carried out on clinically healthy white outbred mice with a group of 10 heads with a live body weight of 12-18 g, who had not previously been exposed to toxic effects and were in the same conditions with appropriate feeding and maintenance conditions. The preparation was administered with water on an empty stomach after a 12-hour fasting diet. The feed was given 2 hours after the preparation. The animals of the experimental group received a 1% solution of potassium humate in the form of a drink freely available instead of water at a dose of 0.1 ml/0.1 l; the control group was given drinking water in similar volumes. The animals were constantly monitored. Attention was paid to behavior, motor activity, thirst, the presence or absence of appetite, the course of pregnancy (if the mice were pregnant), and other physiological parameters.

Production experiments to determine the biological effectiveness of the Gumka-KZ feed additive on the population of farm animals were carried out in farms of Akmola, Karaganda, and Pavlodar regions. Experimental and control groups of cattle were created: meat and dairy calves and crossbreed young ani-mals of 2-3 months, fattening bulls of the Kazakh white-headed breed (Akbas), and young animals of the Simmental breed. The experiment was carried out on healthy animals in the same keeping conditions of detention in a group of 10 heads weighing 100-120 kg. The selection of animals was carried out using the analog pair method. Weakened young animals with signs of metabolic disorders and clinically healthy animals were selected to participate in the experiments. The control and experimental groups of animals received the generally accepted diet. The experimental animals also received a daily preparation of potassium humate in the form of a 1% solution. We have previously selected various concentrations of potassium humate in other experiments on laboratory and agricultural ani-mals, so here is the concentration that showed the best result. The introduction of the feed additive to newborn animals was carried out once in the form of a drink of water or milk daily in the morning before feeding. The rest of the animals re-ceived the feed additive with the feed or in buckets individually with water. The use of feed additives contributed to the appearance of appetite, calves ate food with appetite, drank water and milk completely. The weight of the animals was measured before the experiment and 50 days after the first drinking of potassium humate. Visual monitoring of the condition of the animals was carried out daily, the animals were weighed monthly, and a biochemical blood test was performed before and after the experiment. The results were processed statistically.

Results

To obtain a biologically active feed additive, samples of potassium humate of Kazakh origin obtained from coals mined in Kazakhstan were analyzed by ox-idation and further ultrasonic disintegration, including the coal from the deposits of Saryadyr (1), Sarykol (2), Mamyt (3), Maikube (4), and Shubarkul (5). During the study, the absence of biological activity in sample 1 was observed. Therefore, information on this sample is not provided further.

The results of determining the organoleptic properties of four batches of potassium humate are presented in Table 1.

Table 1 – Organoleptic properties of potassium humate solutions

Indicators	Potassium humate-2	Potassium humate-3	Potassium humate-4	Potassium humate-5
Aggregate state	a thick liquid that leaves oily streaks on the walls	a liquid that leaves slight oily streaks on the walls	a thick liquid that leaves oily streaks on the walls	a thick liquid that leaves slight oily streaks on the walls
Color	brown coal	fulvous coal	fulvous coal	fulvous coal
Smell	when heated, the substance has a pronounced carbon smell/without heating, the smell is weak			
Taste	bitter	bitter	bitter	bitter
Transparency	opaque, the preparation goes off the walls of the container slowly	opaque, the preparation goes off the walls of the container quickly	opaque, the preparation goes off the walls slowly	opaque, the preparation goes off the walls relatively quickly
Sediment	insignificant	insignificant	significant	insignificant
Impurities	none	none	none	none
Foaming during shaking	forms large and small foam bubbles	forms a fine foam	forms a large foam (beer foam) and fine foam	forms a fine foam
pH	13.6	12.39	13.64	13.51

Our studies demonstrated a high biological activity of the potassium hu-mate-4 sample. The characteristics of the potassium humate of the Maykubensky deposit are given in Table 2.

Table 2 – Characteristics of potassium humate (Maykubensky deposit)

Appearance	liquid
Color	from dark brown to black
Smell	weakly expressed coal smell
Weight of dried humic acids, g/100 ml	0.237
The mass of the ash residue of humic acids, g/100 ml	0.040
Mass fraction of free humic acids per analytical state, %	54.86
Alkali concentration in liquid humic acids, g/dm3	22.6
Solubility in water, g/l	easily dissolves at 20°C
Hydrogen index, pH	11-13 units at 20°C
Density, g/cm3 at 20°C	0.96

Analysis of the IR spectra (FITR spectrum) of potassium-4 humate showed that in the spectra of humic acid, there were peaks of tension oscillations charac-teristic of hydroxyl, carboxyl, and benzene rings, and also finer molecular struc-tures (Figure 1).

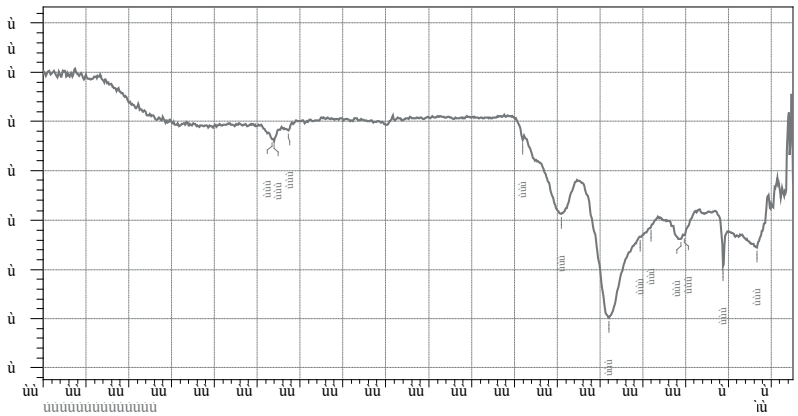


Figure 1 – IR spectra of humic acid (liquid sample of potassium humate-4)

Physico-chemical analysis of basic humate-4 showed that the preparation contained humic substances and mineral compounds and elements. Humic sub-stances in the feed additive were represented by potassium humate, humic, and fulvic acids. As additional components, the feed contained a complex of minerals (sodium (Na): 1,526 mcg/g, magnesium (Mg): 240 mcg/g, potassium (K): 10,997 mcg/g, calcium (Ca): 672 mcg/g, iron (Fe): 1,955 mcg/g, copper (Cu): 73.3 mcg/g, zinc (Zn): 3.60 mcg/g, selenium (Se): 1.16 mcg/g, and others).

The results of sterility testing of four samples of potassium humate and the finished feed additive showed that there was no growth of extraneous microflora in Petri dishes, which indicates their sterility.

Pyrogenicity tests were carried out on rabbits, not albinos, with a body weight of 2.0 to 3.5 kg. In experimental animals with an initial temperature of 37.8 and 38.1°C. Potassium humate was injected intramuscularly into the outer part of the thigh in a volume of 1.5 ml. The final body temperature of rabbits after three hours was 37 and 37.9°C, which indicates the non-pyrogenicity of the preparation.

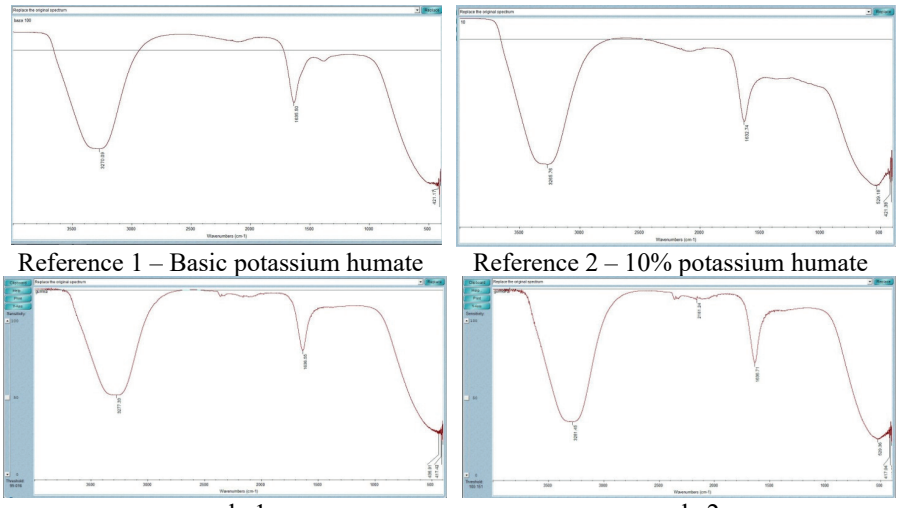
The analysis of the toxicological properties of potassium humate in differ-ent samples of the Maykubensky deposit demonstrated the low toxicity of the preparation. The toxicity and harmlessness of potassium humate were studied on white mice. Observation of animals during the experiment showed the absence of side effects in animals (no refusal of feed, weight loss, absence of case were not-ed). Thus, the sample of potassium humate-4 is sterile and harmless. According to the degree of impact on the body, potassium humate is classified as class 4 toxicity (low-hazard substances).

As a result of oral administration of 1% potassium humate solution (samples 1-4) to white mice, we additionally found that their live weight had increased slightly compared to the control group. The mice became calmer and more active, and their coat became clean and bright white. A particularly noticeable effect was noted when the mice were exposed to potassium humate from the 4th sample.

Thus, it was shown that the use of potassium humate-4 had a positive effect on the growth and development of animals and a stimulating effect on the body, which was manifested by an increase in the live weight of laboratory mice.

Based on the obtained results, we made a feed additive under the patented name Gumka-KZ. Analysis of the organoleptic properties of the finished feed ad-ditive showed that the preparation was a dark brown liquid solution with a weak specific odor, the formation of a slight sediment during storage, and a pH of 11.0±1.0.

IR spectrometry of the Gumka-KZ feed additive on the Nicolet iS 10 IR Fourier spectrometer allowed us to prove the identity of potassium humate. When comparing the reference spectra and the tested substance, it was seen that the positions of the significant bands of both spectra corresponded to each other within 0.5% of the wavenumber scale (Figure 2).



Reference 1 – Basic potassium humate | Reference 2 – 10% potassium humate

sample 1 | sample 2

Figure 2 – Comparative IR analysis of the Gumka-KZ feed additive

A comparative analysis of the IR spectra of the feed additive prototypes and control samples indicated their identity in all samples [16]. The IR spectrum of samples 1 and 2 showed that in the region of 3,300-3,000 cm-1 in the center there were wide peaks at 3,270 cm-1, which were attributed to valence bond fluc-tuations: −OH, −COOH, and H2O. The peaks at 1,637 cm-1 were attributed to the valence oscillations of the −COO group. Accordingly, this indicates at the exist-ence of oxygen-rich functional groups on the surface of pure potassium humate, which contributes to the reaction of complexation or adsorption. These peaks show fluctuations in C-O connected to the potassium ion with ion-ion interaction.

Quantitative analysis of the potassium humate content in the feed additive by UV spectrometry showed the following (Figure 3).

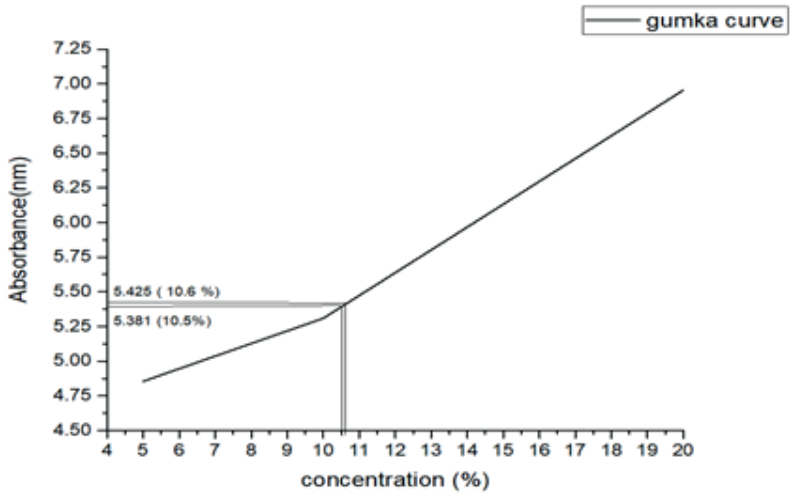


Figure 3 – Concentration of humic substances in the finished feed additive

As can be seen from Figure 3, the concentration of humic acids in two samples of the finished feed additive containing 10% potassium humate was 5.40±0.022, which corresponds to the stated indicators, since the mass fraction of free humic acids in the base solution of potassium humate was 54.86% (Table 2).

Setting up experiments to identify the effect of the Gumka-KZ feed additive based on potassium humate-4 on the growth and development of laboratory animals that received it in the form of a drink for 10 days and observation for a month showed the following: in the experimental group, the total weight increased from 46.1 to 73.7 g, and thus the weight gain was 26.6 g, while in the control group, the total weight increased from 46.5 to 65.6 g. Thus, the total weight of animals in the experimental group increased by 27.6 g, and in group 2 (control) by 19.1 g (Figure 4).

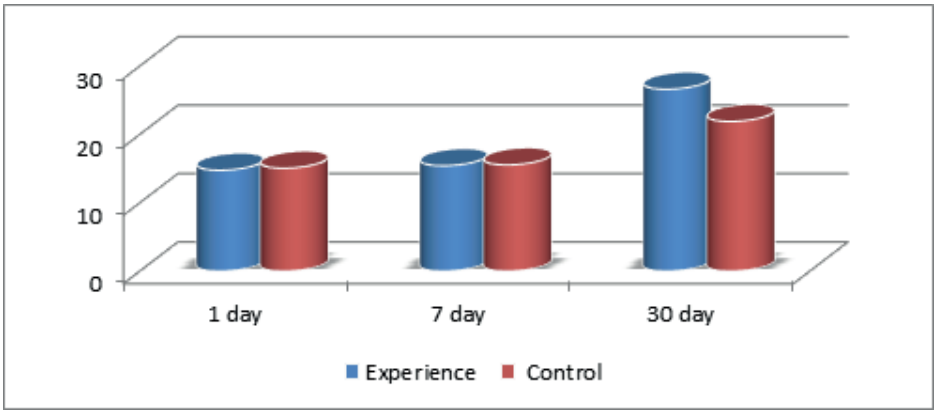


Figure 4 – Weight of laboratory animals that had received potassium humate-4 in the form of a drink

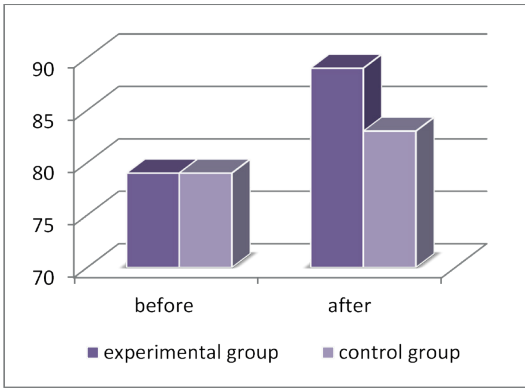
In the next stage, we studied the effectiveness of the feed additive on the population of farm animals in the farms of Akmola, Karaganda, Pavlodar, and Turkestan regions. The effectiveness of the feed additive in production experi-ments was determined on animals that had not previously been exposed to toxic effects and were in the same keeping conditions. The animals were selected by the analog pair method.

We found that the introduction of potassium humate into the calves' diet in addition to the main feed caused an improvement in metabolic processes, accompanied by an improvement in the quality of the calves' coats, increased appetite, and restoration of motor activity (Table 3).

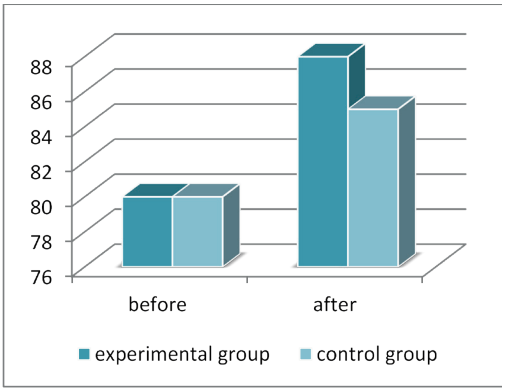
Table 3 – Analysis of comparative data on experimental animals after 30 days

Indicators	Experimental group	Control group
Appearance	healthy, the coat is shiny and bright	healthy, the coat is matte and dull
Behavior	active, mobile	calm
Water intake	within normal limits	within normal limits
Food consumption, appetite	good appetite, the animals eat greedily, within the normal limits	good appetite, the animals eat moderately, within normal limits
Shedding	30 days	47 days

The introduction of the Gumka-KZ feed additive to calves was carried out in the form of a drink with water (to healthy calves once a day and to weakened calves twice a day). The analysis of the obtained data showed the presence of a pronounced biological effectiveness of the feed additive, manifested in the in-crease in the live weight of calves of meat breeds and crossbred calves (Figure 5).



Calves of the Akbas breed, 2 months old Kadam LLP (weakened)



Calves of native breeds, 2 months old Household farm of the Arshaly village (healthy)

Figure 5 – The effect of the Gumka-KZ feed additive on the weight gain in calves

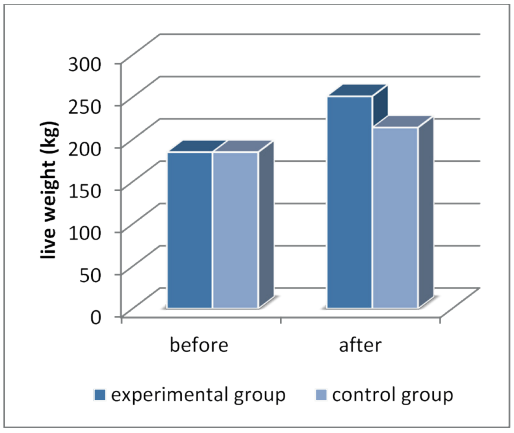
Biochemical analysis of calves' blood before and after drinking the feed additive showed the normalization of indicators to normal or average values (Table 4).

Table 4 – Effect the of feed additive on biochemical parameters of calves receiving the Gumka-KZ feed additive

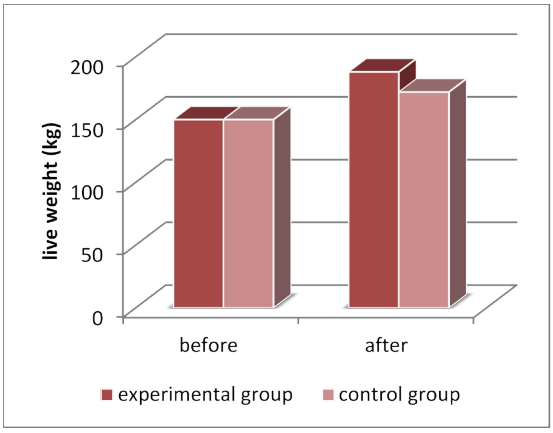
Indicators	Standard	Suckling calves		Weaned calves		Fattening calves	
		Day 1	Day 30	Day 1	Day 30	Day 1	Day 30
Total protein	62,082,0	64,61±1,23	66,28±1,75	61,94±4,7	69,52±3,24	66,2±5,24	71,9±2,33
Creatinine	56-162	55,22±6,19	50,38±5,32	60,97±13,7	55,61±7,15	109,0±31,8	84,45±5,65
Total bilirubin	0,7-14,0	2,22±2,37	1,88±2,61	1,99±2,56	2,11±0,98	1,89±0,63	2,41±0,76
Cholesterol	1,3-4,42	2,34±1,16	1,97±1,43	2,37±1,08	2,16±5,16	3,75±0,64	2,88±0,33
Urea nitrogen	2,8-8,8	3,7±3,53	2,50±0,44	4,21±3,46	4,99±4,57	4,09±0,59	5,28±0,69
Glucose	2,3-4,1	2,14±0,22	2,30±0,84	2,30±0,44	2,58±0,84	0,41±0,14	1,35±0,81
Calcium	2,5-3,13	7,91±2,18	8,26±1,06	9,8±1,55	10,12±0,22	1,63±0,08	2,2±0,50
Alkaline phosphatase	18-153	298,33±5,84	199,63±2,45	166,22±7,2	155±4,85	101,04±30,76	121,35±16,52
Alanine aminotransferase (ALT)	6,9-35,0	4,67±1,71	20,10±1,41	17,33±2,56	25,33±5,41	23,10±4,36	23,29±2,66
Aspartate aminotransferase (AST)	45-110	37,50±3,74	69,54±3,44	42,69±3,71	82,50±8,77	107,30±12,9	103,22±9,15

Clinical observations have shown that on the 7th day of taking the feed ad-ditive based on potassium humate, the calves' coat obtained a noticeable shine and a bright color, and shedding was completed within a month. Clinical signs of metabolic disorders and vitamin deficiency disappeared in animals 20-30 days after they had started receiving the feed additive. Thus, we identified a positive effect of the Gumka-KZ feed additive on the metabolic processes of the bodies of farm animals.

The introduction of potassium humate into the diet of fattening animals of meat and dairy breeds, in addition to the main diet, affected the improvement of metabolic processes, which led to an increase in the live weight of fattening young animals. It was noted that the weight gain in meat animals was higher than in calves of dairy breeds (Figure 6).



Outbred calves Molshylyk-Agro Socio-Entrepreneurial Corporation (SEC) (60 heads)



Calves of the Simmental breed Milk-Product LLP (110 heads)

Figure 6 – The effect of the Gumka-KZ feed additive on the weight gain of fatten-ing young cattle

As can be seen from Figure 6, the feed additive has a pronounced biological activity, contributing to the normalization of increased weight gain in cattle.

To conduct production tests on the use of the feed additive based on potas-sium humate for fattening livestock, groups of bulls and heifers of meat and dairy cattle were formed in the Abai LLP. Out of 355 experimental bulls, 277 heads (78%) represented the Kazakh white-headed breed. Of the remaining 22%, half of the bulls represented animals born from breed transformation, and the second half were bulls of other meat and dairy cattle breeds.

The analysis of the indicators of average daily weight gain in the range of 900-1,000 g was recorded in 175 (49.3%) bulls of the experimental group. In 110 (31%) bulls, the average daily gain in live weight was in the range of 1,000-1,100 g. The average daily increase in live weight from 1,100 to 1,200 g was noted in 70 (19.7%) bulls. In the control group of 75 bulls, an average daily gain of up to 900 g was observed in 37 (49.3%). 18 (24%) bulls had a weight gain of up to 1,000 g. Up to 1,100 g of average daily gain in live weight was noted in 11 (14.6%) animals. Finally, only 9 (12%) bulls had an average daily gain of 1,200 g (Figure 7).

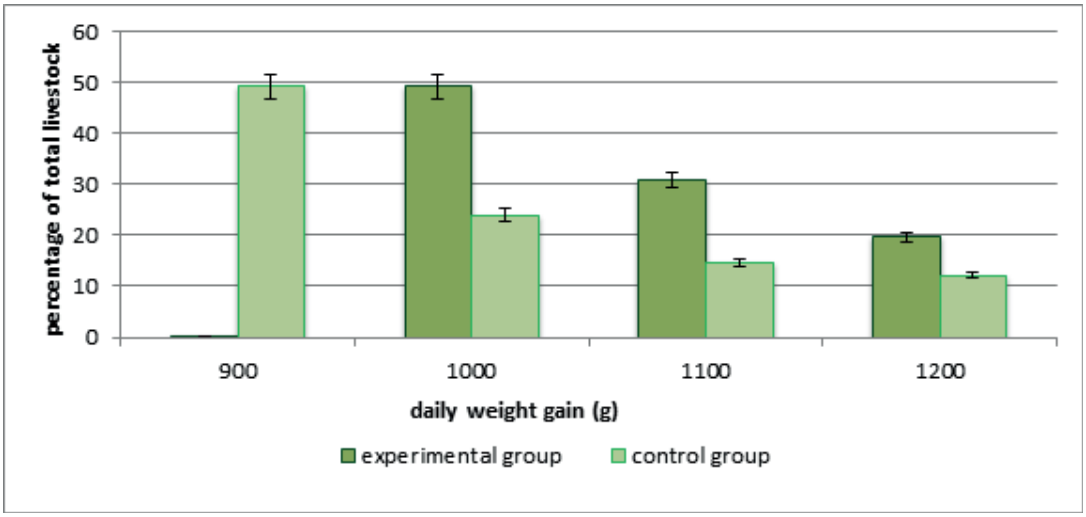


Figure 7 – Indicators of the average daily gain of fattening livestock that had re-ceived the Gumka-KZ feed additive

Comparative data show that in the experimental groups, half of the fatten-ing cattle had an average daily gain of at least 1,000 g, whereas in the control group, only half of the group reached 900 g. The same difference in quantitative and qualitative indicators was observed between the experimental and control groups in terms of average daily weight gain in live weight that equaled 1,100 and 1,200 g. Thus, we found that in the experimental group of fattening animals, the total average daily weight gain was 4-6 kg higher than in the control group. More than 50% of the bulls in the experimental group by the end of the third month of fattening gained a live weight of over 500 kg and were sent for slaugh-ter.

Discussion

In recent years, there has been an increased interest in the use of humic substances in animal diets [17-20]. Humic substances (including humic and fulvic acids) are considered safe and natural feed additives that have a beneficial effect on animal welfare and the quality of animal products. Humic substances (humic acids, fulvic acids, and humins) are natural organic substances found in the soil, formed during the humification of dead organic matter. A rich source of these compounds is oxyhumolite (oxidized brown coal) [21]. Humic acids are the main component of these substances. This fraction is insoluble in acidic solutions (pH < 2), but soluble in solutions with a higher pH. These acids have a high molecular weight from 5,000 to 10,000 Da [22]. They have many physical, chemical, and biological properties that make them suitable for use in animal husbandry and veterinary medicine. They exhibit antioxidant and anti-inflammatory effects and support the work of the gastrointestinal tract of animals, accelerating their growth and simultaneously improving

immunity and reproductive function [23].

Additives of natural origin can be added to feed to improve the growth pa-rameters, animal health, and/or improve the quality of produced meat [24]. In 1999, the European Medicines Evaluation Agency (EMEA) issued a permit for oral administration of humic acids to all animals from which animal products are obtained. In animal husbandry, the addition of humic acids to feed can positively affect all production parameters. Humates, which are part of feed or water for poultry, contribute to the growth of the birds [25].

The ability of humic acids to influence mineral metabolism in animals due to the presence of chelating properties was reported by Rybalka et al. (2020). They demonstrated the ability of humic acid to increase the content of calcium, ionized calcium, and iron, and adjust the content of copper and zinc, as well as increase the activity of alkaline phosphatase in the blood serum of rabbits. They also observed an early effect of humic acid supplementation on an increase in the content of ionized iron and calcium, as well as a later effect on the activity of al-kaline phosphatase and an increase in the content of copper in blood serum; on the distribution of calcium, phosphorus, manganese, copper, and zinc in bone tis-sue [26].

As can be seen from our results, the Gumka-KZ feed additive based on po-tassium humate of the Maykubensky deposit leads to an increase in weight gain in meat and dairy cattle, accelerates the shedding time, improves the condition of the coat, and normalizes the biochemical blood parameters. The weight gain of animals increased by 8.4% in the group of suckling calves, by 7.6% in the weaning group, and by 8.9% in the group of one-year-old calves during pasture keep-ing. Similar results on the positive effect of humic acids are described by Arif et al. (2019), who showed that humic acid plays a favorable role in increasing productivity due to its beneficial effect on the utilization and assimilation of nu-trients [9].

Conclusion

Kazakh potassium humate of the Maykubensky deposit is sterile, non-pyrogenic, non-toxic, and harmless, with an average pH of 11.0-1.0. It also has a biological effect on laboratory animals, increasing the weight of animals in the experimental group by 1.9-2.57 g per month compared to the control group of mice.

The use of the Gumka-KZ feed additive on cattle leads to an increase in weight gain, acceleration of shedding time, improvement of the condition and shiny appearance of the coat, and normalization of blood biochemical parameters. Animals become mobile and active, rumination becomes more active, the growth of animals in the experimental group is visually noticeable, and the average daily weight gain increases by 7.6-8.9%.

Information on funding

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Dear author!

The scientific journal "Bulletin of Science of S.Seifullin Kazakh Agrotechnical Research University: Veterinary Sciences" aims to be included in international databases such as Scopus, Web of Science and AGRIS (International information system for the Agricultural sciences and technology), etc. In this regard, the editorial board of the journal decided to consider and accept for publication from 2023 articles prepared in English.

Basis

In accordance with the order of the Minister of Education and Science of the Republic of Kazakhstan No. 170 dated April 30, 2020, the editorial office of the journal "Bulletin of Science of S. Seifullin Kazakh Agrotechnical Research University: Veterinary Sciences" has developed a website with an online system for submission and review of articles.

In this regard, when submitting an article for publication in a journal, it is necessary to register as an author on the website of the journal and upload the article offered for review on the online platform.

Registration of the author is carried out via the following link: https://bulletinofscience.kazatu.edu.kz/index.php/veterinary-science

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Requirements for publication of scientific articles in the journal "Bulletin of Science of S. Seifullin Kazakh Agrotechnical Research University: Veterinary Sciences"

The editorial board of the journal asks the authors to familiarize themselves with the rules and adhere to them when preparing papers sent to the journal.

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The article should contain only original material, reflecting the results of research of the author/s, previously not published elsewhere.

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Articles in journals (print format)

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Nevin, A. (1990). The changing teacher education special education. Teacher Education and Special Education: The Journal of the Teacher Education Division of the Council for Exceptional Children, 13(3-4), 147-148.

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Jameson, J. (2013). E-Leadership in higher education: The fifth "age" of educational technology research. British Journal of Educational Technology, 44(6), 889-915. DOI:10.1111/bjet.12103

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Bryant, S. J. (1998). European Patent No. EP GB2322334. Munich, Germany: European Patent Office.

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IDENTIFICATION OF WHEAT GENES CONDITIONING RESISTANCE TO PATHOGENIC FUNGI

Aitbay K. Bulashev[1] (ID), Kairat N. Nabiyev[2] (ID)...

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Co-authors: Kairat N. Nabiyeva, e-mail:naruk@mail.ru

Abstract

The author of the article, on the basis of his own research, proves that the presence of wheat resistance genes to pathogenic fungi is a key factor for use in breeding work. The article presents the results of identification of wheat genes Sr32, Bt9 and Bt10 responsible for drought resistance to pathogenic fungi that cause diseases of stem rust, as well as common smut ... [not less than 100 words and not more than 300 words].

Key words: resistance genes; stem rust; hard smut; pathogenic microscopic fungi; electrophoresis; PCR; wheat. (7 words or phrases).

The main text of the article should contain structural elements:

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- Materials and methods;
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