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Efficiency of use of lactic acid bacteria for the treatment of cow endometritis

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Abstract. There is a necessity to find alternative methods to treat endometritis in cows that would not have disadvantages of traditional anti-microbial drugs in particular the occurring of resistance forms of bacteria and limitation of milk usage. The aim of the study was possibility of treating endometritis with a suspension of the probiotic, which includes strain B-2691 of *Lactobacillus acidophilus*. Cows of Ukrainian Black-and-White dairy breed with confirmed endometritis were injected into the uterine cavity with a suspension of the drug (500 billion microbial cells of lactic acid bacteria in 100 cm^3) from one to 3 times with an interval of 6 days between application. The overall effectiveness of treatment of cows of all groups after 3 times of the drug administration was 90%. 100% recovery of cows with subclinical, mild and severe clinical endometritis, as well as 73% recovery of cows with morphological changes in the uterus was observed. Intrauterine injection of the drug (300 billion cells per 100 cm^3 of saline) led to stabilisation of the number of bacteria in the mucus. On the sixth day after administration, the number of bacteria in the uterine mucus of cows with endometritis and of some clinically healthy cows decreased, so in all animals it did not exceed 100 thousand colony-forming units per 1 cm^3. The presence of lactic acid bacteria was detected in all cows. Therapeutic concentrations of *Lactobacillus acidophilus* from 0.5 to 3 billion/cm^3 negatively affected the activity of bull sperm during incubation in a thermostat. At the maximum concentration of lactobacilli, sperm death occurred during the first hour of incubation, and at the minimum concentration – within 2 hours. In the control sample, live sperm were detected after 4 hours of incubation. The probiotic should be used as an alternative method for the treatment and prevention of cow endometritis, but due to the negative effect of therapeutic concentrations of *Lactobacillus acidophilus* on sperm survival, insemination should be performed no earlier than 6 days after administration of the drug, when a decrease in the number of bacteria in the uterine mucus is confirmed

Keywords: cattle; *Lactobacillus acidophilus*; uterine mucus; mobility of sperms; colony-forming units

Introduction

Endometritis is a common disease of dairy cows occurring after calving and causes a deterioration in reproductive capacity. Previous studies have shown that endometritis is related to housing conditions. Appearance of impending calving and duration of preparation for calving depended on the cows keeping, and in fetal membranes of 93% of cows kept tethered, lacked mucins and postpartum catarrhal endometritis were diagnosed (Ugnivenko *et al.*, 2020). In pasture-based housing, catarrhal endometritis was observed in only 4.8% of the population. According to I. Sheldon *et al*. (2019), up to 40% of cows have postpartum uterine diseases. In Holstein cows, cytologically diagnosed endometritis was associated with impaired reproductive function, which was manifested in particular in an increase in the number of open days and a decrease in the percentage of pregnancy after the first insemination. In cows that tested positive for endometritis, in addition to the above-mentioned problems, an increase in the number of inseminations to obtain pregnancy and an increased duration of anestrus after calving were also observed. Endometritis increases the atypical ovarian functioning, in particular the begin and duration of luteal activity. As stated by M. Dickson *et al.* (2020), inflammation in uterus caused changes of mobility and functions of spermatozoa, increase phagocytosis of spermatozoa and zygotes obtained have less possibility to be developed to the stage of blastocyst or even morula.

Negative impact of endometritis on cows' reproductive function causes the necessity of looking for the methods for early diagnosis and treatment of animals. Risks of endometritis occurrence increase under conditions of obstetric care for dystocia, due to increased bacterial contamination, although there is an opinion of I. Sheldon *et al.* (2019) that uterine health depends more on the ability of the endometrium to tolerate pathogens than on the ability to resist bacterial penetration. It was established that uterine microflora of healthy cows and cows with clinical endometritis and sub-clinical endometritis consists of community of 127 different types of Aerobic and Anaerobic bacteria included to 48 families. Quantity of bacteria in the uterine of cows with detected endometritis is essentially lower. Upon the clinical endometritis the level of *Trueperella pyogenes* is increasing. Similar results were obtained in a study by O. Pascottini *et al.* (2020), which noted no significant difference between the microbiome of healthy cows and cows with subclinical endometritis, so, the authors believe that subclinical endometritis is a consequence of dysregulation of inflammation rather than changes in the uterine microbiota. However, in cows with clinical endometritis, a decrease in bacterial diversity in the uterus and the dominance of *Trueperella pyogenes* were found. It should be noted that the presence of *Trueperella pyogenes* in clinical endometritis is associated with a decrease in milk production and fertility after the first insemination, as well as a lengthening of the period from calving to insemination in cows (Paiano *et al.*, 2021). These studies indicate the need to normalise the uterine microflora during clinical endometritis.

The vagina and uterus share a common bacterial community during the postpartum period. In the study of R. Miranda-CasoLuengo *et al.* (2019), changes associated with the development of endometritis were observed on the 7th day after calving, when the vaginal and uterine microbiomes were most similar. The authors of this study identified at least three different types of microbiomes associated with the further development of postpartum endometritis. All of these microbiome types had reduced bacterial diversity. Traditional antimicrobials are used to treat endometritis. Antibiotics are considered to be effective in the treatment of endometritis, but their active use causes the emergence of pathogenic microorganisms' resistance. It has been found by A. Manimaran *et al.* (2019) that *E. coli*, as one of the possible causative agents of endometritis, has a difference in sensitivity to gentamicin and oxytetracycline. Prolonged intrauterine antibiotic therapy for persistent endometritis can lead to fungal endometritis, which significantly complicates treatment and requires specific therapy (Saini *et al.*, 2019). The use of antibiotics can impose restrictions on the use of milk, which leads to additional losses.

To replace antibiotic therapy, the possibility of alternative treatments that involve the use of PGF2α in combination with intrauterine antibacterial drugs, immunomodulators and herbal remedies is being studied (Parmar, 2021). The use of herbal remedies is considered to be one of the priority and affordable methods that have a certain effectiveness. It was found that oral administration of *Aegle marmelos* and *Murraya koenigii* leaf powder reduces bacterial load and inflammation in endometritic cows. *Achyranthes aspera, Azadirachta indica, Ocimum tenuiflorum, Allium sativum, Withania somnifera* and many other plants are also considered effective for solving gynaecological problems in cows (Nikhade *et al.*, 2019). Essential oils of cinnamon, clove, oregano and thyme have been shown to be effective in inhibiting control strains of *Escherichia coli, Fusobacterium necrophorum, Trueperella pyogenes* and *Staphylococcus aureus*, which are considered typical bacteria that cause endometritis. For the prevention of endometritis, positive results were obtained using intrauterine ozone therapy (Escandón *et al.*, 2020). There is evidence found by E. Hussein

& K. Hussein (2022) of positive results in the treatment of bovine metritis and endometritis with biomolecules with silver nanoparticles.

Using all the variety of methods for treating and preventing endometritis, it is important not only to inhibit pathogens in the uterus but also to restore its eubiosis. There is evidence that lactic acid bacteria, which can inhibit pathogen growth, can be used to treat endometritis. Comparison of uterine mucus microbiota profiles made before and after treatment with lactic acid bacteria and antibiotics revealed that lactic acid bacteria can cure endometritis and restore normal physiological state, avoiding the disadvantages of antibiotic treatment, such as a decrease in the amount of beneficial microbiota (Yang *et al.*, 2021).

Among bacterial drugs, probiotics are of traditional interest, as their action is aimed at inhibiting pathogenic microorganisms. Their inclusion in feed and food products helps to inhibit the growth of *Escherichia coli, Listeria monocytogenes*, *Salmonella sp.* and *Bacillus cereus*. According to N. Hashem & A. Gonzalez-Bulnes (2022), the biological activity of probiotics can be a promising alternative to antibiotics for maintaining and restoring eubiosis and reproductive system function, but it should be toned that in-depth studies are needed to determine the mechanisms by which probiotics can affect reproductive processes and to define the specifics of probiotic use during sensitive reproductive periods. The purpose of this study was to investigate the effectiveness of *Lactobacillus acidophilus*-based probiotics for the treatment of cow endometritis and to analyse how they affect the uterine microbiota after administration and what effect this bacterial culture may have on bull germ cells.

Materials and Methods

The use of a probiotic drugs with lactic acid bacteria for the treatment and prevention of cow endometritis was studied. The research was conducted at the State Enterprise Research Farm "Oleksandrivske" and at the Reproduction Biotechnology laboratory of the Institute of Animals Breeding and Genetics named after M.V. Zubets of the National Academy of Agrarian Science of Ukraine, the results of which were analysed and summarised in 2024. As a source of lactic acid bacteria, probiotic "Bovilact" (developed by the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Agrarian Sciences of Ukraine), which contains strain B-2691 *Lactobacillus acidophilus*, which is stored in the Ukrainian Collection of Microorganisms was used. This medicine is used as a probiotic for the treatment and prevention of calf dysbiosis. The product is a light brown powder that contains 50 billion live cells of lactic acid bacteria per 1g. The drug is known to have an antagonistic activity against a wide range of pathogenic and opportunistic microorganisms due to the synthesis of L-lactic acid. In particular, it is highly effective for the treatment of colibacillosis, toxic dyspepsia, salmonellosis and is used to stabilise the microflora of the digestive tract.

The study was conducted on a population of Ukrainian Black-and-White dairy cows. The cows were kept tethered. The cows were fed three times a day. The cows were milked in stalls using milking equipment with a milk pipeline. Every day, the cows were provided with exercise in the feed and exercise area, straw bedding was brought into the stalls, free access to drinking water was provided, and veterinarians and staff had access to the animals the whole time. The conditions of cows and research design met the requirements of the Law of Ukraine No. 3447-IV "On Protection of Animals from Cruelty" (2006).

In a dairy farm, cows were examined for endometritis in the period from 7 to 30 days after calving. Cows were examined by clinical examination and rectal examination. As a result of the examination, 20 cows with indicators of endometritis were identified. Confirmation of the diagnosis of endometritis was provided

by studying the reaction of uterine mucus to sulphur-containing amino acids according to the method of G. Kalinovskiy & G. Podoprigora (1987). According to this method, 4 cm^3 of a 0.5% solution of acetic acid lead was added to a test tube, to which a 20% solution of caustic sodium was dropwise added until a precipitate (lead oxide hydrate) was formed. After 15-20 seconds, the caustic sodium solution was added again until the precipitate disappeared. Then, 1.5-2.0 cm^3 of uterine mucus was added to the test tube, shaken gently and heated without boiling. In the case of endometritis, the mixture gets the colour of strongly brewed tea. Mucus samples were taken with a Korczak spoon. The analysis of uterine mucus was carried out under the conditions of the farm veterinary point. Cows with confirmed endometritis were divided into four groups according to the severity of the disease (Table 1).

Table 1. *Grouping of cows by severity of endometritis*

Group	Number of cows	Clinical indicators
1 (sub-clinical)	6	the cervix and uterus are slightly enlarged, located in the pelvic cavity, there is no fluctuation and no exudate is released
2 (with mild course of the disease)	4	the uterus is enlarged, partially in the pelvic cavity, slightly fluctuating, hypotonic, and there is a slight releasing of exudate
3 (with severe course of the disease)	2	the uterus is located entirely in the abdominal cavity, very enlarged, atonic, significant exudation
4 (with morphological changes in uterus)	8	the uterus is located entirely in the abdominal cavity, very enlarged, atonic, there is a thickening of the uterine walls and their fusion with the peritoneum of the pelvic and abdominal cavity, significant exudation, purulent discharge

Source: developed by the authors

The cows were injected with a suspension of the investigational medical product into the uterine cavity using a rectocervical insemination catheter connected to a Janet syringe. The dosage was 500 billion microbial cells of lactic acid bacteria suspended in 100 cm^3 of sodium chloride saline. The frequency of administration was from one to three times with an interval of 6 days. 6 days after each administration of the drug, cows were examined for indicators of endometritis by analysing uterine mucus for the content of sulphur-containing amino acids (Kalinovskiy & Podoprigora, 1987). The recovery of the cows was recorded and, in this case, treatment was stopped. If it was necessary to continue therapy, the drug was re-administered at the specified dose. At the end of the experiment, the proportion of cows in each group that recovered and the average frequency of drug administration to obtain a positive result were determined. In order to study the effect of the probiotic with lactic acid bacteria on the total bacterial contamination of the uterine from 7 to 30 days after calving, 14 cows were selected, of which 6 were healthy and 8 were sick with endometritis. The diagnosis of endometritis was confirmed after clinical examination by determining the reaction of uterine mucus to sulphur-containing amino acids (Kalinovskiy & Podoprigora, 1987). Uterine mucus was collected with a Korczak spoon in compliance with the rules of asepsis. A sterile vaginal mirror was used to prevent contact between the instrument and other parts of the animal's body. At the same time, mucus samples were taken for bacteriological analysis. On the same day, 300 billion cells of lactic acid bacteria in the form of a suspension in 100 cm^3 of sterile sodium chloride solution were injected into the uterus of cows using a catheter for rectocervical insemination attached to a Janet syringe. Bacteriological studies of uterine mucus were carried out by the method of sowing in the

Boryspil District State Laboratory of Veterinary Medicine (Boryspil, Kyiv region, Ukraine). They determined the total level of bacterial contamination and identified the presence of lactic acid bacteria. According to the degree of bacterial contamination, 5 classes were determined (Table 2). The samples were taken again 6 days after the probiotic administration. Bacterial contamination was determined in the samples and lactic acid bacteria were identified.

Table 2. *Classification of samples by the level of bacterial contamination*

Class	Bacterial contamination level, thousand colony forming units per 1 cm^3	Degree of bacterial contamination
1	0	sterile
2	1-100	insignificant
3	101-2,000	weak
4	2,001-50,000	average
5	over 50,000	strong

Note: gradation of bacterial contamination in bacteriological examination reports
Source: developed by the authors

The biological effect of the investigational medicinal product on bull sperm was studied in the laboratory of beef cattle reproduction at the M.V. Zubets Institute of Animal Breeding and Genetics. Frozen bull semen in pellets was thawed in 1 cm^3 of 2.9% sodium citrate solution and added to tubes with different concentrations of lactic acid bacteria. The concentration of lactic acid bacteria in the test samples was 3, 2, 1 and 0.5 billion per 1 cm^3. The medicine was dissolved in sodium citrate at the specified concentration and then 1 cm^3 of the solution was mixed with dissolved semen. Thawed sperm was used as a control. A 1 cm^3 solution of sodium citrate was added to the control.

The samples were kept in a thermostat for 5 hours. The temperature of the thermostat was 38 °C. Sperm survival was assessed by motility (the number of live cells with straightforward motion in points, on a 10-point scale). Sperm motility was assessed before incubation and every one hour using a Biolam microscope at 200× magnification.

The effectiveness of the use of the probiotic for the treatment of endometritis was assessed by the percentage of recovered cows and the average number of injections of the probiotic suspension to obtain a positive result. The bacterial contamination of uterine mucus from healthy and endometritic cows before and after administration of the probiotic was determined by sowing the samples on meat-peptone agar, followed by incubation at an average temperature of 37 °C for 24-48 hours and counting the number of colonies. When assessing the survival of sperm in the medium with lactic acid bacteria, the incubation time was estimated and the number of sperm with straightforward motion was counted.

Results and Discussion

After injecting a probiotic with a culture of *Lactobacillus acidophilus* bacteria into the uterine cavity of cows suffering from endometritis, it was expected that due to its antagonistic effect, the pathogenic microflora should be suppressed, which eventually contribute to the cows' recovery. Since the medicine is intended to normalise the intestinal microflora, it was not known whether it would have a similar positive effect when administered into the uterine cavity. There was also not clear how effective it will be on different severities of endometritis. A positive result of the treatment of cow endometritis with the probiotic was obtained 6 days after the first administration (Table 3).

Table 3. *Efficiency of treatment of cow endometritis with use of the probiotic*

Indicator	Group of cows according to the severity of endometritis			
	1	2	3	4
Number of cows selected into the group, heads	6	4	2	8
Recovered cows after the probiotic injection from 1 to 3 times, heads	6	4	2	6
-- // --, %	100	100	100	73
Average amount of administrations before recover	1.0	1.5	1.5	2.6

Source: developed by the authors

In the first group, with subclinical endometritis, 6 days after the first administration of the suspension, recovering of all cows was confirmed. Also, recovery of some cows was observed after the first administration in groups 2 and 3. After three injections of the lactic acid bacteria suspension into the uterine cavity, 18 out of 20 cows recovered. The overall positive result of the treatment was 90%. For the majority of cows, one or two doses of the probiotic lead to recovery, and the treatment effectiveness for cows with subclinical, mild and severe endometritis was 100%. The exception was the cows in group 4 (with morphological changes in the uterus). In this group, up to 3 injections of the drug were required to ensure a positive result, and the treatment effectiveness reached 73%. Intrauterine administration of the medicine is in fact a direct contamination with lactic acid bacteria and it affects the balance of microflora. The task was to investigate how the number of bacteria in the uterine mucus of cows was changed after the administration of the probiotic. In particular, the authors were interested in how the drug affects the uterine environment not only in cows with endometritis but also in healthy cows. It was also necessary to study whether, after a single administration of the probiotic, lactic acid bacteria will stay (colonised) in the uterus. In two groups of cows, one of which included clinically healthy cows and the other cows diagnosed with endometritis, the total bacterial contamination of uterine mucus was studied (Table 4).

Table 4. *Effect of intrauterine administration of the drug on the total bacterial contamination of uterine mucus*

Clinical state of the cow	Inventory number of the cow	Class by bacterial contamination		The presence of lactic acid bacteria	
		prior to the administration of the probiotic	after the administration of the probiotic	prior to the administration of the probiotic	after the administration of the probiotic
Healthy	2997	2	2	-	±
	2928	3	2	-	±
	1009	2	2	-	±
	1169	2	2	-	+
	0240	2	2	-	+
	3139	3	2	-	+
Endometritis	0285	4	2	-	±
	2739	4	2	-	±
	1004	4	2	-	+
	2866	4	2	-	±
	2503	4	2	-	±
	2759	4	2	-	±
	2832	4	2	-	±
	2916	4	2	-	+

Note: "-" lactic acid bacteria are not detected; "±" lactic acid bacteria are detected in minor number; "+" clearly determined presence of lactic acid bacteria

Source: developed by the authors

Prior to the administration of a suspension of lactic acid bacteria into the uterine cavity in clinically healthy cows, the bacterial contamination of uterine mucus was at the level of the 2nd and 3rd classes, which corresponds to up to 100 or up to 2,000 thousand colony-forming units per 1 cm^3. Lactic acid bacteria were detected in the selected samples. In cows with endometritis, the bacterial contamination of uterine mucus was much higher and corresponded to the 4th class of contamination (from 2,000 to 5,000 thousand colony-forming units per 1 cm^3). Lactic acid bacteria were not detected in the uterine mucus samples taken in this group.

The administration of a suspension made from the probiotic helped to stabilise the total bacterial contamination of uterine mucus in both groups of cows. 6 days after the administration of the probiotic with lactic acid bacteria into the uterus of cows, the total level of bacterial contamination of uterine mucus of all cows corresponded to the second class (up to 100 thousand colony-forming units per 1 cm^3). Lactic acid bacteria were found in the bacterial cultures of all animals, indicating that they colonised the uterus. Within the groups, in 50% of healthy cows and in 25% of cows with diagnosed endometritis, lactic acid bacteria were clearly detected in large numbers.

The microflora of the uterus of healthy cows, before the administration of the probiotic, did not include lactic acid bacteria. Since the antagonistic activity of the bacteria included in the probiotic caused by the synthesis of L-lactic acid, it was assumed that changes in the microbiome and the appearance of *Lactobacillus acidophilus* in it could lead to changes in the intrauterine environment. Changes in the uterus can affect the fertility of cows due to the influence of lactic acid bacteria on bull sperm. The survival of bull sperm during incubation in semen solvents with the addition of lactic acid bacteria and comparison with the control without lactic acid bacteria was studied in laboratory conditions (Table 5).

Table 5. *Motility (points) of bull sperm during incubation in medium with lactic acid bacteria*

Incubation hours	Control	Concentration of lactic acid bacteria, billion/cm^3			
		3	2	1	0.5
0	4.5	4.1	4.1	4.1	4.0
1	4.5	D	0.5	2.5	3.9
2	2.5		D	D	3.8
3	2.5				D
4	1.0				
5	D				

Note: D – death of all sperms
Source: developed by the authors

At the beginning of incubation, the control sample at 4.5 points in terms of the number of live sperm with straightforward movement was evaluated. During the first hour of incubation, no significant changes were detected. A decrease in the number of live sperm in the control group was observed after 2 hours of incubation. After 5 hours, no live sperm were detected.

Rapid changes in sperm motility were noticed in the experimental samples. The addition of lactic acid bacteria to the tubes for incubating thawed bull semen immediately led to a decrease in the number of live spermatozoa by 0.4-0.5 points compared to the control. A decrease in the number of sperm with straightforward motion was observed in all experimental samples, regardless of the concentration of lactic acid bacteria. During further incubation in a thermostat, the survival of germ cells was affected by the concentration of lactic acid bacteria. In the sample with a concentration of 3 billion/cm^3 of lactic acid bacteria, no live sperm

were detected after one hour of incubation. At concentrations of lactic acid bacteria of 2 and 1 billion/cm^3, the death of all sperm was observed at the second hour of incubation, while in the sample with a concentration of lactic acid bacteria of 0.5 billion/cm^3, sperm activity was completely lost after two hours of incubation. The rate of change in sperm motility in the experimental samples was inversely proportional to the concentration of lactic acid bacteria.

The results confirmed the possible risks of bull sperm death under the influence of lactic acid bacteria that colonised in the uterine environment. However, during the experiment, the number of lactic acid bacteria was in a therapeutic dose and significantly (in the smallest sample, more than 5,000 times) exceeded the number of microorganisms in the uterus of cows, which was detected 6 days after the administration of the drug. Since a decrease in the concentration of lactic acid bacteria is associated with a prolongation of sperm survival during incubation, the negative impact of the probiotic on fertilisation of treated and recovered cows is likely to be negligible.

Traditional treatment of bovine endometritis is complicated by the emergence of antibiotic resistance of common pathogens. In particular, the analysis of *E. coli,* isolated from endometritis of cows revealed its resistance to most antibiotics, so effective treatment of animals required the use of new complex drugs (Shafique *et al.*, 2021; Karatieieva *et al.*, 2024). The use of probiotics for therapeutic purposes allows to avoid further development of pathogen resistance to antimicrobial drugs. In the current study, the probiotic with *Lactobacillus acidophilus* was highly effective. Recovery was observed in 100% of cows in case of disease without morphological changes in the uterus and at the level of 73% among cows with morphological changes in the uterus. The potential of using lactic acid bacteria for the health of the genital organs of cows is of interest in dairy farming. In particular, it was found by S. Peter *et al.* (2018) that *Lactobacillus buchneri* had a positive effect on the health of the uterus, which led to an improvement in the reproductive function of cows. And the presence of *Lactobacillus acidophilus* in vaginal samples led to the suppression of a number of pathogens (*Staphylococcus* spp., *Pseudomonas* spp., *Kocurea* spp. and *Granulicatella* spp.), according to L. Khamees *et al.* (2022). The practice of using lactic acid bacteria can be an effective alternative to antimicrobials, but there are certain caveats. In experiments on the uterine microbiota conducted by M. Wang *et al.* (2018), *Lactobacillus* and *Acinetobacter* were found in dairy cows with subclinical endometritis. The probiotic "Bovilact" is advisable to use for the treatment of cows with endometritis, it can replace antimicrobial drugs, both in conventional and organic livestock, but the study of uterine microbiomes of cows with the inclusion of *Lactobacillus acidophilus* should be continued.

The use of lactic acid bacteria for the treatment of bovine endometritis directly affects other uterine bacteria. There are research results in the literature that indicate the inhibition of certain pathogenic cultures by lactic acid bacteria (Peter *et al.*, 2018). There are also reports of differences in the uterine microbiomes of healthy cows and cows with clinical and subclinical endometritis. As stated by R. Paiano *et al.* (2022), healthy cows have a greater diversity of bacterials, and endometritis diseases significantly reduces it. In modern studies, less attention is paid to changes in the total bacterial colonisation of uterine mucus and the effect of individual bacteria on this indicator. The authors of the current study have found that intrauterine administration of a probiotic with *Lactobacillus acidophilus* led to stabilisation of the total number of bacteria in uterine mucus. In healthy cows, as well as in the case of endometritis, the total bacterial colonisation after 6 days did not exceed 100 thousand colony-forming units per 1 cm^3 and in some cows causes its significant decrease.

It was not possible to evaluate the effect of probiotics with *Lactobacillus acidophilus* on the number of bacteria in the uterine mucus of cows. But there is more information about the effect of such probiotics on the intestinal microflora. In particular, the inclusion of *Lactobacillus acidophilus* in cat food led to decrease in the total number of anaerobes in the faeces and to significant decrease in certain groups of microorganisms. Also, a tendency to a slight decrease in pH and restoration of the typical microbiome after the end of probiotic administration was observed (Marshall-Jones *et al.*, 2006). Earlier studies on humans by A. Lidbeck *et al.* (1987) also showed changes in the total number of bacteria in the intestines and oropharynx and a decrease in the number of *E. coli* during probiotic administration. These studies revealed a decrease in the number of *Lactobacillus acidophilus* after the end of the probiotic administration to the initial level.

Thus, the changes caused by the probiotic after injection into the uterine cavity led to inhibition of pathogenic microflora and normalisation of the number of bacteria in mucus at the level typical for healthy cows. This had a therapeutic effect on endometritis and did not affect negatively healthy cows. The presence of lactic acid bacteria in the uterine mucus 6 days after administration of the probiotic was probably a temporary phenomenon and will not affect the reproductive use of cows.

Endometritis is often observed at the end of the voluntary waiting period. The clinical endometritis reduces the efficiency of cow fertilisation using artificial insemination, although no correlation between cow fertilisation and the presence of certain bacterial species has been found (Ballas *et al.*, 2021). The absence of a confirmed effect of bacteria on cow fertility does not mean that the use of a probiotic with *Lactobacillus acidophilus* will not have negative consequences. The risks that may arise are directly related to the effect of lactobacilli on the viability of germ cells. In the current study, no lactic acid bacteria were observed in the uterine microflora of clinically healthy cows and cows with endometritis. Thus, *Lactobacillus acidophilus* is not a typical representative of the uterine microbiome, so their presence, due to the influence on the pH of uterus and because of other factors, can negatively affect the fertilisation of cows due to sperm death. In particular, a decrease in pH in the uterus as a possible cause of lowering of fertility in cows during high nitrogen concentrations in the blood plasma was reported by M. Rhoads *et al.* (2004). According to S. Hugentobler *et al.* (2004), in cattle, the pH of the oviducts was 7.60 and the uterus – 6.96 and remained stable regardless of the day of the cycle. Optimal level of pH for maintaining the vital activity of sperm is 7...7.5, but changes below 6.5 and above 8 leads to a deterioration in most parameters (Contri *et al.*, 2013). Since the use of "Bovilact" causes lowering of pH, there are risks to the viability of sperm introduced into the uterus during insemination.

There are few data in the literature on the interaction of *Lactobacillus acidophilus* with bull sperm, although there is information on the interaction with other bacteria of the genus *Lactobacillus*. In particular, the addition of a probiotic containing *Lactobacillus crispatus, Lactobacillus gasseri* and *Lactobacillus brevis* to diluted bull semen is recommended as a means of inhibiting *Mycoplasma bovis* and reducing the risk of its transmission during artificial insemination (García-Galán *et al.*, 2020). This study found that after 15 hours of incubation of diluted semen with a bacterial concentration of 3.24...324 million CFU, the pH decreased from 6.93 to 6.73, but did not show how this affected sperm survival. Another study by M. Lenický *et al.* (2022) found that short-term coincubation of dilute sperm with *L. curvatus* and *L. hilgardii* had a beneficial effect on sperm motility, mitochondrial activity, and antioxidant properties. In the current study, coincubation of thawed sperm in a medium with a therapeutic concentration of *Lactobacillus acidophilus* during the first two hours led to sperm

death. It should be noted that the concentration of bacteria in the incubation medium in this case significantly exceeded the number of bacteria registered in other studies, like the one by Z. Mohammed *et al.* (2019), in which conclusions were made. The decrease in the concentration of bacteria in the solution is associated with a lengthening of the period during which the presence of live sperm was observed. Due to the fact that the therapeutic concentration of lactic acid bacteria in the uterus after administration of the investigational probiotic did not last long, and after 6 days the total level of bacterial contamination did not exceed 100 thousand CFU, per 1 cm^3, the authors of the current study believe that the risk of sperm death due to the presence of lactobacilli in the uterus is insignificant. Although the fertility of cows treated for endometritis with the probiotic requires additional analysis.

Conclusions

The use of probiotics with lactic acid bacteria may be an option for alternative treatment of cow endometritis. The introduction into the uterine cavity of a suspension of the probiotic based on the strain B-2691 *Lactobacillus acidophilus* with a therapeutic concentration of 500 billion microbial cells allowed to effectively treat clinical endometritis of mild and severe level. In the case of severe endometritis with morphological changes in the uterus, the effectiveness of three times application of probiotic remained high with the recovery rate of more than 70%.

The introduction of probiotics with lactic acid bacteria into the uterine cavity allowed to reduce the total number of bacteria in the mucus of endometritis and clinically healthy cows in 6 days and stabilise it at a level under 100 thousand CFU per 1 cm^3. In 6 days after the probiotic administration, lactic acid bacteria were detected in the uterus, which indicates their ability to remain viable in the intrauterine environment for a certain period of time.

The presence of *Lactobacillus acidophilus* in therapeutic concentration negative affected the activity of sperm and the duration of time during which they remain viable during joint incubation. So, insemination of cows during intrauterine administration of the probiotic may not be effective. It is advisable to inseminate cows after complete recovery, 6 days after the last administration of the probiotic, when the total number of bacteria in the uterine mucus decreases to the levels typical for clinically healthy cows. In this case the risks of reducing the viability of sperm during insemination is insignificant. Further research should be directed to identifying other cultures and strains of lactic acid bacteria effective while treating the endometritis of cows. Additionally, it is necessary to determine the optimal interval between the last introduction of a suspension with live bacterial culture and the admission of a cow for insemination.

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Conflict of Interest

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Ефективність використання молочнокислих бактерій для лікування ендометритів у корів

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Анотація. Актуальною проблемою є пошук альтернативних методів лікування ендометриту корів, які б не мали недоліків традиційних антимікробних препаратів, зокрема виникнення резистентних форм бактерій та обмеження споживання молока. Метою дослідження було визначити можливість лікування ендометриту суспензією пробіотика, до складу якого входить штам В-2691 *Lactobacillus acidophilus*. Коровам української чорно-рябої молочної породи з підтвердженим ендометритом вводили в порожнину матки суспензію препарату (500 млрд мікробних клітин молочнокислих бактерій у 100 см^3) від 1 до 3 разів з інтервалом 6 діб. Загальна ефективність лікування корів усіх груп після трикратного введення препарату становила 90 %. Спостерігалося 100 % одужання корів із субклінічним, легким та важким клінічним ендометритом, а також 73 % одужання корів із морфологічними змінами в матці. Внутрішньоутробне введення препарату (300 млрд клітин на 100 см^3 фізіологічного розчину) призводило до стабілізації кількості бактерій у матковому слизу. На шосту добу після введення кількість бактерій у матковому слизу хворих на ендометрит і деяких клінічно здорових корів зменшувалась, тому у всіх тварин вона не перевищувала 100 тис.

колонієутворюючих одиниць на 1 см^3. У всіх корів виявлено наявність молочнокислих бактерій. Терапевтичні концентрації *Lactobacillus acidophilus* від 0,5 до 3 млрд/см^3 негативно впливали на активність сперми бугая під час інкубації в термостаті. При максимальній концентрації лактобактерій загибель сперматозоїдів відбувалася впродовж першої години інкубації, а при мінімальній концентрації – впродовж двох годин. У контрольному зразку живі сперматозоїди були виявлені після 4 годин інкубації. Пробіотик доцільно використовувати як альтернативний метод лікування та профілактики ендометриту корів, але через негативний вплив терапевтичних концентрацій *Lactobacillus acidophilus* на виживання сперматозоїдів осіменіння слід проводити не раніше ніж через 6 днів після введення препарату, коли підтверджується зниження кількості бактерій в маткової слизу

Ключові слова: велика рогата худоба; *Lactobacillus acidophilus*; матковий слиз; активність сперматозоїдів; колонієутворюючі одиниці

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Fatty acid composition of cow milk fat under the influence of humic acids

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Abstract. The fatty acid composition of milk from healthy animals is relatively stable and within the appropriate limits. Adding an organic feed mixture based on humic acids to the diet of dairy cows can affect the content of the main components of milk and change its fatty acid composition.

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The study was conducted to determine the effect of an organic feed mixture made on the basis of humic acids on the mass fraction of fat in cow milk and its fatty acid composition. An organic feed mixture made on the basis of humic acids was included in the diet of cows (dosage: 20 g/100 kg of body weight). Milk samples (volume 500 cm^3 each) were taken from cows of the experimental (n = 20) and control (n = 20) groups at the beginning of the experiment and on the 65th day after the mixture was applied. 21 fatty acids were identified and quantified by gas chromatography. It was found that the mass fraction of fat in the milk of cows in the experimental group increased (at $p < 0.05$) 65 days after the modification of the diet, which may be the result of better assimilation of feed ingredients under the influence of humic acids. An increase in long-chain fatty acids was noted: myristic (C14:0) and palmitic (C16:0), but these changes were not statistically significant. An increase in heptadecanoic (C17:0) at $p < 0.01$ and stearic (C18:0) at $p < 0.05$ fatty acids was found. Long-chain fatty acids are synthesised by the absorption of circulating fatty acids, dietary fats absorbed from the digestive tract, and non-esterified fatty acids (NEFA) from mobilised fat stores. The synthesis of heptadecanoic acid (C17:0) is carried out by the bacterial microflora of the rumen, which indicates an increase in the number of bacteria under the influence of humic acids. The change in the isomeric composition of unsaturated fatty acids in milk occurred due to octadecene (elaidic) acid (C18:1) (trans-9), and a tendency to increase the intermediate products of rumen hydrogenation – linoleic acid C18:2 (cis-9:12) was also noted. The biological value of milk fat is characterised by the saturation coefficient, which is determined by the ratio of saturated fatty acids to unsaturated. The saturation coefficient of milk of the experimental and control groups did not significantly differ

Keywords: mass fraction of fat; saturated short-chain fatty acids; unsaturated long-chain fatty acids; humic acids

Introduction

In 2020-2025, more and more attention is paid to the study of the composition and properties of lipids in food products. Fats are the main source of energy, perform a structural function, and are also the main components of cell membranes, ensuring their flexibility and integrity. Fats are also factors in the absorption of vitamins E, A, K, and D, which provide the physiological needs of the body (Comerford *et al.*, 2021; Teter *et al.*, 2023). According to data from Y. Hou *et al.* (2023) and W. Lou *et al.* (2024), fats, including milk fat, play a role in thermoregulation, helping to maintain optimal body temperature in animals. In turn, V. Sadvari *et al.* (2024) and B. Paszczyk & E. Tońska (2025) argue that milk and dairy products, due to the balance of components necessary for the body, play an important role in a healthy daily diet of people. In addition to proteins, carbohydrates, vitamins and minerals, milk contains milk fat. The fat present in milk has a unique property, as it contains about 400 different fatty acids, which makes it the most complex of all natural fats (Sadvari *et al.*, 2025).

Milk fatty acids are synthesised from two sources: feed and the result of microbial activity that occurs in the rumen of cows. Lipids in cow's milk are mainly present in globules in the form of an "oil-in-water" emulsion (Kholif *et al.*, 2021). The fatty acid content of milk is a reliable indicator of its quality. The change in this parameter depends on various factors that must be taken into account when implementing methods for adjusting the fatty acid composition of milk (Iakubchak *et al.*, 2024). I. Hryshchuk *et al.* (2022) studied the effect of different types of nervous activity on the content of saturated fatty acids. In the study of blood plasma, it was found that caproic acid in the normotonic group of cows (1.19 ± 0.01) was 0.15% lower compared to blood plasma taken from cows with a sympathotonic nervous system ($p \leqslant 0.01$) and 0.15% higher compared to vagotonic cows ($p \leqslant 0.001$). The content of

caprylic acid was 0.28% higher in normotonics (1.19 ± 0.05) compared to sympathotonics ($p \leq 0.001$). The content of lauric acid in normotonics (0.54 ± 0.03) is higher than in vagotonics by 0.13% ($p \leq 0.01$), and the content of myristic acid is lower in normotonics (2.62 ± 0.08), compared to sympathotonics by 0.30% ($p \leq 0.001$). As for the content of palmitic acid, it is 2.95% lower in normotonics (17.59 ± 0.46), compared to vagotonics ($p \leq 0.001$). At the same time, the content of arachidic acid is lower in normotonic cows (0.21 ± 0.01), compared to sympathotonics by 0.08% ($p \leq 0.001$). Cows with a normotonic type of nervous system are characterised by the highest content of saturated fatty acids in blood plasma: capric (1.19 ± 0.05), lauric (0.54 ± 0.03), and the lowest – myristic (2.62 ± 0.08) and arachidic (0.21 ± 0.01). The blood plasma of sympathotonic cows is characterised by the highest content of the following saturated fatty acids: caproic (1.18 ± 0.04), myristic (2.92 ± 0.03) and arachidic (0.29 ± 0.01), and the blood plasma of vagotonic cows contains the lowest content of the following saturated fatty acids: caproic (0.88 ± 0.01), capric (0.82 ± 0.3) and lauric (0.41 ± 0.01). Therefore, the tone of the autonomic nervous system of cows is characterised by an indirect role in the metabolism of saturated fatty acids in blood plasma.

This study aimed to determine the fatty acid composition of cow milk fat under the influence of humic acids.

Literature Review

Fats in the body play an important role as an energy and plastic material, and therefore their research by many scientists has been relevant for the past decades. Deficiency of fats in the diet leads to complex pathological consequences, which are accompanied by nervous disorders, decreased immunity and, as a result, the quality of life deteriorates and the natural life expectancy is reduced. As M. Markiewicz-Kęszycka *et al.* (2013) noted, the mentioned processes are associated with lipid metabolism disorders. J. Miciński *et al.* (2012) found that short- and medium-chain fatty acids (C4:0, C6:0, C8:0, C10:0), which are present in milk, are oxidised in the liver to acetyl-CoA and do not affect the activity of low-density lipoprotein receptors. They do not increase the level of lipids in the blood, and therefore do not pose a threat to the development of obesity and cardiovascular diseases.

Milk from healthy cows is characterised by a relatively constant content of fatty acids, which are within natural physiological limits (Gómez-Cortés *et al.*, 2018). Although J. Ruiz *et al.* (2016) note that some fluctuations in the content of individual fatty acids may occur when the animal's diet changes. Thus, the concentration of lactate ($p < 0.05$) in cows that had protein feed (soybean) in the diet decreased by 18%. The mass fraction of butyric acid in the milk of cows increased when the buffer mixture was added to the diet, and the content of acids such as 18:1, 18:2, 18:3 and 20:4 ($p < 0.05$-0.01) decreased. When fat was added to the diet, the proportion of fatty acids such as linoleic (cis-9,12 18:2) ($p < 0.05$) and oleic (cis-9 18:1) increased in milk. When feeding both diets ($p < 0.05$), the buffer feed additive reduced the content of oleic and linoleic acids. In the case of feeding soybeans to cows instead of soybean meal, an increase in the content of the sum of trans-isomers of unsaturated fatty acids in milk ($p < 0.001$) was found by 1.7 times. Moreover, feeding cows additionally with a buffer mixture reduced the content of these acids ($p<0.05$) in milk (Hultyayeva *et al.*, 2017).

Researchers A. Kholif *et al.* (2021) claim that adding a feed supplement containing humic acids to the diet of dairy cows affects the content of the main components of milk and changes the fatty acid profile. A. Teter *et al.* (2021) found that adding humic acids to the diet of cows in combination with mineral supplements increases the mass fraction of fat in milk and enhances its cheese-making properties. The ability of humic acids to change the composition of the intestinal microflora of cows has been scientifically

proven, and this, in turn, improves the digestibility of feed nutrients. And as a result, the fat content increases, and the chemical composition of milk improves (Potůčková & Kouřimská, 2017).

It should be noted that humic compounds are formed as a result of the decomposition of plant and animal residues in the soil and are widely used in agronomy (Alsudays *et al.*, 2024). According to N. Strzałkowska *et al.* (2009), they are a source of fulvic and humic acids, as well as minerals. Humic acids have multidirectional properties, including anti-inflammatory, antibacterial, antiviral and immunomodulatory effects, and also reduce oxidative stress (Abo-Zeid *et al.*, 2017; Hassan *et al.*, 2020). Scientists Y. Kansagara *et al.* (2022) experimentally proved that humic acids stabilise the digestion of nutrients. N. El-Zaiat *et al.* (2018) also reported that the administration of humic acids resulted in an increase in both the fat and protein content of milk. In animals that received humic acids in the diet, the content of total protein, globulin and glucose in the blood increased.

Modern results of scientific research conducted by S. Malyugina & P. Horky (2024) indicate that humic acids, as a natural feed additive for ruminants, play an important role in nitrogen metabolism and stabilisation of protozoa without a negative impact on rumen fermentation. Feed additives of humic nature (potassium humate) are used in cow feeding rations. This activates metabolic processes in the animal body (Angeles *et al.*, 2022; Begma *et al.*, 2024). M. Potůčková & L. Kouřimská (2017) when feeding dairy cows humic substances revealed a significant increase in the content of crude protein, pure protein and casein in milk. In the studies conducted by A. Teter *et al.* (2021) note that adding a humic-mineral feed supplement to the diet of cows increased the fat content in milk and improved the properties of milk for cheese production. Thus, the current task is to conduct a study of the effect of the used supplement containing humic acids on the nutritional value of milk fat in raw milk.

Materials and Methods

The study was conducted during the autumn-winter period of 2022-2024 at the dairy farm "Kolosok" in the village of Kozhenyky, Bila Tserkva district, Kyiv region, Ukraine, on black-and-white cows, which were kept loose in a two-row cowshed that accommodates 100 cows (7.5 m^2 per 1 cow). All cows had free access to water, which they consumed ad libitum. The cows were fed a mixed diet. The main diet consisted of corn silage (63%), haylage (30%), rapeseed meal (1.8%), mixed feed (39% protein), soybean meal (2.1%), straw (0.6%) and tricalcium phosphate (0.2%). Forty cows were preliminarily selected from the herd between the 30th and 120th day of the I or II lactation (average 68 days). This period is characterised by the highest lactation. The average body weight of the cows was 554 ± 47 kg, and the daily milk yield was 25.68 ± 3.12 kg. Milking was carried out twice a day, at 6:00 am and 6:00 pm.

Cows were divided into two groups: experimental (n = 20) and control (n = 20) according to the principle of groups-analogues. Animals of the experimental and control groups were kept in different cowsheds on the same type of diet with ensuring their well-being. Cows of the experimental group were controlled to add an organic feed mixture containing 65% humic acids in terms of dry matter (based on 20 g/100 kg of body weight) to the main diet. The dosage was experimental; it was calculated taking into account the diet and productivity of cows. Organic feed additive is a complex of biologically active substances of natural origin, made from natural raw materials (peat, restructured water, leonardite). It is a liquid dark brown mixture with a weak specific odour, which contains 44.4 humic acids and 24.2% fulvic acids with other ingredients. The feed additive was stored in a sealed package at a temperature of 2°C to 16°C, avoiding freezing and direct sunlight. The manufacturer of the product is the company "Greenat Ecology", Bila Tserkva. The duration of the experiment was 65 days.

At the beginning of the experiment and after its completion, milk for the study was collected during morning milking in accordance with DSTU 3662:2018 (2019). An average sample of freshly milked milk was used for the study and was examined within 2 hours after milking. The fat content in milk was determined in accordance with DSTU ISO 488:2007 (2009). The determination of the fatty acid content in milk was carried out by gas chromatography after fat separation according to the method described in DSTU ISO 1211:2002 (2003). To determine the fatty acid composition of the obtained milk fat, its methylation was carried out according to DSTU ISO 5509-2002 (2003). The saturation coefficient of milk was also determined as the ratio of the sum of saturated fatty acids to the sum of unsaturated fatty acids.

Fatty acids were determined in the food laboratory of the State Enterprise "Kyivoblstandartmetrology" on a GC-2010 gas chromatograph (Shimadzu, Japan), equipped with a flame ionisation detector (FID) and a separate sample introduction system (ratio 1:30). Peak separation was performed on a capillary chromatographic column, 30 m long and 0.32 mm internal diameter, 0.25 μm film (Omegawax model – 320, Supelco Co., EUA). Chromatography temperature conditions: injector (280°C) and detector (260°C). At the beginning of the analysis, the thermostat temperature was 40°C for 3 min, followed by programming at 2.5°C/min (from 41 to 180°C), and subsequently at 2.0°C/min to 210°C, and the last temperature was maintained for 25 min. In this case, helium was used as a carrier gas. To achieve a helium velocity of 25.0 cm/sec, the appropriate pressure was set in the column. For identification and quantitative determination of fatty acid peaks of control and experimental milk samples, they were compared with similar indicators of analytical standards. Heptadecanoic acid (C17:0; Sigma Chemical Co.) was used as an internal standard, added for the purpose of quantitative calculation of fatty acid composition. Studies on the determination of fatty acid composition were carried out in 3 parallel tests. Xcalibur software (version 2.07) was used to read the chromatograms. Analysis of fatty acid content was performed according to DSTU ISO 5508-2001 (2003). Statistical data processing was performed using the Student's t-test. (at $p < 0.1$; $p < 0.05$; $p < 0.01$). The experimental studies were conducted in compliance with the requirements of the European Convention... (1986), as well as the Law of Ukraine No. 3447-IV (2006).

Results and Discussion

The mass fraction of milk fat of cows in the experimental group increased with the addition of an organic feed mixture made on the basis of humic acids to the diet (Table 1).

Table 1. *Milk fat content in cows following the administration of humic acids, %*

Animal groups	Experimental scheme			
	Beginning		End	
	[a]M ± m	[b]Lim	M ± m	Lim
Control	3.3 ± 0.05	2.98 - 4.22	3.5 ± 0.24	3.05 - 3.81
Experimental	3.27 ± 0.28	3.01 - 4.54	4.15 ± 0.21**	3.24 - 5.16

Note: ** – p<0.05; [a]M ± m (M – arithmetic mean, m – standard error of the mean); [b]Lim – limits of fluctuations of the indicator

Source: developed by the authors based on research

An increase in the mass fraction of fat in the milk of cows in the experimental group ($p < 0.05$) was noted 65 days after the modification of the diet, namely, the introduction of humic acids into the diet. The improvement in the synthesis of milk fat may be the result of better

assimilation of feed ingredients under the influence of humic acids. The increase in the fat content in milk under the influence of humic acids occurs due to their ability to change the composition of the intestinal flora and, thus, improve the use of feed nutrients, which positively affects the chemical composition of milk (Mehmet & Songül, 2021). Furthermore, the addition of humic acids to the diet of dairy cows also affected the fatty acid profile and lipid composition of milk. Palmitic and palmitic acids predominated in the milk fat of cows in the experimental and control groups (16:0), stearic (C18:0) and myristic (C14:0) fatty acids (Table 2).

Table 2. *Fatty acid composition of cow's milk under the influence of humic acids, %*

Name indicators	Fatty acid code	Research results	
		research group	control group
Saturated fatty acids			
Butyric acid	C4:0	3.96 ± 0.68	4.22 ± 0.02
Caproic acid	C6:0	2.21 ± 0.13	2.35 ± 0.1
Caprylic acid	C8:0	1.19 ± 0.01	1.28 ± 0.15
Capric acid	C10:0	2.41 ± 0.32	2.78 ± 0.39
Lauric acid	C12:0	2.86 ± 0.45	3.20 ± 0.54
Myristic acid	C14:0	11.02 ± 1.31	10.50 ± 0.91
Pentadecanoic acid	C15:0	1.05 ± 0.12	1.37 ± 0.35
Palmitic acid	C16:0	31.12 ± 2.5	30.69 ± 1.57
Heptadecanoic acid	C17:0	0.46 ± 0.02*	0.22 ± 0.01
Stearic acid	C18:0	11.02 ± 0.66*	9.39 ± 0.059
Arachidic acid	C20:0	0.03 ± 0.02	0.08 ± 0.02
Behenic acid saturated	C22:0	0.08 ± 0.02	0.58 ± 0.002
Monounsaturated fatty acids			
Undecanoic acid	C11:0	0.21 ± 0.02	0.36 ± 0.04
Myristoleic acid	C14:1	1.34 ± 0.06	1.68 ± 0.1
Palmitoleic acid	C16:1	1.95 ± 0.53	2.02 ± 0.59
Heptadecenoic acid	C17:1	1.02 ± 0.59	0.93 ± 0.04
Elaidic acid	C18:1 (trans-9)	1.80 ± 0.06*	1.61 ± 0.05
Oleic acid	C18:1n9c	23.17 ± 3.39	23.79 ± 3.13
Polyunsaturated fatty acids			
Linoleic acid	C18:2 (cis-9:12)	2.36 ± 0.06*	2.20 ± 0.02
Linolenic acid	C18:3 (cis-9.12.15)	0.35 ± 0.02	0.43 ± 0.03
Linoelaidic acid	C18:2 (trans-9:12)	0.36 ± 0.02*	0.32 ± 0.01

Note: * – p<0.1; ** – p<0.05; *** – p<0.01

Source: developed by the authors based on research

Milk fat is characterised by a significant mass fraction of saturated fatty acids. Short-chain saturated fatty acids from C4:0 to C10:0 in the milk of cows of the experimental and control groups, respectively, on average amounted to 9.8 and 10.6% of the total saturated fatty acids. A characteristic feature of milk fat is the presence of butyric acid in milk. This fatty acid is formed in milk by synthesis in the epithelial cells of the udder from the products of microbial fermentation of feed carbohydrates. According to the studies conducted, the mass fraction of butyric acid (C4:0) in the milk of cows of the experimental group ranged from 3.29 to 4.66%, and in the milk of the control group – 3.95-4.05%. Long-chain fatty acids are synthesised by the absorption of circulating fatty acids from dietary fats absorbed from the digestive tract and non-esterified fatty acids (NEFA) from mobilised fat stores (Vašková *et*

al., 2011). In the milk of cows of the experimental group, an increase in long-chain fatty acids was noted: myristic (C14:0) and palmitic (C16:0), but these changes were not statistically significant. Along with this, the percentage of heptadecanoic acid C17:0 ($p < 0.01$) and stearic acid C18:0 ($p < 0.05$) increased.

The change in the isomeric composition of unsaturated fatty acids in milk occurred due to octadecenoic (elaidic) acid (C18:1), trans-9. In addition, a tendency to increase the intermediate products of rumen hydrogenation – linoleic acids C18:2 (cis-9:12). The saturation coefficient of milk of the experimental and control groups did not differ significantly (Table 3). The biological value of milk fat is characterised by the saturation coefficient, which is determined by the ratio of saturated fatty acids to unsaturated ones.

Table 3. *Ratio of fatty acid groups in cow's milk with the use of humic acids (M ± m, n = 20, in %)*

No.	Fatty acid groups	Research group	Control group
1	Saturated fatty acids	67.41 ± 6.33	66.66 ± 4.04
2	Unsaturated fatty acids	32.59 ± 4.85	33.34 ± 4.78
3	Saturation factor	2.07	1.99
4	Monounsaturated fatty acids	29.49 ± 4.65	30.39 ± 3.97
5	Polyunsaturated fatty acids	3.1 ± 0.1	2.95 ± 0.06

Source: developed by the authors based on research

R. Golubets *et al.* (2011) point out the regularity that with a decrease in the saturation index, the proportion of unsaturated fatty acids in milk increases. This is manifested in a more pronounced taste and smell, a softer consistency of butter, and milk fat is more stable during storage. In previous studies, O. Yakubchak *et al.* (2023) found that when feeding dairy cows a feed mixture containing 45% humic and 22% fulvic acids, an increase in the cows' milk productivity (an increase in average daily milk yield) and an increase in the mass fraction of fat and protein in their milk were observed. At the same time, R. Mylostyvyi *et al.* (2023) noted that the fatty acid composition of milk can be influenced by various factors, and the study by O. Yakubchak *et al.* (2021) showed that the organoleptic and physicochemical parameters of raw milk change depending on the season. In particular, in winter, the content of saturated fatty acids in milk increases, while in summer and autumn, the content of unsaturated fatty acids increases, which is associated with the presence of green mass and corn silage in the diet of cows. This confirms the need for a comprehensive approach to assessing factors affecting the fatty acid composition of milk. The authors also indicate that the ratio of fatty acids in the milk of cows characterises the level of their feeding, and this confirms the feasibility of using feed additives to improve the quality of dairy products.

The diverse fatty acid composition of cow's milk is also due to the rumen biohydrogenation of C18 unsaturated fatty acids of feed lipids and the synthesis of fatty acids *de novo* in breast tissue (Markiewicz-Kęszycka *et al.*, 2013). The addition of humic acids to the diet of dairy cows affected the change in the fatty acid profile, namely, the increase in saturated fatty acids: stearic acid (C18:0) at $p < 0.05$ and heptadecanoic acid (C17:0) at $p < 0.001$. Taking into account the fact that the synthesis of heptadecanoic acid (C17:0) is carried out by the bacterial microflora of the rumen, it can be assumed that the number of bacteria increases under the influence of humic acids. Stearic acid, which belongs to long-chain fatty acids, can be desaturated in the mammary gland with the formation of the corresponding

monounsaturated acids. Part of the saturated fatty acids absorbed from the blood is converted into monounsaturated by Δ9-desaturase of the mammary gland. The main substrates of this enzyme are stearic (18:0) acid, which is converted into the trans-9 isomer of C18:1 monounsaturated fatty acids.

Trans fatty acids are negatively perceived by consumers, but it is necessary to distinguish between trans fatty acids of natural origin and those obtained industrially. In the milk of cows in the experimental group, an increase in essential polyunsaturated long-chain fatty acids (linoleic and linoleic acid), which is important for milk as a biologically complete food product. Therefore, feeding dairy cows a feed additive based on humic acids contributes to the change not only of the main components of milk, but also improves its fatty acid composition.

Conclusions

The conducted studies have shown a significant effect of enriching the diet of dairy cows with an organic feed additive based on humic acids on milk quality indicators and its fatty acid profile. It was experimentally established that the use of this additive leads to a statistically significant increase in the mass fraction of fat in the milk of cows of the experimental group ($p < 0.05$) 65 days after diet modification. This phenomenon is explained by the improvement of the assimilation of feed ingredients under the influence of humic acids, which are able to change the composition of the intestinal microflora and optimise metabolic processes in the animal body. Chromatographic analysis of milk of dairy cows that received a feed additive based on humic acids allowed the identification of 21 fatty acids of different classes: 12 saturated, 6 monounsaturated and 3 polyunsaturated fatty acids. Particularly significant were the changes in the profile of long-chain fatty acids, namely a statistically significant increase in the concentration of heptadecanoic acid C17:0 ($p<0.01$) and stearic acid C18:0 ($p<0.05$). At the same time, there was a tendency to increase the content of myristic acid C14:0 and palmitic (C16:0) acids, although these changes did not reach statistical significance. Analysis of the ratio of different groups of fatty acids showed that the saturation coefficient of milk between the experimental and control groups did not differ significantly, being 2.07 and 1.99, respectively. This indicates the preservation of the biological value of milk fat when using the studied supplement.

The results obtained are of important practical importance for dairy farming, as they demonstrate the possibility of targeted modification of the fatty acid composition of milk by using natural biologically active substances. This opens up prospects for the production of dairy products with improved functional properties and increased biological value. In the context of further research, it seems appropriate to comprehensively study the dynamics of changes not only in the fatty acid composition, but also in the amino acid composition of milk with long-term use of feed additives based on humic acids. Promising directions also include the study of the effect of different concentrations and compositions of humic compounds on the metabolism of the mammary gland, the study of seasonal variability in the effectiveness of the additive, as well as the assessment of the economic feasibility of its use in different systems of keeping and feeding dairy cows. Additionally, it is relevant to study the effect of such additives on the organoleptic properties of milk and its technological suitability for processing into various types of dairy products.

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Жирнокислотний склад молочного жиру корів за впливу гумінових кислот

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Анотація. Жирнокислотний склад молока здорових тварин є відносно сталим і знаходиться у відповідних межах. Збагачення раціону дійних корів сумішшю на основі органічних компонентів з додаванням гумінових кислот може впливати на вміст основних компонентів молока та змінювати його жирнокислотний склад. Дослідження проведено з метою визначення впливу органічної кормової суміші, виготовленої на основі гумінових кислот, на масову частку жиру в молоці корів та його жирнокислотний склад. До раціону корів було включено органічну кормову суміш, виготовлену на основі гумінових кислот (дозування: 20 г/100 кг маси тіла). Зразки молока (об'ємом по 500 $см^3$) відбирали від корів дослідної ($n = 20$) та контрольної ($n = 20$) груп на початку досліду та на 65-й день після застосування суміші. Методом газової хроматографії ідентифіковано та кількісно визначено 21 жирну кислоту. Встановлено, що масова частка жиру в молоці корів дослідної групи підвищилася (за $p < 0,05$) через 65 днів після модифікації раціону, що може бути результатом кращого засвоєння кормових інгредієнтів під впливом гумінових кислот. Відзначали підвищення довголанцюгових жирних кислот: міристинової (C14:0) та пальмітинової (C16:0), проте

ці зміни не були статистично достовірними. Виявили підвищення гептадеканової (С17:0) за $p < 0,01$ та стеаринової (С18:0) за $p < 0,05$ жирних кислот. Довголанцюгові жирні кислоти синтезуються шляхом поглинання циркулюючих жирних кислот, харчових жирів, що всмоктуються із травного тракту, і неестерифікованих жирних кислот (НЕЖК) з мобілізованих запасів жиру. Синтез гептадеканової кислоти (С17:0) здійснюється бактеріальною мікрофлорою рубця, що вказує на зростання кількості бактерій під впливом гумінових кислот. Зміна ізомерного складу ненасичених жирних кислот молока відбувалася за рахунок октадеценової (елаїдинової) кислоти (С18:1) (транс-9), а також відзначали тенденцію до збільшення проміжних продуктів рубцевої гідрогенізації – лінолевої кислоти С18:2 (цис-9:12). Біологічна повноцінність молочного жиру характеризується коефіцієнтом насиченості, який визначається відношенням насичених жирних кислот до ненасичених. Під час досліджень не виявлено вірогідної різниці коефіцієнту насиченості молока у корів дослідної та контрольної груп

Ключові слова: масова частка жиру; насичені коротколанцюгові жирні кислоти; ненасичені довголанцюгові жирні кислоти; коефіцієнт насиченості

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Variability of milk urea nitrogen traits and their potential use in dairy cattle

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Abstract. Milk urea nitrogen (MUN) is an important biomarker that reflects the efficiency of protein metabolism in cows, the level of consumption and quality of "input" protein, and the energy balance in the diet. MUN fluctuations are closely related to possible discrepancies in the total mixed ration (TMR), physiological and genetic factors. The aim of the study was to determine the influence of genetic (cow's paternal origin) and paratype (calving year-month) factors on milk yield, quality and biochemical parameters of milk, in particular urea nitrogen concentration, in Holstein cows. The research was based on data from 595 cows kept at the Kolos Agricultural Firm LLC in the Kyiv region (Ukraine). The study was conducted under standard conditions with cows fed "without restrictions" and analysis of productivity indicators and biochemical indicators of milk quality. It was found that the factor "year-month of calving" has the greatest impact on productivity indicators and milk quality parameters, causing seasonal fluctuations associated with minor changes in feeding and microclimate. The influence of the sire (cow's father) on milk yield per lactation was noted – 12.4%, milk fat content per lactation – 13.4%, milk protein content per lactation – 12.9%. The influence of non-genetic factors was quite high for daily milk yield, fat, protein and lactose content, as well as MUN. Even with total use of standard TMR, MUN values varied widely in cows from a minimum of 2.6 mg/dl to a maximum of 32.9 mg/dl, with average values of 12.31 ± 0.24 mg/dl, and depended on the cows' ability to separate the diet, minor changes in the diet, and the level of protein and non-structural carbohydrates. A tendency was noted whereby at high MUN values (>16 mg/dl) the absolute level of such important milk components as fat, protein and lactose content decreases, which reduces the energy value of milk – ECM (energy-corrected milk). The results obtained indicated the need to include overall average samples of MUN across the entire herd to optimise feeding programmes and animal welfare management

Keywords: protein metabolism in cows; energy value of milk; Holstein breed; influence of factors

Introduction

Basic methods of data analysis for predicting milk quantity and quality with options for making the right management decisions on dairy farms are being intensively studied. According to O. Palma *et al*. (2025), recent research has focused mainly on predicting milk yield (29%), detecting lameness (26%) and early detection of mastitis (13%). Based on the analysis of a large amount of data, it has been concluded that analytical data combined with optimisation and modelling methods offer advantages (Matvieiev *et al.*, 2025). Predictive modelling in dairy farming is increasing in practical applications (Ruban & Danshyn, 2024). This increases the relevance of finding reliable and relatively simple-to-determine predictor indicators, especially for use in accurate milk production forecasting and detection of diseases such as mastitis and lameness in cows. M. Štolcová *et al*. (2024) provided data on the problems of negative energy balance (NEB), which is a serious problem in most dairy herds. NEB occurs after calving when cows cannot consume enough dry matter to meet their energy needs at the beginning of lactation, and the breakdown of fat reserves releases non-esterified fatty acids (NEFA) into the blood, high concentrations of which cause health problems such as ketosis, fatty liver syndrome, and susceptibility to infections. S. Ruban & V. Danshyn (2024) pointed to a genetic component (breed influence) on blood biochemical parameters and blood urea nitrogen (BUN) levels. V. Souza *et al*. (2021) have demonstrated a close relationship between blood urea nitrogen and milk urea nitrogen levels. According

to R. Jahnel *et al.* (2023), moderate heritability estimates clearly suggest possible selection for MUN, while near-zero estimates of genetic correlations indicate no risk of undesirable correlated selection responses for other milk traits. At the same time, the assessment of the nature of MUN variability together with other important milk components (fat, protein, lactose content) indicates the possibility of their use for diet adjustment.

Nitrogen use efficiency in lactating dairy cows, defined as nitrogen (N) excretion in milk as a proportion of N consumed, is low, with most of the N consumed being excreted in urine and faeces. Accordingly, A.-L. Craig *et al*. (2022) calculated the average nitrogen use efficiency. Nitrogen losses through evaporation, gaseous ammonia, can lead to acidification of water and soil, and loss of biodiversity (de Vries, 2021). Studies by M. Li *et al.* (2022) have demonstrated differences in rumen microbiota in cows with different nitrogen utilisation efficiency. Similar results were obtained for intensive protein consumption values, where cows used protein more efficiently than those with higher intensive consumption values (Risyahadi *et al*., 2023). According to A. Zeleke *et al.* (2025), reducing the crude protein content in dairy cow diets did not affect microbial composition, diversity, and functional profiles. The crude protein content in the diets of dairy cows can be reduced from 17% to 15% to increase nitrogen use efficiency and reduce nitrogen emissions to the environment.

The absolute values and fluctuations of milk urea nitrogen depend on a large number of internal and external factors, affecting animal health, product quality and creating environmental problems due to increased nitrogen losses in excreta. The study was devoted to assessing the variability of MUN and its relationship with such a complex indicator as ECM, as well as the main components of milk that have a certain nutritional value and determine the economics of production.

Literature Review

In modern breeding programmes, when determining the best animals in terms of lifetime value, traits that characterise milk quality (milk fat and protein, somatic cell count) account for 47.4% of their economic (monetary) significance in the overall selection index (VanRaden *et al.*, 2021). In recent years, milk components have been considered not only as indicators of quality and nutritional value, but also as predictors of health status, control of the correctness of the diet, and indicators of environmental impact (Ruban & Danshyn, 2024). At the same time, milk quality indicators are closely related to the duration of lactation, which, in turn, depends on the age of cows, genetic factors (the influence of the sire), calving season and other paratypic factors that directly affect the formation of the lactation curve and overall animal productivity (Kramarenko *et al.*, 2025). According to L. Musembei *et al.* (2023), it is already necessary to rethink the assessment of raw milk quality through the evaluation of its microbiological parameters to ensure high quality dairy products. M. Mortazavi *et al.* (2025) state that milk urea nitrogen serves as an indicator of protein metabolism in dairy cows, and blood urea nitrogen provides an idea of the overall nitrogen balance and kidney function. Under normal circumstances, almost 28% of the nitrogen consumed by dairy cows is converted into milk components, with 5% excreted as non-protein nitrogen and the rest representing “true” protein. Reducing nitrogen emissions in milk production is crucial due to the impact of livestock farming on the environment, as nitrogen is released from cow excreta (manure, urine) and evaporates into the atmosphere or enters farm water sources (Borshch, 2023).

There are different views on the MUN level for describing the physiological characteristics of the gastrointestinal microbiota and kidneys in cows of different breeds, in different climatic zones and with different diet structures. Holstein cows, according to A. Zeleke *et al*. (2025),

are characterised by lower MUN concentrations compared to others, and the amount and composition of "input" dietary protein are considered to be the main factor influencing MUN concentration (Nousiainen *et al.*, 2004; Wattiaux *et al.*, 2011). This is explained by the peculiarities of urea synthesis by the liver, the level of which depends on the concentration of ammoniacal nitrogen (NH_3-N) produced in the rumen. Cows that consume a diet high in crude protein (CP) usually absorb more NH_3-N through the rumen wall because rumen NH_3-N production exceeds the ability of rumen microorganisms to capture it. Ammonia (NH_3) and nitrogen, ammonia (NH_3-N) are different expressions of the chemical forms of ammonia. The NH_3-N form uses the molecular weight of nitrogen atoms only, while the NH_3 form uses the molecular weight of the entire ammonia molecule (1 nitrogen atom + 3 hydrogen atoms). This may affect certain capabilities of the animal's microbiota, the level of nitrogen digestion, the overall health of the animal, and the quality of animal products (Sadvari *et al.*, 2024). Thus, K. Li *et al.* (2023) conducted a multi-omic analysis (biological analysis based on the use and integration of large amounts of data) on rabbits exposed to high and low concentrations of ammonia under similar environmental conditions to determine changes in the microbiota of the nose and colon, gene expression in the lungs and colon, and muscle metabolic profile. The results showed that ammonia exposure significantly affected the microbial structure, composition, and functional capacity in both the nose and colon, which may influence local immune responses and inflammatory processes. Transcriptome analysis (the set of all transcripts synthesised by a single cell or group of cells, including mRNA and non-coding RNA) showed that genes associated with cell death (MCL1, TMBIM6, HSPB1, and CD74) and immune response (CDC42, LAMTOR5, VAMP8, and CTSB) were differentially expressed in the lungs, while colon genes associated with redox status (CAT, SELENBP1, GLUD1, and ALDH1A1) were significantly up-regulated, meaning that the gene or protein is expressed at a higher level than usual, often due to a specific trigger or signal. This increased expression causes the cell to produce more of the corresponding protein and can lead to changes in cell function or behaviour. Several key differentially abundant metabolites, such as L-glutamic acid, L-glutamine, L-ornithine, oxoglutaric acid, and isolactic acid, have been identified in the muscle metabolome, which may indicate a disruption in the influence of ammonia on amino acid synthesis, nucleotide function, and energy metabolism characteristics.

According to V. Ishler (2023), different MUN ranges and their dynamic changes complicate the requirements for their optimal values. Frequently recommended ranges are between 10 and 14 milligrams per decilitre (mg/dl), while others recommend a range of 8 to 12 mg/dl, which is associated with a protein level in the diet of around 16%. It has been calculated that the change in nitrogen is 2 mg/dl for each percentage point change in diet from 15 to 18.5% protein. MUN values are influenced by the feeding system (total mixed ration) versus herds fed individual feed components, and feeding time relative to milking time. MUN values usually peak 3-5 hours after feeding. In addition, herds that are milked three times a day tend to have higher MUN values than herds that are milked twice a day. Another factor that affects MUN values is breed. Holstein cows usually have lower MUN values than other dairy breeds, such as Jersey cows (Ruban & Danshyn, 2024). However, this may be related to body weight rather than breed. In addition, MUN values tend to be higher in the summer months.

One strategy for interpreting MUN on a specific dairy farm is to evaluate the current diet along with the MUN results from laboratory tests. It is useful to have several MUN values over a period of time to help identify possible problems in the diet or farm management practices. Possible problem areas include high

MUN (>12-14 mg/dl) and especially highly controversial values in repeat samples, which may be related to measurement accuracy and cows' responses to external factors that are considered normal "genotype-environment" responses. According to J. Jakobsen *et al.* (2009) and R. Savickienė & A. Galnaitytė (2024), it is the effect of environmental factors that continues to influence the expression of genetic traits over a period of time. From a practical point of view, according to V. Ishler (2023), low MUN levels (<8-10 mg/dl) indicate a possible protein deficiency, which can occur when the number of bacteria in the rumen decreases, limiting milk production and milk protein yield. High MUN levels (>12-14 mg/dl) may be associated with excess dietary protein or an imbalance of rumen protein, protein fractions and energy, and especially non-structural carbohydrates, which include sugar, starch and pectin (Ruban & Vasilevsky, 2015). In addition, it may be associated with the inclusion of soda in the diet of cows as a preventive measure against ketosis. It is high MUN values that indicate additional feed protein and energy expenditure for the synthesis of additional protein when excess nitrogen is released into the environment.

Thus, milk quality indicators combined with detailed biochemical analysis allow for understanding the characteristics of such processes and identify the main influencing factors. Milk urea nitrogen is one of the convenient and fairly accurate descriptive components that reflects the characteristics of ruminant digestion. This study determined the range of fluctuations in the main indicators of milk quality and MUN, followed by the identification of the influence of genetic and paratypic factors on the observed changes. The presented material was an attempt to subsequently determine the possibilities of using such assessments of the efficiency of feed nitrogen utilisation and animal welfare in dairy cattle farming from the point of view of preserving their health.

Materials and Methods

The research material consisted of milk production data for 595 Holstein cows at the Kolos Agricultural Firm Limited Liability Company in the Kyiv region (Ukraine). The research was conducted between December 2024 and April 2025. When conducting the experimental studies presented in this paper, all manipulations with the cows involved in the studies were carried out in accordance with the basic principles of bioethics, in accordance with the European Convention for the Protection... (1986), Law of Ukraine No. 3447-IV (2006) and Procedure for Conducting... (2012). The farm used a tethered milking system with milk piping. Milking was performed three times a day by one milker for up to 50 cows. Feeding was carried out using a total mixed ration (TMR), the characteristics of which are presented in Tables 1 and 2.

Table 1. *Characteristics of the TMR mixture for Holstein cows*

Diet ingredients	Weight, kg		Percentage	
	physical	dry matter	by physical weight	dry matter
Corn silage	28.000	9.240	49.36	37.5
Grain mixture	8.722	7.967	15.38	32.37
Beer lees (moisture)	5.000	0.924	8.81	3.76
Alfalfa haylage	3.500	1.838	6.17	7.47
Pomace	4.000	0.560	7.05	2.28
Corn grain with increased moisture content	2.20	1.541	3.88	6.2

Table 1. Continued

Diet ingredients	Weight, kg		Percentage	
	physical	dry matter	by physical weight	dry matter
Sunflower meal	1.500	1.385	2.64	5.63
Straw	0.80	0.703	1.41	2.86
Water	2.000	0.001	3.53	–
Beet molasses	1.00	0.45	1.76	1.83
Total	56.722	24.6	10	43

Note: the mixture was calculated for cows weighing 550-650 kg, with a milk yield of 28-30 kg, fat content of 4.00%, protein content of 3.40% and lactose level of 4.68%

Source: developed by the authors based on the results of chemical analysis dated 25 April 2025

Table 2. *Biochemical characteristics of the general mixed diet for dairy cows*

Component	Dry matter content, %	Content, g
CP	15.8380	3,897.6920
NDF	28.9485	7,124.1460
NDF fodder	20.4421	5,030.7430
ADF	19.41	4,777.2170
Sugar	6.1480	1,513.0130
Starch	26.0718	6,416.2060
Soluble fibre	7.9292	1,951.3560
Ash	7.6352	1,879.0090
Ca	0.6520	160.4592
R	0.3987	98.1302
Mg	0.4370	107.5504
K	1.1080	272.6834
Fodder	47.8724	–
Concentrate	52.1276	–
Total carbohydrates	73.1534	18,002.8800
Ammonia	0.4838	119.0553

Note: CP – crude protein; NDF – neutral detergent fibre; ADF – acid detergent fibre

Source: developed by the authors based on the results of chemical analysis dated 25 April 2025

Energy-corrected milk calculations were calculated using the formula by M. Hall (2023) (1):

$$\text{ECM} = [(\text{fat content, \%} \times 383 + \text{protein content, \%} \times \times 242 + \text{lactose content, \%} \times 165.4 + 20.7)/3{,}140] \times \text{milk yield, kg.} \quad (1)$$

The analysis of the qualitative and biochemical indicators of milk was determined using the EKOMILK Bond ultrasonic analyser (Bulgaria). The amount of urea in milk was determined using the diacetylmonoxime method. Its level was judged by the content of the red complex formed by urea with diacetylmonoxime in an acidic environment in the presence of thiosemicarbazide and trivalent iron according to the method of N. Langenfeld *et al.* (2025). The molar concentration of urea (C) in mmol/l was determined from the optical density data of sample A relative to standard B using formula (2):

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

Statistical analysis (descriptive statistics,variance analysis, correlation and regressionanalysis) was performed using the

RStudio-2023.03.0-386 programme. The effects of the year-month of calving, sire (father) and lactation number were analysed using a linear model (3):

$$y_{ij} = a_i + b_j + c_k + e_{ij}, \quad (3)$$

where y_{ij} is the determined factor; a_i is the effect of the i-th year-month of calving; b_j is the effect of the j-th bull-sire (father); c_k is the effect of the k-th lactation number; e_{ij} is the residual.

The degree of influence of factors on the studied traits of beef cattle was calculated using formula (4):

$$\eta^2 = (SSA/SSП)\cdot 100\%, \quad (4)$$

where *SSA* – sum of squares of deviations caused by the factor; *SSП* – total sum of squares of deviations.

Tables 3 and 4 present descriptive statistics of the studied traits.

Table 3. *Descriptive statistics of milk productivity and live weight of cows in terms of standard lactations (n = 595)*

Trait	Min	Max	M ± m	σ2	σ	Cv, %
First lactation						
Yield for 305 days of lactation, kg	2,873	11,348	8,538.3 ± 90.83	1,897,643.5	1,377	16
Amount of milk fat, kg	143	409	288.9	2,028.8	45	15.6
Live weight of cows, kg	396	515	508.3 ± 0.64	284.1	16	3.31
Second lactation						
Yield for 305 days of lactation, kg	3,627	12,083	8,531.9	3,265	1,807	2,162
Amount of milk fat, kg	130	436	290.9 ± 4.6	3,585.5	59.9	20.6
Live weight of cows, kg	521	532	528.4 ± 0.57	292.3	17	3.24
Third lactation						
Yield for 305 days of lactation, kg	2,457	1,210	8,721.4	2,748	1,658	19
Amount of milk fat, kg	134	425	298.7	3,300.5	57.4	19.2
Live weight of cows, kg	532	543	537.1 ± 0.52	298.2	17	3.22
Fourth lactation and older						
Yield for 305 days of lactation, kg	3,364	1,266	7,835.0	3,458	1,859	23.7
Amount of milk fat, kg	168	420	269.0 ± 9.2	3,910	62.5	23.2
Live weight of cows, kg	541	650	620.3 ± 0.49	311.6	17	2.84

Source: developed by the authors based on research

Table 4. *Descriptive statistics of the studied milk indicators (n = 595)*

Feature	Min	Max	M ± m	σ2	σ	Cv, %
Daily yield, kg	10	56	27.7 ± 0.36	70.2	8.4	30
DNMR, %	3.11	10.6	8.74 ± 0.02	0.34	0.58	6.6
Fat content, %	3.17	5.7	4.39 ± 0.08	0.6	0.82	16.7
Protein content, %	2.4	4	3.40 ± 0.01	0.03	0.17	5
Freezing point, °C	0.4	0.7	0.58 ± 0.01	0.001	0.03	5.2
Lactose content, %	0.5	5.8	4.68 ± 0.01	0.10	0.32	6.8
pH	2.1	7.4	7.09 ± 0.01	0.06	0.24	3.4
Titratable acidity, Th	1.5	10.5	6.46 ± 0.04	0.75	0.87	13.5
Conductivity	3.3	8.4	3.98 ± 0.02	0.15	0.39	9.8
MUN* (1st level), 8-11.99 mg/dl	8.7	11	9.90 ± 0.07	1.17	1.0	10.9
MUN* (2nd level), 12-15.99 mg/dl	12	15	13.89 ± 0.10	1.2	1.1	7.9

Table 4. Continued

Feature	Min	Max	M±m	σ2	σ	Cv, %
MUN* (3rd level), 16.0 mg/dl and above	16	32	21.96 ± 0.39	18.5	4.3	19
MUN for the entire sample	2.6	32	12.31 ± 0.24	35.6	5.97	48.5
Live weight of cows, kg	488	650	526.4 ± 0.75	308	17	3
Energy-corrected milk (ECM)	10.3	66	29.13 ± 0.35	70	8.3	28

Note: *MUN (milk urea nitrogen) gradations and number of observations by groups: 1st level – 217 heads; 2nd level – 120 heads; 3rd level – 122 heads

Source: developed by the authors based on research

Based on milk quality indicators, the studied animals were combined into a general sample (Table 4), which made it possible to calculate the influence of such factors as "year-month of calving", "bull-breeder", "lactation number" using a "mixed" model. To determine possible changes in milk composition in relation to MUN levels, this indicator was divided into three levels based on a range of values: Level 1: 8-11.99 mg/dl; Level 2: 12-15.99 mg/dl; Level 3: 16.0 mg/dl and above.

According to DSTU 3662:2018 (2019), the following was additionally included in the milk analysis: (1) dry non-fat milk residue (DNMR) – or the dry mass of milk remaining after fat removal, consisting of proteins, lactose and minerals. SDF can range from 8.0 to 9.5% and is often used as an indicator of milk quality and to calculate dry matter content; (2) milk freezing point (°C), the temperature at which it begins to freeze, averages -0.540°C to -0.570°C, and for higher, first and extra grades -0.520°C; (3) lactose content – plays an important role in the production of dairy products, is about 5 g per 100 ml or 4.6-5.2%; (4) milk pH, which is usually in the range of 6.5-6.7, indicating a slightly acidic environment. A decrease in pH below 6.5 may indicate health problems, and an increase in pH may indicate rumen alkalosis, i.e. a digestive disorder characterised by impaired metabolism and dysfunction of the liver and other organs. An alkaline environment forms in the rumen at an ammonia concentration of more than 16.1 mg%, which leads to a decrease in the number of microorganisms and a decrease in their activity. Total protein in the blood increases to 113 g/l, and the reserve alkalinity of the blood and the pH of milk and urine increase; (5) titrated acidity of milk, or Turner degrees (T) – the number of millilitres of 0.1 M alkali solution required to neutralise the acids in 100 ml of milk. The titrated acidity of freshly milked milk is 16-18°T, with an acceptable value of 15.99-20.99°T.

Results and Discussion

A large number of factors affect milk quality, forcing specialists to find ways to understand the mechanisms of such influence and their significance. Table 5 presents the results of a variance analysis of the influence of such seasonal factors as the year and month of calving, the origin of daughters by sire (progenitor), which is a genetic factor, and the number of lactations.

Table 5. *Impact of year-month of calving, sire (father of cows) and lactation number on milk production and reproduction indicators (n = 595)*

Factor, indicator	Sum of squares of deviations	Number of degrees of freedom	Mean square of deviations	Fisher's F-test	η^2, %
Daily milk yield					
Year-month of calving	15,751	25	630.1	15.424***	80.7
Buhai	2,979	44	67.7	1.657**	15.3

Table 5. Continued

Factor, indicator	Sum of squares of deviations	Number of degrees of freedom	Mean square of deviations	Fisher's F-test	η^2, %
Lactation number	779	6	129.8	3.177**	4
Balance	19,975	489	40.8		
Milk yield per lactation					
Year-month of calving	378,318,037	22	17,196,274	8.509***	24.5
Buhai	191,039,506	44	4,341,807	2.148***	12.4
Lactation number	8,578,626	6	1,429,771	0.707	0.01
Balance	968,079,307	479	2,021,042		
Fat content in milk during lactation					
Year-month of calving	4.260	22	0.19364	49.53***	66
Buhai	0.189	44	0.00430	1.10	3
Lactation number	0.056	6	0.00938	2.40	0
Balance	1.873	479	0.00391		
Amount of milk fat per lactation					
Year-month of calving	318,450	22	14,475	5.988***	18.5
Buhai	229,563	44	5,217	2.158***	13.4
Lactation number	12,340	6	2,057	0.851	0.7
Balance	1,157,855	479	241		
Protein content in milk during lactation					
Year-month of calving	1.0362	22	0.04710	39.351***	59
Buhai	0.0973	44	0.00221	1.847**	5.6
Lactation number	0.0249	6	0.00415	3.468**	1
Balance	0.5733	479	0.00120		
Amount of milk protein per lactation					
Year-month of calving	350,078	22	15,913	7.426***	22
Buhai	206,319	44	4,689	2.188***	12.9
Lactation number	10,720	6	1,787	0.834	0
Balance	1,026,389	479	214		
Service period					
Year-month of calving	8,520,826	23	370,471	141.145***	86
Buhai	130,619	42	3,110	1.185	1
Lactation number	35,538	5	7,108	2.708	0
Balance	1,215,259	463	262		

Note: * – P > 0.95; ** – P > 0.99; *** – P > 0.999

Source: developed by the authors based on research

A significant impact on productivity indicators of factors such as the year and month of calving has been proven in almost all cases (Table 5). Despite the use of a generally mixed diet, which was standard throughout the calendar year, the season of the year had a significant impact on daily milk yield, lactation yield, fat and protein content. The origin of the cows' sires also had a significant impact, although in most cases it was significantly smaller than seasonal fluctuations. The influence of the sire (father of the cow) on lactation yield was 12.4%, milk fat content during lactation was 13.4%, and milk protein content during lactation was 12.9%. Seasonal fluctuations (year-month of calving) had a significant and probable effect on milk quality indicators (Table 6), with minimal influence from genetic factors such as paternal origin.

Table 6. *Influence of the year and month of calving and the sire (father) on milk quality indicators*

Factor	Sum of squares of deviations	Number of degrees of freedom	Mean square of deviations	Fisher's F-test	η^2, %
		Daily milk yield			
Year-month of calving	15,751	25	630.1	15.444***	4
Buhai	2,979	44	67.7	1.659**	7.7
Balance	19,975	489	40.8		
		SZMZ			
Year-month of calving	30.33	25	1.2132	3.914***	0
Buhai	7.33	45	0.1630	0.526	0.02
Balance	160.25	517	0.3100		
		Fat content			
Year-month of calving	4.260	22	0.19364	49.524***	67
Buhai	0.189	44	0.00430	1.100	3
Balance	1.873	479	0.00391		
		Protein content			
Year-month of calving	3.505	2	0.14018	5.488***	19
Buhai	1.032	45	0.02293	0.898	5.7
Balance	13.206	517	0.02554		
		Freezing point			
Year-month of calving	0.0631	25	0.0025236	3.578***	13
Buhai	0.0234	45	0.0005196	0.737	5.08
Balance	0.3647	517	0.0007053		
		Lactose content			
Year-month of calving	7.09	25	0.28375	2.962***	11
Buhai	3.11	45	0.06910	0.721	5.1
Balance	49.52	517	0.09579		
		Conductivity			
Year-month of calving	6.95	25	0.2779	2.113**	7
Buhai	9.33	45	0.2074	1.577	10.6
Balance	68.0	517	0.1315		
		pH			
Year-month of calving	14.569	25	0.5827	16.345***	42
Buhai	1.111	45	0.0247	0.693	3.2
Balance	18.432	517	0.0357		
		Titrated acidity			
Year-month of calving	29	25	1.1638	1.588	6
Buhai	35.1	45	0.7793	1.063	7
Balance	379	517	0.7331		
		MUN			
Year-month of calving	1,982	25	79.28	2.386***	9
Buhai	1,601	45	35.57	1.070	7
Balance	17,179	517	33.2		

Note: * – P > 0.95; ** – P > 0.99; *** – P > 0.999

Source: developed by the authors based on research

It can be stated that there is a significant influence of such organised factors as minor changes in feeding in different seasons and months of the year and even changes in temperature conditions on the farm during different periods of the year. The influence of

non-genetic factors was quite high in terms of daily milk yield, fat, protein and lactose content, as well as MUN. To assess the genetic component of these indicators, the authors believe it is necessary to use not the absolute values of these indicators, but the nature of their change over a certain period of time in terms of genetic groups, linking such changes to the norm of the "genotype-environment" reaction.

The MUN control system was developed in the United States by the Dairy Herd Improvement Association DHI (Dairy Herd Improvement), which provides dairy farmers with information for managing and improving their herds based on the analysis of "input data" that records the characteristics of animal feeding and milk quality indicators (Fig. 1). In addition, DHI includes the collection and analysis of data on milk production and quality, cow health and breeding factors. DHI programmes are often managed by DHIA (Dairy Herd Improvement Associations).

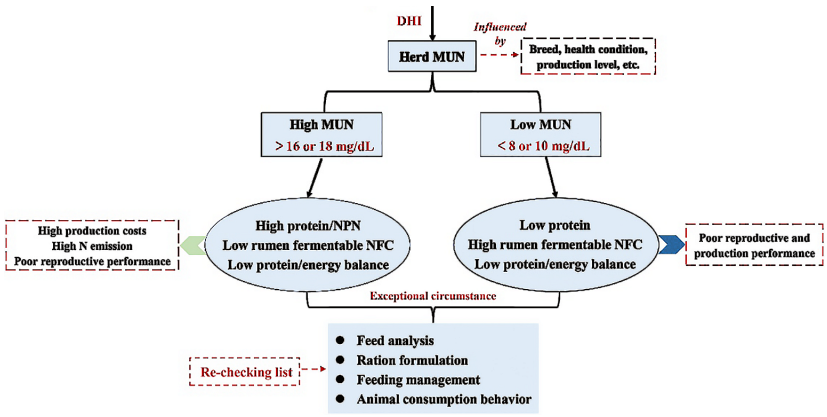

Figure 1. Process for identifying the causes of high or low MUN concentrations, as applied by the consulting company DHI (Dairy Herd Improvement), USA

Note: MUN – milk urea nitrogen; NPN – non-protein nitrogen; NFC – non-fibre carbohydrates

Source: X. Zhao *et al*. (2024)

According to experimental data by V. Ishler (2023), the possible ranges for MUN can complicate their interpretation, as some researchers recommend a MUN range of 10 to 14 milligrams per decilitre (mg/dl), while others recommend a range of 8 to 12 mg/dl. The latter range reflects diets that are formulated according to the protein requirements of cows and have an excellent balance of protein (16%), protein fractions and carbohydrates to capture excess ammonia in the rumen. A study by C. Müller *et al.* (2021) showed that a one percent increase in crude protein in the diet resulted in an increase in MUN concentration of 1.04 and 1.24 mg/dl at milk yields of 40 and 30 kg/day, respectively. One strategy for meaningful interpretation of MUN on a specific dairy farm is to evaluate the current diet and MUN across the entire herd from the milk storage tank. It is useful to have several MUN values to compare with a specific diet as a baseline. Two possible problem areas are high (>12-14 mg/dl) and highly unstable MUN values. Low MUNs (<8-10 mg/dl) indicate a possible protein deficiency in the diet, which can occur when rumen bacteria activity decreases, limiting milk production and milk protein synthesis. High MUN levels (>12-14 mg/dl) may be associated with excess protein in the diet or an imbalance of protein in the rumen, protein fractions and energy (non-structural carbohydrates). These factors may also be associated with reduced milk yield, true protein content

and feeding efficiency. High MUN values indicate a loss of feed protein and cows using more energy to excrete this excess protein through milk and excreta, which means excess nitrogen is released into the environment.

A key factor is ensuring sufficient amounts of available carbohydrates in the rumen to provide energy to the rumen microbiota for converting ammonia into microbial protein. According to X. Zhao *et al.* (2024), MUN control serves as a reliable indicator of the nutritional characteristics of dairy herds due to its impact on reproductive function, health and nitrogen utilisation efficiency, and nitrogen utilisation efficiency itself is associated in ruminants with methane (CH_4), carbon dioxide (CO_2), nitrogen (N_2), and ammonia (NH_4). According to A. Plomaritou *et al.* (2025), dairy cows typically sort TMR, selecting smaller feed particles over longer ones, resulting in higher consumption of highly fermentable carbohydrates and lower consumption of effective fibre than expected, which affects rumen pH and changes in milk composition. The ECM is a comprehensive indicator for determining the commercial attractiveness of milk, as it generally determines the price range for products according to the following formula: the higher the ECM, the higher the price per unit of product. The data in Table 7 reveal the relationship between ECM and the main components of milk, including changes in MUN levels across the three control groups.

Table 7. *Correlation coefficients between ECM and other studied characteristics (n = 595)*

Daily milk yield	Milk fat content	Milk protein content	Lactose content in milk	Live weight of cows	Urea nitrogen content in milk (MUN)
Across the entire sample (n = 595)					
0.9077 ± 0.0177***	0.1127 ± 0.0418**	-0.2180 ± 0.0411***	-0.2172 ± 0.0411***	-0.0511 ± 0.0432	-0.0005 ± 0.0421
With urea nitrogen content in milk of 8-11.99 mg/dl, (n = 207)					
0.8926 ± 0.0315***	0.1412 ± 0.0691*	-0.3054 ± 0.0665***	-0.3155 ± 0.0663***	-0.0461 ± 0.0423	+0.0134 ± 0.0698
With urea nitrogen content in milk of 12-15.99 mg/dl, (n = 112)					
0.9253 ± 0.0362***	0.0351 ± 0.0953	-0.2290 ± 0.0928*	-0.2395 ± 0.0926**	-0.0502 ± 0.0436	+0.1069 ± 0.0948
with urea nitrogen content in milk of 16 mg/dl and above (n = 248)					
0.9371 ± 0.0333***	-0.0034 ± 0.0953	-0.1129 ± 0.0947	-0.1191 ± 0.0946	-0.0451 ± 0.0431	-0.1344 ± 0.0945

Note: * – P > 0.95; ** – P > 0.99; *** – P > 0.999

Source: developed by the authors based on research

There is a logical correlation between milk yield and the main components of milk with virtually no correlation with MUN (data for the entire sample). However, in the variants with an increase in MUN threshold values from 8 to 16 mg/dl and above, there is a tendency towards a negative value of this relationship between MUN and ECM, which was -0.1344 ± 0.0945 (Table 7). Obviously, high MUN values (16 mg/dl and above) are a potential problem area, as they are associated with a decrease in the levels of important milk components such as fat, protein and lactose.

Conclusions

Milk components such as fat, protein, lactose, and milk urea nitrogen (MUN) have a wide range of phenotypic expression, which characterises the complex biochemical processes in the body of ruminants with significant factors influencing such environmental fluctuations. Within the confidence intervals of the analysed sample, the fluctuations (min-max) in the main characteristics, together with their mean values and errors, were as follows: daily milk yield 10.0-56.0 kg (27.7 ± 0.36); fat content

3.17-5.73% (4.39 ± 0.08); protein content 2.4-4.0% (3.40 ± 0.01); lactose content 0.5-5.8% (4.68 ± 0.01); pH 2.1-7.4 units (7.09 ± 0.01); MUN 2.6-32.9 mg/dl (12.31 ± 0.24); live weight of cows 488.0-650.0 kg (526.4 ± 0.75); energy-corrected milk yield (ECM) 10.3-66.6 kg, (29.13 ± 0.35). Within the specified ranges of the experimental sample, no significant correlation was found between the energy value of milk (ECM) and the urea nitrogen content in milk (-0.0005±0.0421).

When selecting cows from the general sample within the MUN values of 8-11.99 mg/dl, (first group – 207 heads) and 12-15.99 mg/dl (second group – 112 heads), the correlation coefficient with ECM indicators was +0.0134 ± 0.0698 and +0.1069 ± 0.0948, respectively (the estimate is unreliable). There was a tendency for high MUN values (>16 mg/dl) to reduce the absolute level of such important milk components as fat, protein and lactose content, which reduces the energy value of milk (ECM). No significant influence of the genetic component (paternal origin) on MUN values was found. The very principle of searching for genetic influence on such fluctuations should be based on the analysis of the dynamics of MUN estimates over time in the context of such genetic groups. In further studies, it is advisable to conduct long-term monitoring of changes in the level of milk urea nitrogen in the daughters of different sires to identify the genetic component of the influence on the efficiency of feed nitrogen utilisation, as well as to create predictive models that combine the genotypic characteristics of cows (by origin), seasonal factors and MUN indicators to optimise breeding programmes, which will make it possible to expand the set of analytical data and, in combination with optimisation and modelling methods, provide specific recommendations for the use of such comprehensive assessments.

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Conflict of Interest

The authors declare no conflict of interest.

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Мінливість ознак азоту сечовини молока та можливості їх використання в молочному скотарстві

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Анотація. Азот сечовини молока (MUN) – важливий біомаркер, який відображає ефективність білкового обміну корів, рівень споживання та якість «вхідного» протеїну, баланс енергії в раціоні. Коливання MUN тісно пов'язані з можливими розбіжностями в загально змішаному раціоні TMR (total mixed ration), фізіологічними та генетичними факторами. Мета роботи полягала у визначенні впливу генетичних (походження корів за батьком) та паратипових (рік-місяць отелення) факторів на молочну продуктивність, якісні та біохімічні показники молока, зокрема концентрацію азоту сечовини, у корів голштинської породи. Матеріалом для досліджень слугували дані 595 корів, що утримувались в умовах ТОВ «Агрофірма "Колос"» Київської області (Україна). Дослід проведено у стандартних умовах при годівлі корів «без обмежень» з аналізом продуктивних показників та біохімічних показників якості молока. Встановлено, що найбільший вплив на продуктивні показники та параметри якості молока має фактор «рік-місяць отелення», що зумовлює сезонні коливання, пов'язані з незначними змінами годівлі та мікроклімату. Відзначено вплив плідника (батька корови) на надій за лактацію – 12,4 %, кількість молочного жиру за лактацію – 13,4 %, кількість молочного білка за лактацію – 12,9 %. Вплив

не генетичних факторів був доволі високим за добовим надоєм, вмістом жиру, білка та лактози, а також MUN. Значення MUN навіть при тотальному використанні стандартного TMR варіювали у корів у широких межах від min 2,6 мг/дл до max 32,9 мг/дл, при середніх значеннях 12,31 ± 0,24 мг/дл, і залежали від можливостей сепарування раціону коровами, незначними змінами раціону, рівнем протеїну та неструктурних вуглеводів. Відзначена тенденція, коли при високих значеннях MUN (16 мг/дл, та >) знижується абсолютний рівень таких важливих компонентів молока, як вміст жиру, білка та лактози, що знижує енергетичну цінність молока – ECM (energy-corrected milk). Отримані результати свідчать про необхідність включення загальних середніх проб MUN по всьому стаду для оптимізації програм годівлі та управління добробутом тварин

Ключові слова: білковий обмін корів; енергетична цінність молока; голштинська порода; вплив факторів

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Effect of water temperature on growth and development of red swamp crayfish *Procambarus clarkii* (Girard, 1852)

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Abstract. One of the most widespread commercial crayfish species in world's aquaculture is the red swamp crayfish *Procambarus clarkii*. The aim of this work was to analyse the effect of temperature on growth parameters of *Procambarus clarkii* grown in the water temperature range from 22.0 to 32.0°C with a temperature step of 2.0°C (6 experimental groups in total). During the experiment, theoretical (analysis, systematisation, comparison), experimental (laboratory) and generally accepted research methods were used. The results clearly demonstrated the inhibitive effect of high water temperatures – 30.0°C (Group 5) and 32.0°C (Group 6) – on the growth and development (length and weight gain, survival rate) of crayfish, while the lowest water temperature in this experiment (Group 1, 22.0°C) contributed to more efficient growth and supported survival of crayfish. For example, the final average weight of crayfish in Group 1 (22.0°C) was 15.20 ± 1.97 g, in Group 2 (24.0°C) the average weight was 13.71 ± 1.35 g, crayfish of Group 3 (26.0°C) reached an average weight of 14.85 ± 1.75 g, Group 4 (28.0°C) – 14.08 ± 2.25 g, and in Group 5 (30.0°C) and Group 6 (32.0°C) the average weight was 9.75 ± 2.33 g and 6.60 ± 0.36 g, respectively, which was 1.5 and 2.3 times less than in Group 1. At the same time, the linear growth was more homogeneous and amounted to 8.00 ± 0.29 cm in Group 1, an average of 7.38 ± 0.29 cm in Group 2, individuals of Group 3 had an average length of 7.66 ± 0.22 cm, in Group 4 – 7.42 ± 0.33 cm, and in high-temperature Groups 5 and 6 the average length was 6.80±0.50 cm and 6.04 ± 0.26 cm, respectively. A tendency to decrease the survival rate of crayfish with increasing water temperature in the groups was established. The survival rate in Group 1 at the end of the study was 58.82%, in Group 2 – 41.18%, Group 3 – 47.06%, Group 4 – 41.18%, Groups 5 and 6 experienced the greatest pressure of the temperature on survival – 35.29% and 29.41%, respectively

Keywords: aquaculture; *Procambarus clarkii*; weight; length; survival; thermoregulation; metabolism

Introduction

Considering the difficult situation in which natural crayfish populations are currently in Ukrainian water bodies, it is becoming increasingly important to breed these species in controlled conditions, thereby reducing pressure on natural populations. However, the biological characteristics (slow growth, demanding water quality, etc.) of native crayfish species limit the possibilities for their effective industrial cultivation. Therefore, in terms of researching new highly productive objects of aquaculture among crustaceans, promising is red swamp crayfish *Procambarus clarkii* (Girard, 1852). The prospects for its cultivation are due to its high adaptability to various environmental conditions (in particular, controlled ones), omnivorousness, rapid growth rate, and flexible life cycle, which allows it to survive in various physical and chemical conditions, etc. In addition, given the trends in the development of aquaculture and its potential in Ukrainian water bodies, this species could ideally fit into the structure of multi-trophic aquaculture, the importance of which is underestimated in Ukraine but deserves attention, given the efficiency of its processes and the high potential for commercial yield per unit area (Honcharova *et al*., 2024).

The natural distribution range of *Procambarus clarkii* is the south-eastern United States, but due to human economic activity, this species has significantly expanded its natural range. A. Ballinger (2022) draws attention to the wide range of introduction of this species outside its natural range – all continents except Australia and Antarctica, which was facilitated, firstly, by the widespread popularity of *Procambarus clarkii*

as a species of ornamental aquaculture during the 20th century (Oficialdegui *et al.*, 2025). Only recently, it was recognised as a potential aquaculture species, being included in the list of the 25 most important aquatic alien species introduced into European freshwater and coastal marine waters (Savini *et al.*, 2010). At the same time, according to A. Nota *et al*. (2024), this invasiveness has its advantages, primarily due to the fact that *P. clarkii*, as a more adaptable species, replaces populations of local crayfish that have been lost for certain reasons, which does not always have a positive effect on local ecosystems. As a farmed species, *P. clarkii* is also extremely popular in global aquaculture. It is successfully used commercially in countries where it has developed a significant population of harvestable crayfish. According to FAO (2021), the vast majority of *P. clarkii* production in aquaculture, which is over 2 million tons per year, is currently in China. The same source indicates that *P. clarkii* is the second most cultivated crustacean species in the world.

However, such rapid and intensive development of red swamp crayfish populations in water bodies around the world is still a cause for concern in terms of the impossibility of fully assessing the impact of its populations on local water bodies. Therefore, potential ways to reduce its possible destructive impact or negative pressure on local water bodies are being studied. It is known that *P. clarkii*, as ectotherms, are not capable of independent thermoregulation, and the determining factor for their successful reproduction and development is precisely the water temperature, and its decrease or increase beyond the species optimum causes enormous stress in their organism. It is low water temperatures, especially during wintering, that inhibit the spread of *P. clarkii* in northern latitudes. Furthermore, it has been established that cold stress affects almost all biochemical and physiological processes in crustaceans, including their behaviour, growth and development, reproduction, metabolism, and immunity (and, accordingly, resistance to disease) (Ren *et al.*, 2021).

The only monitoring study conducted so far by L. Peruzza *et al.* (2015) found that populations of *P. clarkii* populations inhabiting the cold-water Brancolo canal (northern Italy) have unique biological characteristics that allow them to complete their life cycle at temperatures that, according to other studies, should inhibit their growth and development and prevent reproduction (Jiang *et al.*, 2024). The results clearly confirm the adaptation of this population to an atypical temperature environment, characterised by an average annual water temperature of 13.32±0.0°C. However, the fertility of females from the cold-water Brancolo canal was 35.0±7.0 eggs compared to 285.0-995.0 eggs in females that reproduce at optimal water temperatures. It is also interesting that in high latitudes, *Procambarus clarkii* reproduces once a year (at the end of summer) and can carry eggs throughout the winter, while in lower latitudes, the species' life cycle includes year-round reproduction with several peaks per year (Chucholl, 2011; Slusar *et al.*, 2023). The results presented indicate that *P. clarkii* is capable of adapting well to new, cold habitats by changing its life cycle.

Regarding the effect of high temperatures on the cultivation of *Procambarus clarkii*, an interesting study by X. Chen *et al*. (2024) found that groups of crayfish kept at a higher water temperature (30.0°C) had correspondingly higher levels of astaxanthin, which developed a rich bright red colouration of the crayfish shell. Thus, the vast majority of studies are aimed at investigating the characteristics of this species' adaptation to water temperature, but they do not answer the question of whether there is an optimal temperature for rapid linear growth and weight gain, sexual maturation, and high survival under controlled industrial farming conditions, or whether it is possible to influence the productivity of *P. clarkii* using this parameter. In addition, the issue of the invasiveness of this species should not be ignored, as against the backdrop of climate change and reduced biotic

stability of water bodies, including in Ukraine, it may create conditions that will disrupt the stability of freshwater ecosystems and create conditions for the ideal adaptation of this species. Therefore, it is necessary to understand in more detail the mechanism of adaptation of the red swamp crayfish to the environment and the possible ecological barriers to their successful development in natural ecosystems.

For this reason, the objective of this study was to analyse the impact of different temperature regimes on the growth of *Procambarus clarki* on changes in their growth and survival rates. The study was conducted in accordance with Agreement No. BF/37-2021 (state registration number 0121U113569) within the priority thematic area "Innovative solutions for ensuring the sustainable development of agriculture, forestry, and veterinary medicine in conditions of martial law", applied research "River basin management in the context of climate change".

Materials and Methods

To understand the impact of the aquatic environment on the growth characteristics of the red California crayfish, it was important to study the organism's response not only to temperatures within the species' optimal range, but also to temperatures exceeding its tolerance limit. For this study, young *Procambarus clarkii* aged 20-25 days were used. The study was conducted between June and August 2024 at the Aquaculture Technology Laboratory (V. Kondratyuk Laboratory of Aquatic Bioresources and Aquaculture). The crayfish juveniles for the study was obtained from own broodstock of red swamp crayfish kept in the same laboratory. Six groups were formed for the study in the temperature range from 22.0°C to 32.0°C: Group 1 – grown at a water temperature of 22.0°C, Group 2 – 24.0°C, Group 3 – 26.0°C, Group 4 – 28.0°C, Group 5 – 30.0°C, and Group 6 – 32.0°C. These temperatures were partly within the species optimum range and partly outside the tolerance limits of the organisms, creating extreme conditions (>29.0-30.0°C). A total amount of *Procambarus clarkii* which were used in the experiment, were 102 individuals, 17 in each group. The groups were formed by randomly selecting individuals for each group. The initial weight of the crayfish was 0.2±0.05 g on average, with an average length of 0.2±0.05 g. The crayfish were kept in a freshwater model aquarium system consisting of six 100 L tanks with identical water treatment and cultivation conditions (with an equal number of shelters). Water temperature and dissolved oxygen levels were monitored daily at three measurement points: two stationary points using a JBL Thermometer Premium thermometer and an Aqua-Tech HT-1 electronic thermometer with a remote sensor, and the third using a portable AZ-86021 oximeter.

For feeding at different stages of development, following high-protein compound feeds were used: Alltech Coppens TOP, 0.5-0.8 mm (protein: 60.0%, fat: 16.0%, fibre: 0.3%, ash: 10.7%), Alltech Coppens Vital, 0.5-1.2 mm (protein: 46.%, fat: 10.0%, fibre: 0.09%, ash: 11.9%), Alltech Coppens Advance, 1.5 mm (protein: 54.0%, fat: 15.0%, fibre: 0.3%, ash: 12.0%), Aller Aqua Futura, 0.5-1.0 mm (protein: 64.0%, fat: 9.0%, fibre: 0.5%, ash: 13.0%), Aller Aqua Futura, 0.9-2.0 mm (protein: 64.0%, fat: 9.0%, fibre: 0.5%, ash: 13.0%). At the beginning of the third month of rearing, to increase the content of plant components, the daily diet included Zolotaya Rybka (Gold Fish) Flora feed, 1.7-2.5 mm (protein: 30.0%, fat: 5.5%, fibre: 5.0%, ash: 7.0%) in an amount of 20.0% of the daily feed dose, while Coppens accounted for 50.0% and Aller aqua for 30.0% of the daily feed amount. The daily feed ration was determined based on the total weight of the crayfish and varied from 10.0 to 5.0% depending on age, with the feed fraction increasing according to the size of the individuals. In the daily diet, the weight of Coppens and Aller aqua feed had a 1:1 ratio.

The experimental groups were fed twice a day: the specified feed dose was divided into two parts in a ratio of 30:70 – the first part was

administered in the morning, and the second part was administered in the evening. This took into account the natural behaviour of crayfish, which search for food at dusk. The feed was administered at two feeding points located on opposite sides of the aquarium in order to avoid competition or aggressive behaviour by dominant individuals. In general, the same optimal growing conditions (aquarium equipment, feeding, shelter) were created for all six groups, so that only the water temperature differed between the experimental groups.

Intermediate measurements of the length and weight of crayfish were carried out every 10 days. At this time, the number of individuals was also counted to assess their survival rate. Based on the data obtained, the daily feed dose was adjusted. Body weight and length were measured in accordance with general fish biology recommendations using a vernier calliper and measuring tape, as well as TBE-0.21 laboratory scales with a minimum increment of 0.02 g. The conditions for keeping, feeding, and caring for crayfish complied with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986); Regulation (EU) No. 1143/2014 of the European Parliament and of the Council "On the Prevention and Management of the Introduction and Spread of Invasive Alien Species" (2014), in compliance with the bioethical standards of proper treatment of animals enshrined in the Regulation "On the Protection of Animals Used for Scientific or Educational Purposes at the National University of Life and Environmental Sciences of Ukraine" (2023). The research was carried out with basic funding (contract BF/37-2021 dated 02.08.2021) as part of the applied research project "River Basin Management in the Context of Climate Change".

Results and Discussion

According to the results of daily monitoring of the aquatic environment, the actual water temperature in each experimental group differed from the set experimental temperature, often exceeding the studied level, which was primarily due to weather conditions in the middle and end of the hot summer when the study was conducted. All experimental groups were simultaneously exposed to this influence. The actual temperature regime of all experimental groups is presented in Table 1.

Table 1. *Temperature conditions for the experimental groups of Procambarus clarkii*

Group (experimental, t, °C)	Group 1 (22.0°C)	Group 2 (24.0°C)	Group 3 (26.0°C)	Group 4 (28.0°C)	Group 5 (30.0°C)	Group 6 (32.0°C)
Lowest water temperature	21.3°C	23.8°C	25.8°C	27.1°C	29.7°C	30.9°C
Highest water temperature	24.0°C	26.5°C	28.0°C	30.0°C	32.0°C	34.0°C
Average water temperature during the first month 08.06-30.06	22.2°C	24.5°C	26.5°C	28.9°C	30.8°C	32.4°C
Average water temperature during the second month 01.07-31.07	23.3°C	25.7°C	26.7°C	28.8°C	30.6°C	32.8°C
Average water temperature during the third month 01.08-31.08	23.0°C	25.6°C	26.4°C	29.1°C	30.5°C	32.9°C
Average water temperature during the fourth month 01.09-16.09	22.2°C	24.2°C	26.5°C	28.4°C	30.0°C	31.8°C

Source: authors' development

Based on the data of Table 1, each group experienced slight temperature fluctuations (± 2.0°C) during the study period. For example, the highest daily water temperatures were recorded during the second and third months of cultivation, while the lowest water temperatures were recorded in the last weeks of the study (September). As for other important parameters of the

aquatic environment, the content of dissolved oxygen throughout the study period fluctuated within the optimal range for this species: from 5.4 mgO_2/L to 7.7 mgO_2/L; pH – 7.0-8.0. At the beginning of the study, the average weight in all groups was the same and amounted to 0.2 ± 0.05 g, the average body length was 1.5 ± 0.2 cm. The results of the first and all subsequent measurements of the weight of *Procambarus clarkii* carried out every 10 days are presented in Table 2.

Table 2. *Weights of Procambarus clarkii during the study period*

Date	Average M, g (M ± m)					
	Group 1 (22.0°C)	**Group 2 (24.0°C)**	**Group 3 (26.0°C)**	**Group 4 (28.0°C)**	**Group 5 (30.0°C)**	**Group 6 (32.0°C)**
	min/max*	min/max*	min/max*	min/max*	min/max*	min/max*
	μ**	μ**	μ**	μ**	μ**	μ**
18.06	0.25/2.0	0.4/1.9	0.5/1.6	0.3/1.7	0.3/1.5	0.25/0.4
	0.77 ± 0.11	1.02 ± 0.11	1.01 ± 0.09	0.64 ± 0.09	0.58 ± 0.08	0.3 ± 0.01
28.06	0.5/4.6	0.3/3.4	0.6/2.7	0.4/5.1	0.4/2.6	0.3/1
	1.48 ± 0.24	1.61 ± 0.21	1.74 ± 0.16	1.50 ± 0.33	0.89 ± 0.16	0.50 ± 0.07
08.07	1.0/9.8	1.0/5.3	1.5/4.6	1.0/8.1	0.5/4.5	0.3/1.4
	2.68 ± 0.53	2.65 ± 0.33	2.84 ± 0.25	2.15 ± 0.51	1.56 ± 0.31	0.67 ± 0.12
18.07	1.8/14.5	1.0/8.3	1.9/7.5	1.3/13	1.1/7.7	0.5/3.0
	4.07 ± 0.81	3.81 ± 0.55	4.37 ± 0.51	3.37 ± 0.99	2.46 ± 0.71	1.18 ± 0.27
28.07	2.7/15.3	2.2/13.5	2.4/12.5	1.7/14.6	1.4/12.4	1.1/3.2
	5.27 ± 0.88	5.49 ± 0.87	6.08 ± 1.01	4.29 ± 1.19	3.46 ± 1.19	2.07 ± 0.25
07.08	3.4/15.5	2.9/9.6	3.8/18.5	1.9/19.7	1.8/14.4	1.6/4.7
	6.96 ± 1.07	6.53 ± 0.76	8.33 ± 1.6	5.87 ± 1.66	5.78 ± 1.89	3.24 ± 0.38
17.08	4.3/15.9	3.8/14.8	4.3/20.0	2.2/20.5	2.6/20.0	1.6/5.3
	8.45 ± 1.17	8.84 ± 1.48	10.61 ± 1.84	8.01 ± 2.09	6.34 ± 2.33	3.64 ± 0.51
27.08	5.1/19.1	4.2/15.7	5.2/20.8	2.4/20.5	2.6/20	3.0/5.9
	10.55 ± 1.43	9.57 ± 1.25	11.72 ± 1.7	8.25 ± 1.92	7.21 ± 1.95	4.35 ± 0.42
06.09	6.2/19.9	4.9/16.0	6.8/22.0	2.7/20.8	4.1/20.4	3.5/7.1
	12.09 ± 1.5	10.71 ± 1.46	14.06 ± 1.99	9.78 ± 2.64	8.08 ± 2.54	5.61 ± 0.56
16.09	9.3/29.7	6.5/16.6	7.2/21	9.9/22.8	5.7/20.6	5.4/7.4
	15.20 ± 1.97	13.71 ± 1.35	14.85 ± 1.75	14.08 ± 2.25	9.75 ± 2.33	**6.60 ± 0.36**

Note: * – maximum and minimum values; ** – average value; highest and lowest values are highlighted in bold
Source: authors' development

The first measurement of the length and weight of crayfish in the experimental groups 10 days after they were placed in the growing conditions showed a trend of intensive growth in individuals in experimental Groups 1-4 (t range 22.0-28.0°C) (Fig. 1). At the same time, individuals from experimental Group 5 (30.0°C) and especially Group 6 (32.0°C) showed a lag in weight gain. This trend was observed during the first month of cultivation. Thus, at the end of the first month of cultivation, the average recorded weight of individuals in Group 1 (22.0°C) was 4.07 ± 0.81 g, which was 3.86 g more than the initial weight (0.2 ± 0.05 g). The average weight of individuals in experimental Group 2 (24.0°C) increased by 3.61 g relative to the initial weight and was 3.81 ± 0.55 g. The weight of individuals in experimental Group 3 (26.0°C) reached 4.37 ± 0.51 g, which was 4.17 g more than the initial weight and was the largest increase among the experimental groups during the first month of the experiment. In experimental Group 4 (28.0°C), the average weight was 3.37 ± 0.99 g, with an increase of 3.17 g, respectively. Group 5 (30.0°C) reached an average weight of

2.46 ± 0.71 g, and the increase relative to the initial weight was only 2.26 g. Group 6 (32.0°C) was characterised by the lowest average weight among all experimental groups for the first month – 1.18 ± 0.27 g, and accordingly has the smallest increase in average weight relative to the initial weight – only 0.98 g, which was 4.25 times less than the increase in Group 3.

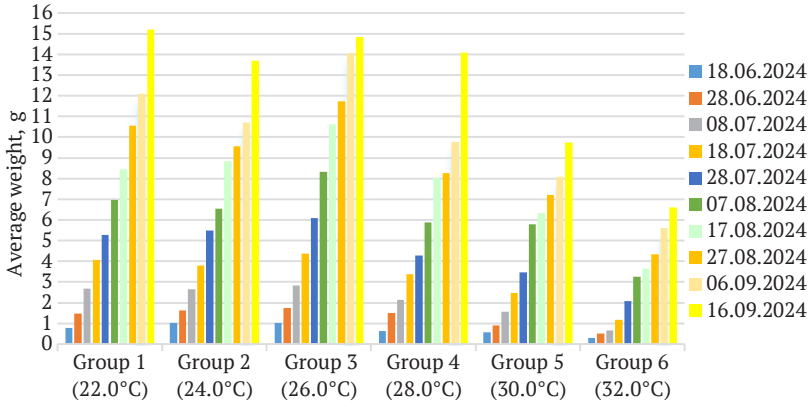

Figure 1. Weight growth of crayfish during the study period

Source: authors' development

The results of the second month of cultivation were also characterised by a rapid increase in body weight in Groups 1-4 (22.0-28.0°C) and moderate growth in Groups 5 (30.0°C) and the smallest in Group 6 (32.0°C). For example, individuals in Group 1 (22.0°C) were characterised by an average increase of 4.39 g during the second month of cultivation. Crayfish in Group 2 (24.0°C) had an average increase of 5.03 g, and Group 3 (26.0°C) again had the largest increase in average weight – 6.24 g. The average weight gain in individuals of Group 4 (28.0°C) during the second month was 4.64 g. Groups 5 (30.0°C) and 6 (32.0°C), which were grown at extremely high temperatures, had an increase of 3.88 g and 2.46 g, respectively, which were the lowest among the experimental groups.

The results of the last month of the study showed that the highest average weight was recorded in Group 1 (22.0°C) and amounted to 15.2 ± 1.97 g with an increase in average weight of 15.0 g relative to the initial average weight. Thus, at the end of the study, Group 1 surpassed Group 3 (26.0°C) in terms of average weight, which had the highest average weight during the first and second months of cultivation. Individuals in Group 2 (24.0°C) reached an average weight of 13.71 ± 1.35 g with an increase of 13.51 g. Crayfish in Group 3 (26.0°C) reached an average weight of 14.85 ± 1.75 g at the end of the study, with a weight gain of 14.65 g. In experimental Group 4 (28.0°C), the final average weight was 14.08 ± 2.25 g with a gain relative to the initial weight of 13.88 g. As for Group 5 (30.0°C), the average weight of its individuals was 9.75 ± 2.33 g with an increase of 9.55 g. Crayfish in Group 6, which were grown at the highest experimental temperature of 32.0°C, had an average weight of 6.6 ± 0.36 g at the end of the study, with an increase of 6.2 g, which was the lowest among all studied groups.

In general, during the study period, significant growth asynchrony was observed in all groups, which is typical for crustaceans. In particular, Figure 2 shows individuals from experimental Group 3 (28.0°C) during the intermediate measurement on August 7, which most clearly demonstrated the variability of linear growth and weight gain within the group: the minimum weight was 3.80 g, and

the maximum was 18.50 g (Fig. 2). A similar pattern was observed in each group, which is a natural feature of crayfish growth and is not related to study conditions.

Figure 2. Asynchronous growth of individuals in Group 3 (28.0°C) during intermediate measurement

Source: authors' photo

Assessment of the nature of linear growth changes in *Procambarus clarkii* during the study also showed a relationship between water temperature and crayfish body length: in Groups 1-4 (22.0-28.0°C), where cultivation took place within the optimal temperature range, there was intense linear growth, while in Group 5 (30.0°C) and especially in Group 6 (32.0°C), where temperatures were extremely high, moderate linear growth was recorded. The results of linear growth measurements for all six groups during the study period are shown in Table 3.

Table 3. *Linear growth of Procambarus clarkii during the study period*

Date	Average L, cm (M ± m)					
	Group 1 (22.0°C)	**Group 2 (24.0°C)**	**Group 3 (26.0°C)**	**Group 4 (28.0°C)**	**Group 5 (30.0°C)**	**Group 6 (32.0°C)**
	min/max*	min/max*	min/max*	min/max*	min/max*	min/max*
	μ**	μ**	μ**	μ**	μ**	μ**
18.06	2.0/4.0	2.0/3.8	2.3/4.0	2.0/4.0	2.0/4.0	1.8/2.3
	2.94 ± 0.14	3.08 ± 0.15	3.02 ± 0.11	2.67 ± 0.13	2.49 ± 0.15	2.03 ± 0.03
28.06	2.5/5.2	2.2/4.7	2.7/4.5	2.5/5.2	2.5/4.6	2.0/3.1
	3.53 ± 0.17	3.58 ± 0.16	3.82 ± 0.15	3.35 ± 0.20	3.06 ± 0.17	2.78 ± 0.13
08.07	3.0/6.8	3.2/5.5	3.9/5.5	3.0/6.0	2.7/5.0	2.2/3.5
	4.35 ± 0.23	4.26 ± 0.16	4.31 ± 0.12	3.74 ± 0.23	3.57 ± 0.19	2.77 ± 0.14
18.07	4.0/7.6	3.2/6.5	4.2/6.0	3.5/7.5	3.0/6.0	2.5/4.5
	4.85 ± 0.24	4.70 ± 0.26	4.99 ± 0.18	4.52 ± 0.33	4.08 ± 0.31	3.17 ± 0.25
28.07	4.0/8.0	4.0/7.2	4.2/7.2	3.5/7.5	3.5/7.5	3.0/4.8
	5.36 ± 0.28	5.30 ± 0.26	5.57 ± 0.33	4.83 ± 0.35	4.57 ± 0.42	3.97 ± 0.19
07.08	4.8/7.5	4.5/7.0	5.0/8.0	4.0/8.0	4.0/7.8	3.6/5.8
	6.04 ± 0.25	5.94 ± 0.28	6.21 ± 0.38	5.50 ± 0.37	5.11 ± 0.42	4.80 ± 0.27
17.08	5.2/8.0	5.1/8.0	5.5/8.5	4.5/8.3	4.8/8.0	3.6/5.8
	6.50±0.30	6.61±0.40	6.87±0.36	6.16±0.46	5.56±0.46	4.95±0.30

Date	Average L, cm (M ± m)					
	Group 1 (22.0°C)	**Group 2 (24.0°C)**	**Group 3 (26.0°C)**	**Group 4 (28.0°C)**	**Group 5 (30.0°C)**	**Group 6 (32.0°C)**
	min/max*	min/max*	min/max*	min/max*	min/max*	min/max*
	μ**	μ**	μ**	μ**	μ**	μ**
27.08	5.8/8.8	5.4/7.7	5.8/8.2	4.5/8.5	4.0/8.0	4.2/5.8
	7.13 ± 0.30	6.80 ± 0.27	7.06 ± 0.27	6.02 ± 0.45	5.56 ± 0.41	4.98 ± 0.23
06.09	6.0/9.0	5.5/8.0	6.2/8.4	4.6/8.5	5.1/8.0	4.9/6.2
	7.41 ± 0.31	6.97 ± 0.32	7.46 ± 0.27	6.37 ± 0.59	5.85 ± 0.45	5.56 ± 0.21
16.09	7.0/10.0	6.2/8.1	6.5/8.3	6.5/8.7	5.8/9.0	5.3/6.7
	8.00 ± 0.29	7.38 ± 0.29	7.66 ± 0.22	7.42 ± 0.33	6.80 ± 0.50	**6.04 ± 0.26**

Note: * – maximum and minimum values of; ** – average value; highest and lowest values are highlighted in bold
Source: authors' development

The fastest linear growth was observed in individuals in experimental Group 1 (22.0°C), which reached 8.00 ± 0.29 cm over three months of the study, increasing their body length by 6.50 cm compared to the initial length (1.5 ± 0.3 cm). The average length of crayfish in Group 2 (24.0°C) increased by 5.88 cm and at the end of the study averaged 7.38± 0.29 cm. At the end of the study, individuals in Group 3 (26.0°C) had an average body length of 7.66 ± 0.22 cm, with an increase of 6.16 cm relative to the initial value. In Group 4 (28.0°C), individuals reached an average length of 7.42 ± 0.33 cm with an increase of 5.92 cm. Crayfish in Group 5 (30.0°C) increased their body length by an average of 5.30 cm and reached an average length of 6.80 ± 0.50 cm. The lowest linear growth results were observed in crayfish from Group 6 (32.0°C): the body length of individuals in this group increased by 4.54 cm, reaching 6.04 ± 0.26 cm at the end of the study period, which was 1.3 times less than in Group 1 (Fig. 3).

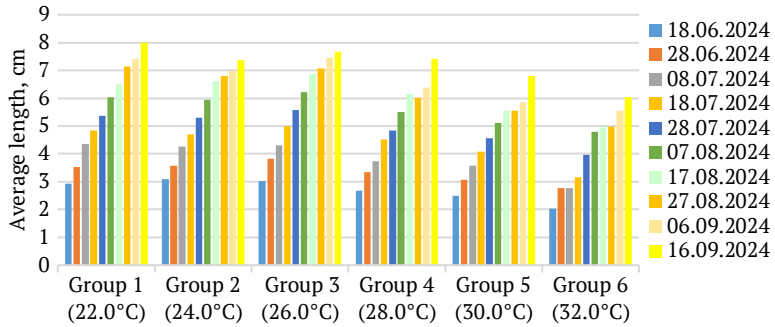

Figure 3. Linear growth of crayfish during the study period
Source: authors' development

Observation of the behavioural reactions of *P. clarkii* during the experimental period showed a typical phenomenon of the cultivation technology of any object in controlled or natural conditions, namely a decrease in the number of individuals, while revealing a direct relationship between water temperature and the number of crayfish in the experimental groups. In this study, the reduction in numbers in some groups was caused by intense cannibalism as a behavioural response to elevated (extremely high) water temperatures (Groups 4-6).

However, it cannot be ruled out that the decline in number could also have been the result of the low vitality of crayfish grown at high temperatures (28.0-30.0°C), since the organism's main resource could have been directed toward thermoregulation, namely cooling the organism, rather than maintaining optimal vital functions. In particular, observations have shown that the highest intensity of mortality and cannibalism was in Groups 5 and 6, which were grown at temperatures beyond the tolerance limits of the organism – 30.0 and 32.0°C, respectively. Table 4 shows general data on changes in the number of *Procambarus clarkii* in six experimental groups throughout the entire study period.

Table 4. *Dynamics of the number of Procambarus clarkii individuals during the study period*

Date	Number of individuals, n*					
	Group 1 (22.0°C)	Group 2 (24.0°C)	Group 3 (26.0°C)	Group 4 (28.0°C)	Group 5 (30.0°C)	Group 6 (32.0°C)
18.06	17.0	17.0	16.0	16.0	16.0	16.0
28.06	17.0	17.0	15.0	14.0	15.0	11.0
08.07	16.0	13.0	13.0	13.0	12.0	10.0
18.07	15.0	13.0	11.0	11.0	9.0	9.0
28.07	14.0	12.0	10.0	10.0	9.0	8.0
07.08	13.0	9.0	9.0	10.0	9.0	7.0
17.08	12.0	7.0	8.0	8.0	7.0	7.0
27.08	11.0	7.0	8.0	7.0	6.0	6.0
06.09	10.0	7.0	8.0	7.0	6.0	6.0
16.09	10.0	7.0	8.0	7.0	6.0	5.0
% survival	58.82	41.18	47.06	41.18	35.29	29.41

Note: * – initial number in all groups – 17

Source: authors' development

It was found that the greatest losses in numbers were observed during the first two months of cultivation, when the juveniles regularly, but asynchronously, moulted (ecdysis), after which, under optimal conditions, the juveniles remained in the metecdysis stage for 1-2 days – a period of formation and strengthening of the new exoskeleton, during which individuals are most vulnerable to cannibalism. However, the experimental groups that were grown at elevated temperatures (Groups 5 and 6) were characterised by a longer period of metecdysis – 2-3 days, remaining vulnerable to environmental factors, primarily cannibalism, for a longer period of time. Thus, survival at the end of the second month of the study underwent significant changes relative to the initial number and amounted to 70.59% in Group 1, 47.06% in Groups 3 and 4, and 41.18% in Groups 2, 5, and 6. The third month of cultivation was characterised by fewer moults (which is a typical feature of crayfish of this age), so survival during the third month remained satisfactory. In particular, in Groups 2, 3, 4, and 5, it was 100% (compared to the second month of cultivation), in Group 1 – 83.3%, and in Group 6 – 71.43%, which indicates a decrease in the intensity of cannibalism due to a decrease in the number of moults in crayfish.

Analysis of the final results of the study showed that the survival rate of crayfish in Group 1 with the lowest cultivation temperature (22.0°C) was 2 times higher than in Group 6 with the highest water temperature (32.0°C) and 1.7 times higher than in Group 5 (30.0°C). At the same time, Groups 2-4 were characterised by approximately the same survival rates of individuals – 41.18-47.06%. This result may indicate an increase in the level of cannibalism and mortality in proportion to the increase in

the cultivation temperature. In general, cannibalism, especially during the moulting period of crayfish, is one of the most acute problems in the artificial cultivation of crustaceans (crayfish, shrimp). Special measures, such as placing shelters or coverings, structuring the space, etc., significantly reduce the intensity of its manifestation, but cannot completely eliminate it (Fedorovych *et al*., 2022a; Ishchuk *et al*., 2024), so this aspect of crayfish farming technology needs to be studied in more detail.

Thus, the studies conducted have examined and established the influence of a wide range of water temperatures on the growth, development, and behavioural responses of the red swamp crayfish under controlled conditions, and it has been established that the optimal temperature range for *P. clarkii* is 22.0 ± 2.0°C. Water temperatures above 28.0°C had a negative inhibitory and, in some cases, lethal effect on crayfish. According to a study by X. Ren *et al.* (2021), as water temperature rises, ectothermic animals need to obtain more energy to maintain an increased metabolism, which leads to a decrease in food consumption rate (in the current experiment, uneaten feed residues were observed precisely in high-temperature groups). However, as the water temperature rises, the rate of catabolism (energy metabolism) in animals exceeds the rate of anabolism (plastic metabolism). This process can be explained in more detail by analysing the work of H. Jiang *et al*. (2024), which studied the effect of different water temperatures (20.0°C – control, 10.0°C and 30.0°C – experimental) on the activity of enzymes (pyruvate kinase (PK), hexokinase (HK) and phosphoenolpyruvate carboxykinase (PEPCK)) in the muscles and hepatopancreas of *P. clarkii*, and changes in the activity of α-amylase (α-AMY) and lipase (LPS) in the intestine and hepatopancreas. Analysing the activity of digestive and metabolic enzymes, H. Jiang *et al*. (2024) found that extremely low (10.0°C) and high (30.0°C) temperatures inhibited their activity levels compared to the control group (20.0°C).

It is obvious that the metabolic and digestive activity of crayfish in the study directly depended on water temperature: extremely high values (above 28.0°C and above, Groups 4, 5, and 6) caused thermal stress, inhibiting the secretion and activity of digestive enzymes and causing metabolic disorders. It is likely that the main expenditure of energy reserves in individuals from high-temperature groups was directed toward thermoregulation, and the activity of digestive enzymes depended on water temperature, affecting the absorption of nutrients from compound feed through various aspects of metabolism, which scaled differently with water temperature.

The optimal water temperature, which was 22.0°C according to the experiment, had a positive effect on the activity of digestive and metabolic enzymes, which manifested itself in the intensive linear-weight growth of individuals, high survival rate, and the appearance of the first females with eggs in this group. At the same time, due to changes in metabolic processes and suppression of digestive enzyme activity, high-temperature Groups 5 (30.0°C) and 6 (32.0°C) were characterised by the lowest linear growth and weight gain and critically low survival rates. The results obtained also coincide with the data of E. Fedorovych *et al*. (2022a), who, having conducted research on the cultivation of various species of crayfish under different temperature and oxygen conditions, found that the best temperature for keeping all the studied species is in the range of 23.0-25.0°C. The negative effect of high temperatures on *P. clarkii* is also described in the work of K. Guo *et al*. (2020), which analysed the intestinal immune response of *P. clarkii* individuals at a temperature of 25.0°C (control) and a stressful temperature of 35.0°C. Based on biochemical analysis of oxidative stress parameters and immune indicators in the intestine, a negative reaction to temperature stress (35.0°C) was established, which manifested itself through increased oxidative stress in the intestine and decreased activity of antioxidant and immune enzymes, which together caused mortality.

An explanation for this result can be found in the work of B. Carreira *et al.* (2017), which studied the feeding of crayfish with different feeds at different temperatures. The results of the studies indicate a decrease in the efficiency of animal feed consumption at higher temperatures, since juveniles fed an animal diet were unable to maintain high growth rates during a prolonged heat wave; and a decrease in the efficiency of plant diets at low temperatures, since juveniles accumulated fewer reserves at lower temperatures when fed a plant-based diet. Similar results regarding an increase in herbivory with increasing water temperature were also investigated by P. Zhang *et al.* (2020), who proved that an increase in temperature increases the herbivory of ectothermic hydrobionts. Along with this, a study of other crustacean species conducted by P. Kovtun & S. Merzlov (2023) demonstrated a significant impact of diet composition, in particular the inclusion of vermiculture biomass, on their productivity.

In the study conducted by the authors of this paper, compound feed with a high protein content (46.0-64.0%) was used to feed crayfish of all groups, including high-temperature ones, and only at the beginning of the third month of cultivation was compound feed with a high fibre content (5.0%) introduced into the crayfish diet in an amount of 20.0% of the daily ration, which was obviously insufficient to maintain normal metabolic processes in individuals from high-temperature Groups (5 and 6). However, this study did not consider the aspect of food selectivity in favour of animal or plant feed for feeding *P. clarkii* at different water temperatures, but the data presented may be useful in future studies for compiling a daily diet for crayfish.

All of the above-mentioned studies lead to one common conclusion: high water temperatures have an undeniable negative impact on crayfish survival, which is also confirmed by this study. It has been found that in juvenile crayfish from high-temperature groups, the metecdysis period (the period after moulting until the formation of a new exoskeleton, which, according to S. Galib *et al.* (2022), is a period of defencelessness for crayfish) lasted 2-3 days, compared to 1-2 days in crayfish at temperatures of 22.0-26.0°C. Thus, individuals from Groups 5 and 6 remained more vulnerable to environmental factors for a longer period of time, primarily to cannibalism, which actively progressed due to the asynchrony of the moulting process itself. The longer period of metecdysis is associated with the functioning of the endocrine system, which regulates the secretion of the substance chitin synthase, which is used to form a new exoskeleton, which was apparently suppressed by high water temperatures. At the same time, the neuroendocrine system of crayfish and related molecules regulate the behaviour of crayfish and physiological processes, including the perception of environmental factors, which could affect the thermotolerance and aggressive behaviour of individuals from high-temperature groups (Ren *et al.*, 2021; Fedorovych *et al.*, 2022b). As crayfish grow, the problem of cannibalism decreases as the frequency of moulting decreases.

As follows, the undoubted advantage of temperature conditions formed in Group 1, which demonstrates the highest percentage of survival and the highest linear growth and weight gain of experimental crayfish, was established. Groups 2-4 are also characterised by satisfactory growth rate, but demonstrate lower survival rates. Also, according to the results of study and analysis of available literature, it was proved inexpedient to grow *P. clarkii* at the temperature conditions formed in Groups 5 and 6, which showed the lowest linear growth and weight gain and the lowest survival rates.

Conclusions

According to the results of the study, the lowest temperatures among the studied ones had a positive effect on the growth and development of *Procambarus clarkii*, while the highest temperature regimes studied, which went beyond the tolerance of the crayfish, showed the lowest

efficiency. So, comparing the results obtained, it was found that the highest growth and survival rates were observed in Group 1, which was grown at the lowest temperature among the studied, namely 22.0°C. Groups 2-4 (24.0-28.0°C) showed slightly lower but satisfactory growth and survival rates, but compared to Group 1, these groups were characterised by a lower survival rate. High-temperature Groups 5 (30.0°C) and 6 (32.0°C) were unable to maintain high growth and survival rates.

The influence of high temperatures was also revealed by the results of the assessment of death and cannibalism as a behavioural response of the organism to factors of the external (high temperature) and internal (metabolic processes, activity of the endocrine and neuroendocrine systems) environment. So, the survival rate of individuals in Group 1 (22.0°C) was 2 times higher than in Group 6 (32.0°C). In addition, to the disadvantages of high-temperature cultivation regimes, one can also add naturally higher energy consumption for water heating, which, with large volumes of cultivation, can be high, and most importantly, unnecessary costs. The results obtained have significant potential for the development of an optimised and scientifically sound technology for the cultivation of *Procambarus clarkii*, and can also serve as a basis for further, more in-depth research. In particular, a promising area for further research is to study the effect of the combination of parameters and growing conditions on the physiological parameters and productivity of *Procambarus clarkii* at different stages of ontogenesis.

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Conflict of Interest

None.

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Вплив температури води на ріст та розвиток червоного каліфорнійського рака *Procambarus clarkii* (Girard, 1852)

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Анотація. В галузі аквакультури одним з найрозповсюдженіших у світі комерційним видом раків є червоний каліфорнійський рак *Procambarus clarkii*. Метою даної роботи був аналіз впливу температурного режиму на параметри росту *Procambarus clarkii* при вирощуванні в діапазоні температур води від 22,0 до 32,0°C з кроком температур 2,0°C (всього 6 дослідних груп). Під час проведення експерименту використано теоретичні (аналіз, систематизація, порівняння), експериментальні (лабораторні) та загальноприйняті у рибництві методи досліджень. Отримані результати чітко продемонстрували пригнічуючий вплив високих температур водного середовища – 30,0°C (Група 5) та 32,0°C (Група 6) – на ріст та розвиток (лінійно-ваговий приріст, виживаність) особин, тоді як найнижча в даному досліді температура води (Група 1, 22,0°C) сприяла більш ефективному росту та підтримувала виживаність раків. Так, кінцева середня маса особин Групи 1 (22,0°C) становила 15,20 ± 1,97 г, у Групі 2 (24,0°C) середня маса складала 13,71 ± 1,35 г, раки Групи 3 (26,0°C) досягли середньої маси в 14,85±1,75 г, Група 4 (28,0°C) – 14,08 ± 2,25 г, а в Групах 5 (30,0°C) і 6 (32,0°C) отримана

середня маса складала 9,75 ± 2,33 г і 6,60 ± 0,36 г відповідно, що в 1,5 та 2,3 раза менше порівняно з Групою 1. Водночас показники середнього лінійного приросту мали більш однорідний характер і становили на кінець дослідження у Групі 1 – 8,00 ± 0,29 см, у Групі 2 – в середньому 7,38±0,29 см, особини Групи 3 мали середню довжину 7,66 ± 0,22 см, у Групі 4 – 7,42 ± 0,33 см, а у високотемпературних Групах 5 і 6 середня довжина складала 6,80 ± 0,50 см і 6,04 ± 0,26 см відповідно. Встановлено тенденцію зниження виживаності особин при збільшені температури води у групах. Так, виживаність у Групі 1 на кінець досліджень становила 58,82 %, у Групі 2 – 41,18 %, у Групі 3 – 47,06 %, Групі 4 – 41,18 %, а Групи 5 і 6 зазнали найбільшого тиску температурного показника на виживаність – 35,29 % і 29,41 % відповідно

Ключові слова: аквакультура; *Procambarus clarkii*; маса; довжина; виживаність; терморегуляція; метаболізм

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Fish spectral classification based on principal component analysis

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Abstract. Spectral detection technology can be used to identify different types of fish flesh and determine the presence of ingredients in samples, which helps to control the authenticity and conformity of products. The aim of this study was to develop and compare the effectiveness of machine learning methods for the classification of different types of fish based on hyperspectral

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data. In this paper, hyperspectral technology was used to obtain spectral data of 328 sampling points in the 400-1,000 nm band of four different fish, and the spectral curves were preprocessed to obtain more accurate and efficient spectral curves. The study systematically compared the preprocessing methods of Savitzky-Golay smoothing and multivariate scatter correction (MSC), and combined the principal component analysis (PCA) method to optimise the k-nearest neighbour (KNN) and support vector machine (SVM) classification models, in order to provide a more effective solution for fish classification. The principal component analysis method was used to reduce the dimensionality of the spectral data. The principal components after dimensionality reduction were compared with the full components, and the training set and test set were randomly generated. The SVM algorithm and the KNN algorithm were used in Python to predict and determine the accuracy of the model. It was shown that the average accuracy of spectral data after preprocessing and dimensionality reduction in the classification of SVM models can reach 97.25%, and the average classification accuracy of KNN models can reach 97.48%, which indicates that different types of fish flesh can be classified. Both models showed that overall, the preprocessing method that combines MSC and PCA is better than the single preprocessing method, highlighting the importance of PCA in removing redundant information and preserving key classification characteristics. When comparing the model performance under the same preprocessing conditions, the accuracy of SVM is higher than that of KNN, indicating that the SVM has greater adaptability to spectral data classification. Through the development and application of spectral detection technology, technological progress and innovation in food testing can be promoted, guiding the entire industry to a safer and more reliable future

Keywords: spectrum; Savitzky-Golay method; multiplicative scatter correction; k-nearest neighbours model; support vector machine algorithm

Introduction

As people's awareness of food safety continues to grow, food quality and authenticity have become the focal points of global concern. The food industry faces issues such as adulteration and counterfeiting. Criminals might seek profits by adding inexpensive or harmful substances to products. Thus, it is essential to develop reliable detection methods to prevent and identify such counterfeiting behaviours. However, traditional food quality detection methods are destructive, cumbersome to operate, and require trained professionals. With the rapid development and integration of image processing, spectral analysis, and food intelligence technologies, the application of hyperspectral imaging technology in food detection has attracted growing attention (Wu *et al.*, 2025). The development and advancement of spectral technologies, particularly the increasing applications of near-infrared spectroscopy, Raman spectroscopy, fluorescence spectroscopy, and other techniques, offer faster, more accurate, and non-destructive approaches for food analysis and testing. L. He *et al.* (2022) employed ultra-high performance liquid chromatography – tandem mass spectrometry (UPLC – MS/MS) to identify serum albumin target peptides in beef and horse meat. The results demonstrated that this method exhibited good linearity and excellent analytical performance within a certain concentration range. B. Li *et al.* (2024) utilised support vector machine, random forest, and LSTM algorithms to establish a classification model by fusing hyperspectral reflectance and transmittance spectral data, and combined with partial least

squares regression to quantitatively analyse the concentration of beef adulterants. They found that the detection accuracy of chicken, duck, and pork adulterated in beef could be improved. The scientific work by I. Palamarchuk *et al.* (2024) used the laser-induced breakdown spectroscopy (LIBS) method for spectroscopic assessment and quantitative analysis of the trace element composition of plant additives to meat products.

Spectral detection technology can analyse numerous samples in a short time, enhancing detection and production efficiencies. Through the development and application of spectral detection technology, technological progress and innovation in food testing can be promoted, guiding the entire industry towards a safer and more reliable future (Melnychuk *et al.*, 2016). Moreover, the importance of standardised quality metrics in food production was emphasised by N. Kim (2021), who proposed a generalised quality indicator method for assessing various objects, including food products. This method integrates multiple quality metrics into a cohesive model, enabling precise and rapid evaluation of food quality, which is critical for ensuring consistency and reliability in non-destructive spectral detection applications.

Fish, being a common food item, its quality and authenticity are of great significance to consumers' health and trust. Fish spectral detection plays a crucial role in ensuring food safety and quality. By analysing the spectral characteristics of samples to identify their components and quality attributes, consumers' health and rights can be safeguarded (Wang *et al.*, 2019). T. Xu (2020) synthesised three gold nanorods (Au NRs) with different aspect ratios using the seed-growth method and, in combination with SERS technology, detected industrial dye drug residues in four types of fish flesh. Z. Chen *et al.* (2021) used these non-destructive methods to assess the freshness of pearl gentian grouper under different storage conditions. G. Tsagkatakis *et al.* (2020) proposed a new approach to fish freshness assessment using information encoded in the spectral profile obtained with a spectral imaging camera. Experimental evaluation on individuals of the Sparidae family (*Boops* sp.) confirmed that the proposed approach is a valid methodology, offering both accuracy and ease of application. Spectroscopic technology can achieve fast and non-destructive fish classification by analysing the absorption or reflection characteristics of biological tissues to electromagnetic waves (Shi *et al.*, 2022; Yuan *et al.*, 2025). However, the original spectrum is easily interfered with by factors such as instrument noise and sample surface scattering, and it is necessary to combine dimensionality reduction techniques such as principal component analysis (PCA) to improve classification accuracy.

The aim of this paper was to provide a more efficient study of fish classification based on spectral analysis. The research objectives included a systematic comparison of preprocessing methods, Savitzky-Golay (SG) smoothing and multivariate scatter correction (MSC), and a combination of PCA to optimise KNN and SVM classification models.

Materials and Methods

All experimental procedures were conducted at the Hyperspectral Imaging Laboratory, School of Food Science and Engineering, Bengbu University (Bengbu, Anhui Province, China) during March 2025. The experimental studies were conducted in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (1986), as well as the Law of Ukraine No. 3447-IV "On Protection of Animals from Cruelty" (2006).

Experimental materials. The fresh fish samples for this experiment were purchased from the Bengbu Fishery. The fish species included Snakehead, Grass carp, Bighead carp, Redfin culter, with 10 individuals of each type.

The length of each fish ranged from 25 to 40 cm. Samples were prepared from the mid-section of freshly slaughtered and cleaned fish, with 2 cm thick cross-sectional slices divided into four groups. Each container was labelled with sample identification and preparation date. Specimens were subsequently refrigerated at 3°C for 24 hours to stabilise physicochemical properties whilst preventing interference from trace microbial activity. Following this stabilisation period, samples were arranged on a motorised stage for spectral acquisition in a controlled-environment chamber maintained at 15°C to minimise wavelength-specific intensity variations caused by thermal effects. After processing to eliminate the interference of irrelevant factors, the fish samples were detected using a hyperspectral imager. Hyperspectral data of different types of fish in the wavelength band from 400 nm to 1,000 nm were collected. Following preprocessing to eliminate extraneous factors, hyperspectral imaging was performed using a GaiaField-V10 system (Jiangsu Shuangli Hepu Technology Co., Ltd) as illustrated in Figure 1. The apparatus comprised: a hyperspectral imager with internal push-broom scanning mode, a CCD camera (696×658 pixels), four 200W halogen lamps, a black background panel, a computer-controlled translation stage, and a light-tight enclosure. Samples were positioned centrally on the motorised stage within the enclosure. Operating within a 400-1,000 nm spectral range, the system acquired data at 0.7 nm/s with 328 output wavelengths. Key specifications included: spectral resolution – 2.8 nm, focal length – 25 mm, spectral sampling interval – 2.34 nm. During acquisition via SpecView software, parameters were set to: exposure time – 30 ms, gain – 1. Sample-to-camera distance was maintained at 55 cm to ensure image clarity. Radiometric calibration was performed using a 99% reflectance standard white reference prior to daily measurements to ensure a linear reflectance response.

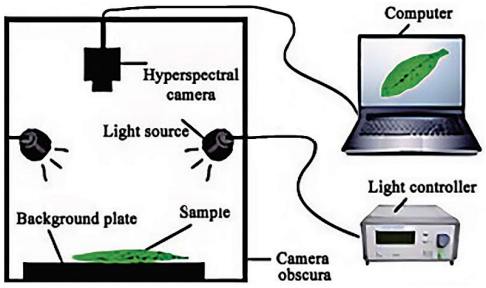

Figure 1. Hyperspectral detection device
Source: developed by the authors

Data processing methods. Hyperspectral image data of fish specimens were acquired in this study. Spectral curves of samples were extracted using ENVI (Environment for Visualising Images) software, yielding spectral information across 328 bands. Subsequent preprocessing of raw spectral data and curves was conducted within the Python 3.9.21 software environment. To mitigate noise and enhance the signal-to-noise ratio, Multiplicative Scatter Correction (MSC) and Savitzky-Golay (SG) smoothing algorithms were applied. The preprocessed data were partitioned into two subsets: one subset underwent dimensionality reduction via Principal Component Analysis (PCA) to efficiently represent features in a lower-dimensional space, while the other subset retained its original dimensionality. This process generated four distinct datasets (MSC+PCA, MSC, SG+PCA, SG), which were subsequently reimported into the Python environment. During model training, each dataset was split into training and testing sets at a 4:1 ratio, employing a cross-validation strategy. Two classification algorithms – Support Vector Machine (SVM) and K-Nearest Neighbours (KNN) – were utilised to classify spectral data from four fish categories. To meet computational and data storage requirements, the software environment was configured as follows: Development platform: PyCharm 2023.1.3 Professional Edition; Deep learning framework: PyTorch 1.12.0+cu113. Key dependencies: numpy

1.23, pandas 1.5.3, matplotlib 3.7.1, scikit-learn 1.2.2, torchvision 0.15.2, tqdm 4.65.0, tensorboard 2.12.2. Models were trained on the training sets, with final performance evaluated through confusion matrices and accuracy metrics on the test sets.

In the field of hyperspectral data preprocessing, two methods are widely used: Savitzky-Golay smoothing and multidimensional scattering correction, which are used to suppress noise and eliminate scattering interference, respectively. SG smoothing is a local weighted average algorithm based on polynomial approximation. By shifting an odd-length window on the spectral curve, a polynomial regression is performed on the data in the window, and the centre value of the approximation curve is used to replace the original value. In this study, the window size was set to 5 points during SG data processing, and the polynomial order was set to 3rd order. During preprocessing, a least-squares polynomial approximation was performed on the data points in each window, and the smoothing value of the centre point of the window was calculated. The edge of the data was processed by mirror expansion. In addition, to eliminate the scattering effect caused by the physical surface characteristics of fish samples (such as particle size and roughness), the multidimensional scattering correction (MSC) method was used to achieve spectral baseline correction.

Fish hyperspectral data usually contains hundreds of continuous bands (400-1,000 nm), which is a typical high-dimensional data feature. Principal Component Analysis (PCA) is a well-established linear dimensionality reduction method. It maps high-dimensional data to a low-dimensional space through orthogonal transformation. It effectively reduces dimensionality while retaining the main information of the data, and it has become an important preprocessing technology for fish hyperspectral data analysis. In this study, the PCA method was used to reduce the dimensionality of the data. First, the principal components were extracted by calculating the covariance matrix, and then the principal components with a cumulative variance contribution exceeding 95% were selected. The data after dimensionality reduction were then trained by KNN and SVM algorithms. This study established eight comparative experimental groups comprising the following specific combinations: SG+PCA+SVM, SG+PCA+KNN, MSC+PCA+SVM, MSC+PCA+KNN, SG+SVM, SG+KNN, MSC+SVM, and MSC+KNN. Among these, four experimental sets required initial dimensionality reduction via PCA prior to input of the data into SVM and KNN models for training. After the 8 experimental groups were preprocessed by SG and MSC, 4 of them were subjected to PCA dimensionality reduction and then substituted into the SVM and KNN models for training. The other 4 groups were not subjected to PCA dimensionality reduction and were directly substituted into the model training. Finally, the accuracy rates obtained from the training of the 8 model combinations were compared and analysed.

Results and Discussion

The original spectral curves of the four fish samples, the spectral curves after SG convolution smoothing, and the spectral curves after multivariate scattering correction (MSC) processing are shown in Figures 2, 3, and 4, respectively. Through comparative analysis, it can be observed that the Savitzky-Golay convolution smoothing algorithm effectively suppressed high-frequency random noise while fully retaining the position and intensity information of the characteristic absorption peak. This method achieved data smoothing through local polynomial fitting. Under the 5-point sliding window parameter setting, it successfully eliminated signal fluctuations caused by instrument noise and environmental interference. Compared with SG processing, the multivariate scatter correction technology

demonstrated a more centralised optimisation effect in spectral preprocessing. By regressing each sample spectrum against the reference spectrum, MSC processing not only effectively eliminated the baseline drift caused by particle scattering effects but also centralised the spectral data. Experimental data indicated that both preprocessing methods can enhance the quality of spectral data.

Prior to model training, the fish hyperspectral data were subjected to both noise-reduction preprocessing and dimensionality reduction using PCA. Figures 5 and 6 show the PCA two-dimensional projection results of fish spectral data after SG and MSC preprocessing. In both figures, the horizontal axis PC1 accounted for more than 86.0% of the variance. In Figure 6, the vertical axis PC2 accounted for 6.2% of the variance, and in Figure 5, the vertical axis PC2 accounted for 10.1% of the variance. The cumulative variance contribution of PC1 and PC2 was high, reaching over 94%. PC1 was the main dimension for differentiating fish samples. Redfin culter showed the highest degree of distinction from other species. However, Bighead carp, Grass carp, and Snakehead had relatively weak distinctions because of the partial overlap of their spectral features. Further refinement was required by combining more dimensions or analysis methods.

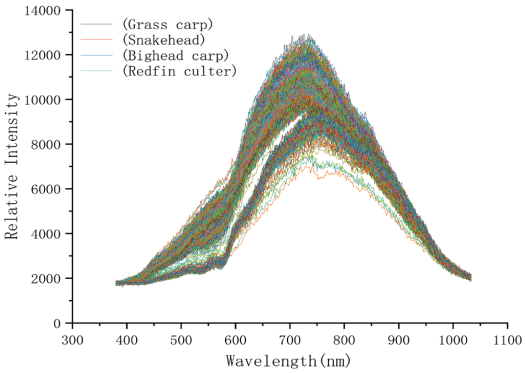

Figure 2. Raw spectral profiles of four fish species

Source: developed by the authors

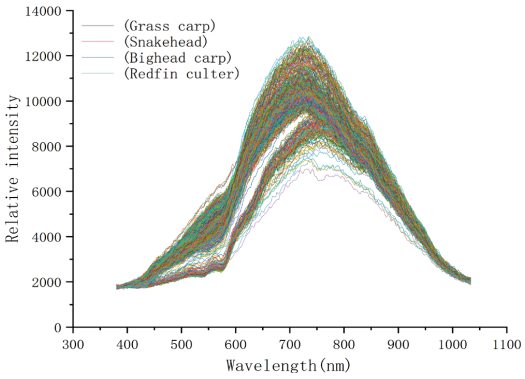

Figure 3. Spectrum after SG treatment

Source: developed by the authors

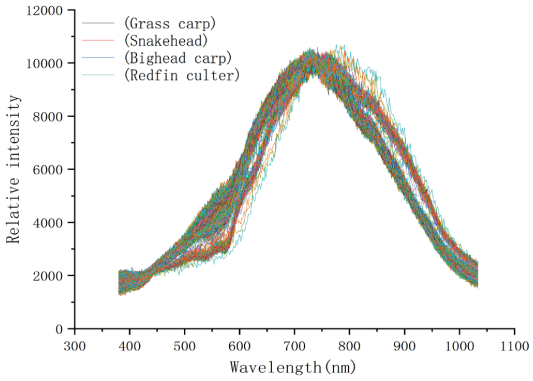

Figure 4. Spectrum after MSC treatment

Source: developed by the authors

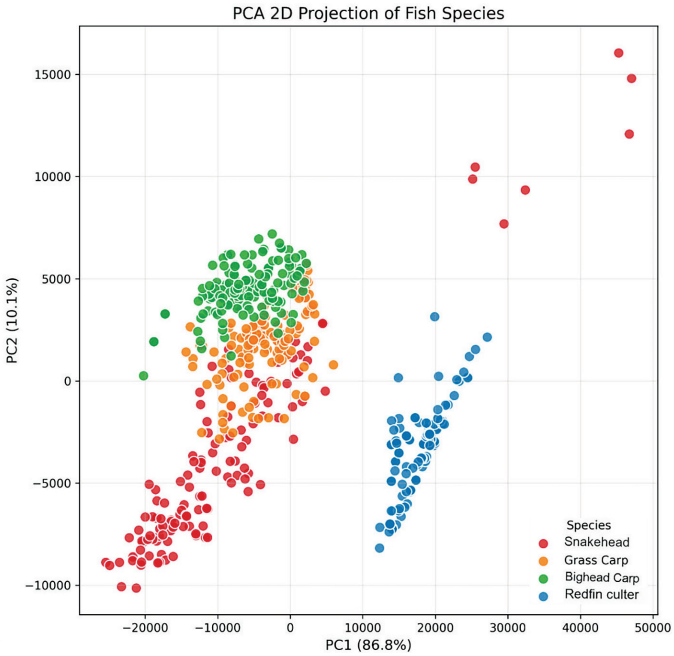

Figure 5. SG-PCA two-dimensional projection of the fish flesh spectrum
Source: developed by the authors

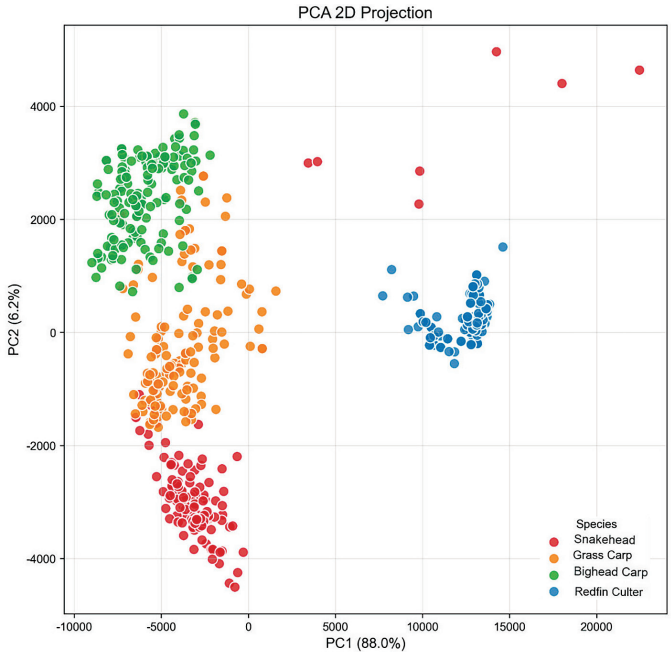

Figure 6. MSC-PCA two-dimensional projection of the fish flesh spectrum
Source: developed by the authors

Table 1 and Table 2 present the cumulative contribution variance statistics after performing PCA dimension analysis. They illustrate the contribution rates of the first 5 principal components when the fish spectral data were processed using SG-PCA and MSC-PCA.

Table 1. *Contribution rates of the top 5 principal components of SG-PCA*

Principal component number	Percentage of variance (%)	Cumulative (%)
PCA1	86.80	86.80
PCA2	10.10	96.90
PCA3	2.50	99.40
PCA4	0.30	99.70
PCA5	0.20	99.90

Source: developed by the authors

Table 2. *Contribution rates of the top 5 principal components of MSC-PCA*

Principal component number	Percentage of variance (%)	Cumulative (%)
PCA1	88.00	88.00
PCA2	6.20	94.20
PCA3	0.90	95.10
PCA4	0.40	95.20
PCA5	0.10	95.30

Source: developed by the authors

As is evident from Table 1, in the SG-PCA combination, the variance contribution rate of the first principal component, PCA1, reached as high as 86.80%. The cumulative contribution rate of the first two principal components is 96.90%, and that of the first 5 principal components was as high as 99.9%. This implied that most of the variance information could be accounted for by the first two principal components, and the first 5 principal components nearly covered all the information. In contrast, in the MSC-PCA combination, the variance contribution rate of PCA1 was 88.00%, which was higher than that of PCA1 in the SG-PCA combination. However, the cumulative contribution rate of the first two principal components was 94.20%, and the cumulative contribution rate of the first 5 principal components was 95.30%, both of which were lower than those in the SG-PCA combination. This indicated that, under the MSC-PCA combination, the explanatory power of the first 5 principal components for the data was slightly weaker, yet the explanatory power of the first principal component was stronger.

From the principal component contribution rates of the two preprocessing methods, it was observed that when the preprocessed spectral data underwent PCA analysis, more than 95% of the original information was retained while the data dimensionality was reduced to fewer than ten. This demonstrated that the combination of preprocessing and PCA effectively eliminated redundant spectral information. As a result, it not only decreased the amount of training data and shortened the training time but also improved the analysis efficiency. The training results are presented in Table 3 and Table 4.

Table 3. *SG classification model training results*

Principal component number	Accuracy	Average accuracy (with or without PCA)	Average accuracy
MSC	97.33%	96.77%	97.25%
SG	96.20%		
MSC-PCA	97.86%	97.73%	
SG-PCA	97.59%		

Source: developed by the authors

Table 4. *KNN classification model training results*

Principal component number	Accuracy	Average accuracy (with or without PCA)	Average accuracy
MSC	97.59%	96.97%	97.48%
SG	96.35%		
MSC-PCA	98.39%	97.99%	
SG-PCA	97.60%		

Source: developed by the authors

The results indicated that in the task of fish spectral classification, the preprocessing effects and classification performances of the SVM and KNN models exhibited significant differences. For the SVM model, the MSC-PCA combined preprocessing demonstrated the best performance, with an accuracy of 98.39% and an average precision of 97.99%. This was significantly better than using MSC alone (97.59%), SG alone (96.35%), or SG-PCA (97.60%), thus verifying the synergistic optimisation effect of MSC correction and PCA dimensionality reduction. When preprocessed individually, the spectral correction effect of MSC (97.59%) was better than that of SG (96.35%). The optimal preprocessing scheme for the KNN model was also MSC-PCA, with an accuracy of 97.86% and an average precision of 97.73%. This was superior to SG-PCA (97.59%), single-use of MSC (97.33%), and SG (96.20%), further validating the effectiveness of the feature extraction of this combination. Both models showed that, overall, the preprocessing method combining MSC and PCA was better than a single preprocessing method, highlighting the importance of PCA in removing redundant information and retaining key classification features. In the comparison of model performances, under the same preprocessing conditions, the accuracy of SVM was generally higher than that of KNN, indicating that SVM had a stronger adaptability to the classification of spectral data.

The results of the research are consistent with the conclusions of other scientists regarding the application of spectral analysis with data processing methods for identifying fish species and assessing the freshness of fish products. M. Rahman *et al.* (2023) used principal component analysis to differentiate the spectra of mackerel meat using fluorescence spectroscopy. Principal component analysis of FFs spectra demonstrated clear discrimination (PC1 + PC2 = 91.7%) between white and dark meat of frozen fish depending on the freshness state. Therefore, the principal component analysis method of dense matter spectra could instantly distinguish the freshness state of white and dark meat in fish products, even in the frozen state. The effectiveness of the fluorescence fingerprinting method combined with PCA for rapid and non-destructive monitoring of fish quality had also been demonstrated in the works of the aforementioned authors. In particular, M. Rahman *et al.* (2022) proposed a method using fluorescent fingerprints for non-destructive prediction of pH in frozen fish fillets. The results showed that the developed method was accurate enough to predict pH

changes in horse mackerel ($R^2 = 0.71$) and spotted mackerel ($R^2 = 0.90$), therefore, it could be used as a fast and non-contact alternative to traditional pH electrodes.

K. Omwange *et al.* (2021) proposed a rapid and non-destructive method for assessing the freshness of *Tribolodon hakonensis* using multispectral imaging combined with multivariate analysis methods. The results achieved an R^2 of 0.94 and an RMSE of 2.42%. Multispectral fluorescence imaging was found to be a powerful tool for non-destructive monitoring of fish freshness during storage. The multispectral imaging method was appropriate for non-destructive detection of quality characteristics not only of fish, but also of other white meat products, such as shrimp, chicken, duck, and goose. Non-destructive methods for examining food products also included fluorescence spectroscopy and colour imaging. Based on machine learning and convolutional neural networks, these techniques could not only be used to determine the freshness and category of white meat through imaging and analysis, but could also be used to detect various harmful substances in meat products (Fan & Su, 2022; Fan *et al.*, 2024).

In the work of Y. Hu *et al.* (2023), a method for fish species authentication based on Raman spectroscopy was proposed. In this case, the PCA method was applied to analyse the data using KNN and SVM models to study the spectral features. At the initial stage of spectrum analysis, SVM models were selected to classify the sample into the group of salmon or non-salmon fish. Secondary classification models based on the PLS-DA or KNN algorithm were applied to classify the sample into one of four salmon species or one of seven non-salmon fish species. The classification model at the first level provided almost 100% accuracy, which allowed for confident identification with an accuracy of >93% at the second level for both fish groups. This fast and reliable fish authentication method was another useful tool for controlling the authenticity of fish, and therefore, for protecting consumer benefits.

In the work of J. Qin *et al.* (2020), a study was conducted to differentiate six types of fish fillets and evaluate the freshness of frozen-thawed fillets. Four types of spectra were obtained – visible and near-infrared reflectance, fluorescence, short-wave infrared reflectance, and Raman spectroscopy. The hyperspectral images of fish fillets were analysed using six models, including KNN and SVM. The highest accuracy was achieved at 100% when using the full VNIR reflectance spectra for species classification and 99.9% when using the full SWIR reflectance spectra for freshness classification. This method was proposed to quickly detect substitution and mislabelling of fish fillets (Do & Wong, 2025). Similarly, H. Li *et al*. (2022) developed rapid detection methods (RPA, RPA-LFD, and real-time RPA) targeting the serC gene of *Edwardsiella ictaluri* in yellow catfish, achieving a detection limit of 10^2 copies/μl within 30 minutes at 38°C. These methods, particularly RPA-LFD, demonstrated high sensitivity and specificity without requiring specialised equipment, offering a practical solution for on-site fish pathogen detection in aquaculture settings. R. Wang (2022) proposed a quantitative detection method for fish paste adulteration based on hyperspectral technology. By comparing and analysing the full-wavelength and simplified regression models, the optimal fish paste adulteration detection model was determined. The results indicated that both the full-wavelength model and the simplified model could effectively detect fish paste adulteration, with the prediction coefficient R^2 greater than 0.90, and the root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) being 1.9989% and 1.7983%, respectively.

In the paper of Y. Feng *et al.* (2023), surimi adulteration detection was used as an example for in-depth research on the performance of different spectral reconstruction algorithms. In the spectral reconstruction process, two main

methods were investigated: the multivariate polynomial least squares regression (PMLR) algorithm and the hierarchical deep learning regression network (HRNet). Experimental results showed that the support vector regression model with standard normal transformation (SNV) preprocessing achieved the best results with a prediction correlation coefficient (R_p) of 0.9830 and a root mean square prediction error ($RMSE_p$) of 3.9544% in the model based on the spectrum reconstruction by the PMLR algorithm. In the model based on the reconstructed spectrum of the HRNet deep learning network, the support vector regression model, also using SNV preprocessing, achieved the best performance with a prediction correlation coefficient of 0.9987 and a root mean square prediction error of 4.0808%. Thus, the PMLR algorithm and the HRNet network reconstruction method based on RGB images not only realised efficient spectral reconstruction but also provided a reliable solution for surimi adulteration detection. Furthermore, Z. Deng *et al*. (2024) reviewed the application of deep learning models, such as convolutional neural networks (CNNs) and multilayer perceptrons (MLPs), in food authenticity, emphasising their superior performance over traditional machine learning for complex data analysis. Their work highlighted the potential of deep learning combined with spectroscopic techniques for rapid and accurate fish authenticity detection, aligning with the high accuracy achieved in the SVM-based spectral classification. In the studies by J. Zhou e*t al.* (2019) it was found that after spectral preprocessing and CARS characteristic wavelength optimisation, the correlation coefficients of the model were 0.961, 0.881, 0.955, and 0.946, respectively, and the cross-validation root mean square errors were 0.049, 1.659, 0.047, and 2.558, respectively, verifying the good predictive ability of the model and providing an effective method for rapid and non-destructive detection of freshwater fish freshness. P. Tian (2023) used spectral and hyperspectral imaging technology, combined with various chemometric methods and image processing techniques, to conduct chemometric analysis on frozen and refrigerated salmon meat and established a prediction model for qualitative analysis. It was found that the correct identification rate of the test samples could reach 97.92%. The use of CARS and SPA feature wavelength extraction could reduce the data dimension, and the correct identification rate of the CARS-CNN model could still reach 93.75%, effectively realising the identification of fresh and frozen-thawed salmon meat.

The study confirmed that combining multivariate scatter correction with principal component analysis yields the most effective preprocessing pipeline for hyperspectral fish classification, achieving the highest accuracy of 98.39% with the SVM model and demonstrating superior performance in noise reduction, feature extraction, and classification compared to SG and individual preprocessing methods.

Conclusions

Aiming at the classification problem of fish spectral data, this study proposed a dimensionality reduction method based on principal component analysis (PCA). The original spectral data were optimised by integrating two data preprocessing techniques, Savitzky-Golay (SG) and multiplicative scatter correction (MSC). This approach effectively enhanced the data quality. The high-dimensional spectral features were reduced to fewer than 10 dimensions while retaining a cumulative variance contribution rate of over 92%, which verified the effectiveness of the dimensionality reduction method in preserving spectral feature information. Based on the dimensionally reduced data, K-nearest neighbours (KNN) and support vector machine (SVM) classification models were constructed separately. The experimental results indicated that when the nearest neighbour parameter of the KNN algorithm was optimised to $k = 4$, it achieved an average classification accuracy of 97.25% on the test set. The KNN algorithm

demonstrated stability on the dataset with a balanced category distribution, but was more sensitive to noise. Through the selection of the radial basis function (RBF) kernel and parameter tuning, the SVM algorithm achieved a classification accuracy of 97.48%. By comparing the combinations of the two preprocessing methods and dimensionality reduction strategies, it was discovered that the choice of the preprocessing method had a significant influence on the classification results. The accuracy of the MSC method for the SVM classifier was 0.53% higher than that for the KNN classifier. PCA not only decreased the computational complexity but also enhanced the model's robustness by removing redundant features.

This study still has certain limitations. For instance, there was an insufficient capability to identify the differences in spectral features among some fish species, or the generalisation ability under extreme lighting conditions remains to be verified. In the future, it would be possible to further explore the combination of a convolutional neural network (CNN), a deep learning model, with spectral features, or introduce multimodal data to improve the classification performance.

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Conflict of Interest

None.

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Спектральна класифікація риб на основі основного компонентного аналізу

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Анотація. Спектральна технологія виявлення може бути використана для розпізнавання різних видів м'яса риби та визначення наявності у зразках інгредієнтів, що допомагає контролювати автентичність та відповідність продукції. Метою дослідження була розробка та порівняння ефективності методів машинного навчання для класифікації різних видів риби на основі гіперспектральних даних. У роботі гіперспектральну технологію використано для отримання спектральних даних 328 точок вибірки у смузі 400-1000 нм чотирьох різних риб, а спектральні криві були попередньо оброблені для отримання більш точних та ефективних спектральних кривих. У дослідженні систематично порівняно методи попереднього оброблення Савицького-Голея згладжування та багатовимірної корекції розсіювання (MSC) та поєднано метод аналізу головних компонентів (PCA) для оптимізації моделей класифікації k-найближчих сусідів (KNN) і машини опорного вектору (SVM) з метою забезпечити більш ефективне рішення для класифікації риб. Метод аналізу головних компонентів використано для зменшення розмірності спектральних даних. Основні компоненти після зменшення розмірності порівняно з повними компонентами, тренувальний набір та тестовий набір згенеровано випадковим чином. Алгоритм SVM та алгоритм KNN використано у Python для прогнозування та визначення точності моделі. Показано, що середня точність спектральних даних після попереднього оброблення та зменшення розмірності у класифікації моделей SVM може досягати 97,25 %, а середня точність класифікації моделей KNN – 97,48 %. Це свідчить

про те, що різні види м'яса риби можуть бути класифіковані. Обидві моделі показали, що в цілому метод попереднього оброблення, який поєднує MSC і PCA, є кращим, ніж один метод попереднього оброблення, підкреслюючи важливість PCA для видалення зайвої інформації та збереження ключових характеристик класифікації. Під час порівняння продуктивності моделі за однакових умов попереднього оброблення встановлено вищу точність SVM, ніж KNN, що вказує на більшу адаптивність SVM до класифікації спектральних даних. Завдяки розробленню та застосуванню спектральної технології виявлення можна сприяти технологічному прогресу та інноваціям у тестуванні харчових продуктів, направляючи всю галузь до безпечнішого та надійнішого майбутнього

Ключові слова: спектр; метод Савицького-Голея; мультиплікативна корекція розсіювання; модель k-найближчих сусідів; алгоритм машини опорного вектору

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Justification of the technology of sausage products using plant functional ingredients

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Abstract. The search for innovative ways to increase the biological value and optimise the recipes of meat products is an urgent task of food technology. The research was devoted to improving the technology of production of cooked sausage products by introducing a vegetable additive based on carrot puree and mushroom powder to improve their functional and technological properties and increase nutritional value. To obtain experimental data, standard methods for determining the chemical, functional and technological, organoleptic properties of the vegetable additive, model minced meat and finished sausage products were used. The research results were processed by statistical analysis methods. In the course of the research, a recipe composition of a vegetable supplement with an optimal ratio of carrot puree (75%) and mushroom powder (25%) was developed, its nutritional value was established: moisture content – 56.68%, protein – 8.56%, fat – 2.35%, carbohydrates – 36.45%, ash – 6.12%, energy value – 192 kcal/100 g. Functional, technological and organoleptic indicators of boiled sausages with different levels of introduction of the vegetable supplement were analysed. A positive effect of the supplement on the water-binding capacity, consistency and reduction of the energy value of sausages was established. The generalisation of the results showed that the recipe with a 25% replacement of meat raw materials (MP3) provided the best balance between the quality, structure and nutritional value of the finished product. The results can be used in meat processing plants to create cooked sausage products with increased functionality and cost-effectiveness

Keywords: vegetable additive; carrot; oyster mushroom; mushroom powder; functional and technological properties; energy value

Introduction

The state of nutrition is a decisive factor among the main environmental factors affecting the body. This factor directly affects the human body throughout life. Therefore, the problem of improving the structure of nutrition as the basis of human life is one of the most important for both Ukraine and other countries worldwide. As emphasised in the work of L. Bal-Prylypko *et al.* (2023), contemporary food must be functional; it should not only satisfy hunger, but also provide additional physiological benefits for the human body. All food can be considered functional to a certain extent, as it provides energy and essential nutrients required to sustain the vital functions of the human body. However, certain dietary ingredients that are not traditionally classified as nutrients may nonetheless confer positive health benefits. Such ingredients are generally regarded as functional, and food products containing these components are classified as functional foods (Essa *et al.*, 2023). Thus, functional food differs from ordinary food in that it must contain a biologically functional component, the usefulness of which for health has been scientifically proven. Biologically active components of food in the process of metabolism are transformed into components of the body's cells, participating in the formation of their structure and functions. These components play a key role in maintaining physical and mental performance, strengthening the immune system, adaptation to external factors, and also affect the general state of health, life expectancy and level of social activity of a person (Granato *et al.*, 2020).

As noted by L. Pruteanu *et al.* (2023), in the process of developing functional food products, it is advisable to pay special attention to their enrichment with components that contribute to improving the work of the main elements of the body's defence mechanisms, in particular the antioxidant defence system, enzymatic

detoxification and cell membrane structures, etc. A promising area for research into the creation of functional products with increased nutritional and biological value is the combination of raw materials of animal and plant origin in accordance with modern requirements of nutritional science. Among animal products, meat and meat products are considered the main components of a healthy and balanced diet. They are concentrated natural sources of complete proteins, as well as other nutrients, such as fats, vitamins and minerals, which are necessary for the processes of growth, development and vital activity of the human body. In addition, meat is rich in biologically active substances and endogenous antioxidants with well-established beneficial properties related to their anti-inflammatory, immunoregulatory and protective activity against oxidative stress. The nutritional profile of meat consists of 75% water, 19% protein, 2.5% fat, 1.2% carbohydrates and 1.65% nitrogen compounds. The quantitative composition of these components is labile and directly correlates with feeding, storage and processing technologies (González-Osuna *et al.*, 2024).

Along with this, a significant amount of meat raw materials with low water-binding capacity are used for the production of meat products, so the use of functional food additives that eliminate this drawback is quite relevant. Modern food additives improve the organoleptic, structural-mechanical and physico-chemical parameters of finished meat products (Hunko *et al.*, 2022). Their application enables the production of goods incorporating substantial amounts of high-fat meat raw materials (Murlykina, 2025). An analysis of contemporary scientific literature indicates an increased interest in the widespread use of non-traditional types of plant raw materials, despite the development of the production of synthetic and refined forms of food additives. In particular, J. Jarnot (2022) established that *Portulaca oleracea* L. is a plant with numerous nutritional and medicinal properties and can be used in sausage production as a food additive to improve nutritional value. According to the researcher, the use of additives may be necessary to extend the shelf life. However, many manufacturers violate the technology and add cheaper substitutes for the main raw material to reduce production costs, while reducing the nutritional value of the product and its quality.

According to literature sources, a complex combination of vegetable and animal proteins is recommended, as meat raw materials contain an excess of essential amino acids, including those that are absent in plant-based raw materials. As noted in the study by V. Vovkotrub *et al*. (2024), in order to enhance the biological value of sausage products, plant-based raw materials play a key role, being considered an inexpensive source of high-quality protein. The most common is the use of soybean proteins, because, unlike animal-derived additives such as powdered milk, casein, egg white and yolk, gelatine, their cost is much lower. In particular, the authors K. Kang *et al.* (2022) developed a model of an emulsion based on soy protein as an alternative to chicken meat for the production of Vienna sausages. This contributed to a significant reduction in their cost. Scientists systematically investigated different levels of meat replacement with soy emulsion and their effect on structural and physicochemical properties. At the same time, this study had limitations in terms of storage, safety, and sensory testing under real consumption conditions, and requires further extensive trials for broader industrial implementation.

Plant raw materials are widely used to increase the nutritional value of sausage products, which are capable of exhibiting complexing and radioprotective properties due to the content of physiologically useful ballast substances, such as cellulose, hemicellulose, pectin, lignin, extensin, cutin, waxes, as well as non-structural polysaccharides such as gums, resins, and alginates. An example of such raw

materials is oyster mushrooms. Indeed, in the study by I. Bandura *et al*. (2021), the content of macro- and micronutrients, amino acids, and vitamins in the mushroom species *Calocybe indica* was identified and thoroughly analysed. The authors emphasised the prospect of using this mushroom as a functional ingredient in the food industry. In the work of A. Mazumder *et al.* (2023) an original technological concept of creating a vegetable emulsified sausage-type product using a biopolymer complex of oyster mushroom (*Pleurotus sajor-caju*) and chickpeas. The authors substantiated the effectiveness of combining mushroom and legume raw materials for forming a protein-fibrous matrix with high hydration properties. The study also included microstructural analysis, which enabled accurate interpretation of changes in textural characteristics resulting from the substitution of animal protein. Despite the high quality of the basic experiments, both studies have certain limitations related to the lack of a complete link "from bioraw materials to finished product" and the lack of long-term studies of the functional safety and stability of the resulting systems.

Additional attention of scientists is also attracted by carrot puree as an affordable source of dietary fibre, β-carotene and mineral elements. Carrots, due to the high content of pectin substances and carotenoids, are able to increase the antioxidant activity of food systems, regulate the texture, moisture retention and colour of sausage products. Specifically, the study by F. Sam *et al*. (2021) demonstrates that the addition of 5-10% carrot paste to frankfurter sausages significantly enhances antioxidant activity and improves sensory attributes, aligning with the concept of functional nutrition. However, the limited period and range of analysed parameters do not allow for a full assessment of the long-term effectiveness and safety of the product. Thus, partial or complete replacement of meat raw materials in sausage products with non-traditional raw materials allows to obtain new and diverse products that can satisfy the needs of people whose consumption choices are limited by cultural conditions, religious beliefs, personal beliefs or health status. However, the introduction of these new ingredients leads to technological problems in production, as well as organoleptic shortcomings in the final products, which must be solved.

The purpose of the research was to substantiate the possibility of improving the technology of cooked sausage products by enriching them with a functional vegetable additive based on mushroom raw materials and carrots. To achieve this goal, the following tasks were formulated: determine the recipe composition of a functional additive made from mushroom raw materials and carrots, determine the effect of a functional plant additive on the physicochemical, functional-technological and organoleptic indicators of model minced cooked sausage products; theoretically justify the recipe amount of a functional additive in the composition of minced sausage.

Materials and Methods

The experimental part of the research was conducted during 2024-2025 on the basis of the laboratory of meat, fish and seafood technology of the Faculty of Faculty of Food Technology and Quality Control of Agricultural Products of the National University of Life and Environmental Sciences of Ukraine. The first stage of the research was to determine the rational amount of ingredients in the composition of the vegetable additive for further use in the technology of cooked sausage products. Carrots of the Chantane variety (manufacturer – LLC "Ahroovoch Ukraine", Zhytomyr region) were used as vegetable raw materials. The root crops were without signs of spoilage, medium in size. After sorting and washing, the carrots were manually peeled, cut into cubes with 10 mm edge length, packed into heat-resistant polymer bags, and vacuum sealed. Heat treatment was carried out using Sous vide technology at a temperature of 80 ± 2°C for 30 ± 5 minutes until softened.

Next, the rubbing was performed in two stages: first on a rubbing machine with sieves with openings of 1.2...1.5 mm, then with sieves with openings of 0.5...0.7 mm. After rubbing, carrot puree was obtained.

The mushroom component of the functional supplement was obtained from the fruiting bodies of the common oyster mushroom (*Pleurotus ostreatus*), grown under controlled conditions at the enterprise LLC "Hrybna Ferma" (Kyiv). Fresh mushrooms were first washed, then sliced to a thickness of 3 mm and dried in a drying oven at 45°C for 12 hours until a residual moisture content of no more than 7% was achieved. The dry raw materials were ground to a powder state in a laboratory crusher and sieved through a sieve with a hole diameter of 1.2...1.5 mm. The resulting mushroom powder had a light brown colour and a pronounced mushroom aroma. The functional vegetable supplement was a mechanically homogenised composition of carrot puree and mushroom powder in various ratios according to the data given in Table 1.

Table 1. *Modelling of the recipe composition of a vegetable additive for the production of cooked sausages*

Ingredients	Proportional composition of ingredients, %				
	MG1	MG2	MG3	MG4	MG5
Carrot puree	90	85	80	75	70
Mushroom powder	10	15	20	25	30

Source: compiled by the authors

The second stage of the study was devoted to the development of a recipe for cooked sausages, taking into account the inclusion of a functional additive. The basis for the new recipe was the minced meat of cooked sausage "Okrema", which included the main ingredients: beef of the 1st grade 60%, semi-fat pork – 25%, pork brisket – 15%, as well as additional ones: table salt 2.5%, coriander 0.1%, fresh garlic 0.3%, sodium nitrite 0.007%, nutmeg 0.05%. The meat raw material was obtained from a certified slaughterhouse (LLC "Miaso Polissia", Zhytomyr region) and met the requirements of DSTU 6030:2008 (2009). Beef – from the front part of the carcass, chilled, without visible defects; Semi-fat pork had a muscle-to-fat ratio of approximately 60:40. A total of five variants of sausage minced meat were produced: one control (traditional, without vegetable additive) and four experimental (MP1-MP4) with a stepwise introduction of vegetable additive in the amount of 15%, 20%, 25% and 30%, respectively (Table 2). The quantity of additional ingredients in the model minced meat formulations was equivalent to that of the selected reference sample. The formulation of the plant-based supplement for incorporation into the model mixtures was determined based on the results of the first stage of the study. The expected product yield was 80% by weight of unsalted raw materials.

Table 2. *Formulation of model minced meat mixtures for the production of cooked sausages*

Component names	Prescription amount of ingredients, %				
	"Okrema"	MP1	MP2	MP3	MP4
Beef, 1st grade	60	60	55	50	45
Pork, semi-fat	25	25	25	25	25
Pork brisket	15	–	–	–	–
Plant-based supplement	–	15	20	25	30
Total	100	100	100	100	100

Source: compiled by the authors

The volume of each batch was 2.5 kg. All samples were prepared according to the same scheme. The production process of the sausage products involved mincing the meat raw materials using a meat grinder with a plate diameter of 3-5 mm, followed by mixing in a bowl cutter with additional ingredients according to the formulation. The functional plant-based supplement, in the form of a homogenate, was added during the mince blending stage (at the second stage of bowl cutting), and the mixture was processed until a uniform consistency was achieved. Then, the minced meat was filled into collagen casings with a diameter of 32 mm using an automatic syringe, and loaves 12...15 cm long were formed. Thermal processing was carried out in a steam-convection oven according to the following regime: roasting at 80°C for 30 minutes, followed by cooking until the internal temperature of the sausage loaf reached 72 ± 1°C (approximately 60 minutes). The sausages were then cooled in a refrigeration chamber to 4°C. Samples were stored for no more than 24 hours before analytical studies.

Standard methods were used to perform analytical studies. determination of chemical, functional and technological properties of the vegetable additive, model minced meats and finished sausage products: water-binding, water-holding, fat-holding, fat-binding capacity, pH value, components of the chemical composition: moisture content, protein, fat, carbohydrates, ash content, organoleptic evaluation. To confirm the reliability of the results obtained, all experimental studies were carried out with three repetitions of each series. For each sample and each indicator (water-binding capacity, fat and water absorption capacity, structural and mechanical characteristics), at least n=5 independent measurements were performed, which provided a sufficient amount of statistical data for further analysis. Statistical processing. The experimental data were analysed using the MS Excel 365 license package with additional add-ins for regression modelling. The significance of differences between mean values was assessed using analysis of variance (ANOVA). When assessing the significance of the results, a standard confidence level of α=0.05 was used.

The hydrogen ion concentration was measured in accordance with DSTU ISO 2917:2001 (2003) using a Testo 205 pH meter. The device provided measurements in the pH range from 0 to 14 units at temperatures from 0 to 60°C, with an accuracy of 0.01 pH units and 0.1°C; the error limit was ±0.02 pH and ±0.4°C. For the analysis, an aqueous extract was prepared at a mince-to-water ratio of 1:10. To this end, 5 g of mince or homogenised sample product was placed in a 250 ml conical flask, followed by the addition of 50 ml of distilled water. Extraction was then carried out for 30 minutes with intermittent stirring. After completion of the process, the extract was filtered and the pH in the filtrate was determined.

The fat absorption capacity of the test samples was determined as follows: 3 g of the test sample of the product and 15 ml of oil were mixed in a laboratory mixer for 1 min at a speed of 50 r/60 s, then the suspension was left in a thermostat at a temperature of 90°C for 20 min. Thereafter, the mixture was centrifuged at 4,000 rpm for 5 minutes. The fugate was drained, its mass F and the content of dry substances in it were determined. The fat absorption capacity was calculated by the formula (1):

$$\mathrm{FAC} = \frac{\mathrm{B}-(\mathrm{F}-m)}{\frac{M\cdot(100-W)}{100}-m}\cdot 100,\%, \qquad (1)$$

where B is the amount of oil poured into the centrifuge tube, ml; M is the weight of the product added to the centrifuge tube, g; F is the mass of the fugate, g; W is the moisture content in the product, %; m is the content of dry substances in the fugate, g. m was determined by the formula (2):

$$m = \mathrm{F}\frac{\mathrm{DM^F}}{100}, \qquad (2)$$

where $\mathrm{DM^F}$ is the dry matter in the sample taken for determination, g.

Water absorption capacity (WAC) was determined by the above method, but distilled water was used instead of oil. The experiment was repeated three times. The arithmetic mean results of the calculations were presented in the form of graphical images. The method for determining the water-binding capacity (WBC) was based on the principle of water release from 300 mg of the sample during 10-minute pressing with a load weighing 1 kg. The evaluation was carried out based on the area of the stain on filter paper, which formed as a result of the absorption of the released liquid by the paper. The contour of the spot resulting from the pressed minced meat was marked with a pencil. The area of the wet zone was calculated as the difference between the total area of the imprint and the area occupied by the mince itself. The content of bound water was calculated by the formula (3):

$$X = \frac{(A-8,4B)\cdot 100}{A}, \quad (3)$$

where X is the content of bound water (WBC), % of total water; A is the total water content in the sample, mg; B is the area of the wet spot, cm^2.

Determination of water-holding capacity (WHC) and fat-holding capacity was performed in the following order according to generally accepted methods described in the work of T. Szmańko *et al*. (2021). The water content of the samples was determined in accordance with DSTU ISO 1442:2005 (2008) by drying in a thermostated drying oven at a temperature of 130°C. The method was based on the property of the tested material to lose hygroscopic moisture when heated to a specified temperature. The protein content was determined by the Kjeldahl method, which is based on the mineralisation of the sample, distillation of ammonia into a sulfuric acid solution with subsequent titration (DSTU ISO 937:2005, 2007). The mineralisation process was carried out by heating the sample with concentrated sulphuric acid in the presence of a copper-sulphate catalyst. As a result of the reaction, the ammonia formed interacted with an excess of sulfuric acid to form ammonium sulphate (4):

$$2NH_3 + H_2S0_4(k) \rightarrow (NH_4)_2S0_4. \quad (4)$$

To determine the amount of ammonia, ammonium sulphate was decomposed by concentrated sodium hydroxide (5):

$$(NH_4)_2S0_4 + 2NaOH \rightarrow 2NH_3 + 2H_20 + Na_2S0_4. \quad (5)$$

The ammonia formed was absorbed by a sulfuric acid solution during titration (6):

$$2NH_3 + H_2S0_{4k} \rightarrow (NH_4)_2S0_4. \quad (6)$$

The excess sulfuric acid was titrated with sodium hydroxide solution, and the amount of ammonia absorbed, or the corresponding mass of nitrogen, was calculated from the volume of bound acid. The fat content was determined by a method based on repeated extraction of fat from a dried sample using a volatile solvent, followed by removal of the solvent and drying of the extracted thimble to a stable mass (DSTU ISO 1443:2005, 2008). Extraction was carried out in a Soxhlet apparatus using dichloroethane as a solvent. The sample, dried to constant weight, was transferred into a paper thimble, which was weighed and placed into the extractor of a Soxhlet apparatus. The extraction time was 4-6 hours.

The ash content was determined as the mass of mineral substances obtained after ashing of organic substances (DSTU ISO 936:2008, 2008). The combustion of the organic part of the test sample was carried out in a muffle furnace at a temperature of 500...800°C. A 5 g portion of the test sample, weighed to an accuracy of 0.0002 g, was placed into a pre-calcined crucible that had been weighed to constant mass. The crucible with the sample was placed in a muffle furnace. Ashing was started at a low temperature, then continued at a temperature of 500...800°C for 1...2 hours. The crucible was cooled in a desiccator and then weighed.

Carbohydrate content was determined as the sum of sugars, starch, and dietary fibre, which were determined according to standard methods (DSTU ISO 13965:2007, 2009; Bandura *et al.*, 2022). The calculation of the energy value of 100 g of product (kcal) was performed according to the formula (7):

$$EV = (C_p \cdot 4) + (C_c \cdot 3.75) + (C_f \cdot 9), \quad (7)$$

where C_P – protein content, %; C_C – carbohydrate content, %; C_f – fat content, %; 4.0 – coefficient for calculating the energy value for proteins (3.75 – for carbohydrates, 9.0 – for fats, kcal).

Organoleptic evaluation of cooked sausage products was carried out in compliance with ethical and methodological requirements that ensure reproducibility of results and compliance with standards (DSTU 4823.2:2007, 2009). The study was conducted with the involvement of a tasting panel consisting of 12 individuals (7 women and 5 men aged between 24 and 60 years), of whom 8 had professional training in food technology, at least three years of experience in sensory evaluation, and had been certified as internal experts within food industry enterprises. The remaining members of the commission were qualified specialists with knowledge of the consumer properties of meat products. The composition of the panel and participation in the study were carried out on a voluntary basis, following informed consent obtained in writing from all participants after they had been briefed on the aim of the study and the tasting conditions, in accordance with the provisions of the WMA Declaration of Helsinki (2013). The evaluation criteria and their limits were determined in accordance with the requirements of DSTU 4436:2005 (2007), using a five-point scale: 5 points – excellent quality, fully compliant with the requirements of the standard, signs are maximally pronounced, no defects; 4 points – good quality, minor deviations in the severity of signs, do not affect the overall impression; 3 points – satisfactory quality, moderately pronounced defects, but the product is suitable for consumption; 2 points – unsatisfactory quality, noticeable defects; 1 point – product unsuitable for consumption.

Each sample was served to the tasters anonymously, under a conditional code, chilled (8...10°C), cut into pieces 1 cm thick and weighing approximately 10 g. All samples were identically prepared, served in disposable tasting dishes, under the same lighting, in a tasting room that meets sanitary and hygienic standards (illumination 600... 800 lux, temperature 20±2°C, neutral interior colours, absence of foreign odours). Between tasting different samples, the tasters were able to neutralise the taste with drinking water and a slice of white bread. The evaluation procedure followed a specific sequence: external appearance was assessed based on structure, density, uniformity of mince filling, and the absence of air pockets or casing defects; cut surface colour was evaluated visually, taking into account homogeneity, intensity, and colour typicality for the respective type of sausage; aroma and taste were assessed through organoleptic analysis after chewing the sample, with attention to the presence or absence of off-flavours or odours, the distinctiveness of the aromatic profile, and saltiness; consistency was evaluated by pressing the sample surface with a finger and during chewing, considering elasticity, firmness, and uniformity. The assessment was carried out individually by filling out tasting questionnaires. All data were summarised in a table and analysed statistically. The reliability of the difference between the estimates for different samples was checked using variance analysis at a significance level of α=0.05.

Based on the results of the questionnaire, a comprehensive evaluation of organoleptic characteristics was carried out. It included determining the total score of model samples S_i and the coefficient of intensity of manifestation of the general impression S_i/S. This coefficient is used for comparative assessment of the

quality of food products, in particular – in tasting studies. It is calculated by the formula (8):

$$S_i/S = \frac{s_i}{s_{max}}, \quad (8)$$

where S_i is the total score obtained by this sample for all organoleptic indicators; S or S_{max} – the maximum possible score. If all 5 indicators are rated 5 points, then:

$$S, S_{max} = 5 \cdot 5 = 25 \text{ points}$$

Based on the results of organoleptic evaluation, conclusions were drawn about the formulation of the tested samples and the quality of the finished product.

Results

Modelling the formulation of a plant-based supplement

The main property of minced meat food systems that affects the quality of finished sausage products is the sorption of water and fat. Water and fat provide key sensory and physiological benefits. Their presence contributes to the formation of taste, texture and appearance of products. From the perspective of consistency, water and fat have a decisive influence on the binding, rheological, and structural properties of meat products and play a crucial role in the formation of meat emulsions within the mince. However, from the standpoint of healthy nutrition, reducing the fat content in food products is considered a promising approach. In turn, a decrease in the fat content in meat products is accompanied by a deterioration in appearance, taste and consistency. Therefore, the most important functional and technological properties were selected as criterion indicators when modelling the recipe quantity of ingredients of a plant-based supplement: water absorption capacity (WAC) and water-holding capacity (WHC), fat absorption capacity (FAC) and fat-holding capacity (FHC) of mixtures. The results of determining these criterion indicators are presented in Figures 1 and 2.

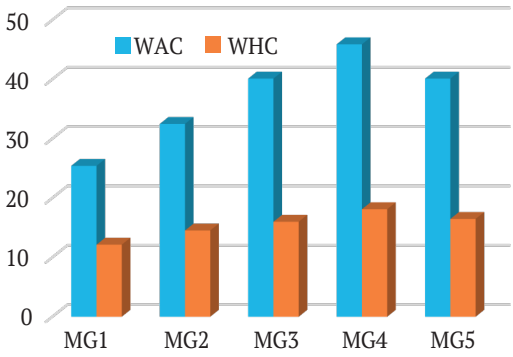

Figure 1. The value of the WAC and WHC of the composite mixtures of the plant-based supplement, %

Source: compiled by the authors

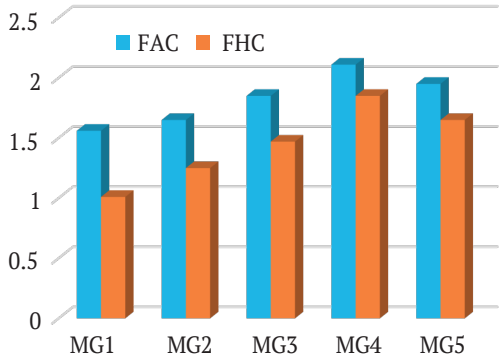

Figure 2. The value of the FAC and FHC of the composite mixtures of the plant-based supplement, g fat/g

Source: compiled by the authors

The obtained results proved that the best functional and technological properties were possessed by the composite mixture of the plant-based supplement MG4, which contained 75% carrot puree and 25% mushroom powder. At the same time, the water absorption and water-holding capacity were 45.89% and 18.11%, respectively, the fat absorption and fat-holding capacity were 2.11 and 1.85 g fat/g, respectively (Figs. 1, 2). A further increase in the mass fraction of mushroom powder in the formulation of the plant-based

supplement was accompanied by a decrease in the numerical values of all functional and technological parameters. Such results are associated with the physicochemical structure of plant polysaccharides that dominate in the composition of mushroom powder and are present in carrot puree.

Water-holding capacity is the amount of water that remains bound to the fibre hydrate after the application of external centrifugal force of gravity or compression (Raghavendra *et al.*, 2006). According to known literature data, the dietary fibres of mushroom powder particles have the ability to retain water by adsorption inside the fibre matrix, which will help preserve the structure of future finished sausage products (Grigelmo-Miguel *et al.*, 1999; Lan *et al.*, 2012). Fat-holding capacity is also the most important technological property, which is associated with the chemical structure of plant polysaccharides and depends on the thickness, surface properties, and hydrophobic nature of the fibrous particles (López-Marcos *et al.*, 2015).

An increase in the percentage of mushroom powder in the formulation of the plant-based supplement led to a higher proportion of its insoluble fraction, which adversely affected the functional and technological properties, resulting in their reduction. Correlation-regression analysis confirmed the existence of a close direct correlation between the concentrations of mushroom powder and the functional and technological properties of the plant-based supplement. The pairwise correlation coefficient between the concentration of mushroom powder in the formulation and the WAC of the plant-based supplement was r = 0.85, and the WHC – r = 0.86. The general regression model of the relationship between the concentration of mushroom powder in the formulation of the plant-based supplement and its WAC is described by the graphical dependence and equation presented in Figure 3, and the WHC – in Figure 4.

The pairwise correlation coefficient between the concentration of mushroom powder in the plant-based supplement formulation and the FAC of the supplement was r=0.88, and for the FHC – r=0.90. The general regression model of the relationship between the concentration of mushroom powder in the plant-based supplement formulation and its FAC is described by the graphical dependence and equation presented in Figure 5, and for the FHC – in Figure 6.

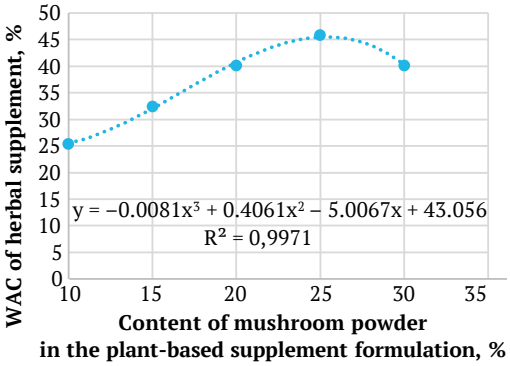

Figure 3. Dependence of the WAC on the content of mushroom powder in the plant-based supplement formulation

Source: compiled by the authors

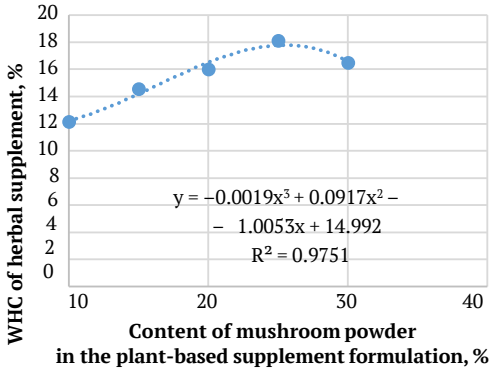

Figure 4. Dependence of WHC on the content of mushroom powder in the plant-based supplement formulation

Source: compiled by the authors

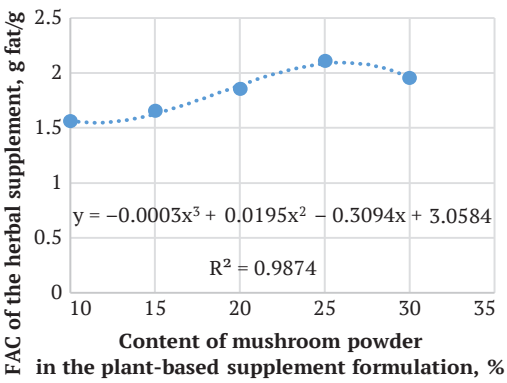

Figure 5. Dependence of FAC on the content of mushroom powder in the plant-based supplement formulation

Source: compiled by the authors

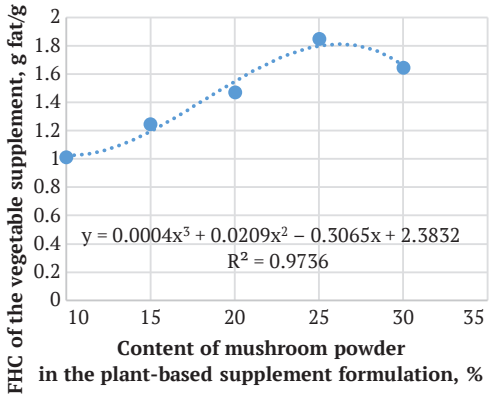

Figure 6. Dependence of FHC on the content of mushroom powder in the plant-based supplement formulation

Source: compiled by the authors

The resulting equations make it possible to predict the key functional and technological properties of the plant-based supplement depending on the mushroom powder content in its formulation. Based on the results obtained, a composite mixture of the plant-based supplement MG4 is recommended for further use, which includes 75% carrot puree and 25% mushroom powder. The chemical composition and calorie content of the developed composite mixture of the plant-based supplement MG4 are given in Table 3.

Table 3. *Chemical composition and caloric value of the composite plant-based supplement mixture MG4*

Chemical composition components	Content, %
Moisture	56.68 ± 0.02
Fat	2.35 ± 0.03
Protein	8.56 ± 0.43
Ash	6.12 ± 0.21
Carbohydrates	36.45 ± 0.14
Caloric value, kcal per 100 g	192.08

Source: compiled by the authors

According to the results of the chemical composition analysis, carbohydrates are the main component. However, it should be noted that the carbohydrate profile of the plant-based supplement consists primarily of so-called complex carbohydrates. The so-called simple carbohydrates, the main representatives of which are sugars, make up a smaller part of them. Their content was 6.56 ± 0.23%. This indicator is generally acceptable, especially for groups of the population suffering from diabetes. In addition, scientists Á. Farkas *et al.* (2020) found that β-carotene, which is contained in carrot puree, inhibits insulin resistance in diabetes. Thus, the developed plant-based supplement demonstrates high technological performance, which may contribute to reducing mass losses of cooked sausage products during thermal processing, promoting the formation of a homogeneous and tender consistency in the final product, and decreasing the number of defective items by minimising moisture and fat release.

Modelling the recipe for cooked sausage products

The formulation modelling of the experimental cooked sausage samples was carried out taking into account the results of organoleptic and physicochemical analyses. The functional and technological properties of the model minced meats are given in Table 4.

Table 4. *Functional and technological properties of model minced cooked sausages with vegetable additive MG4*

Sample name	Moisture content, %	Water-binding capacity, %	Water-holding capacity, %	Fat-holding capacity, g fat/g	pH
"Okrema" (control)	64.0 ± 0.7	81.2 ± 3.5	72.5 ± 2.9	68.5 ± 3.9	5.6 ± 0.1
MP1	66.1 ± 0.24	85.6 ± 2.1	78.2 ± 3.0	74.6 ± 3.3	5.8 ± 0.3
MP2	68.3 ± 1.8	87.7 ± 2.3	81.1 ± 3.7	74.9 ± 3.3	5.9 ± 0.2
MP3	70.1 ± 1.7	90.5 ± 3.6	83.4 ± 2.8	75.2 ± 6.5	6.2 ± 0.1
MP4	72.2 ± 0.9	92.1 ± 2.6	86.1 ± 4.8	75.6 ± 5.8	6.5 ± 0.2

Source: compiled by the authors

The presented research results indicate that the incorporation of the MG4 plant-based supplement – comprising carrot purée and mushroom powder – into model cooked sausage mixtures at levels of 15-30% increases their water-holding capacity by 5.7-13.6% and fat-holding capacity by 6.65-7.58%, which contributes to improved structure in the final products (Table 4). The sensory evaluation of model samples of cooked sausages confirmed that the partial replacement of meat raw materials with a functional vegetable supplement did not worsen their organoleptic indicators. However, increasing the amount of the plant-based supplement to 30% resulted in a pronounced carrot and mushroom flavour and aroma, which is uncharacteristic of cooked sausages and led to a decrease in their sensory evaluation scores (Fig. 7, Table 5).

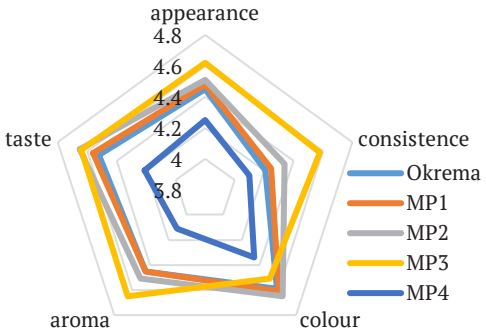

Figure 7. Sensory profile chart of the model cooked sausage samples

Source: compiled by the authors

Table 5. *Results of calculating the areas of the constructed quality profiles of the sausage scoring*

Indicators	"Okrema" (control)	MP1	MP2	MP3	MP4
Consistence	4.21	4.25	4.34	4.58	4.10
Colour	4.59	4.61	4.65	4.51	4.34
Aroma	4.45	4.45	4.51	4.65	4.11
Taste	4.52	4.56	4.65	4.64	4.21
Appearance	4.45	4.48	4.51	4.62	4.25
S	22.22	22.35	22.66	23.0	21.01
S_i/S	0.89	0.89	0.91	0.92	0.84

Note: S – overall score of the experimental samples; S_i/S – coefficient of intensity of manifestation of general impression

Source: compiled by the authors

In particular, the overall score of the experimental samples ranged from 21 to 23 points. The highest total organoleptic score among all samples was recorded for sample MR3 (23.00 points), which contained 25% of the plant-based supplement. This corresponded to a general impression intensity coefficient (S_i/S) of 0.92, indicating the most favourable perception of the sample by the tasting panel. This indicates the best perception of the sample by the tasting panel. Slightly lower values were received by samples MP2 (S_i/S = 0.91), MP1 (0.89) and the control sample "Okrema" (0.89), which indicates the preservation or slight improvement of organoleptic properties when including 15...20% of the plant-based supplement in the formulation. In contrast, sample MP4, which contained 30% of the functional composition, had the lowest index (0.84), which confirms the decrease in consumer characteristics with excessive dosage of the plant-based component. In order to provide a comprehensive characterisation of the final sausage products, their chemical composition and energy value were determined (Table 6).

Table 6. *Chemical composition of finished sausage products*

Model sample	Component content, %					Energy value, kcal
	Moisture	Protein	Fats	Carbohydrates	Ash	
"Okrema" control	64.0 ± 0.7	11.3 ± 1.08	14.94 ± 1.76	0.01 ± 0.01	0.84 ± 0.07	179.32
MP1	66.1 ± 0.24	11.28 ± 1.74	13.02 ± 0.86	1.89 ± 0.09	0.89 ± 0.04	169.43
MP2	68.3 ± 1.8	11.31 ± 1.31	12.11 ± 1.27	1.92 ± 0.16	0.93 ± 0.03	161.43
MP3	70.1 ± 1.7	11.48 ± 1.33	11.08 ± 0.87	1.95 ± 0.28	0.96 ± 0.02	152.36
MP4	72.2 ± 0.9	11.54 ± 1.13	10.22 ± 1.16	1.96 ± 0.12	0.99 ± 0.06	143.79

Source: compiled by the authors

Analysis of the presented data showed that the addition of the plant-based supplement composed of carrot purée and mushroom powder led to an increase in the mass fractions of moisture, protein, carbohydrates, and ash, while simultaneously resulting in a reduction in fat content compared to the control. A noticeable decrease in fat content was accompanied by a decrease in the energy value of finished products. The reduction in fat content and the increase in "beneficial carbohydrates" allow the sausage products with partial substitution of meat raw material by the plant-based supplement to be classified as products with enhanced functional properties.

Discussion

Functional and technological characteristics of minced meat systems are formed under the influence of a complex of physicochemical and sensory factors that determine textural parameters, structural integrity, colour intensity, flavour profile and moisture and fat holding during heat treatment. The results obtained confirmed that the water absorption capacity (WAC) and water-holding capacity (WHC), fat absorption capacity (FAC) and fat-holding capacity (FHC) of the mixtures are the most important technological indicators that determine the quality of cooked sausages. It was found that the composition MG4 (75% carrot puree and 25% mushroom powder) demonstrated the highest values: WAC – 45.89%, WHC – 18.11%, FAC – 2.11 g/g and FHC – 1.85 g/g. With an increase in the proportion of mushroom powder, all indicators declined, which can be attributed to the growing amount of the insoluble fraction, which is less effective in liquid binding. These results are consistent with the findings of L. Korets (2019) and Y. Xu *et al*. (2022), who demonstrated that the incorporation of dietary fibres into meat products significantly improves the water-holding capacity of meat mince. Similarly, in the work of S. Lee *et al.* (2023) found that the fibrous structure

provides stability to emulsions by forming bonds with fat globules, which maintains the structure of the minced meat and reduces moisture loss. J. Feng *et al.* (2025) found that fat-holding capacity correlates with the hydrophobic properties of the fibres – a high proportion of mushroom powder, which is rich in insoluble fibres, can worsen the HFCS, which is also reflected in the indicators for MG5 compositions in the current study. The data obtained in this study also resonate with the results of A. Mazumder *et al.* (2023), in which a significant decrease in HFCS and HFCS was recorded when a high concentration of mushroom powder was added to sausages – similar to observations for MG5.

Particular attention should be given to the role of carrot purée: in the study by J. Richards *et al.* (2024), the addition of 3-4% carrot purée to meat mince improved the water-binding capacity by 8.5-15.7% and increased the yield of the final product by 13.9-22.8%. This indicates a synergistic effect of carrot-mushroom powder, which the authors of the current study confirmed, obtaining the maximum indicators for the MG4 composition. Moreover, S. Yadav *et al*. (2018) found that the addition of dried carrot pomace in combination with wheat bran improves the technological properties of chicken sausages without compromising their sensory qualities.

Correlation-regression analysis of the conducted study confirmed the high statistical significance of the relationship between the concentration of mushroom powder and functional indicators (r = 0.85-0.90). This coincides with the studies of B. Mishra *et al.* (2023), which revealed the dependence of WHC and FHC on the proportion of fibre compounds in sausage systems. Thus, the synergistic combination of carrot fibre and mushroom powder provides a technological compromise between texture, emulsion stability, and juice retention, which is optimised at a ratio of 75:25. Adding 15-30% of the plant-based supplement MG4 to the minced meat of cooked sausages contributed to a significant increase in water-holding capacity (by 5.7…13.6%) and fat-holding capacity (by 6.65…7.58%) compared to the control, which is consistent with the conclusions of X. Li *et al.* (2024). The observed positive effect is attributed to the fact that the dietary fibres in mushroom powder and carrot purée have a low gelatinisation temperature, which is close to the denaturation temperature of meat proteins. Upon reaching this temperature, moisture is released and subsequently absorbed by the protein-carbohydrate complex introduced with the carrot-mushroom supplement.

According to DSTU 4436:2005 (2007), no more than 75% moisture is allowed in cooked sausage products. Adding a vegetable additive contributed to an increase in the moisture content in the products. However, the moisture content of the model samples produced using the classical formulation (control) and the MP1, MP2, and MP3 formulations complied with the requirements for the highest commercial grade, whereas the MP4 formulation corresponded to the standards for first and second commercial grades. The organoleptic evaluation confirmed a consistently high level of consumer properties for samples with 15-25% of the additive, while 30% caused the appearance of a pronounced carrot-mushroom taste and aroma and a decrease in the S_i/S coefficient to 0.84. The results of the correlation analysis confirmed the obtained results. A strong inverse correlation was established between the content of the plant-based supplement and the fat content in the finished sausage products, with a correlation coefficient of r = –0.99. In contrast, strong direct correlations were observed with all other chemical composition parameters: with protein content (r = 0.70), carbohydrate content (r = 0.89), and ash content (r = 0.98). Based on a comprehensive assessment of the functional and technological properties and organoleptic indicators of finished sausage products, the optimal recipe composition was determined to be the composition of MP3, which provides for the introduction of 25% of the vegetable additive.

Conclusions

In the course of the study, a novel solution was proposed to a relevant scientific and applied problem, namely the improvement of the functional, technological, and consumer properties of cooked sausages through the addition of a plant-based supplement formulated from carrot purée and oyster mushroom powder. Taking into account the functional and technological indicators, the optimal recipe composition of the vegetable supplement was substantiated. Its composition includes 75% carrot puree and 25% mushroom powder. The nutritional value of the vegetable supplement was established: moisture content – 56.68%, fat – 2.35%, protein – 8.56%, carbohydrates – 36.45%, ash – 6.12%, energy value – 192 kcal per 100 g. The investigation of organoleptic indicators and functional properties of the finished sausage products confirmed the feasibility of partially replacing meat raw materials with a functional plant-based supplement. Based on experimental studies, it was established that the optimal recipe composition is the MP3 composition, which provides for the introduction of 25% of the vegetable supplement.

The improved technology of cooked sausage products, which involves the use of 25% vegetable supplement of mushroom powder and carrot puree, has advantages over traditional technology – rational use of meat raw materials, the ability to regulate functional and technological properties, effective impact on the energy value of finished products. The prospects of the conducted research are significant for both science and the food industry. The scientific value lies in the introduction of a composite plant-based supplement, based on carrot purée and oyster mushroom powder, as a functional ingredient that enhances the water- and fat-holding capacities of minced meat systems and enriches the product with biologically active compounds. The results obtained create a basis for further research into interfacial interactions in protein-carbohydrate systems and the study of the physiological impact of bioactive components on human health. From a practical perspective, the proposed formulation allows for partial replacement of meat raw materials, reduction of fat content, enhancement of nutritional value, and alignment with current trends in healthy and functional nutrition, making it a promising option for industrial implementation.

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Conflict of Interest

None.

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Обґрунтування технології ковбасних виробів з використанням рослинних функціональних інгредієнтів

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Анотація. Пошук інноваційних шляхів підвищення біологічної цінності та оптимізації рецептур м'ясних продуктів є актуальним завданням харчових технологій. Дослідження було присвячено удосконаленню технології виробництва варених ковбасних виробів шляхом введення рослинної добавки на основі морквяного пюре та грибного порошку

для покращення їхніх функціонально-технологічних властивостей і підвищення харчової цінності. Для отримання експериментальних даних використані стандартні методи визначення хімічних, функціонально-технологічних, органолептичних властивостей рослинної добавки, модельних фаршів та готових ковбасних виробів. Результати досліджень оброблено методами статистичного аналізу. У процесі дослідження розроблено рецептурну композицію рослинної добавки з оптимальним співвідношенням морквяного пюре (75 %) та грибного порошку (25 %), встановлено її харчову цінність: вміст вологи – 56,68 %, білка – 8,56 %, жиру – 2,35 %, вуглеводів – 36,45 %, золи – 6,12 %, енергетична цінність – 192 ккал/100 г. Було проаналізовано функціонально-технологічні та органолептичні показники варених ковбас із різним рівнем введення рослинної добавки. Встановлено позитивний вплив добавки на вологозв'язувальну здатність, консистенцію та зниження енергетичної цінності ковбас. Узагальнення результатів показало, що рецептура з 25 %-вою заміною м'ясної сировини (МРЗ) забезпечувала найкращий баланс між якістю, структурою та харчовою цінністю готового продукту. Результати можуть бути використані на підприємствах м'ясної промисловості для створення варених ковбасних виробів із підвищеною функціональністю та економічною ефективністю

Ключові слова: рослинна добавка; морква; глива; грибний порошок; функціонально-технологічні властивості; енергетична цінність

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Microbiome of Kraft soft cheeses made from raw goat milk during ripening

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Abstract. The orientation of consumers towards healthy food increases the demand for artisanal cheeses made from raw goat milk. The production of such cheeses in Ukraine is carried out on small eco-farms, ensuring the uniqueness of their microbiological composition. The aim of the study was to determine the microbiome of soft goat cheeses Feta and Chèvre as a criterion of quality and safety during the ripening process. The MALDI-TOF method was used for the identification of microorganisms. In brined Feta cheese, on the 7th day, 18th, and 30th months of ripening, no moulds were detected, and the total number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM), as well as yeasts, showed little change. The number of dominant lactic acid bacteria species in Feta cheese, *Lactococcus lactis* and *Lactobacillus paracasei*, depended on the ripening period. Small amounts *of Staphylococcus simulans*, *Serratia liquefaciens*, *Kurthia gibsonii*, *Enterococcus faecalis*, *Enterococcus durans*, and *Bacillus cereus* were also isolated from this cheese. In white mould-ripened Chèvre cheese, the MAFAnM count did not change significantly on the 3rd, 20th, and 42nd days of ripening. The amount of mould in this cheese showed a strong direct correlation, while yeast counts had a strong inverse correlation with the ripening period. The main species of lactic acid bacteria in Chèvre cheese were represented by *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Lactobacillus plantarum*. During ripening, small amounts of *Staphylococcus simulans*, *Serratia liquefaciens*, *Kurthia gibsonii*, *Escherichia coli*, and *Enterococcus durans* were isolated from Chèvre cheese. The primary moulds in Chèvre cheese were *Galactomyces candidus*, *Galactomyces geotrichum*, and *Penicillium halotolerans*. The species and quantitative composition of microorganisms in Feta and Chèvre cheeses indicated their proper quality and safety and can serve as criteria for their authenticity. The research results provided new data on the microbiome dynamics of artisanal soft cheeses made from raw goat milk produced in Ukraine

Keywords: Feta cheese; Chèvre cheese; mesophilic aerobic and facultative anaerobic microorganisms; lactic acid bacteria; moulds; yeasts

Introduction

Cheese is a product of milk processing involving a complex microbiome, which includes bacteria, moulds, and yeasts that are either intentionally introduced as starter cultures or naturally present in the milk (Centeno & Carballo, 2023). Recently, artisanal cheeses made from raw milk of small ruminants, particularly goats, have gained significant value and popularity among consumers. With the development of farming in Ukraine, the production and consumption of goat cheeses have been increasing annually. These cheeses are perceived as homemade and natural products that meet the criteria for healthy nutrition. Moreover, such cheeses are characterised by unique and exceptional flavour richness, which drives consumer demand. The expansion of the assortment of artisanal cheeses made from raw goat milk is associated with the production of a significant number of soft cheeses, which differ in ripening time, texture, and sensory characteristics (Holiachuk *et al.*, 2023). The homeland of soft cheeses that mature with the involvement of noble moulds like *Geotrichum candidum* (teleomorph *Galactomyces candidus*), such as Chèvre, Brie, or Camembert, is France. Moulds play an important role in the ripening of soft cheeses due to their metabolism of proteins, lipids, and organic acids, enabling their growth on the cheese surface and enhancing the development of organoleptic properties (Perkins *et al.*, 2020). Brined cheeses, such as

Feta, originate from Greece, are designed for long-term storage, and can be consumed on their own or paired with other dishes.

Cheeses made from raw milk (especially goat milk) are believed to have a more pronounced and rich flavour compared to those made from pasteurised or microfiltered milk. According to A. Camargo *et al.* (2021), the natural microbiota of raw milk is responsible for the typical sensory properties and flavour development of such cheeses. The dominance of lactic acid bacteria is a characteristic feature of artisanal cheeses, providing them with desirable qualities. However, alongside beneficial microflora, such cheeses can also be contaminated with undesirable microorganisms, which may cause spoilage or even pathogens that pose a risk to consumer health (Margalho *et al.*, 2020). Soft cheeses can carry various microbial hazards, including *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli* (Dos Santos Rosario *et al.*, 2021; Remizova *et al*., 2024).

Due to the lack of standardisation in artisanal production, data on the microbial parameters of soft cheeses made from raw goat milk remain scarce. Microbiological monitoring of the ripening process is particularly important for cheeses with a higher risk to consumers, i.e., those with a short ripening period. This is because artisanal cheeses are produced on small-scale farms, where the level of automation and mechanisation of production processes and environmental control is low. Under such conditions, there is a potential risk of microbial contamination of the milk (raw material) and cheeses during both production and ripening. This, in turn, leads to high variability in the total bacterial count and species diversity of lactic acid microorganisms between batches of soft cheeses, as noted by F. Pasquali *et al.* (2022). The microbiological status at each stage of cheese production can be useful for describing the entire food production chain and specifically assessing the variability of artisanal production criteria (Sadvari *et al.*, 2024). Currently, there are very few scientific studies worldwide devoted to the microbial quality of soft cheeses made from raw goat milk, especially in artisanal production (Silva *et al.*, 2021; Parente *et al.*, 2022). Thus, the aim of this research was to determine the species composition of the microbiota at different stages of ripening of artisanal soft Feta and Chèvre cheeses made from raw goat milk as criteria of quality and safety.

Materials and Methods

The study involved two batches of artisanal cheeses made from raw goat milk: brined Feta cheese and white mould-ripened Chèvre cheese. Under the conditions of the eco-farm "Zhuravka" in Kyiv region, five batches of Feta cheese weighing 5 kg each were produced, as well as 15 wheels of Chèvre cheese, each weighing 180-200 g. The study was conducted during 2022-2024. The milk used for cheese production was obtained from Anglo-Nubian goats raised on natural pastures. To produce Feta cheese, raw goat milk was heated to 33°C, and a mesophilic starter culture MA 11 (Danisco France SAS, France), containing *Lactococcus lactis* and *Lactococcus cremoris*, was added. The mixture was thoroughly stirred and left for 60 minutes. Liquid rennet Rennet Liquid 92/8 (Pamir Service, Kyiv, Ukraine) was then added, mixed thoroughly for 30 seconds, and left to form a curd for 40 minutes. After the curd formed, it was cut into cubes measuring 1 × 5 cm, stirred gently for 20 minutes, and left to rest for 10 minutes to allow whey separation. The curd was transferred into moulds and left for 10-11 hours at a temperature of 20-22°C. Afterward, the cheese was removed from the moulds, cut into pieces weighing 200-300 g, placed into containers, and covered with whey in which 9% salt was dissolved. Care was taken to ensure there was no air between the container lid and the whey (Fig. 1a).

a  b 

Figure 1. Feta cheese in brine aged 30 months (a), Chèvre cheese aged 40 days (b)
Source: created by the authors

The cheese was stored at 8-9°C for up to 30 months. To prepare Chèvre cheese, raw goat milk was slowly heated to 32°C while stirring constantly. A mesophilic starter culture MA 11 (Danisco France SAS, France), containing *Lactococcus lactis* and *Lactococcus cremoris*, was added to the milk. The mixture was thoroughly stirred, and mould cultures AGE M-GC (*Geotrichum candidum*) and AGE M-PC (*Penicillium candidum*) (IGEA Cultures, Italy) were introduced. Liquid rennet Rennet Liquid 92/8 (Pamir Service, Kyiv, Ukraine) was then added, mixed thoroughly for 30 seconds, and left to coagulate for 9-11 hours at a temperature of 20-23°C. After coagulation, the whey was drained, and the dense curd was cut into cubes measuring 1 cm using a long knife. The curd mass was placed in a polyester bag and left for 10-12 hours to drain the whey. Salting of Chèvre cheese was done by adding salt at a rate of 20-25 g per kg of cheese. The salted mass was divided into equal portions weighing 180-200 g and shaped into firm cylinders with a thickness of 3 cm and a diameter of 8-9 cm. The cheese was placed on a drainage mat at a temperature of 20-23°C for drying and turned several times over 24 hours. The dried cheese was transferred to a ripening chamber at a temperature of 6-12°C for 40 days, with the wheels turned once daily (Fig. 1b).

Average samples of Feta cheese for analysis were collected on the 7th day, 18th month, and 30th month of storage, with 200 g taken from five containers. Samples of Chèvre cheese were collected on the 3rd, 20th, and 40th days of ripening, with five wheels sampled each time. Microbiological studies were conducted at the LLC "Expert Centre Biolights" (ISO/IEC 17025 (2017) accreditation), Ternopil, Ukraine. Sterile disposable plastic containers with a volume of 100 cm^3 were used for sampling. All cheese samples were placed in a cooling container at 2°C and delivered to the laboratory after collection. For analysis, 10-fold dilutions were prepared. All studies were performed in triplicate. The quantity of bacteria and fungi in the cheeses was determined in colony-forming units (CFU), and the data are presented in the tables as lg CFU/g. The identification of bacterial and fungal species in the cheeses was performed according to the operational instruction "Working with MALDI-TOF Bruker Biotyper". Microorganisms were isolated and identified using MALDI-TOF, compliant with ISO 16140-1:2016 (2016).

For bacterial enumeration in the cheeses, the following culture media were used: Plate Count Agar M091 (HiMedia, India), *Lactobacillus* MRS Agar M641 (HiMedia, India), M17 Agar Base (HiMedia, India), Blood Agar (BioMérieux, France), Buffered Peptone Water (BPW) (HiMedia, India), Baird-Parker Agar (BPA) (HiMedia, India), Endo Agar (Farmaktiv, Ukraine), *Pseudomonas* Agar (HiMedia, India), *Enterococcus* Agar (Farmaktiv, Ukraine), *Bacillus cereus* Agar

M833 (HiMedia, India), Bismuth Sulfite Agar (BSA) (HiMedia, India), Xylose Lysine Deoxycholate Agar (XLD Agar) (HiMedia, India), HCCA Matrix for mass spectrometry (art. 255344) (Bruker, Germany), and Bacterial Calibration Standard (art. 255343) (Bruker, Germany). For the isolation of yeasts and moulds, Sabouraud Agar (HiMedia, India) was used.

After cultivation on Petri dish media, an isolated colony of microorganisms with a volume of 1-2 µL was selected and applied to the surface of a chip well. After drying, 1 µL of matrix solution was applied to the sample. The chip was transferred to the MALDI-TOF device, and the isolated cultures were identified using MBT Compass MALDI Biotyper 3.1 Compass 1.4 for FLEX-Volume 1 and 2 Software and Manuals (Bruker Daltonik, Bremen, Germany). The study results included bacteria identified with a score value of 2.00 or higher. Statistical analysis of the obtained data was performed using one-way analysis of variance (ANOVA). The dynamics of the mycological indicators of the cheeses were analysed using regression and correlation analyses. Data processing was carried out using Microsoft Excel 2016 combined with XLSTAT software. Data in the tables are presented as x ± SD (mean ± standard deviation). Differences between variants were considered significant at $P < 0.05$ using Tukey's test with Bonferroni correction.

Results and Discussion

Microbiological characteristics of Feta cheese

The number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) in brined Feta cheese was highest at the beginning of the ripening period. By the 18th month, it had decreased and remained at this level until the 30th month (Table 1).

Table 1. *Microbiological indicators of goat Feta cheese during ripening*

Maturation period	MAFAnM	Mould Fungi	Yeast
7 days	8.39 ± 0.14a	< 1a	6.84 ± 0.16a
18 months	6.96 ± 0.32b	< 1a	4.67 ± 0.17b
30 months	7.02 ± 0.29b	< 1a	6.67 ± 0.11a

Note: x ± SD, n = 5, lg CFU/g; different superscript letters indicate values that differ significantly within the same column ($P < 0.05$) according to Tukey's test with Bonferroni correction

Source: created by the authors

Moulds were not detected in Feta cheese; however, yeasts were a consistent component of its microbiome throughout the ripening period, with a slight decrease observed by the 18th month. Unique properties of Feta cheese are associated with its sensory aspects and are determined by the geographical location of the production area, its diverse vegetation, the chemical composition of soils and water, the specifics of the production culture, and historical traditions (Tzora *et al.*, 2021). A distinctive feature of artisanal Feta cheese made from raw goat milk is the relative stability of the total count of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) and yeasts, with the absence of mould fungi throughout the entire maturation period. In contrast, a study by A. Geronikou *et al.* (2023) found that the most dominant yeast species in white brined cheeses, such as Feta, were *Geotrichum candidum*, *Kluyveromyces marxianus*, *Kluyveromyces lactis*, *Debaryomyces hansenii*, *Rhodotorula mucilaginosa*, and *Trichosporon* spp. According to A. Geronikou *et al.* (2023), the mycological characteristics of brined cheeses, which typically have a shelf life of 52 weeks, depend on storage temperature and duration. An increase in yeast counts was observed during the first 12-14 weeks of storage, stabilising afterward within the range of 4.19-7.08 log CFU/g. The dominant

yeast species in white brined cheeses were *Candida zeylanoides* and *Debaryomyces hansenii*, while *Candida parapsilosis*, *Kluyveromyces lactis*, *Kazachstania bulderi*, *Pichia kudriavzevii*, *Pichia fermentans*, *Torulaspora delbrueckii*, *Rhodotorula mucilaginosa*, and *Wickerhamomyces anomalus* were detected less frequently. As expected, the bacterial composition of Feta cheese made from raw goat milk was primarily dominated by lactic acid bacteria (Table 2).

Table 2. *Species composition of bacteria in goat Feta cheese during ripening*

Indicator	Cheese maturation period		
	7 days	18 months	30 months
Lactococcus lactis	5.04 ± 0.18a	3.61 ± 0.13b	< 1c
Lactobacillus paracasei	< 1a	3.59 ± 0.21b	4.11 ± 0.17c
Staphylococcus simulans	1.73 ± 0.26a	< 1b	< 1b
Enterococcus faecalis	< 1a	1.63 ± 0.20b	< 1a
Enterococcus durans	< 1a	2.04 ± 0.19b	< 1a
Serratia liquefaciens	1.69 ± 0.32a	< 1b	< 1b
Kurthia gibsonii	2.01 ± 0.19a	< 1b	< 1b
Bacillus cereus	< 1a	1.03 ± 0.16b	< 1a

Note: x ± SD, n = 5, lg CFU/g; different superscript letters indicate significant differences within the same row of the table ($P < 0.05$) based on the results of Tukey test with Bonferroni correction
Source: created by the authors

In freshly prepared Feta cheese (7 days old), *Lactococcus lactis* was the dominant species. At 18 months of age, the cheese contained both *Lactococcus lactis* and *Lactobacillus paracasei*, while in 30-month-old cheese, only *Lactobacillus paracasei* was present. The species composition of lactic acid bacteria in Feta cheese varied with age, characterised by a decrease in *Lactococcus lactis* from the 7-day-old cheese to the 18-month-old cheese, with its complete absence in the 30-month-old cheese. At the same time, *Lactobacillus paracasei* was not detected in 7-day-old Feta but appeared at 18 months and increased in abundance by 30 months of storage.

The bacterial composition forming the microbiome of Feta cheese made from unpasteurised goat milk and stored for 30 months is quite dynamic, consistent with findings from analyses of similar cheese made from unpasteurised sheep milk (Tsigkrimani *et al.*, 2022). This indicates the presence of bacteria and yeasts in the cheese that are highly resistant to a 9% salt concentration, but not moulds. It is believed that most non-starter lactic acid bacteria are facultative anaerobes resistant to salt and acid, capable of reproducing and continuing their growth in cheese. The dominant bacteria in artisanal Feta cheese made from raw goat milk include two key species: *Lactococcus lactis* and *Lactobacillus paracasei*. The role of these bacteria and their proportions in forming the Feta cheese microbiome during ripening stages of 7 days, 18 months, and 30 months is crucial. Similar types of microorganisms were found in Feta cheese after 3 and 6 months of storage (Tzora *et al.*, 2021). It is indisputable that *Lactobacillus paracasei* contributes to the unique flavour of aged Feta cheese made from unpasteurised goat milk at 30 months of age. In addition, specific strains of *Lactobacillus paracasei*, due to their anti-listerial activity, are considered to have significant practical potential as starter cultures in cheesemaking.

The growth and development of microorganisms during cheese ripening are influenced by physicochemical parameters (water activity, pH, ripening chamber temperature, redox potential), chemical composition (salt content, organic acids, and their ratios), and bacteriocins produced by starter and non-starter cultures of microorganisms. Salt content in the cheese matrix often acts as a preservative with antimicrobial effects (György & Laslo, 2021). Brined cheeses are characterised by the growth of salt-resistant microorganisms, as observed in the microbiome analysis of Feta cheese made from raw goat milk. A small number of *Staphylococcus simulans*, *Serratia liquefaciens*, and *Kurthia gibsonii* were isolated from 7-day-old Feta cheese, but these microorganisms were absent as the ripening period increased. Two species of enterococci, *Enterococcus faecalis* and *Enterococcus durans*, as well as *Bacillus cereus*, were detected only at the 18-month ripening stage (Table 2). A distinctive feature of the microbiome in Feta cheese stored in brine for 30 months is the presence of an almost monocultural population of *Lactobacillus paracasei*. The presence of staphylococci and yeasts in Feta cheese is attributed to their entry from raw milk. In raw goat milk, R. Hoving-Bolink *et al.* (2023) identified dominant bacterial genera such as *Staphylococcus*, *Pseudomonas*, *Lactococcus*, *Microbacteria*, *Acinetobacteria*, and *Corynebacteria*. M. Akinyemi *et al.* (2023) in their studies determined the ratios in raw goat milk. In raw milk, the dominant bacterial genera include *Lactobacillus* (36%), *Streptococcus* (34%), and *Enterococcus* (12%), as well as fungi such as *Kluyveromyces* (28%), *Saccharomyces* (24%), and *Candida* (18%).

One of the reasons for the detection of enterococci in Feta cheese, such as *Enterococcus faecalis* and *E. durans* (which also belong to the group of lactic acid microorganisms), is their widespread presence in the environment and in the milk of small ruminants. It has been proven that the most frequently isolated species from raw sheep milk were *Enterococcus faecium*, *Lactiplantibacillus plantarum*, and *Pediococcus pentosaceus* (Tsigkrimani *et al.*, 2022). Another factor determining the ability of enterococci to persist for extended periods in brined cheese is their resistance to 10-15% NaCl in whey solutions. The role of enterococci in cheesemaking has been actively discussed by researchers. Alongside undesirable effects, M. Tsigkrimani *et al.* (2022) identified 189 strains of lactic acid bacteria isolated from Feta and Kefalograviera cheeses made from sheep milk, including *Enterococcus faecium*, *E. faecalis*, *Lactococcus lactis*, *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*, *Lactiplantibacillus pentosus*, *Latilactobacillus curvatus*, *Lp. plantarum*, *Levilactobacillus brevis*, and *Weissella paramesenteroides*, which tolerate bile salts. This makes them potential candidates for use as starter or adjunct cultures.

The dynamic microbiome composition of Feta cheese observed during study over a long storage period aligns with data from M. Tsigkrimani *et al.* (2022) obtained during the microbiological analysis of Feta cheese made from sheep milk. In Feta cheese, *Lp. plantarum* and *E. faecium* dominated during the early ripening stage, while *Weissella paramesenteroides* prevailed in the late ripening stage. In Kefalograviera cheese, *Levilactobacillus brevis* and *E. faecium* dominated in the early stage, while *W. paramesenteroides* and *E. faecium* were prevalent in the late stage. Another advantage of enterococci over other cheese microorganisms is their ability to grow at temperatures ranging from 10°C to 45°C, in a 6.5% NaCl solution, in a 40% bile solution, and at pH levels from 4 to 9.6. Additionally, they can withstand heating to 60°C for 30 minutes (Graham *et al.*, 2020). In Portugal, Spain, Italy, and Greece, enterococci are highly valued and used in cheesemaking as components of starter cultures to enhance the flavour and aroma of cheese during ripening. Moreover, some enterococci can produce bacteriocins active against spoilage-causing and

pathogenic microorganisms in food (such as *Listeria monocytogenes*) and exhibit probiotic properties, which are compelling arguments for their application in the production of fermented food products (Sioziou *et al.*, 2023).

Regarding the isolation of *Serratia liquefaciens* from Feta cheese made from unpasteurised goat milk during the ripening process, data align with the findings of P. Papadakis *et al.* (2021), who identified unpasteurised milk as a source of this bacterium. In Feta cheese made from unpasteurised goat milk at the 7-day ripening stage, *Kurthia gibsonii* was detected, which belongs to relatively new bacterial species. This bacterium was recently isolated from chickens by L. Lozica *et al.* (2022), as well as from wastewater, and is considered a conditionally pathogenic microorganism, like in the study by M. Romance *et al.* (2024). Considering that Feta cheese production takes place on an eco-farm where not only goats but also other animals, such as chickens, are kept, the latter can be regarded as a potential source of this bacterium. According to standards, Feta cheese must mature for at least two months, but it continues to mature throughout its storage period in whey brine. During this time, bacteria such as *Bacillus cereus* are detected after 18 months, which is uncharacteristic for young cheese and is supported by similar data obtained by A. Tzora *et al.* (2021) in their analysis of Feta cheese after six months of storage. Overall, the microbiome of Feta cheese, made from unpasteurised goat's milk, is dynamic and changes with the age of the cheese, adapting to storage conditions and contributing to the formation of its unique characteristics.

Microbiological characteristics of Chèvre cheese

Unlike Feta cheese, Chèvre belongs to cheeses with a short ripening period. The MAFAnM count in Chèvre cheese did not differ significantly on the 3rd, 20th, and 40th days of ripening. More pronounced changes in the microbiome of this cheese were related to moulds and yeasts. An increase in the number of moulds in Chèvre cheese was observed on the 20th day of ripening, with an increase of 1.73 lg CFU/g and 1.58 lg CFU/g compared to the 3rd day (Table 3).

Table 3. *Microbiological parameters of goat Chèvre cheese during ripening*

Maturation period, days	MAFAnM	Mould fungi	Yeast
3	8.26 ± 0.21a	4.16 ± 0.14a	7.02 ± 0.13a
20	7.97 ± 0.26a	5.89 ± 0.31b	5.63 ± 0.15b
40	8.04 ± 0.12a	5.74 ± 0.25b	5.31 ± 0.18b

Note: x ± SD, n = 5, lg CFU/g; different superscript letters indicate statistically significant differences within a column ($P < 0.05$) based on Tukey test with Bonferroni correction
Source: created by the authors

At the same time, for yeast, an inverse relationship was identified: by day 20 of Chèvre cheese ripening, their quantity decreased by 1.39 lg CFU/g, and by day 40, it dropped by 1.71 lg CFU/g compared to 3-day-old cheese. Correlation analysis of the mycological parameters of Chèvre cheese showed that the number of moulds had a strong positive correlation with the ripening period ($r = 0.704 \pm 0.134$, $P < 0.01$), while the number of yeasts exhibited a strong inverse correlation with the ripening period ($r = -0.948 \pm 0.060$, $P < 0.001$). In both cases, the regression line was expressed as a second-degree polynomial (Fig. 2).

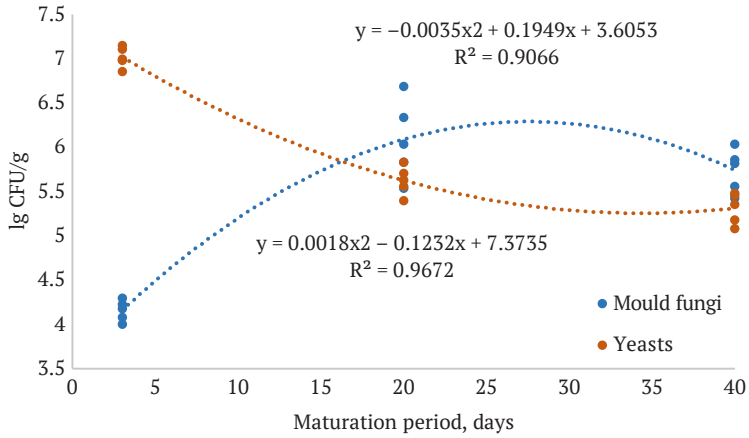

Figure 2. Dependence of mould fungi and yeast counts on the ripening period of Chèvre cheese, n = 15
Source: created by the authors

The species composition of lactic acid bacteria in Chèvre cheese was somewhat similar to that of Feta cheese, particularly regarding *Lactococcus lactis*, which dominated in young cheese and in cheese at the middle of the ripening period (Table 4). At the same time, on day 20 of ripening, three dominant species of lactic acid bacteria were identified in Chèvre cheese: *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Lactobacillus plantarum*. By day 40, only *Leuconostoc mesenteroides* remained dominant. Staphylococci were found only in 3-day-old Chèvre cheese, represented by *Staphylococcus simulans*. On day 20 of ripening, *Serratia liquefaciens* and *Kurthia gibsonii* were isolated from the cheese. By day 40, the bacterial diversity of Chèvre cheese slightly increased compared to the 3-day-old cheese, due to the presence of *Escherichia coli* and one enterococcus species, *Enterococcus durans* (Table 4).

Table 4. *Species composition of bacteria in goat cheese Chèvre during maturation*

Indicator	Cheese maturation period (days)		
	3	20	40
Lactococcus lactis	5.14 ± 0.22a	2.32 ± 0.19b	< 1c
Leuconostoc mesenteroides	< 1a	4.23 ± 0.42b	4.98 ± 0.21b
Lactobacillus plantarum	< 1a	5.08 ± 0.44b	< 1a
Staphylococcus simulans	2.03 ± 0.61a	< 1b	< 1b
Enterococcus durans	< 1a	< 1a	2.06 ± 0.11b
Escherichia coli	< 1a	< 1a	1.61 ± 0.23b
Serratia liquefaciens	< 1a	1.90 ± 0.38b	1.32 ± 0.33b
Kurthia gibsonii	< 1a	1.63 ± 0.22b	< 1a

Note: x ± SD, n = 5, lg CFU/g; different superscript letters indicate values that significantly differed within a row of the table (P < 0.05) based on Tukey test with Bonferroni correction
Source: created by the authors

The ripening of Chèvre cheese is characterised by the formation of a rind involving moulds, which varied in species composition depending on the ripening period. On the 3rd day of ripening, three species of moulds were isolated from Chèvre cheese: one starter species

(*Galactomyces candidus*) and two non-starter species (*Galactomyces geotrichum* and *Penicillium halotolerans*) (Table 5). As the ripening period of Chèvre cheese increased, the species diversity of moulds decreased. Specifically, 20-day-old cheese contained two mould species: *Galactomyces candidus* and *Galactomyces geotrichum*, whereas only the starter culture species *Galactomyces candidus* was identified in 40-day-old cheese. In studies of Mexican artisanal cheeses, P. Cuevas-González *et al.* (2024) identified significantly greater diversity of lactic acid bacteria, including the genera *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Leuconostoc*, *Lactococcus*, *Weissella*, *Pediococcus*, *Carnobacterium*, *Aerococcus*, and *Tetragenococcus*.

Table 5. *Species composition of moulds in goat Chèvre cheese during ripening*

Indicator	Cheese maturation period (days)		
	3	20	40
Galactomyces candidus (*Geotrichum candidum*)	3.57 ± 0.24a	4.98 ± 0.25b	5.74 ± 0.25c
Galactomyces geotrichum	2.19 ± 0.11a	2.61 ± 0.23b	< 1c
Penicillium halotolerans	3.91 ± 0.33a	< 1b	< 1b

Note: x ± SD, n = 5, lg CFU/g; different superscript letters indicate values that significantly differed within a row of the table ($P < 0.05$) based on Tukey test with Bonferroni correction
Source: created by the authors

In freshly made Chèvre cheese from unpasteurised goat milk, the species diversity of bacteria is quite limited. In addition to lactic acid bacteria, staphylococci, including *Staphylococcus simulans*, were isolated from the cheese which was also characteristic of young Feta cheese. *S. simulans* in cheese can originate from milk, as well as from the mucous membranes and skin of goats (Výrostková *et al.*, 2021; Wesołowska & Szczuka, 2023; Aguiar *et al.*, 2024). Currently, the study of the ability of pathogenic and conditionally pathogenic bacteria to synthesise enterotoxins is particularly relevant. As shown in studies by P. Wiśniewski *et al.* (2024), the presence of staphylococci in milk and cheese does not always indicate a risk to human health. Moreover, staphylococci, including *S. simulans*, are capable of exhibiting anti-listerial activity in dairy products, including cheeses (Bockelmann, 2002; Ferrocino *et al.*, 2022).

Considering that both Chèvre and Feta cheese are produced from raw milk of the same goats, it is not surprising that despite differences in production technology and ripening periods, they share a similar species composition, including certain lactic acid bacteria, *Serratia liquefaciens*, *Kurthia gibsonii*, and *Enterococcus durans*. In Chèvre cheese, a small amount of *Escherichia coli* was also isolated on the 40th day of ripening. *E. coli* and coliforms serve as indicators of milk and cheese production hygiene, reflecting faecal contamination of the raw material and final product. The number of indicator microorganisms should not exceed 100 CFU/g, which is the threshold value in many countries (György & Laslo, 2021; Jaramillo-Bedoya *et al.*, 2021). In this case, their levels in the cheese did not exceed the recommended value, indicating proper hygiene practices in this artisanal production. In addition to bacteria, mould fungi play a key role in the ripening of Chèvre cheese, as they contribute to the formation of the rind and the unique sensory characteristics of this cheese. Although studies did not reveal a high species diversity of mould fungi in Chèvre cheese, other researchers have isolated *Galactomyces candidus*, *Candida parapsilosis*, *C. batistae*, *C. sake*, *Debaryomyces hansenii*, *Geotrichum candidum*, *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, *K. lactis*, *Yarrowia lipolytica*, and *Pichia kudriavzevii* from such cheeses (Banjara *et al.*, 2015).

Artisanal cheeses have a heterogeneous microbiota characterised by dynamic population changes and are associated with the geographical location of the region. The soft Italian PDO (Protected Designation of Origin) cheese Robiola di Roccaverano is made from raw goat milk with natural starters. In samples of fresh and aged cheese, a significantly higher diversity of microorganisms was detected, including *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Leuconostoc pseudomesenteroides*, *Lactobacillus brevis*, *Leuconostoc citreum*, along with fungi such as (*Geotrichum candidum*, *Trichosporon coremiiforme*, *Saturnispora silvae*, *Kluyveromyces marxianus*, *Kluyveromyces lactis*, *Yarrowia lipolytica* (Biolcati *et al.*, 2021; Ferraz *et al.*, 2024; Lokes *et al.*, 2024). At various stages of ripening, one to three species of mould fungi were isolated from Chèvre cheese made from unpasteurised goat milk, including one starter species (*Galactomyces candidus*) and two non-starter species (*Galactomyces geotrichum* and *Penicillium halotolerans*). At the end of the ripening period, only one starter fungal species, *Galactomyces candidus*, dominated in Chèvre cheese, which could be attributed to the moisture content of the cheese (Zhang *et al.*, 2022) and its antagonistic effect against other representatives of its mycobiota. Thus, the microbiome of artisanal soft goat cheeses made from unpasteurised milk, such as brined Feta and white noble mould Chèvre, is characterised by a similar species composition of the predominant microorganisms, which may be one of the criteria for determining their quality, safety, age, and comprehensive assessment of authenticity.

Conclusions

The total number of MAFAnM in Chèvre cheese did not depend, and in Feta cheese it was characterised by an inverse dependence on the ripening period. The microbiological characteristics of artisanal soft cheeses made from unpasteurised goat milk, including brined Feta cheese and Chèvre cheese with noble white mould, indicate that the mesophilic aerobic and facultative anaerobic microorganisms throughout their ripening period are primarily composed of lactic acid bacteria. Their population size and species composition are dynamic. The dominant species of lactic acid bacteria in Feta cheese are *Lactococcus lactis* and *Lactobacillus paracasei*, while in Chèvre cheese, they are *Lactococcus lactis, Leuconostoc mesenteroides*, and *Lactobacillus plantarum*.

The ripening of Chèvre cheese involves three species of mould fungi: one starter species (*Galactomyces candidus*) and two non-starter species (*Galactomyces geotrichum* and *Penicillium halotolerans*). With increasing ripening time, the species diversity of mould fungi in Chèvre cheese decreases from three to one of the starter *Galactomyces candidus*. A feature of brine Feta cheese is the absence of mould fungi at all stages of ripening and a stable amount of yeast, while in Chèvre cheese, the number of mould fungi had a direct dependence, and yeast – an inverse dependence on its age. The species diversity of bacteria in Feta and Chèvre cheeses is similar in number of *Staphylococcus simulans*, *Enterococcus durans*, *Serratia liquefaciens* and *Kurthia gibsonii* and depends on the term of their ripening. In both cheeses, species of non-fermented microorganisms were found that are related to the formation of a rich, bright and unique taste and structure. For most sanitary-indicative microorganisms, artisanal cheeses made from unpasteurised goat milk (Feta and Chèvre), produced at an eco-farm in Ukraine, can be considered of good quality and safe for consumers at all stages of ripening. In the future, to assess the quality, safety, authenticity, and age of artisanal goat cheeses Feta and Chèvre, it is planned to study their chemical composition, as well as conduct microstructural analysis.

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Conflict of Interest

None.

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Мікробіом м'яких крафтових сирів, виготовлених із сирого козячого молока, під час дозрівання

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Анотація. Орієнтація споживачів на здорову їжу збільшує попит на крафтові сири, виготовлені з непастеризованого козиного молока. Виробництво таких сирів в Україні здійснюється на невеликих екофермах, що забезпечує унікальність їх мікробіологічного складу. Метою дослідження було визначити мікробіом м'яких козиних сирів Фета і Шевр як критерій якості і безпечності у процесі дозрівання. Для ідентифікації мікроорганізмів використано метод MALDI-TOF. У розсільному сирі Фета на 7-му добу, 18-й і 30-й місяць дозрівання плісеневих грибів не виявляли, а загальна кількість мезофільних аеробних і факультативних анаеробних мікроорганізмів (МАФАМ), а також дріжджів мало змінювалась. Чисельність домінуючих видів молочнокислих бактерій у сирі Фета *Lactococcus lactis* і *Lactobacillus paracasei* залежала від терміну його дозрівання. В незначній кількості із цього сиру виділяли *Staphylococcus simulans*, *Serratia liquefaciens* і *Kurthia gibsonii Enterococcus faecalis* і *E. durans*, а також *Bacillus cereus*. У сирі з білою благородною пліснявою Шевр на 3-тю, 20-ту і 40-ву добу дозрівання чисельність МАФАМ суттєво не змінювалась. Кількість плісеневих

грибів у цьому сирі мала пряму сильну залежність, а дріжджів – обернену сильну залежність від терміну його дозрівання. Основні види молочнокислих бактерій сиру Шевр представлені *Lactococcus lactis*, *Leuconostoc mesenteroides* і *Lactobacillus plantarum*. У процесі дозрівання з сиру Шевр виділяли незначну кількість *Staphylococcus simulans*, *Serratia liquefaciens*, *Kurthia gibsonii*, *Escherichia coli*, *Enterococcus durans*. Основу плісеневих грибів сиру Шевр складали *Galactomyces candidus*, *G. geotrichum* і *Penicillium halotolerans*. Видовий і кількісний склад мікроорганізмів сирів Фета і Шевр свідчить про належну їх якість і безпечність і може служити критерієм їх автентичності. Результати досліджень розкривають нові дані динаміки мікробіому крафтових м'яких сирів з непастеризованого козиного молока, вироблених в Україні

Ключові слова: сир Фета; сир Шевр; мезофільні аеробні і факультативні анаеробні мікроорганізми; молочнокислі бактерії; плісеневі гриби; дріжджі

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