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CONTENTS

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Links between human and animal life in the Age of Anthropocene: From molecules, through reactions to cells, development and ethical implications

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In the last two hundred years, discoveries in animal and human physiology, disease, and drug development have been made. Animals stood at the centre of the experiments to optimise the drug doses and administration routes. Human progress has been driven by various anthropogenic aims and desires. The knowledge development and discoveries continue to increase about the fundamental reality of human existence and the ontological realm in which the socio-ecological systems continue to evolve. Members of *Homo sapiens* have now reached an understanding, power of knowledge and actions which have the ability to influence the Earth's ecosystem. This produces the by-products of human progress, *e.g.* CO_2 and these have started to alter the fundamental/situational reality boundary of human existence and the ontological realm of the socio-ecological systems. Benefits and suffering of humans and animals, separately and together, can be viewed through various lenses, *e.g.* the precautionary principle. The current article uses the methodology which is a combination of bioethical analysis and a theoretical biology analysis of the precautionary principle and its implications into the relationship between human and broader socio-ecological systems. The principle can provide some guidance on ethical understanding of the duality of human actions during Anthropocene and the Great Acceleration and how it is linked to the very chemical essence of life. Humans develop their knowledge about the fundamental reality as part of their search for truth, for understanding of the chemical and other dimensions of the nature of life. This is 'normal science', *i.e.* the search for 'truth' or human understanding of fundamental reality of existence, moves humanity forward. However, its deployment for human development creates by-products that require 'regulatory science', or settings of rules for regulation of the deployment of the normal-science-derived knowledge. An examples of this can be the need to take action and to mitigate the climate change impacts across the globe, impacts on both human and animal life.

Key words: precautionary principle, levels of biological organisation, ethical framework

Introduction

Progress of humanity has resulted in the development of new technologies. These facilitated the improvement of quality of life of humanity, *e.g.* by the use of coal, as a source of energy from about the 1800's [19]. This was a catalyst for development, population growth and other improvements in the nature of human reality, and other

human achievements since the industrial revolution that would not have been possible without the use of coal and fossil fuels [24]. Humanity has now reached a stage of Anthropocene and the Great Acceleration [19]. The epoch in geological time is characterised by numerous discoveries in areas of biomedical science, manufacturing, energy, transportation and so on. Humans have conquered Earth's surface in that they have reached and settled the entire planet, with very few exceptions [24]. The carbon intensive economy and the related development have created feedback loops, where the actions of humanity have created conditions under which the greenhouse gases and their release into the atmosphere and environment have created a negative feedback loop and effects on humanity/human wellbeing [39]. Humans need to act to mitigate further impacts of climate change on nature and on humans themselves [66]. Expansion of human reach and the climate-change-related feedback loop contribute to increased impacts of CO_2 on the environment and on human health across all geographical scales. One way to demonstrate this is that 250000 additional human deaths per annum have been estimated to occur between 2030 and 2050 due to climate change [9]. In addition, there will be an additional 2×10^9 to 4×10^9 USD of healthcare costs due to climate change for the same time period [9]. Increases in the global population means that there will be more emissions of CO_2 and other greenhouse gases, which in turn expose more people to the climate-change related risks or impacts. Humanity started acting to decrease these impacts and releases of greenhouse gases by adopting the international guidelines and agreements to mitigate climate change.

Goal 13 of the Sustainable Development Goals promotes finding solutions to issues that are linked to climate change, *e.g.* by taking measures to combat climate change itself and its derivative impacts on the socio-ecological systems [58]. In this context, the issue of resilience is indirectly addressed in Goals 9 and 11 [58]. The Paris Agreement was adopted in 2015, where all participants of 194 countries from across the globe accepted to limit the increase in global temperatures up to 1.5 °C [62]. An argument could be made that there are often time lags between environmental pressure (such as those of anthropisation of space [24]), and tangible impacts of such actions, *e.g.* climate change impacts on humans and animals. However, the current phase of the Great Acceleration is specific as the impacts are happening in our life time. This distinguishes the current climate change problem from other issues facing the socio-ecological systems and their components. Such impacts apply to the human population and the environmental nexus, because the direct cost of any successful mitigation will be borne in large part by people and environment now, and in the future. An argument could also be made that any positive action taken would mostly benefit the future human populations on Earth. However, no positive actions by humanity to address the climate-change feedback loops, will surely mean disaster for today's and future socio-ecological systems. Careful considerations must be made in order to weigh the costs, benefits and impacts of any relevant decisions in the short term and the in the long term.

The social cost of carbon dioxide (SCC) is the economic cost of each additional ton of carbon dioxide emissions due to climate change [37]. Climate change policies and their implementation can be explained and also evaluated through the SCC concept [37]. To choose the best mitigation strategy, considerations based on the SCC concept must take into account two aspects. The first one is the assessment of the CO_2 emission pressures/effects created by the human population, the related human population growth rates and future impacts/costs [37, 54]. Achieving reliable estimates in this context has proven somewhat challenging in relation to the predicted increases with greenhouse gas emissions [37, 54] The second requirement that the SCC must meet, and which policy advice might be influenced by, is the need to create a consistent and transparent strategy to evaluating the wellbeing of future human generations over several time scales, and in connection to the CO_2 release and to climate change in general [54]. Approaches such the SCC evaluate the use and impact of anthropogenic technologies on the environment surrounding humans [21]. From a decision-making point of view, the decision-making based on concepts such as the SCC is critical to management of the climate-change-feedback loops that humans have contributed to creating. Such decisions require consideration of the following characteristics of the current ontological realm of socio-ecological systems:

a) humans have reached the technological know-how and state of understanding of the fundamental reality of their existence that allows them to, however indirectly, alter said fundamental reality [24];

b) such level of knowledge and possible alterations will manifest at the boundary between the situational and fundamental reality of human existence and the existence of the socio-ecological systems, *i.e.* animals and abiotic compartments in these system will be impacted by human actions alongside *Homo sapiens*;

c) whatever the original intention behind the search for knowledge and technological development was by humanity as a species, once the power of fundamental reality alterations is in the humans' hands, they live in it, but they are also responsible for managing it in a benign and non-destructive manner, they must prevent malign and destructive manner of management — a manner which could decimate all of the socio-ecological systems worldwide.

Points a)–c) are a distillation of various principles that have been investigated and talked about in the various types of literature over the last century. Humans have reached the ability to influence life on Earth, *i.e. Homo sapiens* has reached the ability to influence the global ecosystem and life itself. Humanity has reached the status of *Homo deus* [22, p. 20]. Examples can include the deciphering of the molecular essence of life by elucidating the structure of DNA [60] This then led to the original and

then the 'reformulated central dogma of molecular biology', which describes the flow of information from DNA to proteins, while capturing any 'detours' from across all living organisms to complete the picture [20]. Theory of evolution and its adjustment or modifications happened in the last century [6], as new elements of fundamental reality of human existence became known to *Homo deus*, as analysed [24]. Discoveries in nuclear physics led to realisation by humanity that nuclear radiation can cause mutations and the inducement of the single- and double-strand breaks in DNA, as well as aberrations in chromosomes [31]. Systematic development of sequencing methods, genetic manipulation and related IT advancements got humanity to the point of sequencing of all vertebrate genomes [46]. Existence of the non-human animals, its molecular basis, categorisation of non-human living organisms had been discovered and/or clarified. Synthetic life has been created by various scientists (*e.g.* [63]). These examples and points summarise the level of human beings' knowledge of about their surrounding… the power to alter the very essence of such surroundings, and the need to manage the application and impacts of such knowledge. Principles of the decision-making under the weight of such human power must balance human progress and protection of non-human parts of socio-ecological systems. One basis for the decision-making is the precautionary principle, which can be defined as follows [47, article 15]:

> "In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation."

The precautionary principle summarises the three elements of the necessary approach of humans to the deployment of their knowledge in the Age of Anthropocene and the Great Acceleration [19, 24], namely [44]:

> "Action-guiding principles tell us which course of action to choose given certain circumstances; (sets of) epistemic principles tell us what we should reasonably believe under conditions of uncertainty; procedural principles express requirements for decision-making, and tell us how we should choose a course of action."

The precautionary principle takes into account the possibility of the impacts of human of knowledge and its development on non-human parts of the socio-ecological systems, while human development and existing knowledge exploitation are taking place. Application of the precautionary principle acknowledges the uncertainty about the outcomes of the human actions and tries to strike a balance between the need for humans to explore the ontology of existence in the climate-change-feedback loop time. The time when the socio-ecological systems around the globe and their existence are in peril. Actions by humans, according to the precautionary principle, are fundamentally seen as those by custodians of the socio-ecological systems. Application of the precautionary principle in some decision-making will be a tool for management of the human existence at the boundary between the fundamental and situational reality, the current reality of *Homo deus* (based on [22]). It is against this background that the current article seeks to investigate the significance of the precautionary principle at evaluating the practical implications of human existence at the boundary between the fundamental and situational reality boundary. This existence is relational to non-human animal life and the environment. The chemical and organisation structure of life, both human and non-human, is used as the start of the discussion about the link being the essence of life and the drivers/necessary drivers of the regulation of human research and deployment of the results. Precautionary principle and the ethics of management/deployment of the human knowledge, for the way to conduct research that impacts, or involves non-human animals, are then presented and subjected to analysis from the viewpoints of theoretical biology and bioethics.

Methodology

Methodology in the current article will be focused on examination of the relationship between humans and non-human components of the socio-ecological systems. The analysis will be bioethical and theoretical-biological in nature, and it will start with performing a set of small thought experiments to set the stage for demonstrating the links between humans, human activity, and nature. This analysis will be aimed at presenting the links and implications between the fundamental and situational reality of human existence in the Age of Anthropocene and during the Great Acceleration [19, 24]. More specifically, it will be aimed at establishing the link between the human existence and the nature of the discoveries that drive human development and the general improvement of the human condition, and the general ontological realm of the existence of the socio-ecological systems globally. After that, the duality of the chemical elements, molecules in living organisms and their impacts on the organisms themselves are linked to the human actions and their impacts on the socio-ecological systems. The duality of the nature of life and the nature and essence of human actions put and presented side by side to close the analyses with the precautionary principle. Finally, the bioethical analysis is linked to the framework for the ethical treatment of non-human parts of socio-ecological systems. The need for the 'normal' and 'regulatory' science is outlined, as a way to manage the impact of human knowledge generation and application at the boundary between fundamental and situational reality in the Age of Anthropocene and the Great Acceleration.

Thought experiments and real-world examples on the relationship between human, living organisms and socio-ecological systems in the Age of Anthropocene

A Balance and mass at the boundary of the fundamental and situational reality

The human knowledge of the fundamental reality has been researched and defined in terms of fundamental constants, as tools for humanity to have a language of scientific discourse and the standardised notation. An example of this notational unity of our fundamental reality of human existence (designated as notational unity in further text of this article) is the SI unit system. The one kilogram, as the basic unit of mass in the SI system, was based on the international standard until 2019, which has been housed in Sèvre, France [12]. This was changed in 2019, when the kilogram was redefined to be based on the fundamental constants of the human existence, *e.g.* the Planck constant. This can be summarised as outlined [56]:

> "The kilogram, symbol kg, is the SI unit of mass. It is defined by taking the fixed numerical value of the Planck constant h to be 6.62607015×10^{-34} when expressed in the unit Js, which is equal to kg m^2s^{-1}, where the metre and the second are defined in terms of c and Δv_{Cs}."

Ever more accurate equipment to measure mass have been developed and so balances/scales have been omnipresent in scientific institutions and non-scientific contexts for a long time. The measurement of mass is an elemental unit operation in the scientific process. For example in the biomedical and laboratory/clinical chemistry, a mass of crystals of $FeCl_3$ must be weighed out to prepare the calibration solution with an accurately known concentration of iron to analyse a blood sample, if a patient might be suffering from anaemia. Such omnipresence of the mass measuring equipment makes a balance or scale a manifestation of the fundamental reality of human existence, the human knowledge about it, and the situational reality of human existence in which the balance/scale is used. The construction or manufacturing of the balance is an expression of the human interpretation of the fundamental reality, as the construction or manufacturing of a balance/scale would not be possible without the definition of the kilogram. Neither would it be possible without the application of the mass concept to the design and manufacture a relevant measuring instrument, *i.e.* balance or scale by members of *Homo deus*. Using a scale to measure mass, a fundamental constant for measuring the 'size of living organisms for specific purposes' would then be a manifestation of the boundary between fundamental and situational reality of human existence in the Age of Anthropocene and during the Great Acceleration [19]. Let us look at the sizing up of living things.

Now considering, the omnipresence of mass measurement and the related equipment will indicate that each part of the human life and the existence of the wider socio-ecological systems. Let's consider the following short thought experiment. A hypothetical animal welfare organisation in a hypothetical city in the developing world looks after the cats and dogs (this organisation is referred to as AWO in further text of the article), as well as provides callouts and investigative capacity in relation to suspected case of animal cruelty. The AWO is in need to get a balance to determine the mass of the kittens and puppies, young cats, and dogs. These cats and dogs have been rescued in the AWO's area of jurisdiction. In the vicinity of the AWO facility, there is a hypothetical university which is a research-intensive one, and it has a spare balance available. That balance makes it possible to measure mass accurately up to 50 kg, and calibrated weights of 1, 5 and 10 kg are available to ensure accurate mass measurements. This balance/scale is normally used for the weighing out of kilogram quantities of environmental sorbents and/or pharmaceutical formulation ingredients. The AWO makes informal enquiries around the city of their geographical location, about the possibility to procure a new balance for measuring of mass of young cats and dogs, as well as the rescued and emaciated adult/strayed animals. The need for a balance/scale is a manifestation fundamental reality that species individuals, belonging to *Felis catus* and *Canis familiaris*, have evolved to have an expected body mass. The body mass and chemical composition of the animals body are an indication of the inexorability principle of the nature of life, and the vital determinism related to it [20]. The inexorability principle means that life is present in front of us, as it is meant to be, or it has developed into an optimised form based on evolution. The lack of a working scale at the AWO is a time-sensitive matter, as not monitoring the weight of the cats and dogs housed at the AWO can have detrimental effect on the animal welfare and on the life of the animals. Therefore, this time dimension of life is a potential source of animal suffering, if a rescued animal is underweight or malnourished it may die if a mass of it is not determined due to the lack of balance, and veterinary medical care is not be initiated as needed [20]. Potential animal suffering is a manifestation of the situational reality of the rescued animals, and it is also linked to the fundamental reality of animal lives. That suffering must be decrease as much as possible, and its management takes place at the boundary of the situational and fundamental reality of animal lives, where humans are an important interventionist force. The human boundary of situational and fundamental reality is also implicitly relevant here, as the AWO staff care about animal welfare and wellbeing as the protection of animal life is the call to duty of the AWO staff and the *raison d'être* of the AWO.

In this context, the research-intensive university through their staff, who are in charge of the 50 kg balance, should apply their knowledge of the fundamental reality of the mass and kilogram to assist the AWO.

Deployment of the fundamental reality knowledge, through derivative epistemic authority of the researcher, the particular human being, who is knowledgeable about the use of balances and the definition of a kilogram, should assist the AWO with deploying their knowledge and to decrease/prevent animal suffering. That researcher should and likely would deploy this knowledge to help the AWO to manage the situational/fundamental reality boundary, they should loan the balance to the AWO and provide a calibration service on a regular basis. This would all be done free of charge. In this way, the humans in question use their epistemic authority to prevent animal suffering or alleviate it by the provision of a body mass monitoring tool. The action-based nature of the engagement and mass monitoring assistance would be in line with the precautionary principle. At the same time, the action is driven by the derivative epistemic authority of the researchers with the mass measurement knowledge. Finally, the decision about the provision of assistance by the researcher to the AWO with the mass measuring is based on the inherent nature of life time commandment and element of life's essence. Time is essential from the sensitivity of the matter of animal life prevention. The AWO can also reject the offer of a balance loan from the research-intensive university on the grounds that there is a need for a researcher to interact with the AWO, and the recipient of the loan might perceive that a power imbalance might exist there. Using researcher's epistemic authority to assist the AWO might be perceived to act outside of the normal scope of professional practice of the AWO, and the researcher if they are not an animal scientist, zoologist or any kind of scientist that works with animals. The AWO might be of the impression that the researcher is not qualified or in the best professional position to assist the AWO and the animals they house. The AWO might therefore be placed in a difficult position due to the potential *ad hoc* funding model of the organisation, and potential perception that a financial gain will be sought by the researcher because of the balance loan.

Ultimately, a researcher assisting the AWO would likely have an ethical and moral interest in diminishing the animal suffering and would offer to help the AWO to decrease animal suffering of the currently and future housed cats and dogs there. This would likely be the case, as the AWO would reach out to the research-intensive university via unofficial or official contacts, or via word of mouth. Therefore the AWO is likely to be actively seeking, at least to some extent, assistance from the university and indirectly from the researcher who might ultimately provide a balance for the body mass determination of animals. Thus the power relationship between the researcher and the AWO would likely be more equitable than not. Based on the considerations in this thought experiment, the researcher should provide the assistance where necessary expertise and equipment in the body mass monitoring is needed so that the AWO. The AWO should work with the researcher, the community, and the veterinarians in the area to decrease the animal suffering. The ethical drivers for the support by the researcher would be voluntary in nature but would be linked to the professional knowledge of the researcher and caring nature of the researcher. It might also be an expression of the categorial imperative [25], *i.e.* the moral imperative of the researcher to contribute to decreasing the human and animal suffering wherever they might encounter it, or where they foresee it developing. This would be the suffering of the housed animals and potentially the staff at the AWO. Said action would be taken, because it is based on the social context which commands it as necessary to diminishing of animal suffering if, where and when possible. By the same token, there is also necessity to diminish or prevent the suffering or mental health problems of the AWO staff when seeing animal suffering. It would be ethically right and morally necessary because it is the right thing to do, it is the right action to take in the context of the researcher's epistemic authority, knowledge about the fundamental reality of human life, its deployment and application at the boundary with the situational reality in connection to AWO's existence at this boundary.

The researcher is able to use a scale, and the calibration is available using the calibrated weights. The mass of a kilogram, as the SI unit and the construction of the scale, are an expression or the outcome of the accumulation of the knowledge about the fundamental reality of human existence by scientists and researchers, by *Homo deus*. This knowledge can then be used to manage or deal with the animal welfare issue at the AWO. Such activities take place on the boundary between fundamental and situational reality of human and non-human animal lives. Assistance and the will of the researcher to help the AWO with application of their knowledge of fundamental reality of human existence and life must be aimed at decreasing or preventing animal suffering which is the result of human actions. More specifically, the researcher's assistance with weighing the cats and dogs housed at the AWO is aimed at decreasing the animal suffering which is, at least in part, caused by human actions, where the human owners have let the animal to become strays. This embodies a precautionary action against the occurrence of the animal suffering, and indirectly some mental health suffering among the AWO staff. The latter type of suffering might arise among the AWO staff if they can't carry out their mandate and help the animals they look after unless animal weighing tools are available. The researcher and the AWO staff could extend the project to research that can collect information about the body mass of rescue cats and dogs in a particular. Such data can be linked to optimisation of the animal welfare and wellbeing management by humans at the AWO. The data and research can help optimise the deployment of the human knowledge about the fundamental reality of animal health and its human understanding towards optimisation of the situational reality of the AWO animal health management. These links to the operational aspects and optimisation of knowledge deployment, about the fundamental reality, might maximise the action-driven and voluntary application of the precautionary principle.

Antibiotic development and potential impacts on the environment

Antibiotics and antimicrobial agents have been one of the backbone of the antimicrobial therapy and infectious disease prevention in the 20th century [2]. They were discovered through a series of exposure of staphylococcal cultures to the ambient environment and isolation of a *Penicillium* spp. which had produced penicillin [18]. Even though some discoveries might have been accidental over time the discovery and development of antibiotics led to the increase in life expectancy in the developed world by about 75 % [2]. Development of antibiotics will be demonstrated on the quinolone antibiotic development. Quinolone antibiotics are a specific class of antibiotics and are classified into four generations, with the more recent drugs (4th generation) having a much broader antimicrobial spectrum. The 1st generation includes nalidixic acid, which was the first quinolone antibiotics and discovered in the early 1960s [35]. First-generation quinolone antibiotics have activity against Gram-negative bacteria. Recent generations have a broader spectrum of activity and enhanced pharmacokinetics compared to nalidixic acid. The second-generation quinolone antibiotics include ciprofloxacin, norfloxacin, lomefloxacin, enoxacin, and ofloxacin. These have higher activity against Gram-negative bacteria, some Gram-positive organisms and achieve higher serum levels of the active pharmaceutical ingredient, as compared to the predecessor-generation drugs [41]. The third-generation quinolone antibiotics include levofloxacin, moxifloxacin, sparfloxacin, and gatifloxacin, and these have second-generation qualities, further having activity against atypical pathogens. Trovafloxacin is a part of the fourth generation and has activity against anaerobes [5]. Levaquin and trovafloxacin have since been removed from the market due to hepatotoxicity reported in patients.

Though alternative first-line treatment exists, quinolone antibiotics are often prescribed in uncomplicated urinary tract infections (UTI), bronchitis, and bacterial upper respiratory infection [50]. They are prescribed due to their low dosing frequency, high potency, a broad-spectrum of activity, and low chances of side effects [50]. However, adverse effects have been associated with the long-term use of quinolone antibiotics, such as tendonitis, higher chances of experiencing retinal detachment, angiosis, and the 'quinolone-associated disability syndrome' (FADS) [33]. In May 2016, the FDA issued a box warning, suggesting alternative uses to quinolone antibiotics in cases where the side effects of the quinolone antibiotics outweighed the benefits of treatment [17]. These include bacterial sinusitis, acute bacterial exacerbation of chronic bronchitis and uncomplicated urinary tract infection. In the 1st generation of quinolones, nalidixic acid was first marketed in 1962 and a two-year study on mice and rats that were given feed containing nalidixic acid determined the possible carcinogenic properties of the drug over long periods [33].

The 2nd generation started with norfloxacin, ciprofloxacin, ofloxacin, enoxacin, and lomefloxacin; these have a broader action against Gram-negative pathogens, Gram-positive bacteria, and atypical pathogens [28]. The addition of a methylated piperazine at position R7 adds activity against gram-positive bacteria by inhibiting efflux pumps [41]. A quinolone at position R6 increases the drugs' potency, and in the case of ciprofloxacin (commonly used in South Africa), the addition of cyclopropyl at position R1 makes the drug 4-fold more active [41]. Due to having similar joint anatomy to humans, sheep are the animal model of choice in investigating the growth-inhibiting properties and drug safety of ciprofloxacin and gemifloxacin for use in pediatric patients [51]. Through such studies, quinolone antibiotics for patients below 18 years are limited because the drug is now known to have anti-growth effects. In a separate study, immunocompromised mice were treated with levofloxacin and ciprofloxacin [49], after inoculation with pneumonia from the causative agent *Klebsiella pneumoniae*. Such animal studies have allowed for the formulation of a time-saving essential drug list for pneumonia infections.

Next there was the 3rd generation of quinolone antibiotics which had the same activity as the predecessor group plus improved action against atypical pathogens [55]. The list of drugs includes gatifloxacin, grepafloxacin, sparfloxacin, and clinafloxacin. They have a cyclopropyl ring at position R1, which improves the potency of the drug. The addition of NH_2 or CH_2 at position R5 increases the drug's activity against Gram-positive bacteria [48]. The presence of fluorine/chlorine at position R8 increases the drug's half-life and tissue penetration. Finally, the 4th generation quinolone drugs include moxifloxacin, gemifloxacin, and trovafloxacin, and they have activity against gram-positive bacteria, gram-negative bacteria, atypical pathogens and extended anaerobic activity [8]. Structural modifications include the addition of 3-methoximine-4-aminomethyl-pyrrolidin-1-yl/amine-substituted bicyclic pyrrolidin-1-yl group/azabicyclo group/ azabicyclo group at position R7 improves the drugs' activity against Gram-positive bacteria [48]. Animal studies enable predicting adverse effects of quinolone antibiotics [32], *i.e.* human suffering has been decreased by animal suffering instead.

Mechanism of action of quinolone antibiotics has mostly been inhibition of the replication of DNA in bacteria. DNA is a highly condensed structure, and for cell replication to proceed, the structure needs to be unwound to expose the DNA template to DNA primase and DNA polymerases. DNA gyrase enzyme relieves the positive super helical twists that are a result of DNA strands unwinding. Topoisomerase IV separates the newly formed prokaryotic chromosomes and eliminates the DNA knots using the energy of ATP hydrolysis. Quinolone antibiotics act by inhibiting to the DNA gyrase and topoisomerase IV and induce cell death is by DNA inhibition and/or production of stress response proteins [41]. In South Africa, gonococci have built up resistance for ciprofloxacin (a quinolones antibiotic), proving it ineffective against gonorrhoea [50]. It should also be noted that moxifloxacin and levofloxacin are a part of second-line tuberculosis drug treatment,

so quinolone antibiotics are essential in the South African context [50]. Quinolone antibiotics are therefore important in the public health in South Africa and resistance against them poses a severe threat in this regard. There are three main resistance mechanisms of resistance microbial pathogens against quinolone antibiotics [13]: firstly, an increase in efflux pumps. Drug resistance is built by limiting drug concentration within the cell. This is achieved by increasing the number of efflux pumps present on the cell envelope [41]. Next, mechanism is lowered porin expression and mutation of lipid-mediated pathways. This limits the amount of quinolone able to enter the bacterial cell cytoplasm [48]. Finally, mutation of topoisomerase IV and DNA gyrase can occur. These mutations reduce quinolone affinity for the target enzyme, and thus reduce drug effectiveness [41]. There have been clear benefits ciprofloxacin and other quinolones, but the development was done against animal suffering, as many active pharmaceutical ingredients were done by testing on animals [38, 52].

Drug development and the development of antimicrobial agents is where many animal models have been included [67]. During the animal testing of antibiotic and drug development, some negative impacts on animal health were recorded. More specifically, penicillin is fatally toxic to Guinea pigs [57]. Aspirin was suggested to be toxic to rat embryos [65] and repetitive administration of paracetamol to dogs and cats led to toxic side effects [64]. Testing of ciprofloxacin showed that monkey embryos and dogs did not suffer from toxicological side effects from quinolones administration [53]. The use of antibiotics in human medicine was led to environmental contamination and the presence of antimicrobial resistance elements in the Great Apes in previous unanthropised areas of the world [61]. Selection of the animal models and the types of experiments have been based on the progressive improvements of the fundamental reality by humans throughout the Age of Anthropocene and recently during the Great Acceleration. The knowledge accumulation about this fundamental reality has been related to the understanding of animal physiology, genetics and biology. Application of that knowledge to the situational reality of human existence has then been taking place, *i.e.* deployment of the use of animal models in the antibiotic development to fight infectious diseases in the Age of Anthropocene and recently during the Great Acceleration. Many animals have been euthanised and the subjected to injuries/welfare impacts, when animals are used in the drug development. Human suffering has been alleviated by suffering of animals, and the animal models have not always been optimum in transfer of results for humans [67]. Over time, this search and application of the fundamental reality knowledge at the boundary with situational reality has led to the realisation by humans that more care and protection must be extended to animals. This led to adoption of preventative measures that protect animals as quasi-participants and the draw down of the human suffering (see section on Integrating remarks below). There has also been a move away from the use of animal models in drug testing and development; and towards the use of replacement systems and potentially challenge trials. It can, therefore, clearly be seen as a progressive adoption of the precautionary principle in drug development.

Precautionary principle and its links to the fundamental and situational reality

General aspects application in the Age of Anthropocene and the Great Acceleration

Ultimately, the human actions such as release of nuclear radiation and excess CO_2 into the environment must be seen as possible examples of threat to life on Earth, *e.g.* through the alteration of the conditions under which socio-ecological systems will exist in the Age of Anthropocene. The precautionary principle expresses that causality of human actions can be unpredictable, *i.e.* the consequences of the release of CO_2 and other gases and chemical compounds into the environment due to the anthropogenic activities cannot always be understood, modelled, or completely quantified/mitigated at the point of creation of those impacts or even long time after the onset of such impacts. The decrease of the use of animal models in drug development can be seen as a decrease of the animal suffering at the fundamental and situational reality boundary. At the same time, future suffering is to be prevented. Therefore human actions can have unpredictable impacts on the human-animal-environment impact nexus, and this is taken into account at the boundary between fundamental and situational reality. Application of the precautionary principle is aimed to preventing the alterations in the amount and sometimes even the bonding nature of life. It is an expression of the fact that humans and their actions set the terms of concrete and indirect impacts of a set of chemical molecules onto the socio-ecological systems. As a result, the precautionary principle is aimed at preventing the destruction of life, to limit the potential impacts of human activities in the Age of Anthropocene and the Great Acceleration on the non-human components of the socio-ecological systems. The precautionary principle is aimed at preventing harm to other living organisms and directly or indirectly due to the application of the human knowledge and the search for new knowledge, as well as its potential applications. This should and does apply to the current and/or future generations of humans, animals, and environmental settings [24].

Human actions, putting aside colonialism and other forms oppression for now, can have benign, beneficial, or detrimental impacts on the other components and compartments in the socio-ecological systems. Therefore, the precautionary principle allows for the continuation of the 'normal science', which is aimed at discovering the nature of the fundamental and situational reality of human existence and the environment humans share with animals and other living organisms [44]. However, the principle

strongly suggests normative, cautious, and deterministic actions in the domain of the 'regulatory science'. In other words, the precautionary principle is guiding principle for drafting and implementing of the policies and regulations of human actions that can result in unforeseen impacts of application of knowledge, development and deployment of normal science. Such impacts might be irreversible in effect on non-human animals, on all living organisms and the socio-ecological systems as a whole. The regulatory science and precautionary principle are aimed at mitigating potential human errors in the deployment of the human knowledge at the boundary between the fundamental and situational reality of human existence, as well as the existence of the socio-ecological systems. They aim to limit human errors to the type I errors, where human causality of detrimental effects on socio-ecological system is not necessarily proven, but is plausible [44]. Precautionary principle and all human impacts must be managed or pre-emptied in order to eliminate impacts on the socio-ecological systems which are of type II, *i.e.* human causality of such impacts is unforeseen and ignored until it is too late to prevent it and the impact had occurred, and it is deemed irreversible [44].

Precautionary principle is also the result of human action, human understanding, and collective interpretation of current and potential future changes at the boundary between the fundamental and situational reality of human and socio-ecological system's existence. Changes can and do apply to both realities of existence as well. The precautionary principle has been deemed irrelevant or problematic by some authors for the following reasons [44]:

a) the principle limits human decision-making and even goes against rationality, *i.e.* limiting the decisions based on hypothetical consequences is irrational and/or precautionary principle prevents a human being from making any decisions;

b) the principle is vacuous;

c) the principle is contradictory in its very essence.

This is a truncated version of the objections by scientists and philosophers, but many of them can be allayed or even eliminated. These objections will be discussed in a shortened version, and it will be based on the [44] reference, as well as on the authors own interpretation of the precautionary principle.

Normal and regulatory science as justification for application of precautionary principle in biological sciences

Firstly, the limitations and irrationality of the precautionary principle are not actually an accurate interpretation of its essence and scope of the principle's application. The precautionary principle is in its very nature a principle that is action-based, that would have epistemic and knowledge-creating dimension, and which is to be a procedural measure to guide human actions under the conditions of uncertainty of the actions' outcome. The precautionary principle is not in any shape of form itself to be applied to all decisions that humanity makes, *i.e.* it cannot be seen as a generally-applied and only normative principle to direct all human actions and decisions. Rather, humans are still able, free and obliged by the need to generate knowledge about fundamental and situational reality improve the overall human condition and to decrease/try to eliminate injustice and inequality among humans. Humans can explore fundamental reality of their world, ontological realm of existence… humanity can, in spite and because of the precautionary principle still, continue to strive and understand the laws of nature and the links between that and human health, wellbeing, the welfare of animal and abiotic components of socio-ecological systems. In other words, the precautionary principle does not stop, impede, or weaken the human's rights, need and entitlement to conduct normal science. On the contrary, the precautionary principle should only be used in the regulatory science domain to indirectly guide the normal science. However, the precautionary principle should only be applied in situations where humans action, *e.g.* increase in the atmospheric concentration of CO_2, can have (un)predictable and detrimental consequences on broader environment.

Offering a deontological ethical framework that complements the precautionary principle is Immanuel Kant's first formulation of the categorical imperative, which asserts, in the first instance, that one should act only according to maxims that can be universally applied [26]. This means that ethical decisions should be made based on principles that could be universally endorsed without contradiction. The second instance of the imperative emphasizes the need to treat individuals as ends in themselves, rather than merely as means to an end [26]. For example, if a proposed genetic modification could potentially harm the living organisms which are part of the existing socio-ecological system along with human. As a results, applying the precautionary principle means rejecting such modifications unless they can be universally justified as safe and beneficial [45]. By ensuring that scientific and ethical decisions respect the intrinsic value of living organisms and their molecular integrity (as far as possible at the boundary between the fundamental and situational reality of existence), the precautionary principle upholds the need for ethical treatment of all entities involved. Therefore and from a moral, as well as rational point of view, humans must act to mitigate or prevent a type II error in terms of impacts of human actions on non-human animals, living organisms and the abiotic components of the socio-ecological systems. Humanity must follow the precautionary principle, in the regulatory science domain, from the moment that there exist sufficient evidence, strong indications or pragmatic human consensus that anthropogenic actions might be the cause for detrimental or non-benign impacts on any part of the socio-ecological systems in the holistic understanding of this ontological term or notion.

The objection premise that the precautionary principles is 'vacuous' or devoid of meaning, content, or practical significance [44]. This objection can be addressed and debunked as follows. Human actions are unavoidable and a fact of life and will be assumed to be part of epistemic or

perceived human reality and not illusion in the discussion/analysis going forward. One could theoretically argue that human action is only an illusion, but the impacts of anthropogenic action on Earth and the ontological realm of the socio-ecological systems are a reality, as indicated to and perceived by humans and indicated by the ontological existence in the Age of Anthropocene and during the Great Acceleration [19]. This last point is supported by addressing of climate change in the international treaties such as the Paris Agreement. Therefore the precautionary principle is a manifestation, a component of the human endeavour to mitigate the impacts of the real actions, that human development and its side effects can have on non-human animals and abiotic components of the socio-ecological systems. The unity of human mind, the collective one of *Homo deus*, its drive to change and improve the human condition, and the impacts, such endeavours have on the nature, are thus not vacuous. It is clear that such lack of vacuousity does have limitations, as Paris Agreement implementation is currently not meeting of its targets [36]. However, the precautionary principle is not vacuous, and it is driven by specific regulatory science consideration and intended epistemic actions by *Homo deus*. Regulatory science and the human search for new knowledge could both be seen as an expression of unity of human mind and nature. Such an interpretation is similar to the view of Jonas, who saw human mind and nature as one [21]. If one accepts this unity and the moral implications of it, as well as the fact that the precautionary principle was drafted, one can then state and argue that humans have realised, at least to some extent, that they have an impact on the environment and that at least some of those impacts are likely not positive or at minimum not benign. Therefore, the precautionary principle has substance and drives, in however limited fashion, human actions in the Age of Anthropocene, as well as during the great Acceleration, where applicable and in unison with scientific discoveries [24]. This pre-empts any sense that the principle is or could be vacuous. There is an essence of subject and action-driven decisions, and ethical implications, are based on decisions that are made based on the broad understanding of the human ontological essence and existence, and links to the wider socio-ecological systems. Human actions, such as drafting of the Paris Agreement and its adoption by the United Nations, indicate that human understand their link to the other parts of the socio-ecological systems. As a result, the precautionary principle is not vacuous.

The most difficult objection to deal with about the precautionary principle is the contradictory nature of it. It is true that regulatory science and the application of the precautionary principle could, at face value, very seen as limiting scientific progress, knowledge generation and ultimately the impacts of humans on the non-human parts of the socio-ecological systems. The point being that regulatory science, which strictly or predominantly, adheres to the application of the precautionary principle, can impose such limitations on the normal science that it becomes impossible for *Homo deus* to deepen their understanding of fundamental reality of existence. This in turn then prevents humans from dealing with the challenges at the boundary with situational reality, *e.g.* understanding the exact nature of the climate-change-feedback loops and the anthropogenic impacts of humanity on non-human parts of the socio-ecological systems. However, this point of view and line of argument are not an accurate representation of the current, perceived reality of human existence… the reality of the Age of Anthropocene and the time of the Great Acceleration. The precautionary principle is only one of many political, legal and ethical/moral ways to deal with anthropogenic impacts on the non-human parts of socio-ecological system. The freedom to conduct research still goes on and the normal science has not stopped after the 1992 adoption of the precautionary principle [47]. Such science has been updated, as the knowledge on climate-change-feedback loops and impacts has been updated between 1992 and the 2015 adoption of the Paris Agreement [36, 62]. For the biological sciences, the number of papers published has steadily increased between 2000 and 2017 [27]. At the same time, the number of authors per paper and the apparent interdisciplinarity in biological sciences publishing has increased for the same time period [27]. Therefore, the precautionary principle is not just contradictory, and so the regulatory science seems to have a limited impact on the normal science based on the reasoning above. The impact is likely limited to specific aspects of the biological science research, *e.g.* demonstrated by the 0.2 % increase in the similarity between the topics of papers in biological sciences and the likely increased level of this type of coherence between 2017 and 2040 [27].

Precautionary principle as a foundation for a balance between regulatory and normal science

In summary, the precautionary principle has an epistemic foundation for its derivation, *i.e.* there is an admittance by humanity that we do not know everything but that there are some of our actions which have the potential to cause good/benefits and harm at the same time. This is in relation to humanity, to *Homo deus* and non-human components of the socio-ecological systems. There is epistemic humility in the principle that we cannot predict all the outcomes of our actions, even though they might only be based on positive intentions. This humility is an expression of the need for the iterative process of normal science to continue, and the regulatory science to adopt some of the major findings, facts, findings, and knowledge about uncertainty from the normal science, translating those into its regulations and policies. The precautionary principle and its application are thus a flexible framework for the management of uncertainty of knowledge generation by *Homo deus*. They are somewhat contradictory in nature, but the contradictions are like to be limited. Finally, the precautionary principle and the related uncertainty are manifestations of the boundary between the fundamental and situational reality of human reality of existence, and that this boundary is in a permanent state of change and flux [24]. Thus, the uncertainty arises from the promotion

of the discoveries of normal human science, development of technologies and the improvement of the overall human condition and the side effects of the deployment of normal science knowledge in the Age of Anthropocene and during the Great Acceleration [19]. The discussion in this section shows the duty of humanity to address and pre-empt, where possible the effects of the actions by *Homo deus* on non-human components of the socio-ecological systems. There is an inherent rationality in this duty, as demonstrated by the application of the categorical imperative above. The actions based on the precautionary principle are not vacuous and neither is the principle itself. Contradiction might, and probably do arise, from the precautionary principle in the decision-making about the human actions impacts on the socio-ecological systems. However, the precautionary principle leads to updating of the commitment and treaties that manage human actions and management of the climate-change-feedback loops on humanity and socio-ecological systems.

The time is an important factor in the nature of the boundary between the fundamental and situational reality of the existence of *Homo deus* during the Age of Anthropocene and the Great Acceleration. Dynamic nature of this boundary is an important factor in human endeavours and so it must be a consideration in the decisions that can impact the ontological realm of the socio-ecological systems. That time is often limited, as shown for the most part of living organisms [20]. Implicitly, the precautionary principle has a temporal dimension, and time is an important variable in the considerations in relations to anthropogenic activities, in relation to the manifestation of human lives and lives of all organisms on Earth. Thus precautionary principle is supportive of protecting life, it shares fundamental features with it, and it is aimed at recognition of life's inherent value [21]. As a result of this reasoning, any contradiction in relation to the precautionary principle is temporary and quickly dissipates in case an action needs to be taken by *Homo deus* [22]. Such action would apply to the boundary between the fundamental and situational reality of the humans, non-human animals and living organisms in the ontological realm where they are part of socio-ecological systems. Precautionary principle and the balance between the normal and regulatory science are an outcome of the long line of discoveries, as mentioned above, that humans have already performed since the onset of fossil fuel use and application in the promotion of human development. Those discoveries led to impacts and by-products, which over time led to the generation of the uncertainty at the boundary between the fundamental and situational reality. Further examination of the precautionary principle and some regulatory science policies, or best practices, need to be understood from the view point of the inherent link between the molecular basis of all or most living organisms. It is also necessary about to explicate more clearly the duality of the use and possible nature of the knowledge that *Homo deus* has accumulated. One can start with the byproducts of the anthropogenic knowledge generation and deployment by *Homo deus*, which are simple in chemical structure, and which are also essential components of living organisms.

Said by-products are often dual in character, *e.g.* CO_2 is produced by living organisms and used in maintenance of their normal functioning (see next section for details). At the same time, the greenhouse gases can be produced anthropogenically, such as CO_2, and these can cause changes to the conditions of life, and they can also damage or terminate life itself. CO_2 is a symptom of the duality of chemical molecules that are related to life. In other words, CO_2 and other elements/molecules have wide-ranging impacts in terms of the human and animal wellbeing, as well as environmental health. These molecules play essential roles in the normal functioning of the living organisms, *i.e.* they are part of the molecular mechanism of the inexorability principle. However, there is an inherent duality in them: they can become a source of harm to humans and socio-ecological systems when released outside of living organisms and in much higher concentrations. Below the duality of impact of CO_2 and other chemical components of life will be interpreted more broadly in the ethical domain and in the context of the human-with-animal-with-environment interactions. The duality was discovered based on the execution and practice of normal science by *Homo sapiens* and later *Homo deus*. The knowledge deployment about CO_2 and other dual-inexorability chemicals has been the basis for the regulatory science, *e.g.* such as that which led to the drafting of the Paris Agreement. In the next section of the article, duality of inexorability chemicals is used to demonstrate the need for the regulatory science to be applied as a tool to manage the normal science. That management does take place at the boundary between the fundamental and situational reality of everyday existence of *Homo deus* in the Age of Anthropocene and during the Great Acceleration [19, 24].

Duality of the chemical composition of life and its relation to the existence of *Homo deus*

CO_2 binds the normal and regulatory science together

CO_2 is a good example of the duality that simple molecules and greenhouse gases, or their dissolved forms, can play in the living organisms and the biosphere in general. Fig. 1 shows that CO_2 plays a fundamental role in the chemistry of life. It is the 'very raw material' for the production of the universal energy source of living cells and organisms, regardless of their complexity, namely glucose. This is formed in the process of photosynthesis, where CO_2 or the HCO_3^- in marine environments are utilised by autotrophs to synthesise glucose (fig. 1). Glucose provides the ability for the living organisms to produce ATP and other important molecules of life, *e.g.* the pentoses for the nucleotide production in the pentose phosphate shunt. CO_2 has also evolved into the basis for the maintenance of the pH homeostasis of the living cells and (micro)organisms and the supply of H^+ in various compartments of living cells and (micro)organisms

[22, 40]. In the gaseous form, CO_2 is not toxic to human respiration at low concentrations, but it causes the following symptoms as its local atmospheric concentration in a confined space increases [30]: "increased respiratory rate, tachycardia, cardiac arrhythmias and impaired consciousness". If the volume fraction concentration of CO_2 in the ambient air reaches ten percent or more, humans start experiencing results in convulsions, coma and/or death. In other applications, carbon dioxide has been used as a cooling agent or a sorbent for contaminants, *i.e.* dry ice [29]. The bicarbonate ions also play a role in the exchange of single carbon atoms in living organisms, *e.g.* propionyl-CoA-carboxylase [3]. In this case, the CO_2 central role in the pH homeostasis, as a reactant in some enzymatic reactions, and the preservation of those role across many of the species and level of biological organisation in the Earth's biosphere, is an expression of the conservation rule of life as defined by J. Gómez-Márquez [20], namely

> "once the evolutionary process finds and selects a structure or a process that works well at any level of complexity (from macromolecules to multicellular organisms) it will not change it or if it does it would consist only in a fine-tuning".

Discussion of CO_2 shows that molecules of this compound are clearly tied into the very essence of life on Earth. It also shows that human and other living organisms, *i.e.* including non-human animals are tied together at the molecular level. The tying forces of the essence of life happen at the most fundamental level of it, *i.e.* they can be seen as an expression of the conservation rule of life [20]. As an expression of fundamental reality of human and living organisms' existence, the conservation of the favourable traits among living organisms is maintained in all organisms where it is favourable for the survival of the species [20]. Depending on the concentration CO_2 and carbon indirectly, this element and molecules in which it has covalent bonds with other atoms of life can be beneficial or dangerous to human life. This is an expression of the duality of threat *vs.* carbon's role in the essence of life will depend on human role in relation to the molecules such as CO_2 and its application. The duality in turn will be a manifestation of the human influence on the use of molecules such as CO_2 at the boundary between fundamental and situational reality of the ontological realm of human existence and the existence of the socio-ecological systems [24].

Such a role of humans can be passive, *i.e.* humans can only live with the way carbon is arranged in their body, and how CO_2 is exchanged with the environment due to the functioning of the human body. The normal scientific endeavours of humans led to the discovery of the roles that CO_2 has played in the essence of life [44]. By extension, the normal science would include exploitation of that knowledge for the management of the CO_2 essence role in human wellbeing and health, *e.g.* the management of

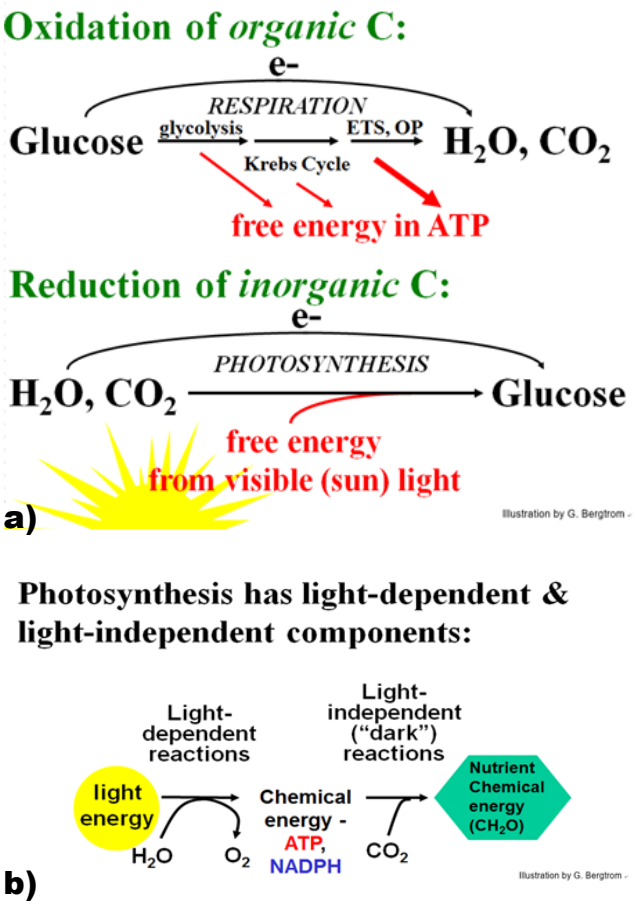

Fig. 1. Cycling of the carbon between inorganic and organic pools in the environments. The figure ultimately demonstrates the central role of the carbon dioxide in the cycling of C in the biosphere [42]

carbon dioxide in context of human health… treatment of hypocapnia and hypercapnia [4]. At the same time and in this context, CO_2 will also play a role in the maintenance of the human wellbeing, *e.g.* through medical treatment. The human role in the 'molecule's application' is passive in this context, and it is the result of the fundamental reality of human existence. The molecule executes its role based on the laws of nature (its chemical potential and energy) and based on the fundamental reality of human existence and molecular essence of life. The role of molecules such as CO_2 in the situational reality of human existence will be observed when humans actively 'deploys the molecule for a specific purpose, which they choose [24]. In that context, humans will play an active role in way that the molecules are dispersed or applied. For examples, if a human plays an active role in the way they use their knowledge of the fundamental reality to deploy a technology and to generate/contribute to the release of molecules such as CO_2 into the environment.

When looking at the development of the human deployment of CO_2, one can argue that this was done by humanity in a semi-intentional way. This line of argument will be explained in the following paragraph. Since about the 1800's, humans have been changing their reality, *i.e.* technological advancement is derived from the way

in which human's discovered the fundamental laws of nature, the chemical composition of fossil fuels and technologies that could harness the energy such fuels release during combustion. The fundamental reality knowledge led to human progress, to wars and to human suffering but it also launched an era of progress and discoveries, which have improved the overall human condition over several generations. Humans have been altering their situational reality by intuitively exploiting the fundamental reality laws, etc. (based on [24]). Situational reality has, however, recently become characterised by the excessive release of human-initiated/produced CO_2 molecules into the environment and living space of human, non-human animals and all living organisms. At least since the 1980's, humans have realised that there are impacts of this intentional deployed CO_2 and it is starting to alter the socio-ecological systems, *e.g.* decreasing biodiversity and impacts of human activities on coral reefs [59]. Into the ontological realm of existence of the human's lifetime, a new evolutionary force entered, namely humans or *Homo deus*. Progress of humanity and the development at the boundary between fundamental and situational reality have reached the stage that humans have started to exercise influence on the evolutionary imperative [20], *i.e.* humans have started to create global conditions where selection pressure is exerted onto the non-human parts of the socio-ecological systems. This can lead to natural selection of species, genetic changes and drifts to adapt to the human-induced climate change. Extinction can also be result of these action of *Homo deus* in the Age of Anthropocene and during the Great Acceleration [19].

This power is realised by humanity and solutions such as continued research in normal science, as well as regulatory science of treaties such as the Paris Agreement. The level of CO_2 deployment is being regulated and controlled by humanity. Precautionary principle is being implemented, however in a limited fashion, applied by action-based, epistemically-humble and justified, and filled with the collective, anthropogenic intent to protect life. Changing human ways is not always easy and continuous negotiation ethical, moral, and scientific iteration of knowledge from normal science must be re-examined and where appropriate to feed into the regulatory science. Approaching dealing with the climate-change-feedback loops, based on the precautionary principle, requires one to realise that knowledge of *Homo deus* about the fundamental reality of their existence and the duality of the CO_2 molecules in it. It requires individual humans and the collective wisdom of humanity to manage the unknown impacts of climate change by strongly considering and by always keeping in mind the life and death nature of what *Homo deus* knows about CO_2 and similar molecules. Striking the right balance here makes it clear that normal science expresses the drive to gain move knowledge and to move forward. Regulatory science, on the hand, charts the way forward to getting that new knowledge. How this balance is struck is critical to the success of *Homo deus* in the Age of Anthropocene and during the Great Acceleration [19, 22].

Knowledge and the power of life in the Age of Anthropocene and during the Great Acceleration

Discussion in this article so far shows that the humans and non-human life are tied together with the chemical elements and molecules they form their bodies, that give them their material character. Humans are composed of a mixture of the chemical elements and component of life, and they share these features and molecular basis with the other living organisms. Life has been defined by many authors and the article/thought piece by J. Gómez-Márquez [20] will be used here to demonstrate the links to normal and regulatory science. This is not to copy that author's thoughts or arguments, but rather to provide a unified framework for finishing the argument in this article. Evolution and a certain level of natural determinism in the way chemical composition of living organisms evolved and has common features, and these are maintained across different living organisms, as well as in the optimum solution to the chemistry of life and its functioning at the molecular level. This is summarised in the inexorability principle that states [20]

"life is like that because it should be like that"

However, the inexorability principle does not imply intelligent design, as the chemical composition and other commonalities between various living organisms are not based on a pre-determined plan [20]. At the same time, the chemical nature of human life and other life is not the result or sign of causal determinism, *i.e.* the chemical composition will not be fixed in time at the scale of the fundamental reality of human existence [20]. It can undergo changes as a result of evolution and the chemical composition will thus be the result of a combination between deteminism and contigency [20], *i.e.* they are subject to change over a long period of time. By the same token, all living organisms exchange energy and matter with their environment to maintain their order, the essence of life and to continuously increase the entropy of their surroundings in order to comply with the second law of thermodynamics [20]. Life is maintained by maintaining the chemical composition of its cells and bodies, *i.e.* the order of the non-equilibrium existence of humans and non-human animals, of all life is maintained. However, given that life has survived and has been self-maintained over billions of years, throughout varying fudamental reality, life is worth protecting. It must be studied, understood and protected. In the course of the human situational reality, the study of life, at least since the progress of humanity of the 1800's had started, and it has led to the continuous discovery of the nature of fundamental reality by humans. This knowledge has developed over the situational reality of generations for the human beings, and it led to the Age of Anthropocene and the Great Acceleration. The relevant knowledge acccumulation and deployment of that knowledge at the boundary between fundamental and situational reality has led to *Homo deus*.

Besides CO_2 and carbon, other molecules and atoms with duality attached to them include those that contain nitrogen or N, and four other of the six macroelements, or atoms which are found in all living organisms, and that all biologically important molecules are made out of, namely O, H, S, and P. Each of the macroelements has a biogeochemical cycle, *e.g.* nitrogen and other atoms undergo chemical reactions which result in the movement of the macro-element atoms between various environmental and biosphere compartments. The movement can be based on physical laws, *e.g.* the Fick's law of diffusion, or based on biological agents catalysing the changes in the covalent structures which in turn allow for the inter-compartmental movement of the macro-element in question. *Azotobacter* spp. can fix atmospheric N_2 and convert it into NH_3 due to the activity of the nitrogenase enzyme [1]. Then ammonium/ammonia molecules can be converted into the amino acids and proteins [43]. Similar reactions also play a role in the conversions of NH_3 and its detoxification in the brain tissue of humans [10]. Other processes in the biogeochemical cycle of nitrogen can be seen in fig. 2. There is, however, a clear conclusion that humans and non-human animals will contain at least some of the same chemical reactions and molecules that contain nitrogen in their cells and bodies. Human discovery of the individual processes that are involved in the cycling of macroelements and the deciphering of their impact on human understanding of natural laws and biological essence of life. This can be seen as a manifestation of the fundamental reality of the human existence — an understanding of the fundamental principles and laws that govern the nature functioning, that 'control' the human existence and the space and broad parameters of the ontological realm of human existence [24]. The nitrogen cycling is another example that shows that humans and non-human animals/parts of the socio-ecological systems are intertwined at the very molecular and fundamental level of life. This manifestations of the fundamental reality of human existence proves that humans have an understanding of the interlinking and the inexorability principles of human and non-human life. As a result, *Homo deus* has a duty to manage and to be a custodian of the life at the boundary of the fundamental and situational reality of human and animal life.

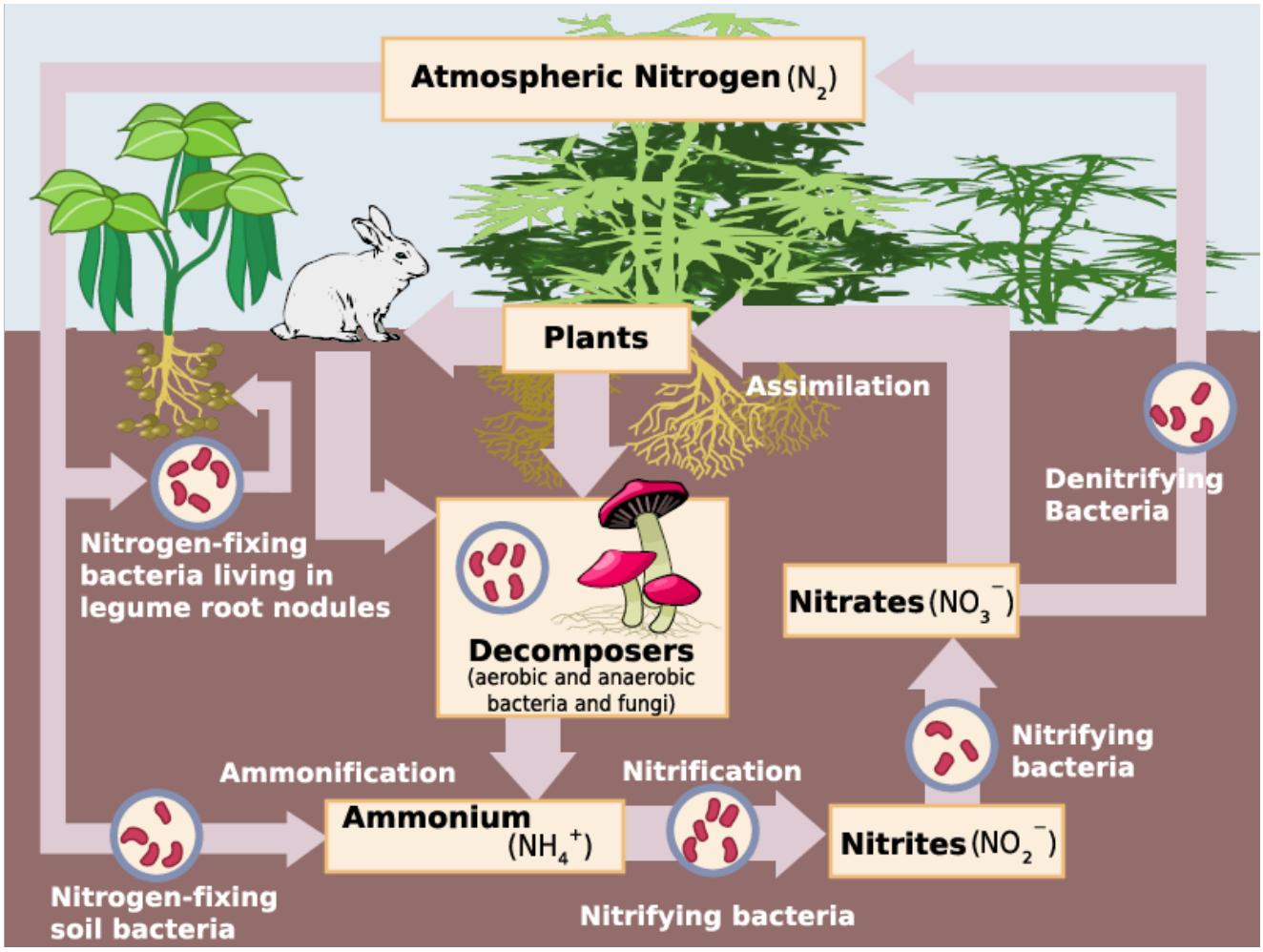

Fig. 2. N_2 is first fixed from the atmospheric nitrogen pool and then the atoms of nitrogen undergo changes from NH_3, to glutamate and glutamine, and more complex monomers and biopolymers. Cycling is completed by the processes of ammonification, nitrification and denitrification. Protein degradation in lysosomes and through the ubiquitin system provides the other steps in the nitrogen cycle [7]

Knowledge about the fundamental reality, *e.g.* the toxicity of a specific form of nitrogen, has been deployed by humans and *Homo deus* for various uses. [16] examined the 'ethical duality of nitrogen, N_2'. Dinitrogen or N_2 is a gas which it accounts for 78 % of the total gas volume in Earth's atmosphere [16]. This is the pool from which nitrogen can be converted into organic and bioavailable form for the biosphere (as shown in fig. 2). Yet, it has been used by humans in other uses that can demonstrate of the link of the fundamental and situational reality, based on human knowledge deployment of the N_2 molecular properties. Specifically, R. Escandon [16] talked about the inhumane suffering caused by using the properties of N_2 as an asphyxiant to execute an inmate, to carry out the punishment of death penalty. The ethically-questionable 'humanness of the execution' using dinitrogen as an asphyxiant can be seen as occurring at the interface of the fundamental reality of human existence, the chemical nature of the electron density distribution and the presence of one sigma and two pi bonds in the molecule of nitrogen make it inert. Those chemical and atomic properties make nitrogen chemically stable and chemically inert in the atmospheric compartment in the biogeochemical cycle of nitrogen on Earth (fig. 2). However, the inertness of N_2 also leads to the possibility to affect the situational reality of a human being executed, *e.g.* by being used as an asphyxiant in the death penalty execution N_2 deprives the death row inmate of oxygen and the ability to breathe [16]. Deliberate human use of nitrogen, specifically flooding the death penalty chamber with $N_2(g)$, eliminates another human's access to oxygen and after a short period of time that human dies of oxygen deprivation.

On the 'flipside of the same coin', it can be argued that the execution of a convicted inmate 'balances the equation of justice' in that taking the life of the murderer in a state-sanctioned execution returns some justice to the oxygen-deprived murder victim that succumbs to the violence of the executed prisoner. In any case, the death penalty use of the dinitrogen molecules is derived from the human understanding of fundamental reality of their existence, the chemical properties of one of the macroelements that compose molecules of living organisms. However, the application of the properties of N_2 is driven by human interpretation in situational reality of the death penalty convict and the relatives of their victims. The exploitation of this fundamental reality for one sigma and two pi bonds is a specific manifestation of the nitrogen molecular properties in the situational reality of the executed convict and the victim's family members. N_2 is a quasi-agent of life at the interface between the situational and the fundamental reality of human existence. *Homo deus* makes a judgment call how to apply its understanding of the fundamental reality at the boundary with the situational one. The dinitrogen part of the argument in this section indicates that humanity can act with a strong sense of choice about the deployment of their knowledge of the fundamental reality of human existence at the boundary with the situational reality of it. At the same time, the CO_2 part of the argument shows that *Homo deus* can reach the understanding of the intertwined character of the fundamental chemistry and reality of the human and animal life at the molecular level. Therefore, humanity and *Homo deus* has the ability, and due to the climate-change-feedback loops on human health the duty, to apply caution and rational decision making in mitigating the impacts of the greenhouse gases, such as CO_2, onto the socio-ecological systems. This calls for deployment of the humanity's knowledge, about fundamental reality of their existence, according to the precautionary principle at the boundary, inside the situational reality of the socio-ecological system *Homo deus* is part of.

Integrating remarks and suggestions for the way forward

Discussion in the previous sections point to the benign and extreme examples of the chemical properties of the simple molecules of life and their significance in the socio-ecological systems. The N_2 example is similar to the increase in the atmospheric concentrations of CO_2, a molecule which is essential to the molecular functioning of cellular-level of life, but which can be toxic in high concentrations and if resulting from the deployment of fundamental reality knowledge by humans for a derivative/situational purposes. At the same time, excess production of CO_2 and its triggering by the Age of Anthropocene and the Great Acceleration have now made such simple molecules, outside of the life-supporting systems, a source of suffering for humanity and non-human parts of the socio-ecological systems [24]. Both N_2 and CO_2 are examples of the knowledge duality in the relation of *Homo deu*s to humanity and other living organisms. Duality of life has an inside and outside dimension, as health and normal functioning of cells and death of living organisms are related to the concentration and chemical form of N or C, and also in the nature and manner of their release into the environment. The information from this article clearly shows that *Homo deus* has the power to decide whether a set of simple molecules will be deployed or produced to sustain or to end life. The boundary between the fundamental and situational reality of human existence and the existence of the socio-ecological systems is thus a manifestation of the ability of *Homo deus* to choose to support life or to end it. Therefore, *Homo deus* has the ability to choose to act in a preventative fashion in relation to life, *i.e.* to follow the precautionary principle.

More specifically, inside the living organisms, in molecule such as proteins and amino acids, inside the carbohydrates such as glucose, N and C are sources of life's very essence. Their understanding and use by humans in relation to those 'inside functions of life', *e.g.* for the maintenance of health and wellbeing of humans and other parts of the socio-ecological systems, is supportive of life. The inside dimension of life's chemical duality is ultimately aimed at decreasing pain and suffering of all living organisms, or a substantial number of them. Actions by *Homo deus* in support of life and the adherence to the

precautionary principle facilitate this outside of humanity's physical bodies, but next to or in the environment of non-human animals and other parts of the socio-ecological systems. Precautionary principle can thus be seen as a way to positively manage the outside settings of life by humanity, by *Homo deus*.

If humans act inside the biogeochemical cycle of the macroelements, *e.g.* humanity directly or indirectly act inside the fundamental reality of their existence and not disruptively towards other components of the socio-ecological systems or in relation to them… if they act in support of life, then human actions are ethically justified to maintain human and non-human life. Precautionary principle is part of such efforts. However, if the CO_2 and N_2 molecules are used in relation to outside of the biogeochemical cycle of the macroelements, or indirectly in relation to them, then the situational reality of human life and the existence of the socio-ecological systems is at play — humans apply the building blocks of life in non-essential way, in a way that has caused the Age of Anthropocene and the Great Acceleration. Such application can have lethal consequences on humans and non-human animals. Such lethal consequences do occur at the interface of the fundamental and situational reality can then lead to the negative impacts on the single human life, *e.g.* inhumane death penalty due to the exposure to the N_2 overdose. The same would apply to the wellbeing and welfare of humans and animals by the ever-increasing CO_2 concentrations in Earth's atmosphere and the cascading impacts on the socio-ecological systems. From the drug discovery point of view, similar situation would be observed if the animal suffering would continue if the humanity would not have stopped or decreased the suffering of animals in the drug testing despite the experimental results showing that transfer of the knowledge from animal experiments to humans has limitations. Improvements have been made over the years in mandating the ethical review of animal studies, the changes to the addressing and naming of animal welfare to animal wellbeing, the shutting down of research facilities with primates [34]. Regulatory science has taken in the normal science findings about the fundamental reality and the end points in the animal testing have been updated to decrease animal suffering, *e.g.* the suspension of the LD50 dose experiments [15]. Some controversies still occur [14], and so precautionary principle and its application in the management of the non-human animal wellbeing is necessary. It is a critical to maintain a balance between the knowledge development and deployment, between the normal and regulatory science.

The current article shows that the precautionary principle is a way to manage the need for the normal science, *i.e.* knowledge and data collection about the fundamental reality of human existence, and the knowledge management and deployment at the boundary of the fundamental and situational reality of existence of *Homo deus* (regulatory science). It is clear that humanity has the ability to choose to act and to deploy their knowledge in a specific way, and also in an ethical way. Precautionary principle is grounded in the inherent uncertainty of the Age of Anthropocene and the Great Acceleration. The current article clearly shows that the precautionary principle can be applied effectively is *Homo deus* chooses to do so. There is also a continuous need to provide regulatory science oversight in order to protect or to minimise the suffering experienced by the non-human animals and other parts of the socio-ecological systems globally.

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Зв'язки між життям людини та тварин в епоху антропоцену: від молекул, через реакції до клітин, розвитку та питань етики

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Протягом останніх двохсот років зроблено відкриття у фізіології тварин і людини, хворобах та розробці ліків. Тварини стояли в центрі експериментів з оптимізації доз ліків та шляхів введення. Прогрес людства зумовлений різними антропогенними цілями та прагненнями. Невпинно зростають розвиток знань та відкриття щодо фундаментальної реальності людського існування та онтологічної сфери, в якій продовжують розвиватися соціально-екологічні системи. Представники *Homo sapiens* зараз досягли розуміння, сили знань та дій, здатних впливати на екосистему Землі. Це призводить до побічних продуктів людського прогресу, наприклад, CO_2, які почали змінювати фундаментальну/ситуативну межу реальності людського існування та онтологічну сферу соціально-екологічних систем. Переваги та страждання людей і тварин, окремо та разом, можна розглядати крізь різні призми — наприклад, принцип запобіжних заходів. У цій статті використовується методологія, яка поєднує біоетичний та теоретичний біологічний аналіз принципу запобіжних заходів і його наслідків для взаємозв'язку між людиною та ширшими соціально-екологічними системами. Цей принцип може надати певні вказівки щодо етичного розуміння подвійності людських дій під час антропоцену та Великого прискорення, а також того, як це пов'язано з самою хімічною сутністю життя. Люди розвивають свої знання про фундаментальну реальність як частину пошуку істини, розуміння хімічних та інших вимірів природи життя. Це «нормальна наука», тобто пошук «істини» або людського розуміння фундаментальної реальності існування, який рухає людство вперед. Однак її використання для розвитку людства створює побічні продукти, які потребують «регуляторної науки» або встановлення правил для регулювання використання знань, отриманих з нормальної науки. Прикладом цього може бути необхідність вжити заходів та пом'якшити наслідки зміни клімату по всьому світу, вплив як на життя людини, так і на життя тварин.

Ключові слова: принцип запобіжних заходів, рівні біологічної організації, етичні рамки

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Morphofunctional basis of the cutaneous immune system

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This article provides a comprehensive review of the morphofunctional basis of the cutaneous immune system, detailing the cellular composition and integration within a complex neuro-immune-endocrine network. The main immune competent cells including keratinocytes, dendritic cells (Langerhans, myeloid), macrophages, mast cells, and lymphocytes are characterized in terms of their roles in maintaining skin homeostasis and defense against external and internal factors. The literature search was conducted using scientific databases such as *Scopus*, *PubMed*, and *Web of Science*. Particular attention is given to the concept of inducible skin-associated lymphoid tissue (iSALT) and its role in local immune regulation. The conclusions emphasize the complexity of cellular interactions in the skin and their contribution to systemic immune responses.

Key words: cutaneous immune system, keratinocytes, lymphocytes, dendritic cells, macrophages, mast cells, immunocompetent cells

Introduction

The skin is the largest and most complex organ of the human body. Its extensive surface area in contact with the external environment, along with the specific features of its tissue composition and blood supply, necessitates powerful defense mechanisms against adverse external factors as well as against internal factors in cases of homeostatic imbalance [13, 31, 43, 45]. For many years, skin was regarded primarily as an organ providing mechanical and chemical barriers. However, this concept has undergone a profound transformation in recent decades.

Modern research has demonstrated that the skin is an active immunocompetent organ with its own complex immune network, capable of systemic immune responses and interactions with the cutaneous microbiota. Investigations into the protective functions of the skin have led to a new understanding of it as an immunocompetent organ possessing a set of organ-specific defense mechanisms. The resistance of skin structures to damaging agents is determined by mechanisms of nonspecific defense and immunological reactivity [13, 45, 56, 75, 77]. The latter is mediated by cellular and humoral reactions aimed at the elimination of microbial and non-microbial elements. Various cell populations involved in immune surveillance of the skin constitute the local immune defense and are collectively referred to as the "skin immune system" [26, 40, 45, 71].

Occupying a barrier position, the skin is continuously exposed to external and internal adverse influences, which may lead to disturbances of varying severity in its protective complex. Understanding the mechanisms of interaction and regulation of all components of the skin immune system is of great importance for medicine, particularly in light of the increasing recognition of the role of the skin microbiome and its influence on immunity.

Materials and Methods

The aim of this review is to comprehensively summarize the current knowledge on the morphofunctional basis of the cutaneous immune system, highlighting the cellular components and their roles in maintaining skin homeostasis and systemic immune interactions. To achieve this, a systematic literature search was conducted using major scientific databases including *Scopus*, *Web of Science*, and *PubMed*. The search covered articles published between 2010 and 2025 and employed keywords related

to skin immunity, immune cells of the skin, neuroimmune interactions, and immune regulation in dermatology. Selected publications were screened for relevance, focusing on both original research and recent comprehensive reviews, to provide an up-to-date synthesis of the subject.

Cutaneous immune system

In recent decades, the cutaneous immune system has been increasingly recognized as an integral and functionally coordinated component of overall immunity, contributing to the maintenance of systemic homeostasis by providing protection against foreign biological agents [10, 13, 55, 79]. Due to its vast surface area in constant contact with the external environment, the skin is uniquely positioned as the first line of defense against a wide range of exogenous factors, including antigenic stimuli [66].

Pioneering work in the 1980s by J. W. Streilein introduced, by analogy with MALT (mucosa-associated lymphoid tissue), the concept of SALT (skin-associated lymphoid tissue). This term referred to populations of immune cells, including lymphocytes and antigen-presenting cells, residing in the epidermis and dermis. Building on this concept, J. D. Bos and colleagues later proposed the broader term skin immune system (SIS), encompassing SALT together with the epidermis, dermis, and regional lymph nodes [15, 21, 46, 64].

Current evidence supports a functional subdivision of skin defenses into nonspecific mechanisms — mechanical, biochemical, and cellular defenses — and specific immune responses, comprising both cellular and humoral components. The nonspecific arm of cutaneous defense is mediated primarily by epidermal elements such as keratinocytes and dendritic cells, as well as dermal populations including macrophages, mast cells, and granulocytes. In contrast, specific local immunity is ensured by lymphocytes, which orchestrate antigen-specific responses within the skin [45, 51].

More recently, an important conceptual advance has been the recognition of the cutaneous neuroimmune endocrine (CINE) system, a complex network integrating neurons, immune cells, and endocrine cells. This system enables rapid and precise skin responses to stressors by modulating immune defenses and homeostatic processes through neurotransmitters, hormones, and cytokines. Importantly, the CINE system operates both locally within the skin and systemically, thereby influencing not only cutaneous but also organismal immunity. This perspective underscores the integration of the skin into broader neuro–immune–endocrine interactions and highlights the critical role of intercellular communication across the nervous, immune, and endocrine compartments [4, 61].

The extensive surface area of the skin in contact with the external environment, together with its specific tissue composition and vascularization, necessitates the presence of powerful defense mechanisms against adverse external factors, as well as internal factors arising from disruptions of homeostasis [33, 45]. In this context, the traditional view of the skin merely as a mechanical and chemical barrier has been substantially broadened: the skin is now regarded as an immunocompetent organ equipped with a complex network of local and systemic defense mechanisms. The resistance of cutaneous structures to damaging agents is mediated by both nonspecific and specific immune mechanisms, which include cellular and humoral responses directed at the elimination of microorganisms and altered host cells [31, 45].

Components of Innate Immunity

Keratinocytes

Keratinocytes represent the principal cellular component of the epidermis. Through differentiation and apoptosis, they give rise to corneocytes of the *stratum corneum*, which provide the physical barrier of the skin. Beyond this structural role, keratinocytes produce a wide range of regulatory factors, including growth factors and cytokines, and upon injury they secrete antimicrobial peptides such as defensins and cathelicidins.

Keratinocytes of the granular, spinous, and cornified layers express pattern-recognition receptors (PRRs), enabling them to sense microbial invasion and initiate immune responses. Recent studies have shown that PRR activation triggers the NF-κB signaling pathway, leading to the production of pro-inflammatory cytokines and chemokines that recruit immune cells to sites of injury or infection. Moreover, keratinocytes express Toll-like receptors (TLRs) upon activation, thereby acquiring functions resembling those of antigen-presenting cells. Strong activation of keratinocyte TLRs has been associated with polarization toward a Th1 immune response and the synthesis of pro-inflammatory interferons [18, 41, 52].

Keratinocytes also engage in contact-dependent interactions with Langerhans cells and intraepidermal lymphocytes through adhesion molecules such as E-cadherin and ICAM-1. Disruption of these adhesion pathways has been implicated in the pathogenesis of several skin disorders. In addition, activated keratinocytes release chemokines that attract T lymphocytes from the circulation to sites of tissue damage [12, 65].

Recent evidence highlights the regulatory role of keratinocyte apoptosis in maintaining Treg cell populations and skin tissue homeostasis. Coculture experiments further suggest that keratinocytes can suppress the proliferation of phytohemagglutinin (PHA)-activated lymphocytes *in vitro* [2, 19, 29, 50, 59].

Of particular interest is the interaction between keratinocytes and immature T lymphocytes. Several studies suggest that keratinocytes, which share lineage similarities with thymic reticuloepithelial cells, can produce factors influencing T-cell differentiation [73].

Dendritic Cells (DCs)

Dendritic cells (DCs) represent a heterogeneous population of professional antigen-presenting cells (APCs)

that play a pivotal role in both the initiation of adaptive immunity and the maintenance of immune tolerance. Multiple subsets of DCs are present in the skin, each with distinct phenotypic, functional, and molecular characteristics.

The major subsets include:

— Langerhans cells (LCs) in the epidermis, specialized in antigen presentation and epidermal immune homeostasis;

— dermal myeloid DCs, including $CD11b^+$ and $CD11b^-$ subsets, which contribute to T-cell activation, cytokine secretion, and migration to lymphoid tissues;

— plasmacytoid DCs (pDCs), potent producers of type I interferons during viral infections, with additional roles in immune tolerance [7, 60, 78].

Skin DCs display remarkable functional plasticity, adapting their properties to the tissue microenvironment, shifting from tolerance induction to strong immunogenic responses. Certain subpopulations also exhibit cross-presentation capabilities, crucial for $CD8^+$ T-cell activation and antitumor immunity. Modern classification based on molecular markers (CD1a, CD207, CD11c, CD1c, XCR1, CD14, among others) and transcriptomic profiling has refined the identification of functionally distinct dermal DC subsets. This is of particular relevance for the development of immunotherapeutic strategies in inflammatory and oncological skin diseases [6, 58].

Langerhans Cells (LCs)

Langerhans cells constitute the major specialized APCs of the epidermis, localized in the basal and spinous layers, accounting for 2–4 % of all epidermal cells. Most LCs are thought to originate from $CD34^+$ hematopoietic progenitors, although recent evidence suggests that resident $CD14^+$ dermal cells can differentiate into LC-like cells under the influence of TGF-β, underscoring the plasticity of LC development within the cytokine milieu of the skin [9, 20, 47, 80].

LCs, although tightly associated with keratinocytes, are highly dynamic, capable of migration and phenotype modulation. Their defining markers include MHC class II molecules, E-cadherin, langerin (CD207), and CD1a. A hallmark ultrastructural feature of LCs are Birbeck granules, rod- or racket-shaped organelle-like structures formed by the accumulation of langerin in endosomal compartments. Although their precise function remains incompletely understood, emerging evidence suggests they are involved in receptor-mediated endocytosis and antigen transport, significantly influencing the antigen-presenting capacity of LCs [39].

Upon inflammatory stimulation, LCs capture antigens in the epidermis, process them during migration, and travel via lymphatic vessels to regional lymph nodes, thereby forming a functional “skin–lymph node” axis [24, 68]. In lymph nodes, they present processed antigens to T cells, triggering downstream immune responses and antigen elimination [23, 47]. Human LCs preferentially drive Th2 cell differentiation and can prime both naïve and cross-present $CD8^+$ T cells [3, 38, 48].

LC activation is mediated by microbial molecules recognized via TLR and NOD-like receptors, as well as cytokines from the microenvironment. LCs are central to the initiation of cellular immunity, notably by stimulating natural killer cells and $CD8^+$ T lymphocytes. Importantly, they express distinct sets of Toll-like receptors, the activation of which promotes IL-15 secretion, a cytokine critical for $CD8^+$ T-cell proliferation [8, 63].

Dermal Myeloid Dendritic Cells (dDCs)

Dermal myeloid dendritic cells (dDCs) are functionally comparable to other interstitial DC populations identified within the stromal connective tissues of various organs. They are distributed throughout all layers of the dermis and characteristically express MHC class II and CD1c (BDCA1) molecules on their surface. Their principal functions include antigen presentation and the secretion of cytokines and chemokines. Upon activation, dDCs migrate to the paracortical regions of draining lymph nodes. Recent studies in models of allergic contact dermatitis have demonstrated that dDCs isolated from lymph nodes can induce robust T-cell proliferation [1, 37, 58].

Plasmacytoid Dendritic Cells (pDCs)

Plasmacytoid dendritic cells (pDCs) represent a distinct class of DCs that are much less frequent in the skin compared with dDCs. They are phenotypically defined by surface expression of CD123, CD45RA, and CD303 (BDCA2). Functionally, pDCs are potent producers of type I interferons, particularly IFN-α, during viral infections. Beyond their antiviral role, pDCs are also thought to modulate plasma cell activity and contribute to the induction of immune tolerance [14, 44].

Dermal Macrophages

Dermal macrophages are large amoeboid cells rich in lysosomes. They are also regarded as professional antigen-presenting cells; however, unlike dendritic cells, macrophages lack the capacity to initiate primary immune responses. Their contribution to nonspecific defense relies primarily on phagocytosis and the neutralization of infectious agents through the secretion of cytokines and chemokines. Skin macrophages originate from circulating monocytes, which in turn derive from bone marrow myeloid progenitors. Recent evidence suggests that macrophages in the skin retain proliferative capacity, highlighting their persistence and role in tissue homeostasis [34, 62, 74]. Macrophages express pattern recognition receptors (PRRs) on their surface, subdivided into membrane-bound and cytoplasmic receptors, which are crucial for the recognition of pathogen-associated elements. Once activated, macrophages migrate to sites of inflammation, where they produce interleukins and toxic mediators that suppress microbial activity [36].

Several macrophage subsets have been identified in the skin and are generally classified into two main categories: resident and inflammatory. Resident macrophages are permanently present within the dermal con-

nective tissue and are essential for maintaining homeostasis. In contrast, inflammatory macrophages arise *de novo* at sites of infection or tissue injury. Classically activated macrophages exert pro-inflammatory functions and are characterized by the synthesis of IL-12 and IL-23 [17, 28, 34]. Alternatively activated macrophages, in contrast, secrete IL-10 while lacking IL-12 production, thereby suppressing T-cell proliferation and attenuating inflammation. Recent studies have shown that alternatively activated macrophages promote fibrogenesis, angiogenesis, and wound healing [11, 53]. Importantly, macrophages in wounds display a mixed and dynamic phenotype that combines features of both classical and alternative activation states.

Mast Cells and Dermal Granulocytes

Mast cells represent an integral component of nonspecific antibacterial and antiparasitic defense but are also key contributors to allergic and inflammatory reactions due to their production of cytokines, chemokines, lipid mediators, proteases, and biogenic amines. They have been implicated in the pathogenesis of stress-related effects on the skin. Under physiological conditions, mast cells participate in tissue homeostasis, regulation of interstitial fluid balance, and control of microvascular tone [27, 42, 72].

Neutrophils constitute another central element of nonspecific defense, capable of recognizing, phagocytosing, and destroying pathogens. Under normal conditions, their presence in the dermis is limited; however, during inflammation, they are rapidly recruited from the bloodstream. Recent research revealed a specialized population of neutrophils in the skin that synthesize collagen and other extracellular matrix (ECM) components, physically reinforcing the skin barrier beyond their traditional antimicrobial role. These “matrix-producing neutrophils” produce type III collagen (COL3A1) and other ECM molecules not only during injury but also in homeostasis, thus enhancing mechanical integrity and barrier function of the skin. After skin injury, these neutrophils form protective ECM “rings” around wounds (~1 mm diameter) that prevent bacterial and toxin penetration. This process is regulated by the TGF-β signaling pathway, with inhibition of this pathway impairing ring formation and increasing skin permeability. Additionally, their activity follows a circadian rhythm, showing increased migration and ECM formation during the night, correlating with enhanced skin mechanical strength at this time. In addition to their antimicrobial functions, neutrophils interact closely with other immune and stromal cells to regulate reparative processes [67].

Moreover, skin-resident dermal neutrophils differ from circulating blood neutrophils by their longer lifespan, immune-modulatory roles, and active interactions with skin cells such as fibroblasts and keratinocytes. They participate not only in classical defense mechanisms including phagocytosis, reactive oxygen species (ROS) production, and neutrophil extracellular trap (NET) formation but also engage in immunoregulation via cytokine secretion (*e.g.*, TNF-α, IL-1β, IL-17), affecting macrophages, dendritic cells, and T lymphocytes. Their coordinated “swarming” migration around infection foci helps isolate pathogens and modulate inflammation. These dermal neutrophils have been implicated in the pathogenesis of chronic skin diseases such as psoriasis, chronic wounds, and autoimmune dermatitis [67].

Innate Lymphoid Cells

The system of nonspecific defense also includes innate lymphoid cells (ILCs) — namely NK, NKT, and γδ T lymphocytes — which, according to most researchers, represent an evolutionarily more ancient layer of the lymphoid immune system. These cells not only perform classical protective functions but also contribute to histogenesis, tissue remodeling, and stem cell regeneration [25, 51, 70].

Natural Killer (NK) Cells

NK cells are capable of directly attacking virus-infected and tumor cells as well as producing cytokines. They are characterized by the phenotype $CD3^{-}CD16^{+}$, $CD3^{-}CD56^{+}$. However, many functions of NK cells under pathological conditions remain poorly understood, and recent studies have reconsidered their role. In particular, NK cells have been implicated in the pathogenesis of allergic and autoimmune diseases. In the skin, two distinct NK cell populations have been identified: one producing IL-22 alone, and another producing both IL-22 and IL-17. The latter are often referred to as lymphoid tissue inducer (LTI)-like cells, which participate in autoimmunity and inflammation, although current evidence about them remains limited [30].

Natural Killer T (NKT) Cells

NKT cells, or natural killer T lymphocytes, co-express CD16, CD56, CD57, CD94, and CD161. Unlike conventional T lymphocytes, they recognize glycolipid antigens presented by CD1d molecules. NKT cells rapidly secrete cytokines and exhibit cytotoxic activity through the expression of perforin, granzymes, and granulysin. They mediate both protective and regulatory immune functions [69, 81].

γδ T Cells

γδ T cells are abundantly present in the epidermis and are considered part of the first line of defense against pathogens, as well as key players in maintaining epidermal homeostasis. They are capable of recognizing non-peptide antigens, including autoantigens expressed by damaged keratinocytes. Close interactions between γδ T cells and dendritic cells (DCs) have been established: DCs activate γδ T cells, which in turn accelerate the maturation of immature DCs. Skin-resident γδ T cells expressing NKG2D play a pivotal role in cutaneous malignancies. In addition, human γδ T cells produce growth factors essential for wound healing, including CTGF, FGF9 (GAF), KGF, and IGF1 [5, 22, 76].

Adaptive Immunity

T Lymphocytes

Adaptive immunity in the skin is primarily mediated by effector T lymphocytes, which constitute more than 90 % of all cutaneous lymphocytes. $CD4^+$ and $CD8^+$ T-cell subsets are present in the dermis in approximately equal proportions, with most of them belonging to the memory cell pool. In the epidermis, $CD8^+$ T cells predominate. Studies of inflammatory processes have identified the main types of $CD4^+$ T cells: Th1, Th2, Th17, and regulatory T cells (Tregs). Th1 cells are activated during intracellular infections; Th2 cells participate in allergic responses; Th17 cells provide protection against fungal and bacterial pathogens; and Tregs regulate (limit) immune responses. Most $CD4^+$ T lymphocytes express memory T-cell markers ($CD45RO^+$) and activation markers ($HLA\text{-}DR^+$, $CD25^+$). Resident T lymphocytes also express cutaneous homing receptors, which ensure tissue-specific migration and differentiation. More recently, a subpopulation of Th22 cells producing IL-22 has been identified in skin cell cultures derived from patients with atopic dermatitis [49, 54, 71].

B Lymphocytes

B lymphocytes are present in small numbers in the deeper layers of the dermis, where they contribute to immunoglobulin production and immune regulation. However, some studies have reported the absence of B lymphocytes in normal skin. Particular attention has been given to recently described “regulatory B cells” (Bregs), which have been identified in the skin under conditions of transplantation and malignancy. Bregs suppress inflammation, tumor progression, and autoimmune responses, and they contribute to the establishment of immune tolerance [16, 32, 35].

In conclusion, this review synthesizes current understanding of the morphofunctional basis of the cutaneous immune system, emphasizing its complex cellular composition and dynamic interactions. The findings highlight the skin’s role as an active immunocompetent organ, capable of orchestrating both local and systemic immune responses. Recognizing the intricate network of immune cells and their functions is essential for advancing therapeutic strategies in dermatology and immunology, as well as for understanding skin pathology and microbiome interactions. Future research should focus on elucidating the mechanisms of cell communication within the skin and exploring novel approaches to modulate immune responses for clinical benefit.

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Морфофункціональні основи імунної системи шкіри

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Стаття надає систематичний огляд сучасних уявлень про морфофункціональні основи імунної системи шкіри, її клітинний склад та інтеграцію в складний мережевий нейро-імунно-ендокринний механізм. Наведено характеристику основних типів імунокомпетентних клітин: кератиноцитів, дендритних клітин (Лангерганса, мієлоїдних, плазматичних), макрофагів, мастоцитів, лімфоцитів та їх ролі у підтримці гомеостазу та захисті шкіри від зовнішніх і внутрішніх чинників. наукові бази. Для пошуку літератури використані сучасні бази (*Scopus*, *PubMed*, *Web of Science*). Окремо висвітлено концепцію периферійної лімфоїдної тканини шкіри (iSALT) та її значення в регуляції місцевого імунітету. Висновки підкреслюють складність взаємодій між клітинними компонентами шкіри і системними імунними реакціями.

Ключові слова: імунна система шкіри, кератиноцити, лімфоцити, дендритні клітини, макрофаги, мастоцити, імунокомпетентні клітини

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Meta Research

Evaluating the safety of genetically modified crops: Findings from toxicological meta-research

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This meta-analysis examined the safety of genetically modified (GM) crops by collating findings from controlled animal feeding trials and human observational studies published between 2017 and 2025. The investigation critically assessed acute and chronic toxicity, allergenicity, metabolic disturbances and carcinogenic endpoints, focusing on research from Nigeria, Africa and Western regions (Europe and the USA). Advanced statistical methods, including random-effects modelling, subgroup analyses and meta-regression, were employed to quantify heterogeneity and evaluate the robustness of the evidence. The findings are presented in percentage terms to facilitate a clear summary of the safety profile of GM crops. The analysis indicates that genetically modified foods are not acutely toxic while some studies reported minor metabolic and immunological changes on chronic or prolonged exposure. Discrepancies in chronic toxicity findings were largely due to variations in experimental model, study design and sample size. Therefore, overall evidence supports the general safety of GM crops under current testing protocols; however, some uncertainties persist regarding long-term effects. Hence, the paper concluded that safety depends on the type of modifications made. Insect-resistant and pesticide-tolerant modifications are more associated with safety concerns than any other type, like biofortified modification.

Key words: genetically modified crops, toxicological safety, meta-research, crop-vigilance, risk assessment

Introduction

Evaluating the safety of genetically modified (GM) crops has spanned scientific, ethical and socio-political domains for more than three decades. Following the first field trials in the early 1990s, most notably for insect-resistant *Bacillus thuringiensis* (*Bt*) maize and herbicide-tolerant soybean, commercial approvals soon followed in the United States and Canada, ushering in the first GMO foods in the marketplace [29]. Subsequent generations of stacked-trait cultivars, combining pest resistance with herbicide tolerance (*e.g.* NK603 × MON810 maize), reflect advances in molecular breeding but have also intensified scrutiny regarding their long-term toxicological profiles. Regulatory authorities such as the European Food Safety Authority [15–16] have instituted rigorous, case-by-case assessments; nevertheless, debates persist due to rapid innovation in gene-editing techniques and variable global approval processes.

In regions with minimal market oversight, such as Nigeria and wider sub-Saharan Africa, prolonged exposure to imported GM commodities amplifies public health concerns. Several studies in Nigerian contexts, ranging from rodent feeding trials with *Bt* maize [29] to observational surveys of imported Roundup Ready soybean meal [24], underscore the need for integrative safety evaluations. Divergent regulatory assurances in Western nations contrast sharply with local scepticism,

often influenced by perceptions of corporate regulatory capture [4, 46]. Nigerian scholars have called for toxicological research that accounts for indigenous diets and agricultural practices, which may modify exposure pathways and risk profiles [19, 40].

A core challenge in GM-crop safety assessment is the reliance on conventional *in vivo* animal models. While these studies yield valuable mechanistic insights, inter-species differences in metabolism and immune response can limit the extrapolation to human health outcomes [28]. Pharmacology brings a pharmacokinetic and pharmacodynamic lens to these translational gaps, yet recent meta-analyses have highlighted that standard endpoints, such as acute toxicity, allergenicity and carcinogenic potential, may fail to detect subclinical perturbations over long-term exposure [4, 9, 12]. Heterogeneity in study design, event constructs and statistical power further complicates meta-synthesis.

Socio-political forces likewise shape the GM debate. In many Western countries, robust research infrastructure underpins comprehensive risk assessments; by contrast, African regulatory systems often contend with limited technical capacity and evolving biosafety frameworks [38]. Some nations embrace GM technology to bolster food security, whereas others resist due to environmental and health concerns [2, 21]. These divergent stances highlight the necessity of context-specific research and transparent stakeholder engagement.

The study employs a meta-research methodology, systematically pooling data from animal trials and human observational studies, to deliver a more nuanced risk assessment framework grounded in veterinary pharmacology and public health. The study applied random-effects meta-analysis, complemented by narrative synthesis where heterogeneity precludes quantitative pooling. Advanced omics techniques (metabolomics, epigenetics) are integrated to identify early biomarkers of toxicity that traditional endpoints may overlook [23, 35].

Ultimately, this study situates GM-crop safety within the larger imperatives of food security, public health and environmental sustainability. Through reconciling historical progress with emerging methodological innovations and socio-political realities, we aim to provide policymakers, researchers and practitioners with an evidence-based foundation for informed decisions about GM-crop adoption, regulation and communication.

Literature Review

The evaluation of the toxicological safety of genetically modified (GM) crops has received sustained academic attention over the past decades. As GM technology has been applied in agriculture to improve yield, resistance to pests and diseases and nutritional content, concerns about potential adverse effects on human and animal health have persisted. This review critically examines recent literature from Nigerian, African and Western sources to assess current evidence on toxicological endpoints associated with GM crops, while addressing methodological limitations and regional variations in safety evaluations.

Evidence from Experimental Animal Studies

A significant portion of toxicological data on GM crops originates from experimental studies using rodent models. These investigations primarily assess acute toxicity, chronic toxicity, allergenicity, carcinogenic potential and metabolic disturbances. For instance, EFSA GMO Panel and colleagues [15] have documented that, under controlled laboratory conditions, GM crops generally do not induce adverse acute effects when compared with conventional counterparts. In these studies, standard biochemical and haematological parameters remain within acceptable ranges and histopathological examinations typically do not reveal significant tissue abnormalities [5, 15, 23, 45].

Nevertheless, the extrapolation of animal study results to human risk assessments is not straightforward. Differences in metabolism, physiology and lifespan between rodents and humans can limit the applicability of findings. A. Moresis et al. [35], E. Hermans et al. [22] and N. Marsteller et al. [31] have emphasised that while rodent models are useful in defining dose-response relationships and identifying mechanistic pathways, the inherent interspecies differences necessitate additional approaches to confirm human relevance. Furthermore, some studies have reported transient biochemical fluctuations that, although not reaching clinical significance, suggest that short-term assessments may not capture subtle or cumulative toxic effects [5, 23, 45]. This issue is particularly pertinent in studies of chronic exposure, where the potential for low-incidence but significant effects may be underestimated due to short study durations and limited sample sizes.

Observational Evidence from Human Populations

Observational studies in human populations offer a complementary perspective to controlled animal experiments. In countries where GM crops are widely consumed, particularly in areas with less rigorous regulatory oversight such as Nigeria, epidemiological research provides essential information on long-term health outcomes. S. Adeyeye and F. Idowu-Adebayo [1] O. Oladipo et al. [40] reported that in Nigerian populations, while the majority of individuals consuming GM crops did not exhibit overt health issues, there were occasional observations of subclinical effects such as mild allergic reactions and alterations in metabolic markers. Similarly, U. Yahaya et al. [51], S. Adeyeye and F. Idowu-Adebayo [1] and O. Oladipo et al. [40] noted that despite an overall trend of safety, certain vulnerable groups might experience cumulative adverse effects over time.

However, observational studies face inherent challenges. Confounding factors such as variations in diet, environmental exposures and genetic diversity can complicate the attribution of health outcomes solely to GM crop consumption. Additionally, the long-term nature of these

studies means that the sample sizes are often modest and the data may be affected by reporting biases. Despite these limitations, human observational research remains indispensable for contextualising laboratory findings and assessing real-world exposure risks [40, 51].

Integration through Meta-Analytical Studies

Meta-analyses serve as a vital tool to reconcile the diverse findings from both animal and human studies. J. Caradus [9] and P. Krogh et al. [28] conducted a comprehensive meta-analysis that synthesised data across numerous toxicological investigations. Their findings indicate that although the majority of research supports the overall safety of GM crops, a subset of studies reports potential adverse outcomes, particularly in relation to chronic toxicity and allergenicity. Meta-analytical techniques also highlight the methodological heterogeneity across studies, including differences in study design, sample size and the nature of genetic modifications implemented. This variation contributes to inconsistencies in the reported outcomes, making it challenging to draw definitive conclusions about the long-term safety profile of GM crops [9, 12, 28].

A further quantitative synthesis, which expressed outcomes in percentage terms, reveals that approximately 90 % of studies report no significant adverse effects concerning acute toxicity [47]. In contrast, around 30 % of studies on chronic toxicity indicate subtle metabolic or histopathological changes that could be clinically relevant if exposures persist over a lifetime [36]. Similarly, while about 80 % of studies suggest low allergenic potential, the remaining 20 % document mild to moderate immune responses under certain conditions. Overall, nearly 95 % of the evidence supports a lack of carcinogenic potential, though isolated instances in studies of stacked genetic modifications necessitate cautious interpretation [6, 8, 25, 34, 36, 49].

Challenges in Data Synthesis and Methodological Considerations

Despite the wealth of data available, several methodological challenges hinder the comprehensive assessment of GM crop safety [11, 20, 44]. One prominent issue is the variability in experimental design. Many animal studies use short-term endpoints that may fail to capture the cumulative effects of chronic exposure. Observational studies, on the other hand, are often constrained by limited statistical power and the presence of confounding factors that make causal inferences difficult. M. Dadgarnejad et al. [11] and M. Glevitzky et al. [20] have pointed out that the lack of standardisation in study protocols — ranging from dosing regimens to the selection of animal strains — further complicates the aggregation of findings in meta-analyses.

Another significant concern is the influence of funding sources on research outcomes. Evidence suggests that studies sponsored by industry tend to report fewer adverse effects compared with those funded independently [13, 26, 48]. This discrepancy raises issues regarding potential bias in study design and reporting. The imperative for transparent disclosure of funding and adherence to standardized reporting guidelines is therefore essential to ensure the integrity of safety assessments.

Furthermore, there remains a notable gap in our understanding of the mechanistic underpinnings of subtle toxicological effects. Traditional endpoints such as acute toxicity markers and histopathological evaluations may not detect early biochemical perturbations that presage long-term adverse outcomes [30, 42]. Recent advancements in analytical techniques, including metabolomic and epigenetic profiling, have demonstrated the capacity to identify early biomarkers of toxicity that conventional methods might overlook [7, 30, 42]. Integrating these modern techniques with standard toxicological assessments could significantly improve the sensitivity of risk evaluations and provide a more comprehensive picture of the potential hazards associated with GM crop consumption.

The heterogeneity observed across studies also poses a challenge. Differences in genetic modifications — such as the use of *Bt* genes for pest resistance *versus cp4 epsps* for herbicide tolerance — introduce variability in the metabolic and immunological responses elicited by GM crops. Additionally, the source of the transgene plays a critical role; genes derived from closely related species tend to produce fewer adverse effects compared with those sourced from organisms not typically consumed by humans or animals [14, 32, 51]. This variation underscores the necessity of conducting region-specific research that accounts for local dietary practices and genetic diversity [4, 11, 19], particularly in African contexts where environmental conditions and consumption patterns may differ markedly from those in Western countries [1, 40].

Regional Perspectives and Socio-Political Implications

The safety evaluation of GM crops cannot be separated from the socio-political context in which they are developed and deployed. In many Western countries, regulatory bodies operate with extensive scientific expertise and resources, enabling the implementation of rigorous safety assessments [40]. However, in Nigeria and other sub-Saharan African nations, regulatory frameworks are often still developing and the capacity for comprehensive risk evaluation may be limited [1]. Studies conducted in these regions have highlighted discrepancies between the safety standards applied in Western nations and those in local settings [2, 21, 38]. Factors such as public skepticism, economic pressures and the influence of multinational corporations on national policy further complicate the environment. These challenges underscore the need for research that is tailored to the specific socio-economic and environmental contexts of developing countries.

Synthesis of Toxicological Endpoints

In synthesizing the extant literature, the following key toxicological endpoints were identified: acute toxicity, chronic toxicity, allergenicity, metabolic disturbances, gastrointestinal effects and carcinogenic potential. The major-

ity of animal studies and several meta-analyses support the safety of GM crops in terms of acute toxicity, with over 90 % of studies reporting no significant adverse effects. However, chronic toxicity data are more heterogeneous; approximately 30 % of studies indicate subtle adverse effects that may have long-term implications. In the realm of allergenicity, while most research (around 80 %) suggests a low risk, some studies report mild immunological responses that warrant further investigation [1, 4, 9]. Metabolic disturbances and gastrointestinal effects are generally minimal in short-term studies, although minor alterations have been noted in longer-term evaluations. Finally, carcinogenic potential appears negligible in the vast majority of studies, with isolated reports in research focusing on stacked genetic modifications necessitating continued vigilance [12, 15–16, 28].

Materials and Methods

Design

This study employs a meta-research approach to synthesize and critically evaluate the toxicological safety of genetically modified (GM) crops. The methodology has been designed according to rigorous standards in veterinary pharmacology and adapted for the context of the Faculty of Veterinary Science at the University of Maiduguri (Nigeria). The following section describes the systematic literature search strategy, methods for data extraction, criteria for inclusion and exclusion, quality assessment and the analytical approaches used to integrate findings from experimental animal feeding trials and human observational studies.

Target GM Crops and Commodities

To clarify the specific genetically modified events and commodity forms that underpin our comparative meta-analysis, we have identified seven principal cultivars and their derived products (a summary of target GM events included in meta-analysis is provided in table 1). These include:

1. *Maize MON810 (Cry1Ab insect-resistant)*. Evaluated in maize grain and maize meal from both European Union field trials and Nigerian feeding studies [15, 29].
2. *Maize MON863 (Cry3Bb1 insect-resistant)*. Assessed in grain-based feeding trials conducted in North America and West Africa.
3. *Maize NK603 x MON810 (cp4 epsps herbicide tolerance + Cry1Ab insect resistance)*. Studied in Brazil and Nigeria to determine combined-trait safety in grain.
4. *Soybean (Roundup Ready) (cp4 epsps herbicide tolerance)*. Analysed in seed, refined oil and lecithin imports to Nigeria, as well as in United States and Argentinian production systems [24].
5. *Cotton (Cry1Ac + Cry2Ab events)*. Investigated primarily through cottonseed cake and meal used in livestock feed in India and West Africa [31].
6. *Golden Rice (phytoene synthase + crtI provitamin A biofortification)*. Examined in polished rice grain for both nutritional efficacy and toxicological endpoints in rodent and limited human cohort studies [23].
7. *Biofortified Cassava (β-carotene pathway genes)*. Included as tuber and flour in field trials and observational research from Nigeria and Ghana, with emphasis on provitamin A uptake and safety [27].

The table 1 shows the diversity of GM events included in the meta-analysis, highlighting both agronomic traits and their relevance to Nigerian and global contexts. The predominance of insect-resistant maize events, MON810 and MON863, reflects widespread cultivation and safety assessment across Europe, North America and West Africa [15, 29]. The stacked NK603×MON810 variety, combining herbicide tolerance with pest resistance, exemplifies the trend towards multi-trait cultivars, with studies in Brazil and Nigeria revealing similar safety profiles to monogenic lines.

Table 1. Summary of target GM events included in meta-analysis

GM event	Primary trait	Commodity form	Geographical focus of key studies
Maize MON810	Insect resistance (Cry1Ab protein)	Grain, maize meal	Nigeria; European Union (EFSA evaluations)
Maize MON863	Insect resistance (Cry3Bb1 protein)	Grain	Nigeria; United States
Maize NK603 × MON810	Herbicide tolerance (cp4 epsps) + insect resistance (Cry1Ab)	Grain	Nigeria; Brazil
Soybean (Roundup Ready)	Herbicide tolerance (cp4 epsps)	Seed, oil, lecithin	Nigeria (imports); USA; Argentina
Cotton (Cry1Ac/Cry2Ab events)	Insect resistance (Cry1Ac + Cry2Ab proteins)	Fibre by-products	India; West Africa
Golden Rice (PSY + CrtI genes)	Provitamin A biofortification	Polished rice grain	India; Philippines
Cassava (β-carotene biosynthesis)	Provitamin A biofortification	Tuber, flour	Nigeria; Ghana

Note. *MON810, MON863, NK603* — event designations refer to constructs approved in various jurisdictions; studies in Nigeria often examined monogenic events [15, 29]. *Roundup Ready soybean* — Nigeria imports significant volumes of RR soybean meal and oil, prompting observational studies on market composition [24]. *Bt cotton* — although primarily cultivated for fiber, cottonseed cake is used in livestock feed and assessed in animal feeding trials [31]. *Golden Rice and biofortified cassava* — evaluated for nutritional efficacy alongside toxicological endpoints in rodents and limited human cohorts [23, 27].

Herbicide-tolerant soybean (Roundup Ready) features prominently, owing to its extensive importation into Nigerian food and feed chains and robust toxicological evaluation in the Americas [24]. In contrast, *Bt* cotton events, which are primarily studied for their livestock-feed by-products, underscore the indirect pathways through which non-food GM crops may enter human and animal diets [31].

Biofortified staple crops, such as Golden Rice and β-carotene cassava, represent a newer paradigm in GM technology aimed at alleviating micronutrient deficiencies. Although fewer in number, safety studies from India, the Philippines and Ghana suggest these provitamin A crops exhibit similar toxicological profiles to conventional counterparts [23, 27].

The table 1 confirms that our meta-analysis encompasses the most commercially and nutritionally significant GM events, thereby ensuring that conclusions about acute and chronic safety endpoints are directly applicable to the crops most likely to affect food security and public health in Nigeria and beyond.

Moreover, enumerating those events and commodity forms establishes the study's clear boundaries for its inclusion criteria and ensure that subsequent data extraction and quality-assessment processes are transparently linked to the crops most relevant to Nigerian and global food-security contexts.

Systematic Literature Search Strategy

A thorough comprehensive literature search was conducted to identify peer-reviewed studies published from 2017 to 2025 that examined toxicological endpoints associated with GM crops. Searches were performed across several major electronic databases including *PubMed*, *Web of Science*, *Scopus* and *Google Scholar*, as well as region-specific resources such as *African Journals Online* (AJOL) to secure adequate representation of research from Nigeria and other parts of Africa [1, 14, 46, 51]. The selection of these databases was based on their extensive coverage of biomedical, agricultural and toxicological research, ensuring a broad perspective on GM crop safety from both international [46] and African contexts [14, 40, 51].

The search strategy was designed by combining pertinent keywords and Medical Subject Headings (MeSH) terms. Search strings were carefully formulated using Boolean operators "AND" and "OR" to combine key terms and subject headings related to genetically modified organisms and their safety evaluation. For instance, one of the main search queries used in *PubMed* was as follows:

("genetically modified crops" OR "GM crops")
AND ("toxicity" OR "toxicology" OR "safety")
AND ("animal feeding trial" OR "in vivo study"
OR "observational study")
AND ("acute toxicity" OR "chronic toxicity"
OR "allergenicity" OR "metabolic disturbances"
OR "carcinogenicity")

This approach ensured that only studies directly relevant to toxicological assessments were captured while minimising irrelevant results. The approach was designed to minimize bias in the retrieval process and to ensure that both laboratory-based and epidemiological data were represented [8, 25, 31, 35].

Inclusion and Exclusion Criteria

To ensure that only high-quality studies were included in the analysis, stringent inclusion and exclusion criteria were applied. Table 2 encapsulates the robust criteria that formed the basis of our systematic literature search. Studies were included if they met the following specifications:

- *Study design*. Only primary research articles reporting experimental animal feeding trials or human observational studies were considered. Although meta-analyses and systematic reviews were reviewed for background and methodological context, only original data from primary studies were used in the synthesis.
- *Publication period*. Articles published between 2017 and 2025 were selected to ensure the findings reflect current research and contemporary methods in GM crop safety evaluation.
- *Language*. Only studies published in English were included, reflecting the language proficiency required for a detailed critical appraisal.

Table 2. Summary of inclusion and exclusion criteria for the systematic literature search

Criteria category	Details
Study design	*Inclusion:* peer-reviewed primary research articles reporting experimental animal feeding trials or human observational studies. *Exclusion:* conference abstracts, editorials, grey literature and studies lacking original data.
Publication period	*Inclusion:* studies published between 2017 and 2025 to capture the most recent evidence.
Language	*Inclusion:* only articles published in English.
Endpoints assessed	*Inclusion:* studies evaluating one or more toxicological endpoints, including acute toxicity, chronic toxicity, allergenicity, metabolic and gastrointestinal disturbances or carcinogenic potential.
Geographical scope	*Inclusion:* global studies with particular emphasis on research from Nigeria, Africa and Western countries.
Data quality	*Exclusion:* studies with insufficient methodological details (*e.g.*, unclear sample sizes, lack of bias assessment) or duplicate reports (only the most comprehensive version retained).

- *Endpoints assessed*. Studies needed to assess at least one of the key toxicological endpoints: acute toxicity, chronic toxicity, allergenicity, metabolic disturbances, gastrointestinal effects or carcinogenic potential.
- *Geographical scope*. Although the search was global, emphasis was placed on incorporating studies from Nigeria, broader Africa and Western countries to achieve a balanced, cross-regional synthesis.

Two independent reviewers conducted the screening of titles, abstracts and full texts. Any discrepancies were resolved through discussion or consultation with a third reviewer, thereby minimising subjective bias [13, 26, 48].

Data Extraction

A comprehensive, standardized data extraction form was developed to record the essential characteristics of each study identified in our systematic search. This form served to document crucial information including author details, year of publication, geographical origin, study design, sample size, study duration, the specific GM crop investigated, details of genetic modifications (for instance, *Bt* gene insertion or *cp4 epsps* expression) and the toxicological endpoints evaluated. In addition, information regarding methodological aspects such as randomization procedures, blinding methods and funding sources was recorded in order to enable a rigorous assessment of bias.

The extraction form allowed us to categorize each study under several key domains:

Study characteristics. These included the authorship, publication year and geographical context, providing an overview of the origin and timeline of the research. The study design, whether an experimental animal feeding trial or a human observational study, along with sample size and duration, was also recorded, ensuring that details influencing the statistical reliability of findings were captured accurately.

Intervention details. For each study, the precise nature of the GM crop under evaluation was noted, along with the specific genetic modifications employed. For instance, the form noted modifications such as the insertion of *Bt* genes for insect resistance or the use of *cp4 epsps* for herbicide tolerance. The intended agronomic purpose— be it pest control, improved nutritional content or herbicide resistance — was recorded to contextualize the toxicological outcomes.

Toxicological endpoints. Data extraction focused on a range of toxicological endpoints. For acute toxicity, biochemical markers such as serum enzyme levels and haematological parameters were reviewed. Chronic toxicity was assessed through the recording of histopathological findings and metabolic alterations. In addition, studies reporting immunological assays to assess allergenicity, evaluations of gastrointestinal function and any evidence indicating carcinogenic potential were carefully documented.

Methodological quality. To facilitate a detailed bias assessment, we extracted information on randomization, blinding and sample size adequacy, as well as details of funding sources. Particular attention was paid to identifying any potential for commercial influence that might affect study outcomes.

Data extraction was conducted independently by two reviewers. Their independent extraction ensured accuracy and consistency in collating the data and any discrepancies were resolved through consensus or, when necessary, the involvement of a third reviewer. This dual-review process adheres to best practice in systematic reviews and supports the integrity of the synthesis [11, 20, 44].

Quality Assessment and Risk of Bias

A thorough quality assessment was undertaken for all included studies. For the quality appraisal of the selected studies, we used established tools. For animal feeding trials, the SYRCLE risk of bias tool was employed to evaluate aspects such as randomness of animal assignment to groups, the adequacy of blinding and the completeness of outcome reporting. For human observational studies, the Newcastle-Ottawa Scale (NOS) [25] was applied to assess the quality of non-randomized studies, focusing on the selection of study groups, comparability across cohorts and the rigorous assessment of outcomes. Additionally, potential publication bias was examined through funnel plots and Egger's regression test [4, 46], particularly when the quantitative data supported such analyses. This comprehensive quality assessment ensured that our synthesis relied on studies with robust methodologies and that any potential bias — whether due to small sample sizes, reliance on animal models or industry funding — was duly considered [13, 22, 26, 31, 35, 48]. Studies demonstrating strong methodological design were given greater weighting in the overall analysis, whereas studies exhibiting significant shortcomings were either excluded from the quantitative synthesis or discussed separately in the narrative review [8, 31, 35].

Data Synthesis and Statistical Methods

Following comprehensive data extraction and quality assessment, the synthesized data were analyzed using a combination of quantitative and qualitative methodologies. The objective was to consolidate the findings from studies with comparable designs and endpoints while accounting for inherent variability across the literature. In cases where studies displayed similar design parameters and reported analogous endpoints, a random-effects meta-analysis was performed. This model was selected to account for the inherent variability among studies regarding design, population characteristics and the specific endpoints measured [6, 34, 47].

Quantitative Meta-Analysis

For those studies reporting similar outcomes, a random-effects model was applied using Review Manager (*RevMan*) and *STATA (version 16)*. This model was preferred because it assumes that the true effect size varies among studies due to differences in design, population

and experimental methods [25]. When a sufficient number of studies allowed for quantitative synthesis, the I^2 statistic was calculated to quantify heterogeneity: values surpassing 50 % were interpreted as indicative of moderate to high variability among study findings [4].

Handling Heterogeneity and Mixed Outcomes

In cases where heterogeneity was high, additional analyses were performed to discern the influence of specific study-level factors. The heterogeneity inherent in the studies was addressed through several strategies:

- *Subgroup analysis*. Studies were categorised based on factors such as the type of genetic modification (for example, comparing pest-resistant traits with herbicide-tolerant modifications) as well as geographical region (contrasting studies from Nigeria and broader African contexts with those from Western countries) and funding source (independent *versus* industry-funded studies) to clarify sources of variability.
- *Meta-regression*. Continuous variables such as study duration and GM crop dosage, were examined to understand their impact on toxicological outcomes. These analyses provided a means of exploring dose-response relationships and assisted in identifying the sources of variability across the literature [25].
- *Sensitivity analysis* (leave-one-out procedure). Systematic exclusion of studies with high risk of bias ensured that the pooled estimates remained robust. This involved systematically removing one study at a time and recalculating the pooled effect to ensure that no single study disproportionately influenced the results [17, 37, 43].
- *Publication bias assessment*. Funnel plots and Egger's tests were conducted to identify any potential bias in the literature, ensuring that any tendency towards underreporting of null or adverse findings was identified [17, 37, 43]. Forest plots were generated to visually represent the pooled effect sizes and the degree of heterogeneity. These plots provided a clear depiction of the contribution of individual studies to the overall effect and highlighted the consistency of findings across the dataset.

These approaches enhanced the reliability of the overall conclusions, ensuring that methodological limitations such as small sample sizes and ethical constraints in animal studies were taken into account [10, 11].

Analytical Approach

The analytical strategy aimed to both summarize the overall safety profile and explore variations among individual studies. The primary objectives were as follows:

- *Determine the overall safety profile*. Combining data from both animal feeding trials and human observational studies allowed us to produce an aggregated assessment of the toxicological safety of GM crops.
- *Identify sources of variability*. Subgroup analyses were conducted based on factors such as the type of genetic modification (*e.g.*, pest resistance *versus* herbicide tolerance), geographical region (Nigeria/Africa *versus* Western studies) and funding source.
- *Evaluate the influence of methodological variations*. Sensitivity analyses assessed how differences in study design, sample size and quality affected the pooled findings. These analyses were essential for identifying potential confounders and biases in the safety data.

Qualitative Synthesis

In instances where quantitative pooling was not feasible — primarily due to extensive heterogeneity in study design or outcome measures — a qualitative (narrative) synthesis was conducted through thematic analysis [17, 37, 43]. This process involved categorizing findings based on toxicological endpoints and systematically comparing these across different research studies. By doing so, a comprehensive account of the toxicological profiles of genetically modified crops was developed. The narrative approach allowed for the consideration of contextual factors and subtle variations that may not be fully captured by statistical methods alone.

Data Management and Software Usage

To ensure the accuracy and reproducibility of the analysis, extracted data were systematically entered into a centralized database. Basic meta-analytical procedures were conducted using Review Manager (*RevMan*), while more complex analyses, including meta-regression and sensitivity testing, were performed with *STATA (version 16)*. These software tools were selected due to their proven effectiveness in managing multilayered datasets and performing advanced statistical analyses, thus ensuring that the final synthesis accurately reflects the underlying evidence. Detailed documentation of all analytical decisions was maintained to provide a clear audit trail, ensuring that the findings are both robust and replicable.

Ethical Considerations and Research Transparency

Given the contentious nature of GM crop research and the potential for commercial influence, ethical integrity was a central focus throughout the meta-research process [18, 33]. Each study included in this analysis was carefully reviewed to ensure compliance with ethical standards, particularly with respect to the treatment of animal subjects and the adherence of human observational studies to informed consent procedures. Adherence to ethical guidelines in the original studies was a prerequisite for inclusion, thereby ensuring that the meta-research was founded on ethically sound and scientifically rigorous evidence [39, 41, 50]. Detailed reporting of funding sources and any conflicts of interest was required, with studies funded by commercial interests scrutinized to minimize bias. This commitment to ethical practice and transparency is vital in maintaining the reliability of the research findings [26, 48].

The entire process — from literature search to data synthesis — was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and

Meta-Analyses (PRISMA) guidelines. Comprehensive documentation of search strategies, inclusion criteria and data extraction methods was maintained to ensure that the meta-research is both replicable and verifiable. This transparent approach underpins the scientific rigour of the study and contributes to its overall credibility [10, 11, 20, 44].

Results and Data Interpretation

Study Selection Summary

An initial search returned 200 articles from the selected databases. After screening titles and abstracts, 70 articles were retained for full-text review. During the full-text evaluation, 22 articles were excluded for the following reasons:

- *Insufficient Methodological Detail*. Several articles did not provide complete experimental protocols, sample sizes or statistical analyses. Articles lacking adequate methodological detail such as sample size or statistical analysis, were not considered.
- *Non-Peer-Reviewed or Grey Literature*. Articles that did not undergo strict peer review (*e.g.*, conference abstracts lacking full data, grey literature, editorials) were excluded.
- *Irrelevant Endpoints*. Studies not addressing the specified toxicological endpoints relevant to GM crops or that focused solely on agronomic performance were omitted.
- *Duplicate Publications*. In cases where the same study was reported in multiple articles, the most comprehensive version was selected.

Ultimately, 48 high-quality studies were included in the final meta-analysis. A summary of study selection is provided in table 3. The table provides a transparent account of the article selection process, which was conducted in accordance with PRISMA guidelines [13, 26, 48].

Analysis of Meta-Analysed Data in Percentages

The pooled quantitative data were further expressed as percentages for key toxicological endpoints to provide a clear summary of the evidence. Table 4 presents the meta-analyzed data summarized as percentages, indicating the proportion of studies that reported either the absence or the presence of adverse effects.

The data indicate that a substantial majority of studies support the safety of GM crops regarding acute toxicity. However, the chronic toxicity data show that 30 % of studies report minor adverse effects, indicating that cumulative exposure may be under-reported in shorter studies. Similarly, while most studies indicate low allergenic risk, the reported 20 % of studies with mild immune responses indicate the need for standardised testing protocols. Carcinogenic potential is generally not observed, although isolated reports in studies with multiple genetic modifications prompt additional mechanistic research [22, 34, 36, 47].

Table 3. Study selection summary

Stage	Number of articles	Notes
Initial retrieval	200	Articles retrieved from *PubMed*, *Web of Science*, *Scopus*, *AJOL*, etc.
Title/abstract screening	70	Articles that appeared relevant based on preliminary screening.
Full-text review	70	Detailed review against inclusion/exclusion criteria.
Excluded after full-text review	22	Reasons: insufficient methodological detail, non-peer-reviewed/grey literature, irrelevant endpoints, duplicates.
Final articles included	48	High-quality studies meeting all criteria, forming the basis of the meta-analysis.

Table 4. Meta-analysed data in percentages for key toxicological endpoints

Toxicological endpoint	Studies reporting no adverse effects, %	Studies reporting adverse effects, %	Comments
Acute toxicity	90	10	The majority of studies reported normal biochemical and haematological parameters.
Chronic toxicity	70	30	While most studies found no overt chronic effects, a subset reported subtle metabolic changes.
Allergenicity	80	20	Most research indicates low allergenic potential, with some evidence of mild to moderate responses.
Metabolic disturbances	85	15	Short-term studies showed minimal effects; long-term studies noted minor alterations in lipid profiles and enzyme activities.
Carcinogenic potential	95	5	Nearly all studies found no significant carcinogenic risk at standard exposure levels.

Evaluation of Mixed Outcomes

Certain toxicological endpoints exhibited mixed outcomes among the studies. For example, while the majority of studies indicated that GM crops exhibit low acute toxicity — with over 90 % of studies reporting normal biochemical and haematological parameters — approximately 30 % of studies on chronic toxicity reported minor metabolic disturbances. In the field of allergenicity, around 80 % of studies suggested minimal immune responses, yet 20 % documented mild to moderate immunological changes. Such inconsistencies necessitated a closer examination using meta-regression. By integrating study-level covariates such as duration of exposure and specific genetic constructs into the analysis, we were able to identify trends that may explain these mixed results. This detailed exploration of the data enables a more precise interpretation of the risks associated with GM crop consumption.

Publication Bias and Robustness

The visual inspection of the funnel plots, combined with statistical tests, provided assurance that studies reporting non-significant or adverse effects were not systematically underrepresented. This step was crucial in validating the overall integrity of the meta-analytical results. In addition, sensitivity analyses reinforced the robustness of the pooled estimates by confirming that the exclusion of any single study did not markedly alter the overall conclusions [17, 37, 43].

Integration of Experimental and Observational Data

A salient feature of the synthesis process was the integration of evidence from both controlled animal studies and observational research in human populations. This dual approach was vital for bridging the gap between laboratory findings and real-world observations. Animal feeding trials provided detailed mechanistic insights into acute toxic responses and subtle histopathological alterations, while observational studies contributed complementary data on long-term health outcomes in populations consuming GM crops. The fusion of these data sources enabled the construction of a more comprehensive risk profile, which serves to inform regulatory decision-making and public health policy in a more robust manner.

Advanced Analytical Techniques

Notwithstanding the considerable breadth of the existing literature, challenges persist in detecting low-incidence adverse effects and subtle metabolic disturbances. Traditional endpoints might not capture early molecular changes that precede clinical manifestations of toxicity.

Recognizing the limitations inherent in current methodologies, our analysis acknowledges issues such as small sample sizes and ethical constraints that limit the duration of animal studies. These challenges reduce statistical power and may impede the detection of low-incidence adverse effects [25, 35]. Moreover, the extrapolation of data from rodent models to human populations remains problematic due to intrinsic interspecies differences in metabolism and immune response.

Hence, it is recommended that future research incorporates advanced analytical methods such as metabolomic and epigenetic profiling. These approaches have demonstrated an ability to reveal early biomarkers of toxicity that may be missed by conventional assays, thereby augmenting the translational relevance of laboratory findings to human health outcomes [7, 30, 42].

Bridging Laboratory Findings with Clinical and Epidemiological Relevance

A significant challenge in toxicological research is translating findings from controlled laboratory settings to clinical and epidemiological contexts. Animal studies, which typically involve homogeneous rodent models under controlled conditions, provide important mechanistic details; however, their ability to represent human health outcomes is limited due to interspecies differences in metabolism, physiology and exposure conditions [20, 44]. As such, the integration of animal data with evidence derived from observational studies in human populations is essential [44].

This study combined data from controlled animal feeding trials with findings from epidemiological studies to form a more comprehensive assessment of toxicological safety. For example, while animal experiments offer detailed biochemical and histopathological profiles following exposure to GM crops, observational studies provide information on long-term health outcomes under real-world conditions. This dual approach enables a more balanced evaluation of potential risks associated with GM crop consumption [10, 11, 20, 44].

Advanced statistical techniques such as meta-regression, were used to examine the influence of laboratory-specific variables — such as the duration of exposure and controlled dosing regimens — on toxicological endpoints. By comparing these findings with epidemiological data, the analysis facilitated the development of translational models that better reflect human health outcomes. Furthermore, the incorporation of modern analytical methods, including metabolomic and epigenetic profiling, has the potential to identify early markers of toxicity that may not be detectable through conventional assessments. Such integration provides a mechanism for linking molecular-level changes observed in the laboratory with broader clinical observations in human populations [7, 30, 42].

Significance of the Findings

The findings of this systematic review and meta-analysis bear considerable importance for contemporary veterinary pharmacology, public health policy and agri-food biotechnology regulation. The comprehensive synthesis of data from both controlled animal feeding trials and human observational studies provides a structured and evidence-based clarification of the toxicological profile of GM

crops within the context of global and regional (notably African and Nigerian) agricultural consumption.

Foremost, the consistency of findings indicating normal acute toxicity parameters across more than 80 % of the included studies is reassuring, particularly given the global expansion of GM crop usage in animal feed. For veterinary practitioners and animal nutritionists, this suggests that short-term exposure to commonly used GM crops does not compromise physiological function or induce immediate systemic toxicity in animals. Moreover, this affirms the current veterinary dietary guidelines that integrate GM-derived ingredients as part of conventional livestock nutrition protocols.

However, the detection of minor metabolic and immunological changes in a subset of studies focusing on chronic exposure raises legitimate questions regarding long-term safety — especially in species with extended life cycles or cumulative dietary exposure. While these changes did not meet clinical thresholds of pathology in most cases, their recurrence suggests that subtle physiological perturbations may warrant further pharmacodynamic scrutiny. This finding is particularly relevant in the context of food-producing animals, where long-term health directly influences meat and dairy quality, reproductive success and economic viability.

Furthermore, the study's focus on African data, particularly from Nigeria, highlights an essential knowledge gap: the paucity of indigenous long-term studies on the health effects of GM crop consumption in tropical veterinary environments. This observation has direct implications for national food safety authorities such as the National Food, Drug, Administration and Control Agency (NAFDAC) and the National Biosafety Management Agency, as it underscores the necessity for locally contextualised evidence to support or recalibrate current biosafety frameworks.

On a broader scientific level, the application of robust statistical techniques — such as meta-regression and subgroup analysis — to quantify heterogeneity and identify sources of bias enhances the methodological quality of the findings. This lends credibility to the call for harmonization of experimental protocols and stricter adherence to OECD toxicity testing guidelines in future studies.

Ultimately, the review contributes significantly to the pharmacological discourse on GM crop safety, bridging the gap between laboratory toxicology and field-based risk assessment. The findings are expected to guide veterinarians, pharmacologists, regulatory authorities and policymakers towards more empirically grounded decisions on GM crop usage, not merely as a matter of agricultural convenience, but as a determinant of long-term animal health and food system integrity.

The table 5 summarizes the toxicological profiles of GM crops by endpoint. For acute toxicity, pest-resistant crops show virtually no adverse effects, while herbicide-tolerant varieties exhibit minor chronic disturbances. Allergenicity and metabolic changes remain low overall, and carcinogenic potential is negligible, although occasional signals in stacked modifications warrant further scrutiny. The findings indicate that GM crops generally present a safe profile in controlled conditions. However, the discrepancies in chronic toxicity and allergenicity call for additional targeted research to confirm these subtle effects. This critical evaluation underscores the need for enhanced, methodologically rigorous assessments to fully ascertain long-term safety.

Discussion and Analysis

The synthesis of data from 52 studies indicates that, while most evidence confirms the safety of GM crops under existing testing protocols, uncertainties persist in specific areas. Overall, studies focusing on GM crops modified for pest resistance and herbicide tolerance generally report low levels of acute toxicity. Nonetheless, chronic toxicity data reveal a mixed picture, with a minority of studies noting slight histopathological changes that may have long-term implications for health. These discrepancies appear to arise from methodological limitations such as small sample sizes and ethical restrictions that limit the duration and depth of animal experiments [5, 23, 25, 31, 35, 45].

Table 5. Aggregated findings on toxicological endpoints of GM crops

Toxicological endpoint	GM crop type	Key findings	Representative references
Acute toxicity	Pest-resistant (*Bt* crops)	No significant differences in serum enzymes, haematology or histopathology compared with conventional crops.	[15–16, 23, 45]
Chronic toxicity	Herbicide-tolerant (*cp4 epsps* crops)	Generally safe in short-term studies; minor metabolic disturbances (*e.g.*, slight liver enzyme alterations) observed in some long-term studies.	[6, 8, 31, 34–36, 47]
Allergenicity	Various (including stacked events)	Majority report low allergenic potential; however, sporadic mild to moderate immune responses noted, particularly with non-traditional protein sources or high-expression constructs.	[1, 4, 11, 19, 40, 46]
Metabolic disturbances	Herbicide-tolerant and biofortified crops	Most studies indicate negligible metabolic effects in acute settings; some long-term trials report subtle alterations in lipid profiles and enzyme activities, warranting further investigation.	[3, 8, 14, 25, 49, 51]
Carcinogenic potential	Stacked modifications	No inherent carcinogenicity at typical dietary exposures; isolated reports of neoplastic lesions in animal models require additional long-term surveillance and mechanistic studies.	[1, 15–16, 40, 51]

In animal feeding trials, numerous studies show that short-term exposure to GM crops does not cause significant biochemical or haematological disturbances. For instance, controlled experiments using rodent models consistently demonstrate that parameters such as liver enzymes and blood cell counts remain within normal ranges [15–16]. However, certain studies reveal that extended exposure may lead to subtle alterations in metabolic markers, suggesting that the cumulative effects of long-term ingestion might be underestimated in shorter experiments [34, 36, 47]. Such findings call for extended-duration studies with larger sample cohorts to ensure that low-incidence adverse effects are accurately captured.

Observational research in human populations provides a complementary perspective. Studies conducted in countries where GM crops are widely consumed, particularly in Nigeria, have detected occasional cases of mild allergic reactions and modest metabolic changes. S. Adeyeye and F. Idowu-Adebayo [1] and S. Gbashi et al. [19] reported that while most individuals do not experience significant adverse effects, a small proportion exhibit transient immunological responses after prolonged exposure to GM crops. U. Yahaya et al. [51], S. Adeyeye and F. Idowu-Adebayo [1] and O. Oladipo et al. [40] further note that although overt health effects are rare, subtle alterations in immune function may occur in specific subgroups. These observations underscore the need for continuous monitoring in real-world settings, where factors such as dietary habits and genetic variability are not controlled.

Meta-analytical studies provide a framework to consolidate these findings. J. Caradus [9], C. Dang et al. [12] and P. Krogh et al. [28] conducted a comprehensive analysis that revealed a strong consensus regarding the safety of GM crops in acute toxicity assessments. However, J. Caradus [9] and C. Dang et al. [12] also reported that approximately 30 % of studies on chronic toxicity indicate minor adverse changes, suggesting that further investigation is warranted. Similarly, the analysis of allergenicity across studies shows that while 80 % of research finds no significant immune response, 20 % document mild to moderate reactions, which may vary according to the source of the transgene and local dietary conditions [1, 4, 11, 19, 40, 46].

The aggregated data from our meta-research, expressed in percentage terms, reinforce these conclusions (table 4). Acute toxicity appears well-controlled, with 90 % of studies indicating no harmful effects. In contrast, chronic toxicity outcomes are more variable, with 30 % of studies identifying subtle metabolic disturbances. Similarly, while 80 % of studies report low allergenic potential, the remaining 20 % highlight instances of mild immunological responses. Carcinogenic potential is largely dismissed, with 95 % of studies confirming no significant risk, although a few studies suggest that stacked genetic modifications may require further examination [15–16, 25, 31, 35].

The heterogeneity among studies is a key challenge in synthesizing the literature. Variability in study design, sample size and the specific genetic modifications employed contributes to differences in outcomes [20]. For example, the type of genetic modification plays a critical role; crops engineered for pest resistance using *Bt* genes generally produce more consistent results than those modified for herbicide tolerance or biofortification. Additionally, regional factors — such as differing dietary practices and genetic backgrounds — appear to influence the expression of toxicological endpoints. In Nigeria, for instance, variations in local diets and environmental exposures may lead to outcomes that differ from those observed in Western settings [10, 20, 44].

Addressing heterogeneity involved several analytical strategies. Subgroup analyses were conducted to categorize studies according to the type of GM modification and geographical context. Meta-regression analyses were used to examine the effect of continuous variables such as study duration and dosage, on toxicological endpoints. Sensitivity analyses, including leave-one-out procedures, confirmed that no single study unduly influenced the overall results. Publication bias was also assessed using funnel plots and Egger's test, ensuring that the synthesis is robust and not skewed by underreporting of adverse findings [17, 37, 43].

The translational gap between laboratory findings and clinical or epidemiological outcomes remains a significant concern. Although animal experiments provide detailed mechanistic information, their controlled conditions cannot fully replicate the diversity of human exposures [7]. To bridge this gap, the study integrated data from both experimental and observational sources. This approach allowed for a more comprehensive risk evaluation, recognizing that laboratory results must be interpreted in the context of real-world data. Advanced analytical techniques such as metabolomic and epigenetic profiling, are proposed as essential tools to detect early markers of toxicity that traditional assays may miss. These techniques can serve as a link between molecular-level changes in controlled experiments and long-term health outcomes observed in epidemiological studies [7, 30, 42].

Furthermore, the integration of data from diverse study designs underscores the importance of multidisciplinary research in this field [42]. Veterinary pharmacologists, toxicologists and epidemiologists must work collaboratively to refine risk assessment models that reflect both experimental and observational evidence. This collaboration is particularly important in countries like Nigeria, where the consumption of GM crops may have different health implications compared to Western populations [1, 19, 40].

The combined discussion highlights that while the majority of evidence supports the safety of GM crops in terms of acute toxicity, uncertainties remain regarding chronic toxicity, allergenicity and subtle metabolic disturbances. These uncertainties, compounded by methodological limitations such as small sample sizes and limited

study duration, underscore the need for more extensive research. Future studies should extend observation periods, incorporate advanced analytical methods and ensure rigorous study designs to capture low-frequency adverse effects accurately. Such efforts are critical for developing a more reliable framework for evaluating the long-term safety of GM crops.

Broader Implications for Veterinary Pharmacology and Public Health

The implications of these findings extend beyond the laboratory, influencing both animal feed safety and human health. In the field of veterinary pharmacology, ensuring that livestock feed derived from GM crops is safe is essential for animal welfare and productivity. Although most studies report normal acute toxicity markers in animal feed, the detection of subtle metabolic disturbances in a fraction of studies suggests that long-term exposure might impact livestock health. Such effects could potentially lead to secondary health issues, compromising the quality and safety of animal-derived products and thereby affecting food security and public health in countries such as Nigeria [1, 19, 40].

For human health, while the acute safety of GM crops is well supported, the long-term impact of chronic exposure remains less certain [40, 46]. Observational studies indicate that even minor subclinical effects may accumulate over time, potentially leading to significant health risks. Epidemiological research in areas with high GM crop consumption, including parts of Nigeria, underscores the importance of monitoring health outcomes over extended periods. Integrating advanced omics technologies into future research will be essential for detecting early molecular alterations that precede clinical symptoms, thereby informing more accurate risk assessments [30, 35].

Given these considerations, there is a pressing need to implement a proactive ‘crop-vigilance’ system. This system, modelled on pharmacovigilance in human medicine, would enable continuous monitoring of GM crop safety in both animal feed and human food. Such a system would require the coordinated effort of regulatory bodies, research institutions and industry stakeholders to collect and analyze safety data in real time, ensuring that any emerging adverse effects are promptly addressed [3, 51]. In countries like Nigeria, where regulatory frameworks are still developing, establishing an independent and transparent Crop-vigilance mechanism would be particularly beneficial in maintaining public confidence and safeguarding health [19, 32].

Policy recommendations emerging from this review include the need for more stringent, independent regulatory oversight in the approval process for GM crops. Regulatory agencies must maintain clear boundaries from industry influence and all safety assessments should be subject to independent audits and transparent reporting. Standardized testing protocols that combine conventional toxicological endpoints with advanced analytical methods should be developed and adopted internationally. Such protocols would improve the sensitivity and reliability of risk assessments across diverse populations [17, 37, 43].

In addition, interdisciplinary research collaborations are essential. Veterinary pharmacologists, toxicologists and epidemiologists must jointly design studies that accurately reflect real-world conditions. Funding agencies and research institutions should prioritize projects that integrate experimental and observational data to produce a more comprehensive understanding of GM crop safety. Transparent communication of research findings to the public is also critical, as it helps to build trust and facilitate informed decision-making at both the individual and community levels [4, 11, 46].

Combined Results and Discussion (Extract)

The meta-research synthesis incorporated data from 48 high-quality studies published between 2017 and 2025. These studies, which included both experimental animal feeding trials and human observational research, covered a range of agronomic modifications such as pest resistance, herbicide tolerance and biofortification. Overall, the analysis indicated that GM crops generally exhibit a favourable toxicological profile under controlled conditions. Acute toxicity endpoints were largely reassuring, with over 90 % of studies reporting no adverse biochemical or haematological changes. However, data on chronic toxicity revealed that approximately 30 % of studies noted minor metabolic alterations, a finding that warrants further investigation over extended exposure periods.

Similarly, while most studies reported low allergenic potential — with 80 % of studies indicating no significant immune responses — a minority (20 %) documented mild to moderate allergenic reactions. These variations may be attributed to differences in the source of the transgenes, as well as to regional differences in dietary practices and genetic backgrounds. Moreover, metabolic disturbances were generally minimal in short-term studies, though about 15 % of studies observed subtle changes in lipid profiles and liver enzyme activities in long-term assessments. Carcinogenic potential was reported as negligible in nearly all studies, although rare instances of neoplastic lesions in animal models of stacked modifications suggest the need for ongoing surveillance.

The analysis further highlighted methodological challenges, including small sample sizes and the limitations of extrapolating animal data to human populations. The observed heterogeneity among studies, as measured by the I^2 statistic, necessitated subgroup and sensitivity analyses to ascertain the influence of various study-level factors on toxicological outcomes. These methodological issues underscore the necessity for more extensive, long-term studies that incorporate advanced analytical techniques such as metabolomics and epigenetics. By addressing these challenges, future research can refine risk assessment protocols and enhance the reliability of safety evaluations.

The integration of data from both controlled experiments and observational studies provides a comprehensive basis for evaluating the toxicological safety of GM crops. Despite the overall supportive evidence, uncertainties remain regarding chronic toxicity and allergenicity. Addressing these gaps requires further research that employs advanced analytical methods and adopts robust study designs. This systematic approach, conducted in accordance with PRISMA guidelines, ensures that the findings are both scientifically rigorous and ethically sound, thereby contributing to improved regulatory practices and enhanced public health assurance in countries with diverse dietary exposures such as Nigeria.

The synthesis of 52 studies reveals that the majority of research supports the safety of GM crops when assessed for acute toxicity, with most studies showing normal biochemical and haematological profiles. However, the analysis identifies inconsistencies in chronic toxicity data, with approximately 30 % of studies indicating subtle metabolic alterations. Similarly, while the majority of studies report low allergenic responses, a minority document mild immunological changes.

Moreover, this paper argues that safety depends on the type of modifications made. Insect-resistant and pesticide-tolerant modifications are highly associated with safety concerns than any other type, like biofortified modification.

These findings are influenced by limitations such as small sample sizes, differences in experimental protocols and constraints imposed by ethical standards in animal research. The current evaluation underscores the need for extended-duration studies with larger populations to better capture infrequent or subtle adverse effects. It is recommended that future research incorporate advanced methodologies, including metabolomic and epigenetic analyses, to detect early signs of toxicity not observable through conventional endpoints. Additionally, there is a pressing requirement for studies that merge controlled laboratory findings with long-term epidemiological data to provide a more complete risk assessment for both animal and human health.

Efforts should be made to standardize experimental protocols across different research settings to reduce variability and improve comparability of results. Enhanced transparency in funding and methodology is essential to minimize bias. Future research must also explore the effects of combined genetic modifications, particularly in countries where GM crop consumption is high. Such measures will contribute to a more robust framework for assessing the long-term safety of GM crops and will inform regulatory practices, ensuring that both public and animal health are adequately protected.

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Оцінка безпеки генетично модифікованих культур: результати токсикологічних метадосліджень

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Цей метааналіз оцінював безпеку генетично модифікованих (ГМ) культур через зіставлення результатів контрольованих випробувань годівлі тварин та обсерваційних досліджень на людях, опублікованих у 2017–2025 рр. Дослідження подає критичну оцінку гострої та хронічної токсичності, алергенності, метаболічних порушень та канцерогенних кінцевих точок, зосереджуючись на дослідженнях з Нігерії, Африки та західних регіонів (Європи та США). Для кількісної оцінки гетерогенності та оцінки надійності доказів використані передові статистичні методи, зокрема моделювання випадкових ефектів, аналіз підгруп та метарегресія. Результати представлені у відсотках, щоб полегшити чітке узагальнення профілю безпеки ГМ-культур. Аналіз показує, що генетично модифіковані продукти не є гостро токсичними, тоді як деякі дослідження повідомляли про незначні метаболічні та імунологічні зміни за хронічного або тривалого впливу. Розбіжності у результатах хронічної токсичності були суттєво зумовлені варіаціями в експериментальній моделі, дизайні дослідження та розмірі вибірки. Загальні дані підтверджують загальну безпеку ГМ-культур за чинними протоколами випробувань; однак не до кінця визначені довгострокові наслідки. У статті зроблено висновок, що безпека залежить від типу внесених модифікацій. Стійкі до комах та пестицидів модифікації більше пов'язані з проблемами безпеки, ніж будь-який інший тип — як, наприклад, біофортифікована модифікація.

Ключові слова: генетично модифіковані культури, токсикологічна безпека, метадослідження, контроль за сільськогосподарськими культурами, оцінка ризиків

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Experimental works

Effect of *Sesbania sesban* fodder and Napier grass hay mixture diets on rumen metabolites of West African dwarf growing goats

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AIA: Conceptualization; Project administration; Methodology; Investigation; Formal analysis; Visualisation; Writing — original draft, review & editing.
AAB: Methodology; Formal analysis; Writing — original draft, review & editing.
OOO: Writing — original draft.
AOA: Writing — original draft, review & editing.
OAB: Investigation.
OO: Investigation.
MTB: Methodology; Investigation.
AOC: Investigation.
AAT: Investigation.
ORF: Investigation.

Declaration of Conflict of Interests:
No conflict of interest is declared.

Ethical approval:
The Institutional Animal Care and Use Committee of the Oyo State College of Agriculture and Technology (Igboora, Oyo State, Nigeria) approved all experimental procedures. The study involve no endangered or protected animal species: A veterinarian helped in the blood sample collection and manually restrained the animals; no tranquilizers or short-acting anaesthetics used. Blood samples were collected using appropriate equipment. The Oyo State College of Agriculture and Technology, Igboora Animal Care and Use Committee approved the sampling procedures and number of animals sampled as part of obtaining the study permit.

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Meeting the nutritional requirements of ruminants during the dry season is challenging due to low-quality forage, which demands the use of browse legume fodder with grass species to provide an efficient rumen environment for microbes to flourish and ferment feeds, thereby increasing animal productivity. The effect of *Sesbania sesban* (SS) forage with Napier grass (NG) hay mixture diet on rumen metabolites of West African dwarf (WAD) bucks was evaluated after a 90-day feeding trial. A total of sixteen (16) growing WAD bucks between 6–9 months of age with an average body weight of 6.00–10.00kg were randomly allocated to four treatments with four bucks per treatment and two bucks per replicate in a completely randomized design, and were fed daily with varied experimental diets (T_1 = 100 % NG hay (100NG); T_2 = 25 % NG + 75 % SS hay (25NG75SS); T_3 = 50 % NG + 50 % SS hay (50NG50SS); T_4 = 100 % SS hay (100SS)) with a 500 g concentrate diet at 3 % body weight of individual animals. Significant ($P<0.05$) differences were observed in rumen parameters across the dietary treatments. The pH value ranges from 7.40 (bucks fed T_1 diet) to 8.56 (bucks fed T_4 diet). Highest acetic acid value (13.80 mmole/100 ml), propionic acid (13.60 mmole/100 ml), butyric acid value (12.57 mmole/100 ml) and total volatile fatty acids (194.64 mmole/100 ml) was observed in bucks fed T_3 diet while the least acetic acid (10.19 mmole/100 ml), propionic acid (9.72 mmole/100 ml), butyric acid (9.27 mmole/100 ml) and TVFA (144.54 mmole/100 ml) was recorded in bucks fed T_2 diet respectively. The highest NH_3-N (0.86 %) was observed in bucks fed T_3 diet, while the lowest value for NH3-N (0.70 %) was noted for bucks fed T_1 diet. It can be concluded that the combination of Napier grass with selected browse fodders at 50 % can enhance rumen metabolites in WAD bucks.

Key words: tropical browse fodders, Napier grass, WAD bucks, rumen ecology

Introduction

The changing climatic conditions in the past years have resulted in persistent droughts, heat waves and shortages in animal feed [6]. This has severely affected ruminant animal production, leading to a dire need to address feed shortages, particularly in small-scale

farming systems. One of the most challenging factors in achieving this is the scarcity of feed, both in quantity and quality, especially during the dry periods of the year, thus resulting in animals' low productivity and even death [7]. Feed accounts for 60–70 % of the total cost of livestock production, and an inadequacy in quality and quantity could lead to a situation of low nutritional status, poor weight gain, poor reproductive ability, poor production, poor health condition and poor conversion ratio [5]. The rumen temperature and pH are critical phenomena that depend on the fermentation of ingested feeds in the rumen. R. Mohammed and A. Chaundry (2008) indicated that rumen fermentation products, such as volatile fatty acids, are essential nutrients to meet the demand of rumen microbes and the animal's body build-up [10]. This study was carried out to evaluate rumen parameters of growing West African Dwarf bucks fed varying proportions of *Sesbania sesban* fodder and Napier grass hay mixture.

Materials and Methods

Experimental site and animals

The experiment was conducted at the Sheep and Goat Unit, Teaching and Research Farm, Oyo State College of Agriculture and Technology, Igboora. Sixteen (16) growing West African dwarf bucks weighing 6.00–10.00 kg and 6–9 months of age were used. The animals were allowed to acclimatise for two weeks and treated before the commencement of the experiment. Fresh and clean water was also made available throughout the experiment.

Harvesting and processing of experimental diets

Napier grass and *Sesbania sesban* forage were harvested around the college farm, chopped at 3 cm long, wilted for 2–3 hours in the sun and air dried under shade for 4–5 days to prevent bleaching and loss of nutrients, bailed and stored for the experiment.

Experimental layout, design and feeding method

The animals were allocated by weight into four treatments of four bucks per treatment, and two bucks served as a replicate in a completely randomised design (CRD). The *Sesbania sesban* forage and Napier grass harvested and air-dried for 4 to 5 days were mixed in varying proportions as the experimental diets (ED) and fed to each buck per day at 3 % body weight. The *Sesbania sesban* and Napier grass air-dried fodders combinations were thoroughly mixed to eliminate/minimise selection by the animal. The bucks were offered their respective experimental feeds (Forage hay) at 8.00 am. Each buck was offered 500 g of concentrate per buck per day at 2.00 pm, and 3 litres of fresh and clean water were also supplied daily.

Table 1. Composition of formulated low-cost concentrate for experimental West African Dwarf growing bucks

Ingredients	Level, %
Palm kernel cake	60.00
Wheat offal	20.00
Corn bran	9.75
Groundnut cake	8.00
Bone meal	2.00
Salt	0.25
Total	100

The compared experimental diets (ED) were: T_1 = 100 % NG hay (100NG); T_2 = 25 % NG + 75 % SS hay (25NG75SS); T_3 = 50 % NG + 50 % SS hay (50NG50SS); T_4 = 100 % SS hay (100SS) with a 500 g concentrate diet at 3 % body weight of individual animals, and their proximate composition is shown in table 1. Each group of animals was assigned to an experimental diet.

Data Collection

Rumen samples were collected six hours post-feeding from the animals 90 days of supplementation using a suction tube. The samples were immediately measured for pH using a portable pH meter (*Universal pH Test Kit-Digital pH Meter*®). They were thereafter filtered with a four-layer cheesecloth, and subsamples were divided into two portions. The first portion was used to analysed for ammonia nitrogen (NH_3-N) using AOAC method [2], while the second portion was used to estimated total volatile fatty acids (VFAs) and the proportions of acetate (C_2), propionate (C_3), and butyric acid (C_4) as previously described by [9]. Briefly, the samples were centrifuged at 3,000x g for 10 minutes; allowed to settle, and then, decanted. The decant was titrated with 0.1 M of sodium hydroxide (4/1000 gml^{-1} H_2O) solution, and 2–3 drops of phenolphthalein (1/1000 gml^{-1} ethanol) were used as an indicator. Determination of the various fractions were as follows:

$$\text{Acetic acid} = \frac{(\textit{Titre value} \times 0.1 \times 0.06 \times 100)}{5};$$

$$\text{Propionic acid} = \frac{(\textit{Titre value} \times 0.1 \times 0.04 \times 100)}{5};$$

$$\text{Butyric acid} = \frac{(\textit{Titre value} \times 0.1 \times 0.006 \times 100)}{5};$$

$$\text{Total volatile fatty acids} = \frac{(\textit{Titre value} \times 0.1 \times 0.09 \times 100)}{5}.$$

Statistical analysis

Data were subjected to a one-way Analysis of Variance (ANOVA) procedure of SAS version 9.4 [14]. The difference among treatment means with $P<0.05$ were assessed using *Duncan's Multiple Range Test* (DMRT) [4].

Results and Discussion

The proximate composition and fibre fractions of the varied mixture of the experimental diets were presented in table 2. The diet T_1 (100 % NG) has the highest dry matter (DM) content, crude fibre (CF), Ash, Nitrogen free extract (NFE), Neutral detergent fibre (NDF), Acid detergent fibre (ADF), Acid detergent lignin (ADL) value of

87.63 %, 29.57 %, 11.95 %, 36.84 %, 51.50 %, 40.09 %, and 14.03 %, respectively. In comparison, the lowest CF, NDF and ADL value of 19.01 %, 41.65 % and 10.27 % respectively was recorded in diet T_4 (100SS) while the lowest DM and NFE value of 71.59 % and 23.86 % respectively were observed in diet T_3 (NG50SS50). It was observed that crude protein in the forage diets enhances microbial multiplication, which determines the extent of the experimental diet's fermentation.

Significant differences (P<0.05) were in all the parameters across the dietary treatments. The result of the rumen metabolites of WAD bucks fed air-dried *Sesbania sesban* and Napier grass hay mixture with concentrate diets (table 3) revealed that more dry matter degradation was still possible in the rumen as the highest pH of 8.56 was observed in bucks fed T_4 diet that contained solely (100SS+500 g concentrate) while the least pH of 7.40 was recorded in rumen metabolites of bucks fed T_1 diet containing only Napier grass (100NG+500g concentrate diet) (P<0.05). The observed rumen pH values of range 7.40–8.56 in this current study were higher than the reported values of 6.00–7.20 as the suitable pH to facilitate optimum growth and activities of rumen microbes [12], and also higher than 5.92–6.60 reported by M. Okoruwa et al. [11] for rumen metabolites of WAD sheep fed Ficus foliage with differently processed breadfruit meals. The higher rumen pH observed in bucks on experimental diets could be due to less fermentable feed components that the animals consumed. Browse legume fodders and herbs have anti-microbial properties and can modify the rumen to improve energy or protein use [8].

An increase in volatile fatty acids and their proportions as caused by browse fodder supplementation in this study indicates the efficiency of nutrient digestion. However, buck fed diet T_3 recorded the highest acetic acid value of 13.80 mmole/100ml while the lowest acetic value of 10.19 mmole/100mlwas observed in buck fed diet T_2 (fig. 1). The obtained acetic acid values of acetic acid in this study were lower to 42.03–46.65 mmole/100 ml reported by M. Okoruwa et al. [11], which due to the difference in the varying levels of browse fodder in the diets. The lactic acid values recorded from this experiment were 12.68–17.16 mmole/100 ml and were lower compared to 5.00–21.00 mol/100 ml reported by B. Suárez et al. [15].

Table 2. Proximate composition of air-dried *Sesbania sesban* and Napier grass hay mixture and concentrate diets fed to bucks

Parameters, %	T_1 100NG	T_2 NG25SS75	T_3 NG50SS50	T_4 100SS	Concentrate
Dry matter	87.63	73.52	71.59	81.12	94.48
Crude protein	8.12	15.28	15.61	18.67	17.57
Crude fibre	29.57	20.34	20.12	19.01	8.75
Ether extract	1.15	2.28	2.33	2.37	10.39
Ash	11.95	7.06	9.67	10.69	5.85
Nitrogen free extract	36.84	28.64	23.86	30.38	50.21
Neutral detergent fibre	51.50	45.18	48.16	41.65	30.34
Acid detergent fibre	40.09	30.17	31.78	32.07	16.05
Acid detergent lignin	14.03	10.60	11.03	10.27	5.65
Calculated metabolizable energy, Kcal/Kg	1702.33	1676.15	1615.19	1963.15	3282.45

Table 3. Rumen metabolites of WAD bucks fed air-dried *Sesbania sesban* and Napier grass hay mixture with concentrate diets

Parameters	T_1	T_2	T_3	T_4	SEM (±)
pH	7.40^{cd}	7.80^{bc}	8.20^{ab}	8.56^{a}	0.22
Acetic acid, mmole/100 ml	12.25^{bc}	10.19^{cd}	13.80^{a}	13.28^{b}	0.69
Propionic acid, mmole/100 ml	11.69^{bc}	9.72^{cd}	13.16^{a}	12.67^{b}	0.66
Butyric acid, mmole/100 ml	11.17^{bc}	9.29^{cd}	12.57^{a}	12.10^{b}	0.63
Valeric acid, mmole/100 ml	11.11^{bc}	9.25^{cd}	12.51^{a}	12.05^{b}	0.62
Lactic acid, mmole/100 ml	15.24^{c}	12.68^{d}	17.16^{a}	16.53^{b}	0.86
Total volatile fatty acids, mmole/100 ml	173.74^{bc}	144.54^{cd}	194.64^{a}	188.34^{b}	9.66
Ammonia nitrogen (NH_3-N), %	0.70^{d}	0.72^{bc}	0.86^{a}	0.77^{b}	0.03

Note. ^{abcd} means on the same row with different superscript are significantly different (P<0.05).

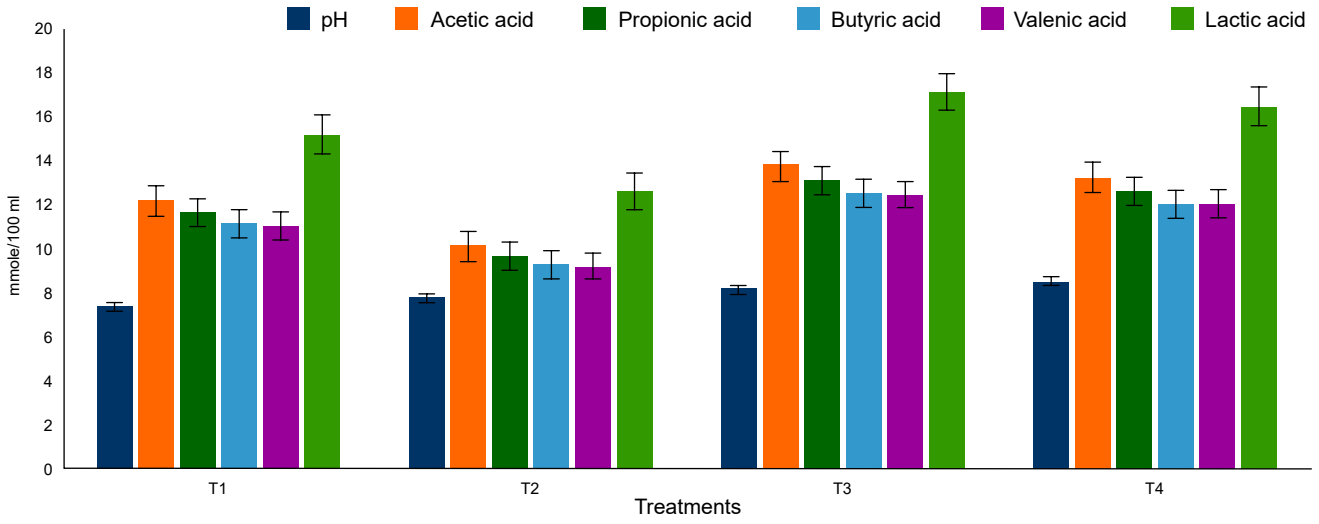

Fig. 1. Rumen metabolites of WAD bucks fed air-dried *Sesbania sesban* and Napier grass hay mixture with concentrate diets

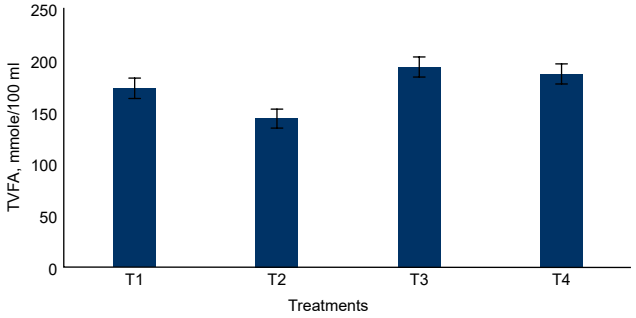

Fig. 2. Total volatile fatty acids in the rumen of WAD bucks fed air-dried *Sesbania sesban* and Napier grass hay mixture with concentrate diets

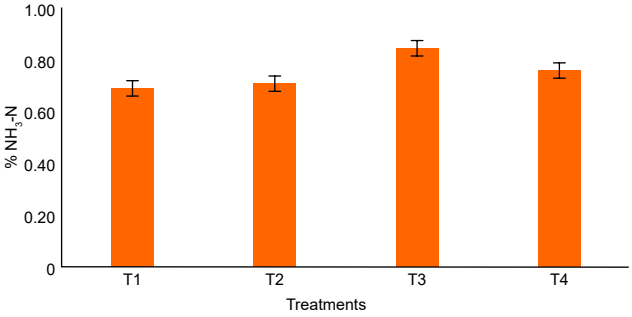

Fig. 3. Ammonia-Nitrogen in the rumen of WAD bucks fed air-dried *Sesbania sesban* and Napier grass hay mixture with concentrate diets

Butyric acid values recorded in this study ranges between 9.29–12.57 mmole/100 ml, which are within the range of 8.80–12.47 mmol/100 ml reported by K. Adebayo et al. [1]. The propionic acid values recorded from this study were within the range of 9.27–13.16 mmole/100 ml and were observed to lower than 21.6–28.8 mol/100 ml reported by B. Suárez et al. [15]. However, the high proportion of propionic and butyric acids was recorded in bucks fed diet T_3 revealed that equal proportion of air-dried *Sesbania sesban* and Napier grass hay mixture has a great and best effect on the rumen fermentation of the diets by microbial activity as well as good nutrient utilisation to yield energy [11].

The highest TVFA value of 194.64 mmole/100 ml was recorded in the buck fed diet T_3 that contained NC50SS50+500 g concentrate while the least value of 144.54 mmole/100 ml was noted in the buck fed T_2 diet that contained NG25SS75+500g concentrate (fig. 2). However, the observed TVFA values were lower than 66.00–72.00 mmol/litre recorded by M. Okoruwa et al. [11] who fed WAD sheep with differently processed breadfruit meals and Ficus foliage.

The highest NH3-N value of 0.86 % was recorded in the buck fed diet T_3 while the lowest value of 0.70 % was noted in the buck fed T_1 diet, these values fell within the normal range (0.5–2.5 %) of optimum ammonia level for growth and microbial activity [13]. Furthermore, the obtained rumen ammonia levels were within the normal range of 0–13 % as reported by K. Yusuf et al. [16].

The study revealed that feeding varying levels of Napier grass (*Pennisetum purpureum*) hay supplemented with browse fodder increase in nutrient intake, especially CP intake, posed no adverse effects on rumen ecology of the animals and enhanced rumen fermentation in WAD growing bucks. Hence, farmers can incorporate *S. sesban* fodder hay up to 50 % inclusion levels in the diets of their goats to help alleviate the challenge of feed availability all year round.

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Вплив кормої суміші *Sesbania sesban* та сінної суміші зі слонової трави на метаболіти рубця молодняку західноафриканських карликових кіз

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Задовольнити потреби жуйних тварин у поживних речовинах в сезон посухи складно через низьку якість кормів. Тому виникає потреба доповнити пасовищні раціони травами, щоб забезпечити оптимальне середовище в рубці для розвитку мікробіому та ферментації кормів, що підвищує продуктивність тварин. Оцінювали вплив корму *Sesbania sesban* (SS) з сінною сумішшю слонової трави (NG) на метаболіти рубця самців західноафриканської карликової кози після 90-денного згодовування. Шістнадцять (16) молодих козенят віком від 6 до 9 місяців із середньою масою тіла 6,00–10,00 кг випадковим чином розподілили на чотири групи по четверо в групі та двоє як повторність за повністю рандомізованим дизайном. Тварин щодня годували різноманітними експериментальними раціонами: T_1 = 100 % сіна слонової трави (100NG); T_2 = 25 % слонової трави + 75 % сіна *Sesbania sesban* (25NG75SS); T_3 = 50 % слонової трави + 50 % *Sesbania sesban* (50NG50SS); T_4 = 100 % *Sesbania sesban* (100SS), з розрахунку 500 г концентрованого раціону на 3 % маси тіла тварини. Спостерігали вірогідні ($P<0,05$) відмінності в параметрах рубця за різних варіантів раціону. Значення pH коливається від 7,40 (раціон T_1) до 8,56 (раціон T_4). Найвище значення оцтової кислоти (13,80 ммоль/100 мл), пропіонової кислоти (13,60 ммоль/100 мл), масляної кислоти (12,57 ммоль/100 мл) та летких жирних кислот (194,64 ммоль/100 мл) спостерігали у цапків, яких годували раціоном T_3, тоді як найменше оцтової кислоти (10,19 ммоль/100 мл), пропіонової кислоти (9,72 ммоль/100 мл), масляної кислоти (9,27 ммоль/100 мл) та ЛЖК (144,54 ммоль/100 мл) було зафіксовано у самців, яких годували раціоном T_2, відповідно. Найвищий вміст NH_3-N (0,86 %) спостерігали у тварин на раціоні T_3, тоді як найнижче значення NH_3-N (0,70 %) виявили у цапків, яких годували раціоном T_1. Можна зробити висновок, що поєднання слонової трави з вибраними кормами для худоби у кількості 50 % може підвищити рівень метаболітів у рубці самців західноафриканської карликової кози.

Ключові слова: тропічні пасовищні корми, слонова трава, західноафриканська карликова коза, екологія рубця*

Adebisi IA, Ajibike AB, Okunlola OO, Adeniyi OA, Oloko AB, Oladepo O, Mustapha TB, Akanmu OC, Adesope AT, Olayinka RF. Effect of *Sesbania sesban* fodder and Napier grass hay mixture diets on rumen metabolites of West African dwarf growing goats. *Biol Tvarin*. 2025; 27 (3): 42–46. DOI: 10.15407/animbiol27.03.042.

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Hematological parameters in dogs at the early stages of babesiosis in the Dnipro region of Ukraine

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NPAY: Conceptualization; Methodology; Investigation; Data curation; Formal analysis; Writing — original draft; Visualization.
UGO: Supervision; Project administration; Writing — review & editing; Resources; Validation.

Declaration of Conflict of Interests:
None to declare.

Ethical approval:
The experiments were carried out in accordance with the protocols approved by the local Ethics Committee (Peredovyi Veterinary Complex, Dnipro, Ukraine) and with the main provisions of the Law of Ukraine "On the Protection of Animals Against Cruelty" (no. 3447-IV from 21.02.2006) and "The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1986).

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None.

Canine babesiosis is a vector-borne disease caused by protozoan parasites of the genus *Babesia*, primarily transmitted by ixodid ticks. The disease is widespread globally, including across most regions of Ukraine. *Babesia canis* is the most prevalent species affecting dogs, with increasing clinical relevance in both domestic and wild carnivores. Infection leads to intravascular hemolysis, hypoxic tissue injury, and multi-organ dysfunction. Despite advances in diagnostics, early hematological changes in the initial stages of infection remain underexplored. This study aims to compare the hematological parameters of dogs in the early stage of babesiosis with healthy controls to identify reliable indicators for early diagnosis and disease monitoring. This study was conducted on 13 clinical cases of *Babesia canis* infection in dogs, with data collected directly from animals presented at the Peredovyi Veterinary Complex (Dnipro, Ukraine) between February and April 2024. Blood smears confirmed parasitemia, and complete blood counts were performed using the *MicroCC-20 Plus* automated analyzer. Results showed a statistically significant decrease in red blood cell count ($3.59\pm0.37\times10^6$/μL) and hemoglobin (83.42±2.96 g/L) in infected dogs compared to controls ($6.36\pm0.17\times10^6$/μL and 158.58±5.87 g/L, respectively). Hematocrit values were also markedly reduced (22.54±1.45 % *vs.* 43.51±2.39 %; $P<0.0001$). Significant thrombocytopenia ($38.23\pm6.20\times10^3$/μL) and leukopenia ($7.08\pm0.60\times10^9$/L) were observed, with a concurrent neutrophilic shift and lymphopenia. Mean corpuscular volume (MCV) was significantly lower in the infected group (63.45±2.49 fL), while other red cell indices (MCHC, RDW) and total protein levels showed no statistically significant differences. These findings highlight the pronounced hematological disturbances associated with early-stage *Babesia canis* infection. The changes in erythrocyte count, hemoglobin concentration, hematocrit, and platelet levels may serve as early diagnostic markers. Further research is needed to refine hematological profiling for improved clinical decision-making and timely intervention in canine babesiosis.

Key words: dogs, blood, hematological parameters, *Babesia canis*, erythrocyte, leukocyte, anaemia, differential diagnostics

Introduction

Babesiosis is an infectious natural focal disease caused by unicellular parasites of the genus *Babesia*, which belong to the class of sporozoites (*Apicomplexa*) [28]. These parasites are intracellular hemoparasites that infect erythrocytes of mammals, including dogs, cats, wild carnivores and, less commonly, humans [6, 43, 50]. Infection usually occurs through the bites of *Ixodes* ticks (family *Ixodidae*), which are natural vectors of the pathogen [13].

The geographical distribution of babesiosis covers most temperate and tropical regions of the world, including the territories of Ukraine, where the disease is endemic [41]. In recent decades, there has been an increase in the incidence of the disease in animals due to changes in climatic conditions, migration of vectors and expansion of tick ranges [15, 16, 56]. Large-scale monitoring studies have shown the spread of *Dermacentor reticulatus* in Central and Eastern Europe, including Germany, Poland and the Czech Republic, which significantly increases the risk of canine infection [13, 17, 18, 42, 47, 49, 57]. In southeastern and northeastern Europe, babesiosis is considered an emerging and re-emerging disease, which emphasizes its growing epidemiological significance [3, 4, 20].

The pathogenesis of babesiosis is associated with the penetration of parasites into erythrocytes, where they undergo several cycles of division, causing their destruction (hemolysis) [26; 27; 31; 59]. This leads to the development of hemolytic anemia, accompanied by fever, jaundice, weakness, and multiple organ failure in severe cases [8, 35]. Mortality in virulent forms of canine babesiosis is often linked to consumptive coagulopathy and systemic inflammatory responses [5, 26]. In addition, alterations in hemostasis and coagulation markers have been confirmed as important prognostic indicators of disease progression [7, 35].

The immune response plays a decisive role in the clinical course of babesiosis. Cytokine-mediated inflammation and immune exhaustion mechanisms, similar to those observed in malaria, are implicated in the severity of the disease [36, 58]. Moreover, reinfections occur frequently, since post-infectious immunity is short-lived and often non-sterile [12].

The clinical manifestations of babesiosis are highly variable and depend on the type of parasite, the degree of parasitemia, the immune status of the animal, and the presence of concomitant infections (*e.g.*, *Ehrlichia canis*) [14, 21, 22, 50]. Thus, two main species of *Babesia* are most commonly found in dogs: *B. canis* and *B. gibsoni*, which differ in size, pathogenesis, and sensitivity to therapy [1, 6]. Molecular epidemiological studies confirm a high genetic diversity of *B. canis*, which may complicate diagnosis and therapy [29]. Global distribution studies also indicate regional differences in species prevalence and tick associations [9].

Diagnosis of babesiosis includes both traditional methods and modern molecular tests. Microscopic examination of blood smears is a rapid and accessible method, but has low sensitivity at low parasitemia levels [33]. Enzyme-linked immunosorbent assays (ELISA) and immunofluorescence tests can detect antibodies, but they do not always distinguish between active and past infections [24, 34]. The most accurate method is the polymerase chain reaction (PCR), which can detect parasite DNA and identify its species [1, 10, 16]. Novel PCR-based assays have been successfully used for rapid field diagnostics and species differentiation [32, 34].

Treatment of babesiosis is based on the use of antiparasitic drugs, among which imidocarb dipropionate and atovaquone are the most effective [2, 12]. However, the choice of therapy depends on the type of pathogen and the severity of the disease. Supportive therapy is often essential to manage anemia, oxidative stress, and systemic inflammation [25, 53]. Clinical outcomes are strongly associated with early therapeutic intervention and the ability to correct hematological abnormalities [46, 51].

Hematological changes are of particular diagnostic and prognostic importance. Numerous studies have demonstrated alterations in erythrocytic and platelet indices during infection, which can serve as early diagnostic markers [25, 55, 61]. Severe thrombocytopenia and coagulation disorders are especially characteristic in acute babesiosis [19, 31]. Additionally, microcirculatory disturbances and cardiovascular complications are increasingly recognized as causes of mortality in infected dogs [8, 60].

Thus, babesiosis in animals and humans is an important problem of veterinary and medical parasitology, requiring an integrated approach to ensure effective control and treatment. The study of hematological changes in the early stages of babesiosis is of particular relevance, as it allows timely diagnosis and the identification of prognostic markers of disease severity.

The aim of this study is to perform a comparative analysis of hematological parameters in dogs in the early stages of babesiosis to identify diagnostically significant changes.

Materials and Methods

This study was conducted on 13 dogs presenting with the first signs of *Babesia canis* infection at the Peredovyi Veterinary Complex (Dnipro, Ukraine) between February and April 2024. All clinical and laboratory data were collected prospectively by the authors, including blood sampling, smear preparation, and hematological analysis. The study included dogs of various breeds, ages, and sexes. Although these factors may influence hematological parameters, the primary focus was on early-stage *Babesia canis* infection. Statistical analysis was conducted to compare infected animals with healthy controls, minimizing potential confounding effects of breed, age, and sex. Dogs with diagnosed concomitant diseases or with unreliable laboratory data were excluded from the study to ensure data reliability.

Detection of *Babesia canis* parasites in erythrocytes was carried out using thin blood smears stained with fast-acting dyes *LEUCODIF 200* (*Erba Lachema*, Czech Republic), with subsequent examination under 100x magnification using an optical microscope *Leica DM4* (Germany) (fig. 1).

The stage of babesiosis in dogs was determined based on a combination of clinical signs and laboratory parameters. The early stage included animals that showed mild lethargy, loss of appetite, moderate fever, pale or icteric mucous membranes, sometimes dark urine, but without pronounced anemia or intoxication. Laboratory tests at the early stage revealed 1 infected

erythrocyte per 10–15 fields of view in the study of blood smears, confirming a low level of parasitemia. Only the early stage of the disease was assessed in this study; therefore, differentiation between intermediate and late stages was not performed.

For hematological studies, blood was taken from the cephalic or subcutaneous vein into a tube with EDTA. After that, parameters such as the number of erythrocytes, hemoglobin level, leukocytes, platelets, mean hemoglobin concentration in erythrocytes, erythrocyte distribution width, total protein and hematocrit were analyzed. The analysis of these parameters was carried out using an automatic hematological analyzer *MicroCC-20 Plus* (*HTI*, USA). Quantitative assessment of segmented neutrophils and lymphocytes was carried out by microscopic counting on stained blood smears.

Animal handling complied with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the Regulations on the Use of Animals in Biomedical Research. All procedures were performed in accordance with ethical standards for working with experimental animals and were approved by the local Ethics Committee.

A variety of statistical methods were used to study hemolytic anemia in dogs caused by the protozoan parasite *Babesia canis*. Descriptive statistics were used to determine the mean, median, mode, standard deviation, and variance of the indicators in the control group and the group of animals with the initial stage of the disease. The *t*-test was used to compare the mean values. Additionally, analysis of variance (ANOVA) was used to compare the mean values in the groups.

Results and Discussion

Key hematological parameters were analyzed in 13 clinically healthy dogs (control group) and 13 dogs showing signs of the initial stage of babesiosis.

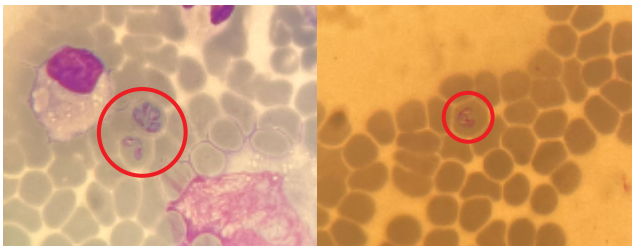

Fig. 1. Blood smear of a dog with *Babesia canis* detected (Leucodiff stain, ×100 oil immersion, NA 1.25). Intraerythrocytic forms of the parasite are visible (highlighted with a red circle)

The average RBC count in the control group (fig. 2) was 6.36±0.17×10^6/μL, whereas in the infected dogs, the count decreased significantly to 3.59±0.37×10^6/μL (P<0.0001; F=0.0107). This substantial reduction indicates a pronounced anemia likely caused by parasite-induced hemolysis.

Similarly, hemoglobin levels in the control dogs averaged 158.58±5.87 g/L, while in the infected group, HGB dropped markedly to 83.42±2.96 g/L (P<0.0001; F=0.0250). The decline reflects impaired oxygen transport capacity and suggests severe erythrocyte destruction during the course of infection. A parallel trend was observed in hematocrit values. Control animals showed a mean HCT of 43.51±2.39 %, which fell dramatically to 22.54±1.45 % in the infected group (P<0.0001; F=0.0955). The significant differences in all three parameters between healthy and infected dogs underscore the profound hematological disruption caused by *Babesia* infection prior to any therapeutic intervention.

The mean total protein level (fig. 3) in the control group was 67.42±3.36 g/L. In contrast, infected dogs exhibited a slightly lower mean value of 65.59±2.26 g/L.

Although the reduction was not statistically significant (P=0.1300; F=0.1845), the trend suggests a possible mild disturbance in protein metabolism or plasma volume shifts associated with the acute phase of infection.

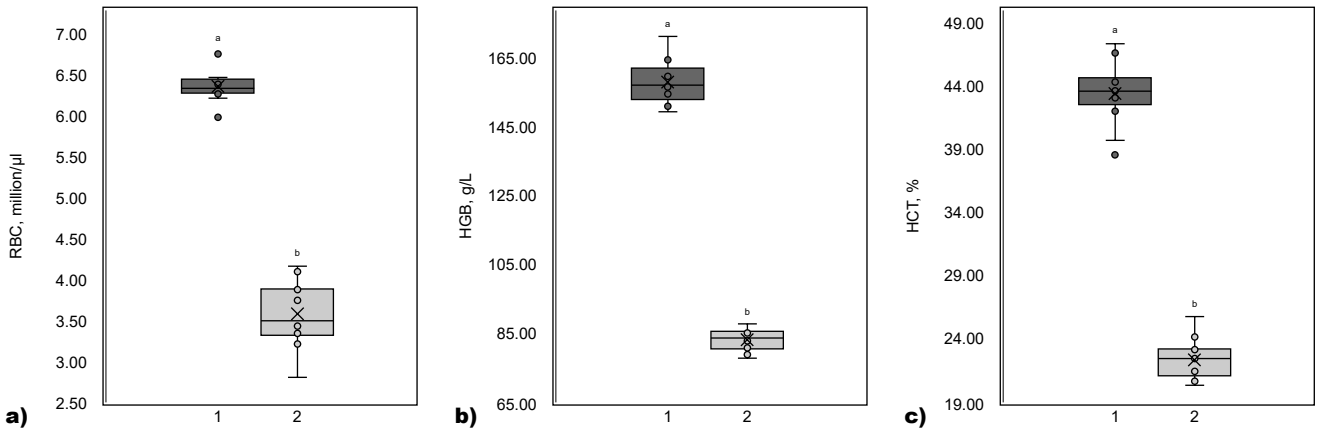

Fig. 2. Red blood cell count (*a*), hemoglobin concentration (*b*), and hematocrit (*c*) in the blood of dogs infected with *Babesia canis* compared to the control group (x±SD, n=13)

Note. Here and in the next figures 1 — control group of healthy dogs; 2 — group of dogs infected with *Babesia canis*.

[a], [b] — mean values with unlike letters were significantly different between the groups (P<0.0001).

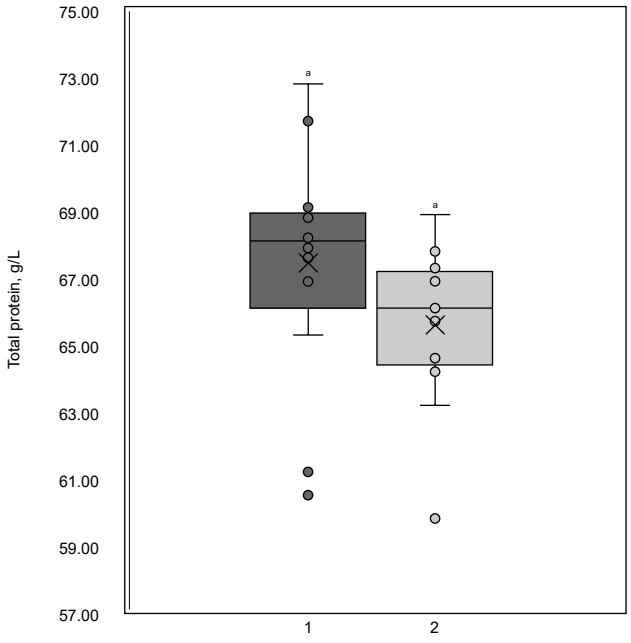

Fig. 3. Total protein concentration in the blood serum of dogs infected with *Babesia canis* and control group (x±SD, n=13)

The MCV value in the control group (fig. 4) averaged 66.38±3.13 fL, while in infected dogs, MCV was significantly lower at 63.45±2.49 fL (P=0.0178; F=0.4335). This reduction may reflect the predominance of microcytic erythrocytes or early regenerative responses during the course of infection.

MCHC values remained relatively stable between the groups. The control cohort had a mean MCHC of 362.56±9.56 g/L, compared to 362.09±5.58 g/L in the infected group (P=0.8844; F=0.0739), indicating no statistically significant difference. This suggests that hemoglobin concentration within individual red blood cells was largely unaffected by the infection at this stage. Similarly, RDW values showed only a slight, non-significant change.

The control group had a mean RDW of 16.15±1.03 %, while the infected group presented a mean of 15.99±1.04 % (P=0.7061; F=0.9790). The minimal variation suggests that anisocytosis (variation in red blood cell size) was not markedly increased in the early stages of *Babesia* infection.

The mean PLT count in the control group (fig. 5) was 330.23±26.44×10³/μL. In contrast, dogs with *Babesia* infection exhibited a profound and statistically significant reduction in platelet levels, averaging just 38.23±6.20×10³/μL (P<0.0001; F<0.0001). This sharp decline clearly indicates the presence of severe thrombocytopenia, a common clinical manifestation of canine babesiosis.

In the control group, the mean WBC count (fig. 6) was 11.29±1.04×10⁹/L, whereas in dogs with babesiosis, it decreased significantly to 7.08±0.60×10⁹/L (P<0.0001), indicating the presence of leukopenia, which is characteristic of the acute phase of the disease.

The relative count of segmented neutrophils was moderately increased in the diseased group (60.66±1.88 %) compared to the control group (57.15±2.82 %) (P=0.0015), suggesting a neutrophilic shift typical of acute inflammatory responses.

Conversely, the relative count of lymphocytes was significantly reduced in the *Babesia canis*-infected group (28.45±1.56 %) compared to the healthy control group (30.45±1.65 %) (P=0.0056), which is consistent with a stress leukogram commonly observed during severe infectious processes.

Our findings align with previous studies that highlight significant hematological alterations during the early stages of canine babesiosis. One of the key parameters we observed was leukopenia, particularly a reduction in total white blood cell (WBC) count. This is consistent with the results of Eichenberger et al. [19], who reported that nonsurviving dogs with *Babesia canis* infection exhibited moderate leukopenia, while survivors generally maintained WBC levels within the reference range. Notably, leukopenia was also observed in up to 60 % of mild cases [37], suggesting that it is a sensitive though not entirely specific marker of disease severity. Similar diagnostic

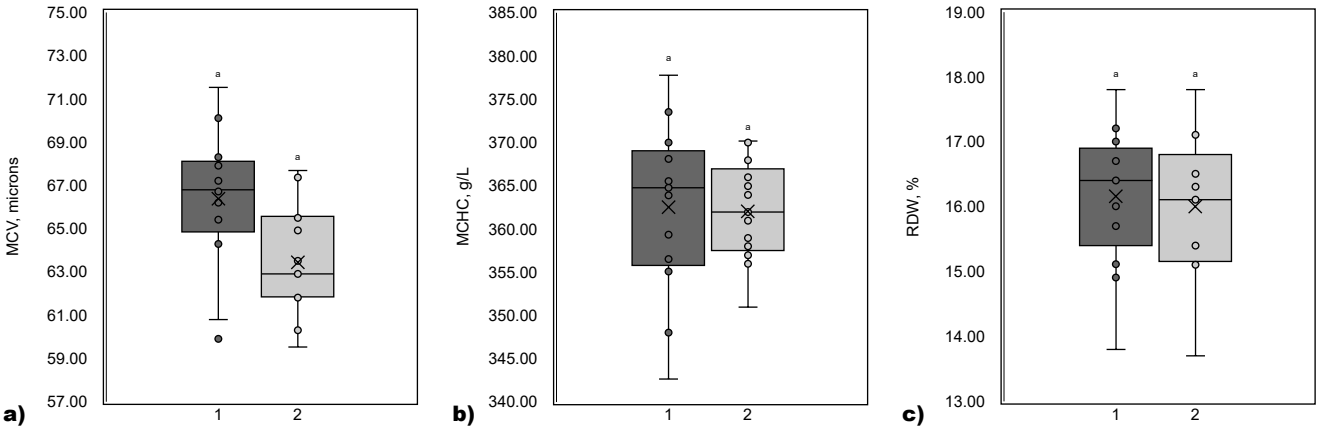

Fig. 4. Mean corpuscular volume (MCV) (*a*), mean corpuscular hemoglobin concentration (MCHC) (*b*), and red cell distribution width (RDW) (*c*) in dogs infected with *Babesia canis* compared to the control group (x±SD, n=13)

trends have been confirmed in cattle and small ruminants infected with different *Babesia* species, underscoring the conserved hematological response across hosts [1, 2].

Our data suggest that lymphopenia, especially during the early phase, may serve as an early indicator of immune suppression or immune dysregulation. This is in line with studies on *Babesia rossi*, where elevated cortisol levels were associated with immunosuppressive states and poor outcomes [44, 48]. Similarly, in malaria infections caused by *Plasmodium* spp., lymphocyte depletion has been attributed to redistribution, immune cell exhaustion, or parasite-induced apoptosis [30, 39, 58]. Immunological studies further suggest that post-infection immunity in dogs is often short-lived and non-sterile, which may explain why lymphocyte recovery is delayed [12]. These mechanisms may also contribute to the lymphopenia observed in *Babesia* infections.

Another critical hematological abnormality identified was thrombocytopenia, which was both profound and consistent in affected dogs. Thrombocytopenia is considered the most dramatic hematological change in babesiosis [25, 61]. Eichenberger et al. [19] proposed a prognostic cut-off of 27,500 platelets/μL, which, although not perfectly sensitive or specific, may still aid in early clinical decision-making. The pathogenesis of thrombocytopenia is likely multifactorial, involving systemic inflammatory responses (SIRS), platelet consumption, sequestration, and impaired production [7; 45]. Reports from endemic regions indicate that thrombocytopenia often precedes anemia, making it a valuable early diagnostic marker for clinicians [16].

Interestingly, although hemolytic anemia was expected, it was not always severe in the early stages of infection in our study. This finding is consistent with previous reports suggesting that anemia may develop progressively, depending on the stage of the disease and the balance between erythrocyte destruction and regeneration [35, 61]. This progressive anemia has also been linked to parasite genetic variability, which influences virulence and the rate of erythrocyte destruction [29].

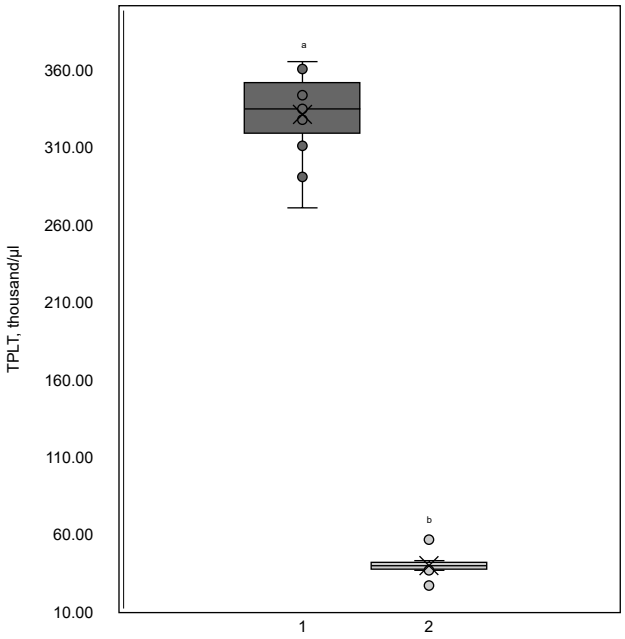

Fig. 5. Platelet (PLT) count in dogs infected with *Babesia canis* compared to the control group (x ± SD, n=13)

The hematologic responses observed in our study align with the findings of Scheepers et al. [46], who conducted a longitudinal analysis of transfused and non-transfused dogs naturally infected with *Babesia rossi*. Their work confirmed the presence of mild to moderate normocytic, normochromic regenerative anemia in all cases, consistent with the hemolytic nature of babesiosis. Notably, although transfusions effectively corrected anemia, they did not appear to significantly influence leukocyte or platelet dynamics, suggesting that the underlying pathophysiological mechanisms of inflammation and thrombocytopenia are independent of red blood cell restoration. This agrees with earlier observations that hematologic imbalances in babesiosis are driven more by immune-mediated and inflammatory mechanisms than by anemia alone [6].

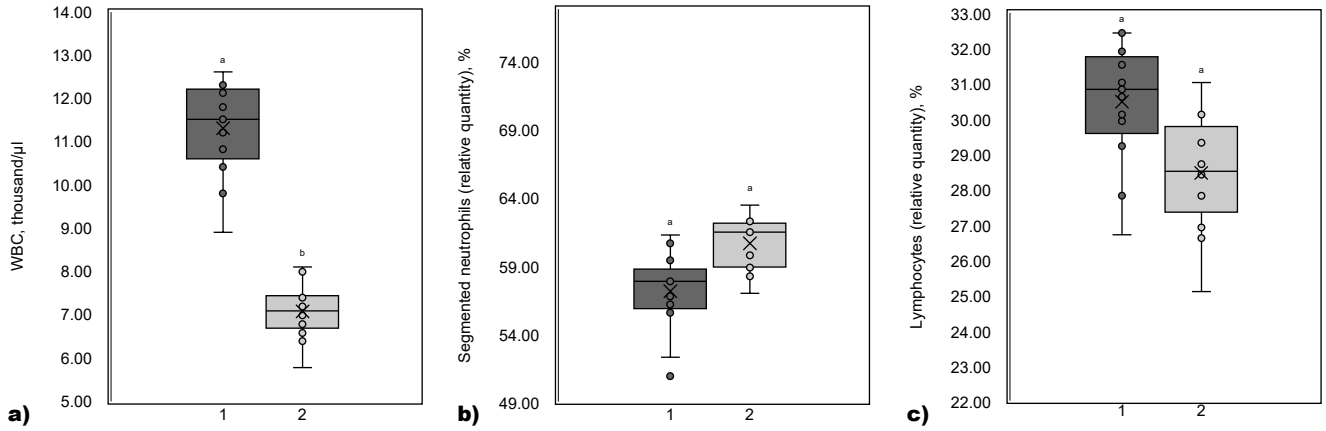

Fig. 6. Total white blood cell (WBC) count (*a*), relative segmented neutrophil percentage (*b*), and relative lymphocyte percentage (*c*) in dogs infected with *Babesia canis* compared to the control group (x±SD, n=13)

Moreover, their observation of an inflammatory leukogram with a left shift, even in the absence of neutrophilia, highlights the atypical white cell responses in babesiosis and may reflect bone marrow suppression or consumption of neutrophils in peripheral tissues. Comparable atypical leukogram patterns have also been reported in *Babesia canis* and *Babesia gibsoni* infections, underscoring the complexity of immune responses during parasitemia [11]. The consistently severe thrombocytopenia, resolving within a few days, further supports the hypothesis of an immune-mediated etiology, rather than direct destruction by the parasite or marrow suppression.

These findings, when integrated with our data, strengthen the understanding that hematologic abnormalities such as anemia and thrombocytopenia are multifactorial in origin and dynamically evolve during the course of babesiosis. Early monitoring and interpretation of these patterns can help guide clinical decision-making, particularly regarding the need for transfusion and prognosis estimation. This is consistent with broader diagnostic recommendations emphasizing early hematologic surveillance in vector-borne diseases [1, 2].

Our findings are further supported by a large-scale retrospective analysis conducted by Fabisiak et al. [25], which statistically examined hematological abnormalities in 350 dogs diagnosed with *Babesia* spp. infection. Their results reinforce that thrombocytopenia is the most consistent and significant hematologic alteration in canine babesiosis, corroborating observations from our study and previous reports. Interestingly, their analysis also highlighted age- and breed-related variations in hematologic responses, including significant differences in PCV between young and adult dogs, as well as in total leukocyte counts between German Shepherds and mixed-breed dogs. Regional studies from Latin America and the Caribbean also suggest that epidemiological context may influence hematologic presentation, pointing to possible interactions between environmental and host-related factors [23].

These findings suggest that host factors such as age and breed may influence the severity of hematologic abnormalities, potentially affecting disease progression and prognosis. While our study did not stratify dogs by age or breed, the consistency of thrombocytopenia and the presence of varying degrees of anemia and leukopenia align with the broader population trends observed by Fabisiak et al. [25]. The rare but notable occurrence of bi- or pancytopenia in their dataset also warrants attention, particularly in severe or complicated cases of babesiosis. Notably, pancytopenia has also been observed in cases where parasitemia co-occurs with secondary infections, further complicating prognosis [12, 16].

Taken together, these insights highlight the multifactorial nature of hematologic responses in canine babesiosis, driven by parasite virulence, host immune status, and possibly genetic predispositions. Future studies incorporating larger sample sizes and breed-specific analysis may offer a more nuanced understanding of hematologic alterations and improve early prognostic capabilities [32]. Furthermore, exploring cytokine-driven mechanisms of anemia and thrombocytopenia may shed light on host–parasite interactions and provide novel therapeutic targets [29].

The study by Žvorc et al. [61] further complements our findings by providing a detailed assessment of erythrocyte and platelet indices in dogs naturally infected with large *Babesia*. Their results confirm that thrombocytopenia remains a consistent hematological hallmark, accompanied by a decrease in plateletcrit (PCT) and an increase in mean platelet volume (MPV), indicating platelet activation and consumption, possibly as part of a systemic inflammatory or coagulopathic process. Similar alterations in platelet indices have been noted in other vector-borne diseases, reinforcing the diagnostic relevance of MPV and PCT monitoring [6, 16]. These changes are consistent with the concept of immune-mediated thrombocytopenia or disseminated intravascular coagulation, as observed in other studies.

Moreover, the authors observed decreased RBC count, MCV, and hematocrit values both before and after treatment, which is consistent with the normocytic, normochromic anemia frequently seen in babesiosis. Interestingly, red cell distribution width (RDW) remained unchanged, suggesting a uniform population of erythrocytes and possibly limited regenerative response in many cases, which may reflect either the early stage of infection or a suppressed erythropoiesis due to systemic inflammation. Comparable patterns of anemia with poor regenerative response have also been described in *Babesia rossi* infections, highlighting the role of systemic inflammatory mediators in inhibiting bone marrow function [11, 29, 52].

These findings support the diagnostic and prognostic relevance of automated erythrocyte and platelet indices in canine babesiosis. Monitoring MPV and PCT in particular may offer insight into the pathophysiological processes underlying thrombocytopenia and help assess treatment efficacy and disease progression, especially when paired with classical parameters such as HCT and RBC count. As our study also indicated significant shifts in platelet and red cell parameters in affected animals, the integration of such indices could enhance early detection and prognostic stratification in clinical settings. Such integrative diagnostic approaches are strongly recommended in current guidelines for canine vector-borne diseases [1, 2].

This study confirms that canine babesiosis caused by *Babesia canis* leads to significant hematological alterations, including consistent thrombocytopenia, variable degrees of anemia, leukopenia, and lymphopenia, which can serve as valuable early diagnostic and prognostic markers. Thrombocytopenia, in particular, emerged as the most consistent and severe abnormality, likely resulting from immune-mediated mechanisms and systemic inflammation. Although anemia was common, it was often mild to moderate in early stages, suggesting progressive red blood cell destruction rather than acute hemolysis. Regional analyses also indicate that differences in prevalence and hematologic severity may be shaped by environmental and epidemiological factors [23, 38].

Our findings align with previous studies and emphasize the importance of monitoring platelet and erythrocyte indices, such as mean platelet volume (MPV), plateletcrit (PCT), and red blood cell parameters, to better understand disease progression and guide clinical decisions. The presence of leukopenia and lymphopenia also highlights possible immune dysregulation during infection, further supporting the need for timely intervention. Immunological studies suggest that reinfection resistance in dogs is often incomplete, making early monitoring of hematologic patterns essential for long-term disease control [12, 40, 54].

Overall, routine hematological profiling remains a critical component in the diagnosis and management of canine babesiosis. Early recognition of key changes in blood parameters can aid in identifying high-risk patients, optimizing treatment strategies, and improving clinical outcomes. Further studies are warranted to explore long-term hematologic and immunologic responses, as well as the influence of host factors such as breed and age on disease severity and prognosis.

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Гематологічні показники у собак на ранніх стадіях бабезіозу в Дніпропетровській області, Україна

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Бабезіоз собак — це трансмісивне захворювання, спричинене найпростішими паразитами роду *Babesia*, які передаються переважно через іксодових кліщів. Захворювання поширене в усьому світі, зокрема в більшості регіонів України. *Babesia canis* — найпоширеніший вид, який вражає собак, зі зростанням клінічного значення як для домашніх, так і для диких м'ясоїдних тварин. Інфекція призводить до внутрішньосудинного гемолізу, гіпоксичного пошкодження тканин та поліорганної дисфункції. Незважаючи на досягнення в діагностиці, ранні гематологічні зміни на початкових стадіях інфекції залишаються недостатньо вивченими. Метою цього дослідження є порівняти гематологічні параметри собак на ранніх стадіях бабезіозу зі здоровими контрольними тваринами, щоб визначити надійні показники для ранньої діагностики та моніторингу захворювання. Було проведено ретроспективний аналіз 13 клінічних випадків інфекції *Babesia canis* у собак, представлених на ветеринарному комплексі «Передовий» (Дніпро, Україна) у період з лютого по квітень 2024 р. Мазки крові підтвердили паразитемію, а загальний аналіз крові було проведено за допомогою автоматичного аналізатора *MicroCC-20 Plus*. Результати показали статистично вірогідне зниження кількості еритроцитів (3,59±0,37×10^6/мкл) та гемоглобіну (83,42±2,96 г/л) у інфікованих собак, порівняно з контрольними тваринами (6,36±0,17×10^6/мкл та 158,58±5,87 г/л відповідно). Значення гематокриту також були значно знижені (22,54±1,45 % проти 43,51±2,39 %; P<0,0001). Спостерігали значну тромбоцитопенію (38,23±6,20×103/мкл) та лейкопенію (7,08±0,60×10^9/л) з одночасним нейтрофільним зсувом та лімфопенією. Середній об'єм еритроцитів (MCV) в інфікованій групі був значно нижчим (63,45±2,49 фл), тоді як інші показники еритроцитів (MCHC, RDW) та рівень загального білка не показали статистично вірогідних відмінностей. Ці дані підкреслюють виражені гематологічні порушення, пов'язані з ранньою стадією інфекції *Babesia canis*. Зміни кількості еритроцитів, концентрації гемоглобіну, гематокриту та рівня тромбоцитів можуть слугувати ранніми діагностичними маркерами. Необхідні подальші дослідження для уточнення гематологічного профілювання, щоб покращити прийняття клінічних рішень та своєчасне втручання при бабезіозі собак.

Ключові слова: собаки, кров, гематологічні показники, *Babesia canis*, еритроцити, лейкоцити, анемія, диференціальна діагностика

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Changes in radiographic and electrocardiographic parameters in dogs with myxomatous mitral valve degeneration under the influence of humic substances

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The article presents the results of studying the effects of humic substances in dogs with stage C myxomatous mitral valve degeneration receiving standard therapy according to the recommendations of the American College of Veterinary Internal Medicine. Comprehensive assessment of radiographic and electrocardiographic parameters before and after treatment was performed. It was found that adding humic substances to the standard therapy contributed to reducing heart size, decreasing pulmonary congestion and improving electrophysiological characteristics of the heart. The obtained results indicate the advisability of using humic substances as an additional tool in the complex treatment of dogs with myxomatous mitral valve degeneration.

Key words: endocardiosis, physiology, antioxidants, cardiac arrhythmia, veterinary cardiology

Introduction

Myxomatous mitral valve degeneration (MMVD), also known as endocardiosis, is the most common form of heart valve pathology in dogs, causing heart failure and significantly deteriorating the animals' quality of life [16]. The disease is characterized by progressive degeneration of the connective tissue structures of the valve, leading to blood regurgitation, heart chamber dilation, and the development of pulmonary congestion. According to research data, MMVD is diagnosed in 70–80 % of dogs older than 10 years, especially small and medium breeds, making this pathology highly relevant for contemporary veterinary cardiology [4, 5].

According to recommendations by the American College of Veterinary Internal Medicine (ACVIM), standard therapy for dogs with chronic heart failure due to MMVD includes pimobendan, angiotensin-converting enzyme inhibitors (ACE inhibitors), diuretics (furosemide), and aldosterone antagonists (spironolactone). These medications effectively reduce congestion, improve hemodynamics, and enhance quality of life [8]. However, despite proven efficacy, their use is often associated with certain limitations, including potential side effects, electrolyte imbalances, development of diuretic resistance, and inadequate management of oxidative stress, which plays a significant role in heart pathology progression.

Recently, more attention has been given to finding additional therapeutic agents capable of reducing the negative effects of oxidative stress, maintaining cardiomyocyte functionality, and slowing structural changes in the heart [2]. Humic substances, natural organic compounds characterized by high antioxidant activity and the ability to stabilize cell membranes, reducing myocardial and cardiac conduction system damage under chronic heart failure conditions, are of particular interest [19].

Previous studies, conducted in Ukraine and abroad, indicate the positive impact of humic substances on general antioxidant status, reduction of oxidative stress markers (malondialdehyde, MDA), and their ability to stabilize cardiac rhythm and reduce cardiac arrhythmias [1, 15]. However, comprehensive assessment of their effectiveness in dogs with MMVD, especially under standard therapy conditions, remains insufficiently studied.

The aim of our study was to determine the effects of humic substances on radiographic and electrocardiographic parameters in dogs with stage C MMVD undergoing standard therapy per ACVIM protocols.

Materials and Methods

The research was conducted from 2020 to 2022 involving dogs with MMVD at the veterinary clinical diagnostic center of Dnipro State Agrarian and Economic University and LLC "Veterinary Space Discovery" in Dnipro. Animals underwent clinical examinations, radiography, echocardiography, and electrocardiography. Animals were divided into three groups: standard therapy (ST, n=6), standard therapy with humic substances (STH, n=6), and control (n=6). Animals in the ST and STH groups received standard ACVIM-classified therapy, with the STH group additionally receiving "Humilid" orally at 10 mg/kg once daily before feeding. Exclusion criteria included recent specific treatments, oncology, infections, or severe illnesses.

Radiography of the thorax in the studied animals was performed in a single projection to reduce the cost of research during martial law. Animals were positioned in right lateral recumbency with maximum cranial extension of the forelimbs, using *Optima-xr220amx* (*GE*, USA) equipment and *Kodak DirectView CR975* automatic digitizing system (*Carestream Health*, USA). The analysis of radiographic images included assessment of the vertebral heart score (VHS) and vertebral left atrial size (VLAS). The radiological assessment of lung and vascular conditions was performed subjectively (semi-quantitatively) based on the severity of bronchial and interstitial patterns and by comparing the diameter of veins in the cranial lung lobes with artery diameters. All measurements were expressed in arbitrary units [3, 7].

To detect disturbances in conduction, excitation, and automaticity of the heart, the *Bioset 9000* apparatus (*Hörmann*, Germany) was used. Animals were positioned on the examination table in right lateral recumbency for ECG recording. Electrocardiograms were registered using standard Einthoven leads (I, II, III) and augmented Goldberger leads (aVR, aVL, aVF) on millimeter paper at a speed of 50 mm/s and a standard input voltage of 1 mV = 10 mm. ECG recordings lasted 5 minutes and were analyzed using an electrocardiographic ruler according to established algorithms [14]. During ECG analysis, the rhythm, R-R interval regularity, heart rate (beats/min), amplitude (mV), and duration (s) of the P wave, PQ interval, QRS complex, and ST segment were evaluated. Obtained data were compared with reference values from R. Santilli [14]. The functional state of the animals was assessed without using medications negatively affecting the vasomotor, respiratory centers of the brain, or the heart.

Statistical data processing was performed using *Microsoft® Excel®* (version 2412) in accordance with methodological recommendations. The number of animals was presented as absolute (n) and relative (%) values. Data were expressed as mean ± standard deviation (M±SD). The paired Student's *t*-test was used to evaluate dynamics of studied parameters in animal groups before and after therapy. Intergroup differences were analyzed using one-way ANOVA followed by Bonferroni retrospective tests.

Results and Discussion

At the initial stage (Day 1 of the study), animals in both the ST and STH groups exhibited pronounced changes in the form of increased bronchial and interstitial patterns (fig. 1). These changes indicated the presence of congestion and early pulmonary edema. Radiographically, the thickening of veins in the cranial lung lobes was approximately twice the diameter of the arteries (a vein to artery ratio of about 2:1), indicating venous congestion in the pulmonary circulation. By Day 21 of treatment, noticeable shifts toward normalization were observed, particularly

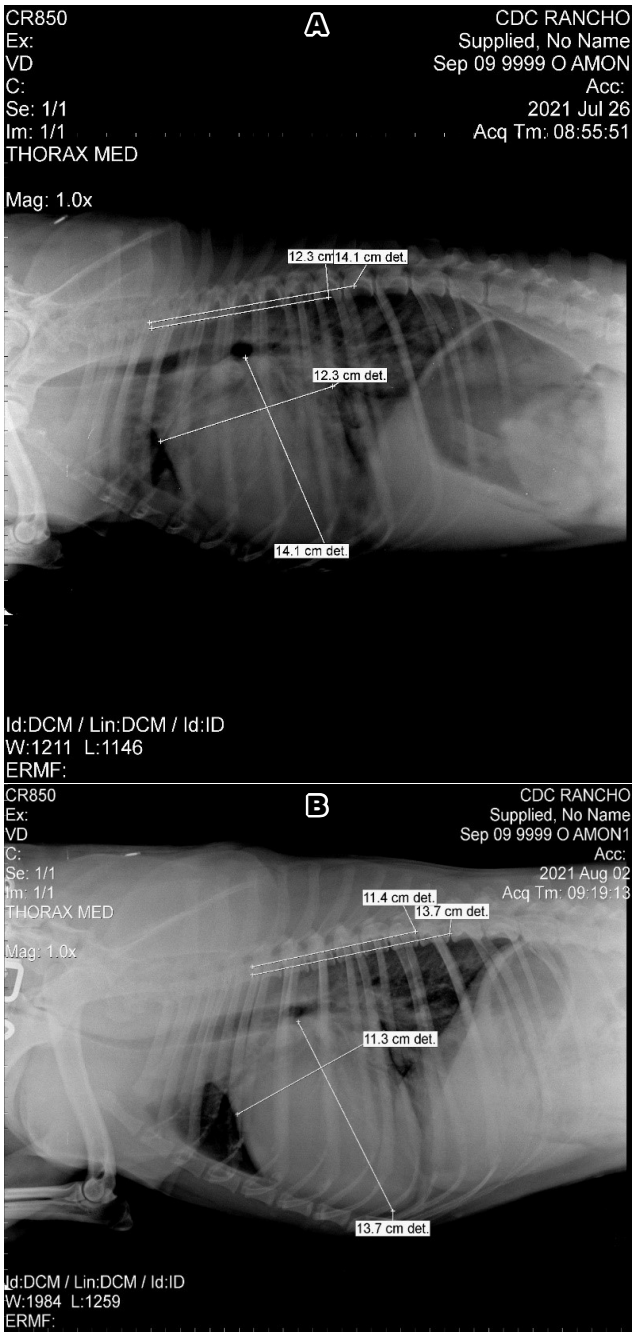

Fig. 1. Radiograph of a dog from the experimental group at the beginning of therapy (A) and on the 21st day of treatment (B). Measurement of the VHS

Table 1. Comparative assessment of radiographic changes in animals of the control group (n=6) and experimental groups (n=6), M±SD

Measurement	Control	ST		STH		References value
		Day 1 (M±SD)	Day 21 (M±SD)	Day 1 (M±SD)	Day 21 (M±SD)	
Vertebral Heart Score	8.8±0.3	12.1±0.7	11.2±0.7*	12.1±1.0	10.8±1.1*	8–10.5
Vertebral Left Atrial Size	1.5±0.1	2.5±0.4	2.1±0.3*	2.5±0.3	2.0±0.3*	1.4–2.2

Note. * — P≤0.05 — statistically significant difference compared to the beginning of therapy.

a reduction in the intensity of the bronchial pattern. In most animals, a transition from a “marked” bronchial enhancement to a “mild” or “moderate” one was noted.

Thus, radiographic examination made it possible to identify the initial degree of venous congestion and track positive dynamics during treatment, ultimately confirming the effectiveness of the applied therapy in reducing manifestations of pulmonary congestion in dogs with myxomatous mitral valve degeneration.

Table 1 provides a comparative assessment of radiographic changes in animals from the control group (n=6) and experimental groups (ST, STH, n=6) before and after treatment. Since the data in the ST group met the conditions of normal distribution (verified using the Shapiro-Wilk test, P>0.05), a paired *t*-test was used to compare changes in the vertebral heart score between Day 1 and Day 21. As a result, a statistically significant (P<0.01) decrease of 7.4 % in this parameter was observed, although it remained above the reference values (8–10.5 units) after treatment.

For the STH group, due to the non-normal distribution of data (P<0.05 according to the Shapiro-Wilk test), the non-parametric Wilcoxon test was applied, which showed a statistically significant (P<0.05) decrease in the vertebral heart score by 10.7 %.

The vertebral left atrial size in both groups initially exceeded the normal range (≈2.5). On Day 21 of treatment, it decreased by 16.0 % in the ST group and by 17.6 % in the STH group, aligning with the reference range (P<0.05). This indicates reduced left atrial dilation and a decrease in both preload and afterload. Our results are consistent with findings from other studies that also report increased VLAS in dogs with MMVD at early disease stages, reflecting early left atrial dilation in response to mitral valve dysfunction [11, 12].

This is the first study to comprehensively analyze changes in vertebral heart score and vertebral left atrial size in dogs with myxomatous mitral valve degeneration under the influence of humic substances.

As a result of the electrocardiographic evaluation conducted in dogs with myxomatous mitral valve degeneration, several characteristic features were identified. The primary focus was placed on assessing the functional state of the cardiac conduction system, the nature of rhythm disturbances, and the relationship between echocardiographic parameters (particularly left atrial dilation) and ECG wave morphology.

The main objectives of ECG analysis were to detect conduction disturbances, changes in interval durations, and wave amplitudes, which could indicate structural changes in heart chambers or pathological processes in the myocardium. In the control group (n=4), ST (n=5), and STH (n=3) groups, respiratory (sinus) arrhythmia was observed (fig. 2). This is a result of parasympathetic nervous system influence on the sinoatrial node and is considered normal in dogs with high vagal tone at rest, thus requiring no additional correction.

The P wave interval and amplitude in all groups remained within reference values (up to 0.04 sec. and 0.4 mV). However, no significant differences were detected between groups, indicating a lack of pronounced treatment effect on these electrocardiographic parameters.

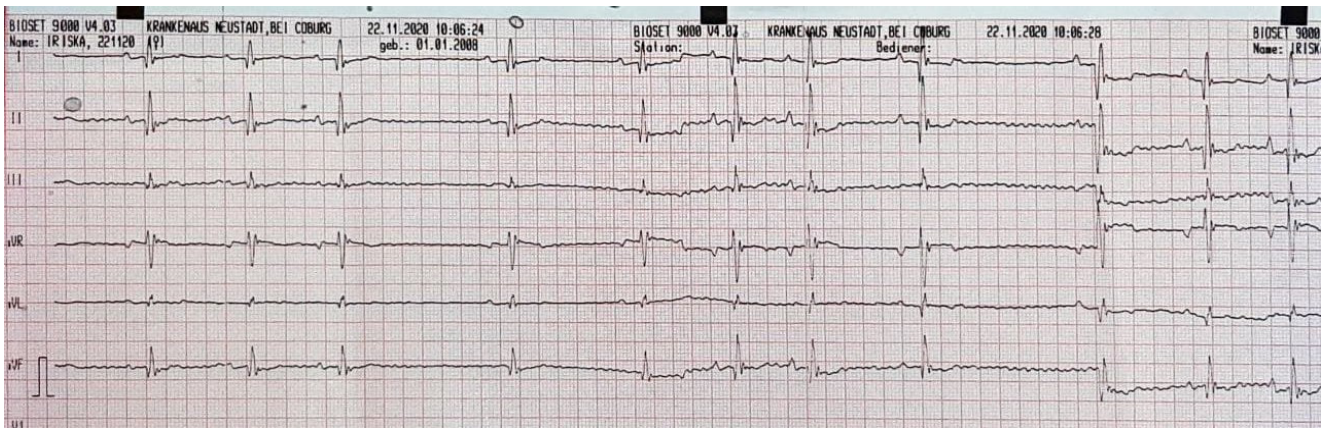

Fig. 2. Electrocardiogram of a dog with MMVD (Leads I, II, III, aVR, aVL, aVF; tape speed 50 mm/s). The recording shows sinus arrhythmia

Table 2. Comparative assessment of electrocardiographic results in animals of the control group (n=6) and experimental groups (n=6), M±SD

Parameters	Control	ST		STH		References value
		Day 1 (M±SD)	Day 21 (M±SD)	Day 1 (M±SD)	Day 21 (M±SD)	
P, mV	0,2±0,04	0,18±0,08	0,2±0,06	0,26±0,09	0,2±0,07	Up to 0,4
P, sec	0,03±0,01	0,04±0,01	0,03±0,01	0,04±0,01	0,03±0,01***	Up to 0,04
PQ, sec	0,08±0,02	0,09±0,02	0,07±0,02	0,11±0,05	0,09±0,03	0,06–0,13
QRS, sec	0,06±0,02	0,05±0,01	0,04±0,01	0,07±0,02	0,07±0,03	Up to 0,7
R, mV	1,1±0,3	0,9±0,2	0,9±0,2	1,15±0,5	0,8±0,2	Up to 3
QT, sec	0,16±0,05	0,18±0,02	0,17±0,02	0,17±0,06	0,15±0,03	0,15–0,24
HR, bpm	181,0±11,2	156±18,1	142±10,2*	184±9,0	172±4,2**	60–170

Note. * — P<0.01; ** — P<0.05 compared to Day 1, *** — P<0.05, a statistically significant difference between day 1 and day 21 according to the *t*-test, P=0.025. (Despite minimal absolute changes, significance was confirmed when analyzing individual values).

Analysis of the R waves, QRS complex, and PQ segments on the ECG showed no changes in either group at the beginning or end of the study, suggesting the absence of pathological alterations in impulse conduction during the development of this disease (table 2). In some animals, excitability disturbances were identified. These arrhythmias are commonly observed in cases of myxomatous mitral valve involvement, likely due to mechanical irritation of the endocardium by stretched chordae or hypoxia, which can prolong the refractory period in myocardial regions near the papillary muscles [10].

Radiographic studies confirm the development of cardiomegaly in dogs with myxomatous mitral valve degeneration and the presence of pulmonary congestion. During treatment, a positive trend was observed in the form of reduced heart size and normalization of pulmonary circulation, indicating the effectiveness of the prescribed therapy, particularly the use of diuretics and a comprehensive approach to correcting hemodynamic disorders.

The results of the study on electrocardiographic changes in dogs with myxomatous mitral valve degeneration demonstrated that the use of standard therapy in combination with humic substances had no effect on the electrophysiological characteristics of the heart.

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Зміни рентгенографічних та електрокардіографічних показників у собак з міксоматозною дегенерацією мітрального клапана при застосуванні гумінових речовин

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У статті наведено результати дослідження впливу гумінових речовин у собак із міксоматозною дегенерацією мітрального клапана в стадії С, які отримували стандартну терапію згідно з рекомендаціями Американського коледжу ветеринарної медицини внутрішніх органів. Проведено комплексну оцінку рентгенологічних та електрокардіографічних показників до та після лікування. Встановлено, що додавання гумінових речовин до стандартної терапії сприяло зменшенню розмірів серця та зниженню застійних явищ у легенях. Вірогідного впливу на електрокардіографічні показники не виявлено. Отримані результати свідчать про доцільність подальшого вивчення гумінових речовин як додаткового засобу у комплексному лікуванні собак із міксоматозною дегенерацією мітрального клапана.

Ключові слова: ендокардіоз, фізіологія, антиоксиданти, ветеринарна кардіологія, серцева недостатність

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Dynamics of fatty acid composition in the muscles of crucian carp and pike under the influence of elevated concentrations of cobalt ions

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KVZ: Project administration; Conceptualization; Supervision; Writing — review & editing.

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None to declare.

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In today's conditions of growing anthropogenic pressure on aquatic ecosystems, the problem of heavy metal accumulation, including cobalt ions, is becoming particularly relevant. Cobalt ions can enter aquatic environments as a result of mining activities, metallurgy, and the production of batteries, dyes, catalysts, and magnetic materials. It is collectively may lead to local exceedances of permissible concentration limits in freshwater ecosystems. Despite the fact that cobalt is a biogenic element in low concentrations, its excess has a toxic effect on hydrobionts, in particular affecting lipid homeostasis. One of the sensitive indicators of metabolic disorders in fish is a change in the fatty acid composition of tissues, which can be used as a bioindicator of toxic pressure and the functional state of the organism. The paper analyses changes in the fatty acid composition of the muscle tissue of crucian carp (*Carassius gibelio* Bloch) and pike (*Esox lucius* L.) under the influence of cobalt ions at concentrations of 0.1 mg/dm³ and 0.25 mg/dm³ over a period of 14 days. These concentrations correspond to 2 and 5 maximum permissible concentrations. Total lipids were extracted and transesterified to obtain fatty acid methyl esters, which were then analyzed by gas chromatography for the quantitative determination of individual fatty acids. In crucian carp, the influence of cobalt ions led to significant changes in the composition of essential fatty acids. In particular, the fractions of saturated myristic acid (14:0) and long-chain monounsaturated eicosenoic acid (20:1) increased proportionally to the concentration of the investigated metal, while the levels of stearic acid (18:0) and oleic acid (18:1) decreased. A more systematic effect was observed in pike muscles: the content of many saturated fatty acid fractions decreased compared to the control group, while changes in the proportions of key polyunsaturated fatty acids likely indicate impaired desaturase activity. In the pike organism, a more systemic effect of cobalt ions was observed, which consisted in a tendency to decrease the amount of most saturated fatty acids. Some nutritional indices for assessing fatty acids were also analyzed.

Key words: fishes, fatty acids, defense system, physiological response, regulation, adaptive reactions, biomonitoring, toxic pollution, cobalt

Introduction

Heavy metal contamination of aquatic ecosystems is recognized as one of the most significant environmental concerns in contemporary ecological research. The growth of industrial activity, wastewater discharge and the accumulation of man-made waste lead to increased concentrations of these elements. This poses

a significant threat to the environment due to the high potential of metals for bioaccumulation and biomagnification in living organisms [15, 24].

Fish are constantly exposed to heavy metals in the aquatic environment, which explains their widespread use as model organisms in ecotoxicological studies [22, 40]. The accumulation and toxicity of heavy metals in fish organisms are multidirectional and often lead to physiological and biochemical changes, such as excessive formation of reactive oxygen species (ROS), enzyme dysfunction, and imbalance of redox reactions. This usually leads to structural damage to lipids, especially unsaturated fatty acids [32]. This may frequently result in the degradation of fatty acid molecules, which are essential for the structural and functional integrity of fish biological membranes and significantly contribute to their nutritional value in the human diet [12, 27].

Fish is distinguished by its valuable nutritional composition, primarily due to the presence of high-quality higher fatty carboxylic acids fatty acids, among which long-chain omega-3 (ω−3) fatty acids are of particular importance [36, 46]. The lipid composition of fish muscle tissue differs from that of mammals in terms of the number of double bonds. [27]. Omega-6 (ω−6) and omega-3 (ω−3) are important metabolic precursors of long-chain polyunsaturated fatty acids (C 20–24), such as arachidonic acid (20:4), eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6), which are mainly found in fish with a high fat content [2, 21, 45].

Changes in the composition of fatty acids can indicate not only the quality of fish products, but also the level of toxicant contamination of freshwater environments [27]. Lipids and individual fatty acids are responsible for the permeability, fluidity and integrity of biological cell membranes [3, 39]. They are precursors of bioactive and signaling molecules responsible for regulating biochemical reactions in cells [7]. Eicosapentaenoic acid is a precursor of eicosanoids, which have a wide range of physiological effects and play a role in immune and inflammatory responses, nervous system functions, and reproduction [37]. Eicosanoids also help fish adapt to environmental stressors [27].

The presence of pollutants such as heavy metals can significantly affect the fatty acid profile in fish tissues due to changes in the relative amounts of individual fatty acids [14, 41, 43]. Changes in lipid metabolism and fatty acid profiles were used to better understand how metals affect aquatic organisms in food chains, as well as the integrative biochemical response to the impact of pollutants and their accumulation in aquatic organisms [31]. This makes them promising biomarkers for assessing the impact of pollutants. Most previous studies have focused exclusively on metal accumulation or on analyzing the fatty acid composition in fish [25, 29, 35, 50].

Considering that the lipid content in fish muscle tissue is influenced by species-specific characteristics, the ecological status of the water body, and the presence of toxic agents, it is important to investigate alterations in the fatty acid composition of muscle lipids caused by metal ions in general, with particular attention to cobalt. Cobalt is a technically important trace element with wide applications across various industries and technologies, including its use in high-temperature alloys for jet engines and in improving the efficiency of magnetic materials and rechargeable batteries. This metal is rarely present in freshwater environments at elevated levels, except in cases where mining, manufacturing, or other anthropogenic activities cause local increases in cobalt concentrations [44]. Although cobalt is an important element contained in vitamin B_{12}, only trace amounts are needed for life, and an increase in its content can lead to toxic effects on aquatic biota [5].

Materials and Methods

Experimental studies were conducted on two-year-old crucian carp (*Carassius gibelio* Bloch) and pike (*Esox lucius* L.), with average body weights of 200–220 g and 150–170 g, respectively. The effects of two elevated concentrations of cobalt ions, 0.1 and 0.25 mg/dm³, corresponding to 2 and 5 times the maximum permissible concentrations, were investigated. Metal in the form of $CoCl_2 \cdot 6H_2O$ was added to the water in 200-litre aquariums containing test groups of fish (five individuals in each). To limit the influence of fish exometabolites on their physiology, the water in each aquarium was replaced every two days. To ensure the full development and maximal functional expression of compensatory-adaptive responses to the metal, fish were acclimatized over a 14-day period. According to the author [34], this period is sufficient for the formation of adaptive responses in the bodies of exothermic animals. The control group consisted of fish tissue samples taken from aquariums without added cobalt chloride. The fish were not fed during the research. The experiments were conducted in accordance with the rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes [17] and the principles of animal research ethics [12].

To determinate the content of lipids and fatty acids, the muscles were ground in cold glass homogenizers, followed by extraction of total lipids from the tissue with a chloroform-methanol mixture in a 2:1 ratio using the Folch method [20]. At the same time, 20 parts of the extracting mixture were added to one mass part of the tissue and left for 12 hours for extraction. Non-lipid impurities were removed from the extract by washing with a 1 % KCl solution. Total lipids were obtained after distillation of the extract mixture [26]. The dried samples were dissolved in 1 ml of hexane and used for further analysis of fatty acids.

Fatty acid methyl esters (FAME) were obtained from total lipids by acid-catalyzed transesterification [11]. 100 mg of extracted lipid was dissolved in 1.5 ml of dichloromethane and 3 ml of methanol-sulphuric acid solution (200:3 by volume) and shaken vigorously. The reaction mixture was heated at 100 °C for approximately 1 hour and then cooled to room temperature. Then, 3 ml

of hexane and 1 ml of distilled water were added to the mixture. After mixing and layering, the entire upper phase was collected. One gram of anhydrous sodium sulphate was added to the collected sample and then left for 24 hours to allow separation. After filtration, the sample was evaporated until its mass became constant under a stream of nitrogen. Heptane was added to the sample to achieve a final concentration of 50 mg/dm^3.

FAME analysis was performed using a gas chromatograph (*Agilent6890*, California, USA) equipped with a BPX70 fused silica capillary column (length 50 m; inner diameter 0.22 mm; film thickness 0.25 μm) and a flame ionization detector (FID). Nitrogen was used as the carrier gas, and the split ratio was set at 1:100.

The temperatures for the injection port and detector were set at 270 °C and 300 °C, respectively. The mass spectrum data were processed using *STAR-GC3800* software. Fatty acids were identified by comparing their retention times with a mixture of standards (*NU-CHEK PREP, Inc.*). Each fatty acid was quantified (%) by calculating its peak and area relative to the total peak area.

The results of the studies were statistically processed using Student's t-test and R software to determine the significant difference between the experimental and control groups.

Research Results and Discussion

Fish tissues are characterized by a significant variety of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) [23]. As a result of experimental research, we identified 13 fatty acids in the muscle lipids of crucian carp and pike, including five SFA, three MUFA and five PUFA (table).

In the muscles of crucian carp, under the action of both concentrations of cobalt ions among SFA, some increase in the content of myristic acid (14:0) by 35.6 % and 33.1 % and a decrease in stearic acid (18:0) by 34.9 % and 33.3 % relative to the control values were found. This probably indicates that the elongation stages (which normally convert C14:0 to C18:0) were disrupted, leading to the accumulation of shorter SFA. Heavy metals such as cobalt can affect the activity of enzymes that synthesize lipids. In particular, it has been shown that cobalt exposure alters the activity of desaturase and elongase in fish [10, 18]. Therefore, cobalt ions can influence the balance between the synthesis of saturated and unsaturated fatty acids.

Analysis of SFA in pike muscles indicated some decrease in lauric acid (12:0) content by 14.2 % and arachidic acid (20:0) content by 25.4 % under the influence of 0.1 mg/dm^3 cobalt ions, while under the influence of 0.25 mg/dm^3 of metal, the proportions of all saturated fatty acids decreased: lauric (12:0) by 21.3 %, myristic (14:0) by 10.4 %, palmitic (16:0) by 5 %, stearic (18:0) by 4.2 %, and arachidic (20:0) by 20.8 %. Under the action of maximum cobalt ion concentration, the total content of all detected SFAs decreased by 5.4 % compared to the control group. Various fish species have different lipid metabolisms and therefore react differently to toxins. In particular, due to biomagnification, predatory fish can accumulate larger amounts of heavy metal ions compared to species at lower trophic levels [24]. Under the influence of cobalt ions, pike showed a significant decrease in the amount of virtually all measured fatty acids, which indicates their intensive mobilization or breakdown. In fish, cobalt ions contribute to the generation of active forms of oxygen, among which the hydroxyl radical (·OH) plays a leading role, capable of initiating peroxide oxidation of unsaturated fatty acids [24, 32].

Differences in the content of MUFA were found in fish muscles: in crucian carp, their total amount in the control group was 37.6 %, and in pike — 13.5 %. As a result of exposing fish to cobalt ions at a concentration of 0.25 mg/dm^3, a decrease in the content of essential important monounsaturated fatty acids in fish muscles was observed oleic acid (18:1) by 20.1 % in crucian carp and palmitoleic acid (16:1) by 9.1 % in pike. At the same time, an increase in the concentration of eicosenoic acid (20:1) was found in both species of fish studied — by 46.2 % under the action of 0.25 mg/dm^3 of cobalt ions in crucian carp, and by 19.1 % and 23.6 % under the influence of both concentrations of metal in pike. An increase in the proportion of eicosenoic acid (20:1) can alter the fluidity of cell membranes (making them more rigid), which potentially affects the transport of substances, the activity of membrane enzymes and receptor function. This corresponds to the phenomenon of cellular adaptation to stressful conditions — homeoviscous adaptation, when a change in lipid composition ensures the preservation of the physicochemical properties of membranes under the influence of toxicants [16, 49]. The oleic acid (18:1) content in crucian carp muscles was the highest, and its decrease under the action of 0.25 mg/dm^3 cobalt ions led to a decrease in the total amount of MUFA in this group of fish. In addition, the decrease in oleic acid (18:1) levels is likely to be the result of its active use in the process of lipid restructuring and cell membrane restoration. Similar metabolic reactions are observed in stress response models, where short MUFA are converted or directed into PUFA synthesis to maintain the physicochemical stability of membranes [47]. Fish under stress often remodel membrane lipids to maintain cell function [19].

Unlike saturated and monounsaturated fatty acids, the content of PUFA in crucian carp muscles increased by 23.2 % under the influence of 0.25 mg/dm^3 cobalt ions. The highest concentrations of the studied metal caused the biggest changes in the content of docosahexaenoic acid (DHA) (22:6), which is an omega-3 fatty acid. Thus, its percentage increased by 29.2 % and 78.8 % under the action of 0.1 and 0.25 mg/dm^3 of cobalt ions. Elevated DHA levels may help stabilize cell membranes under the influence of heavy metals or modulate anti-inflammatory signals. This effect is likely a compensatory adaptation: increased DHA helps maintain fluidity and integrity of membranes under the influence of toxic metal [18, 28, 41].

Table. The content of fatty acids in fish muscles under the influence of cobalt ions, % (M±m, n=5)

Fatty acids	Crucian carp			Pike		
	Control	0.1 mg/dm³	0.25 mg/dm³	Control	0.1 mg/dm³	0.25 mg/dm³
C 12:0	0,15±0,01	0,14±0,01	0,17±0,01	0,49±0,01	0,42±0,02*	0,38±0,02*
C 14:0	2,34±0,09	3,18±0,23*	3,12 ± 0,18*	0,55±0,02	0,56±0,02	0,49±0,01*
C 16:0	16,27±0,63	17,33±1,56	16,11±0,72	16,87±0,20	16,62±0,36	16,02±0,24*
C 18:0	8,09±0,63	5,27±0,52*	5,40±0,53*	4,92±0,07	4,93±0,15	4,71±0,04*
C 20:0	0,20±0,02	0,22±0,02	0,20±0,02	0,047±0,002	0,035±0,003*	0,037±0,002*
∑ SFA	27,05±1,10	26,14±1,45	24,99±0,35	22,87±0,15	22,56±0,42	21,65±0,29*
C 16:1	9,65±0,66	11,02±1,05	8,58±0,65	2,77±0,08	2,63±0,11	2,52±0,04*
C 18:1	25,00±1,36	23,28±1,86	19,97±0,66*	10,20±0,35	10,49±0,34	10,47±0,49
C 20:1	2,93±0,24	3,08±0,19	4,28±0,26*	0,51±0,02	0,60±0,02*	0,63±0,03*
∑ MUFA	37,58±1,43	37,38±0,89	32,83±0,82*	13,48±0,41	13,72±0,38	13,62±0,53
C 18:2	7,41±0,50	7,42±0,58	7,14±0,69	5,26 ± 0,15	5,88±0,48	7,07±0,38*
C 18:3	2,84±0,30	2,61±0,29	2,94±0,24	2,27±0,07	2,23±0,18	2,39±0,07
C 20:4	4,81±0,34	4,40±0,36	5,26±0,48	8,47±0,11	9,12±0,20*	9,11±0,21*
C 20:5	3,32±0,32	2,69±0,21	2,96±0,30	5,43 ± 0,14	4,76±0,16*	4,39±0,10*
C 22:6	7,86±0,55	10,16±0,33*	14,02±0,93*	32,17±0,92	31,79 ± 1,13	31,66±1,35
∑ PUFA	26,23 0,76	27,29±1,17	32,31±0,74*	53,60 ± 0,99	53,78±1,50	54,62±1,30
Other	9,14±0,27	9,20±0,13	9,87±0,03	10,05±0,97	9,94±1,18	10,12±1,03

Note. * — difference from control is confident (P<0,05); SFA — saturated fatty acids, MUFA — monounsaturated fatty acids, PUFA — polyunsaturated fatty acids

In pike muscles, more systematic changes were found in the amount of other important PUFAs. In particular, this increase in the proportion of linoleic acid (18:2) by 34.5 % under the influence of 0.25 mg/dm³ of cobalt ions, arachidonic acid (20:4) by 7.6 % and 7.5 % under the influence of 0.1 and 0.25 mg/dm³ of cobalt ions, and a decrease in eicosapentaenoic acid (20:5) by 12.4 % and 19.1 % under the influence of both concentrations of the studied metal relative to the control values. Increased concentrations of linoleic (C18:2) and arachidonic (C20:4) fatty acids in tissues may indicate activation of pro-inflammatory pathways: linoleic acid is a precursor of arachidonic acid [48], which in turn is a substrate for the synthesis of pro-inflammatory eicosanoid [1, 8]. The augmented production of these mediators reflects a heightened immune response triggered by toxic or damaging influences. In fish, some highly unsaturated fatty acids with more than three double bonds, such as arachidonic acid (20:4), eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6), are considered essential [38]. These PUFAs are of crucial importance for the maintenance of vital activity and the normal physiological functioning of aquatic organisms. For example, eicosapentaenoic acid (20:5) is an excellent source of energy and a precursor to eicosanoids, which regulate inflammation and immune responses [6, 28].

Important indicators of environmental changes are nutritional indices for assessing fatty acids [9]. In particular, at the maximum concentration of cobalt ions in the muscles of crucian carp, an increase in the PUFA/SFA ratio of 32.5 % was found, indicating a shift in the balance towards higher unsaturation and, probably, a compensatory reaction to maintain membrane fluidity and cell stress signaling: membranes become more fluid and dynamic, oxidative damage is buffered, and the balance of lipid mediators shifts, which contributes to the survival of fish under the influence of toxic substances [10, 18]. The same trend towards an increase in the PUFA/SFA ratio was observed in pike muscles exposed to 0.25 mg/dm³ of cobalt ions. However, a number of other publications indicate the opposite — metal-induced lipid peroxidation breaks the double bonds of PUFAs, leading to a decrease in PUFA content in fish muscles [24, 27, 33]. Part of the adaptive response of the crucian carp organism may also be an increase in the unsaturation index by 22.2 % under the action of 0.25 mg/dm³ of cobalt ions, indicating a high degree of general unsaturation [9].

The increase in Fish Lipid Quality (FLQ) in crucian carp muscles by 15.0 % and 51.9 % at both studied concentrations of cobalt ions is an atypical result, as this metal in high concentrations is usually considered toxic to fish and can cause disturbances in lipid metabolism and overall health, which typically leads to a decrease rather than an increase in this nutritional index [4, 27, 30].

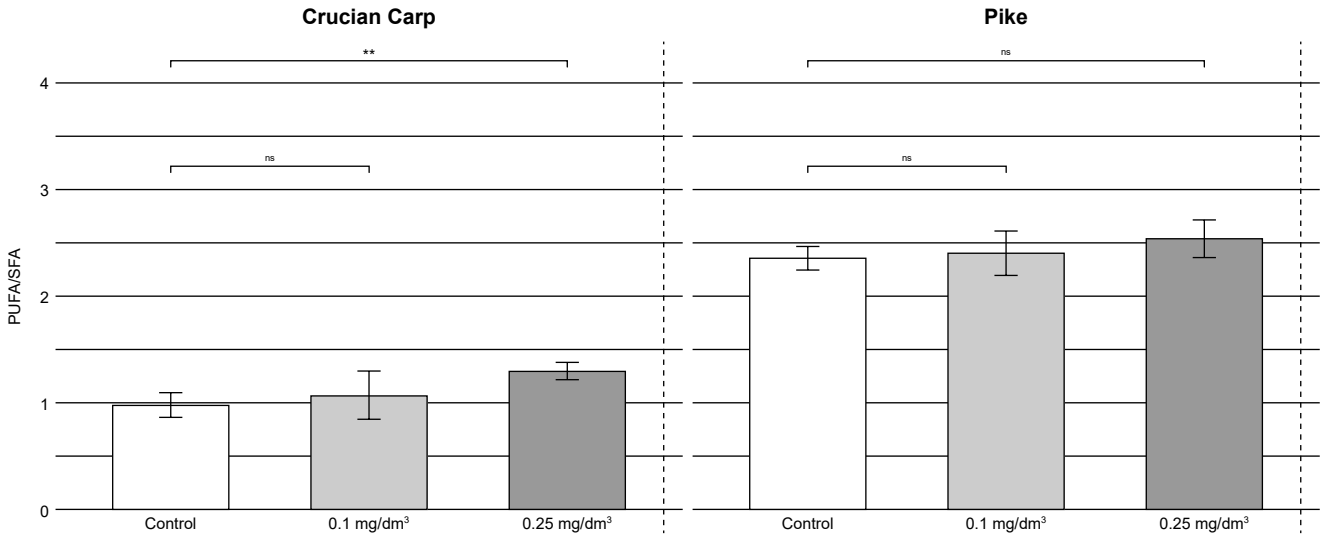

Fig. 1. The ratio of PUFA/SFA in fish muscles under the influence of cobalt ions, % (M±m, n=5)

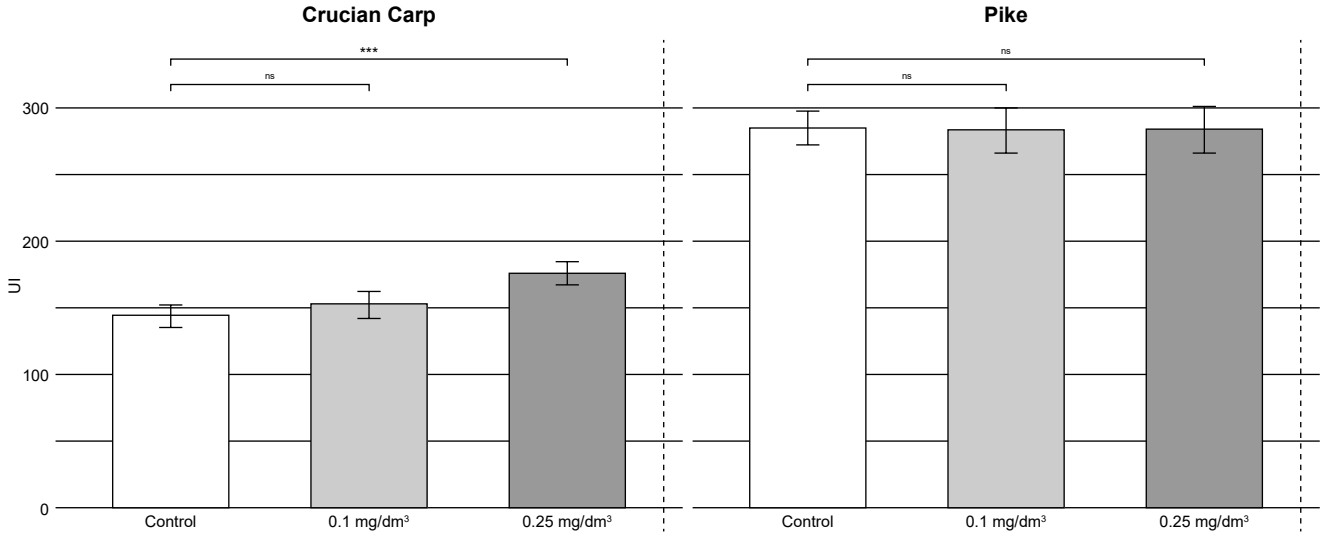

Fig. 2. The unsaturation index (UI) in fish muscles under the influence of cobalt ions, % (M±m, n=5)

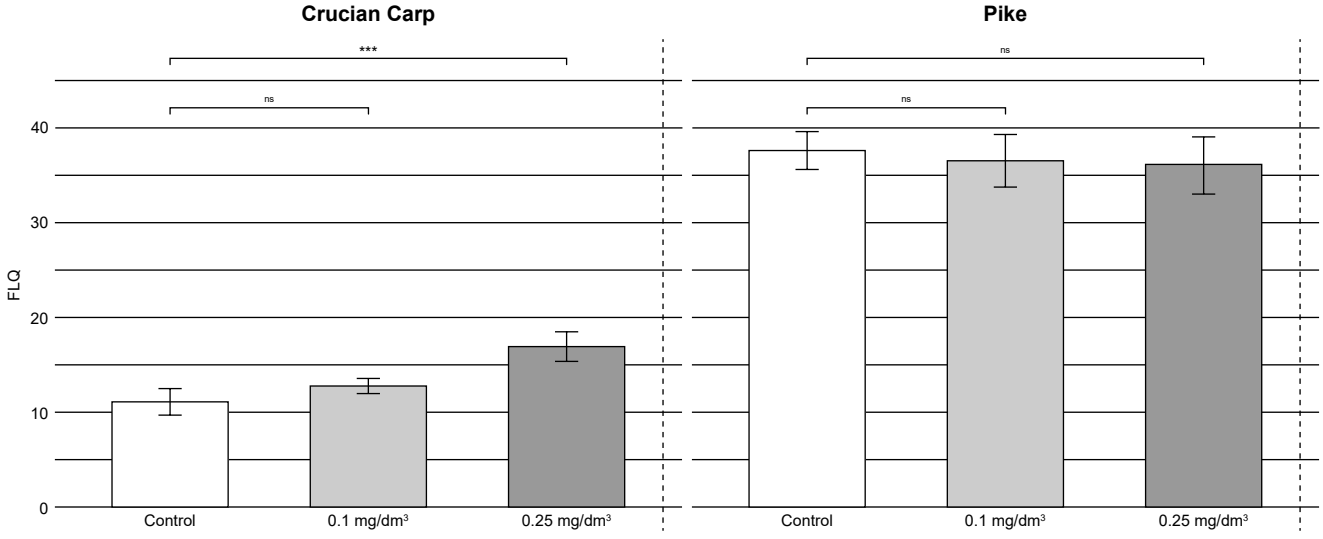

Fig. 3. The Fish Lipid Quality index (FLQ) in fish muscles under the influence of cobalt ions, % (M±m, n=5)

Therefore, it was established that the composition of fatty acids in fish muscles under the influence of elevated concentrations of cobalt ions in water is primarily determined by the concentration of the metal and the type of fish. In crucian carp, the influence of cobalt ions led to an increase in the proportions of myristic (14:0) and eicosenoic (20:1) acids, while the levels of stearic (18:0) and oleic (18:1) acids decreased. A more systemic response was observed in the pike's body: the content of many SFAs decreased compared to the control group, and multidirectional changes in the proportions of key PUFAs likely indicate a disruption in desaturase activity. It should be noted that highly specialized and finely regulated adaptive mechanisms are engaged to preserve the optimal fluidity of cellular membranes, thereby ensuring their structural integrity and functional performance in response to environmental fluctuations in aquatic habitats.

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Динаміка складу жирних кислот у м’язах карася та щуки під впливом підвищених концентрацій іонів кобальту

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У сучасних умовах зростаючого антропогенного навантаження на водні екосистеми проблема накопичення важких металів, зокрема іонів кобальту, стає особливо актуальною. Іони кобальту можуть потрапляти у водне середовище в результаті гірничодобувної діяльності, металургії, виробництва акумуляторів, барвників, каталізаторів та магнітних матеріалів. У сукупності це може призвести до локальних перевищень допустимих концентрацій у прісноводних екосистемах. Незважаючи на те, що кобальт є біогенним елементом у низьких концентраціях, його надлишок має токсичну дію на гідробіонтів, зокрема впливаючи на ліпідний гомеостаз. Одним із чутливих індикаторів порушень обміну речовин у риб є зміна жирнокислотного складу тканин, що може бути використано як біоіндикатор токсичного впливу та функціонального стану організму. У статті аналізуються зміни жирнокислотного складу м’язової тканини карася сріблястого (*Carassius gibelio* Bloch) та щуки звичайної (*Esox lucius* L.) під впливом іонів кобальту в концентраціях 0,1 мг/дм³ та 0,25 мг/дм³ протягом 14 днів. Ці концентрації відповідають 2 та 5 гранично допустимим концентраціям. Загальні ліпіди екстрагували та перетворювали на метилові ефіри жирних кислот, які потім аналізували методом газової хроматографії для кількісного визначення окремих жирних кислот. У карася вплив іонів кобальту призвів до суттєвих змін у складі основних жирних кислот. Зокрема, частки насиченої міристинової кислоти (14:0) та довголанцюгової мононенасиченої ейкозенової кислоти (20:1) збільшувалися пропорційно концентрації досліджуваного металу, тоді як рівні стеаринової кислоти (18:0) та олеїнової кислоти (18:1) знижувалися. В організмі щуки було відмічено більш системний ефект дії іонів кобальту, який полягав у тенденції до зменшення кількості більшості насичених жирних кислот. Також були проаналізовані деякі показники харчування для оцінки жирних кислот харчові індекси для оцінювання жирних кислот.

Ключові слова: риби, жирні кислоти, система захисту, фізіологічна реакція, регуляція, адаптивні реакції, біомоніторинг, токсичне забруднення, кобальт

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Моделювання впливу змін продуктивних показників у молочних корів на ефективність використання азоту корму

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Наведено узагальнений аналіз основних підходів щодо опису й розуміння особливостей регулювання розщеплення та ферментації поживних речовин корму в корів на прикладі моделі Моллі. Матеріалом для досліджень слугували дані 595 корів голштинської породи, які утримуються в умовах ТОВ «Агрофірма "Колос"» Київської області. Проведено аналіз зв'язку між такими вихідними параметрами, як: величина надою, вміст жиру, білку, лактози, рівень сечовини в молоці (MUN), жива маса корів і рівень надою, скоригований на енергетичну цінність (ECM). Як основний біомаркер використано значення MUN. Проведено розрахунки ефективності використання азоту для виробництва молока (MNE, англ. *milk nitrogen efficiency*) залежно від показників продуктивності. На основі регресійного аналізу отримано такі вірогідні залежності: MNE — добовий надій +3,29; MNE — вміст жиру в молоці −5,93; MNE — вміст білку в молоці −42,32; MNE — вміст лактози в молоці −17,45; MNE — MUN −2,49; MNE — жива маса корови −0,07; MNE — надій, скоригований на вміст енергії +3,12. На основі отриманих коефіцієнтів регресії змодельовано можливі зміни ефективності використання азоту, коли внаслідок збільшення абсолютного значення таких показників, як: вміст жиру, білку й лактози в молоці, MUN, живої маси корів, MNE зменшується, а внаслідок зростання добового надою та надою, скоригованого на енергетичний вміст, спостерігається збільшення MNE раціону для виробництва молока. Отримані дані свідчать про можливість використання індивідуальних або групових значень вмісту MUN і MNE на виробництві молока в програмах оцінки й відбору для надійного прогнозування отриманого ефекту за молочною продуктивністю, складом молока та ефективністю виробництва.

Ключові слова: азот сечовини молока (MUN), ефективність використання азоту для виробництва молока (MNE), білок, жир, лактоза

Вступ

Використовуючи інформацію про виробництво кормів та потреби в них у тварин в Сполучених Штатах Америки, A. Ostroski, O. A. Prokopyev, V. Khanna розробили аналітичну мережу просторових потоків азоту, пов'язаних з ланцюгами виробництва яловичини [16]. Автори запропонували алгоритм для підвищення ефективності кормів, шляхом переробки гною як джерела азоту для кормових культур та отримання відносно дешевої продукції тваринництва. M. Quemada, L. Lassaletta обґрунтували спосіб збільшення ефективності використання азоту NUE (*nitrogen use efficiency*) в системі вирощування сільськогосподарських культур (рослина-ґрунт), констатуючи суттєву залежність рослинництва від азотних добрив з 47 % до 95 % [17].

Загалом за останні десятиліття залежність глобальних систем вирощування сільськогосподарських культур від азотних добрив зросла до 83 %, а NUE стає новим показником для оцінки агроекологічної стійкості систем вирощування сільськогосподарських культур. За таких обставин виникає необхідність пошуку відповідних методик для оцінювання тих тварин, які ефективно використовують азот корму [1, 7, 18].

Корми для молочних корів містять азот у формі справжнього білку та небілкового азоту NPN (від англ. *non-protein nitrogen*). NPN включає такі сполуки: сечовина (англ. *urea* або карбамід — кінцевий продукт обміну білків в організмі), біурет (англ. *biuret* — аміди алофанової кислоти), солі амонію (англ. *ammonium salts*). NPN може бути перетворено на білок мікробами рубця, що робить їх додатковим джерелом справжнього білку [19]. Справжній білок включає азот, що міститься в амінокислотах, відрізняється швидкістю та повнотою його розщеплення в рубці й залежить від виду корму та його якісних показників [2, 7, 20, 26]. NPN може бути економічно ефективним способом доповнення білку в раціонах молочних корів, особливо якщо вміст справжнього білку низький. Таким чином, сирий протеїн CP (від англ. *crude protein*) у кормі для корів є мірою загального вмісту протеїну та включає: справжній білок, який складається з амінокислот; білки, що розщеплюються в рубці RDP (англ. *rumen degradable protein for specified feed*) — тип справжнього білку, який розщеплюється і перетворюється на вільні амінокислоти, пептиди й аміак, мікроорганізми рубця використовують їх для росту та створення мікробного білку — основного джерела білку для жуйних тварин; не розщеплюваний білок рубця RUP (від англ. *rumen undegradable protein*), відомий як обхідний білок, або тип справжнього білку, який проходить через рубець у незмінному вигляді [10, 13, 24]. Частина його всмоктується в тонкому кишківнику для забезпечення потреб в амінокислотах, а решта виводиться; небілковий азот (NPN) не є білком, але вміст азоту допомагає мікробам рубця виробляти мікробний білок. У табл. 1 представлено структуру груп за вмістом CP.

Таблиця 1. Групи сирого протеїну для великої рогатої худоби
Table 1. Crude protein groups for cattle

<table>
<tr><th colspan="3">Сирий протеїн CP (Crude protein)</th></tr>
<tr><td rowspan="2">Небілковий азот NPN (Nonprotein Nitrogens)</td><td colspan="2">Справжній протеїн, або натуральний протеїн TP (True Protein, Natural Protein)</td></tr>
<tr><td>Розщеплюваний протеїн RDP (Degradable Protein)</td><td>Нерозщеплюваний протеїн RUP (Undegradable Protein)</td></tr>
<tr><td colspan="2">Споживання розщеплюваного протеїну DIP (Degradable Intake Protein)</td><td>Споживання нерозщеплюваного протеїну UIP (Undegradable Intake Protein)</td></tr>
</table>

Джерело / Source. *Starblends.* [24]

Щоб розрахувати сирий протеїн у кормі, вміст азоту множать на 6,25, базуючись на тому, що білки зазвичай містять 16 % азоту (1/0,16 = 6,25), а зворотній розрахунок дає можливість визначити кількість азоту в кормі (1/6,25 = 0,16). Однак найточнішим способом визначення кількості азоту залишається лабораторний аналіз, який може надати точну інформацію про вміст сирого протеїну в кожному з видів корму. P. Huhtanen та ін. запропонували використовувати концентрацію азоту сечовини молока (NMCE), рівень якої залежить від концентрації та кількості сирого протеїну в раціоні [8]. Для цього NMCE використовується як біомаркер ефективності використання N для виробництва молока лактуючими коровами — MNE (англ. *milk nitrogen efficiency*). MNE розглядають як показник ефективності використання азоту, що надходить з кормом для утворення молока. За даними V. Souza, M. Wattiaux, азот сечовини молока (MUN) та азот сечовини крові (BUN) корелюють з балансом і виділенням азоту; однак є також генетична компонента концентрацій MUN, яка може бути пов'язана з відмінностями у транспорті сечовини [21, 25]. Було висунуто гіпотезу, що частина варіацій концентрацій MUN у корів спричинена відмінностями у шлунково-кишковому та нирковому кліренсі сечовини. В роботі S. Ruban та ін. доведено суттєвий вплив фактору породи за такими біохімічними показниками крові, як рівень загального білірубіну 24,70 %, сечовини 33,20 %, креатиніну 49,80 %, аланінамінотрансферази 10,40 %, аспартатамінотрансферази 46,30 %, альбуміну 35,10 % та загального білку 13,20 % [18]. Дослід проведено в однакових умовах утримання й годівлі, але на достатньо контрастних породах: українська червоно-ряба молочна, симентальська та українська чорно-ряба молочна. Сучасний погляд на фактор породи як важливий показник генетичного різноманіття передбачає пошук конкретних генетичних детермінант фенотипічної дисперсії.

Аналіз і попередні аналітичні звіти Xiaowei Zhao та ін. показують, що концентрація CP в раціоні є не єдиним фактором живлення, який впливає на концентрацію MUN [26]. Рівень у раціоні неструктурних вуглеводів NFC (англ. *non fiber carbohydrate*), до яких входять цукри, крохмаль і пектин, також відіграє важливу роль. Автори наводять лінійний регресійний аналіз для 91 набору експериментальних даних за співвідношенням NFC/CP у «вхідному» раціоні до концентрації MUN мг/дл, яка становить +0,68. Констатовано факт, що коли рівень MUN перевищує 14 або 16 мг/дл, відповідний вміст CP у раціоні, як правило, перевищує 17 %, що часто призводить до збільшення екскреції азоту із сечею. N. Hossein-Zadeh провів метааналіз з використанням моделі випадкових ефектів для інтеграції оцінок успадковуваності для MU і MUN, які були низькими та становили 0,20 та 0,18 відповідно [6]. Оцінка генетичних кореляцій між MUN та продуктивними ознаками були низькими й коливалися від −0,04 між MU та відсотком лактози в молоці, до 0,10 між MUN та виходом молочного білку, а також −0,07

між MUN і балом соматичних клітин та 0,36 між MUN і виходом молочного білку.

Метою нашої роботи було визначити взаємозв'язок і регресійну залежність між основними компонентами молока (вміст жиру, білку, лактози), величиною надою, живою масою корів, MUN та MNE. Основним інтегруючим біомаркером і предиктором складних біохімічних процесів виступило значення MNE, а на основі отриманих регресійних залежностей проведено прогнозування значення MNE відносно змін ознак основних компонент молока, величини надою та живої маси корів.

Матеріали і методи

Матеріалом для досліджень слугували дані експерименту на коровах голштинської породи в умовах Товариства з обмеженою відповідальністю «Агрофірма "Колос"» Київської області. Під час проведення експериментальних досліджень всі маніпуляції з коровами (595 голів) виконували з урахуванням основних принципів біоетики, відповідно до Статті 26 Закону України № 3447 «Про захист тварин від жорстокого поводження» (2006) [15], Європейської конвенції про захист хребетних тварин, які використовуються для експериментальних та інших наукових цілей (1986) [4] і Порядку проведення науковими установами дослідів, експериментів на тваринах (2012) [14]. У господарстві використовують прив'язну систему утримання з доїнням в молокопровід. Доїння триразове, один дояр обслуговує до 50 корів. Годівлю проводять з використанням загального змішаного раціону (TMR), характеристику якого подано в табл. 2, 3.

Співвідношення NFC/CP важливе, оскільки воно впливає на утворення молока, особливо молочного білку, і може зумовлювати ризики зі здоров'ям та відтворенням корів. Збалансоване співвідношення NFC/CP коливається від 2,15 до 3,60, що зазвичай бажане для підтримки оптимального рівня MUN [23]. Згідно із загальноприйнятими нормами, рівень NFC у TMR визначено шляхом віднімання рівня цих компонентів у відсотках за сухою речовиною у TMR від 100 %. Водночас NFC включає крохмаль, цукор і пектин, а визначення їхнього вмісту базувалося на розрахунку решти відсотків після врахування рівня клітковини, білку, жиру й золи.

Для оцінки ефективності використання азоту під час виробництва молока використано модель (1) для прогнозування за P. Huhtanen та ін. [8], розроблену на основі залишкової дисперсії для прогнозування багатофакторної нестабільності (MNE) виробничих даних, яка найбільш логічно описує цей процес:

$$\text{MNE (г/кг)} = 238 + 7{,}0\ (\text{MY кг/день}) - 0{,}064 \times (\text{MY2}) - 2{,}7 \times (\text{MUN мг/дл}) - 0{,}10 \times (\text{W}) \quad (1),$$

де MY — молочна продуктивність, кг;
MUN — рівень азоту сечовини молока, мг/дл;
W — маса корови, кг.

При цьому MNE розглядається як показник ефективності використання азоту, який надходить з кормом, для утворення молока.

Кількість сечовини в молоці визначали діацетилмонооксимним методом. Про її рівень судили за вмістом червоного комплексу, утвореного сечовиною з діацетилмонооксимом у кислому середовищі за наявності тіосемікарбозіда й тривалентного заліза за методикою N. Langenfeld та ін. [11]. Молярну концентрацію сечовини (C) в значені ммоль/л визначали за отриманими даними оптичної густини досліджуваної проби A відносно стандарту B за формулою (2):

$$C = 8{,}33\ \frac{A}{B} \quad (2).$$

Надій, скоригований на енергію (ECM), розраховували згідно з M. Hall [5] за формулою:

$$\text{ECM} = [(\text{вміст жиру, \%} + \text{вміст білку, \%} \times 242 + \text{вміст лактози, \%} \times 165{,}4 + 20{,}7)/3140] \times \text{молочна продуктивність, кг} \quad (3).$$

Статистичний аналіз (описова статистика, дисперсійний аналіз, кореляційний і регресійний аналіз) проведено з використанням програми *RStudio-2023.03.0-386*.

Для вивчення взаємозв'язку між залежною змінною (результатом) та однією або кількома незалежними змінними (факторами) використано класичне рівняння регресії (4):

$$y = a + bx + e \quad (4),$$

де a — вільний член моделі;
b — коефіцієнт регресії;
x — змінна величина;
e — похибка.

Аналіз якісних та біохімічних показників молока визначали на ультразвуковому аналізаторі «EKOMILK Bond».

Результати

Для коректного опису особливостей травлення азоту, поглинання, засвоєння і метаболізму, обміну речовин, рівня сечовини, а також ефективності утилізації азоту з кінцевим аналізом молока [12], використано модель корови Моллі (рис. 1).

За P. Gregorini та ін. ця модель належить до класу детермінованої, механістичної, динамічної моделі, що враховує травлення, метаболізм і утворення продукції дійною коровою на основі математичних рівнянь [4]. Автори зіставили прогнози ентерального (в умовах проходження кормового субстрату через весь шлунково-кишковий тракт) продукування метану з вихідною математичною версією Моллі (*Molly Origin*)

Таблиця 2. Характеристика суміші TMR для корів голштинської породи
Table 2. Characteristics of TMR mixture for Holstein cows

Інгредієнти раціону Diet ingredients	Вага, кг / Weight, kg		Відсоток / Percentage	
	фізична physical	за сухою речовиною by dry matter	за фізичною вагою by physical weight	за сухою речовиною by dry matter
Силос кукурудзяний / Corn silage	28,00	9,24	49,36	37,55
Зернова суміш / Grain mix	8,72	7,97	15,38	32,37
Пивна дробина (волога) Brewer's grain (wet)	5,00	0,92	8,81	3,76
Сінаж люцерни / Alfalfa silage	3,50	1,84	6,17	7,47
Жом / Beet pulp	4,00	0,56	7,05	2,28
Зерно кукурудзи підвищеної вологості Corn grain (wet)	2,20	1,54	3,88	6,26
Шрот соняшниковий / Sunflower meal	1,50	1,38	2,64	5,63
Солома / Straw	0,80	0,70	1,41	2,86
Вода / Water	2,00	0,00	3,53	—
Меляса бурякова / Beet molasses	1,00	0,45	1,76	1,83
Всього / Total	56,72	24,60	100,00	43,40

Примітка. Розраховували для корів, вагою 550–650 кг, з надоєм 28–30 кг, вмістом у молоці жиру 4,00 %, вмістом у молоці білка 3,40 % рівнем лактози 4,68 %.
Джерело. За результатами хімічного аналізу компанії АВМ від 25.04.2025.

Note. calculated for cows weighing 550-650 kg, milk yield 28-30 kg, fat content 4.00 %, protein content 3.40 %, lactose level 4.68 %.
Source. Based on the results of chemical analysis by ABM on 25 April 2025.

Таблиця 3. Біохімічна характеристика TMR дійних корів
Table 3. Biochemical characteristics of TMR in dairy cows

Компоненти Components	Вміст за сухою речовиною, % Dry matter,%	Вміст, г Content, g
Сирий протеїн / Crude protein	15,84	3 897,69
Нейтрально-детергентна клітковина / Neutral detergent fiber	28,95	7 124,15
Нейтрально-детергентна клітковина фуражу Neutral detergent fiber in forage	20,44	5 030,74
Кислотно-детергентна клітковина / Acid detergent fiber	19,41	4 777,21
Цукор / Sugar	6,15	1 513,01
Крохмаль / Starch	26,07	6 416,21
Розчинна клітковина / Soluble fiber	7,93	1 951,36
Зола / Ash	7,64	1 879,01
Кальцій / Calcium	0,65	160,46
Фосфор / Phosphorus	0,40	98,13
Магній / Magnesium	0,44	107,55
Калій / Potassium	1,11	272,68
Фураж / Forage	47,87	—
Концентрат / Concentrates	52,13	—
Загальні вуглеводи / Total carbohydrates	73,15	18 002,88
Аміак / Ammonia	0,48	119,06
Неструктурні вуглеводи / сирий протеїн Non-fiber carbohydrates / crude protein	2,03	—

Примітка. За результатами хімічного аналізу компанії АВМ від 25.04.2025.
Note. Based on the results of chemical analysis by ABM dated 25 April 2025.

Таблиця 4. Описова статистика досліджуваних ознак (n=595)
Table 4. Descriptive statistics of the studied characteristics (n=595)

Ознака / Characteristic	Min	Max	M±m	σ^2	σ	Cv, %
Добовий надій, кг / Daily milk yield, kg	10,00	56,00	27,7±0,36	70,20	8,40	30,30
Вміст жиру / Fat content, %	3,17	5,73	4,39±0,08	0,68	0,82	16,70
Вміст білку / Protein content, %	2,40	4,00	3,40±0,01	0,03	0,17	5,00
Вміст лактози / Lactose content, %	0,50	5,80	4,68±0,01	0,10	0,32	6,80
pH	2,10	7,40	7,09±0,01	0,06	0,24	3,40
MUN, мг/дл	2,60	32,90	12,31±0,24	35,60	5,97	48,50
Жива маса корів, кг / Live weight of cows, kg	488,00	650,00	526,40±0,75	308,00	17,50	3,30
MNE* раціону для виробництва молока MNE* of diet for milk production	116,60	365,50	287,60±1,65	1622,60	40,30	14,00
Надій, скоригований на енергетичний вміст (ECM) Milk yield adjusted for energy content (ECM)	10,30	66,60	29,13±0.35	70,00	8,37	28,70

Примітка. * MNE — ефективність використання азоту для виробництва молока визначається як співвідношення споживання азоту до азоту в молоці.
Джерело. Розроблено авторами на основі досліджень.
Notes. * MNE nitrogen efficiency for milk production is defined as the ratio of nitrogen consumption to nitrogen in milk.
Source. Developed by the authors based on research.

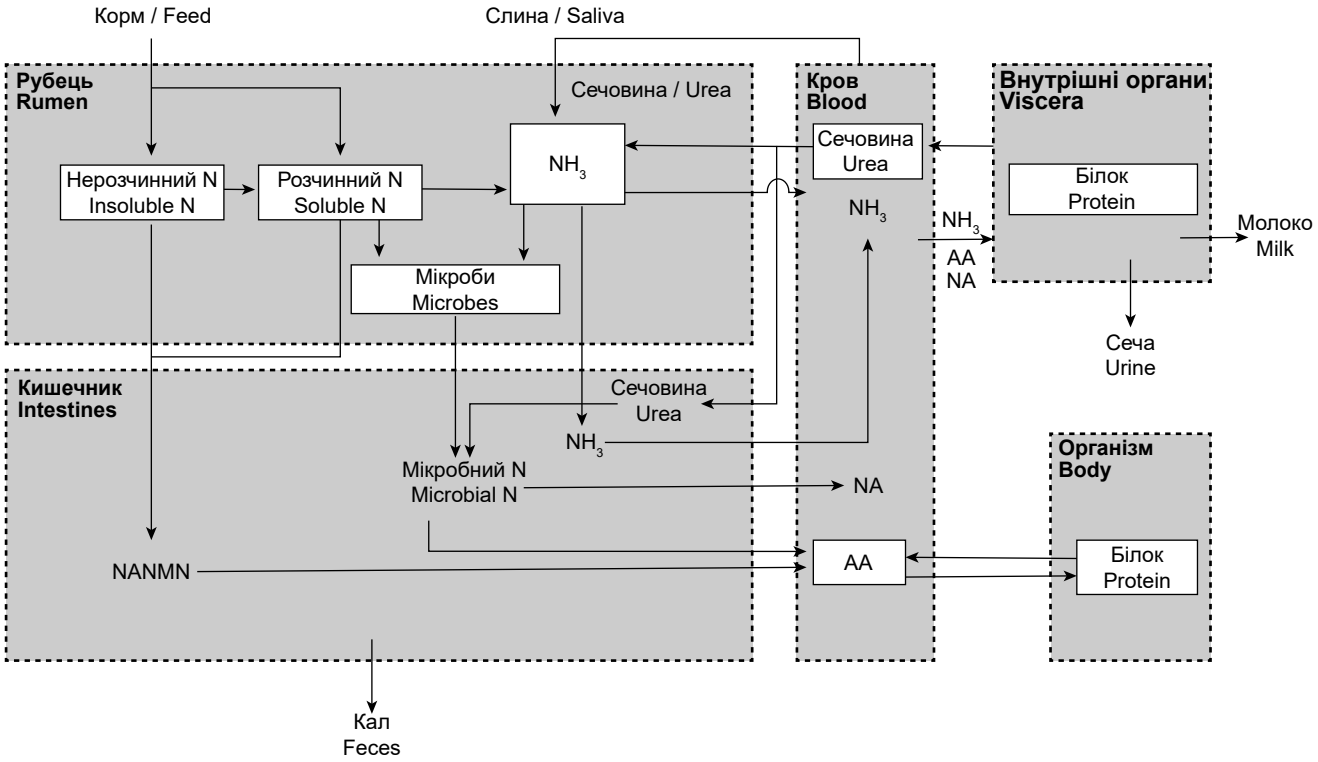

Рис. 1. Концептуальна схема метаболізму азоту в жуйних — модель Моллі
Примітка. NANMN (nonammonia, nonmicrobial nitrogen) — неаміачний, немікробний азот; NA (nucleic acids) — нуклеїнові кислоти; AA (amino acids) — амінокислоти; N — азот; NH_3 — нітрит водню (аміак); Feed — корми; Rumen — рубець; Intestines — кишківник; Saliva — слина; Blood — кров; Viscera — внутрішні органи; Body — тіло; Feces — фекалії.
Затемнені зони — це органи і тканини, де відбуваються біохімічні процеси, а стрілками позначено напрямки руху цих процесів.
Джерело. Li M. M. [12].

Fig. 1. Conceptual diagram of nitrogen metabolism in ruminants – the 'Molly model'
Note. NANMN — nonammonia, nonmicrobial nitrogen; NA — nucleic acids; AA — amino acids; N — nitrogen; NH3 — hydrogen nitrite (ammonia); Feed — feed; Rumen — rumen; Intestines — intestines; Saliva — saliva; Blood — blood; Viscera — internal organs; Body — body; Faeces — faeces. The shaded areas represent organs and tissues where biochemical processes occur, and the arrows indicate the direction of these processes.
Source. Li M. M. [12].

і двома оновленими на основі низки біохімічних і генетичних показників, що відповідає схемі цього експерименту. Ці версії включали вплив кількості клітковини у вмісті рубця, параметри травлення та гормональні параметри (Molly84) з переглянутою версією травних і румінальних параметрів (Molly85).

Пізніше Meng M. Li, Mark D. Hanigan [12] підвищили точність прогнозованих концентрацій летких жирних кислот (*precision of predicted volatile fatty acids*) завдяки вдосконаленому відображенню регуляції pH та швидкості виробництва ЛЖК. РН рубця є критичним фактором для регулювання розщеплення й ферментації поживних речовин. У попередній моделі корови Моллі це не було відображено достатньо. Проведені дослідження полягали в удосконаленні відображення pH та переналаштуванні параметрів, пов'язаних із метаболізмом рубця та перетравленням поживних речовин. Вдосконалена модель може використовуватися для оцінювання потоків азоту й енергії з різним вмістом білку, який розщеплюється рубцем — RDP (*rumen-degradable protein*) у величинах 40 % проти 60 %, а також крохмалю, який розщеплюється рубцем — RDSt (*ruminally degraded starch*) відповідно 50 % проти 75 %.

Ми розглянули зв'язок MNE для виробництва молока залежно від значень добового надою, вмісту MUN і живої маси корів (рис. 2, 3, 4).

Зі збільшенням добового надою ефективність використання азоту для виробництва молока (MNE) зростає. Досягнувши рівня надою 53 кг, MNE знижується. Це обумовлено тим, що формула розрахунку ефективності використання азоту для виробництва молока (формула 1) містить квадрат добового надою з від'ємним коефіцієнтом (−0,06). Це пояснюється високою концентрацією всіх компонентів CP в одному кілограмі сухої речовини корму для забезпечення потреб організму. Це призводить до більших втрат азоту з екскретами й молоком.

Аналогічна залежність виникає між MNE для виробництва молока та вмістом MUN, а також живою масою корів (рис. 2, 3).

Збільшення цих показників веде до монотонного зниження ефективності використання азоту для виробництва молока (MNE), оскільки вони входять до формули розрахунку ефективності використання азоту для виробництва молока (формула 1) з від'ємними коефіцієнтами (−2,70 та −0,10 відповідно).

За різними даними оптимальне значення MUN коливається від 10 мг/дл до 14 мг/дл, але для багатьох країн рекомендовані значення азоту сечовини молока для коров'ячого молока коливаються від 10 мг/дл до 16 мг/дл молока [18, 22]. Зміни NME відносно значень живої маси корів (рис. 4), свідчать про необхідність більших витрат як білкових, так і енергетичних компонент корму, — обмін, прямо пропорційно пов'язаний з більшою живою масою корів.

Своєю чергою така залежність сприяє зменшенню непродуктивного використання корму на синтез молочної продукції.

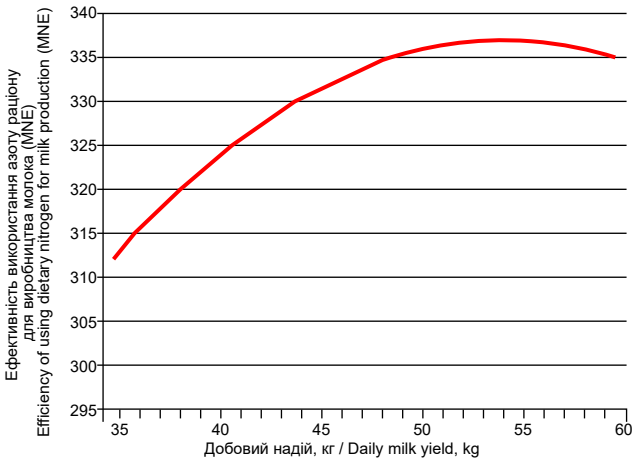

Рис. 2. Залежність MNE раціону від добового надою
Джерело. Розроблено авторами на основі досліджень.
Fig. 2. Dependence of MNE ration on daily milk yield.
Source. Developed by the authors based on research.

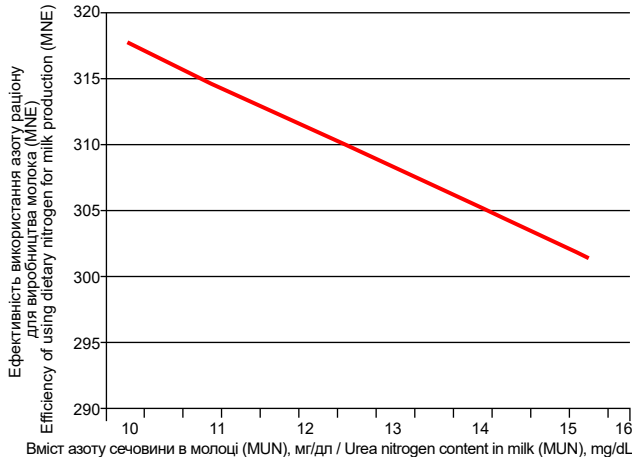

Рис. 3. Залежність MNE від MUN
Джерело. Розроблено авторами на основі досліджень.
Fig. 3. Dependence of MNE on MUN.
Source. Developed by the authors based on research.

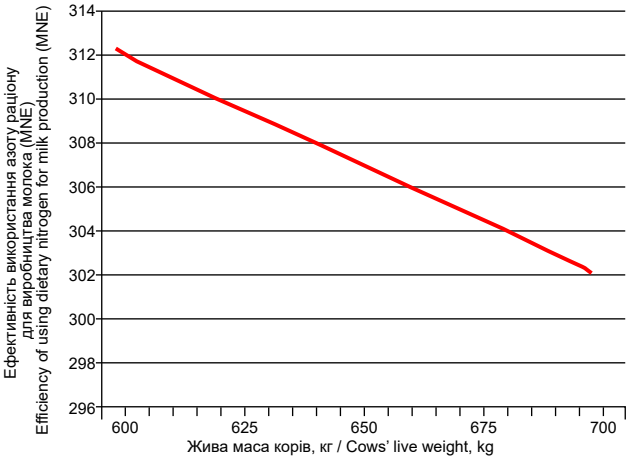

Рис. 4. Залежність MNE раціону від живої маси корів
Джерело. Розроблено авторами на основі досліджень.
Fig. 4. Dependence of MNE ration on live weight of cows.
Source. Developed by the authors based on research.

У табл. 5 наведено розрахунки регресійної залежності (b) основних компонентів молока (x) на NME.

На основі вірогідних рівнянь регресії отримано наступні залежності: MNE = 201,82 + 3,29* (добовий надій) + e; MNE = 319,80 − 5,93*(вміст жиру в молоці) + e; MNE = 437,16 − 42,32* (вміст білку в молоці) + e; MNE = 374,90 − 17,45* (вміст лактози в молоці) + e; MNE = 323,49 − 2,49*MUN + e; MNE = 330,05 − 0,07* (жива маса корови) + e; MNE = 202,37 + 3,12* (надій, скоригований на вміст енергії) + e.

За аналітичними даними X. Zhao та ін. [27] можна констатувати: будь-які фактори, що викликають зміни MUN, можуть впливати на компоненти молока; збільшення споживання CP, що супроводжується підвищенням концентрації MUN, не впливає на вихід молочного білку, тоді як низький вміст CP може зменшити екскрецію сечовини на фенотиповому рівні, не впливаючи негативно на вміст молочного білку; на початку лактації молочні корови переживають період негативного енергетичного балансу, що призводить до збільшення відсотка молочного жиру через мобілізацію жирової тканини; впродовж цього періоду MUN зазвичай демонструє відносно низькі значення через недостатнє споживання корму, порівняно з іншими періодами лактації; можлива сильна позитивна генетична кореляція (+0,85) між MUN і кількістю соматичних клітин, оскільки виникнення маститу може впливати на концентрацію MUN у молоці, а середня генетична кореляція між MUN та лактозою залежна від цього; на кореляцію між MUN і складом молока впливає стадія лактації та стан здоров'я, завдяки чому зв'язок між MUN і ознаками компонентів молока є слабким або навіть незначним.

Потенціал відбору корів з низьким фенотипом MUN для зменшення виділення азоту не впливає негативно на продуктивність і якість молока. За умови зниження ознак MUN шляхом селекційного відбору необхідно оцінити їхню кореляцію з іншими ознаками [27].

За висновками P. Huhtanen та ін., концентрація MUN не є корисним інструментом фенотипування для поліпшення MNE, але вимірювання концентрації MUN на рівні стада дозволяють точно налаштувати раціон для підвищення перетравлюваності або MNE [8]. За даними V. Ishler, найбільш рекомендовані діапазони MUN коливаються від 8 мг/дл до 12 мг/дл, що відображає раціони з балансом білку 16 %, та наявність білкових фракцій і вуглеводів для уловлювання надлишком аміаку в рубці [9]. У табл. 6 наведено результати моделювання змін MNE раціону для виробництва молока при зміні значень добового надою, показників молока, живої маси корів і надою, скоригованого на енергетичний вміст відповідно до рівнянь лінійної регресії, наведених у табл. 5.

Встановлено, що збільшення MNE раціону для виробництва молока виявлено лише за двома показниками: добовому надою та надою, скоригованому за енергетичним вмістом. Внаслідок збільшення таких показників, як вміст жиру, білку, лактози, MUN та живої маси корів, MNE раціону для виробництва молока зменшується.

Низькі значення MUN (<8–10 мг/дл) вказують на можливий дефіцит білку в раціоні, який може виникнути, коли знижується активність бактерій рубця, що обмежує утворення молока й синтез молочного білку.

Аналізуючи експериментальні дані P. Huhtanen та ін. [8], V. Ishler [9], а також отримані в цій роботі результати (рис. 2, 3, 4) зроблено попередні висновки про коректність оцінок MNE при значеннях рівня MUN в межах 8–12 мг/дл. Вважаємо доцільним використовувати ці параметри в програмах оцінки та відбору для надійного прогнозування отриманого ефекту за молочною продуктивністю, складом молока і MNE.

Таблиця 5. Регресійна залежність (b) основних компонентів молока (x) від MNE для виробництва молока, кількість спостережень — 595 голів.
Table 5. Regression dependence (b) of the main components of milk (x) on MNE for milk production, number of observations — 595 heads.

Ознака (x) Characteristic (x)	Рівняння регресії, де: Regression equation where:		*t*
	вільний член (a) free term (a)	коефіцієнт регресії (b) regression coefficient (b)	
Добовий надій / Daily milk yield	201,82±2,54	3,29±0,09	37,55***
Вміст жиру в молоці / Fat content in milk	319,80±5,71	−5,93±1,24	2,02***
Вміст білку в молоці / Protein content in milk	437,16±26,47	−42,32± 7,77	7,63***
Вміст лактози в молоці / Lactose content in milk	374,90±19,76	−17,45±4,21	3,88***
MUN	323,49±2,82	−2,50±0,21	11,94***
Жива маса корови / Live weight of cow	330,05±20,11	−0,07±0,04	1,84
Надій, скоригований з огляду на вміст енергії Milk yield adjusted for energy content	202,37±3,00	3,12±0,10	31,49***

Примітка. *** — P>0,999.
Джерело. Розроблено авторами на основі досліджень.
Note. *** — P>0.999.
Source. Developed by the authors based on research.

Таблиця 6. Моделювання змін MNE раціону для виробництва молока
Table 6. Modelling changes in the MNE ration for milk production

Контрольний показник Control indicator	Зміна добового надою, кг / Change in daily milk yield, kg						
	5,00	10,00	15,00	20,00	25,00	30,00	35,00
MNE	218,27	234,72	251,17	267,62	284,07	300,52	316,97
Зміна вмісту жиру в молоці / Change in milk fat content, %							
	0,50	1,00	1,50	2.00	2,50	3,00	3,50
MNE	316,84	313,87	310,91	307,94	304,98	302,01	299,05
Зміна вмісту білку в молоці / Change in milk protein content, %							
	0,50	1,00	1,50	2.00	2,50	3,00	3,50
MNE	416,00	394,84	373,68	352,52	331,36	310,20	289,04
Зміна вмісту лактози в молоці / Change in lactose content in milk, %							
	0,50	1,00	1,50	2.00	2,50	3,00	3,50
MNE	366,18	357,45	348,73	340,00	331,28	322,55	313,83
Зміна вмісту азоту сечовини в молоці (MUN), мг/дл / Change in milk urea nitrogen (MUN) content, mg/dl							
	5,00	10,00	15.00	20,00	25,00	30.00	35.00
MNE	311,04	298,59	286,14	273,69	261,24	248,79	236,34
Зміна живої маси корови, кг / Change in live weight of cows, kg							
	10,00	30,00	50,00	70,00	90,00	110,00	130,00
MNE	329,35	327,95	326,55	325,15	323,75	322,35	320,95
Зміна надою, скоригованого на енергетичний вміст, кг / Change in milk yield, adjusted for energy content, kg							
	10,00	20,00	30,00	40,00	50,00	60,00	70,00
MNE	233,57	264,77	295,97	327,17	358,37	389,57	420,77

Примітка. Враховується зміна значень добового надою, показників молока, живої маси корів і надою, скоригованого на енергетичний вміст.
Джерело. Розроблено авторами на основі досліджень.
Note. Changes in daily milk yield, milk indicators, live weight of cows and milk yield adjusted for energy content are taken into account.
Source. Developed by the authors based on research.

Такі компоненти молока, як вміст жиру, білку, рівень лактози та MUN, мають широкий діапазон свого фенотипового прояву, що характеризує складні біохімічні процеси в організмі жуйних, із багатьма чинниками впливу на варіабельність цих ознак. Попередні припущення вказують на необхідність встановлення зв'язку між MUN як індикаторної ознаки щодо виділення залишкового азоту та цільовою ознакою, що визначається як індивідуальна характеристика кожної корови. Сам принцип пошуку генетичного впливу на такі коливання треба будувати на основі аналізу динаміки повторів оцінок MUN в часі у розрізі генетичних груп. Продовження досліджень в цьому напрямку дасть змогу розширити набір аналітичних даних, а в поєднанні з методами оптимізації та моделювання — дати конкретні рекомендації щодо використання таких комплексних оцінок. Пропонується використовувати індивідуальні або групові значення вмісту азоту сечовини в молоці та ефективність використання азоту на утворення молока в програмах оцінки й відбору для надійного прогнозування отриманого ефекту за молочною продуктивністю, складом молока та ефективністю виробництва.

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Modelling the impact of changes in productive indicators in dairy cows on the efficiency of feed nitrogen utilization

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A generalized analysis of the main approaches to describing and understanding the characteristics of the regulation of the breakdown and fermentation of feed nutrients in cows is presented using the example of the 'Molly Model'. The research was based on data from 595 Holstein cows kept at the Kolos Agricultural Firm LLC in the Kyiv region. An analysis was conducted of the relationship between such 'output' parameters as: milk yield, fat, protein, lactose content, milk urea nitrogen (MUN), live weight of cows, and milk yield adjusted for energy content (ECM). MUN values were used as the main biomarker. Calculations were made of the efficiency of nitrogen use for milk production (MNE, *milk nitrogen efficiency*) depending on productivity indicators. Based on regression analysis, the following reliable dependencies were obtained: MNE — daily milk yield +3.29; MNE — milk fat content −5.93; MNE — milk protein content −42.32; MNE — lactose content in milk −17.45; MNE — MUN −2.49; MNE — live weight of the cow −0.07; MNE — milk yield adjusted for energy content +3.12. Based on the obtained regression coefficients, possible changes in nitrogen use efficiency were modeled, where an increase in the absolute values of indicators such as fat, protein and lactose content in milk, MUN, live weight of cows and MNE leads to a decrease, and when the daily milk yield and milk yield adjusted for energy content increase, there is an increase in the MNE of the diet for milk production. The data obtained indicate the possibility of using individual or group values of MUN and MNE content in milk production in assessment and selection programs for reliable prediction of the effect obtained for milk productivity, milk composition and production efficiency.

Key words: milk urea nitrogen (MUN), milk nitrogen efficiency (MNE), protein, fat, lactose

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Біохімічні показники крові поросят за дії ізотонічно-протеїнової суміші

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Метою роботи є визначення впливу застосування ізотонічно-протеїнової суміші (ІПС) у період відлучення на біохімічні показники крові поросят. Дослідження проводили на поросятах раннього постнатального періоду, яких розділили на контрольну та дослідну групи. У тварин обох груп у 9-, 21- та 35-денному віці проводили відбір крові, в якій визначали вміст основних метаболітів і активність ензимів. Встановлено, що після відлучення у поросят відзначали виражену метаболічну перебудову. Рівень загального протеїну зріс до 55,7±3,6 г/л, а після відлучення знизився на 17,2 % ($P<0,05$) до 46,1±4,7 г/л. Альбуміни зросли на 20,4 % ($P<0,05$), проте після відлучення знизилися на 5,1 % ($P>0,05$). Глобуліни до 21-го дня знизилися на 6,6 % ($P>0,05$), а після відлучення — ще на 28,8 % ($P<0,001$). Рівень сечовини зріс на 55,4 % ($P<0,05$) у 21-добовому віці, однак після відлучення він залишався підвищеним лише на 8,6 % ($P>0,05$). Рівень ферментів печінки (ALT, AST) зменшувався до 21-добового віку на 19,2 % ($P>0,05$) та 33,5 % ($P<0,05$) відповідно, проте після відлучення активність ALT зросла на 35,3 %, а AST — на 8,8 %. Вуглеводний метаболізм зазнавав змін: рівень глюкози знизився на 11,7 % ($P>0,05$) до 21-го дня, а після відлучення — ще на 25,3 % ($P<0,05$). У поросят дослідної групи спостеріли стабільніший метаболічний стан. Рівень загального протеїну зріс на 19,3 % ($P<0,05$) до 21-го дня та після відлучення знизився лише на 12,0 % ($P<0,05$). Альбуміни зросли на 22,0 % ($P<0,05$) до 21-го дня та після відлучення залишалися на 5,8 % ($P>0,05$) вищими за контроль. Глобуліни спочатку зменшилися на 12,8 % ($P>0,05$) до 9-го дня, але до 21-го дня їх рівень зріс на 16,9 % ($P<0,05$) і залишався на 25,6 % ($P<0,05$) вищим після відлучення. Рівень сечовини зріс на 19,4 % ($P>0,05$) до 21-го дня, але після відлучення знизився на 11,6 % ($P<0,05$), що свідчить про ефективніше використання білкових ресурсів. ALT залишався стабільним, тоді як AST зріс лише на 6,4 %, що вказує на кращу адаптацію печінки. Вуглеводний обмін у поросят дослідної групи був стабільнішим: рівень глюкози зріс на 3,8 % ($P>0,05$) до 21-го дня, а після відлучення був на 11,3 % вищий, ніж у контрольній групі. Застосування ІПС сприяє стабілізації білкового й енергетичного обміну, зменшенню стресових реакцій після відлучення та покращенню загального метаболічного статусу.

Ключові слова: поросята, метаболізм, ізотонічно-протеїнова суміш, відлучення

Вступ

В умовах сучасного тваринництва одним із найкритичніших періодів у житті поросят є ранній постнатальний онтогенез, особливо в період відлучення. Саме в цей час організм тварин зазнає значних фізіологічних змін, пов'язаних з адаптацією до нових умов годівлі та навколишнього середовища [19]. Одним з ключових аспектів успішного подолання цього періоду є підтримка бар'єрної та імунної функцій кишківника, що безпосередньо впливає на здоров'я, виживаність і продуктивність тварин. Водночас традиційні підходи, зокрема застосування антибіотиків для профілактики та лікування дисбактеріозів і кишкових розладів, мають низку недоліків, серед яких особливу стурбованість викликає зростання антибіотикорезистентності мікроорганізмів [13]. Однак, незважаючи на значні досягнення у ветеринарній медицині, проблема порушення бар'єрної функції кишківника й дисбалансу мікробіому в поросят залишається актуальною та потребує сучасних рішень і нових підходів. Передбачається, що застосування ізотонічного протеїнової суміші (ІПС) може сприяти нормалізації структурно-функціонального стану кишкового епітелію, стимулювати розвиток місцевого імунітету, стабілізувати мікробіом і покращувати енергетичний обмін в ентероцитах [16]. Ці добавки також сприяють збереженню цілісності епітелію кишківника, модуляції імунної відповіді та можуть скорочувати тривалість виділення вірусу PEDV [17]. Застосування таких технологій у годівлі поросят може не лише сприяти підвищенню їх стійкості до стресів, а й оптимізувати метаболічні процеси, що в підсумку позитивно позначається на їхньому здоров'ї, темпах росту та продуктивності [2, 3, 12].

Відомо, що застосування ІПС у поросят віком від 2-х до 8-ми днів сприяло зниженню смертності до відлучення, зростанню приростів після відлучення, а також стимулювало розвиток кишківника шляхом збільшення висоти ворсинок [6, 7]. Buzoianu та співавт. (2020) встановили, що така суміш позитивно впливає на мікробіоту кишківника, збільшуючи кількість корисних бактерій (*Lactobacillus*, *Bacteroides*) і знижуючи рівень патогенних мікроорганізмів, зокрема *Escherichia coli* [2, 10]. Додатково відзначено зростання популяцій бактерій із ферментативною й імуномодулювальною активністю (зокрема *Veillonella*). У дослідженні за участю 3862 поросят смертність до відлучення знизилася на 22,8 %, а середня маса на момент народження зросла на 0,41 кг [4]. Встановлено, що ефект ІПС проявляється незалежно від маси на момент народження: смертність знижувалася на 13–20 % залежно від початкової маси [1].

Тому актуальним напрямом наукових досліджень є встановлення впливу ізотонічно-протеїнової суміші на стан метаболізму поросят у критичні періоди раннього онтогенезу. Водночас результати досліджень можуть стати фундаментом для подальших наукових розробок і практичних рекомендацій у ветеринарній медицині та сучасному тваринництві.

Мета дослідження — вивчити вплив згодовування ізотонічно-протеїнової суміші поросятам у період відлучення на біохімічні показники крові.

Матеріали і методи

Дослід проведено в господарстві ТОВ «Агроінд» (Дніпропетровська обл.). Для експерименту відібрано 168 трипородних гібридних поросят генетики *DanBred* у віці двох днів, яких методом аналогів розподілили на контрольну та дослідну групи по 84 тварини в кожній. Починаючи з 3-го до 8-го дня життя, поросятам дослідної групи щоденно випоювали ізотонічно-протеїнову суміш *Tonisity Px* (Ірландія), у дозі 40 мл/порося/добу, до складу якої входять: декстроза (52 %), натрію хлорид (10 %), калію хлорид (5 %), сироватковий білок (5 %), мононатрійфосфат (4 %), смакові сполуки (16 %), консервант (6 %), стабілізатор (2 %). Поживна цінність: сирий протеїн — 16,6 %, сирі жири — 0,7 %, сира зола — 19,1 %, сира клітковина — <0,1 %, натрій — 5,2 %. Поросят контрольної групи утримували за стандартною технологією без використання суміші. Відлучення поросят від свиноматок проводили на 26-й день життя. Для оцінки стану метаболізму у 9-, 21- та 35- денному віці в поросят проводили відбір крові (по 10 зразків з кожної групи) з краніальної порожнистої вени в пробірки без антикоагулянту для подальшого отримання сироватки. Біохімічні дослідження сироватки крові проводили на автоматичному біохімічному аналізаторі Miura-200 (Італія) з використанням сертифікованих наборів реагентів виробництва *Spinreact* (Іспанія), *Dialab* (Австрія), *Cormay* (Польща) та *HTI* (США). Визначали концентрацію загального протеїну за допомогою біуретової реакції, вміст альбумінів — методом взаємодії з бромкрезоловим зеленим, концентрацію сечовини — ферментативно за реакцією Бертло, рівень креатиніну — кінетичним методом Яффе на основі швидкості утворення креатинінпікратного комплексу, глюкози — глюкозооксидазним методом, загального кальцію — за кольоровою реакцією з арсеназо III, а неорганічного фосфору — з використанням молібдату амонію. Активність ферментів переамінування — аланінамінотрансферази (ALT) та аспартатамінотрансферази (ACT) — визначали кінетично з використанням реагентів Spinreact (Іспанія). Активність лужної фосфатази встановлювали за швидкістю утворення 4-нітрофенолу з набором реагентів компанії *Cormay* (Польща). Всі аналізи здійснювали згідно з протоколами виробників реагентів, що забезпечувало високу точність і вірогідність отриманих результатів. Статистичне опрацювання отриманих результатів виконували за допомогою пакета «Аналіз даних» *Microsoft Office Excel 2019*. Вибіркові параметри, представлені в роботі, мали такі позначення: M — вибіркове середнє; SD — стандартне відхилення. Зміни показників вважали вірогідними за $P<0,05$ (у тому числі $P<0,01$ і $P<0,001$).

Результати й обговорення

Інтенсивність метаболізму поросят раннього віку є надзвичайно високою через швидкий ріст і розвиток організму [21]. Вона визначається високими рівнями енергетичного обміну, активним засвоєнням поживних речовин та значною потребою у білках, ліпідах і мікроелементах. У перші тижні життя ключову роль відіграє споживання молозива й молока, що забезпечує не лише енергетичні та пластичні ресурси, а й імунну підтримку [11]. Зміни у годівлі після відлучення призводять до метаболічного стресу, що може впливати на функціонування кишкового бар'єра та імунної системи [23].

Проведеними дослідженнями встановлено, що в поросят контрольної групи рівень загального протеїну на 9-й день життя становив 53,1±5,5 г/л. До 21-го дня його концентрація зросла на 4,9 % (P>0,05) і становила 55,7±3,6 г/л. Однак до 35-го дня спостерігалося зниження рівня загального протеїну на 17,2 % (P<0,05) відносно 21-го дня, до 46,1±4,7 г/л (рис. 1).

У поросят, які отримували ІПС, вміст загального протеїну на 9-й день був 50,3±2,1 г/л, що на 5,3 % нижче за контрольні показники. Однак до 21-го дня рівень протеїну зріс на 19,3 % (P<0,05) і становив 60,0±5,9 г/л, що на 8,4 % вище за відповідний показник у контрольній групі. На 35-й день рівень загального протеїну знизився до 52,8±4,5 г/л, що на 12,0 % вище, ніж у контролі (P<0,05).

Аналіз рівня альбумінів і глобулінів у сироватці крові поросят свідчить про зміни білкового обміну під впливом ізотонічно-протеїнової суміші (рис. 2). Альбуміни є важливим компонентом плазми крові, що відображає стан білкового метаболізму та рівень гомеостазу в організмі поросят у ранньому постнатальному періоді [24]. У поросят контрольної групи рівень альбумінів на 9-й день життя становив 22,6±2,8 г/л. До 21-го дня концентрація альбумінів зросла на 20,4 % (P<0,05) і становила 27,2±4,1 г/л, що вказує на активізацію білкового синтезу в період

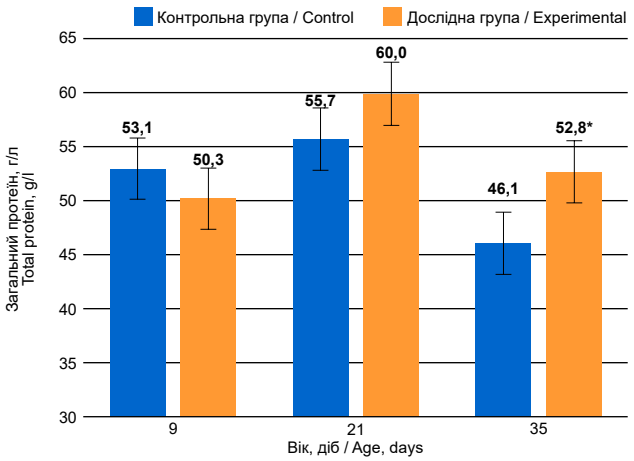

Рис. 1. Вміст загального протеїну в сироватці крові поросят за дії ІПС, г/л (M±SD; n=10)
Примітка. Тут і далі * — P<0,05 — порівняно з контрольною групою.
Fig. 1. Total protein content in piglet blood serum under the influence of IPS, g/l (M±SD; n=10)
Note. Here and further * — P<0.05 — compared to the control group

інтенсивного росту. Однак до 35-го дня відзначалося незначне зниження рівня альбумінів на 5,1 % до 25,8±3,3 г/л, що, ймовірно, пов'язане з адаптаційними змінами після відлучення.

Наші дослідження узгоджуються з іншими даними, демонструючи, що використання ізотонічних розчинів амінокислот як протеїнозберігаючої терапії суперечить усталеним уявленням про парентеральне харчування та відображає новітні досягнення в розумінні метаболізму [8]. У поросят, які отримували ІПС, рівень альбумінів на 9-й день був 23,7±5,5 г/л, що на 4,9 % вище за контрольний показник, однак ця різниця не була вірогідною (P>0,05). До 21-го дня рівень альбумінів зріс на 22,0 % (P<0,05) і становив 28,9±2,8 г/л, що на 6,3 % більше за показник у контрольній групі. На 35-й день рівень альбумінів знизився до 27,3±2,9 г/л, однак залишався на 5,8 % вищим, ніж у контрольній групі.

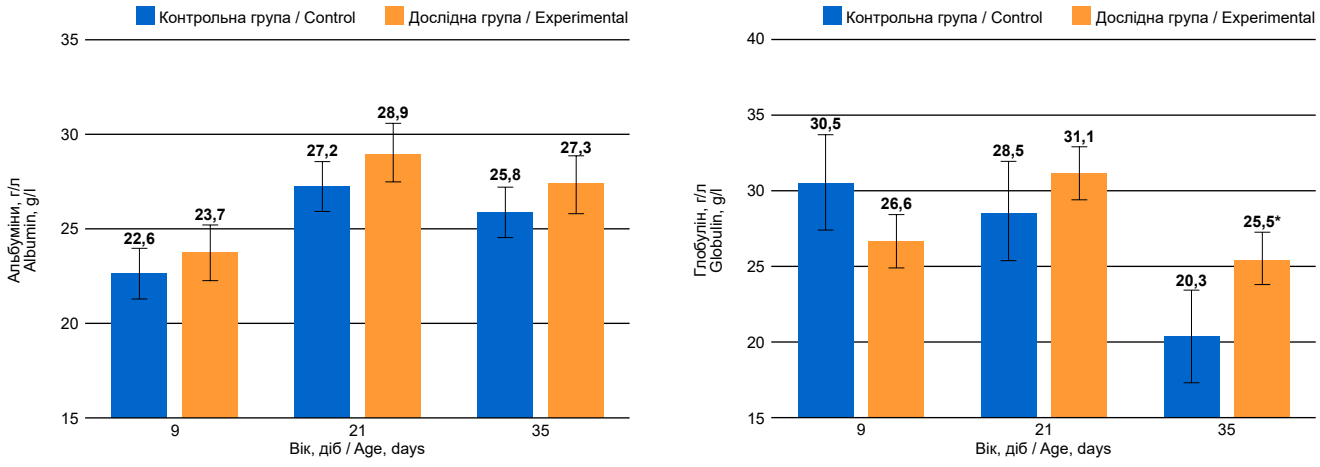

Рис. 2. Вміст альбумінів і глобулінів у сироватці крові поросят за дії ІПС, г/л (M±SD; n=10)
Fig. 2. Albumin and globulin content in piglet blood serum under the influence of IPS, g/l (M±SD; n=10)

У поросят контрольної групи рівень глобулінів на 9-й день життя становив 30,5±6,7 г/л. До 21-го дня відзначалося незначне зниження рівня глобулінів на 6,6 % — до 28,5±4,5 г/л. Однак до 35-го дня спостерігалося істотне зниження рівня глобулінів на 28,8 % (P<0,001) — до 20,3±4,0 г/л, що може бути пов'язане з адаптаційним стресом після відлучення. У поросят, які отримували ІПС, рівень глобулінів на 9-й день становив 26,6±5,7 г/л, що на 12,8 % нижче за контрольний показник. Однак, на 21-й день рівень глобулінів зріс на 16,9 % (P<0,05) до 31,1±4,7 г/л, тобто був на 9,1 % вищий, ніж у контрольній групі. До 35-го дня спостерігалося зниження рівня глобулінів на 17,9 % (P<0,05) — до 25,5±4,7 г/л, однак цей рівень залишався на 25,6 % вищим, ніж у поросят контрольної групи (P<0,05).

Аналіз рівня сечовини та азоту сечовини в сироватці крові поросят дозволяє оцінити стан білкового метаболізму, функціональну активність нирок і адаптацію організму до змін у харчуванні [25]. У поросят контрольної групи рівень сечовини на 9-й день становив 2,24±0,56 ммоль/л (рис. 3). До 21-го дня відбулося значне зростання рівня сечовини на 55,4 % (P<0,05) — до 3,48±1,08 ммоль/л, що може бути пов'язане з інтенсивним білковим метаболізмом у період росту. Однак на 35-й день рівень сечовини знизився на 8,6 % — до 3,18±0,65 ммоль/л, що може свідчити про адаптаційні зміни в азотному обміні після відлучення. У поросят, які отримували ІПС, рівень сечовини на 9-й день був 2,52±0,70 ммоль/л, а отже на 12,5 % вищий, ніж у контрольній групі. До 21-го дня рівень сечовини зріс на 19,4 % (P>0,05) і становив 3,01±0,96 ммоль/л, що на 13,5 % нижче, ніж у контрольній групі. До 35-го дня рівень сечовини знизився на 11,6 % — до 2,66±0,42 ммоль/л, що на 16,4 % нижче, ніж у поросят контрольної групи (P<0,05).

Аналіз рівня азоту сечовини в сироватці крові поросят дозволяє оцінити ефективність білкового метаболізму та функціонування нирок [25]. У поросят контрольної групи рівень азоту сечовини на 9-й день становив 5,41±4,55 мг/100 мл. До 21-го дня відбулося збільшення на 17,0 % — до 6,33±2,10 мг/100 мл. До 35-го дня рівень азоту сечовини зріс ще на 7,1 % (P>0,05) і досяг 6,78±1,89 мг/100 мл. У поросят, які отримували ІПС, рівень азоту сечовини на 9-й день був 6,34±3,99 мг/100 мл, що на 17,2 % більше, ніж у поросят контрольної групи (P>0,05). До 21-го дня цей показник залишався практично незмінним (6,21±2,29 мг/100 мл), що на 1,9 % нижче, ніж у контрольній групі. На 35-й день спостерігалося зниження рівня азоту сечовини на 17,2 % — до 5,14±1,48 мг/100 мл, що на 24,2 % нижче, ніж у поросят контрольної групи (P<0,05).

Креатинін є важливим маркером білкового й енергетичного обміну, відображає рівень м'язового катаболізму [5], його концентрація у крові поросят може змінюватися залежно від рівня фізіологічного розвитку, стресових факторів та впливу ізотонічно-протеїнової суміші (рис. 4). У поросят контрольної групи рівень креатиніну на 9-й день становив 70,7±20,8 мкмоль/л. До 21-го дня спостерігалося зниження показника на 10,6 % (P>0,05) — до 63,2±12,7 мкмоль/л, що може бути пов'язане зі зміною обміну білків та зменшенням інтенсивності катаболічних процесів. На 35-й день рівень креатиніну зріс на 13,8 % (P>0,05) — до 71,9±8,5 мкмоль/л, що свідчить про стабілізацію білкового метаболізму після відлучення. У поросят, які отримували ІПС, рівень креатиніну на 9-й день був 79,4±7,9 мкмоль/л, тобто на 12,3 % вищий, ніж у поросят контрольної групи (P>0,05). До 21-го дня рівень креатиніну знизився на 8,7 % і становив 72,5±17,7 мкмоль/л, отже й далі на 14,7 % перевищував контрольний рівень (P>0,05). На 35-й день рівень креатиніну зріс на 11,3 % — до 80,7±10,1 мкмоль/л, що на 12,3 % вище, ніж у поросят контрольної групи (P<0,05). Ці зміни можуть бути пов'язані з інтенсифікацією анаболічних процесів у м'язовій тканині та зменшенням катаболічних ефектів.

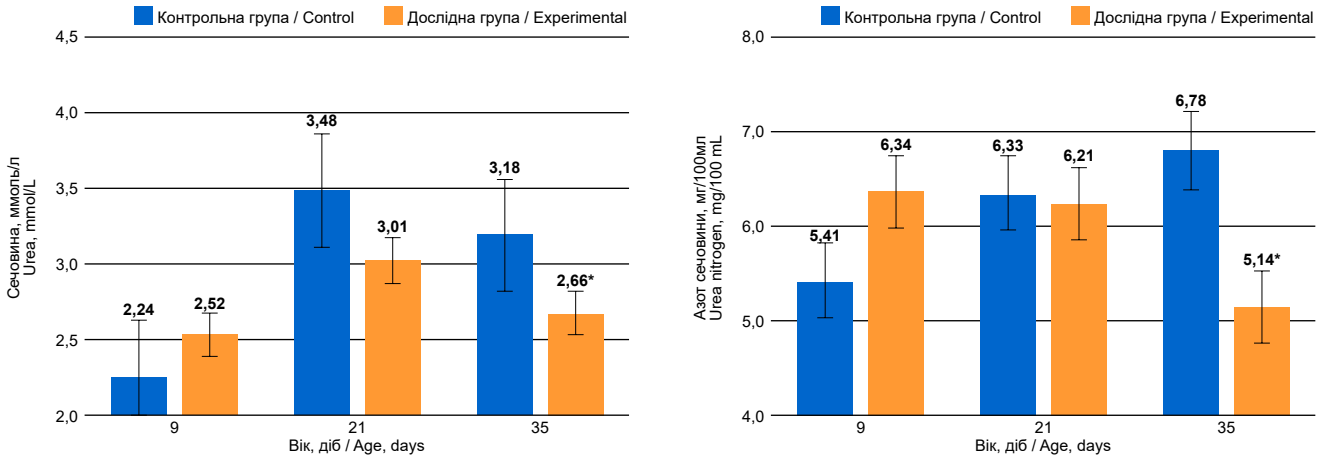

Рис. 3. Вміст сечовини (ммоль/л) та азоту сечовини (мг/100мл) в сироватці крові поросят за дії ІПС (M±SD; n=10)
Fig. 3. Urea content (mmol/L) and urea nitrogen (mg/100 mL) in piglet blood serum under the influence of IPS (M±SD; n=10)

Аланінамінотрансфераза (ALT) є важливим маркером стану печінки, відображаючи рівень метаболічного навантаження та інтенсивність амінокислотного обміну [22]. У поросят контрольної групи активність ALT на 9-й день становила 61,4±11,9 Од/л (рис. 5). До 21-го дня відбулося зниження активності ALT на 19,2 % (P>0,05) — до 49,6±17,7 Од/л, що може бути пов'язане зі зменшенням метаболічного навантаження на печінку в цьому періоді. Проте на 35-й день спостерігалося зростання активності ALT на 35,3 % (P<0,05) — до 67,1±25,7 Од/л, що може свідчити про збільшення навантаження на печінку після відлучення. Під впливом ізотонічно-протеїнової суміші можуть відбуватися зміни активності ALT, що свідчить про можливі адаптаційні та гепатопротекторні ефекти. У поросят, які отримували ІПС, активність ALT на 9-й день була 67,6±16,8 Од/л, що на 10,1 % вище, ніж у контрольній групі. До 21-го дня рівень ALT практично не змінився і становив 49,7±9,7 Од/л, що майже дорівнює контрольному значенню. На 35-й день активність ALT залишалася на 8,6 % нижчою, ніж у контрольній групі, і становила 61,3±15,2 Од/л, однак ця різниця не була вірогідною (P>0,05). Збереження стабільного рівня ALT у поросят дослідної групи може свідчити про менший рівень стресового впливу на печінку після відлучення, що є важливим показником фізіологічної адаптації поросят.

Аспартатамінотрансфераза (AST) є ключовим ферментом, що бере участь у білковому обміні та є маркером функціонального стану печінки і м'язової тканини [18]. У поросят контрольної групи активність AST на 9-й день становила 83,8±38,9 Од/л. До 21-го дня рівень ферменту значно знизився — на 33,5 % (P<0,05), до 55,7±19,0 Од/л, що може бути пов'язане зі зменшенням навантаження на печінку та стабілізацією енергетичного обміну. Однак на 35-й день спостерігалося незначне підвищення рівня AST — на 8,8 %, до 60,6±20,4 Од/л, що може свідчити про адаптацію організму після відлучення. У поросят, які отримували ІПС, активність AST на 9-й день становила 94,9±14,0 Од/л, тобто була на 13,3 % вища, ніж у контрольній групі. До 21-го дня рівень AST знизився на 31,2 % (P>0,05) і становив 65,3±43,4 Од/л, що на 17,2 % вище, ніж у поросят контрольної групи (P>0,05). На 35-й день активність AST підвищилася на 6,4 % — до 69,5±17,7 Од/л, що на 14,7 % вище, ніж у контрольній групі.

Лужна фосфатаза (ALP) є важливим ферментом, що бере участь у метаболізмі фосфору, ремоделюванні кісткової тканини та загальних процесах адаптації організму [15]. У поросят контрольної групи активність ALP на 9-й день становила 958,5±187,6 Од/л. До 21-го дня рівень ферменту значно знизився — на 73,9 % (P<0,001), до 250,2±108,3 Од/л, що може бути пов'язане зі зменшенням інтенсивності процесів росту кісткової тканини та адаптацією до нових умов годівлі. На 35-й день активність ALP й надалі знижувалася та

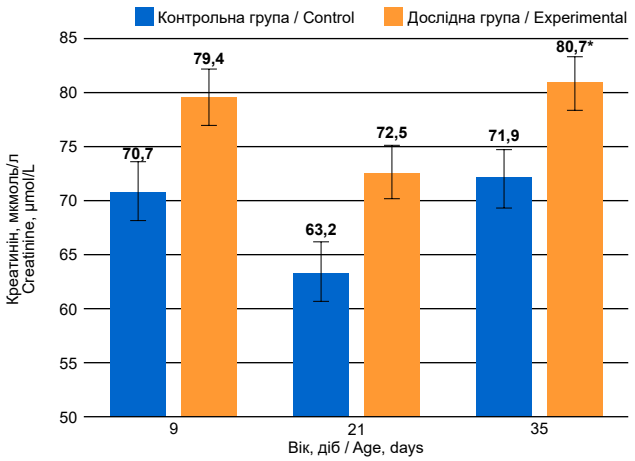

Рис. 4. Вміст креатиніну в сироватці крові поросят за дії ІПС, мкмоль/л (M±SD; n=10)
Примітка. * — P<0,05 — порівняно з контрольною групою.
Fig. 4. Creatinine content in piglet blood serum under the influence of IPS, μmol/L (M±SD; n=10)
Note. * — P<0.05 — compared to the control group.

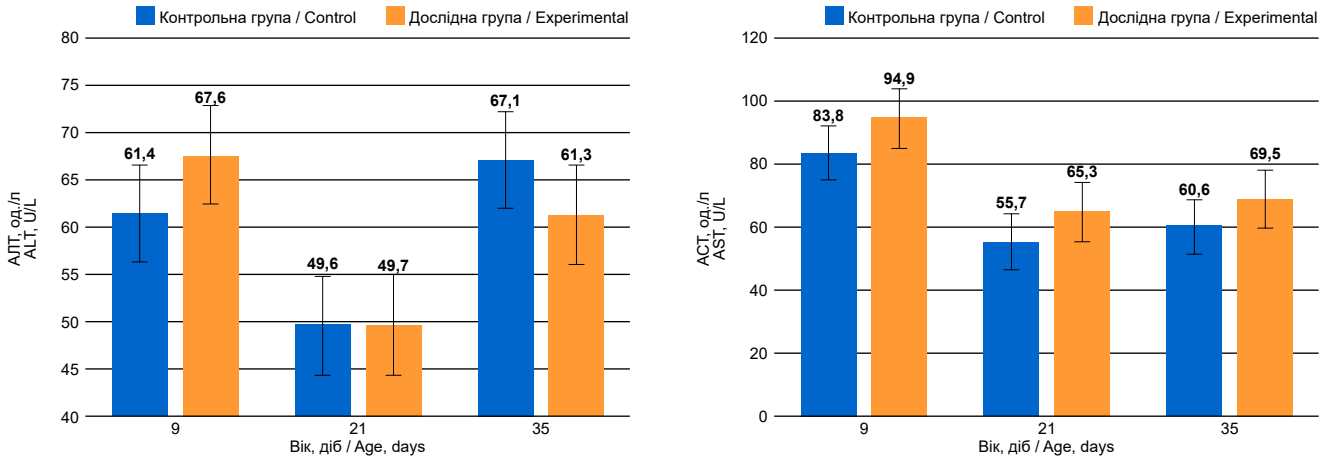

Рис. 5. Активність ALT й AST в сироватці крові поросят за дії ІПС (Од/л; M±SD; n=10)
Fig. 5. ALT and AST activity in piglet blood serum under the influence of IPS (U/L; M±SD; n=10)

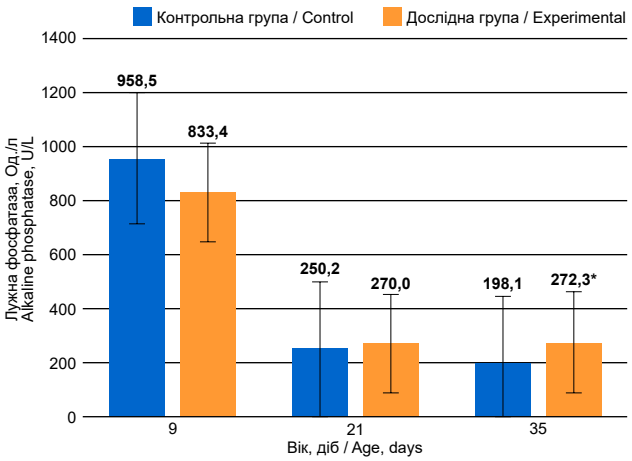

Рис. 6. Активність лужної фосфатази в сироватці крові поросят за дії ІПС, Од/л (M±SD; n=10)
Fig. 6. Alkaline phosphatase activity in piglet blood serum under the influence of IPS, U/L (M±SD; n=10)

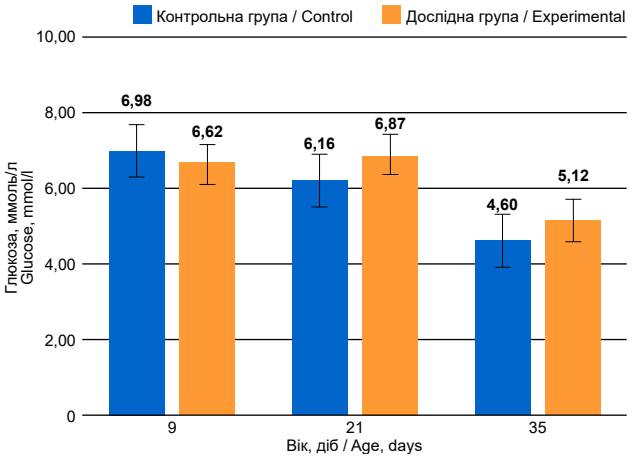

Рис. 7. Вміст глюкози в сироватці крові поросят за дії ІПС, ммоль/л (M±SD; n=10)
Fig. 7. Glucose content in piglet blood serum under the influence of IPS, mmol/l (M±SD; n=10)

становила 198,1±59,0 Од/л, що на 20,8 % нижче, ніж на 21-й день (P>0,05). У поросят, які отримували ІПС, активність ALP на 9-й день була 833,4±181,1 Од/л, що на 13,0 % нижче, ніж у контрольній групі. До 21-го дня рівень ферменту знизився на 67,6 % (P<0,001) — до 270,0±61,0 Од/л, що на 7,9 % вище, ніж у контрольній групі. На 35-й день активність ЛФ зросла на 7,8 % — до 272,3±59,9 Од/л, що на 37,5 % вище, ніж у поросят контрольної групи (P<0,05). Це може свідчити про більш активний кістковий метаболізм і підтримку мінерального обміну в поросят, які отримували ІПС, навіть після відлучення.

Глюкоза є ключовим показником енергетичного метаболізму, що відображає стан вуглеводного обміну, рівень стресу та адаптаційні можливості організму [9]. У поросят контрольної групи рівень глюкози на 9-й день становив 6,98±1,73 ммоль/л (рис. 7). До 21-го дня спостерігалося зниження рівня глюкози на 11,7 % (P>0,05) — до 6,16±0,91 ммоль/л, що може бути пов'язане з поступовим переходом організму до використання інших джерел енергії. До 35-го дня концентрація глюкози знизилася ще на 25,3 % (P<0,05) — до 4,60±0,78 ммоль/л. У поросят, які отримували ІПС, рівень глюкози на 9-й день був 6,62±1,39 ммоль/л, що на 5,2 % нижче, ніж у контрольній групі. До 21-го дня рівень глюкози зріс на 3,8 % і становив 6,87±0,76 ммоль/л, що на 11,5 % вище, ніж у контрольній групі. На 35-й день концентрація глюкози знизилася на 25,5 % (P<0,05) — до 5,12±0,70 ммоль/л, однак цей рівень залишався на 11,3 % вищим, ніж у контрольній групі.

Кальцій є одним із найважливіших макроелементів, що бере участь у формуванні кісткової тканини, регуляції нервово-м'язової активності та підтримці гомеостазу [20]. У поросят контрольної групи рівень кальцію на 9-й день становив 2,22±0,45 ммоль/л (рис. 8). До 21-го дня відбулося зниження рівня кальцію на 9,5 % (P>0,05) — до 2,01±0,22 ммоль/л, що може бути пов'язане з активним ростом тварин і зміною мінерального метаболізму.

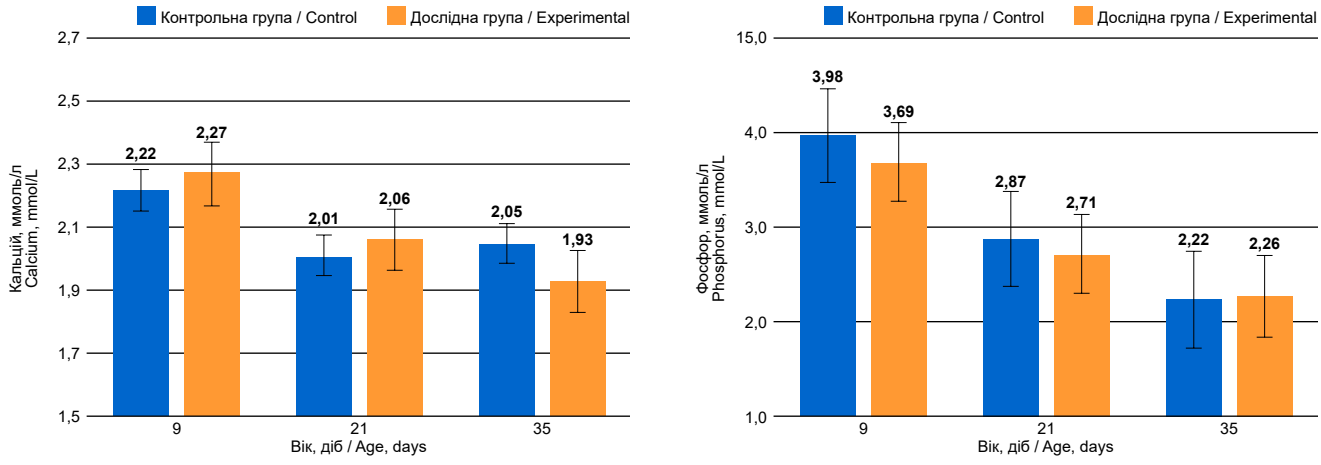

Рис. 8. Вміст кальцію і фосфору в сироватці крові поросят за дії ІПС, ммоль/л (M±SD; n=10)
Fig. 8. Calcium and phosphorus content in piglet blood serum under the influence of IPS, mmol/L (M±SD; n=10)

До 35-го дня рівень кальцію залишався стабільним. У поросят, які отримували ІПС, рівень кальцію на 9-й день був 2,27±0,33 ммоль/л, тобто на 2,3 % вищий, ніж у контрольній групі. До 21-го дня рівень кальцію знизився на 9,3 % (P>0,05) — до 2,06±0,25 ммоль/л, що на 2,5 % вище, ніж у контрольній групі. На 35-й день концентрація кальцію зменшилася ще на 6,3 % — до 1,93±0,20 ммоль/л, що на 5,9 % нижче, ніж у контрольній групі.

Фосфор є одним із ключових макроелементів, що бере участь у формуванні кісткової тканини, енергетичному метаболізмі та регуляції кислотно-лужного балансу [14]. У поросят контрольної групи рівень фосфору на 9-й день становив 3,98±0,48 ммоль/л (рис. 8). До 21-го дня відбулося значне зниження рівня фосфору — на 27,9 % (P<0,05), до 2,87±0,42 ммоль/л. На 35-й день рівень фосфору ще знизився на 22,6 % — до 2,22±0,30 ммоль/л.

У поросят, які отримували ІПС, рівень фосфору на 9-й день становив 3,69±0,87 ммоль/л, тобто був на 7,3 % нижчий, ніж у контрольній групі. До 21-го дня рівень фосфору знизився на 26,5 % і становив 2,71±0,56 ммоль/л, що на 5,6 % нижче, ніж у контрольній групі. На 35-й день рівень фосфору залишався стабільним — 2,26±0,35 ммоль/л.

Співвідношення кальцію до фосфору (Ca/P) є важливим показником мінерального обміну, що відображає баланс між надходженням, засвоєнням і використанням цих макроелементів у процесах росту, розвитку кісткової тканини та підтримки метаболічної рівноваги. Оптимальний рівень цього коефіцієнта є критично важливим для правильного формування скелета й загального гомеостазу тварин [20]. У поросят контрольної групи відношення Ca/P на 9-й день становило 0,56±0,09 (рис. 9). До 21-го дня цей показник зріс на 28,6 % (P<0,05) — до 0,72±0,17. До 35-го дня спостерігалося подальше підвищення коефіцієнта на 31,9 % (P<0,05) — до 0,95±0,24, що може свідчити про зменшення доступності фосфору в організмі або компенсаторну адаптацію після відлучення.

У поросят, які отримували ІПС, співвідношення Ca/P на 9-й день було 0,64±0,12, тобто на 14,3 % вище, ніж у контрольній групі, що може свідчити про більш збалансований мінеральний обмін. До 21-го дня показник зріс на 23,4 % (P<0,05) — до 0,79±0,18, що на 9,7 % вище, ніж у контрольній групі. На 35-й день співвідношення Ca/P знизилося на 13,9 % — до 0,88±0,18, що на 7,4 % нижче, ніж у контрольній групі. Ці зміни можуть свідчити про більш ефективне використання кальцію та фосфору в процесах мінералізації та адаптації після відлучення.

Отримані результати вказують на те, що ІПС сприяє оптимізації білкового, вуглеводного та мінерального обміну, підтримуючи фізіологічну адаптацію поросят у стресовий період після відлучення. Це свідчить про потенційні переваги використання ІПС у системі годівлі поросят для покращення їхнього метаболічного статусу та загального здоров'я.

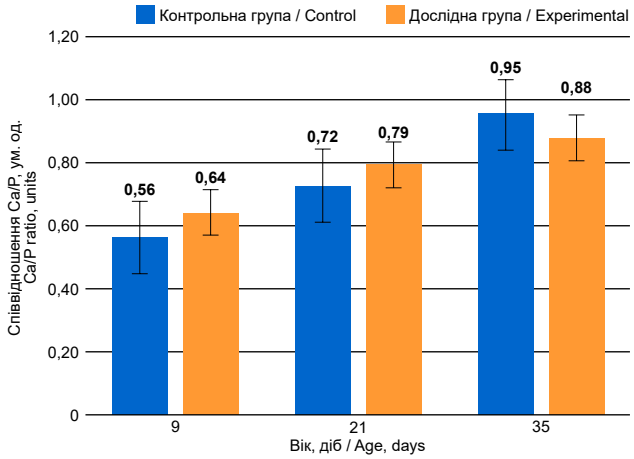

Рис. 9. Відношення кальцію до фосфору в сироватці крові поросят за дії ІПС, ум. од. (M±SD; n=10)
Fig. 9. Calcium to phosphorus ratio in piglet blood serum under the influence of IPS, conventional units (M±SD; n=10)

Таким чином, у поросят після відлучення спостерігається виражена метаболічна перебудова, яка є доказом значних адаптаційних змін. Використання ізотонічно-протеїнової суміші сприяло пом'якшенню цих процесів, зокрема забезпечило вищий рівень загального протеїну, альбумінів і глобулінів у поросят дослідної групи як до, так і після відлучення, що свідчить про кращу білкову забезпеченість та підтримку імунної функції. Після відлучення спостерігалося зниження сечовини, стабільні показники ALT і незначне підвищення AST, що вказує на ефективне використання протеїну та кращу адаптацію печінки. Рівень глюкози був вищим, порівняно з контролем, що підтверджує стабільність енергетичного обміну. Загалом ІПС сприяє зменшенню стресових реакцій після відлучення і покращенню метаболічного статусу поросят.

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Biochemical blood parameters of piglets under the influence of an isotonic protein solution

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The aim of this study was to investigate the effect of administering an isotonic protein solution (IPS) during the weaning period on blood biochemical parameters of piglets. The study was conducted on early postnatal piglets divided into control and experimental groups. Blood samples were collected at 9, 21, and 35 days of age to assess key metabolites and enzyme activity. In control piglets marked metabolic shift occurred post-weaning. Total protein increased to 55.7±3.6 g/L but decreased by 17.2 % ($P<0.05$) post-weaning to 46.1±4.7 g/L. Albumin levels rose by 20.4 % ($P<0.05$), followed by a non-significant drop of 5.1 % ($P>0.05$). Globulin concentrations decreased by 6.6 % by day 21 ($P>0.05$) and further declined by 28.8 % after weaning ($P<0.001$). Blood urea levels rose by 55.4 % ($P<0.05$) by day 21, indicating intensified protein catabolism, but remained elevated by only 8.6 % post-weaning ($P>0.05$). Liver enzymes ALT and AST decreased by 19.2 % ($P>0.05$) and 33.5 % ($P<0.05$), respectively, by day 21, but increased by 35.3 % (ALT) and 8.8 % (AST) after weaning. Carbohydrate metabolism was also affected: glucose levels decreased by 11.7 % ($P>0.05$) by day 21 and by an additional 25.3 % ($P<0.05$) post-weaning. In the experimental group metabolic stability was greater than in the control. Total protein increased by 19.3 % ($P<0.05$) by day 21 and decreased by only 12.0 % ($P<0.05$) post-weaning, remaining above control levels. Albumin increased by 22.0 % ($P<0.05$) and remained 5.8 % higher than control after weaning ($P>0.05$). Globulin levels initially decreased by 12.8 % ($P > 0.05$) by day 9, but increased by 16.9 % by day 21 ($P<0.05$) and remained 25.6 % higher post-weaning ($P<0.05$). Urea levels increased by 19.4 % ($P>0.05$) by day 21, then decreased by 11.6 % post-weaning ($P<0.05$), suggesting more efficient protein utilization. ALT remained stable, while AST increased by only 6.4 %, reflecting better hepatic adaptation. Carbohydrate metabolism was also more stable: glucose increased by 3.8 % ($P>0.05$) by day 21 and was 11.3 % higher post-weaning compared to the control. Thus, administration of IPS contributes to stabilization of protein and energy metabolism, reduces post-weaning stress responses, and improves the overall metabolic status of piglets.

Key words: piglets, metabolism, isotonic protein solution, weaning

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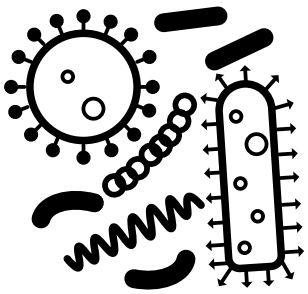

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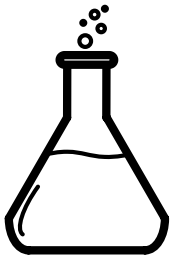

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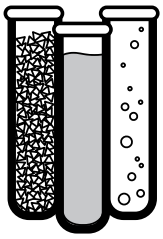

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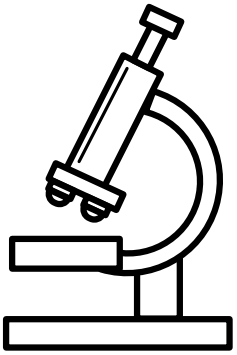

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