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ЗМІСТ

CONTENTS

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The effect of protected choline on metabolic processes, performance, and health of cows and calves: A review

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Abstract. Following calving, cows frequently experience an energy deficit, leading to the mobilisation of fat reserves and rendering them more susceptible to metabolic disorders such as fatty liver and ketosis. Given the increasing demands for dairy productivity and product quality, identifying effective strategies to enhance energy balance and metabolic health has become critically important. One promising approach that has garnered significant attention is the inclusion of rumen-protected choline in dairy cow diets. This review aimed to assess and summarise the effects of rumen protected choline supplementation on milk performance, metabolic well-being, and reproductive outcomes in cows. The review encompassed studies conducted from 2000 to mid 2024, focusing on parameters such as dry matter intake, milk yield, blood metabolites, incidence of metabolic disorders, and reproductive traits. Research findings indicated that rumen protected choline can enhance milk quality and yield; however, its effects on metabolic health and reproductive functions remain inconsistent. An increase in dry matter intake has been observed, which helps reduce energy imbalance during early lactation and contributes to higher milk yield. However, findings related to metabolic health have been inconsistent, potentially due to variations in experimental conditions. Rumen-protected choline has demonstrated a positive effect in reducing the incidence of ketosis and mastitis, although

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its impact on reproductive performance has shown mixed results. The available data suggest that rumen-protected choline may improve colostrum quality, positively influencing calf health. Studies emphasised the importance of adequate choline intake during pregnancy and postpartum for proper foetal development. Further research is required to optimise the use of rumen-protected choline in dairy farming. Given the potential benefits and variability of outcomes associated with the use of rumen-protected choline in the dairy industry, its effects warrant further investigation

Keywords: dairy farming; metabolic balance; metabolic disorders; ketosis; reproductive performance; colostrum

Introduction

Post-calving cows commonly experience a negative energy balance, prompting the active utilisation of internal energy reserves. This increases the likelihood of developing metabolic disorders: ketosis and fatty liver syndrome. Given the constant increase in demands for milk production and product quality, the development of effective methods to improve energy balance and metabolic health in cows is crucial (Zeisel & Holmes-McNary, 1991; Jayaprakash *et al.*, 2016).

Reduced dry matter intake in the postpartum period leads to insufficient energy supply for cows to sustain milk production. This causes a negative energy balance, which in turn triggers the mobilisation of fat reserves in the form of non-esterified fatty acids, which enter the bloodstream as an energy source (Bollatti *et al.*, 2020). The limited ability of the liver to process non-esterified fatty acids or convert them into esterified fatty acids (triacylglycerols) within very low-density lipoproteins increases the risk of liver disorders in early lactation. An excessive amount of nonesterified fatty acids overwhelms hepatic metabolic mechanisms, which can lead to ketosis with increased formation and secretion of ketone bodies, including beta-hydroxybutyric acid, acetoacetic acid, and acetone. The accumulation of triacylglycerols in the liver also increases the risk of developing "fatty liver" pathology. Elevated non-esterified fatty acids and betahydroxybutyric acid contribute to oxidative stress, inflammatory processes, and immune system dysfunction, increasing the risk of infections and negatively impacting fertility. One promising strategy that has attracted the attention of researchers is the inclusion of rumen-protected choline (RPC) in cow diets.

Choline is a crucial component that aids in the transport of fatty acids. Specifically, choline is a constituent of phosphatidylcholine, a component of cell membranes. Phosphatidylcholine and lysophosphatidylcholine play a key role in cell function and structure. Additionally, choline is involved in fatty acid metabolism in the liver and helps transport fat in the form of very low-density lipoproteins. Thus, choline is a primary lipotropic component for dairy cows, capable of reducing hepatic triacylglycerol levels in the postpartum period. The choline present in the diet is rapidly degraded by rumen microorganisms, resulting in less than 20% bioavailability. To improve the utilisation of choline by lactating cows, the only effective method is its application in a form protected from rumen degradation. In this case, choline containing choline chloride under a fatty acid coating has gained popularity as a feed additive (Humer *et al.*, 2019). A growing interest in the potential of choline to improve lipid metabolism and increase milk production has led to active research into rumen-protected choline supplementation in cows in recent decades. However, the results of studies evaluating the effects of these supplements on performance, metabolic well-being, and reproductive parameters have been inconsistent.

Studies have shown that the use of rumen-protected supplements positively influences dry matter intake. However, this effect often correlates with increased milk yield, which does not always lead to improved energy balance and metabolic status in cows. The primary objective of this review is to systematically collate existing research on the impact of such supplements on key performance indicators and the metabolic health of animals and to analyse the results of similar studies conducted since 2000. This includes assessing their impact on dry matter intake, milk volume, blood metabolite levels, frequency of metabolic disorders, and reproductive outcomes. Analysis of these aspects can provide a new understanding of the effectiveness of supplements in supporting the health of cows in the early stages of lactation, which is essential for improving their overall well-being and productivity.

In 2010, the first meta-analysis was conducted to evaluate the impact of dietary supplements protected from rumen degradation on dry matter intake, milk volume, and the level of fat and protein in milk (Sales *et al.*, 2010). E. Humer *et al.* (2019) repeated a similar meta-analysis, adding 27 more studies. Since new data has emerged since then, this study aimed to assess the available information on the value of rumen-protected supplements for influencing various indicators, including dry matter intake, productivity, and health-related factors in cows of different productivity levels. Additionally, the authors studied the impact of using such supplements on calf health and colostrum quality.

The impact of rumen-protected choline on dry matter intake, body weight, and blood metabolite levels

A study by E. Humer *et al.* (2019) demonstrated that the addition of rumen-protected choline did not significantly alter dry matter intake in cows before calving. However, post-calving, a mean increase of 0.79 kg/day in dry matter intake was recorded ($p < 0.01$). This increase correlated with a 1.05 kg/day rise in milk yield ($p = 0.02$). Further analysis revealed increased protein and fat content in milk ($p \leqslant 0.05$), but the qualitative characteristics of protein and fat remained unchanged ($p \geqslant 0.78$). This suggests a potential positive impact of protected choline on the overall productive performance of dairy cows, particularly milk quantity and nutritional properties.

Other studies did not reveal a clear relationship between choline dosage and changes in dry matter intake and milk production. In only 5 cases was a decrease in dry matter intake observed (from -0.9 to -6%), while in 27 cases, an increase of up to 14% was recorded due to the addition of choline (Shahsavari *et al.*, 2016). Regarding milk production, in 8 cases researchers noted a decrease, but in 29 cases, an increase of up to 38% was observed. These results indicate that the quantitative indicators of milk production are not always influenced by a specific dosage of rumenprotected choline.

Data regarding the impact of rumen-protected choline on body weight (BW) and body condition score (BCS) is limited. Most researchers have not found significant changes in these parameters post-calving or have recorded potential negative effects from the application of rumenprotected choline. This may be partially explained by the lack of detailed information or insufficient data quality in some studies.

Overall, the results of meta-analyses confirm that rumen-protected choline positively affects dry matter intake and milk production in the transition period, which can be beneficial for overall increases in dairy cow productivity. However, the effect of rumen-protected choline dosage does not demonstrate a clear dependence on the supplement volume (Zenobi *et al.*, 2022). Most studies have not recorded a significant impact of rumen-protected choline on economically important traits of dairy cattle. For example, P. Elek *et al.* (2013) and A. Pineda & F. Cardoso (2015) did not note the impact of using choline in the diet on the body weight of dairy cows. Other authors also did not see a positive impact on the body condition of cows after calving (Cetin *et al.*, 2018). J. Hartwell *et al.* (2000) research, on the contrary, indicates possible negative effects of choline on body weight and body condition. Therefore, this issue requires additional research for a deeper understanding of these aspects.

The blood metabolic profile serves as a crucial indicator of overall health and productivity in animals, making the study of the impact of rumen-protected choline on this parameter critically important for optimising diets and enhancing dairy production efficiency. An analysis of the available data by E. Humer *et al.* (2019) revealed that the overall response of metabolites to the addition of rumen-protected choline was inconsistent and did not demonstrate significant changes in most key metabolic indicators. No interactive effect was found between milk production level and the addition of rumen-protected choline on the blood metabolic profile. Overall, the levels of rumen-protected choline supplementation did not significantly affect all parameters studied.

No interactive effect was found between milk production level and the addition of rumenprotected choline on the blood metabolic profile. This suggests that the impact of rumen-protected choline on blood metabolites is independent of cow productivity, and the effects of the feed additive do not vary depending on the level of milk production. The dosage of rumenprotected choline supplementation did not alter the blood parameters studied. This confirms the conclusion of the absence of a clear relationship between the dosage of rumen-protected choline and blood metabolite levels. The level of non-esterified fatty acids in the blood of cows receiving rumen-protected choline also did not show statistically significant changes in the meta-analysis ($P = 0.69$). This indirectly indicates that the addition of rumen-protected choline does not affect the mobilisation of fat from fat reserves, which may indicate the stability of fat metabolism in animals.

The analysis also revealed no reduction in the level of beta-hydroxybutyric acid in cows receiving rumen-protected choline ($p = 0.74$). Beta-hydroxybutyric acid is a marker of ketosis, and its levels typically increase with negative energy balance or metabolic problems. The absence of changes may indicate that rumen-protected choline has no significant impact on the energy status of animals. Blood glucose levels also did not show significant changes ($p \geqslant 0.46$). Glucose is a critically important energy source for dairy cows, and its stable level may indicate that rumenprotected choline does not affect the main metabolic processes associated with energy regulation. Analysis of cholesterol levels did not show significant changes with the addition of rumen-protected choline ($p \geqslant 0.46$). Cholesterol is significant for the synthesis of hormones and cytoplasmic membranes, and its stable level may indicate that rumen-protected choline does not have a significant impact on fat metabolism.

Thus, studies demonstrate that the addition of rumen-protected choline does not have a significant impact on the levels of key blood metabolites in dairy cows, including non-esterified fatty acids, beta-hydroxybutyric acid, glucose, and cholesterol. The absence of clear changes in these metabolites suggests that rumen-protected choline probably does not affect the metabolic status of cows within the studied dosage range. These results are important for further research and optimisation of diets for dairy cows, as the blood metabolic profile is critical for assessing the overall health and productivity of animals.

The impact of RPC on postpartum diseases, reproductive performance of cows, and calf development

Postpartum diseases such as ketosis, mastitis, metritis, and postpartum hypocalcaemia pose a significant challenge to dairy production as they can impact productivity, animal health, and economic outcomes. Ketosis is one of the most common postpartum diseases, characterised by increased levels of ketone bodies in the blood due to an energy deficit. In a study conducted by F. Lima *et al.* (2012), cows received 15 grams of choline chloride daily in a rumen-protected form, starting 25 days before calving and continuing until 80 days of lactation. This resulted in a decrease in the incidence of ketosis and mastitis, suggesting a potential positive impact of long-term use of this choline on the health of cows in the early lactation period. However, with short-term application (only 21 days before calving), conflicting results were observed, including an increase in cases of metritis and hypocalcaemia. The overall incidence of ketosis and mastitis was lower in the group that received rumen-protected choline for a longer duration.

S. Davidson *et al.* (2008) studied second and later-lactation dairy cows that received 40 grams of rumen-protected choline from 21 to 91 days of lactation and recorded a decrease in the incidence of ketosis. As the researchers did not conduct a biometric analysis due to the limited number of observations, this suggests potential positive effects of high doses in specific conditions but requires further confirmation. Most studies, including those by R. Van Saun & C. Sniffen (2014), did not find a statistically significant decrease in the incidence of ketosis with the use of rumen-protected choline. It is important to note that the overall risk of ketosis in these studies was very low, which may explain the lack of additional positive effects from the supplement.

Mastitis is another significant postpartum disease that can significantly impact the productivity of dairy cows. The study by F. Lima *et al.* (2012) showed a decrease in cases of mastitis with the long-term use of rumen-protected choline. However, short-term application did not demonstrate significant improvements in the incidence of mastitis, which may indicate a dependence of the effect on the duration of application. Regarding metritis and hypocalcaemia, the results were contradictory. The study recorded an increase in cases of metritis and hypocalcaemia with short-term application of rumen-protected choline, which indirectly suggests a possible negative effect or changes in metabolic processes in cows that received the supplement for only a short period. This could be an important factor when developing application recommendations.

A meta-analysis by E. Humer *et al.* (2019) showed that the effects of rumen-protected choline on postpartum diseases are inconsistent and depend on the dosage and duration of its application. Long-term use can reduce the incidence of ketosis and mastitis, while short-term application can lead to contradictory results. To better understand the optimal conditions for the application of rumen-protected choline and its impact on various aspects of cow health after calving, it is important to conduct research that will help in developing more effective feeding and management strategies to improve the health and productivity of dairy cows.

The ability to reproduce is a critical factor for the efficiency of dairy production, as it directly affects productivity and economic outcomes. Studies on the impact of RPC on the reproductive performance of dairy cows demonstrate varied results, requiring detailed analysis to determine its potential benefits and limitations. The aforementioned meta-analysis revealed the ambiguity of the impact of RPC on the reproduction of dairy cows, with effects depending on specific conditions of application and research parameters. Results from different studies have demonstrated both positive and negative effects of RPC on the reproductive performance of cows.

M. Ardalan *et al.* (2010) and C. Furken & M. Hoedemarker (2014) noted a favourable impact of RPC on reproduction, specifically, an increase in the frequency of cyclicity and fertility in cows receiving RPC. This may be related to improved metabolic status and reduced stress, creating more favourable conditions for the restoration of a normal reproductive cycle after calving. However, the study by F. Lima *et al.* (2024) revealed an increase in the frequency of cyclicity in multiparous cows on the 61st day of lactation when using RPC, but in primiparous cows, the opposite effect was recorded. This may indicate an ambiguous impact of RPC on different groups of cows or at different stages of lactation. A. Pirestani & M. Aghakhani (2017) recorded a negative impact of RPC on reproductive performance, which contrasts with the results of other studies. The negative effect may be due to the specific conditions of the study or individual reactions of cows to RPC.

Different studies used different dosages of RPC and durations of application, which could have affected the results. For example, short-term or inadequate dosing of RPC may not provide a sufficient impact on reproductive performance, while long-term or excessive application may have negative consequences. Individual biological differences between cows, such as stress level, health status, genetic factors, and feed status, can affect the response to RPC. This explains why some studies demonstrate positive effects, while others demonstrate negative ones. The impact of RPC may vary depending on the stage of lactation and reproduction at which it is applied, for example, the effects of RPC may be different for primiparous cows and cows with different lactations.

RPC is a valuable supplement for dairy cows, positively influencing their productivity, health, and reproductive performance. However, its benefits extend beyond the health of adult cows. The consumption of RPC can also significantly impact the health and development of calves. Research demonstrates that a deficiency of choline in pregnant and lactating cows can adversely affect embryogenesis and subsequent foetal development. An epigenetic approach highlights the importance of adequate choline intake for the proper formation and function of genes in offspring, influencing their growth, development, and immune system (Jiang *et al.*, 2014).

The results of studies by M. Zenobi *et al.* (2022) confirm the positive impact of RPC on calf growth and health. Their research analysed various aspects of the influence of RPC on calves, and the results showed significant benefits for the health and growth of calves receiving choline from their mothers. The study found that calves born to cows that consumed RPC during the dry period and then received colostrum from such cows had higher body weight compared to calves that did not receive additional choline. At 50 weeks of age, the body weight of calves receiving RPC was 339 kg, which is 5.9% more than that of calves that did not receive choline (320 kg). This result indicates a positive effect of RPC on calf growth, likely due to improved metabolism and reduced stress factors. This study also showed that the consumption of RPC positively affects calf survival under stress conditions. The study considered four groups of calves that received different variants of choline intake: only in utero, only through colostrum, or both through colostrum and in utero. The control group of calves that did not receive choline either in utero or from colostrum demonstrated the highest mortality rate under stress conditions. Other groups of calves that received choline through one or both pathways showed significantly better results in terms of survival. This suggests that additional choline intake improves the resistance of calves to stress factors and has a positive impact on their health.

The impact of rumen-protected choline on milk yield, colostrum quantity and quality

Beyond indirect pathways, there is also a hypothesis suggesting a potential direct impact of RPC on milk production. As choline's lipotropic properties help reduce liver fat accumulation, it contributes to improved milk production in cows during the postpartum period. Another potential direct effect is choline's function as a methyl donor, which increases the level of available methionine (Shahsavari *et al.*, 2016). This is supported by research from S. Davidson *et al.* (2008), who recorded improved milk production in multiparous cows fed low-methionine diets when supplemented with RPC. Regarding metabolic health, the authors suggested that RPC could positively influence milk production by improving overall animal health. Additionally, a reduction in hepatic triacylglycerols (TAG) may contribute to improved gluconeogenesis in the liver (Mach *et al.*, 2013; Du *et al.*, 2023). Research has shown an increase in glucose transporter mRNA and a decrease in peak mRNA levels of pyruvate carboxylase in cows receiving 14.4 g of choline chloride in the form of RPC daily from 21 days before calving to 42 days of lactation, indicating improved carbohydrate metabolism. Thus, scientists have suggested that high-producing cows, which are at a higher risk of lipid-related diseases, may benefit more from adding RPC. Later, this theory was refuted, as research by J. Pires & R. Grummer (2009) found no link between the response to the supplement dosage and productivity. They noted that no joint effect was recorded between the supplement and the productivity potential.

Increased milk yield is also associated with higher milk protein and fat content, as demonstrated by studies by M. Garg *et al.* (2012). However, the increase in milk components with the addition of RPC, as noted, is a result of an increase in total milk volume, as fat and protein content remained stable. Previously, it was believed that choline could positively influence milk fat synthesis due to its role in the production of phospholipids, which facilitate the absorption and transport of lipids. A. Baldi & L. Pinotti (2006) found that in early lactation, milk fat originates from plasma NEFAs (non-esterified fatty acids), and RPC can reduce the mobilisation of fat stores, decreasing the concentration of NEFAs. Studies have shown that RPC can affect lipid absorption in the intestine and the secretion of hepatic TAGs. Their subsequent entry into the mammary gland may be neutralised by reduced NEFA availability for milk synthesis. It was also proposed that RPC could increase milk protein content by providing methyl groups and supporting methionine levels (Jayaprakash *et al.*, 2016).

Colostrum is critically important for the health of newborn calves. Since the cow's placenta does not allow the passage of immunoglobulins (Ig), the newborn calf's immune protection depends on the acquisition of colostral immunoglobulins through colostrum within the first few hours after birth. Colostrum not only provides immune protection but also has a long-term impact on calf performance, as it provides nutrients and bioactive compounds such as growth factors, antimicrobial peptides, immunoglobulins, and choline. Insufficient high-quality colostrum can necessitate the use of colostrum replacers or lower-quality colostrum, which can negatively impact the health of the newborn. Recent studies show that adding rumen-protected choline to the diet of cows in late lactation can affect the quantity and quality of colostrum.

A study by M. Zenobi *et al.* (2022) demonstrated that the administration of 60 g/day of RPC (12.9 g/day of choline ions) led to an increase in colostrum volume to 9.4 kg in cows receiving the supplement, compared to 8.7 kg in the control group. Immunoglobulin G (IgG) levels in colostrum significantly increased to 702 g/cow, which is 43% higher than in control cows (490 g/cow). The IgG concentration increased to 86.9 g/L, which is a statistically significant improvement ($P = 0.03$). In contrast to these results, J. Bollatti *et al.* (2020) did not find significant changes in the quantity or quality of colostrum when adding RPC. The average IgG concentration in colostrum was 118 g/L, which is 50% higher. However, the overall effect of RPC on colostrum in this study was not confirmed.

T. Swartz *et al.* (2022) investigated both the recommended and 1.5 times higher doses of RPC. The results showed that cows given RPC produced 85% more colostrum (6.3 kg compared to 3.4 kg in the control group, $P < 0.01$). Despite the increase in colostrum volume, the quality as measured by Brix refractometry and somatic cell count did not change ($P = 0.29$; $P = 0.09$, respectively). Additionally, the output of choline metabolites increased, indicating an increase in the total choline content in colostrum by 100%. A study by H. Holdorf *et al.* (2023) also showed a positive effect of RPC on colostrum quantity. Cows receiving RPC produced 5.4 kg of colostrum, which is 59% more compared to 3.4 kg in the control group ($P = 0.04$). Importantly, the quality of colostrum, specifically the concentration of IgG, remained unchanged with the use of RPC.

The precise mechanisms by which RPC influences IgG content and overall colostrum quality remain yet to be fully elucidated. Promising avenues for future research could include the impact of RPC on mammary gland cell proliferation, immunoglobulin transport from plasma to epithelial cells, and lipid metabolism. It is possible that RPC stimulates an increase in the number of mammary gland cells, leading to greater colostrum production. Increased choline levels may enhance the efficiency of immunoglobulin transport from plasma to the mammary gland. Through its influence on lipid metabolism, RPC may also improve colostrum quality. As a methyl donor, choline can influence the regulation of genes responsible for the structural integrity and function of mammary gland cells.

Overall, the consumption of rumen-protected choline positively influences the quantity and quality of colostrum in dairy cows, especially when administered before calving. Results demonstrate a significant increase in colostrum volume and, in some cases, immunoglobulin concentrations, which can improve the health of newborn calves. However, some studies do not confirm this effect, highlighting the need for further research into the mechanisms of RPC's impact on colostrum. Improving the quality and quantity of colostrum is a crucial indicator of a successful healthy transition cow program, which can positively influence the long-term productivity and health of offspring.

The epigenetic aspect of choline's influence

A deficiency in choline during gestation and lactation can negatively affect epigenetic mechanisms, which in turn can impact the development and health of offspring. Choline is a vital methyl donor, essential for the synthesis of folic acid and other crucial molecules that regulate gene expression. Insufficient choline intake plays a detrimental role in epigenetic regulation and, consequently, has a negative impact on the growth, development, and immune response of calves. Therefore, the consumption of rumen-protected choline has a positive effect not only on the productivity and health of adult cows but also on their calves. RPC can improve calf growth and survival under stressful conditions, highlighting the importance of choline in ensuring optimal development and health of offspring. Considering the epigenetic aspects of choline deficiency underscores its significance in maintaining normal gene expression and foetal development. Further research should focus on optimising RPC dosage and investigating the mechanisms of choline's influence on epigenetic changes to ensure maximum benefits for the health and productivity of both adult cows and their offspring.

Although the addition of RPC did not affect dry matter intake (DMI) before calving, it was subsequently found that RPC contributed to an increase in postpartum DMI by 0.79 kg/day ($p < 0.01$). Considering the energy value and protein content of the lactating diet, this increase corresponds to approximately 5.4 MJ of net energy for lactation (NEL) and 123 g of protein per day. It is expected that improved energy and nutrient intake contributed to a reduction in negative energy balance (NEB) early in lactation. Limited dry matter intake early in lactation is a significant factor determining the degree of NEB and increases the likelihood of developing metabolic disorders (Grummer, 2008; Pascottini *et al.*, 2020). It is anticipated that this effect will manifest in improved

NEB, expressed as a smaller mobilisation of fat reserves, reduced weight loss, and lower levels of NEFAs and BHBA (beta-hydroxybutyrate) in the blood, as well as higher levels of glucose and cholesterol, or an increase in milk production. From the perspective of metabolic regulation, humoral mechanisms in early lactation direct energy resources towards milk synthesis rather than reducing energy mobilisation. Indeed, the addition of RPC contributed to an increase in milk production by an average of 1.05 kg per day. The underlying mechanism suggests that improved energy intake at a level of 5.4 MJ NEL may lead to the production of milk with a higher fat content (4%) (Bakr & Mohamed, 2020). Therefore, it can be assumed that the increase in dry matter intake stimulated milk synthesis in cows that received RPC.

Beyond these indirect effects of RPC on milk synthesis, there is also a hypothesis suggesting a potential direct impact. This direct effect may be linked to the lipotropic action of choline, which aids in the removal of fat from the liver (Du *et al.*, 2023). This was previously considered a key mechanism for improving milk production in cows receiving RPC postpartum. Another potential direct effect is its role as a methyl donor, ensuring elevated levels of available methionine (Shahsavari *et al.*, 2016). This is supported by research from S. Davidson *et al.* (2008), which demonstrated improved milk production in multiparous cows fed low-methionine diets when supplemented with RPC.

The indirect impact of RPC on milk production can also be attributed to improvements in overall health. Additionally, the reduction in liver triglycerides can positively influence gluconeogenesis (Mach *et al.*, 2013). In this context, studies have shown increased levels of glucose transporter mRNA and decreased peak levels of pyruvate carboxylase mRNA in cows receiving 14.4 g of choline chloride as RPC daily, from 21 days prepartum to 42 days of lactation. This indicates improved carbohydrate metabolism in these cows. Considering these aspects, it can be hypothesised that cows with higher productivity and those at higher risk of lipid-related diseases may benefit more from RPC. J. Pires & R. Grummer (2009) established that the response to RPC does not correlate with the productive potential of cows. Increased milk yield resulted in higher volumes of milk protein and fat.

However, the increase in the calculated milk components with RPC application is a result of the increased milk production, as the fat and protein content remained unchanged. It was believed that choline could enhance milk fat synthesis, as it is used for the production of phospholipids that facilitate the absorption and transport of lipids. Thus, it was initially hypothesised that choline could enhance milk fat synthesis. However, in early lactation, a significant portion of milk fat originates from plasma NEFAs. RPC can reduce fat mobilisation, leading to decreased NEFA levels. A. Baldi & L. Pinotti (2006) suggested that RPC might influence intestinal lipid absorption, hepatic TAG formation, and subsequent transfer to the mammary gland. Nevertheless, these changes could be offset by reduced NEFA levels, limiting their availability for milk synthesis. Thus, decreased fat mobilisation might impact milk production by limiting the availability of necessary components. J. Sales *et al.* (2010) proposed a different mechanism, suggesting that RPC provides methyl groups and helps conserve methionine, thereby increasing milk protein content. However, in the study by E. Humer *et al.* (2019), an improvement in milk protein content was observed, although the effect of RPC on its level was not confirmed. Other studies have shown improvements in milk composition with RPC supplementation, including increased protein, total solids ($P < 0.01$), and fat content ($P = 0.09$), although milk yield, corrected for fat or total solids, was unaffected ($P \geqslant 0.43$). Supplementation with RPC during the transition period has been shown to increase serum haptoglobin and insulin levels, and improve milk composition without affecting overall milk yield (Leiva *et al.*, 2015). Generally, RPC is incorporated into the diets of dairy cows during late gestation and early lactation to improve lipid metabolism and support overall animal health. In this context, RPC is believed to enhance the synthesis of phosphatidylcholine and VLDL (very lowdensity lipoproteins) during the transition period, thereby improving lipid metabolism (Snider *et al.*, 2018; Hedtke & Bakovic, 2019). Phosphatidylcholine plays a crucial role in controlling the rate of VLDL secretion and influences the liver's susceptibility to TAG accumulation. Its function in this process involves regulating lipid transport mechanisms and the formation of hepatic fat deposits (Shahsavari *et al.*, 2016). Thus, low dietary choline bioavailability coupled with increased demands for VLDL export due to elevated NEFA uptake creates a risk of fatty liver in dairy cows. Consequently, RPC supplementation during the transition period is believed to facilitate the secretion of TAG from the liver.

While some studies indicate a positive effect of RPC on reducing hepatic TAG levels (Elek *et al.*, 2013), this effect has not been consistently confirmed. Since average TAG concentrations, especially in high-producing cows, fluctuate postpartum, this may influence the potential effect of RPC. Given the substantial variation in TAG levels among cows, large numbers of animals per group are necessary to accurately determine the effect. This minimises the impact of individual variations and ensures the reliability of results.

Regarding hepatic TAG, the response to RPC supplementation in terms of blood metabolite formation has been inconsistent. Some studies have observed decreased blood NEFA levels and increased glucose concentrations (Soltan *et al.*, 2012). However, other studies have not shown an overall impact of RPC on the blood metabolic profile. Potential reasons for these discrepancies include variable paratypic factors such as feeding conditions and physiology, leading to significant heterogeneity in experimental mean values. Additionally, the techniques employed in individual studies can significantly influence the final conclusions.

Several studies have indicated a reduction in cases of ketosis in cows receiving RPC both before and after calving. These studies have documented a positive effect of the supplement on the postpartum health of animals (Davidson *et al.*, 2008). Elevated levels of BHBA and ketones can impair the function of immune cells, increasing the susceptibility of dairy cows to infections. Research by F. Lima *et al.* (2024) found a decrease in mastitis cases in cows with a lower risk of ketosis due to the use of RPC, suggesting the potential benefits of improved lipid metabolism for reducing the risk of mastitis. Furthermore, reduced incidences of mastitis, as observed by M. Ardalan *et al.* (2010) and a decrease in somatic cell count in the study by A. Pirestani & M. Aghakhani (2017), were observed in cows receiving RPC, even in cases where ketosis was not detected.

Despite these positive results, several other studies have found either no effects or conflicting results (Furken & Hoedemarker, 2014). The research by F. Lima *et al.* (2024) suggested that RPC may play a significant role during periods of negative energy balance and substantial lipid mobilisation. Positive effects on disease incidence were only observed when RPC was administered both before and after calving. Further research is needed to better understand the potential health benefits of RPC and to delve deeper into the mechanisms of choline action. This will allow for a more accurate assessment of RPC's impact on animal health and reveal all aspects of its function. To achieve more precise results, standardisation of experimental conditions, including an increase in the number of animals, may be required, as well as further investigation of metabolic pathways related to lipid mobilisation, liver function, and milk component synthesis, among other aspects. Lipid metabolism is crucial in the process of adaptation to lactation and is therefore critical for reproductive function. Cows with elevated blood ketone levels postpartum have shown delayed cyclicity and reduced pregnancy rates. Thus, the application of RPC may improve reproductive function by reducing the likelihood of diseases that exacerbate energy deficits and impact the animal's physical condition, or through a direct effect of choline on reproductive tissues. M. Ardalan *et al.* (2010) and C. Furken & M. Hoedemarker (2014) noted improved reproductive performance in cows receiving RPC, but other studies have documented negative consequences (Pirestani & Aghakhani, 2017). Overall, the impact of RPC dosage was not apparent.

A. Baldi & L. Pinotti (2006) confirmed that the dosage of RPC choline did not have a significant impact on milk production. One possible reason for the lack of a dose-dependent effect is the wide variation in the characteristics of RPC sources and their resistance to rumen degradation. However, even in studies using the same product, dose-dependent effects were not observed. The absence of such effects may also be due to a large number of variables, such as diet composition, administration methods, lactation stage, and the productive potential of the animals. Additionally, the response to RPC may vary depending on the availability of methyl group sources, such as methionine, or other cofactors involved in methylation, such as folic acid and vitamin B12. Therefore, further research is needed to conduct statistical analyses that account for variations between studies and to accurately determine the effects of dietary RPC on animal health and reproductive performance. The increase in postpartum dry matter intake by 0.79 kg/day ($p < 0.01$) is a significant result as it contributes to reducing the negative energy balance (NEB) in early lactation, which is critical for the metabolic health of cows (Grummer, 2008). The increased dry matter intake provides an additional 5.4 MJ NEL and 123 g of protein per day, which is expected to improve metabolic status and milk production. Indeed, the results showed an average increase in milk production of 1.05 kg/day, which corresponds to the expected effect of increased energy intake (Bakr & Mohamed, 2020).

RPC can influence milk production due to the lipotropic properties of choline, which helps reduce fat accumulation in the liver. Additionally, this effect may be related to choline's methyldonating ability, which contributes to increased methionine levels in the body (Shahsavari *et al.*, 2016). The results of studies by F. Brüsemeister & K.-H. Südekum (2006) and O. Pascottini *et al.* (2020) confirm that RPC improves milk production in cows with dietary methionine deficiencies. Although some beneficial effects of RPC on productivity have been confirmed, the results regarding metabolism have been inconsistent. Several studies have noted a reduction in hepatic TAG and improvements in metabolic parameters, such as decreased NEFA and BHBA levels and increased glucose and cholesterol, while other studies have found no significant changes in the blood metabolic profile (Soltan *et al.*, 2012). This may be due to the high variability in physiological and feeding conditions of the experiments, as well as differences in experimental designs.

Regarding postpartum health, RPC is reported to reduce the incidence of ketosis and mastitis, which may be linked to improvements in lipid metabolism. However, not all studies support these findings, and the effects can be variable depending on individual circumstances. Regarding reproductive performance, research has not demonstrated consistent benefits of RPC in improving reproductive functions, although some studies indicate potential positive effects (Ardalan *et al.*, 2010; Furken & Hoedemaker, 2014). Potential benefits of RPC for improving colostrum quality and quantity have been noted. Specifically, some studies have shown increased colostrum volume and IgG concentration with RPC supplementation (Elek *et al.*, 2013). This can improve calf health and development, as high-quality colostrum provides essential immune factors for newborns (Jiang *et al.*, 2014).

RPC is typically added to the diet of dairy cows during late gestation and early lactation to improve lipid metabolism processes. This helps to optimise fat metabolism and overall animal health. In this case, RPC is believed to increase the production of phosphatidylcholine and VLDL, leading to improved lipid metabolism (Snider *et al.*, 2018). Phosphatidylcholine plays a key role in regulating the rate of VLDL export and reduces the risk of TAG accumulation in the liver (Shahsavari *et al.*, 2016). Therefore, low choline bioavailability in the diet coupled with increased demands for VLDL export due to increased NEFA uptake can create conditions for the development of fatty liver in dairy cows. Thus, the inclusion of RPC in the diet of transition cows can aid in the secretion of TAG from the liver. Several studies have shown the effect of RPC on reducing hepatic TAG (Mach *et al.*, 2013). Therefore, potentially, the average concentration of TAG may vary in the postpartum period.

Conclusions

A systematic review of current research into the effects of RPC supplementation on the productivity and health of dairy cows and calves has revealed several key findings that allow for an assessment of the efficacy of this supplement in dairy farming. Protected choline is an important supplement for dairy cows, exhibiting a positive impact on productivity, health, and reproductive performance. However, its influence extends beyond the health of adult cows. The analysis confirmed some expected positive effects of RPC, while also revealing certain inconsistencies and contradictions that require further investigation and clarification. One of the most significant findings is the increase in postpartum dry matter intake, which contributes to reducing negative energy balance (NEB) in early lactation. The increased dry matter intake improves the metabolic status of cows and leads to an increase in milk production. This effect confirms the positive impact of RPC on milk production through increased energy intake. This effect may be partially due to the lipotropic action of choline, which helps to remove fat from the liver, as well as methyl donation, which provides a higher level of methionine in the diet.

The impact of rumen-protected choline on the metabolic health of cows has been inconsistent. While some studies confirm a reduction in hepatic triglycerides (TAG) and improvements in metabolic parameters such as decreased NEFA and BHBA levels, and increased glucose and cholesterol, other studies have found no significant changes in the blood metabolic profile. This variability can be explained by differences in the physiological and dietary conditions of the experiments, as well as different experimental designs. RPC has shown a positive effect on reducing cases of ketosis and mastitis, which is likely related to improved lipid metabolism. However, the results regarding reproductive performance are controversial. Some studies have shown the potential benefits of RPC for reproductive functions, while others have not recorded significant changes. This may be due to the variability of RPC sources and experimental conditions.

RPC has been shown to positively influence the quality and quantity of colostrum, as evidenced by increased colostrum volume and IgG concentration in some studies. This can contribute to improved health and development of calves by providing essential immune factors for newborns. However, research results vary, and some studies have not found significant changes in the quantity or quality of colostrum, although IgG concentrations were higher. Research suggests that choline deficiency during gestation and lactation can negatively impact embryogenesis and subsequent foetal development. Studying this aspect from an epigenetic perspective highlights the importance of adequate choline intake for proper gene formation and function in offspring, which in turn affects their growth, development, and immune system.

The results of this systematic review suggest that rumen-protected choline has potential benefits for the productivity and health of dairy cows, particularly in increasing postpartum dry matter intake and milk production, as well as improving colostrum quality. The significance of RPC supplementation for improving metabolic health, preventing postpartum diseases, and reproductive performance remains controversial. Further research should focus on a deeper understanding of the mechanisms of action of RPC, optimisation of dosage, and clarification of its impact under different conditions. It is also important to consider variations in experimental conditions and apply standardised protocols to ensure the reliability and consistency of results.

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Conflict of Interest

None.

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Вплив захищеного холіну на метаболічні процеси, продуктивність і здоров'я корів і телят: огляд літератури

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Анотація. Після отелення корови часто стикаються з енергетичним дефіцитом, що призводить до використання жирових запасів і робить їх більш схильними до порушень обміну речовин, таких як жирова печінка та кетоз. З урахуванням зростаючих вимог до молочної продуктивності та якості продукції, пошук ефективних стратегій для покращення енергетичного балансу та метаболічного здоров'я стає критично важливим. Одна з потенційних стратегій, яка привернула значну увагу, – це додавання холіну, захищеного від руйнування в рубці, до раціону корів. Мета цього огляду полягала в оцінці та узагальненні впливу добавок захищеного від розпаду в рубці холіну на продуктивність молока, метаболічне благополуччя та репродуктивні результати у корів. Огляд охопив дослідження з 2000 року до середини 2024 року, зосереджуючи увагу на споживанні сухих речовин, обсязі виробництва молока, метаболітів у крові, частоті метаболічних захворювань і репродуктивних якостях. Дослідження показали, що захищений від розпаду в рубці холін може сприяти поліпшенню якості молока та його продуктивності, хоча вплив на метаболічне здоров'я та репродуктивні функції є неоднозначним. Зокрема, спостерігалося зростання споживання сухої речовини, що допомагає зменшити енергетичний дисбаланс на початку лактації та сприяє збільшенню обсягу виробленого молока. Однак результати досліджень щодо метаболічного здоров'я показали суперечливі дані, що можуть бути зумовлені різними умовами експериментів. Холін, захищений від руйнування в рубці, позитивно вплинув на зменшення випадків кетозу та маститу, проте репродуктивні показники демонстрували неоднозначні тенденції. Відповідно до отриманих даних, захищений від розпаду в рубці холін може покращувати якість молозива, що позитивно впливає на здоров'я телят. Дослідження підкреслили, що для належного розвитку плоду важливе адекватне вживання холіну коровами як під час вагітності, так і після отелення. Необхідні додаткові дослідження для вдосконалення використання захищеного від розпаду в рубці холіну в молочному скотарстві. Враховуючи потенціал та варіабельність результатів від використання захищеного від розпаду в рубці холіну в молочному скотарстві, його вплив в даній галузі потребує додаткового вивчення

Ключові слова: молочне скотарство; метаболічний баланс; метаболічні захворювання; кетоз; репродуктивні показники; молозиво

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Fishery and biological evaluation of breeders of Galician and scaleless inbred types of the Ukrainian frame breed carp

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Abstract. Identifying the most optimal combinations of existing carp breeds enables achieving a desirable balance between growth, development, reproductive performance, resistance to various disease agents, and adaptability to cultivation conditions. Therefore, this study aimed to conduct a fishery and biological evaluation of breeders belonging to the Galician and the newly developed scaleless Lebedyn factory line inbred types of the Ukrainian frame carp breed. The research was carried out at the state enterprise Experimental Farm "Nyvka". For the evaluation of external and productive characteristics, breeders of the Galician and scaleless Lebedyn factory line inbred types of the Ukrainian frame carp breed were selected. Based on measurement indicators, selection indices were calculated, including the condition factor, relative mass index, body length-to-height ratio, girth index, head index, and body thickness index. For the reproductive assessment of females, individual working fecundity and the average egg weight were measured. An analysis was conducted on the characteristics of male sexual products, including ejaculate volume, overall motility, the proportion of spermatozoa exhibiting linear progressive and circular movements, and the percentage of non viable spermatozoa. The findings revealed that females of the scaleless and Galician types demonstrated superior high-backed indices and relatively smaller heads based on morphological traits. However, no significant differences were observed in critical parameters such as body mass, body length-to-height ratio, and condition factor. Under factory reproduction conditions, females of both inbred types exhibited positive responses to hormonal stimulation. Fertility indicators for Galician-type females showed a working fecundity range of 476.0-576.0 thousand eggs, with an average relative fecundity of 89.55 ± 2.25 thousand eggs/kg. In scaleless females, working fecundity ranged from 372.0 to 543.6 thousand eggs, with an average

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relative fecundity of 74.83±2.89 thousand eggs/kg. The practical significance of these findings lies in their application to further research on the cultivation and performance evaluation of hybrid offspring produced through reciprocal crossbreeding, compared to the original forms

Keywords: Lebedyn factory line; carp external appearance; reproductive indicators; selective breeding in fish farming

Introduction

In 2021, the Food and Agriculture Organization of the United Nations (FAO, 2024b) adopted the "Blue Transformation" program, a key priority enshrined in the FAO Strategic Programme for 2022-2031. This program aims to maximise the potential of aquatic resources to ensure food security and to sustain these achievements for the continued sustainable development of aquaculture as a sector. According to FAO production data on aquaculture, published in March 2024 reports, 731 statistical units, technically identified as species of aquatic organisms cultured in aquaculture, were reported, which is more than the 652 units reported in 2022. The list includes 564 species identified at the species level and 7 interspecific hybrids; 99 groups of species identified at the genus level and 61 groups identified at the family level or higher. Given this biodiversity of aquatic organisms cultured in aquaculture, carp accounted for approximately 51.6% (31,788 thousand tonnes) in 2022 (FAO, 2024a).

Carp has been a dominant aquaculture species in Ukraine for centuries, with records of its cultivation dating back to the 14th-16th centuries (Hrytsynyak *et al.*, 2021). Scientifically based selective breeding of local Ukrainian carp populations began in earnest between 1927 and 1930, initiated by O. Kuzoma (Grynzhevsky & Sherman, 2006; Martseniuk & Martseniuk, 2021). Consequently, scientific research into the selective breeding of Ukrainian carp has been ongoing for nearly a century. These studies aim to improve the productive traits of carp through cross-breeding individuals with not only desirable characteristics but also maximal genetic diversity, a strategy that remains relevant today (Gjedrem & Baranski, 2009; Janssen *et al.*, 2017; Kurinenko *et al.*, 2024). The technological approaches to fish farming (intensification level) and reproductive strategies (natural or induced spawning) impose specific requirements on the expression of economically important traits in selected breeding units, as highlighted by C. Boyd *et al.* (2020). For instance, research on Nesvych carp has yielded impressive results in terms of growth and reproductive performance, which, coupled with resistance to *Aeromonas*, could be used as a breed characteristic, as noted by H. Kurinenko *et al.* (2024).

The Galician inbred type of the Ukrainian frame carp breed is characterised by key selective traits such as winter hardiness (87%) and fecundity (1,181 thousand eggs at 6-8 years of age). Conversely, the genetic basis of the scaleless carp from the Lebedyn factory line provides high productive qualities, including rapid growth, vitality, high fecundity, early maturation, winter hardiness, and resistance to bacterial diseases. Moreover, the scaleless carp type, according to the Ministry of Agrarian Policy order No. 24/4, 2010, meets all European standards for market appearance – a meaty body, body length-to-height ratio, and scaleless. Therefore, the parental broodstocks are characterised by improved productive traits and increased resistance of offspring to adverse environmental factors. Building on the previous research of the research team at the Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine on the aforementioned selective stocks, the author of this study aims to conduct a fishery and biological evaluation of breeders of the Galician and new scaleless Lebedyn factory line inbred types of the Ukrainian frame carp breed and to assess their combining ability in terms of heterosis expression under reciprocal crosses in the offspring. Simultaneously, the study aimed to develop practical applications of current data on the genetic characteristics of structural units of Ukrainian carp breeds in breeding and commercial fish farming.

Materials and Methods

The research was conducted at the state enterprise Experimental Farm "Nyvka", located in the Polissia region of Ukraine, between 2023 and 2024. For the assessment of external appearance and productive traits, breeders (8 females and 10 males) of the Galician (GFC) and scaleless Lebedyn factory line (SLFL) inbred types of the Ukrainian frame carp (*Cyprinus carpio*) were selected. In spring, the wintering ponds were drained, and the experimental groups were inventoried. Galician frame carp breeders were tagged with electronic intramuscular tags on the left side under the dorsal fin.

Following individual assessment, the broodstock of the parental forms underwent morphometric and reproductive evaluation. Fisheries indicators were determined according to the guidelines for conducting selective breeding work with pond fish (Tomilenko *et al.*, 1995). The assessment of breeders for morphometric traits was carried out during bonitation, and for reproductive traits during the spawning season.

The following measurements were used as external appearance indicators:

W, kg – body weight of the individual fish;

l, cm – body length from the tip of the snout to the beginning of the caudal fin ray rosette;

C, cm – head length from the tip of the snout to the end of the gill cover;

H, cm – maximum body height of the individual fish;

O, cm – body girth at the highest point of the body of the individual fish;

Br, cm – maximum body width.

Based on the measurements, the following selection indices were calculated: condition factor (C_F), relative weight index (*W/l*), body length-to-height ratio (*l/H*), girth index (*l/O*), head index (*l/C*), and body thickness index (*l/Br*). For the reproductive assessment of females, individual fecundity and average egg weight were measured (Martseniuk & Martseniuk, 2020). For a more comprehensive characterisation of females, V. Nikandrov & N. Shindavina (1995) proposed using the reproductive index, which serves as an integrated indicator measured by the ratio of the total weight of the selected eggs to the unit body weight of the female. Egg density was calculated using the formula of V. Zhukinsky & I. Dyachuk (1964):

$$d = \frac{6P}{\pi D^3}, \quad (1)$$

where d is the egg density, mg/mm^3; P is the egg weight, mg; D – egg diameter, mm.

Egg size and weight were determined using a standard method in ichthyology: samples were fixed in a 4% formalin solution. For the reproductive assessment of males, a microscope connected to a computer with the Sperm Vision program was used. Analysis of male reproductive products included: ejaculate volume, overall activity, percentage of spermatozoa with progressive linear and circular movements, and percentage of non-viable spermatozoa. Kinetic parameters of spermatozoa were determined using the computer-assisted sperm analysis (CASA) system Sperm Vision by setting the parameters of sperm movement (Yaremchuk & Sharan, 2012; Özgür & Okumuş, 2019).

For the biometric processing of the obtained data, MS Excel 2016 was used with built-in statistical functions. In the process of processing experimental data, the following were calculated: M (arithmetic mean) and $\pm$ m (standard error of the mean), σ (standard deviation), and C_V (coefficient of variation). The following symbols were used in the table to denote the level of significance: t_d (Student's t-test) and P (probability) (Mincer *et al.*, 1991): P⩽0.95, P⩾0.95, P⩾0.99 compared to the control group.

Results and Discussion

In selective breeding to improve the qualities of inbred types of scaleless carp, the assessment of their external appearance measurements plays a crucial role. Comparing the main external appearance characteristics provides an opportunity to determine whether there are significant differences between the breeders of the Galician and scaleless Lebedyn factory line inbred types of the Ukrainian freme carp. During bonitation (Table 1), it was found that the average weight of SLFL females was 6.09 kg. The minimum weight of a female was 5.4 kg, and the maximum was 7.2 kg. The weight of GFC females was 5.84 kg, ranging from 5.1 to 6.2 kg.

According to Table 1, there is a significant difference in certain external appearance characteristics between SLFL and GFC female carp, specifically in the body length-to-height ratio, head index, and thickness index. Table 2 also shows a significant difference in the external appearance characteristics of male SLFL compared to male GFC in terms of commercial length and thickness index.

Table 1. *Weight and external appearance characteristics of female carp (n = 8)*

Indicator		Origin		t_d	P
		SLFL	GFC		
Weight (W), kg	$M \pm m$	6.10±0.20	5.84±0.19		
	σ	0.56	0.54	0.85	⩽0.95
	C_V, %	9.22	9.24		
Commercial length (l), cm	$M \pm m$	57.75±0.80	57.70±1.02		
	σ	2.25	2.88	0.00	⩽0.95
	C_V, %	3.9	4.98		
Condition factor (C_F)	$M \pm m$	3.16±0.09	3.04±0.11		
	σ	0.26	0.31	0.81	⩽0.95
	C_V, %	8.07	10.17		
Relative weight index (W/l), g/cm	$M \pm m$	105.31±2.53	101.00±2.38		
	σ	7.16	6.73	1.16	w⩽0.95
	C_V, %	6.79	6.66		
Body length-to-height ratio (l/H)	$M \pm m$	2.64±0.06	2.83±0.04		
	σ	0.18	0.10	2.39	**⩾0.95**
	C_V, %	6.90	3.61		
Girth index (l/O)	$M \pm m$	1.12±0.03	1.17±0.01		
	σ	0.11	0.04	1.22	⩽0.95
	C_V, %	2.69	3.49		
Head index (l/C)	$M \pm m$	4.37±0.08	3.89±0.06		
	σ	0.23	0.17	4.48	**⩾0.99**
	C_V, %	5.27	4.25		
Thickness index (l/Br)	$M \pm m$	5.23±0.13	4.65±0.07		
	σ	0.37	0.21	3.65	**⩾0.99**
	C_V, %	7.09	4.43		

Note: $M \pm m$ – arithmetic mean and standard error; σ – standard deviation; C_V – coefficient of variation; t_d – Student's t-test criterion; P – probability

Source: compiled by the author based on the method by O. Mincer *et al.* (1991)

Table 2. *Weight and external appearance characteristics of male carp (n = 10)*

Indicator		Origin: SLFL	Origin: GFC	t_d	P
Weight (W), kg	$M \pm m$	4.47 ± 0.13	4.85 ± 0.16	1.78	≤0.95
	σ	0.41	0.50		
	C_V, %	9.24	10.33		
Commercial length (l), cm	$M \pm m$	52.1 ± 0.58	54.2 ± 0.53	2.48	**≥0.95**
	σ	1.85	1.69		
	C_V, %	3.54	3.11		
Condition factor (C_F)	$M \pm m$	3.16 ± 0.09	3.04 ± 0.006	0.96	≤0.95
	σ	0.30	0.20		
	C_V, %	9.36	6.70		
Relative weight index (W/l), g/cm	$M \pm m$	85.59 ± 2.10	89.35 ± 2.31	1.14	≤0.95
	σ	6.63	7.30		
	C_V, %	7.75	8.18		
Body length-to-height ratio (l/H)	$M \pm m$	2.76 ± 0.03	2.74 ± 0.03	0.08	≤0.95
	σ	0.11	0.09		
	C_V, %	3.99	3.29		
Girth index (l/O)	$M \pm m$	1.13 ± 0.05	1.07 ± 0.04	0.03	≤0.95
	σ	0.11	0.09		
	C_V, %	9.48	8.58		
Head index (l/C)	$M \pm m$	3.91 ± 0.05	3.90 ± 0.04	0.08	≤0.95
	σ	0.5	0.13		
	C_V, %	3.96	3.27		
Thickness index (l/Br)	$M \pm m$	5.13 ± 0.15	4.32 ± 0.10	4.35	**≥0.99**
	σ	0.47	0.31		
	C_V, %	9.11	7.12		

Note: $M \pm m$ – arithmetic mean and standard error; σ – standard deviation; C_V – coefficient of variation; t_d – Student's t-test criterion; P – probability

Source: compiled by the author based on the method by O. Mincer *et al.* (1991)

Therefore, the results of the study have shown that SLFL and GFC females have higher body length-to-height ratio indices and smaller head indices. However, no significant differences were found in important parameters such as weight, condition factor, and body length-to-height ratio.

The development of the reproductive system in carp is determined by both genetic factors and environmental influences (Martseniuk & Martseniuk, 2021). It is known that temperature plays a key role in this process. For example, according to I. Valdebenito *et al.* (2015), in southern regions with a hot climate, carp mature much faster and at a significantly lower individual weight than in northern regions with a cooler climate and shorter growing season. At the same time, sexual maturation in carp is accompanied by a slowdown in growth rate. According to research by Y. Tuchapskyy (2013) in the Forest-Steppe zone, the turning point in the maturity coefficient for frame carp females occurs only in the fourth year of life. At this time, the period of trophoplasmic growth of the gonads ends, and their mass in four-year-olds reaches 20.6% of the body weight.

In all the females studied, the gonads were at Stage IV of maturity and, under favourable weather conditions, successfully spawned under controlled reproduction conditions (Table 3). All responded positively to hormonal stimulation. The fecundity indices for GFC females were as follows: absolute fecundity ranged from 476,000 to 576,000 eggs, and relative fecundity averaged 89.55 ± 2.25 thousand eggs/kg. In SLFL females, absolute and relative fecundity ranged from 372,000 to 543,600 eggs and 74.83 ± 2.89 thousand eggs/kg, respectively. A significant difference ($P \geqslant 0.95$) was observed in the weight of selected eggs, with 678.0 ± 16.25 g for GFC compared to 588.0 ± 38.94 g for SLFL. For relative fecundity and reproductive index, a high level of significant difference was also observed at $P \geqslant 0.99$ in favour of GFC females. The egg density in SLFL females at 0.75 ± 0.03 mg/mm^3 was slightly higher than that in GFC females – 0.68 ± 0.01 mg/mm^3, but the difference was not significant.

Table 3. *Characteristics of reproductive indices of female carp (n = 5)*

Indicator		Origin		t_d	P
		GFC	SLFL		
Weight (W), kg	$M \pm m$	5.84 ± 0.223	6.12 ± 0.25	0.73	⩽0.95
	σ	0.51	0.56		
	C_V, %	8.86	9.22		
Mass of selected eggs, g	$M \pm m$	678.0 ± 16.25	588.0 ± 38.94	1.91	**⩾0.95**
	σ	36.33	87.10		
	C_V, %	5.36	14.81		
Working fecundity, thousand eggs	$M \pm m$	522.4 ± 22.0	459.1 ± 30.15	1.52	⩽0.95
	σ	49.33	67.42		
	C_V, %	9.44	14.68		
Relative working fecundity, thousand eggs/kg	$M \pm m$	89.55 ± 2.25	74.83 ± 2.89	3.59	**⩾0.99**
	σ	5.02	6.47		
	C_V, %	5.61	8.65		
Reproductive index, %	$M \pm m$	11.65 ± 0.26	9.59 ± 0.440	3.80	**⩾0.99**
	σ	0.59	0.90		
	C_V, %	5.07	9.42		
Egg weight, mg	$M \pm m$	1.30 ± 0.04	1.28 ± 0.04	0.35	⩽0.95
	σ	0.09	0.8		
	C_V, %	6.55	6.12		
Egg diameter, mm	$M \pm m$	1.54 ± 0.01	1.49 ± 0.03	1.48	⩽0.95
	σ	0.02	0.07		
	C_V, %	1.59	4.63		
Egg density, mg/mm^3	$M \pm m$	0.68 ± 0.01	0.75 ± 0.03	1.79	⩽0.95
	σ	0.03	0.07		
	C_V, %	4.87	9.26		

Note: $M \pm m$ – arithmetic mean and standard error; σ – standard deviation; C_V – coefficient of variation; t_d – Student's t-test criterion; P – probability

Source: compiled by the author based on the method by O. Mincer *et al.* (1991)

In selective breeding, the evaluation of breeders based on sperm quality and fertilisation ability is crucial (Kuts *et al.*, 2021). The quality of male sexual products is one of the most important factors ensuring successful fertilisation and subsequent embryo development. The study of sperm motility has become widely used in reproduction technology, as this method allows for the determination of the quality of the obtained gametes, the timely detection of anomalies, and the prevention of inefficient fertilisation (Fauvel *et al.*, 2010). Among current research, a relevant direction is the study of the quality characteristics of sperm concentration, sperm density, and sperm activity (motility). The weight of the studied GFC males ranged from 5.2 to 6.1 kg (average 5.52 ± 0.16 kg), and for SLFL males, it was 4.5 to 5.6 kg (average 5.02 ± 0.22 kg) (Table 4).

Table 4. *Characteristics of reproductive indices of male carp (n = 5)*

Indicator		Origin		t_d	P
		GFC	SLFL		
Weight (*W*), kg	$M \pm m$	5.52 ± 0.16	5.02 ± 0.22	1.61	≤0.95
	σ	0.37	0.50		
	C_V, %	6.62	9.90		
Ejaculate volume, mm³	$M \pm m$	14.74 ± 0.65	15.62 ± 0.90	0.71	≤0.95
	σ	1.46	2.10		
	C_V, %	9.87	12.88		
Overall activity, %	$M \pm m$	87.20 ± 1.36	87.60 ± 1.33	0.19	≤0.95
	σ	3.03	22.97		
	C_V, %	3.8	3.39		
Relative content of spermatozoa with progressive linear movement, %	$M \pm m$	64.74 ± 2.84	67.40 ± 1.58	0.73	≤0.95
	σ	2.84	3.52		
	C_V, %	6.34	5.23		
Relative content of spermatozoa with circular movement, %	$M \pm m$	24.16 ± 2.68	21.90 ± 2.27	0.58	≤0.95
	σ	5.99	5.08		
	C_V, %	24.81	23.21		
Relative content of immotile spermatozoa, %	$M \pm m$	11.18 ± 1.16	10.70 ± 0.94	0.29	≤0.95
	σ	2.60	2.10		
	C_V, %	23.26	19.59		

Note: $M \pm m$ – arithmetic mean and standard error; σ – standard deviation; C_V – coefficient of variation; t_d – Student's t-test criterion; *P* – probability

Source: compiled by the author based on the method by O. Mincer *et al.* (1991)

The results of the study showed that the average ejaculate volume of GFC males was 14.74 ± 0.65 mm³, while for SLFL males it was 15.62 ± 0.90 mm³. The total number of active spermatozoa was 87.20 ± 1.36% and 87.60 ± 1.33%, respectively. However, the relative content of spermatozoa with circular movement was 10.03% higher in GFC males compared to SLFL males. However statistical calculations did not reveal a significant difference between the reproductive parameters of males of both inbred types.

The usefulness of evaluating animals based on their external appearance in selective breeding has been proven by long-standing Ukrainian and international practice (Tomilenko, 2001). Selective breeding to form highly productive stocks of breeders of various carp breeds, productivity directions, characterised by sufficient productivity and survival, and adequately adapted to the requirements of intensive farming technology, is impossible without a systematic evaluation of animals based on their external appearance (Jeney & Bekh, 2020). The process of creating and further improving the Ukrainian frame carp breed was based on target external appearance parameters. Particular attention was paid to shaping the desired body shape in fish. Galician carp were better adapted to the harsh climatic conditions of Central Europe than other European carp (Krasnopolska, 2021).

In comparison with similar studies conducted on the Nesvych inbred type of Ukrainian frame and scaled carp breeds (Kurinenko *et al.*, 2024), notable distinctions were observed. The average body length-to-height ratio (l/H) of frame females was 2.96% higher than that of males, reaching 2.7 units, with a range of 2.68-2.72 units, while for males it was 2.78 units, with a range of 2.732.89 units. In scaled females, the body length-to-height ratio (l/H) was also at a level of 2.70 units. The girth index (l/O) in females ranged from 1.10 to 1.21 units, with an average value of 1.18 units for frame carp individuals and 1.17 units for scaled individuals. In males, the range of this index was 1.14-1.20 units, with an average value of 1.16 units for both frame and scaly individuals. The average weight of selected eggs in frame females was 530.0 ± 34.76 g, and in scaly females, it was 535.0 ± 31.12 g (Kurinenko *et al.*, 2024).

According to B. Grishin (2021), breeders from the Antoninsko-Zozulenets (AZFC) and Lubin (LFC) inbred types of the Ukrainian frame carp met the expected morphometric values. Specifically, the body length-to-height ratio of 5-6-year-old AZFC breeders was 2.73-2.78, and the girth index was 1.17-1.26. LFC breeders of the same age were more body length-to-height ratio with indices of 2.65-2.68 and 1.16-1.19, respectively. The condition factor of males in both lines significantly exceeded that of females – 3.14-3.73 versus 3.02-3.06. At the same time, the relative fecundity of females of different origins was similar – 88.4-98.7 thousand eggs/kg. However, the relative fecundity of eight-year-old females was lower – 88.4-90.4 thousand eggs/kg – compared to five-year-old females – 96.1-98.7 thousand eggs/kg. On average, for both age groups, the relative fecundity of AZFC females was 92.4 thousand eggs/kg, and for LFC females – 91.9 thousand eggs/kg.

Therefore, the studied breeders of the Galician and scaleless Lebedyn factory line inbred types of carp were not inferior in terms of both morphometric and reproductive indicators. Thanks to such selective breeding, a progressive heterogeneous structure of Ukrainian breed groups is formed, taking into account both regional aquaculture conditions and intensification practices.

Conclusions

In the study comparing breeders of the Galician and scaleless Lebedyn factory line inbred types of Ukrainian frame carp, significant differences were found in the following external appearance characteristics: body length-to-height ratio ($P \geqslant 0.95$), head index ($P \geqslant 0.99$), and thickness index ($P \geqslant 0.99$). Between the male carp of the SLFL and GFC lines, there was a significant difference in commercial length ($P \geqslant 0.95$) and thickness index ($P \geqslant 0.99$).

Under factory reproduction conditions, females of both inbred types responded positively to hormonal stimulation. The fecundity indices of GFC females were as follows: absolute fecundity ranged from 476,000 to 576,000 eggs, and relative fecundity averaged 89.55 ± 2.25 thousand eggs/kg. In SLFL females, absolute and relative fecundity ranged from 372,000 to 543,600 eggs and 74.83 ± 2.89 thousand eggs/kg, respectively. A significant difference ($P \geqslant 0.95$) was observed in the weight of selected eggs, with 678.0 ± 16.25 g for GFC compared to 588.0 ± 38.94 g for SLFL. For relative fecundity and reproductive index, a high level of significant difference was also observed at $P \geqslant 0.99$ in favour of GFC females.

Regarding the reproductive indicators of the males, the average ejaculate volume of GRC males was 14.74 ± 0.65 mm^3, while for SLFL males it was 15.62 ± 0.90 mm^3. The total number of active spermatozoa was 87.20 ± 1.36% and 87.60 ± 1.33%, respectively. Statistical analysis did not reveal any significant differences between these reproductive parameters of males from both inbred types. Further research should focus on the quality of offspring from various combinations of the original forms.

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Valuable Invertebrates, Candidate of Agricultural Sciences, Senior Researcher.

Conflict of Interest

None.

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Рибницько-біологічна оцінка плідників галицького та малолускатого внутрішньопородних типів української рамчастої породи коропа

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Анотація. Пошук найоптимальніших поєднань наявних порід коропа дозволяє досягти бажаного балансу між ростом, розвитком, репродуктивними показниками, стійкістю до різноманітних збудників хвороб та умовами культивування риби. Тому метою виконання даних досліджень була рибницько-біологічна оцінка плідників галицького та нового малолускатого лебединської заводської лінії внутрішньопородних типів української рамчастої породи коропа. Дослідження проводилися на державному підприємстві «Дослідне господарство «Нивка»». Для проведення оцінки екстер'єрних та продуктивних показників були обрані плідники галицького та малолускатого лебединської заводської лінії внутрішньопородних типів української рамчастої породи коропа. За показниками промірів було розраховано селекційні індекси: коефіцієнт вгодованості, індекс відносної маси, індекс високоспинності, індекс обхвату, індекс голови, індекс товщини тіла. Для репродуктивної оцінки самиць вимірювали індивідуальну робочу плодючість і середню наважку ікринки. Проведено аналіз показників статевих продуктів самців: об'єм еякуляту, загальна активність; відсоток сперматозоїдів з прямолінійно-поступальним та манежним рухами, а також неживих сперматозоїдів. За результатами дослідження екстер'єрних параметрів встановлено, що самиці малолускатого та галицького типів виявилися вищими за показником високоспинності, а за індексом голови – з меншою головою. При цьому достовірної різниці за такими важливими показниками, як маса, індекс високоспинності та коефіцієнт вгодованості, не зафіксовано. За умов заводського відтворення самки обох внутрішньопородних типів позитивно реагували на гормональну стимуляцію. Показники плодючості у самок галицького типу були наступними: робоча плодючість – в межах 476,0-576,0 тис. ікринок, а відносна плодючість в середньому склала 89,55 ± 2,25 тис. ікринок/кг. В самиць малолускатого типу робоча і відносна плодючість перебувала в межах 372,0-543,6 тис. ікринок та 74,83 ± 2,89 тис. ікринок/кг відповідно. Практична значимість результатів дослідження полягає у подальшому отриманні та визначенні ефективності вирощування помісних нащадків за умов реципрокних схрещувань у порівнянні з вихідними формами

Ключові слова: лебединська заводська лінія; екстер’єр коропа; репродуктивні показники; селекційно-племінна робота в рибництві

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Productivity of Holstein cows with early insemination of heifers

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Abstract. The age at first insemination is a critical factor influencing the duration of the non productive period, subsequent milk yield, and reproductive performance of cows. Accordingly, the study aimed to determine the impact of early insemination age on cows' milk productivity

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and reproductive capacity. The research was conducted using Holstein cows and carried out at Rozhnivka-Agro LLC, located in the Ichnia District of Chernihiv Region. The findings revealed that the live weight of the experimental animals at first calving showed no significant deviations, remaining within a 3.3% range. During the first lactation, the shortest service period was observed in animals inseminated at 13 months of age, with differences of 4.5 (Group I ↓) and 3.9 days (Group III ↓). The highest insemination index, reaching 3.1, was recorded across all experimental groups during the third lactation. The highest insemination index of 3.1 across all experimental groups was observed during the third lactation. Milk productivity was analysed daily by lactation months, considering milk fat and protein yields. The results indicated no significant differences in milk yield during the first lactation across all groups. However, during the second lactation, a general upward trend in milk yield was noted in all groups. Notably, cows inseminated at 13 months of age exhibited 4% and 1% higher milk productivity compared to Groups I and III, respectively. The peak milk yield across all groups occurred during the third lactation. The lowest milk yield was recorded in cows inseminated early (at 12 months), with differences of 7.8% (Group II ↑) and 6.9% (Group III ↑). Regarding lactation-specific fat content, Group II (13 months) demonstrated higher fat content during the third lactation by 0.23%, 0.13%, and 0.05% compared to the first, second, and fourth lactations, respectively. No significant differences were observed in milk protein content, which ranged between 3.20% and 3.23%. Thus, reducing the age of first insemination can enhance the economic utilisation of animals without compromising their reproductive performance or adversely affecting subsequent milk productivity

Keywords: gestation; milk yield; live weight; service period; insemination index

Introduction

The productive lifespan of cows is significantly shorter than their natural lifespan, averaging approximately 3-4 years in high-yielding dairy cows. Common reasons for culling include reduced milk production, injuries, low reproductive performance, and complications following calving. The productive life of an animal serves as an indicator of milk production efficiency and is measured by the number of lactations. The findings of V. Valchev *et al.* (2020) demonstrated that cows with later calving ages (30 months or more) faced a higher risk of culling at an early age, particularly during their first lactation. According to their compelling conclusions, reducing this risk would lead to improved outcomes in extending the productive lifespan of cows.

High reproductive performance plays a crucial role in the profitability of dairy farming. Reduced fertility and reproductive disorders are associated with decreased milk production (Talukder *et al.*, 2022). According to P. Tamboli *et al.* (2022), the highest-yielding cows also exhibit the highest rates of infertility, which is often a primary reason for culling animals from the herd. The age at first calving is a critical factor for obtaining replacement heifers. This parameter significantly influences the subsequent reproductive performance and milk productivity of cows. Proper management of this factor can result in high animal productivity and substantial profitability for the farm.

Opinions among researchers regarding the optimal age for first calving vary, though it is generally considered to be around 24 months. According to M.C. McCarthy *et al.* (2022), achieving this target helps reduce the non-productive period, facilitates earlier returns on investment, and lowers rearing costs for replacement heifers. Farmers incur losses exceeding USD

100 per month for feed and maintenance for each animal if the first insemination is delayed. The age at first calving is directly influenced by the age at first successful insemination, as the gestation period is relatively fixed at an average of 285 days and is challenging to alter (Steele *et al.*, 2021). H. Atashi *et al.* (2021) and P. Tamboli *et al.* (2022) argue that earlier calving accelerates genetic progress and reduces the generation interval. Consistent growth plays a crucial role in preparing heifers for insemination and future lactation. O. Dorobăț *et al.* (2019) demonstrated a link between increased live weight and earlier sexual maturity. To ensure calving occurs without complications, heifers must meet specific live weight and growth criteria before their first insemination. Consequently, the age at first insemination depends on the growth rate, as sexual maturity cannot be achieved until the animal reaches the required weight. Research by B. Vargas-Leitón *et al.* (2023) indicates that earlier calving may negatively impact subsequent fertility. Therefore, replacement heifers must rapidly attain adequate body weight before their first calving. The adoption of intensive heifer-rearing technologies has enabled earlier insemination, allowing for both milk production and offspring at a younger age.

Early calving is generally associated with risks such as complications during and after calving, as well as reduced reproductive performance and milk productivity in cows. The most common issue during calving is the disproportion between the foetus and the dam, typically caused by an excessively large calf and/or a small pelvic size. However, A. Kraevskiy *et al.* (2020) found that animals inseminated at 20 months had the highest incidence of calving complications (50%), whereas heifers inseminated at 14-17 months experienced nearly half that rate (25.8%). Similarly, H. Kusaka *et al.* (2022) reported that early calving did not have adverse effects but instead resulted in improved reproductive performance. They observed that the frequency of difficult calvings and stillbirths was more closely linked to the weight of the heifer rather than the age at first calving. Their further studies indicated that calving at an earlier age enhanced both productivity and survival rates, while later calving negatively impacted these parameters (Kusaka *et al.*, 2023). Research by S.G. Pishchan (2022) on Brown Swiss cows supports the conclusion that calving at 22.7 months results in a 7.33% higher milk yield compared to animals that calved at 25.5 months. Conversely, M.P. Turiello *et al.* (2020) argue that delaying the age of calving can lead to increased milk yields, though this approach also raises costs due to the extended heifer-rearing period. According to P. Tamboli *et al.* (2022), reducing the age of first calving could lower costs, reduce the production cost per unit, and improve the profitability of dairy enterprises.

Consequently, this study aimed to determine the relationship between the early insemination of Holstein heifers and their subsequent milk productivity and reproductive performance.

Materials and Methods

The study utilised Holstein cows as the research material. It was conducted at the Rozhnivka-Agro LLC in the Ichnia District of Chernihiv Region. Animal groups were formed based on age, live weight, lineage, and the order of oestrus onset. The live weight of the animals at the time of insemination ranged from 380 to 430 kg. Depending on the age at first successful insemination, the cows were divided into three groups: Group I (12 months), Group II (13 months), and Group III (14 months). Each Group comprised 15 animals. During the study period (2019-2023), culling occurred. By the beginning of the third lactation, Group I had 13 animals, Group II had 14, and Group III had 14. By the start of the fourth lactation, the numbers were as follows: Group I – 10 animals; Group II – 10 animals; Group III – 11 animals. All animals were housed and fed under similar conditions. They were kept free-stall in barns, with an implemented uniform feeding system, which was sufficient to achieve a milk yield of 10,000-11,000 kg. A twice-daily milking schedule was implemented on the farm. Milk yield, along with fat and protein content, was measured monthly during control milking sessions. Fat and protein content in the milk were determined using the Ecomilk device. Reproductive performance indicators included the service period, calving interval, age at first calving, insemination index, and reproduction coefficient. Reproductive performance was assessed during the first, second, and third lactations, while milk productivity was evaluated from the first to the fourth lactation. The fifth lactation was excluded from the analysis as most animals had been culled from the study groups by that time.

The data were obtained from the livestock and breeding records, as well as from the Orsek herd management system. Heifers and cows were artificially inseminated using the recto-vaginal method with semen from a Holstein sire breed bull. Insemination occurred after the onset of oestrus, which was determined visually. Pregnancy was confirmed one month later via ultrasound. The live weight of the newborn calves was recorded by weighing them immediately after birth. Heifers were weighed before insemination and calving. The age at first calving was calculated by subtracting the cow's birth date from the date of her first calving. The calving interval was defined as the time between the first and second calving dates. The service period was calculated as the interval from calving to successful insemination.

The reproductive performance coefficient (RPC) was calculated using the formula:

$$RPC = \frac{365}{CI}, \quad (1)$$

where 365 is the number of days in a year; CI is the calving interval between two consecutive calvings.

The insemination index was defined as the number of inseminations per successful pregnancy.

The fertility index (*FI*) of the cows was calculated using the formula by J. Dokhi:

$$FI = 100 - (C + 2CI), \quad (2)$$

where *C* is the age of the cow at her first calving.

For statistical analysis, MS Excel software was used.

The study adhered to the guidelines for animal welfare as outlined in the European Convention on the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (1986).

Results and Discussion

A decline in reproductive performance in livestock farming is one of the major challenges. In this regard, current research focused on determining the relationship between the age at first insemination and subsequent reproductive performance (Table 1). The data obtained revealed that the duration of gestation across all groups during the three lactations was between 276-279 days, which is within the physiological norm. Analysis of the service period indicated that the lowest value of 92.7 days was recorded in the Group inseminated at 13 months, which was 9.1 and 11.1 days lower compared to the groups inseminated at 12 and 14 months, respectively. Notably, Group II also had the highest reproductive performance coefficient, at 0.99, while in the I and III groups, this value was slightly lower at 0.97 and 0.96, respectively. With an optimal calving interval of 365 days, all experimental groups showed an increase in this indicator: Group I increased by 14.6 days, Group II by 3.79 days, and Group III by 17.2 days. Comparison between groups showed that the interval was longest in animals inseminated at 14 months (Group III), with a difference of 2.6 days compared to Group I and 13.41 days compared to Group II. There were no significant differences in the insemination index between Groups I and III, with the lowest index observed in Group II (2.0).

Table 1. *Reproductive performance indicators of the experimental groups*

Group	Gestation	Service period	Insemination index	Calving interval	Reproductive coefficient
First lactation					
12 months	277.8 ± 1.33	101.8 ± 4.84	2.8 ± 0.34	379.6 ± 3.90	0.97 ± 0.01
13 months	276.09 ± 1.03	92.7 ± 4.20	2.0 ± 0.28	368.79 ± 4.20	0.99 ± 0.01
14 months	278.4 ± 0.80	103.8 ± 5.7	2.8 ± 0.40	382.20 ± 6.2	0.96 ± 0.01
Second lactation					
12 months	278.8 ± 0.90	108.9 ± 7.22	2.9 ± 0.40	387.7 ± 7.55	0.95 ± 0.02
13 months	277.9 ± 1.36	104.4 ± 6.76	2.7 ± 0.30	382.30 ± 7.46	0.96 ± 0.02
14 months	279.4 ± 1.10	108.3 ± 7.9	2.9 ± 0.40	387.7 ± 8.00	0.95 ± 0.01
Third lactation					
12 months	277.2 ± 0.79	106.8 ± 5.68	3.1 ± 0.30	384 ± 5.97	0.95 ± 0.01
13 months	276.4 ± 0.70	108.7 ± 10.80	3.1 ± 0.40	385.1 ± 10.60	0.96 ± 0.01
14 months	278.1 ± 0.78	107.8 ± 11.37	3.1 ± 0.43	386.8 ± 11.48	0.96 ± 0.01

Source: developed by the authors

During the second lactation period, the service period in Group II was 4.5 and 3.9 days shorter compared to Groups I and III, respectively. It is also worth noting that in this group, the insemination index was 6.9% lower. The calving interval in animals of Groups I and III was the same, at 387.7 days, which is 22.7 days longer than the optimal interval and 5.4 days longer than in Group II. Analysis of the key reproductive performance indicators in cows during the third lactation shows that there were no significant differences in the service period. The difference between Group I and Group II was 1.9 days, between Group II and Group III it was 0.9 days, and between Group I and Group III it was 1 day. The calving interval in Group II was 1.1 days longer than in Group I, while Group III had a calving interval that was 2.8 days longer than Group I and 1.8 days longer than Group II.

Another important indicator that characterises the reproductive performance of animals is the reproductive coefficient. The highest value of this indicator was observed in Group II during the first lactation (0.98), with a tendency for it to decrease in Groups I and III (0.96). When analysing the data for Group II across different lactations, it is evident that the service period was shorter during the first lactation, at 97.7 days, which is 6.4 and 11 days shorter compared to the first and third lactations, respectively. In Groups I and III, there was a tendency for the service period to increase during the second lactation. In Group I, it was longer by 7.1 days, and in Group III, it was longer by 4.5 days compared to the first lactation. In the third lactation, these groups showed a slight reduction in the service period, by 1-2 days.

The analysis of the dynamics of the insemination index shows that in all three groups, this indicator increases up to the third lactation, reaching 3.1. The dynamics of the calving interval across lactations mirrored the dynamics of the service period. To prevent dystocia, which could negatively affect subsequent reproductive performance and milk production in heifers, it is essential to monitor the heifers' body weight prior to the first insemination. According to the research by L. Han *et al*. (2021), heifers with higher body weight at first calving had higher milk production during the first lactation compared to lighter heifers. It is necessary for animals to meet the recommended body weight norm of 550-625 kg for Holstein heifers at first calving. In the study, animals inseminated at 12.12 months had an average body weight of 403.5 kg, which was only 3.3 and 7.5 kg lower than those inseminated at 13.1 and 14.2 months, respectively. A slight difference in body weight was also observed at first calving, with the difference between Groups II and III being 2 kg, while exceeding Group I's weight by 18.3 and 20.3 kg, respectively. The difference in the birth weight of the calves was minimal, only 1 kg (Table 2).

Table 2. *Live weight and age at first calving*

Group	Age at first insemination, month	Live weight at first insemination	Age at first calving	Live weight at first calving	Calf birth weight	Fertility index
Group I	12.12 ± 0.01	403.5 ± 4.20	21.04 ± 0.15	592.7 ± 10.6	40.1 ± 0.8	52.94 ± 0.32
Group II	13.1 ± 0.03	406.8 ± 3.20	22.14 ± 0.03	611 ± 11.1	41 ± 1.1	53.60 ± 0.03
Group III	14.2 ± 0.01	411 ± 4.50	23.26 ± 0.01	613 ± 12.0	41.1 ± 0.6	51.57 ± 0.4

Source: developed by the authors

The fertility index was calculated using the Dokhi formula. Cows in Group II exhibited a higher fertility index (53.60) compared to animals inseminated at earlier (12 months) or later ages (14 months), with differences of 1.2% and 3.8%, respectively. A crucial aspect of this study was to analyse the milk yield of cows concerning the age at insemination. One of the primary indicators of milk yield is the average daily milk production, which allows for an analysis of the lactation curve.

Table 3 shows that the lowest average daily milk yields were observed in the first and tenth months of lactation in Group I – 35.1 and 24.1 kg, respectively; in Group II – 27 kg and 22.6 kg; and Group III – 34 and 24.8 kg. Notably, Group II had the lowest values. Furthermore, in this group, a sharp increase in milk yield of 11.4 kg was observed in the second month of lactation, marking the peak of lactation. From the third month onwards, yields levelled off, with a difference of only 2.2 kg between the third and seventh months. However, a steep decline of 10.8 kg began in the eighth month. In the other two groups, there was a gradual increase in milk yield, with peaks (38.4 and 41.4 kg) observed in the third month of lactation. From the third month onwards, there was a gradual decline, with a difference of 14.3 kg and 16.6 kg between the third and tenth months, respectively. The average daily milk yield over 305 days of lactation was the same for all three groups and amounted to 33.3 kg.

Table 3. *Average daily milk yield over 10 months of lactation*

Group	Months of lactation									
	1	2	3	4	5	6	7	8	9	10
First lactation										
Group I	35.1±1.52	37.6±2.43	38.4±1.54	37.9±0.97	36±0.93	33.4±1.45	31.2±1.60	30.1±2.26	29.2±2.39	24.1±2.57
Group II	27±1.08	38.4±1.68	37.3±1.10	37.40.70	37.4±0.82	36.6±0.70	35.1±0.93	33.4±1.44	27.7±1.38	22.6±1.16
Group III	34±1.79	37.1±1.87	41.4±1.62	38.8±1.44	34.4±1.30	33.9±1.23	31.2±0.95	29.1±1.08	27.4±1.27	24.8±1.21
Second lactation										
Group I	29.5±1.56	38.4±1.59	45.1±1.25	43.9±1.46	43.3±1.58	37.9±1.59	31.3±1.48	28.9±1.90	24.2±1.19	19.1±0.71
Group II	39.7±2.00	44.6±2.00	43.3±1.44	41.4±1.33	39.7±1.16	35.9±1.35	30.7±1.09	26.1±0.88	26.2±1.30	21.3±0.99
Group III	30.9±1.8	36.1±1.1	45.9±1.2	43.2±1.6	40.1±1.7	38.4±1.8	35.9±1.6	33.6±1.5	28.1±1.9	23.4±2.4
Third lactation										
Group I	36.3±1.36	46.9±1.59	42.6±1.53	42.9±1.21	41.7±1.06	37.3±1.03	34.3±0.91	31.2±0.98	26.8±0.92	20.3±1.05
Group II	40.4±0.82	49.7±0.86	47.2±0.86	46.3±0.71	43.9±0.83	41.2±0.91	37.9±1.04	32.5±1.35	29.1±1.26	22.5±1.22
Group III	38.5±1.68	45.5±1.29	46.5±1.02	43.41.02	43.2±1.10	41.6±1.38	38.4±1.82	34.3±1.6	30.4±1.78	25.1±1.45
Fourth lactation										
Group I	34.5±1.6	42.7±1.2	46.3±1.3	41.8±1.46	37.1±1.75	33.9±1.54	34.5±1.31	32.2±1.48	31±1.45	26.6±1.44
Group II	40.4±2.13	49.7±1.46	47.2±1.82	46.3±1.62	43.9±1.81	41.2±2.41	37.9±2.96	32.5±3.78	29.1±3.56	22.5±3.99

Table 3. Continued

Group	Months of lactation									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Fourth lactation										
Group III	39.4±2.46	45.5±2.55	43.4±1.67	42.5±1.88	38.2±1.73	38.8±1.7	36.9±1.34	34.9±1.37	32.7±1.42	25.5±2.52

Source: developed by the authors

The dynamics of milk yield during the second lactation show that the highest yield in the first month was in Group II, at 39.7 kg, which was 10.2 and 8.8 kg higher than Groups I and III, respectively. In the animals of this group, peak lactation was observed in the second month (44.6 kg), and from the third month onwards, yields began to decline, with a difference of 23.3 kg between the second and tenth months. In Groups I and III, peak lactation occurred in the third month, with a difference of 16.4 kg and 15 kg between the first and third months, respectively. When analysing the lactation curves of all three groups, it can be observed that Groups II and III had more regular curves with a gradual decline in milk yield. In Group I, the curve was more irregular, with a sharp decrease of 5.4 kg in the sixth month.

The changes in milk yield during the third lactation indicate a greater uniformity of milk production compared to previous lactations. In Group II, peak production occurred in the second month (49.7 kg), followed by a gradual decline until the tenth month. The lactation curve of this Group corresponds to Type I (strong, persistent lactation with high yields). In Group I, milk yields were less uniform, with the peak lactation (45.5 kg) also occurring in the second month, but a gradual decline was observed starting from the fifth month. The dynamics of Group III show that maximum production occurred in the third month.

The characteristics of the fourth lactation indicate that the highest milk yields were observed in Groups II and III (46.8 and 46.3 kg, respectively). Animals in Group I exhibited their peak milk production in the second month of lactation (45.5 kg). The average daily milk yield of Group II was higher than that of Groups I and III from the first to the seventh month, and from the seventh month onwards, yields declined sharply. The dynamics of milk production in Group II indicate a sharp decrease starting from the third month, followed by a levelling off from the sixth month.

An analysis of the milk yield of the experimental animals over a 305-day lactation period indicates that, in the first lactation, the difference in milk yield among all three groups was within 1%. Milk production in the second lactation was highest in the experimental Group II, although the difference between this Group and Group III was less than 1%. In Group I, the milk yield over a 305-day lactation period was 4% lower compared to Groups II and III. Figure 1 shows that the dynamics of milk production in the experimental groups remained similar until the third lactation. In Group I, the milk yield was 7.8% and 6.9% lower compared to Groups II and III, respectively. An analysis of the fourth lactation reveals a decrease in milk yield in Groups II and III, while in Group I, a slight increase was observed compared to the previous lactation. The difference between the groups was 4.7% (between Groups I and II) and 4.5% (between Groups III and I).

An important aspect of dairy cow productivity is the fat and protein content of milk. Analysis of protein content shows that there were no significant changes in the experimental animals from the first to the fourth lactation. In all groups, the protein content of milk was within the range of 3.20-3.23% (Table 4). The dynamics of fat content in milk indicate a trend towards increased fat content with each subsequent lactation in every group. In Group I, the highest content was observed in the fourth lactation, which was 0.17%, 0.15%, and 0.02% higher compared to the first, second, and third lactations, respectively. In Group II, the highest fat content was during the third lactation and exceeded the first, second, and fourth lactations by 0.23%, 0.13%, and 0.05%, respectively. In Group III, as in Group II, the maximum content was during the third lactation, with differences between lactations of 0.13% (first lactation), 0.14% (second lactation), and 0.07% (fourth lactation). A comparative analysis between the groups shows that the highest fat content was in Group II.

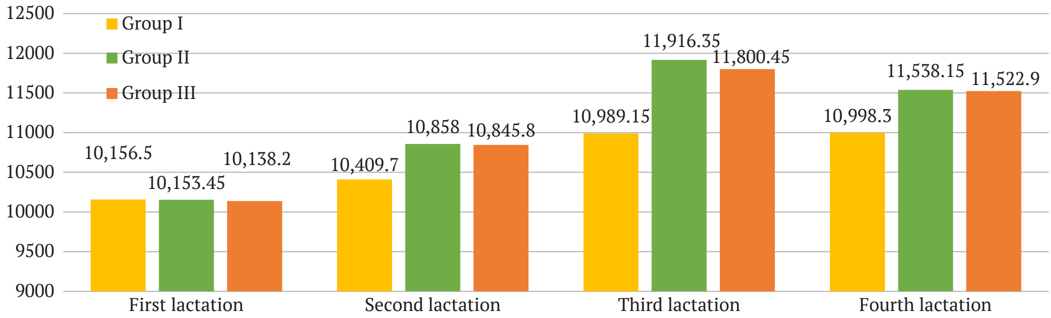

Figure 1. Milk productivity over 305 days of lactation

Source: developed by the authors

Table 4. *Protein and fat content in the milk of experimental animals across lactation*

Lactation	Group I		Group II		Group III	
	Fat content in milk	Protein content in milk	Fat content in milk	Protein content in milk	Fat content in milk	Protein content in milk
First lactation	3.69±0.07	3.22±0.01	3.68±0.1	3.20±0.01	3.75±0.12	3.21±0.01
Second lactation	3.71±0.1	3.21±0.02	3.78±0.12	3.22±0.02	3.74±0.08	3.20±0.01
Third lactation	3.84±0.2	3.21±0.01	3.91±0.4	3.22±0.01	3.88±0.14	3.21±0.02
Fourth lactation	3.86±0.1	3.20±0.01	3.86±0.09	3.23±0.02	3.81±0.08	3.22±0.01

Source: developed by the authors

When analysing the correlation between milk productivity and reproductive performance, it is important to note that there is a strong connection between these indicators. In this study, the relationship between milk productivity over 305 days of lactation and the indicators of reproductive performance, namely service period and reproductive coefficient were examined (Table 5).

Table 5. *Correlation between milk yield and reproductive performance indicators*

Group	Milk yield over 305 days of lactation/service period			Milk yield over 305 days of lactation/reproductive coefficient		
	First lactation	Second lactation	Third lactation	First lactation	Second lactation	Third lactation
Group I	+0.126	+0.031	-0.004	+0.137	+0.002	-0.087
Group II	+0.029	+0.643	-0.257	+0.520	-0.295	+0.135
Group III	-0.070	+0.306	-0.097	-0.450	-0.691	+0.256

Source: developed by the authors

In all three groups, during the first lactation, there was a negligible correlation between the service period and milk yield. In the second lactation, there was a moderate positive correlation in the Group of animals inseminated at 13 months and a low correlation in the other two groups. In the third lactation, a low negative correlation was observed between the indicators in all groups. Analysis of the relationship between milk yield over 305 days of lactation and calving interval showed that in Group I, it was insignificant and fluctuated between r = +0.137 and +0.087 depending on the lactation. In Group II, the correlation was moderately positive in the first lactation, while in the second lactation, it was low and negative. In Group III, the correlation coefficient was negative in both the first and second lactations and was at a moderate level.

Numerous researchers investigating the impact of age at first insemination on milk yield have noted a strong correlation between these two factors, including J. Pytlewski & I.R. Antkowiak (2021) and K.A. Copas Medina *et al*. (2022). However, there is limited literature reporting the negative impact of age at first insemination on animal fertility. This study found that animals inseminated at 13 months had a shorter service period of 11.1 days compared to those inseminated at 14 months, and this difference was statistically significant. Additionally, it is worth noting that in animals of this group, from the first to the fourth lactation, there was no increase in reproductive problems, as was the case in animals inseminated at 14 months. S.G. Pishchan (2022) also reported that, according to their research, heifers had satisfactory reproductive performance regardless of age at first insemination. G.S. Sharapa *et al*. (2021) determined that the highest fertility was observed in animals inseminated at 12-14 months, while older animals had a lower conception rate. In younger animals (12-14 months), the average service period was 102 days, while in older animals (15+ months) it was 121-136 days.

The insemination rate did not show significant variations across the groups, indicating the absence of a negative impact of early insemination on subsequent fertility. These findings are supported by recent studies conducted on Holstein cows. N.T. Eastham *et al.* (2018) reported that animals that calved at an earlier age (22 months) had better survival rates and milk production. Additionally, calving at this age did not negatively affect the reproductive performance of the animals.

Since mammary gland development occurs before the first calving, it is crucial to maintain adequate heifer growth. However, research by L. Han *et al.* (2021), H. Kusaka *et al.* (2022), and A.V. Demchuk & L.P. Ponko (2022) suggests a negative impact of excessive weight gain on mammary gland development, leading to excessive fat deposition in the animals. In this study, heifers selected for insemination had sufficient live weight. The average live weight in Group I was 403.5 kg, which was significantly lower by 2.7% and 5.3% compared to animals inseminated at 13 and 14 months, respectively. It should also be noted that at first calving, the animals also did not have a significant difference in weight; the live weight in the experimental groups was within the range of 592.7-613 kg.

The impact of age at first calving on milk yield has been extensively studied by researchers both in Ukraine and abroad, and despite this, it remains a relevant topic. This is because this indicator is a significant factor influencing a cow's lifetime milk production. By reducing the age at first calving, it is possible to decrease maintenance costs and increase the productive life of the animals (Karlova *et al.*, 2018).

A.L. Shulyar (2019) notes that insemination at too young an age can lead to health problems in animals, complications during pregnancy and calving, and consequently, low productivity and culling rates. According to her research, the optimal age for first insemination should be 16-18 months, and the corresponding age at first calving should be 25-27 months. Heifers inseminated before 16 months had lower milk yields. Research by British scientists N.T. Eastham *et al*. (2018) indicates that cows that calved before 22 months had lower milk yields throughout their productive life compar ed to those that calved at 22 months or later. However, H. Kusaka *et al*. (2023) reported that, regardless of the age at first calving, there was no significant difference in milk yield during the first lactation, but in subsequent lactations, the milk yields of these animals decreased. Meanwhile, K.A. Copas Medina *et al*. (2022) believe that milk yield also depends on the live weight of the animals at first insemination and calving. If the live weight is low, then the milk yield in the first lactation is also lower, but it levels out in subsequent lactations.

Some of the current findings align with previous research, as there were no significant differences in 305-day milk yields during the first lactation across all three groups. However, the Group of animals that calved at 21 months had the lowest milk yields in the subsequent three lactations, while cows that calved at 22 months, conversely, had the highest yields. The difference between these groups was 4% in the second lactation, 7.8% in the third, and 4.7% in the fourth. Thus, age at first insemination had a greater impact on milk yield than live weight, as the youngest animals had a lower live w eight but their milk yield did not increase significantly with age. The research of S.G. Pishchan (2022) also shows that heifers inseminated at 13.4 months had milk yields 6.83% and 5.90% higher compared to those inseminated at 16.3 and 18.9 months, respectively. He also noted that animals with early insemination had a significant advantage in terms of milk fat and protein content. S.S. Kramarenko (2024) confirms that the highest content of these components in milk was observed in cows that were inseminated at a young age (13-15 months). The results of this study suggest that the age at first insemination did not have a significant impact on milk fat content, as there was no significant difference between Groups I, II, and III. However, it should be noted that the highest value for this indicator (3.91) was in Group II during the third lactation.

Therefore, research into the impact of age at first insemination on animal productivity remains relevant, as there is a divergence of opinions among different authors regarding the optimal age for this parameter. According to the results of the conducted study, the optimal age for first insemination is 13 months, as cows in this Group exhibited the highest milk productivity.

Conclusions

In summary, based on the research findings, it can be concluded that insemination at 13-14 months does not have a negative impact on the reproductive capacity and productivity of animals. On the contrary, it increases the productive lifespan of the animals. However, it should be noted that the study had a small sample size. Analysis of reproductive performance indicators showed that Group I had the shortest service period during the first lactation, with differences of 9.1 and 11.1 days compared to Groups I and III, respectively. This Group also had the highest conception rate at 53.6% (Group I ↓ – 1.2%, Group III ↓ – 3.8%). The insemination index tended to increase with each lactation, with the highest value across all groups, regardless of insemination age, observed in the third lactation at 3.1. There were no significant differences in milk yield between the experimental groups during the first lactation. However, starting from the second lactation, cows with earlier insemination (at 12 months) had lower milk production. The highest milk yield in all groups was observed during the third lactation. In Group II, the 305-day milk yield was 11,916.35 kg, which was 7.8% and 1% higher compared to Groups I and III, respectively. Peak lactation, regardless of the age at first insemination, occurred in the second or third month of lactation. Groups II and III exhibited the most stable lactation curves, corresponding to Type I (strong, persistent lactation with high yields). The dynamics of fat content in milk showed an increase up to the fourth lactation. Groups II and III had the highest fat content in the third lactation, at 3.91% and 3.88%, respectively. In Group I, the highest fat content was observed in the fourth lactation (3.86%). Throughout the experimental period, the number of animals in the groups changed due to culling. At the end of the fourth lactation, there were 7 animals in Group I, 7 in Group II, and 8 in Group III. The highest number of culled animals was observed during the fourth lactation. Future research will be conducted on other dairy breeds common in Ukraine. Additionally, the impact of age at first insemination on productive lifespan and the profitability of milk production will be assessed.

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Conflict of Interest

None.

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Продуктивність корів голштинської породи за умов раннього осіменіння телиць

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Анотація. Вік першого осіменіння є важливим фактором, від якого залежить тривалість непродуктивного періоду, подальша молочна продуктивність та плодючість тварин. У зв'язку з цим метою статті було встановити вплив раннього віку осіменіння на молочну продуктивність та відтворну здатність корів. Для проведення дослідження використовували корів голштинської породи. Дослідження було здійснене на базі ТОВ «Рожнівка-Агро» в Ічнянському районі Чернігівської області. За результатами дослідження було встановлено, що жива маса піддослідних тварин при першому отеленні не мала значних відхилень і була в межах 3,3 %. У першу лактацію найкоротший сервіс-період був у тварин яких осіменяли в 13 місяців, різниця була 4,5 (група I ↓) та 3,9 днів (група III ↓). Найвищий індекс осіменіння 3,1 у всіх дослідних групах був у період третьої лактації. Молочну продуктивність досліджували за добу по місяцях лактації та виходу молочного жиру і білка. За отриманими результатами було встановлено, що в першу лактацію у всіх групах надої не мали значної різниці, у другій лактації спостерігається тенденція до їх зростання у всіх групах. Проте варто відзначити, що у тварин, яких осіменяли у 13 місяців, молочна продуктивність була вища на 4 % та 1 % порівняно з I та III групою. Пік в усіх дослідних групах спостерігався на третю лактацію. Найнижчий надій був у корів, які мали раннє осіменіння (12 місяців), різниця становила 7,8 (група II ↑) та 6,9 % (група III ↑). В розрізі лактацій вміст жиру у II групі (13 місяців) був вищим у період третьої лактації на 0,23; 0,13; 0,05 % порівняно з першою, другою та четвертою. Значних відмінностей у вмісті білка в молоці не спостерігалось – він був у межах 3,20-3,23 %. Таким чином, зменшуючи вік першого осіменіння, можна досягти вищого економічного використання тварин без зниження їхньої відтворної здатності та негативного впливу на подальшу молочну продуктивність

Ключові слова: тільність; надій; жива маса; сервіс-період; індекс осіменіння

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Changes in unsaturated fatty acids in milk under vegetative regulation in dairy cattle

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Abstract. The fatty acid content of milk serves as a reliable indicator of its quality. Changes in this parameter depend on various factors, which must be considered when implementing methods to adjust the fatty acid composition of milk. This study aimed to determine the influence of the autonomic nervous system in dairy cows on the fatty acid composition of milk. The research was

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conducted on Ukrainian Black-and-White dairy cows. Experimental groups were formed using electrocardiography to establish the tone of the autonomic nervous system. Fatty acid analysis was performed via gas chromatography, with lipid extraction conducted using the Folch method. It was observed that the proportion of myristoleic acid in the experimental group of vagotonic cows was 6.9% higher than that of normotonic cows ($P < 0.05$). The content of palmitoleic acid in the milk of vagotonic cows, according to chromatographic analysis, was 11.6% higher ($P < 0.01$) and in sympathotonic cows 5.8% higher ($P < 0.05$) compared to normotonic cows. The oleic acid content in the milk of vagotonic cows was 4.2% higher ($P < 0.01$) and in sympathotonic cows 1% higher ($P < 0.05$) compared to normotonic cows. The linoleic acid content in the milk of vagotonic cows was 6.1% higher ($P < 0.001$) and in sympathotonic cows 31.7% higher ($P < 0.001$) than that of normotonic cows. Conversely, the α-linolenic acid content in vagotonic cows was 38.5% lower ($P < 0.01$) and in sympathotonic cows 34.6% lower ($P < 0.01$) than in normotonic cows. These findings indicated that the experimental group of normotonic cows had the lowest levels of unsaturated fatty acids in milk

Keywords: ruminants; lipids; chromatography; cow's milk; milk production

Introduction

Quality control of dairy products is a priority for every farm because, according to G. Gużewska *et al.* (2024), maintaining stable and high profits depends significantly on qualitative and quantitative indicators. Timely identification of changes in milk's constituent elements and making appropriate adjustments prevents considerable economic losses, as milk is produced daily on dairy farms. For the production of milk and dairy products, raw materials must be of high quality, assessed through various technological processes, including chemical and physicochemical factors, such as the fatty acid profile, fat content, protein-to-fat ratio, urea content, acidity, dry matter content, and evaluation of milk fat globules by size and density. One of the most common methods for assessing product quality is determining fatty acid composition via chromatography (Wu *et al.*, 2023).

Modern milk production increasingly focuses on optimising initial milk quality indicators rather than merely increasing total volume and fat content, though these remain relevant objectives. According to M. Salvian *et al.* (2023), the trend in dairy production is shifting away from considering milk weight alone and towards a more detailed emphasis on gross energy value. This approach enables a better assessment of product quality, particularly for raw materials sourced from other countries. Since each country has distinct native breeds and variations in feed and pasture conditions, the effective evaluation of dairy products should be based on comprehensive data (Ali *et al.*, 2023).

Various feed additives are used to improve the quality of the final product, and they exhibit a measurable degree of effectiveness. Improving the fatty acid composition of milk is among the primary objectives, with particular emphasis often placed on unsaturated fatty acids due to their beneficial impact on energy balance and their role in lipid metabolism regulation. It should be noted that the concentration of unsaturated and saturated fatty acids in milk may change during technological processing. According to R. Fan *et al.* (2023), heat treatment processes for milk, including pasteurisation and ultra-high temperature treatment, significantly affect its nutritional components. Studies of milk and dairy products subjected to heat treatment revealed a tendency for an increase in saturated fatty acids. When exposed to high temperatures, the ratio of long-chain fatty acids decreased, while the proportion of medium-chain fatty acids increased. These changes in the fatty acid composition of milk and dairy products underscore the need to develop strategies for enhancing the content of unsaturated fatty acids to improve the nutritional value of raw materials after heat treatment. When examining methods for correcting lipid metabolism, factors regulating these processes must be considered. One example is the autonomic nervous system, which plays a crucial role in maintaining homeostasis within the body.

The study of mechanisms to improve metabolic processes in cows has the potential to significantly increase livestock productivity and enhance product quality to meet consumer demands. Moreover, research into the mechanisms underlying the regulation of metabolic processes will support the development of more effective solutions to challenges encountered in production.

Literature Review

Y. Gao *et al.* (2024) note that milk is an essential component of the daily diet due to the presence of over ten major nutritional components, including protein, lipids, vitamins, and minerals. Depending on the type of animal, the composition of milk and the ratio of key components vary. The presence of these essential nutritional components ensures optimal development of systems such as the nervous and immune systems, which significantly enhance the value of dairy products. D. Brozić *et al.* (2024) emphasised that achieving high productivity in a dairy herd and ensuring the production of quality raw materials require active attention to animal development, even during the foetal stage. Well-developed livestock will ensure a steady supply of high-quality products, ultimately increasing farm profitability. Balanced nutrition for cows during pregnancy, including the mandatory inclusion of high levels of fatty acids, improves the development and formation of the foetus, particularly the central nervous and immune systems. Additionally, it should be noted that after calving, the cow's body must contain sufficient highenergy compounds such as lipids to supply the newborn with adequate energy.

During the cow's growth and development, it is essential to balance lipid intake in the diet because hormonal and metabolic changes can lead to oxidative stress or significant mobilisation of body reserves, which, according to X. Sun *et al.* (2023), can impair lactogenesis and reduce productivity. To address this issue, various nutritional supplements based on unsaturated fatty acids or probiotics are frequently used to enhance rumen digestion. L. Phuoc Thanh *et al.* (2023) found that feed additives containing substantial amounts of polyunsaturated fatty acids resulted in a reduction in saturated fatty acids compared to the control group. Additionally, a decrease in the atherogenicity and thrombogenicity indices was observed. Overall, the total concentration of volatile fatty acids in the rumen decreased, leading to improved initial milk quality.

N. Urrutia *et al.* (2023) and A. Mohammed *et al.* (2024) investigated the use of dietary fatty acid supplements, specifically those with a high concentration of omega-3 polyunsaturated fatty acids, by incorporating flaxseed into the feed. According to the initial results of lipid metabolism studies, the levels of fatty acids in the blood showed significant improvement, and an enhancement in rumen digestion was also observed. Chromatographic analysis of the fatty acid composition of lipids revealed a reduction in the omega-6 to omega-3 fatty acid ratio. In terms of total fatty acid concentration, a significant decrease in both short-chain and long-chain saturated fatty acids was observed. Moreover, an increase in milk production was reported in cows consuming feed enriched with polyunsaturated fatty acids.

In the study of metabolic processes, in addition to examining the organic substances that contribute to overall homeostasis, attention is often directed towards the systems that regulate these processes. A. Furlan & P. Petrus (2023) highlighted an important mechanism within organised biological systems: the connection between the brain and the body in regulating metabolic processes. It has been established that any process in a living organism is influenced by corrective systems, with the neurohumoral system playing a central role. This system encompasses a multifaceted network of components that process, analyse, and regulate numerous interrelated physiological processes. One component frequently emphasised concerning homeostasis regulation is the autonomic nervous system. This system, with its subdivisions – the sympathetic and parasympathetic nervous systems – controls processes such as digestion and the fundamental mechanisms of nutrient synthesis and breakdown. G. Mishra & K.L. Townsend (2023) emphasised the close relationship between the autonomic nervous system and lipid metabolism. They noted that as a result of the direct influence of this nervous system on nutrient synthesis and resynthesis, the sympathetic nervous system mobilises lipids from adipose tissue when energy-rich substances are required. This process is exemplified by the body's response to stress factors.

Materials and Methods

The research was conducted on the dairy farm of Obriy LLC from 2023 to 2024, involving five cows of the Ukrainian Black-and-White dairy breed in a single experimental group. Experimental groups of animals were formed using a variational pulse oscillometric study based on the Baevsky method, employing a Heart Mirror IKO electrocardiograph (Hungary, Innomed). Based on the electrocardiogram results, the following indicators were determined: heart rate interval between R-R segments on the recorded tape, expressed in mode (Mo). The percentage value of the mode indicator, representing the most frequent occurrence in a segment of 100 heartbeats, was calculated and reflected as the mode amplitude (AMo). The difference between the maximum and minimum mode values at selected points, corresponding to the variation range (Δx), was also determined. Subsequently, key values were calculated: the autonomic balance index (ABI), which represents the difference between the mode amplitude and the variation range and characterises the influence of the sympathetic and parasympathetic nervous systems; the autonomic rhythm index (ARI), which reflects the influence of the sympathetic nervous system, was calculated using equation (1):

$$ARI=1\div(Mo\times\Delta x). \quad (1)$$

The stress index (SI), representing the animal's stress state and characterising the tension of the autonomic nervous system, was determined using equation (2):

$$SI=AMo\div(2\times Mo\times\Delta x). \quad (2)$$

The statistical analysis of the initial parameters of the variability-pulse study identified the tone of the autonomic nervous system, which corresponded to normotonia, vagotonia, and sympathotonia tones of the autonomic nervous system. Based on the obtained individual characteristics of autonomic regulation, three experimental groups were formed: normotonics, vagotonics, and sympathotonics. Blood samples were collected in the morning, four hours after feeding, in compliance with aseptic and antiseptic procedures. Samples were taken from the jugular vein using a sterile syringe containing heparin. A heparin solution was prepared in a proportion of 3 drops of 1% heparin per 10 mL of blood. The samples were transported in a thermocontainer maintained at a temperature of +4°C. Blood plasma was separated by centrifugation at 2,000 rpm for 10 minutes.

The preparation of blood plasma for the analysis of the fatty acid composition of milk via gas chromatography was performed according to the Folch method. Centrifuged blood plasma was transferred into a flask with a lapped lid, followed by the gradual addition of methanol and chloroform in a ratio of 2:1. The volume of these solutions was adjusted to the raw material under study at a ratio of 1:20. The flask was thoroughly shaken and left for 12 hours to allow extraction. After this period, the contents of the flask were filtered through filter paper. To the filtrate, 0.74% KCl was added at a ratio of 1:5 relative to the solution. After standing for 12 hours, the solution separated into two layers, with the lower layer consisting of chloroform containing dissolved lipids. The lower layer was removed, and the extracted lipids were obtained using a reflux evaporator (Folch *et al.*, 1957).

The chromatographic analysis was conducted using a Trace GC Ultra chromatograph (USA) equipped with a flame ionisation detector. The samples were chromatographed under the following conditions: detector temperature of +260°C and column temperature ranging from +140°C to +240°C. A sample of the test material was introduced into the chromatograph using a TriPlus autosampler, with an injection volume of 1 μL. The analysis time for each test sample was approximately 65 minutes. Peaks on the chromatogram were evaluated relative to the standard sample, Supelco 37 Component FAME Mix (USA). Statistical processing of the obtained data was performed using Microsoft Excel, with the significance of differences in evaluation indicators assessed via Student's t-test at $P < 0.05$, $P < 0.01$, and $P < 0.001$ levels.

All manipulations involving cattle during the experimental studies presented in this research adhered to the fundamental principles of bioethics, in compliance with Article 26 of the Law of Ukraine "On the Protection of Animals from Cruelty" (2006), the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986), and the "General Ethical Principles for Animal Experiments" adopted by the First National Congress on Bioethics (Procedure for conducting..., 2012).

Results and Discussion

According to the results of the chromatographic analysis of milk from the experimental group of sympathotonics, the following unsaturated fatty acids were identified: myristoleic, palmitoleic, oleic, linoleic, and α-linolenic acids (Table 1).

Table 1. *Statistical analysis of unsaturated fatty acids in milk from cows with normotonia*

Indicators	NVO	SE	M	SD	A	Min	Max
C14:1 Myristoleic acid	5	0.02	1.31	0.05	0.74	1.26	1.38
C16:1 Palmitoleic acid	5	0.02	1.55	0.05	-0.39	1.47	1.61
C18:1n9c Oleic acid	5	0.03	22.75	0.07	-0.52	22.65	22.84
C18:2n6c Linoleic acid	5	0.01	3.12	0.02	-0.21	3.09	3.15
C18:3n3 α-linolenic acid	5	0.01	0.26	0.03	-0.61	0.21	0.29

Notes: NVO – number of valid observations, SE – standard error, M – mean value, SD – standard deviation, A – asymmetry, Min – minimum value, Max – maximum value
Source: authors' development

After processing the chromatogram, it was found that the content of myristoleic acid ranged from 1.26% to 1.38%, with an average of 1.31 ± 0.02%. Myristoleic acid in the experimental group of cows with a balanced sympatho-vagal balance exhibited a difference of 0.12% between the maximum and minimum values, indicating a slight variation in the initial results of the experimental group. The percentage of palmitoleic acid ranged from

1.47% to 1.61%, with an average of 1.55 ± 0.02%. Palmitoleic acid in the experimental group of cows with a balanced sympatho-vagal balance exhibited a difference of 0.14% between the maximum and minimum values, again reflecting slight variation in the initial results. The percentage of oleic acid ranged from 22.65% to 22.84%, with an average of 22.75 ± 0.03%. Oleic acid in the experimental group of cows with a balanced sympatho-vagal balance showed a difference of 0.19% between the maximum and minimum values, indicating minimal variation. The percentage of linoleic acid ranged from 3.09% to 3.15%, with an average of 3.12 ± 0.02%. Linoleic acid in the experimental group of cows with a balanced sympatho-vagal balance exhibited a difference of 0.06%, reflecting slight variation. Finally, the percentage of α-linolenic acid ranged from 0.21% to 0.29%, with an average of 0.26 ± 0.02%. α-linolenic acid in the experimental group of cows with a balanced sympatho-vagal balance showed a difference of 0.06%, indicating minimal variation in the initial results.

According to the results of chromatographic studies of milk from cows with sympathotonia, the following unsaturated fatty acids were identified: myristoleic, palmitoleic, oleic, α-linolenic, and linoleic acids (Table 2).

Table 2. *Statistical analysis of unsaturated fatty acids in milk from cows with sympathotonia*

Indicators	NVO	SE	M	SD	A	Min	Max
C14:1 Myristoleic acid	5	0.04	1.27	0.08	-0.94	1.14	1.36
C16:1 Palmitoleic acid	5	0.03	1.64	0.06	0.35	1.57	1.72
C18:1n9c Oleic acid	5	0.03	22.89	0.08	-0.71	22.78	22.98
C18:2n6c Linoleic acid	5	0.04	4.11	0.10	1.53	4.03	4.28
C18:3n3 α-linolenic acid	5	0.01	0.17	0.03	-0.69	0.12	0.21

Notes: NVO – number of valid observations, SE – standard error, M – mean value, SD – standard deviation, A – asymmetry, Min – minimum value, Max – maximum value
Source: authors' development

After processing the chromatogram, it was found that the content of myristoleic acid ranged from 1.14% to 1.36%, with an average of 1.27 ± 0.04%. Myristoleic acid in the experimental group of cows with sympathotonia exhibited a difference of 0.22% between the maximum and minimum values, which indicated a slight variation in the initial results of the experimental group. The percentage of palmitoleic acid ranged from 1.57% to 1.72%, with an average of 1.64 ± 0.03%. Palmitoleic acid in the experimental group of cows with sympathotonia exhibited a difference of 0.15%, reflecting a slight variation in the initial results. The percentage of oleic acid ranged from 22.78% to 22.98%, with an average of 22.89 ± 0.03%. Oleic acid in the experimental group of cows with sympathotonia exhibited a difference of 0.20%, indicating minimal variation. The percentage of linoleic acid ranged from 4.03% to 4.28%, with an average of 4.11 ± 0.04%. Linoleic acid in the experimental group of cows with sympathotonia exhibited a difference of 0.25%, indicating slight variation. Finally, the percentage of α-linolenic acid ranged from 0.12% to 0.21%, with an average of 0.17 ± 0.01%. α-linolenic acid in the experimental group of cows with sympathotonia exhibited a difference of 0.09%, indicating minimal variation in the initial results.

According to the results of the chromatographic study of milk from the experimental group of vagotonics, the following unsaturated fatty acids were identified: myristoleic, palmitoleic, oleic, linoleic, and α-linolenic acids (Table 3).

Table 3. *Statistical analysis of unsaturated fatty acids in milk from cows with vagotonia*

Indicators	NVO	SE	M	SD	A	Min	Max
C14:1 Myristoleic acid	5	0.02	1.40	0.04	-0.03	1.35	1.44
C16:1 Palmitoleic acid	5	0.04	1.73	0.08	-0.58	1.64	1.80
C18:1n9c Oleic acid	5	0.03	23.71	0.06	0.09	23.64	23.79
C18:2n6c Linoleic acid	5	0.02	3.31	0.05	-0.40	3.24	3.37
C18:3n3 α-linolenic acid	5	0.01	0.16	0.02	-1.03	0.12	0.18

Notes: NVO – number of valid observations, SE – standard error, M – mean value, SD – standard deviation, A – asymmetry, Min – minimum value, Max – maximum value
Source: authors' development

After processing the chromatogram, it was found that the content of myristoleic acid ranged from 1.35% to 1.44%, with an average of 1.40 ± 0.02%. Myristoleic acid in the experimental group of cows with vagotonia exhibited a difference of 0.09% between the maximum and minimum values, which indicated a slight variation in the initial results of the experimental group. The percentage of palmitoleic acid ranged from 1.64% to 1.80%, with an average of 1.73 ± 0.04%. Palmitoleic acid in the experimental group of cows with vagotonia exhibited a difference of 0.16%, reflecting a slight variation in the initial results. The percentage of oleic acid ranged from 23.64% to 23.79%, with an average of 23.71 ± 0.03%. Oleic acid in the experimental group of cows with vagotonia exhibited a difference of 0.15%, indicating minimal variation. The percentage of linoleic acid ranged from 3.24% to 3.37%, with an average of 3.31 ± 0.02%. Linoleic acid in the experimental group of cows with vagotonia exhibited a difference of 0.13%, indicating slight variation. Finally, the percentage of α-linolenic acid ranged from 0.12% to 0.18%, with an average of 0.16 ± 0.01%. α-linolenic acid in the experimental group of cows with vagotonia exhibited a difference of 0.06%, indicating minimal variation in the initial results.

When assessing the fatty acid composition of milk from cows with varying autonomic regulation, it was found that the content of myristoleic acid in animals from the experimental group exhibiting a predominance of vagotonia was higher than in those with a predominance of normotonia by 6.9% ($P < 0.05$) (Fig. 1).

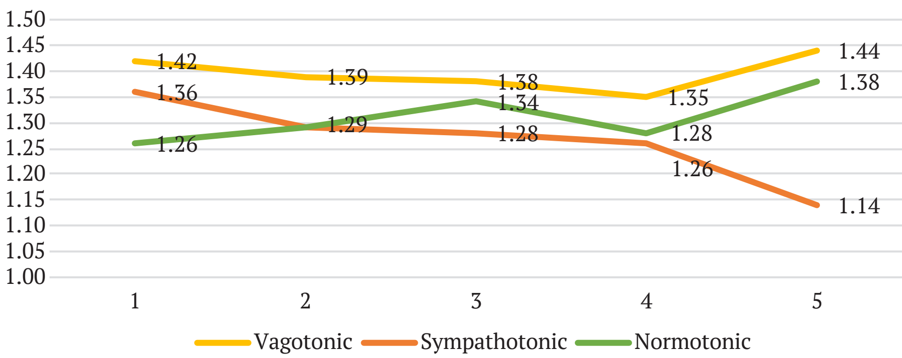

Figure 1. Percentage of myristoleic acid in cow's milk under different autonomic nervous system tones
Note: colours indicate different tones of the autonomic nervous system, respectively
Source: authors' development

When assessing the fatty acid composition of milk from cows with varying autonomic regulation, it was found that the content of palmitoleic acid in animals from the experimental group with a predominance of vagotonia was higher than in those with a predominance of normotonia by 11.6% (P < 0.01), and higher in animals with sympathotonia by 5.8% (P < 0.05) (Fig. 2).

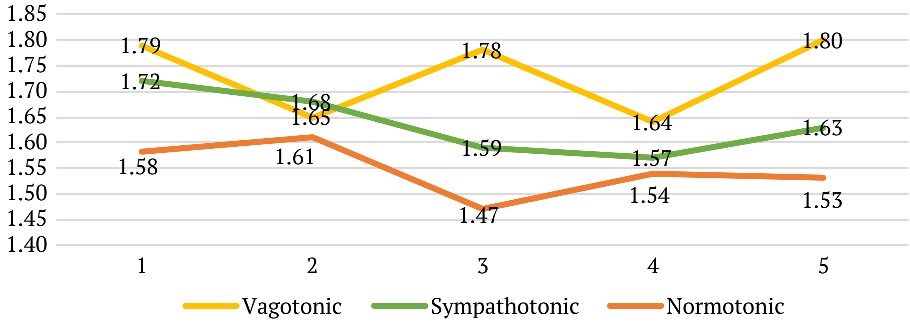

Figure 2. Percentage of palmitoleic acid in cow's milk under different autonomic nervous system tones
Note: colours indicate different tones of the autonomic nervous system, respectively
Source: authors' development

When assessing the fatty acid composition of milk from cows with varying autonomic regulation, it was found that the content of oleic acid in animals from the experimental group with a predominance of vagotonia was higher than in those with a predominance of normotonia by 4.2% (P < 0.01), and higher in animals with sympathotonia by 1% (P < 0.05) (Fig. 3).

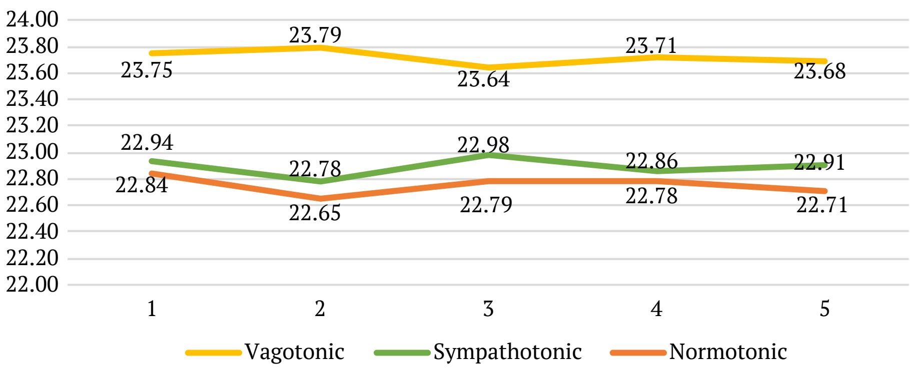

Figure 3. Percentage of oleic acid in cow's milk under different autonomic nervous system tones
Note: colours indicate different tones of the autonomic nervous system, respectively
Source: authors' development

When assessing the fatty acid composition of milk from cows with varying autonomic regulation, it was found that the content of linoleic acid in animals from the experimental group with a predominance of vagotonia was higher than in those with a predominance of normotonia by 6.1% (P < 0.001), and higher in animals with sympathotonia by 31.7% (P < 0.001) (Fig. 4).

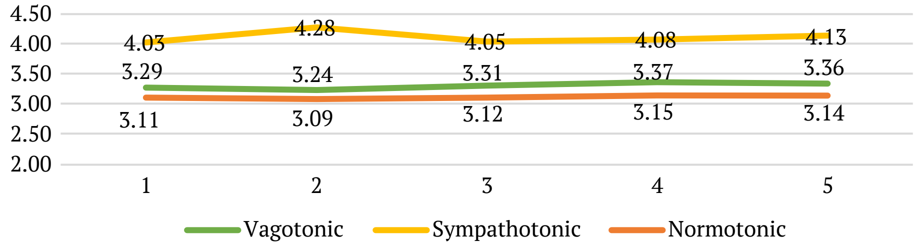

Figure 4. Percentage of linoleic acid in cow's milk under different autonomic nervous system tones

Note: colours indicate different tones of the autonomic nervous system, respectively

Source: authors' development

When assessing the fatty acid composition of milk from cows with varying autonomic regulation, it was found that the content of α-linolenic acid in animals from the experimental group with a predominance of vagotonia was higher than in those with a predominance of normotonia by 38.5% ($P < 0.01$), and higher in animals with sympathotonia by 34.6% ($P < 0.01$) (Fig. 5).

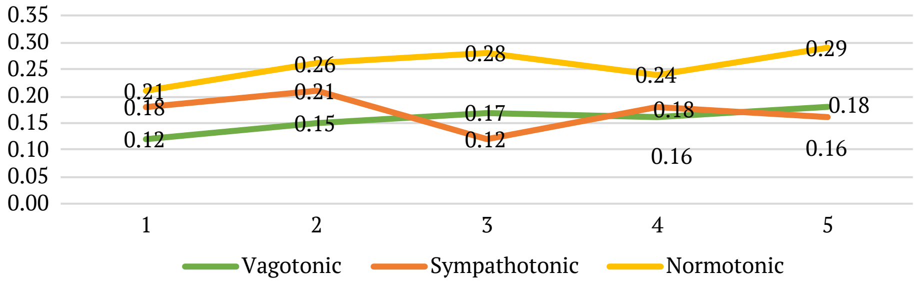

Figure 5. Percentage of α-linolenic acid in cow's milk under different autonomic nervous system tones

Note: colours indicate different tones of the autonomic nervous system, respectively

Source: authors' development

The milk fat profile is a crucial component in evaluating the quality of dairy products. Therefore, improving the composition of milk plays a key role in the management of dairy cattle. C. Loza *et al.* (2023) established that a significant percentage of the composition of milk fat depends on the level of fatty acid synthesis in the animal's body, which is significantly influenced by the feeding diet. To address the challenge of improving cow productivity and the quality of dairy products, various methods for modifying the fatty acid composition of milk through adjustments to the cows' feeding diet have been developed. S.B. Beyzi & C.Ç. Dallı (2023) formulated a cow feeding ration incorporating additives such as fish oil and microalgae. According to their results, there was an increase in the content of C16:0 and C18:1, alongside a decrease in the concentration of C18:0 ($P < 0.05$). However, there were no significant changes in the indicators of the fatty acid composition of milk, nor was there any effect on ruminal digestion parameters or blood cholesterol levels.

D. Rico *et al.* (2023) employed linseed oil to improve the fatty acid profile of milk and stabilise oxidative processes. The use of linseed oil at different dosages affected the levels of cis9, cis-12 linoleic acid and cis-9, cis-12, cis-15 α-linolenic acid, which increased proportionally to the amount of linseed oil added ($P < 0.01$). Notably, the concentrations of tetradecanoic and palmitic fatty acids decreased inversely. However, the increase in unsaturated fatty acids heightened the risk of oxidative processes in milk, which negatively impacts dairy producers. M. Neofytou *et al.* (2023) used olive oil production by-products, namely olive cake, to improve lipid metabolism in animals. The inclusion of this component had a positive effect on milk composition, as it led to an increase in monounsaturated fatty acids, proportional to the concentration of olive cake included. A decrease in saturated fatty acids from C4:0 to C16:0 was also observed. However, no changes were recorded in the total milk yield or milk fat and protein content.

R. Pierce *et al.* (2024) investigated the dietary supplementation of cottonseed in cows to improve milk yield and the fatty acid profile. Their research established that the addition of cottonseed to the diet of cows did not enhance milk fat content or improve milk productivity in multiparous cows. However, a significant increase was observed in first-lactation cows, reflected in a marked rise in milk fat proportional to the volume of the applied food supplement. A. Shazly *et al.* (2023) conducted a study on the use of food additives such as soybean oil, linseed oil, and their combination to regulate fatty acid metabolism in animals, subsequently influencing the lipid profile of milk. Their findings revealed that the content of unsaturated fatty acids, such as C18:1 and C18:2, increased, while the percentage of saturated fatty acids, including C4:0, C14:0, and C16:0, decreased. The study concluded that there was an improvement in the ratio of unsaturated to saturated fatty acids.

Despite the positive effects of vegetable-based food additives on the fatty acid composition of milk, it is worth noting that high levels of unsaturated fatty acids are not always advantageous for dairy products. The shelf life of milk is significantly shortened due to oxidative processes, negatively impacting farm productivity. Furthermore, when incorporating various nutritional supplements into the diet of high-yielding cows, the role of the rumen microbiota must be considered, as highlighted by L. Coates *et al.* (2023). According to H. Hou *et al.* (2023) and W. Lou *et al.* (2024), it is essential to consider the predictive modelling of the fatty acid composition of milk to enhance the effectiveness of methods aimed at improving lipid metabolism in animals. The authors emphasise that successful predictions of fatty acid metabolism require extensive laboratory research, which must be integrated with advanced techniques such as high-precision liquid chromatography. However, these methods are financially burdensome for the agricultural sector.

When evaluating scientific literature on the correction and study of the lipid profile of milk, it is important to note that while some methods yield positive results in influencing the fatty acid composition, excessive inclusion of unsaturated fatty acids can result in negative consequences, including impaired digestibility of these fatty acids. Furthermore, disturbances in stable metabolism may adversely affect the rumen microflora, potentially leading to excessive carbonation of its contents. It should also be noted that in most scientific studies, limited attention is given to the systems involved in correcting lipid metabolism. Neglecting this aspect can significantly impact the reliability of baseline data when evaluating the exchange of energetically valuable substances, which may lead to considerable variability in results, complicating the statistical analysis of laboratory findings. The studies conducted by M. Hur *et al.* (2023),

W. Ren *et al.* (2023) and E. Li *et al.* (2024) provide a strong example of how the autonomic nervous system influences lipid metabolism, particularly the role of its components in lipogenesis and lipolysis. By analysing the results of milk fatty acid profiles using gas chromatography, these authors demonstrated a clear difference in the content of unsaturated fatty acids among the experimental groups. Specifically, depending on the influence exerted by the divisions of the autonomic nervous system – namely, the sympathetic and parasympathetic nervous systems – the intensity of metabolic processes in cows' bodies vary. This variation subsequently affects the lipid content of milk, which is synthesised in the mammary gland under the regulation of the body's homeostatic control systems.

Based on the obtained results, the authors emphasise the importance of analysing the activity of the autonomic nervous system to better predict lipid metabolism in cows' bodies and optimise feeding rations to address differences in metabolic processes.

Conclusions

The influence of the divisions of the autonomic nervous system, namely the parasympathetic and sympathetic nervous systems, on the parameters of unsaturated fatty acids, has been established, reflecting differences in the metabolic processes of lipid exchange in cows with varying sympatho-vagal balance. According to the initial data, differences were observed among the experimental groups of animals in the content of palmitoleic acid, myristoleic acid, linoleic acid, oleic acid, and α-linolenic acid.

It was determined that in cows with a predominance of vagotonia, the content of myristoleic acid was higher by 6.9% ($P < 0.05$) compared to animals with a balanced sympathovagal balance. Palmitoleic acid levels were higher in cows with a predominance of sympathotonia by 5.8% ($P < 0.05$) and in cows with a predominance of parasympathetic nervous system influence by 11.6% ($P < 0.01$) compared to the experimental group with normotonia. Oleic acid levels were higher in cows with a predominance of sympathotonia by 1% ($P < 0.05$) and in cows with a predominance of vagotonia by 4.2% ($P < 0.01$) compared to the experimental group with a balanced sympatho-vagal balance. Linoleic acid levels were higher in cows with a predominance of sympathotonia by 31.7% ($P < 0.001$) and in cows with a predominance of vagotonia by 6.1% ($P < 0.001$) compared to the experimental group with a balanced sympatho-vagal balance. Conversely, α-linolenic acid levels were lower in cows with a predominance of sympathotonia by 34.6% ($P < 0.01$) and in cows with a predominance of vagotonia by 38.5% ($P < 0.01$) compared to the experimental group with a balanced sympatho-vagal balance.

The future prospects for subsequent studies involve the use of biologically active additives, including nanopreparations, to improve lipid metabolism and cow productivity, while considering individual characteristics related to the tone of the autonomic nervous system.

Acknowledgements

None.

Conflict of Interest

None.

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Зміни показників ненасичених жирних кислот у молоці за вегетативної регуляції в корів молочного напрямку

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Анотація. Вміст жирних кислот у молоці є гарним показником якості продукції. Зміна даного показника залежить від багатьох факторів, що варто враховувати при застосуванні методів корекції жирнокислотного складу молока. Мета дослідження полягала у визначенні впливу на показники жирнокислотного складу молока автономної нервової системи в організмі корів молочного спрямування. Дослідження виконували на коровах породи українська чорно-ряба молочна. Формування дослідних груп виконувалося за допомогою електрокардіографії і встановлення тонусу автономної нервової системи. Жирнокислотний аналіз виконувався за допомогою газової хроматографії, екстракцію ліпідів виконували за методикою Фолча. Встановлено, що відсоткове співвідношення мірістоолеїнової кислоти у дослідної групи ваготоніків було більше в порівнянні з коровами, що мають нормотонію, на 6,9 % ($P < 0,05$). Вмісту пальмітолеїнової кислоти за результатами хроматографічного дослідження в молоці корів було більше у тварин з ваготонією на 11,6 % ($P < 0,01$) та у симпатотоніків на 5,8 % ($P < 0,05$) відносно корів з нормотонією. Вмісту олеїнової кислоти за результатами хроматографічного дослідження у молоці корів було більше у тварин із ваготонією на 4,2 % ($P < 0,01$) та у симпатотоніків на 1 % ($P < 0,05$) відносно корів із нормотонією. Вмісту лінолевої кислоти за результатами хроматографічного дослідження у молоці корів було більше у тварин із ваготонією на 6,1 % (P < 0,001) та у симпатотоніків на 31,7 % (P < 0,001) відносно корів із нормотонією. Вмісту α-ліноленової кислоти за результатами хроматографічного дослідження у молоці корів було менше у тварин із ваготонією на 38,5 % (P < 0,01) та у симпатотоніків на 34,6 % (P < 0,01) відносно корів із нормотонією. Згідно з отриманими результатами варто відмітити, що дослідна група нормотоніки мала найменші показники вмісту ненасичених жирних кислот у молоці

Ключові слова: жуйні; ліпіди; хроматографія; коров'яче молоко; виробництво молока

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Meat quality estimates of rabbit as affected by feeding and processing kind

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Abstract. Rabbit meat is of high demand in Nigeria due to its valued nutritional composition and pleasant sensory quality. The purpose of this study was to evaluate the impact of processing methods and feeding type on the nutritional quality of rabbit meat. Three sets of weaned rabbits were subjected to three types of feeding regimes and processing methods and evaluated for vitamins, cholesterol, and proximate composition in a Randomised Complete Block Design experiment. Mean vitamin B1 varied from 0.092 mg $(100\ g)^{-1}$ for cooked rabbit fed with forage to 0.105 mg $(100\ g)^{-1}$ for uncooked rabbit fed with forage with all variances being almost zero. The result of the cluster analysis of the vitamin contents of the rabbit meat indicated that roasted- rabbit fed with forage and cooked rabbit fed with forage and concentrate clustered at the earliest agglomeration distance of 0.128. This was followed by the clustering of cooked rabbit fed with forage and cooked

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rabbit fed with concentrates at the agglomeration distance of 1.941 and the farthest agglomeration distance of 24.973 was obtained for cooked rabbit fed with forage and roasted rabbit fed with forage. Mean protein of the rabbit meat fell between 17.971 for uncooked rabbit fed with concentrates and 31.489 for cooked rabbit fed with forage and concentrates and the variances forms 2 groups (homoscedastic and independent). The results of the general linear model analysis returned $F_{(8,18:0.05)} = 34.58$, 55.839.90, 17.75, 23.11, 20.85, 12.32 and 12.36 for ash content, protein, moisture content, fat, crude fibre, carbohydrate and energy that were significant ($P < 0.05$). Roasting and foraging as established in the present study enhanced nutritional quality of rabbit meat and these offer practical value by addressing consumer demand, advancing quality standards in agricultural production, and recommending optimal processing methods

Keywords: nutrients; homoscedastic; variance; rabbit keeping enterprise; rabbit farming

Introduction

Rabbit meat produced in both intensive and extensive farming systems is valued for its unique properties and high nutritional value. Rabbit is considered a meat producing animal based on its short life cycle, gestation period, and feed conversion ratio. The technological properties and nutritional profile of rabbit are of industrial value as rabbit can be a good raw material for processing, and can be incorporated into value added products for consumers' satisfaction and healthy living.

As reported by T. Daszkiewicz & A. Gugołek (2020), rabbit meat is considered healthier than the other meat traditionally used in the meat products industry due to its specific nutritional characteristics of low fat with a favourable proportion of saturated, monounsaturated. and polyunsaturated fatty acids, low cholesterol and sodium. It is very high in protein (over 30% and approximately 80% of the energy value) with a high share of essential amino acids (lysine, sulphur-containing amino acids, threonine, valine, isoleucine, leucine, and phenylalanine) minerals and vitamins (potassium, phosphorus, selenium, and vitamin B) compared with most types of available meat for consumption, as reported by W. Akindele & A. Adeosun (2022). The consumption of rabbit meat, according to S. Amer *et al.* (2019), has been recommended as a healthier source of dietary protein in the developing countries. Rabbit meat has a high content of B vitamins, in particular B3 and B12 and a low content of sodium, purines and uric acid, according to B. Suvajdžić *et al.* (2023). Rabbit meat has been found to be useful in production of functional foods by B. Pang *et al.* (2020).

Effect of diets composition and feeding systems on the nutritional quality of rabbit meat have been documented by S. Siddiqui *et al.* (2023) and M. Teye *et al.* (2020). This has led to geared efforts in modifications in rabbit diets to meet its adequate nutritional value. This can allow positive exploitation of nutritional aspects of rabbit meat which well fits the current consumer demand for a low-fat meat with a high unsaturation degree of fatty acids and low sodium, cholesterol, and haeme-iron levels. According to G. Frunză *et al.* (2023), it also contribute to balanced diet with possible preventive effects on chronic non-deficiency diseases like obesity, type 2 diabetes, cancer, and cardiovascular diseases. Similarly, feeding practices have been found to play a crucial role in determining the nutritional composition, flavour, and overall quality of meat. Different feed types can affect the growth rate, carcass characteristics, and fat content, ultimately influencing consumer preferences. Additionally, the processing methods employed post-harvest can further modify the meat's sensory attributes, shelf life, and safety.

According to S. Kumar *et al.* (2023), meat quality is determined by several factors, including: nutritional qualities, such as the right amounts of proteins, lipids, and bioactive compounds; sensory attributes, like appearance, texture, and flavour; health benefits, which are dependent on fat and saturated fatty acid (SFA) content, mineral content, and technological factors like processing. Variability in vitamin, cholesterol, nutrient and proximate content of rabbit meat as affected by feed and cooking styles can have significant implications for human nutrition. Understanding how different feeds and cooking methods impact these rabbit meat qualities can help inform dietary choices and promote better health outcomes. Additionally, this research can also provide valuable insights for producers and chefs looking to maximise the nutritional value of rabbit. The purview of this study would cover both the nutritional and health factors including the mineral content, vitamins and cholesterol content, and proximate composition of the rabbit meat as affected by processing and feeding types. The purpose of this study was to assess the effect of three types of feeding regimes and cooking type on the nutritional quality of rabbit meat to improve the potential of rabbit keeping enterprise (RKE).

Materials and Methods

Three sets of weaned Hyela Rabbit at the age of five (5) months were obtained from Onileola Rabbit Farm in Ede, Osun State, Nigeria. The rabbits were housed in semi closed wire cages in three tiers batteries pen made from aluminium nets and poles in the rabbitry. The environmental conditions were adequately monitored to ensure welfare of the rabbits according to Standard guidelines. All the cages were equipped with feeding hoppers and drinking nipples. Each tier of cages was built over separate fecal and urine collectors to maintain high level hygiene and safe collection of rabbit waste for laboratory analyses. The rabbits were fed on three types of feeding regime. The first group of rabbits was fed on forages like Moringa Leaves (Moringa oleifera, Centro/Butterfly peas (*Centrosema pubescens*); Wild sunflower (*Aspilia africana*); Sweet potato Vines and Leaves (*Ipomea batatas*); Sunflower (*Helianthus annuus*); and Milkweed (*Euphorbia heterophyla*). The second group was fed on concentrates called Rabbit Pellet. The concentrate contains Wheat bran, Groundnut meal, Palm kernel meal, Soyabean meal, Cassava peel, Maize, Rice hulls, Sodium Bicarbonate, Salt (Sodium Chloride), Limestone, L-lysine HCl, Toxin Binder, DL-methionine, and Vitamin Premix. While the third group was fed on combination of forage and concentrates, respectively. The rabbits were fed for 90 days after which one rabbit from each group was slaughtered according to the standard guidelines for euthanising rabbits after 24 hour fasting (Institutional Animal Care and Use Committee (IACUC), United States, and Guidelines for the safe handling of wildlife and wildlife products as established by Z. Greatorex *et al.* (2021) and Regulation (EU) 2019/1010 of the European Parliament and of the Council (2019). The slaughtered rabbits were skinned and appropriate parts were packaged and iced in freezer for subsequent processing and laboratory analysis. The favourable environmental conditions were adequately monitored and all the cages were equipped with feeding hoppers and drinking nipples. The study lasted 90 days after which a rabbit from each set was culled and slaughtered according to the standard guidelines for euthanising rabbits after 24h fasting. The slaughtered rabbits were skinned at the slaughter slab of the farm and appropriate parts were packaged and iced in freezer for subsequent laboratory analysis. The study employed a Randomised Complete Block Design that comprise of three feeding regimes (forage, concentrates, and forage with concentrates) as treatments and three processing types (cooking, roasting, and raw) as the blocks. Two parts of meat flesh of thigh of each of the rabbits was subjected to cooking and roasting while the third part was left unprocessed (raw). The experimental materials were designated as RRFF – roasted rabbit fed with forage, RRFC – roasted rabbit fed with concentrates, RRFFC – roasted rabbit fed with both forage and concentrates; CRFF – cooked rabbit fed with forage, CRFC – cooked rabbit fed with concentrates, CRFFC – cooked rabbit fed with both forage and concentrates; URFF – unprocessed rabbit fed with forage, URFC – unprocessed rabbit fed with concentrates, and URFFC – unprocessed rabbit fed with both forage and concentrates.

Proximate composition and mineral content of the rabbit samples were determined using AOAC (2020) method while the cholesterol content was determined using Liebermann-Burchard method described by T. Daszkiewicz & A. Gugołek (2020). The experimental materials were designated as RRFF – roasted rabbit fed with forage, RRFC – roasted rabbit fed with concentrates, RRFFC – roasted rabbit fed with both forage and concentrates, CRFF – cooked rabbit fed with forage, CRFC – cooked rabbit fed with concentrates, CRFFC – cooked rabbit fed with both forage and concentrates, URFF – unprocessed rabbit fed with forage, URFC – unprocessed rabbit fed with concentrates, and URFFC – unprocessed rabbit fed with both forage and concentrates. Data on vitamin A, B1, B2, E, cholesterol, proximate composition including ash content, protein, moisture content, fat, crude fibre, carbohydrate, energy, and mineral components (P, Na, K, Ca, Fe, and Zn) were ethically collected from the animals. The data were subjected to descriptive and inferential statistics, cluster analysis, and general linear model (GLM) analysis using SAS (version 13). The main source of variation for the GLM analysis were the processing and feeding type with 2 (3-1) degree of freedom for both treatment and block while mean of the significantly different variables were separated using Duncan Multiple Range Test (DMRT).

Results

Overview of the data for the rabbit meat parameters (vitamins and cholesterol)

The results of the descriptive statistics analysis of the vitamins and cholesterols contents of the rabbit meat showed that no two samples had mean values for vitamin A that were comparable. The mean vitamin A values were homoscedastic (same variances) and ranged from 0.393 mg (100 g)$^{-1}$ for RRFC to 0.916 mg (100 g)$^{-1}$ for CRFC (Table 1).

Table 1. *Descriptive statistics of the vitamins and cholesterol of the rabbit meat*

Sample	VITA (mg/100 g)		VITB1 (mg/100 g)		VITB2 (mg/100 g)		VITE (mg/100 g)		CHOLE (mg/100 g)	
	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance
CRFF	0.725	0.00013	0.092	0.00001	0.265	0.00005	0.133	0.0000012	30.780	2.56551
RRFF	0.566	0.00013	0.104	0.00000	0.327	0.00005	0.146	0.0000015	23.790	4.01595
URFF	0.432	0.00013	0.105	0.00000	0.335	0.00005	0.093	0.0000015	61.570	0.47472
CRFC	0.916	0.00000	0.095	0.00000	0.319	0.00005	0.109	0.0000005	54.256	0.04340
RRFC	0.393	0.00013	0.103	0.00000	0.314	0.00005	0.137	0.0000005	37.151	0.25228
URFC	0.539	0.00000	0.099	0.00000	0.727	0.00009	0.104	0.0000022	64.088	0.29387
CRFFC	0.572	0.00013	0.102	0.00000	0.453	0.00002	0.125	0.0000022	52.325	0.63296
RRFFC	0.452	0.00013	0.095	0.00000	0.289	0.00014	0.132	0.0000094	30.779	17.76620
URFFC	0.758	0.00000	0.102	0.00000	0.662	0.00006	0.096	0.0000020	42.947	0.68359

Note: CRFF – cooked rabbit fed with forage, RRFF – roasted rabbit fed with forage, URFF – unprocessed rabbit fed with forage, CRFC – cooked rabbit fed with concentrate, RRFC – roasted rabbit fed with concentrate, URFC – unprocessed rabbit fed with concentrate, CRFFC – cooked rabbit fed with forage and concentrate, RRFFC – roasted rabbit fed with forage and concentrate, URFFC – unprocessed rabbit fed with forage and concentrates
Source: experimental data

According to Table 1, the mean vitamin B1 varied from 0.092 mg $(100\ g)^{-1}$ for CRFF to 0.105 mg $(100\ g)^{-1}$ for URFF, with all variances being almost zero. The samples CRFF, RRFF, CRFC, and RRFC were homoscedastic, while URFC, CRFFC, RRFFC, and URFFC were heteroscedastic. The mean vitamin B2 fell between 0.265 mg $(100\ g)^{-1}$ for CRFF and 0.727 mg $(100\ g)^{-1}$ for URFC (Table 1). Mean vitamin E ranged from 0.093 for URFF to 0.146 obtained for both RRFF while the variances were very low and ranged between 0.5×10^{-7} for CRFC and 0.94×10^{-6} for RRFFC. Some of the vitamin E samples of the rabbit meat were the same (Table 1). RRFF had the least cholesterol of 23.790 while the highest cholesterol of 64.088 was obtained for URFC and no 2 rabbit meat samples returned the same cholesterol values (Table 1). The variance was inversely related to the mean cholesterol and they are heteroscedastic. The results indicate that processing (roasting or cooking) has significant impact on the vitamin B1 and B2 and cholesterol obtained from the rabbit meat. The recorded values of vitamin B1, vitamin B2, and vitamin E in the present study further emphasise the potential of rabbit meat as a valuable dietary source.

General linear model (GLM) analysis and mean separation of the vitamins and cholesterols of the rabbit meats

The general linear model analysis of the vitamins and cholesterol of the rabbit meat returned means vitamins that are statistically significant ($P < 0.05$ – Table 2). The $F_{(8,18:0.05)}$ = 1,012.72, 85.84, 1,467.65, 371.91 and 213.71 obtained respectively for vitamin A, B1, B2, E, and cholesterol were significant. Mean vitamin A were partitioned into eight significantly different classes. Mean vitamin A obtained for rabbit meat of CRFC (0.916) was significantly the highest and it is higher than mean vitamin A obtained for rabbit meat of URFC (0.758 – Table 2). The least mean significantly different vitamin A was obtained for RRFC (0.393) and was significantly lesser than mean significantly different mean vitamin A obtained for sample URFF (0.432). Mean vitamin B1 obtained for sample URFF was significantly the highest and not statistically different from mean vitamin B1 obtained for RRFF. Mean vitamin B1 obtained for CRFF (0.092) was significantly the least and it is statistically lesser than mean vitamin B1 (0.095) obtained for both RRFC and RRFFC.

Table 2. *General linear model analysis and mean separation of the vitamins and cholesterol*

Treatment	VIT.A	VIT.B1	VIT.B2	VIT.E	CHOLESTEROL
F-statistics	1,012.72**	85.84**	1,467.65**	371.91**	213.71**
DF	8	8	8	8	8
CRFF	0.725^{c}	0.092^{f}	0.265^{g}	0.133^{c}	30.780^{e}
RRFF	0.566^{d}	0.104^{ab}	0.327^{de}	0.146^{a}	23.790^{f}
URFF	0.432^{g}	0.105^{a}	0.335^{d}	0.093^{g}	42.947^{c}
CRFC	0.916^{a}	0.095^{e}	0.319^{e}	0.109^{e}	54.256^{b}
RRFC	0.393^{h}	0.103^{bc}	0.314^{e}	0.137^{b}	37.151^{d}
URFC	0.539^{e}	0.099^{d}	0.727^{a}	0.104^{f}	64.088^{a}
CRFFC	0.572^{d}	0.102^{c}	0.453^{c}	0.125^{d}	52.325^{b}
RRFFC	0.452^{f}	0.095^{e}	0.289^{f}	0.132^{c}	30.779^{e}
URFFC	0.758^{b}	0.102^{c}	0.662^{b}	0.096^{g}	61.570^{a}

Note: CRFF – cooked rabbit fed with forage, RRFF – roasted rabbit fed with forage, URFF – unprocessed rabbit fed with forage, CRFC – cooked rabbit fed with concentrate, RRFC – roasted rabbit fed with concentrate, URFC – unprocessed rabbit fed with concentrate, CRFFC – cooked rabbit fed with forage and concentrate, RRFFC – roasted rabbit fed with forage and concentrate, URFFC – unprocessed rabbit fed with forage and concentrates
Source: experimental data

Duncan Multiple Range Test (DMRT) partitioned mean vitamin B2 and E into 7 significantly different classes. As per vitamin B2, mean vitamin B2 obtained for URFC (0.727) was significantly the highest and higher than mean vitamin B2 obtained for URFFC meat (0.662 – Table 2). Mean vitamin B2 for CRFF meat was significantly the least (0.265) and it is significantly lesser than vitamin B2 for rabbit meat RRFFC (0.289 – Table 2). Other rabbit meat samples formed significantly different classes between these 2 groups (the significantly highest and significantly lowest classes). Conversely, mean vitamin E for RRFF meat was significantly the highest (0.146) and was significantly higher than vitamin E for RRFC meat (0.137). Vitamin $E_{(CRFF,\ CRFFC\ \&\ RRFFC)} = 0.132 >$ vitamin $E_{(CRFC)} = 0.109 >$ Vitamin $E_{(URFC)} = 0.104 >$ vitamin $E_{(URFF\ \&\ URFFC)} = 0.094$ & 0.096 (Table 2). Highest mean cholesterol (64.088 and 61.57) was obtained for both URFC meat and URFFC meat respectively and were significantly higher than mean cholesterol obtained for CRFC (54.256) and CRFFC (52.325 – Table 2).

The least significantly different mean cholesterol was obtained for RRFF (23.79) while other rabbit meat samples formed independent classes between these 2 groups (highest and least significantly different groups – Table 2). It is evident that rabbit meats fed with forage enhance/retain vitamin B1 and vitamin E with reduced cholesterol. Neither roasting nor cooking enhances any of the vitamins as the raw meats were found to be richer in the vitamins evaluated.

Overview of the data for the rabbit meat parameters (proximate content)

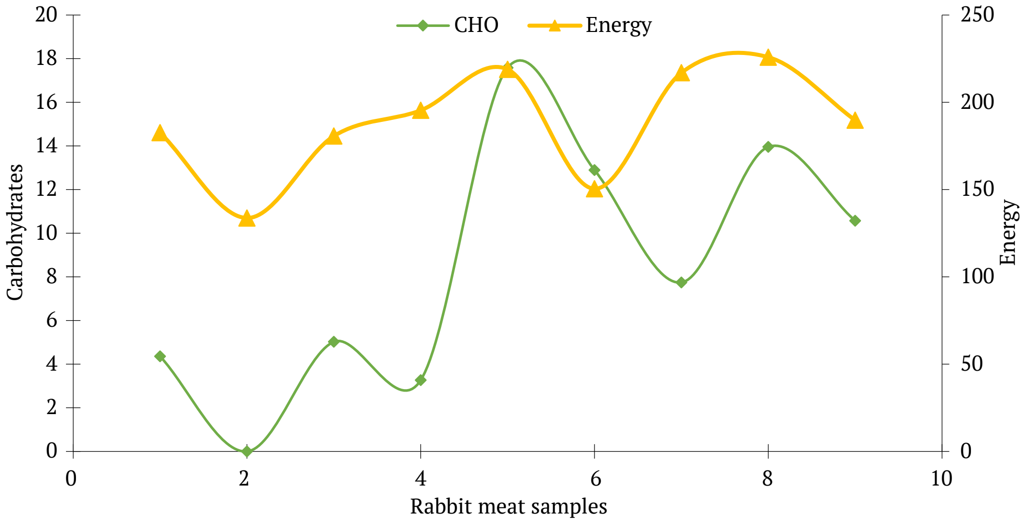
The results of summary statistics analysis for proximate composition indicated that most of the meat samples (CRFF, RRFF, CRFFC, URFF, URFC, and URFFC) returned same ash content of 1.500 and 0.500. Rabbit meat samples in these 2 groups have zero variances while others not in the group sample are homoscedastic (with the same variance of 0.083 – Table 3). The mean ash content obtained for the homoscedastic meat samples were 1.667, 1.833, and 1.667 for CRFC, RRFC, and RRFFC, respectively. The unprocessed rabbit meats returned the least ash content implying that they might likely contain the lower minerals than the processed rabbit samples. Mean protein of the meat falls between 17.971 for URFC and 31.489 for CRFFC. Rabbit meat samples CRFF, RRFF, RRFC, CRFFC, and RRFFC returned the same variance of 0.001, while rabbit samples URFF, URFC, and URFFC returned the same variance of 0.002 (Table 3).

Table 3. *Summary statistics of the proximate component analysis of the rabbit meat*

	_ASH		_PROTEIN		_MOISTURE		_FAT		_CRUFIB	
Sample	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance
CRFF	1.500	0.000	30.041	0.001	58.500	0.280	5.000	0.000	0.600	0.010
RRFF	1.500	0.000	26.740	0.001	56.200	1.240	3.000	0.500	12.060	182.200
URFF	0.500	0.000	26.740	0.002	62.333	0.103	3.000	0.000	0.333	0.003
CRFC	1.667	0.083	31.332	0.000	56.667	0.023	6.333	0.333	0.733	0.003
RRFC	1.833	0.083	27.380	0.001	48.500	12.130	4.333	0.333	0.367	0.003
URFC	0.500	0.000	17.971	0.002	65.367	13.103	7.333	1.333	0.267	0.003
CRFFC	1.500	0.000	31.489	0.001	31.489	0.001	6.667	0.333	0.433	0.003
RRFFC	1.667	0.083	30.512	0.001	48.367	27.123	5.333	0.333	0.167	0.003
URFFC	0.500	0.000	20.358	0.002	61.000	0.002	6.667	0.333	6.667	0.333

Note: CRFF – cooked rabbit fed with forage, RRFF – roasted rabbit fed on forage, URFF – unprocessed rabbit fed with forage, CRFC – cooked rabbit fed with concentrate, RRFC – roasted rabbit fed with concentrate, URFC – unprocessed rabbit fed with concentrate, CRFFC – cooked rabbit fed with forage and concentrate, RRFFC – roasted rabbit fed with forage and concentrate, URFFC – unprocessed rabbit fed with forage and concentrates
Source: experimental data

The most fatty rabbit meat sample was the URFC (7.333 mg/100 g) and was followed by both CRFFC and URFFC with 6.667 mg/100 g. The least fatty rabbit meat was 3.000 mg/100 g obtained for both RRFF and URFF. This indicates the significance of forage feeds in fats reduction and subsequently making it healthy type of feed for animals which will subsequently impact healthiness to the humans that consume the meat. There is no significant trend in the fat content of the rabbit thus neither the processing nor feeding effects on rabbit meat is discernible in the present study. The rabbit meat samples (RRFF) returned very high crude fibre of 12.060 mg/100 mg and majority of the rabbit meat samples were homoscedastic (with variance of 0.003). The least crude fibre (0.167 mg/100 mg) was obtained for RRFFC (Table 3). The range of the carbohydrate content obtained in the present study was from 3.26% for CRFF to 17.58% for RRFC while the energy content fell between 150.46 Kcal for URFC and 225.867 for RRFFC (Fig. 1).



(1 = CRFF; 2=RRFF; 3 = CRFC; 4 = RRFC; 5= CRFFC; 6 =RRFFC; 7 = URFF;8 = URFC; 9 = URFFC)

Figure 1. Mean carbohydrates and energy obtained for different rabbit meat samples

Note: CRFF – cooked rabbit fed with forage, RRFF – roasted rabbit fed with forage, URFF – unprocessed rabbit fed with forage, CRFC – cooked rabbit fed with concentrate, RRFC – roasted rabbit fed with concentrate, URFC – unprocessed rabbit fed with concentrate, CRFFC – cooked rabbit fed with forage and concentrate, RRFFC – roasted rabbit fed with forage and concentrate, URFFC – unprocessed rabbit fed with forage and concentrates

Source: experimental data

General linear model analysis and mean separation of the proximate component of the rabbit

The results of the general linear model (GLM) analysis returned significant results for all the proximate composition variables. The statistics, $F_{(8,18:0.05)}$ = 34.58, 55,839.90, 17.75, 23.11, 20.85, 12.32 and 12.36 obtained for ash content, protein, moisture content, fat, crude fibre, carbohydrate, and energy are significant ($P < 0.05$ – Table 4). Mean ash of the rabbit meats were partitioned into 3 significantly different groups including 1) RRFC with mean ash of 1.833 was significantly different from; 2) CRFFC, CRFF, and RRFF having mean ash of 1.500; and 3) URFF, RRFC, and URFFC having mean ash of 0.500 (Table 4). Protein content of rabbit meat sample, CRFFC (31.489) was significantly higher than protein content obtained for CRFC (31.332). Protein content of other rabbit meat follows the trend of – RRFFC (30.512) greater than CRFF (30.041) greater than RRFC (27.380) greater than RRFF (26.740) greater than URFF (25.143). The least significantly different mean protein was obtained for rabbit sample URFC (17.971) and it was significantly lesser than protein content of URFFC (20.358 – Table 5).

Table 4. *General linear model of the proximate component analysis of the rabbit meat*

Treat	ASH	PROTEIN	MOISTR	FAT	CRUDE FIBRE	CHO	Energy
F-statistics	34.58**	55,839.9**	17.75**	23.11**	20.85**	12.32**	12.36**
DF	8	8	8	8	8	8	8
RRFC	1.833[a]	27.380[e]	48.500[f]	4.333[b]	0.367[ed]	17.587[a]	218.867[a]
CRFC	1.667[ab]	31.332[b]	56.667[cd]	6.333[a]	0.733[a]	4.359[d]	195.400[b]
RRFFC	1.667[ab]	30.512[c]	48.367[f]	6.667[a]	0.167[g]	13.955[ba]	225.867[a]
CRFFC	1.500[b]	31.489[a]	52.167[ef]	6.667[a]	0.433[cd]	7.745[dc]	216.933[a]
CRFF	1.500[b]	30.041[d]	58.500[bcd]	5.000[b]	0.600[b]	4.359[d]	182.600[b]
RRFF	1.500[b]	26.740[f]	56.2[de]	3.000[c]	0.500[bc]	12.060[bc]	182.000[b]
URFF	0.500[c]	25.143[g]	62.333[ab]	3.000[c]	0.333[def]	5.024[d]	180.667[b]
URFC	0.500[c]	17.971[i]	65.367[a]	7.333[a]	0.267[efg]	12.060[bc]	150.467[c]
URFFC	0.500[c]	20.358[h]	61.000[abc]	6.667[a]	0.233[fg]	10.575[bc]	189.733[b]

Note: CRFF – cooked rabbit fed with forage, RRFF – roasted rabbit fed with forage, URFF – unprocessed rabbit fed with forage, CRFC – cooked rabbit fed with concentrate, RRFC – roasted rabbit fed with concentrate, URFC – unprocessed rabbit fed with concentrate, CRFFC – cooked rabbit fed with forage and concentrate, RRFFC – roasted rabbit fed with forage and concentrate, URFFC – unprocessed rabbit fed with forage and concentrates
Source: experimental data

Duncan Multiple Range Test partitioned the moisture content into 2 independent classes of the highest moisture content – URFC (65.367%) and the least moisture content (RRFC – 48.5% and RRFFC – 48.367%). Other rabbit meat sample returned moisture content that formed intermediary classes between the group 2 (of highest and least moisture content – Table 4). Duncan Multiple Range Test partitioned mean fat content into 3 significantly different classes. This includes the highest significantly different class – URFC (7.333), RRFFC, CRFFC, and URFFC (6.667), and CRFC (6.333); the average class consisting of both CRFF (5.00) and RRFC (4.333); the least significant class – URFF and RRFF (3.00). Mean crude fibre obtained for CRFC (0.733) was significantly the highest and it is higher than mean crude fibre obtained for CRFF (0.600) while mean crude fibre returned for RRFFC (0.167) was the least (Table 4). Mean crude fibre obtained for other rabbit meat formed intermediary classes between these classes.

The trend of carbohydrate of the rabbit meat is such that RRFC (17.587) ⩾ RRFFC (13.955) ⩾ RRFF and URFC (12.06). Both URFF and CRFC (4.359) were the least significantly different carbohydrate (Table 4). Three significantly different groups were formed by the Duncan Multiple Range Test for the energy content of the rabbit meat. Mean energy obtained for RRFFC (225.867), RRFC (218.867), and CRFFC (216.933) were significantly higher than mean energy obtained for rabbit meat CRFC (195.400), URFFC (189.733), RRFF (182.2), CRFF (182.600), and URFF (180.667). Mean energy obtained for rabbit meat URFC (150.467) was significantly the least (Table 5). These disparities might have been caused by factors such as age, diet, and breed of the rabbits which can also impact the energy content of the meat.

Table 5. *Descriptive statistics of the mineral content of the rabbit meat*

Sample	**Phosphorous**		**Na**		**K**	
	Mean	Variance	Mean	Variance	Mean	Variance
CRFF	499.333	1.333	35.998	0	201.009	0
RRFF	355	25	69.309	0.045	368.738	0
URFF	215.667	1.333	50.626	0.045	199.456	7.234
CRFC	260	25	39.686	0	174.607	7.236
RRFC	371.667	8.333	67.957	4.533	284.874	0
URFC	202	7	46.938	0.045	201.009	0.045
CRFFC	115.333	0.333	39.809	0.045	185.464	7.373
RRFFC	333.333	108.333	54.313	0.045	354.761	0
URFFC	305.333	20.333	46.938	0.045	41.267	0
	Ca		**Fe**		**Zn**	
Samples	Mean	Variance	Mean	Variance	Mean	Variance
CRFF	41.081	0.104	21.262	0	10.763	0
RRFF	48.162	5.106	39.003	0	19.744	0
URFF	41.267	0	21.098	0.081	10.68	0.021
CRFC	24.308	0.104	18.469	0.081	9.349	0.021
RRFC	46.672	0.104	30.133	0	15.253	0
URFC	41.267	0	21.262	0	10.763	0
CRFFC	41.081	0.104	19.618	0.082	9.931	0.021
RRFFC	35.676	0	37.525	0	18.996	0
URFFC	22.576	0.081	22.576	0.081	11.428	0.021

Note: CRFF – cooked rabbit fed with forage, RRFF – roasted rabbit fed with forage, URFF – unprocessed rabbit fed with forage, CRFC – cooked rabbit fed with concentrate, RRFC – roasted rabbit fed with concentrate, URFC – unprocessed rabbit fed with concentrate, CRFFC – cooked rabbit fed with forage and concentrate, RRFFC – roasted rabbit fed with forage and concentrate, URFFC – unprocessed rabbit fed with forage and concentrates
Source: experimental data

Overview of the data for the rabbit meat parameters (mineral content)

The phosphorous in the rabbit was found to range between 115.333 for CRFFC and 499.333 for CRFF and they were all heteroscedastic except RRFF and CRFC that were homoscedastic (Table 5). The variance ranged between 0.333 for CRFFC and 108.333 for RRFFC and none was zero. The trend of phosphorous obtained in the present study indicated that forage fed rabbit meat have significantly higher phosphorous than the concentrate fed counterpart. Mean sodium ranged between 35.998 for CRFF and 69.309 for RRFF with many homoscedastic groups. The variances obtained for the sodium can be partitioned into 2 groups – 0.000 for CRFF and CRFC, and 0.045 for other rabbit samples (Table 5). The results of the descriptive analysis of the mean potassium of the rabbit means indicated that RRFF returned the highest potassium of 368.738 (mg/100 g) while the least potassium (174.6) was obtained in CRFC. Most of the rabbit samples (0.56) returned zero variance while others were heteroscedastic (Table 5).

Mean calcium of the rabbit meat ranged from 24.30 obtained for CRFC to 48.162 for RRFF with 3 groups of variances – the heteroscedastic (RRFF and URFFC), the zero variance (URFF, URFC and RRFFC) and homoscedastic (CRFF, CRFC, RRFC, CRFFC – Table 5). Mean iron falls between 18.469(unit) for CRFC and 39.003 for RRFF with both zero variance for CRFF, RRFF, RRFC, URFC and RRFFC and homoscedastic variance (0.081) for URFF, CRFC, CRFFC, and URFFC (Table 5). Highest mean zinc (19.744) was obtained for RRFF while the least mean zinc (9.931) was obtained for CRFFC and some rabbit meat samples (URFF and

CRFC = 10.680: CRFF and URFC = 10.763) have the same mean zinc (Table 5). More of the rabbit meat samples (0.56) returned zero variance while the remaining – URFF, CRFC, CRFFC, and URFFC were homoscedastic (having the same variance of 0.021 – Table 5). These findings suggest that roasting effectively preserves certain minerals in rabbit meat, positioning it as the superior method of preparation.

General linear model (GLM) analysis and mean separation of the mineral content of the rabbit meat

The results of the general linear model of the mineral component of the rabbit meat indicated that there exist significant differences between the means obtained for the mineral component. The statistics, $F_{(8,18:0.05)}$ = 1,734.67, 800.13, 5,145.92, 234.17, 5,145.92, and 5,145.92 obtained for phosphorous, sodium, potassium, calcium, iron, and zinc are significant ($P < 0.05$ – Table 6). Mean phosphorous was partitioned into 8 significantly different groups by DMRT. Mean phosphorous obtained for rabbit meat of CRFF (499.333) is greater than that of RRFC (371.667). The least significantly different mean phosphorous was obtained for rabbit meat CRFFC (115.333) and it is significantly lesser than mean phosphorous obtained for rabbit meat URFC (202.00 – Table 6).

Table 6. *General linear model analysis of the mineral content of the rabbit meat*

Treat	PhMg	Na	K	Ca	Fe	Zn
F-Statistics	1,734.67**	800.13**	5,145.92**	234.17**	5,145.92**	5,145.92**
DF	8	8	8	8	8	8
CRFF	499.333[a]	35.998[g]	201.009[e]	41.081[c]	21.262[e]	10.763[e]
RRFF	355.000[c]	69.309[a]	368.738[a]	48.162[a]	39.003[a]	19.744[a]
URFF	215.667[g]	50.626[d]	199.456[e]	41.267[c]	21.098[e]	10.680[e]
CRFC	260.000[c]	39.686[f]	174.607[g]	24.308[e]	18.469[g]	9.349[g]
RRFC	371.667[b]	67.957[b]	284.874[c]	46.672[b]	30.133[c]	15.253[c]
URFC	202.000[h]	46.938[e]	201.009[e]	41.267[c]	21.262[e]	10.763[e]
CRFFC	115.333[i]	54.313[c]	185.464[f]	41.081[c]	19.618[f]	9.931[f]
RRFFC	333.333[d]	54.313[c]	354.761[b]	35.676[d]	37.525[b]	18.996[b]
URFFC	305.333[e]	46.938[e]	213.434[d]	41.267[c]	22.576[d]	11.428[d]

Note: CRFF – cooked rabbit fed with forage, RRFF – roasted rabbit fed with forage, URFF – unprocessed rabbit fed with forage, CRFC – cooked rabbit fed with concentrate, RRFC – roasted rabbit fed with concentrate, URFC – unprocessed rabbit fed with concentrate, CRFFC – cooked rabbit fed with forage and concentrate, RRFFC – roasted rabbit fed with forage and concentrate, URFFC – unprocessed rabbit fed with forage and concentrate
Source: experimental data

Mean phosphorous in other rabbit meat samples were found in between the two groups (of highest and the least). Mean sodium obtained for the rabbit meat, RRFF (69.309) is significantly higher than that mean sodium returned for rabbit meat RRFC (67.957). The next significantly different mean sodium of the rabbit meat was obtained for both CRFFC and RRFFC (54.313) and are significantly higher than mean sodium obtained for rabbit meat URFF (50.626). The least significantly different mean sodium (35.998) was obtained for rabbit meat CRFF and it is significantly lesser than mean sodium obtained for rabbit meat CRFC (39.686 – Table 6). The trends of mean potassium partitioning for rabbit meat are such that; RRFF (368.738) > RRFFC (354.761) > RRFC (284.874) > URFFC (213.434) > URFC (201.009) > > URFF (199.456) > CRFFC (185.464) > CRFC (174.607 – Table 6).

The mean calcium of the rabbit meat, RRFF (48.162) remained significantly the highest and it is higher than mean calcium of the RRFC (46.672). Mean calcium of the RRFC was in turn significantly higher than the one obtained for CRFF, URFF, URFC, CRFFC, and URFFC (41.267) while the next significantly different mean calcium was obtained for RRFFC (35.676). The least significantly different mean calcium was obtained for CRFC (24.308 – Table 6). Mean iron returned for rabbit meat RRFF (39.003) remained the highest and it is significantly higher than mean iron obtained for rabbit meat RRFFC (37.525) which is, in turn, significantly higher the mean iron of the rabbit meat RRFC (30.133). Mean iron in the rabbit meat URFFC (22.576) is the next significantly different class and it is higher than the mean iron obtained for CRFF, URFC (22.262), and URFF (21.098). The least mean iron was obtained for CRFC (18.469) and it is significantly less than mean iron of the CRFFC (19.618 – Table 6). DMRT partitioned mean zinc into 7 significantly different classes which are mean zinc obtained for RRFF (19.744) which is significantly the highest and was followed by mean zinc of the RRFFC (18.996) and it is, in turn, significantly different from the mean zinc returned for RRFC (15.253). Mean zinc obtained for URFFC was the next significantly different class and it is different from the mean zinc obtained for URFC, CRFF (10.763), and URFF (10.680 – Table 6). The least significant mean zinc was obtained for CRFC (9.349) and it is significantly less than 9.931 obtained for CRFFC.

Discussion

The calcium content in this study is higher than those obtained (2.7-9.3) by A. Dalle Zotte & Z. Szendrő (2011). The values of the calcium fall within the USDA threshold of 18mg (United States Department of Agriculture, Nutrient lists from standard reference legacy, 2018). Similarly, the iron (Fe) of the rabbit meat in this study is higher than the recommended 4.8mg of the USDA and what was obtained (1.1-1.3) by A. Dalle Zotte & Z. Szendrő (2011).

The inverse relationship between the mean cholesterol and its variance established in the present study might be responsible for their heteroscedasticity. It is evident that processing (roasting or cooking) has significant impact on the vitamin B1 and B2, and cholesterol obtained from the rabbit meat. This indicates that the methods of processing used can greatly affect the nutritional composition of the meat. This finding is important for researchers and consumers in order to make informed decisions about the preparation and consumption of rabbit meat. This is in accord with the findings of M. Nutautaitė *et al.* (2023) that established relationships between mean and variance of some rabbit meat variables. The mean comparison of vitamin B2, E, and cholesterol levels in various rabbit meat samples of this study provide further valuable insights into the nutritional benefits of forage-fed rabbit meat. R. Gál *et al.* (2022) emphasised the role of rabbit meat as a functional food due to its high vitamin B content, aligning with the current findings that forage-fed rabbits can enhance these levels. In addition, the function of diet in improving the nutritional profile of rabbit meat has been substantiated in various studies (Siudak & Pałka, 2022). Mean proteins (obtained in the present study) are in agreement with mean proteins obtained for livestock meats including rabbit by B. Suvajdžić *et al.* (2023) and S. Siddiqui *et al.* (2023). The protein content obtained in the present study are higher than protein content in rabbit meat reported by A. Gbenge *et al.* (2021), J. Ingweye *et al.* (2021), and M. Suliman *et al.* (2019).

Moisture content has been found to reduce with different types of processing (Akindele & Adeosun, 2022). The moisture contents obtained in the present study were lower than 69.6-79.3% obtained for rabbit in the report of S. Siddiqui *et al.* (2023) and in agreement with the moisture content of rabbit meat in B. Suvajdžić *et al.* (2023). The dissimilitude in the moisture content of rabbit meat in S. Siddiqui *et al.* (2023) and the present study could not be unconnected to differences in the rabbit meat samples and feeding regime in both studies. The fat content obtained in the present study (3.0-7.333) compared favourably with J. Adu *et al.* (2019) and it is higher than the recommended 3.5 g of the USDA and the fat content obtained by S. Siddiqui *et al.* (2023). Forage diets have been established to contain more crude fibre than the concentrated feed (Akindele & Adeosun, 2022) and this coincides with the findings of the present study. In addition, the crude fibre obtained in the present study is slightly lesser than the values established by A. Gbenge *et al.* (2021). This disparity might have been conditioned by differences in the treatments to which rabbit were subjected in the 2 studies. Forage diet is better than concentrate diet in terms of promoting healthy digestion and providing a more natural source of nutrients for livestock. The carbohydrates provided by complete forage diets as established in this study is thus in order for non-harmful development of the rabbit and it is in agreement with Z. Siudak & S. Pałka (2022). The ash content found in this study is comparable to that reported by J. Ingweye *et al*. (2021) and lesser than the ash content obtained by A. Gbenge *et al.* (2021). The ash content can influence the overall nutritional balance of the meat. Higher ash content can indicate a richer mineral profile, which is essential for consumer health (Siddiqui *et al.*, 2023). This is particularly important in diets where meat is a primary protein source.

Forage fed rabbit returned higher crude fibre than rabbits fed with concentrates. This might be conditioned by the high fibre content of the original feed (forage) fed to the rabbit. Forage diets have been established to contain more crude fibre than the concentrated feed (Akindele & Adeosun, 2022). The higher moisture content obtained in the raw unprocessed rabbits may be attributable to the unprocessed nature of the samples while the lower moisture content obtained for the processed samples might be conditioned by the effect of the processing (cooking and roasting) on the meat samples and this coincides with reports by B. Pang *et al.* (2020). Processing and feed type influences carbohydrate content of the rabbit in the present study. The nutritional content of rabbit meat has been established to be correlated with the nutritional values of its feed intake (Yadav *et al.*, 2022). It is obvious from this study that roasted rabbit meat returned higher potassium than cooked rabbit meat and this is attributable to leaching of the minerals into the water during cooking. It has been established that minerals (like calcium, sodium, phosphorous, and zinc) can be lost during cooking (Yadav *et al.*, 2022). It was also established that the least of these minerals is lost during roasting (Dal Bosco *et al.,* 2001). Roasting clearly retained some of the minerals of the rabbit meat, hence making it the best among the rabbit meat and it is in concord with H. Abdel-Naeem *et al.* (2021). The phosphorous content obtained in the present study is higher than the high phosphorous content reported by H. Abdel-Naeem *et al.* (2021) and higher than phosphorous of any other meat in the same report. The zinc content of the rabbit meat in this study was moderately higher than those of J. Ingweye *et al.* (2021) and A. Menon *et al.* (2023). These findings indicate that rabbit meat could potentially be a valuable source of essential minerals such as calcium, iron, and zinc in the diet.

Lastly, the goal of meat quality estimates of rabbit as affected by feeding and processing kind is to evaluate how feeding strategies and processing methods influence the overall quality attributes of rabbit meat. These qualities are critical for enhancing the marketability and health benefits of rabbit meat consumers. Understanding these influences can guide producers in optimising feeding regimens and processing techniques to improve meat quality, thereby increasing consumer demand and satisfaction.

Conclusions

To enhance the promotion of rabbit meat consumption, a robust strategy involves the development of comprehensive information and educational programmes that highlight rabbit meat as a nutritious, flavourful, and premium food option. This can be effectively accomplished by delivering objective and transparent information regarding rabbit meat production, with a particular focus on critical aspects such as safety, quality, and animal welfare. As emphasised in the current study, addressing these issues not only fosters consumer trust but also encourages informed dietary choices, ultimately positioning rabbit meat as a viable alternative in the meat market. Engaging stakeholders through workshops, cooking demonstrations, and community outreach can further amplify awareness and appreciation for rabbit meat, driving its acceptance and integration into mainstream culinary practices. Emphasising the environmental sustainability of rabbit meat production can play a pivotal role in enhancing its consumption. The current study illustrates how methods such as roasting and foraging not only improve the nutritional quality of rabbit meat but also align with sustainable practices. This research significantly enriches understanding of rabbit meat's nutritional value, health benefits, and culinary versatility. It has been demonstrated that rabbit meat serves as a lean protein source, characterised by low cholesterol levels and an abundance of essential nutrients, including iron and B vitamins. Moreover, the study meticulously documents innovative processing and feeding methods that broaden the culinary applications for both chefs and home cooks. Research into the most suitable forage species for optimum rabbit productivity is recommended for future study.

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Conflict of Interest

There is no special interest to declare for this project.

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Оцінка якості м'яса кролика залежно від типу годівлі та методів переробки

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Анотація. М'ясо кролика має високий попит у Нігерії завдяки цінному поживному складу та приємним смаковим якостям. Метою цього дослідження було оцінити вплив методів обробки та типу годівлі на поживну якість кролячого м’яса. Три групи відібраних кроликів були піддані трьом типам режимів годівлі та методів переробки м’яса, яке оцінювалось за вмістом вітамінів, холестерину та основними поживними складниками в умовах експерименту за схемою повного рандомізованого блоку (Randomised Complete Block Design experiment). Середній рівень вітаміну B1 коливався від 0,092 мг на 100 г для вареного м’яса кролика, якого годували рослинним кормом, до 0,105 мг на 100 г для сирого м’яса кролика з тим же типом годівлі, при цьому відхилення були майже нульовими. Результати кластерного аналізу вмісту вітамінів у кролячому м’ясі показали, що печене м’ясо кролика, якого годували рослинним кормом, і зварене м’ясо кролика, якого годували комбінованим кормом, утворювали кластер на найменшій відстані агломерації 0,128. Найбільша відстань агломерації 24,973 була отримана для звареного кролика, якого годували рослинним кормом, і печеного кролика з таким самим раціоном. Середній вміст білка в м'ясі кролика знаходився в діапазоні від 17,971 для сирого кролика, якого годували концентратами, до 31,489 для смаженого кролика, якого годували кормами та концентратами, а дисперсії утворюють 2 групи (гомоскедастичні та незалежні). Результати аналізу загальної лінійної моделі показали $F_{(8,18:0.05)}$ = 34,58; 55,839,90; 17,75; 23,11; 20,85; 12,32 і 12,36 для вмісту золи, білка, вологи, жиру, сирої клітковини, вуглеводів і енергії, які є значущими (P < 0,05). Методи обробки та годування, як встановлено в даному дослідженні, покращували поживну якість кролячого м’яса, що має практичне значення для задоволення попиту споживачів, удосконалення стандартів якості в аграрному виробництві та розробки оптимальних методів обробки

Ключові слова: поживні речовини; гомоскедастичність; дисперсія; кролівницьке підприємство; кролівництво

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Research and application of nozzles with inclined inlet channels for jet sulphitators in the sugar industry

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Abstract. The paper was devoted to the study of the process of liquid spraying by nozzles used in jet sulphitators in the sugar industry or as separate equipment. Liquid sprayers play a major role in ensuring efficient operation of heat and mass transfer equipment due to the creation of a significant contact surface. When designing or auditing its technical integrity, it is necessary to have reliable hydrodynamic characteristics of sprayers. Research in this area is relevant. The purpose of the study was to establish all the characteristics of liquid flow in the elements of the nozzle and spray swath using comprehensive research (experimental and computer) for the possibility of intensifying the operation of equipment, in particular, sulphitators for the sugar industry. To achieve this goal, experimental (studies of injectors and ejectors on a hydraulic stand) and CFD studies of these objects in the modules of the ANSYS software suite (version: ANSYS 2020 R2) were conducted. The dependence of the nozzle flow rate on the liquid pressure and the number of supply channels for different types of injectors (jet, centrifugal-jet) has been experimentally established. It has been experimentally confirmed that the uniformity of liquid distribution in the nozzle swath with two supply channels was high and sufficient for practical applications. In the CFD study of the characteristics of the nozzle spray swath, it was proposed to reduce the design area to the cut cone, which reduced the calculation time. When modelling the hydrodynamics of the liquid in the nozzle, numerically characteristic velocities were determined both in the nozzle elements and in the outlet. It was established that there was a vacuum in the root zone reaching 865 Pa for this nozzle at a liquid inlet pressure of 0.25 MPa, which led to gas ejection into the swath. The above calculation methodology was relevant for determining the characteristics of injectors, in the development and design of new sprayers with a given capacity and a spray swath, used both as active ejector nozzles and as independent equipment for dispersing process liquids in the food industry

Keywords: sugar production; liquid sulphitation; spray nozzle; flow rate; CFD modelling; hydrodynamics; spray swath

Introduction

Liquid spraying is an extremely widespread process in almost all areas of human activity. It has found use starting from growing plants (irrigation), in emergency situations (extinguishing fires), during technological processes in the chemical, food, and aerospace industries. The proposed nozzles with inclined supply channels were developed for the needs of the sugar industry, namely for sulphitation of water and sugar solutions in jet sulphitation tanks, deammonisers of water vapour condensates. These processes are necessary in the production of crystalline sugar, ensuring the production of a high-quality product. However, for the efficient operation of the equipment, injectors with high both consumption, dispersion and operational characteristics must be used. No such studies were conducted for the needs of the sugar industry. It should also be added that knowledge about the characteristics of sprayers is required not only for sugar, but also for any industry where heat and mass transfer processes are carried out according to the technological process.

The injectors had to have high reliability, since the liquids were not previously cleaned of dirt, which led to the loss of their performance. A special feature of the nozzles is that the potential energy of the compressed liquid is converted into kinetic energy, followed by the formation of liquid droplets. Such transformations are accompanied by a significant increase in the contact surface of the phases and an intensification of mass transfer. When carrying out technological processes of heat and mass transfer in food, chemical, and other industries, increasing the contact surface of phases is a powerful means of increasing the amount of substance transferred. Usually, the mass transfer coefficients also increase simultaneously due to the formation of a new interface, its rapid renewal, and a decrease in the size of the boundary layer for the interfacial transition of reagents. In two-phase liquid-gas media, an effective way to increase the surface is to spray the liquid using injectors (sprayers) of various designs. The contact surface (droplet diameter) is different for different types of nozzles and depends on the physical properties of the liquid. Thus, the paper by E. Stallbaumer-Cyr *et al*. (2024) presented experimental data on determining the flow rate, volume diameter, etc. The findings were illustrated with images to confirm the results. However, these data are true only for this type of nozzle under study, and when the dimensions change, or the ratios between dimensions change, the characteristics change, which requires new research.

R. Sijs *et al*. (2023) noted that sprayers are of great importance for many applications, with the drop size being a crucial parameter. It was shown that the average drop size is determined by the area of the nozzle opening, as in the case of conventional flat fan nozzles, but it is necessary to consider the additional pressure drop in the Venturi chamber. This parameter allows reusing the ratios that were obtained for flat fan nozzles.

To reduce air dust, H. Han *et al*. (2023) compared the operation of injectors (and established the optimal phase supply pressures to obtain droplets of minimum size. According to E. Verbiawan *et al*. (2023), to accelerate the evaporation of seawater in salt production along with conventional methods, it is recommended to use liquid spray technology. Jet nozzles are also known to be used to intensify hydrodynamic mixing in tanks. Research by T. Oluwadero *et al*. (2023) aimed at establishing the main characteristics of the spray swath, but the general disadvantage of these studies is that if the conditions for conducting experiments change, it is necessary to repeat them. This is a common disadvantage of conducting a physical experiment.

For the sugar industry, the transition of sulphitation of liquids from bubbling-type apparatuses to jet apparatuses took place in the 1970s, which was progressive at that time. However, the design of the devices took place without a proper scientific and practical basis, which provoked quite serious shortcomings in their operation. One of the main elements that has a significant impact on the operation of the jet sulphitation unit is the design of the sprayer used to disperse the liquid. It has been practically proven that for effective operation of sulphitators, it is necessary to have a centrifugal-jet sprayer. Such sprayers were subsequently proposed, but required a comprehensive study to be able to use them as an active ejector nozzle.

The use of liquid dispersion in combination with the high intensity of processes allows creating extremely efficient equipment that combines a number of advantages: simplicity, compactness, low specific energy consumption. Therefore, the relevance of the subject matter is beyond doubt. The study by S. Kim *et al.* (2023) experimentally showed that the nozzle design has a decisive influence on the formation of the spray swath, its filling with sprayed liquid droplets, the spray angle, and the diameter of liquid droplets. Therefore, the experimental part of the study on swath development is an important stage in clarifying the theory of jet decay and understanding the mechanism of swath development. Along with experimental research and software development, simulation modelling of the spray process plays a significant contribution to understanding the mechanism of swath development. However, it is possible to obtain data that is quite difficult to obtain experimentally. The accuracy of data acquisition depends on the correct configuration of all software modules, the accuracy of performing a three-dimensional model of the object under study, etc.

The purpose of this study was a comprehensive investigation of the process of liquid spraying with nozzles with inclined supply channels. Identification of the influence of the design of injectors on the spray swath, in particular, on the uniformity of liquid distribution over the cross-section of the swath, the angle of the swath. This will improve the equipment for the liquid sulphitation process in the sugar industry: increase the use of SO_2, improve the processing quality of sugar solutions. To achieve this goal, the following tasks were set: to create laboratory installations for conducting research and methods for conducting them; to produce research objects with accuracy that meet the requirements of practical operation; to conduct physical modelling of the spraying process; to conduct simulation modelling of the liquid flow in the elements of the nozzle and its spray swath; to validate the results.

From the scientific originality of the obtained results of a comprehensive study of fluid hydrodynamics in the nozzle elements with inclined supply channels and the spray process, the following should be noted: the dependence of the nozzle flow rate on geometric parameters and the number of supply channels at a liquid supply pressure of up to 0.4 MPa was obtained; it was proposed to perform the design zone of the spray swath in the form of a cut cone, which will reduce the time of machine calculation by reducing the number of calculation cells.

Literature Review

Studies of the liquid spray process were mainly performed for low-performance injectors used in fuel spraying. The results of research in this area can be found in the papers by R. Wu *et al*. (2024), A. Shulhin *et al*. (2022). The main requirement for fuel injectors was to obtain small droplets, and such liquids were pre-prepared by filtration. For them, the theory of fluid flow processes in the twisting chamber, the nozzle, which is a cylindrical channel, was developed, and the theory of decay of a cylindrical jet into liquid droplets was also proposed (Xu *et al*., 2022).

Much less studies have considered the process of spraying significant volumes of liquids not cleaned of contaminants or crystalline formations in it with centrifugal or centrifugal-jet nozzles. When installing them inside a hollow container, one of the key requirements is to evenly fill the cross-section with liquid droplets. This is provided only by centrifugal jet nozzles, which have high consumption characteristics, a filled spray swath, and a fairly fine fractional composition of droplets. Such recommendations can be found in the paper by S. Park & K. Park (2022). To improve the dispersed composition of drops, the nozzle designs are complicated, and additional structural elements are installed that ensure the formation of axial and tangential liquid jets. Such injectors were investigated by H. Han *et al*. (2020) and it was shown that the hydraulic supports of injectors increase at the same time. That is, reducing the size of droplets occurs due to a decrease in the reliability of the nozzle and an increase in energy consumption for spraying.

A variety of purposes also require a variety of sprayer designs. The researchers focused only on hydraulic type injectors, namely centrifugal-jet type injectors, which have proven themselves well in many branches of the economy due to their advantages, such as simplicity of design, although as the tasks that are assigned to sprayers become more complex, according to T. Lee *et al*. (2021), reliability decreases; the possibility of creating a significant phase contact surface, which is important when conducting heat and mass transfer processes in intensive mode. When the liquid is dispersed by nozzles (centrifugal, centrifugal-jet), there is a significant increase in the contact surface of the phases, which leads to a proportional increase in the amount of substance transferred. Droplet size measurements were performed for nozzles of different designs and it was shown that the contact surface increases by an order of magnitude, as found by X. Zhu *et al.* (2021); relatively low energy losses. Thus, the nozzle flow rate (the ratio of the actual flow rate to the theoretical one) in jet injectors reaches 0.95, in centrifugal-jet injectors – 0.85 (Vambol *et al.*, 2020).

Solving industrial problems that arise during technological processes leads to the creation of innovative injectors that ensure maximum efficiency of the process flow. Some of these injectors are injectors with an inclined arrangement of the supply channels relative to its axis (Mudavath & Arumuru, 2023). They were developed for spraying crude process fluids during sulphitation processes in sugar production. For their design, when scaling, the question arose of providing a more complete understanding of the hydrodynamics of the liquid in the elements of the nozzles, the processes of forming the spray swath and obtaining the dependences of the main characteristics of the nozzles, which will allow them to be designed with the specified properties. The characteristics of the injectors can be obtained experimentally, which requires the availability of experimental samples, stands for research, and requires time to conduct and process the obtained data. However, when switching from prototypes to industrial designs, compliance with the results is not always guaranteed, which requires additional experiments.

Modern computer technology and related programmes allow us solving these problems and significantly reducing the time for conducting such studies and reducing the cost of such studies while obtaining reliable results. Computer modelling allows both visualising and obtaining reliable numerical values of various values in any control sections of the studied research objects (nozzles) and in the spray swath, and based on the results obtained, create energy-efficient equipment. It is especially important to carry out such (CFD – computational fluid dynamics) modelling when designing injectors with specified characteristics of the spray swath, or when studying new sprayer designs. Changes in dimensions, their ratios, and design features require experimental confirmation, since these changes can cause significant deviations from the predicted characteristics.

In recent years, computer modelling of nozzle operation has become widespread. The first studies on numerical modelling related to establishing the characteristics of the spray swath of centrifugal nozzles. As the calculation model improved, the data obtained were refined, new tasks were solved, and new data was obtained. Centrifugal-jet injectors were considered, in which the decay of the liquid jet occurred when two flows interact: centrifugal and axial along the nozzle axis (von Helldorff & Micklow, 2019; Santosh *et al.*, 2023).

It should be noted that despite the progressive development of CFD research methods, accurate modelling of any processes, including modelling the liquid sputtering process, is an extremely difficult task. This is due to the fact that all processes in nature occur sequentially or in parallel with the main process under study and are complicated by secondary processes that affect one another. For the liquid spray process, J. Haidl *et al.* (2021) considered the temperature at which spraying occurs. The physical parameters of the liquid, cavitation phenomena, primary and secondary decay of droplets due to interaction with each other, deformation, variable aerodynamic drag, heat transfer between phases, and the establishment of an equilibrium state were considered by K. Kramm *et al.* (2023) and Y. Xu *et al.* (2024).

In CFD modelling of fluid dynamics in nozzle elements and the spraying process, important elements of the settings are the correct setting of boundary conditions, a minimal but sufficient choice of the study area with a breakdown into elements of the computational grid, etc. (Li *et al.*, 2024). Therefore, setting up the calculation modules of the programme to display adequate results is quite an important stage of research.

Materials and Methods

Designs of the injectors under study. For the experimental study, injectors with one (Fig. 1) and two (Fig. 2) inclined supply channels. One of the advantages of such injectors is the ability to adjust the flow rate of liquid in a wide range due to the simultaneous change in the area of the supply channels and the radius of twisting of the liquid in the nozzle body.

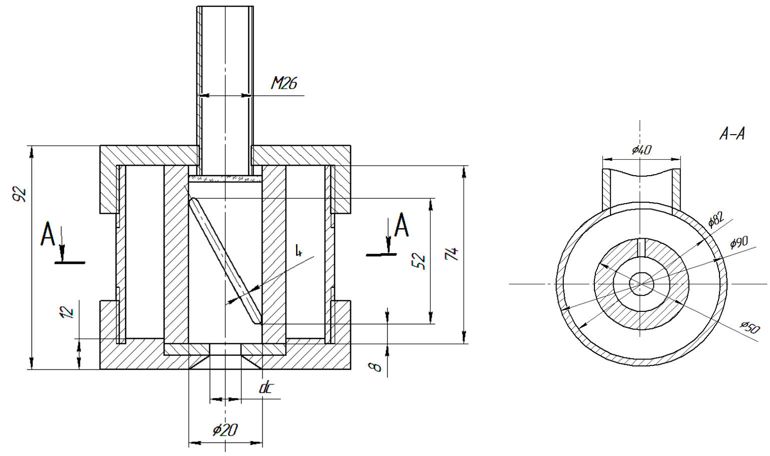

Figure 1. Nozzle with one inclined supply channel
Source: developed by the authors

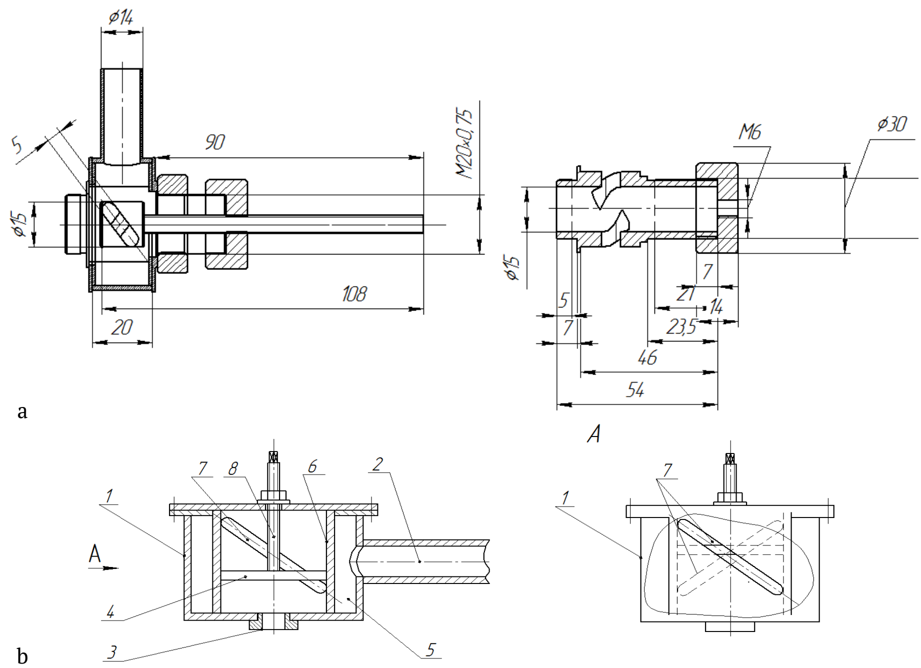

Figure 2. Nozzle with two inclined supply channels: a – structures, b – drawing
Source: developed by the authors

Nozzle (Fig. 2b) consists of a housing 1, to which a supply pipe 2 is connected, through which liquids are supplied inside the housing. At one end of the nozzle body there is an injector 3, and at the opposite end there is a plunger 4 with the possibility of axial movement by means of a screw 8 during its rotation. The supply pipe 2 is connected to the intermediate chamber 5 of the nozzle. The inner cylinder 6 of this chamber has slotted channels 7, which are made at an angle α to the longitudinal axis of the nozzle. There are two such channels on different sides of the axis of the inner cylinder 6.

The flow of liquid under pressure through the supply pipe 2 enters the intermediate part of the nozzle 5 and through the symmetrical slotted supply channels 7 of the inner cylinder 6 is fed into the vortex chamber (VC) of the nozzle. Due to the inclined placement of slotted channels relative to the axis of the housing, the liquid enters the vortex chamber at different distances from the axis and has a different moment of the amount of movement (1):

$$M = q \cdot v_{in} \cdot R, \tag{1}$$

where q – weight of liquid entering the nozzle vortex chamber in this cross-section; v_{in} – speed of the liquid trapped in the vortex chamber; R – swirling radius of the liquid in the control section of the inlet channel.

Depending on the position of the plunger, both the flow rate of liquid and the nature of the flow of liquid from the nozzle jet changes. When the plunger is in the lower position, the liquid enters the vortex chamber tangentially through the slotted channels and has the maximum radius and moment of the amount of movement, which is typical for a centrifugal nozzle (there is an air vortex along the nozzle axis, the spray swath is hollow, and the flow coefficient has a small numerical value). When the plunger is in the middle position, then in addition to the tangential flow, there is a liquid flow with zero twisting radius relative to the vortex chamber axis, that is, the liquid enters the twisting chamber with different moments of the amount of movement (from maximum to zero) and a spray swath is formed at the outlet of the nozzle, which is characteristic of a centrifugal jet nozzle.

In the upper position of the plunger (the slotted channels are completely open), the liquid entering the vortex chamber through the upper and lower parts of the supply channels have the maximum torque, but are directed towards each other. The resulting moment of rotation is zero, which is typical for a jet nozzle: the angle of the spray swath is insignificant, the flow rate of the nozzle takes a larger numerical value.

For this study, injectors were manufactured with one liquid supply channel to the vortex chamber (channel width 4 mm) and a vortex chamber diameter of 20 mm (Fig. 1). Replaceable nozzle jet diameters are 4; 5; 8 mm. Other design dimensions are shown in Figure 2a. The area of the inlet channel was changed by moving the plunger. Nozzles with two inclined supply channels had a twisting chamber diameter of 15 mm and nozzle diameters of 4, 5, 8 mm. Other nozzle sizes were selected according to the recommendations for their design, conditions of geometric similarity, and pressure characteristics of the pump unit. A spillway stand was created to conduct experiments (Fig. 3), which consists of a measuring tank 1, a pump 2, a liquid flow meter 3, a pressure gauge 4, an adjustment tap 5, and a test nozzle 6. The liquid flow rate was determined using a rotary flow meter of the KV-1.5 type, the accuracy class – 1.5. The liquid pressure was monitored with an OBM1-160 pressure gauge, accuracy class – 1.5.

The flow rate was determined using the equation (2):

$$\mu_t = \frac{Q_a}{Q_t} = \frac{V/\tau}{f_n \cdot \sqrt{2 \cdot g \cdot \Delta P}}, \tag{2}$$

where Q_a, Q_t – actual and theoretical fluid flow rates, respectively; V – volume of liquid during the experiment; τ – experiment time; f_n – spray nozzle jet area; ΔP – liquid pressure drop, m.

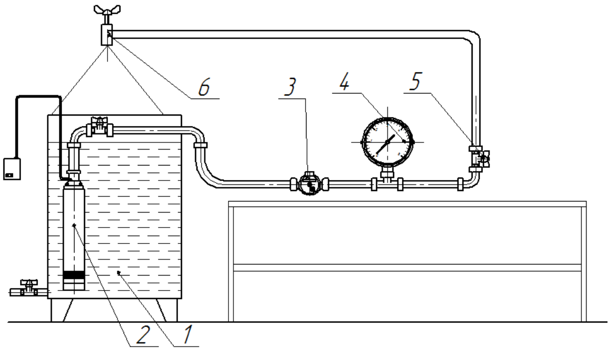

Figure 3. Nozzle test stand

Source: developed by the authors

An important characteristic of the nozzles is the uniform distribution of liquid in the spray swath. To determine this characteristic, a four-beam sampler was manufactured, in which the rays are placed at an angle of 90^0 with test tubes installed through 50 mm. Injectors with one and two supply channels were examined. Experimental data were processed using Mathcad.V15.M050, a Microsoft Office Excel table processor, graphs were created in CurveExpert-Pro-2.2.0, a Microsoft Excel table processor.

Methods of conducting a physical experiment. The study of injectors on the hydraulic stand provided for preparatory operations, including adjusting the verticality of the nozzle position, installing the pressure gauge so that its axis coincides with the middle of the nozzle mixing chamber. In the experimental studies, water was used as the working fluid to fill the container. After the working cycle, the nozzle water returned to the same tank, that is, the working fluid circulated in a closed loop, and its physical properties were the same for all experiments. This allowed excluding the influence of these factors on the results of experiments. The physical properties of the liquid from temperature were estimated based on tables of the thermophysical state of water and water vapour. Changing the operating modes of the injectors was carried out in any order (randomisation of the experiment) by adjusting the liquid pressure with a control valve, which was controlled by a pressure gauge. At least three experiments were performed on each mode. Each experiment lasted two minutes. The indicators of liquid and gas flow meters at the beginning and end of experiments, and the liquid pressure, were recorded.

Methodology of computer modelling of fluid hydrodynamics in the nozzle and spray swath. CFD modelling of the spray process and liquid flow in the nozzle elements, the specificity of which is the presence of inclined supply channels, was relevant. This will allow obtaining the required nozzle characteristics when designing nozzles of various capacities and sizes. Analysis of the software used to solve problems of this type and its functionality showed that the ANSYS software suite with the CFX hydro-gas dynamics module (ANSYS 2020 R2 version) is most widely used for modelling hydrodynamics and sputtering processes.

Physical model of the nozzle. For the simulation, a sprayer with inclined supply channels was selected in the plunger position, which leaves 60% of the height of the supply channels open. In this position, as the studies have shown, the liquid adopts the centrifugal jet type of flow and is characterised by a uniform distribution of the formed droplets over the cross-section of the spray swath, and the flow coefficient takes a high value. This distribution of liquid is most favourable for hollow absorbers, when its entire volume takes part in the mass transfer processes occurring in it. The characteristic dimensions of the nozzle under study were: the diameter of the nozzle channel – 4 mm, its length – 4.5 mm, and the radius of the twisting chamber – 7.5 mm.

Design area for calculating the movement of liquid in the elements of the nozzle and spray swath. In the three-dimensional Autodesk Inventor system, a design area has been created for modelling the hydrodynamics of liquids in sprayer elements with inclined supply channels. A separate area in the form of a cut-off cone was allocated for studying the movement of liquid at the outlet of the nozzle channel (Fig. 4).

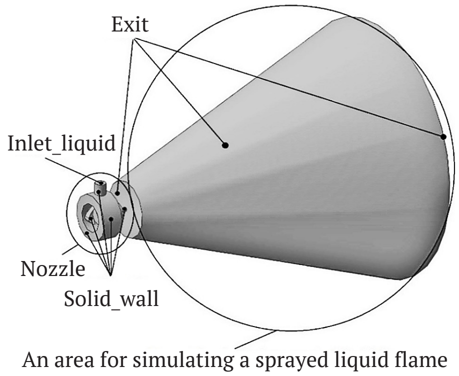

Figure 4. Calculation area and surfaces with boundary conditions
Source: developed by the authors

The design area for modelling a liquid spray swath using the method is created in the form of a cube or cylinder (Kumar *et al.*, 2022). In this case, a fairly significant calculation area was formed with an overestimated calculation zone. To optimise the numerical calculation by reducing the number of calculated cells, the authors suggest using the calculated area in the form of a truncated cone. Thus, a significant part of the calculated volume was removed, which lead to a decrease in the number of calculated cells, and therefore to a reduction in the computer calculation time.

For modelling the selected nozzles with inclined channels, the following dimensions of the design zone were established: the diameter of the design zone at the top of the cone (at the level of the end section of the nozzle) was 50 mm, the angle at the top was 38°, which coincides with the spray angle of the nozzle at this position of the plunger, the diameter of the design zone at the base of the cone – 225 mm. The created calculation area file was transferred to the Design Modeler calculation block of the CFX module in the ANSYS 2020 R2 software suite.

Calculation grid. Based on the calculation area in the mesh block, a calculation grid was automatically created with 288,380 calculation cells of tetraidal shape (the average size of the cell edge – 4 mm). The design grid of the spray swath was clarified by placing small cells in the area with a high concentration of liquid (near the nozzle channel). Larger calculation cells were placed near the outer walls. For a correct computer calculation, the number of cells and their size in the calculated area were the most important indicators of the accuracy of the simulation results. To solve problems related to calculating the size of droplets and the spray mechanism for these dimensions of the design zone, the number of design cells should be within 20 million, which requires powerful computer software.

Configuring calculation parameters. For this study, the problem type was established – non-stationary. The duration of the computer calculation, i.e., the time of stay of the liquid in the calculated area, was 0.5 seconds, and the step between iterations – 0.001. The properties of working media for the following environmental parameters are loaded from the ANSYS library database: atmospheric pressure (101,325 Pa), temperature 25°C. To solve such problems using the Navier-Stokes equations describing the motion of a viscous incompressible fluid, it is recommended to choose *k-ε* a turbulence model that provides a trade-off between the accuracy of results, the duration of machine calculations, and the requirements for the calculation grid. When modelling the swath of liquid sputtering into an unlimited space with a gas phase, the multiphase Euler model "Mixture" was used, in which the phases were considered as mutually penetrating continuums (mass and energy transfer occur without interference). The water surface tension coefficient v ($v = 0.072$ N/m) was determined (from the phase property library), and the drop resistance coefficient was assumed to be constant $C_d = 0.44$, which corresponds to the car model and turbulent motion of liquid droplets in the gas phase, assuming that the drop is spherical. The choice of the coefficient of resistance to liquid droplets should be sufficiently justified, since its numerical value depends on the mode of movement of drops (numbers *Re*) and numbers *We*, which characterises the stability of the drop shape. In the conditions of droplet flight, Weber's number is variable along the length, as is the criterion *Re* due to the resistance to droplet movement.

The initial conditions for computer calculation were the gas phase velocity equal to zero, atmospheric pressure, and volume fraction of air equal to 1 in the calculated region. The boundary conditions of simulation modelling are the area of liquid Inlet to the "Inlet_liquid" nozzle, the cylindrical and end walls of the "Solid_wall" nozzle, and the liquid outlet from the "Exit" computational area. Surfaces with applied boundary conditions are shown in Figure 4. The turbulence parameters of the model were determined: $k = 0.05$, $\varepsilon = 10$, and the value of the volume fraction of water 1 and air 0 was set. For the boundary condition for phase exit from the "Exit" computational area, the "Opening" condition type was set and the pressure was set to atmospheric pressure. The convergence criterion of results was set at 0.0001. With such phase parameters and boundary conditions, the 3D model was sent to the next calculation unit solution of the CFX module. Some specific settings were set that correspond to the technical capabilities of the computer. The possibility of performing calculations with double accuracy was established.

When the convergence criterion was reached at the level of 0.0001, the results were sent to the CFD-Post PostProcessor storage, where they were processed and printed in the form of graphic images, colour gradations, digital objects, or any other (combined) results. For liquid sprayers with inclined supply channels, important calculation results are the liquid flow lines in the nozzle elements, the liquid velocity in the control sections of the nozzle, the volume concentration of the liquid or gas phase in the design zone, the pressure distribution in the cross-section of the spray swath and at different distances from the nozzle.

Results

Experimental study of injectors with one and two supply channels. The nozzles were tested on a hydraulic test stand (Fig. 3): 1. With one inclined supply channel (Fig. 1). The area of the inlet channel was changed by moving the plunger. 2. With two inclined supply channels (Fig. 2). Addiction μ (flow rate) from A (geometric characteristics) for injectors with one supply channel $\mu = f(A)$ is shown in Figure 5. Flow rate for the jet nozzle ($A = 0$) and centrifugal-jet ($0 < A < 1$) depends on the ratio of geometric dimensions of the nozzle ($^{d_c}/D_{vc}$) D_{vc} – diameter of the nozzle vortex chamber). As follows from the above graph, as the numerical value of the geometric characteristic of the nozzle increases from 0 (jet flow) to 1 (centrifugal-jet flow of liquid from the nozzle jet), the flow coefficient decreases.

Flow rate μ for the range of values of a geometric characteristic $A < 1$ expressed by an equation of the form (3):

$$\mu = B \cdot (\frac{d_c}{D_{vc}})^n, \tag{3}$$

where B – coefficient; n – degree indicator.

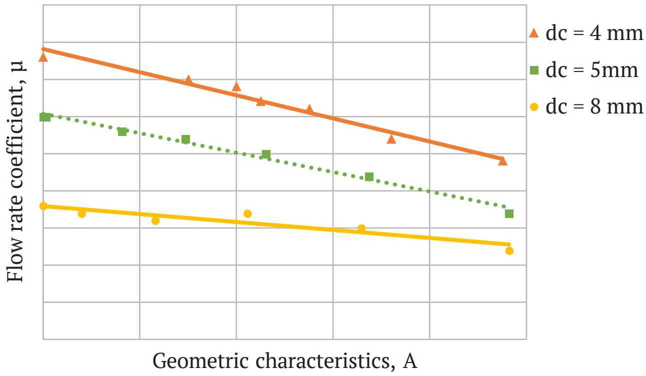

Figure 5. Dependence of µ (flow rate) from A (geometric characteristics of the nozzle)
Source: developed by the authors

The dependence of the flow coefficient μ for nozzles with a single inlet channel on the geometric simplex is known in the form (4):

$$\mu_\partial = 0.545 \cdot \left(\frac{d_c}{D_{vc}}\right)^{-0.206}, \qquad (4)$$

The amount of energy loss due to friction and local resistances during flow through the nozzle elements Σδ defined from the expression (5):

$$\mu_\partial = 0.545 \cdot \left(\frac{d_c}{D_{vc}}\right)^{-0.206}. \qquad (5)$$

A new dependence of the nozzle flow rate on the pressure of the liquid supply to the nozzle of a nozzle with one inlet channel was established for different areas of the inlet channel (different heights of the open channel) (Fig. 6). As the pressure of the liquid increases, and therefore its speed in the nozzle jet, hydraulic losses increase, which leads to a decrease in the flow rate. Within the limits of the studied pressures, the reduction in the flow coefficient was not significant.

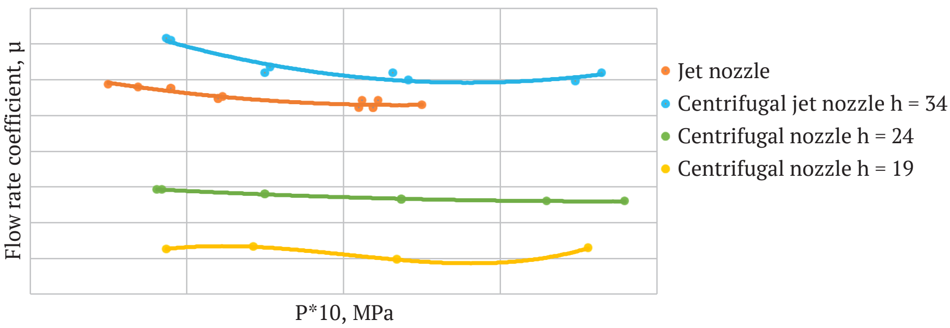

Figure 6. Dependence of the nozzle flow rate (μ) from the pressure of the liquid in the nozzle of the nozzle (P)
Note: input channel height $h = 52$ mm
Source: developed by the authors

According to this graph, in the range of studied pressures, the liquid flow rate at a given height of the open inlet channel depends only on the pressure. The flow coefficient in the studied pressure range can be assumed to be constant when calculating the performance of injectors. It has been experimentally established that the number of inclined supply channels also affects the energy efficiency of injectors: injectors with two supply channels have a higher flow rate, which is associated with a decrease in the speed of the input flow into the mixing chamber of injectors and losses in the mixing chamber itself. A graph of the dependence of the flow coefficient on the liquid inlet pressure for injectors with fully open channels (jet nozzle) is shown in Figure 7.

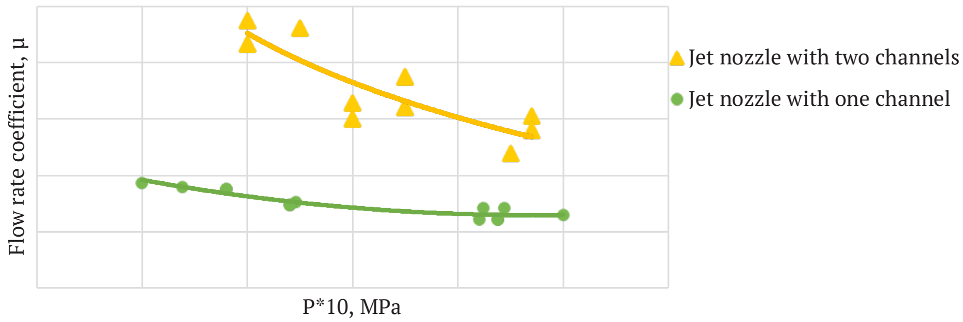

Figure 7. Dependence of the nozzle flow rate (μ) from the pressure of the liquid in the nozzle of the nozzle (P) when the channels are fully open (jet nozzle)
Source: developed by the authors

Considering the number of supply channels, the dependence for the nozzle flow rate will take the form (6):

$$\mu_\partial = n \cdot 0.545 \cdot (\frac{d_c}{D_{vc}})^{-0.206}, \qquad (6)$$

where n – coefficient that considers the number of supply channels; $n = 1$ – for injectors with one supply channel; $n = 1.1$ – for injectors with two supply channels.

The relationship $\mu = f(A)$ for centrifugal jet nozzles with a nozzle diameter of 5 mm and 65% open inlet channels is shown in Figure 8.

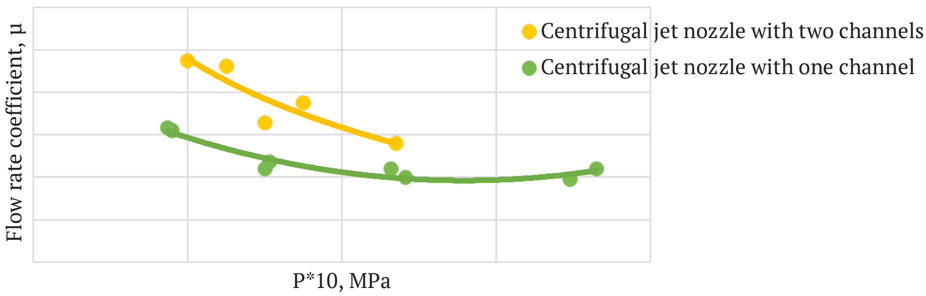

Figure 8. Dependence of the flow rate (μ) from the pressure of the liquid (P) for centrifugal jet injectors with one and two supply channels that are open by 65%
Source: developed by the authors

An important criterion for the effective operation of equipment, in particular ejectors, is the uniform distribution of liquid droplets over the cross-section of the spray swath. Dependence of relative irrigation density q_{rel} (the ratio of the mass of the liquid in the studied section to the maximum) from the relative radius R_{rel} (the ratio of the distance from the axis of the spray swath to the annular section under study to the maximum radius of the torch in this section) for a nozzle with one supply channel is shown in Figure 9, with two supply channels in Figure 10.

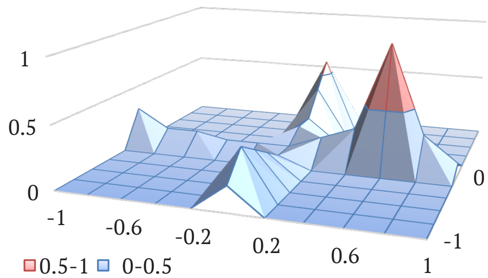

Figure 9. Dependence of the relative irrigation density (q_{rel}) from the relative radius (R_{rel}) for a nozzle with one supply channel at $P = 0.2$ MPar
Source: developed by the authors

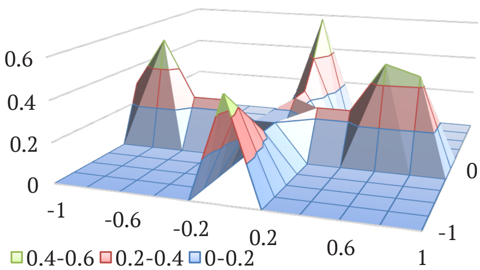

Figure 10. Dependence of the relative irrigation density (q_{rel}) from the relative radius (R_{rel}) for a nozzle with two supply channels at $P = 0.2$ MPa
Source: developed by the authors

The study of all injectors was carried out at the height of the open part of the input channels of 65% (centrifugal jet flow). The liquid pressure in the nozzle jet was 0.2 MPa. The results show that the number of input channels significantly affects the uniformity of liquid distribution over the swath cross-section. With one supply channel (Fig. 9) there was a significant uneven distribution of liquid: on the side of the supply channel, the irrigation density was higher. With two symmetrical supply channels (Fig. 10), the irrigation density in the annular sections was uniform.

CFD-modelling of liquid flow in the elements of the nozzle and spray swath. Modelling of the liquid flow in the nozzle elements was carried out at a liquid flow rate (mass) of 0.2 kg/s, which corresponds to the liquid pressure in the supply pipeline of 0.25 MPa with a nozzle diameter of 4 mm. Figure 11 shows a graph of the distribution of the liquid velocity in the nozzle elements along the axes. The speed of the liquid in the supply pipe reached a numerical value of 2.6 m/s, which is quite high, but it is within the recommended limits for the flow of liquid in pressure pipelines. In slotted channels, due to a decrease in the area, the fluid velocity increases to 2.7 m/s. The liquid in the vortex chamber has a tangential velocity that lies in the range of 1.7...2.6 m/s, and an axial velocity that is maximum along the nozzle axis – 2.2 m/s and 0.8 m/s near the walls of the vortex chamber.

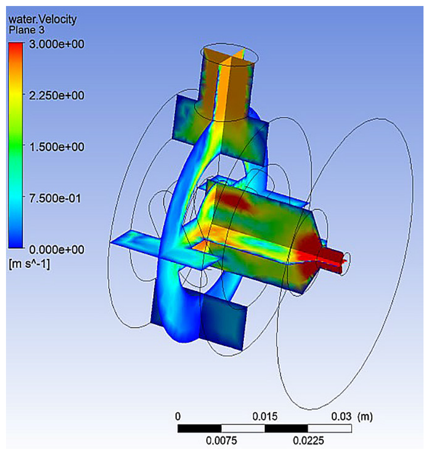

Figure 11. Fluid velocity in nozzle elements with inclined supply channels
Source: developed by the authors

In the nozzle channel, the liquid velocity increases to 19.2 m/s in Figure 12 shows a graph of the velocity distribution in the nozzle. A laminar layer is placed near the walls of the nozzle channel at a distance of up to 0.5 mm from the solid wall, in which the speed change is subject to a linear dependence. The presented data are difficult to obtain experimentally, since the dimensions of the pressure measuring probes are commensurate with the dimensions of the zone in which the velocity is measured.

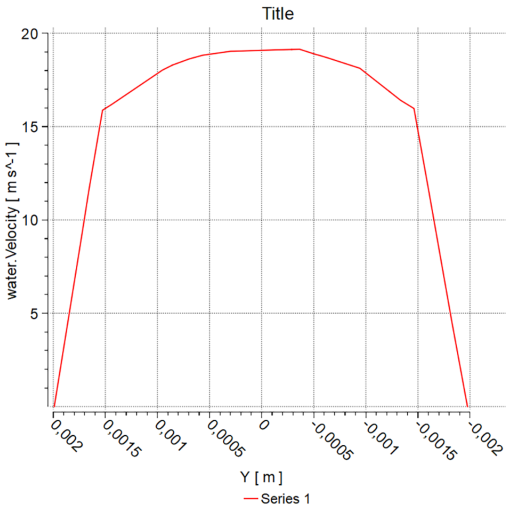

Figure 12. Graph of the dependence of the liquid velocity in the nozzle channel on its radius of the liquid velocity in the outlet section of the nozzle of a centrifugal jet nozzle with inclined supply channels

Source: developed by the authors

Analysis of the latter dependence shows that in the nozzle channel there was an asymmetry in the distribution of the fluid velocity relative to the axis, which suggests that the design of inclined supply channels was inaccurate (there was an asymmetry). This disadvantage can be corrected by performing a longer nozzle channel, in which the speeds relative to the axis are equalised. To visualise the flow of liquid in the nozzle elements, Figure 13 shows the current lines. Due to the inclined location of the inlet channels, the liquid has both a pronounced tangential flow near the walls of the twisting chamber and an axial flow along the nozzle axis. The interaction of flows leads to the development of a general flow with tangential movement of the liquid near the walls and axial in the centre of the nozzle channel.

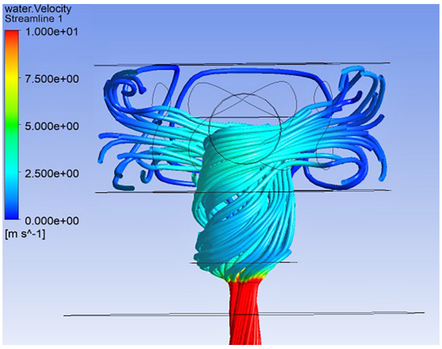

Figure 13. Development of a centrifugal jet spray swath in the nozzle when the input channels are tilted

Source: developed by the authors

Simulation of a liquid spraying swath into an unlimited space. The next logical stage of research was to determine the regularities of the development of a liquid spray swath at the outlet of the nozzle channel. The distribution of the volume concentration of liquid in the calculated zone (Fig. 14). The spray swath angle was 33^0, and the uniformity of the liquid concentration over the cross-section was achieved at a distance of about 150 mm (cross-section *I-I*).

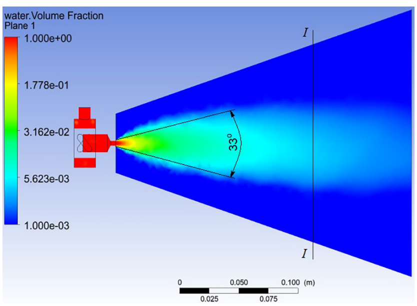

Figure 14. Volume concentration of liquid in the computational area

Source: developed by the authors

Using computer modelling, the pressure distribution over the cross-section of the sprayed liquid swath in the control sections from the nozzle channel section was established (Fig. 15).

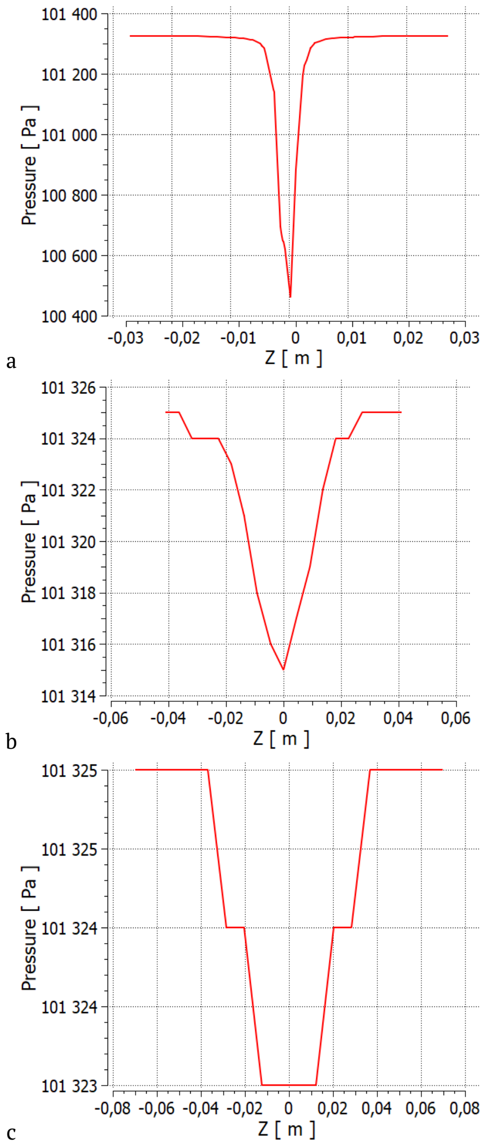

Figure 15. Dependence of the liquid pressure in the nozzle spray swath on the radius of the control sections at a distance from the nozzle channel cut-off

Note: a – 5mm; b – 50mm; c – 150mm

Source: developed by the authors

It was established that when the liquid is sprayed by the nozzle inside the nozzle swath, a vacuum occurs, and it is variable along the length of the swath: the maximum along the axis in the root zone at a distance of 5 mm from the nozzle channel, decreases with distance from the axis and with distance from the nozzle channel. If the distance from the nozzle channel is more than 150 mm, there is no vacuum along the axis of the spray swath. Experimental confirmation of this result of simulation modelling was obtained during the study of the spray swath of centrifugal jet nozzles. It was established that the maximum vacuum along the axis of the spray swath reaches 10 Pa. The obtained data on the pressure distribution in the nozzle spray swath using CFD modelling were obtained for the first time. This explains the ejection of the gas phase inside the spray swath, which is important when explaining ejection processes in jet apparatuses.

Validation of research results on the consumption characteristics of injectors. The results obtained experimentally and according to computer modelling data for one of the most important parameters of injectors – the flow coefficient (the ratio of the actual liquid flow rate to the theoretical one) are compared.

The actual volume flow rate of the liquid was calculated by the equation (7):

$$Q_r = \frac{m_r}{\rho_r \cdot \tau}, \qquad (7)$$

where m_l – weight of liquid during the experiment (50 kg), ρ_l – liquid density (1,000 kg/m^3), τ – experiment time.

The theoretical fluid flow rate was as follows (8):

$$Q_\tau = v_\tau \cdot f_j, \qquad (8)$$

where v_τ – theoretical rate of liquid leakage from the nozzle, f_j – nozzle jet area.

From the balance equation of energies (the potential energy of the fluid pressure is converted into kinetic energy without loss) in the form of the Bernoulli equation (9):

$$\frac{p}{\rho_r \cdot g} + \frac{v_r^2}{2 \cdot g} = const, \quad (9)$$

where p – pressure of liquid in the nozzle, g – acceleration of gravity.

The theoretical velocity of the liquid in the nozzle jet was calculated (10):

$$v_r^t = \sqrt{\frac{2 \cdot p}{\rho_r}} = \sqrt{\frac{2 \cdot 0{,}25 \cdot 10^6}{10^3}} = 22.36 \text{ m/s}. \quad (10)$$

Actual flow rate:

$$\mu = \frac{Q_r}{Q_\tau}. \quad (11)$$

Processing of experimental data made it possible to determine the dependence of the flow coefficient on the main geometric simplex of the nozzle (d_j/D_n) in the form of (12):

$$\mu = 0.545 \cdot \left(\frac{d_j}{D_n}\right)^{-0{,}206}. \quad (12)$$

The flow coefficient was calculated using the following formula for a nozzle with a jet diameter $d_j = 4$ mm and vortex chamber $D_n = 15$ mm. Numeric value: $\mu = 0.716$.

The actual flow rate can be directly calculated by considering the actual second flow rate of liquid through the nozzle and the fluid flow rate for the ideal nozzle (excluding resistances) (13):

$$\mu = \frac{Q_r}{Q_\tau} = \frac{0{,}2}{22{,}36 \cdot 3{,}14 \cdot (0{,}002)^2} = 0.71. \quad (13)$$

Determination of the nozzle flow rate using an empirical equation and directly from the experiment gave the same results for the studied nozzle with inclined supply channels.

The experimental value of μ was compared with the value obtained by CFD modelling. The average velocity of the liquid in the nozzle jet was determined from the condition that the area of the figure describing the instantaneous velocity of the liquid was equal to the area of the rectangle, the ordinate of which was the average velocity of the liquid in the nozzle. According to this method of calculating the average speed, it was equal to $v_m = 15.09$ m/s. The liquid consumption based on the simulation results was:

$$Q_m = v_m \cdot f_j = 15.09 \cdot 3.14 \cdot 0.002^2 = 0.1895 \text{ m}^3\text{/s},$$

and the flow rate was equal to $\mu = 0.696$.

The relative error in finding the flow rate from experimental data and computer modelling was 3.6%. Such comparative calculations of flow coefficients were also performed for other injectors under study.

Discussion

When modelling the process of spraying liquid with a nozzle, an important step is to choose the turbulence model that will be used in calculations. The presented studies were conducted with a standard *k-ε* model that provides for a trade-off between accuracy and calculation time. When performing a numerical study of the spraying process by N. Ayuba & G. Lopes (2023) model verification was performed using *k-ε* Realisable, *k-ε RNG*, standard *k-ω, k-ω SST*, Reynolds Stress Models, Boussinesq, Large Eddy Simulation on the possibility of application in simulation modelling. As a result, it was concluded that the standard model *k-ε* can be used with sufficient accuracy to describe tasks of this type. R. Wu *et al.* (2024) modelling of diesel fuel sputtering at high pressures using a turbulence model *k-ε* and a good agreement with experimental data is shown. The choice of a model in computer modelling was the subject of the work of A. Metsue *et al.* (2024), which compares the accuracy of calculations for six turbulence models. It was established that *k-ε RNG* and *k-ω SST* models better describe the flow structure in the flow part of the ejector, and *k-ω SST* model better describes the process of mixing two streams. J. Xiao *et al.* (2022) presented the results of modelling ejectors using *k-ω SST* and standard *k-ω* and *k-ε* turbulence models. It was established that the most reliable results are achieved when modelling the operation of ejectors on a standard k-ε model. Thus, the presented data indicate that the application of the standard turbulence model k-ε with numerical calculations of the spray swath provides a fairly high-quality picture of the process and reliable numerical results, which is also confirmed by the presented results.

One of the most important parameters for modelling sputtering or ejection processes in jet apparatuses is to set the required number of calculated cells. On the one hand, the larger the number of cells involved in the calculation process, the more accurate the result. However, this increases the requirements for the technical side of computers and the calculation time. The basic rule when creating a calculation grid is the type of problem that is solved during the calculation process. Thus, to simulate the process of forming a swath and determine its dispersed composition, the cell size should be smaller than the size of droplets. For different types of injectors and pressures at which liquid dispersion occurs, the choice of the number of calculated cells requires an individual approach based on a priori information about the geometric size of droplets. In this case, the maximum number of cells was selected.

In the case of modelling a liquid spray swath and the need to obtain consumption characteristics, a significantly smaller number of cells is sufficient without reducing the accuracy of the results. There are known studies of a spray swath when operating an ejector with a number of cells of 25,820 (Nguyen *et al.*, 2024). The researchers concluded that there are enough cells for the general characteristics of the spray swath (visualisation) without detail, but the reliability of quantitative characteristics of the phases in the swath is small. Y. Hou *et al.* (2022) performed ejector modelling and comparison of calculations in a calculated grid of 53,980 cells and 107,580 cells. It was found that the error of the simulation results – 4.45%. The researchers did not specify how much the simulation data correspond to the experimental data. According to the results of studies of the spray swath, with such a large number of cells, a contour image with indistinct borders can be obtained.

When setting the problem of determining the dispersed composition of droplets formed during spraying, the number of calculated cells should be such that its average size does not exceed the geometric size of the droplets. For example, when determining the drop size of a centrifugal nozzle with a nozzle diameter of 0.94 mm and the liquid pressure in the nozzle of 0.2 MPa, the volume-surface diameter of the drops d_{32} – 130 microns, which corresponds to a geometric drop diameter of 98 microns. In other words, this is the minimum size of the calculated cell, but considering the polydispersity of liquid droplets, the cell size should be significantly smaller (at least twice).

There is a known modelling of the process of droplet development during the spraying of liquids with the number of computational cells of the order of 10 million, which allows establishing a qualitative and quantitative picture of the spraying process at different moments of time and droplet sizes, which was confirmed by experimental data of K. Vhora *et al.* (2024) with an error in the range of 11...27%. In this study, the number of design cells in the spray swath area of the nozzle was 288,380. Tetraidal cells (the average size of the cell edge – 4 mm). This number of cells is generally sufficient to determine the main characteristics of the spray swath (spray angle) and establish the distribution of phase concentrations in it along the length and cross-section, that is, to obtain a high-quality visual picture of the development of the swath and its numerical values (phase velocity, pressure, concentration distribution).

It should also be added that simulations were performed with both a larger and smaller number of calculated cells. When the calculated zone was divided into fewer than 50 thousand cells, the simulation results were not accurate. If the number of cells exceeded 500 thousand, the calculation accuracy increased, but its duration increased almost 5 times and amounted to 9...10 hours. The correctness of the calculations performed was confirmed by experimental data.

The results of numerical modelling for determining the pressure in the spray swath at different distances from the nozzle are confirmed by numerous studies, for example, J. Xiao *et al.* (2022), who analysed the operation of an ejector with a cylindrical mixing chamber based on the Navier-Stokes equation in the CFD system. Analysis of the experiment shows that the pressure in the vortex chamber gradually decreases due to the recirculation of the flow and penetration of atmospheric air from the outlet neck. Similar data for injectors with inclined supply channels were obtained as a result of simulation modelling: the maximum vacuum along the axis in the basal zone at a distance of 5 mm from the nozzle channel and decreases with distance from the end of the nozzle channel. Thus, CFD modelling in the modules of the ANSYS software suite (version ANSYS 2020 R2) demonstrates significant potential for application tasks for determining the characteristics of the spray swath and allows visualising the spray process and predicting droplet sizes with sufficient accuracy with appropriate adjustment of the calculated grid. This method deserves further development and improvement, demonstrating its versatility and practical usefulness.

Conclusions

A comprehensive study of nozzles with inclined supply channels, which are used as active ejector nozzles in jet sulphitators in sugar production, was carried out. The hydrodynamics of liquid movement in the nozzle elements was established and the process of forming a spray swath was studied. The dependence of the liquid flow coefficient on the geometric parameters of the nozzle and the number of supply channels was experimentally established: a nozzle with two symmetrically placed supply channels relative to the axis had a 20...25% higher flow coefficient. In addition, with two supply channels, the irrigation density of the spray swath cross-section was significantly improved.

A numerical study of the liquid spray process by injectors was carried out using the Ansys 2020 R2 software suite. The standard *k-ε* model with the number of computational cells 288,380 was used for modelling and it was proposed to reduce the computational zone to a truncated cone, which would optimise the calculation time and provide a better picture of the spray swath and its quantitative characteristics. A computer study of hydrodynamics in the form of liquid flow lines in nozzle elements with inclined channels showing the stages of development of a centrifugal jet flow of liquid was carried out. A numerical distribution of the fluid velocity in the end section was found on the nozzle section, which was maximum along the nozzle axis of 19.2 m/s, and a laminar layer was located near the walls of the nozzle channel at a distance of up to 0.5 mm from the solid wall. Computer modelling has obtained new results confirming the presence of a low-pressure zone along the spray swath axis, which is maximum in the basal zone, decreases as it moves away from the nozzle end and disappears at a distance of 150 mm from it. The results of the nozzle flow rate coefficient were validated. The relative error in determining the nozzle flow rate during CFD modelling compared to experimental results does not exceed 5%.

Further research will focus on visualising and determining the quantitative characteristics of the liquid flow in the nozzle elements and the characteristics of the spray swath of other types of nozzles and when scaling. Such data will help to design injectors of various capacities with specified characteristics, to achieve an efficient mode of operation of equipment for the food industry.

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Conflict of Interest

The authors of the study performed the work equally and have no mutual claims of authorship.

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Дослідження та застосування форсунок з нахиленими підвідними каналами для струминних сульфітаторів цукрової промисловості

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Анотація. Стаття присвячена дослідженню процесу розпилення рідини форсунками, що застосовуються в струминних сульфітаторах цукрової промисловості або як окреме обладнання. Розпилювачі рідин відіграють основну роль в забезпеченні ефективної роботи тепломасообмінного обладнання внаслідок створення значної поверхні контакту. При проектуванні або аудиті його технічної досконалості необхідно мати достовірні гідродинамічні характеристики розпилювачів. Роботи такого направлення є актуальними. Метою статті було встановлення всіх характеристик течії рідини в елементах форсунки та факелу розпилення за допомогою комплексного дослідження (експериментального та комп'ютерного) для можливості інтенсифікації роботи обладнання, зокрема сульфітаторів для цукрової промисловості. Для досягнення поставленої мети було використано експериментальні (дослідження форсунок та ежекторів на гідравлічному стенді), CFD-дослідження зазначених об'єктів в модулях програми ANSYS (версія: ANSYS 2020 R2). Експериментально встановлено залежність коефіцієнта витрати форсунок від тиску рідини та кількості підвідних каналів для різних типів форсунок (струминної, відцентрово-струминної). Експериментально підтверджено, що рівномірність розподілення рідини в факелі форсунки з двома підвідними каналами була високою та достатньою для практичного застосування. При CFD-дослідженні характеристик факелу розпилення форсунок було запропоновано зменшити розрахункову область до зрізаного конусу, що дозволило скоротити час розрахунку. При моделюванні гідродинаміки рідини в форсунці визначено чисельно характерні швидкості як в елементах форсунки, так і в соплі. Встановлено наявність у прикореневій зоні розрідження, що досягала 865 Па для даної форсунки при тиску рідини на вході 0,25 МПа, що приводило до ежектування газу всередину факела. Наведена методологія розрахунку актуальна при визначенні характеристик форсунок, при розробці, проектуванні нових розпилювачів з заданою продуктивністю та факелом розпилення, що застосовуються і як активні сопла ежекторів, і як самостійне обладнання для диспергування технологічних рідин харчової промисловості

Ключові слова: виробництво цукру; сульфітація рідини; розпилювач; коефіцієнт витрати; CFD-моделювання; гідродинаміка; факел розпилення

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Contemporary trends in broiler meat production in North America: A literature review

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Abstract. Global broiler meat production is increasing at a faster rate than any other type of meat. The United States is the largest producer of broiler meat worldwide. In 2022, the USA, together with Canada and Mexico, accounted for 20% of the total global broiler meat production. This study aimed to analyse and summarise the literature on contemporary trends in broiler chicken production technologies in the USA, Canada, and Mexico. The study employed methods of analysis, synthesis, and content analysis. It was found that among the North American countries, the USA ranks first in both the number of chickens and broiler meat production. Globally, in 2022, the total chicken population was 26,561,634.0 thousand birds. China held the first position (5,185,477.0 thousand birds), with the USA in fifth place (1,528,000.0 thousand birds), Mexico in eighth (611,202.0 thousand birds), and Canada in 29th (173,942.0 thousand birds). In the global

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rankings of broiler meat producers in 2022, the USA was first, Mexico seventh, and Canada 22nd. A consistent increase in broiler meat production was observed in North American countries over the period 2018-2022. In 2022, the USA produced 20,652,970.91 tonnes of broiler meat, Canada 1,332,967.57 tonnes, and Mexico 3,668,552.28 tonnes. The leading producers of meat products in the USA and across North America are Tyson Foods, Cargill, and Bell & Evans. The most common broiler breeds used in North American poultry farming are Cobb and Ross. A comparative analysis of the performance of the Cobb-500 and Cobb-700 breeds indicated that Cobb-500 broilers outperform in terms of live weight. Overall, Cobb-700 broilers exhibit slower growth rates, with a production cycle extending up to 63 days. The predominant production method involves raising broilers on litter-based floor systems. Researchers from South American universities are conducting various studies that address contemporary challenges in meat poultry production. These studies focus on issues such as housing, feeding, welfare, health, and carcass quality of broilers

Keywords: USA; Canada; Mexico; chickens meat; cross; live weight; feeding

Introduction

Over the past 50 years, the poultry industry worldwide has undergone rapid development, and the progress achieved has been substantial. Significant scientific advancements have focused on enhancing the understanding of poultry science, facilitating the rapid transition from backyard production to industrial-scale farming, reducing the cost of broiler meat, and addressing cultural and religious restrictions on chicken consumption (Siegel, 2023). T. Mcdougal (2022) highlights that poultry meat consumption has increased in nearly all countries and regions, driven by its relatively low price, desirable texture, and higher protein content compared to lower fat content. Global poultry meat consumption is projected to increase to 154 tonnes by 2031. This long-term shift towards poultry meat is expected to continue. In countries with middle and low-income populations, poultry meat has become a more affordable alternative to other meats. As a result, the availability of protein from poultry is projected to increase by 16% by 2031, accounting for 47% of protein consumed from meat sources, followed by pork, lamb, and beef. However, in high-income countries where per capita consumption is already high, demand is expected to level off or even decline, given ageing populations and a greater emphasis on diversified diets. The short-term shift in meat consumption from food service to home cooking, observed during the COVID-19 pandemic, is anticipated to be temporary, with consumers returning to their previous spending habits. Global meat supply will increase to meet growing demand, reaching 377 million tonnes by 2031. The global expansion of poultry farming means that, according to forecasts, the majority of the growth in meat production will come from China, followed by the USA, Brazil, and India.

Broiler production plays a pivotal role in meeting the global demand for poultry meat, which is essential to feed a continually growing population. In 2022, North American countries accounted for 20% of total broiler meat production. Scientific support for the broiler industry in North America is provided by researchers from the USA, Canada, and Mexico. They conduct research in various areas. For instance, J. Purswell *et al.* (2021) demonstrated that feeder size can influence broiler growth, especially during the early stages of rearing. J. Jespersen *et al.* (2024) determined the optimal levels of starch, oil, and amino acids in diets for broilers infected with coccidiosis. A. Myers & S. Rochell (2024) evaluated the impact of energy content in starter diets on nutrient digestibility and growth performance of broilers, using different coccidiosis control programs. L. Aloui *et al.* (2024) investigated the effects of heat stress on the hypothalamic gene expression profile related to water homeostasis in broilers. Scientists S. Sgavioli *et al.* (2023) assessed the behaviour of broiler chickens subjected to heat stress at different times of the day and found that birds spent more time feeding in the morning, regardless of age and rearing temperature. G. Baldi *et al.* (2020) studied factors influencing the high pH of the pectoralis major muscle in broilers affected by wooden breast condition.

The findings of S. Zhou *et al.* (2024) indicate that Cobb-700 broilers with a medium growth rate (weight gain <50 g/day) reach a market live weight of 3 kg at 52 days of age, while slowergrowing birds (weight gain 50-60 g/day) reach this weight at 62 days. Given that broiler carcass colour is a significant factor for poultry consumers in various countries, including Mexico, L. Pantoja & O. Gonzalez (2020) characterised the factors influencing skin pigmentation, highlighting genetics, nutrition, and diseases as key contributors. R. Urtecho-Novelo *et al.* (2021) studied the ethology, health, and productivity of Hubbard cross broilers under tropical Mexican conditions. L. Bean-Hodgins & E. Kiarie (2021) highlighted the mandatory restrictions on the use of medically important antibiotics in broiler chicken production in Canada. S. Che *et al.* (2022) provided data on the prevalence of breast muscle myopathies and associated risk factors in broiler chickens raised on farms in Ontario, Canada. The research of H. Sammari *et al.* (2023) confirmed the presence of numerous broiler carcass quality issues by examining 206 breast fillets purchased from grocery stores in Quebec and assessing them for various defects. Myopathy of the breast muscles was detected in 48.5% of these fillets. J. Lee & M. Mienaltowski (2023) highlight that the increased growth rates and sizes of broilers disrupt certain biochemical processes in the bird's body, leading to inflammation and hypoxia, and subsequently to the formation of foamy cells from macrophages and the deposition of fat along muscle tissues in the form of white stripes.

Considerable attention has been given to broiler welfare issues in North American poultry farms. For example, M. Anderson *et al.* (2021) demonstrated the need to enrich the environment for broiler chickens to improve their welfare. The results of the study by T. Shynkaruk *et al.* (2023) indicate that broiler welfare is compromised at higher stocking densities, as birds experience reduced productivity, poorer litter quality, and increased foot pad lesions. P. Regmi (2024) reports that in Canada, the recommended stocking density for broilers should not exceed 31 kg/m^2, while in the USA, this figure is considered optimal within the range of 32-44 kg/m^2. I. Chan *et al.* (2022) argue that reducing chicken consumption can help avoid potential trade-offs between animal welfare and environmental protection.

This study aimed to analyse FAOSTAT data and synthesise the findings of scientific research conducted by North American scientists between 2019 and 2024 regarding broiler chicken meat production technology in the USA, Canada, and Mexico. The study employed content analysis, analysis and synthesis, and comparative analysis. The authors analysed and adapted data from sources such as FAOSTAT (2024); Cobb 500 Broiler Performance & Nutrition Supplement (2022); and Cobb 700 Broiler Performance & Nutrition Supplement (2020).

Contemporary state of broiler meat production in the USA, Canada and Mexico

M. Jordan (2020), Executive Director of LEAP Market Analytics, analysed broiler meat exports from the USA to Canada and Mexico over the recent period, starting in 2012. The two largest US trading partners in North America (Canada and Mexico) are reliable and important partners for broiler meat exports. The author cites data from the US Department of Agriculture Economic Research Service (USDA ERS), according to which, in 2019, just over a quarter of the approximately 7.1 billion pounds of broiler meat exported from the USA to all countries was sent to Canada and Mexico. Over the past five years, exports to Mexico totalled nearly 7.2 billion pounds, while shipments to Canada amounted to nearly 1.7 billion. The US broiler industry has expanded its presence in both Canada and Mexico in recent years. Trade peaked in 2014 when total exports reached 1.9 billion pounds, representing 4.9% of total domestic broiler meat production that year. Challenges arose when industry workers had to contend with trade restrictions imposed in response to the outbreak of highly pathogenic avian influenza. Exports of poultry meat to Canada and Mexico declined further in 2017 as the US administration implemented antagonistic policies towards these countries. In that year, combined shipments of broiler meat from the USA to Canada and Mexico fell short of 1.7 billion pounds, or slightly less than 4.0% of total domestic production. While shipping broiler meat to China and other countries may seem advantageous, as it could improve trade dynamics and generate significant export revenue in a relatively short time, the expert believes that the USA would be safer to focus on positive relations with historically reliable and geographically proximate trading partners. US poultry companies, struggling to offload surpluses of boneless, skinless chicken breasts, which have been consistently selling for less than 1 USD per pound since September 2019 and are sitting in cold storage in large quantities, undoubtedly welcome the US-Mexico-Canada Agreement (USMCA), which will provide expanded access, primarily to the Canadian market, for poultry products.

H. Unveren & J. Luckstead (2020) constructed a comprehensive supply chain model for the US broiler industry. This model was developed to analyse the impact of tariffs on corn and soybean meal imposed by China and changes to Canada's tariff-rate quota proposed under the USMexicoCanada Agreement. The first scenario assumes that a decrease in feed prices will increase supply, which will contribute to lower production costs for breeding companies and broiler farms. The second scenario suggests that broiler meat exports to Canada will increase at the expense of exports to Mexico.

Characterising the current state of poultry farming, it should be noted that, according to FAOSTAT (2024), the global stocks chickens in 2023 was 27,223,471.0 thousand heads. China ranks first in terms of stocks chickens (5,217,362.0 thousand birds), the USA is fifth (1,526,000.0 thousand birds), Mexico is eighth (621,072.0 thousand birds), and Canada is 30th (171,487.0 thousand birds). For comparison, Ukraine ranks 32nd with a stocks chickens of 165,468.0 thousand birds. The authors investigated the trend in the stocks chickens in North America (Table 1). It should be noted that FAOSTAT, when indicating the stocks chickens in North America, only considers data for the USA and Canada (FAOSTAT, 2024).

Table 1. *Stocks chickens in North America, thousand birds*

Year	Northern America	USA	Canada	Mexico
2019	1,699,609.0	1,530,000.0	169,609.0	580,829.0
2020	1,707,555.0	1,537,000.0	170,555.0	591,596.0
2021	1,692,711.0	1,522,000.0	170,711.0	604,682.0
2022	1,703,099.0	1,532,000.0	171,099.0	609,506.0
2023	1,697,487.0	1,526,000.0	171,487.0	621,072.0

Source: developed by the authors for FAOSTAT (2024)

An analysis of the presented data reveals that the USA has the largest poultry population. Mexico ranks second. Overall, the poultry population in Canada and Mexico has shown an increasing trend from 2019 to 2023. Compared to 2019, the US stocks chickens decreased by 0.1% in 2023 (Fig. 1).

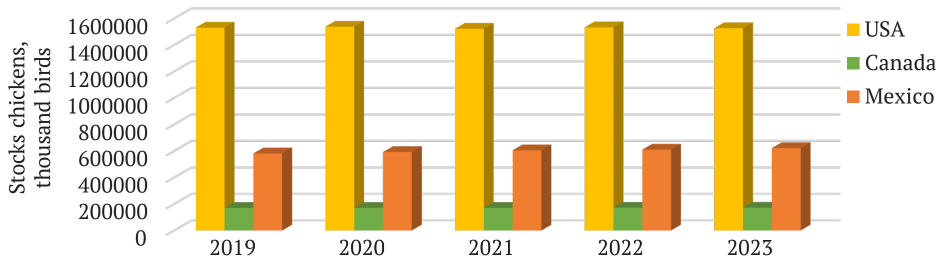

Figure 1. Changes in the stocks chickens in North America, thousand birds
Source: developed by the authors for FAOSTAT (2024)

Regarding poultry meat production, the world produced 123,631,334.7 tonnes in 2023 (FAOSTAT, 2024). The USA ranked first (19,901,890.0 tonnes), followed by Brazil (14,833,000.0 tonnes), China (14,800,000.0 tonnes), Mexico (3,888,207.9 tonnes), and Canada (1,415,926.0 tonnes) in the seventh and 24th positions respectively. The authors also analysed broiler meat production in North American countries (Table 2) (FAOSTAT, 2024).

Table 2. *Broiler meat production in North American countries, tonnes*

Year	USA	Canada	Mexico
2019	20,197,090.5	1,331,571.0	3,476,622.2
2020	18,814,264.0	1,305,207.0	3,578,694.1
2021	19,230,123.0	1,334,020.0	3,668,551.3
2022	19,841,239.0	1,370,800.0	3,781,735.3
2023	19,901,890.0	1,415,926.0	3,888,207.9

Source: developed by the authors for FAOSTAT (2024)

An analysis of broiler meat production shows that, as with the stocks chickens, the USA is the leading producer, followed by Mexico. Overall, there has been an increase in meat production over the five-year period examined in Canada and Mexico, while in the USA, production increased only from 2021 to 2023 (Fig. 2).

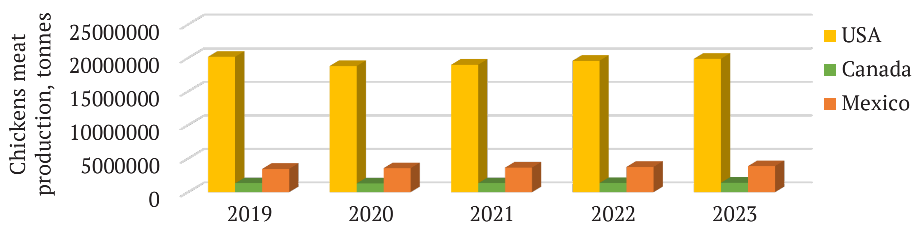

Figure 2. Dynamics of chickens meat (fresh or chilled) production in North America, tonnes
Source: developed by the authors for FAOSTAT (2024)

According to the US Department of Agriculture (USDA), broiler meat production is projected to grow steadily until 2033, after a significant decline in the poultry population in 2022 and 2023 due to outbreaks of highly pathogenic avian influenza (Hein, 2024).

S. Beets (2019) highlights that chicken meat, or broiler, production in the United States is a vertically integrated industry where integrator corporations control all aspects of the business. Primarily due to a series of mergers and acquisitions, a duopoly has formed. The two dominant integrator corporations, Pilgrim's Pride and Tyson Foods, are highly profitable, and their executives and shareholders benefit from the corporations' financial success. However, many local farmers who raise chickens for meat and sell to integrators (wholesale buyers) receive minimal benefits from the integrators' financial success, as farmers face a multitude of financial, environmental, social, and health problems. Many producers find it difficult to abandon an occupation that requires a significant initial investment or debt of hundreds of thousands of dollars. Another issue with large-scale broiler production is air pollution and the creation of unfavourable conditions for people living nearby. Several demographic, economic, educational, health, and quality-of-life indicators in 50 states of the USA were analysed to gain insight into the ethics of the broiler industry and why some states produce significantly more broilers than others.

A. Pescatore *et al.* (2019) report that the USA is the world leader in chicken consumption, reaching a level of 90 pounds per capita. To meet this demand, the country produces approximately 9 billion chickens annually, providing over 50 billion pounds of broiler meat. The broiler market is segmented as follows: 55% of sales are attributed to retail and 45% to the food service sector. Any changes in the production system can impact the sustainability and economic efficiency of the poultry industry. Over the past 50 years, broiler chickens have significantly changed due to genetic improvements and enhanced diets. However, rapid growth and increased productivity have also led to problems such as muscular myopathies and skeletal disorders. In response to these challenges, some producers have begun transitioning to slower-growing chicken genotypes, which yield less than 50 grams of average daily weight gain. The popularity of such approaches is growing among restaurants and stores, including Whole Foods, Panera Bread, Chipotle, Noodles and Co., and Quiznos. They prefer poultry raised according to Global Animal Production (GAP) standards. For example, the company Bell and Evans implements slower-growing chickens through their proprietary breed (cross) Das Klassenbester. The shift towards slower-growing chickens and GAP standards could significantly impact the entire poultry meat production industry.

The Canadian poultry sector plays a significant role in the Canadian economy. Canada's broiler industry benefits both urban and rural economies. As a driving force in the Canadian economy, 2,877 poultry farmers and 185 poultry processors create jobs, increase prosperity, and help sustain agricultural communities in every province of the country. Specifically, the sector: supports 101,900 jobs and contributes 8 billion USD to Canada's gross domestic product. It pays 1.9 billion USD in taxes, and purchases 3 million tonnes of feed, thereby supporting other farmers. Canadian poultry farmers are a stabilising factor for rural regions of Canada and create a variety of jobs not only in agriculture and processing but also in transportation, retail, restaurants, and more (Good for Canada, 2021).

In 2021, Canada had 2,823 chicken producers who produced 1.3 billion kilograms of chicken meat (in eviscerated carcasses), 60% of which was produced in Ontario and Quebec. Overall, in 2021, chicken production in Canada generated 3.3 billion USD, accounting for 4.0% of cash receipts in farming operations. In 2021, Canada also had over 78 primary processing establishments, 40 of which are federally inspected, along with many other processors whose business depends on chicken supplies. In 2020, chicken was the most consumed animal protein in Canada with consumption of 34.4 kg per capita, slightly below the 2019 level. This was the first annual decline in a decade, attributed to the impact of the COVID-19 pandemic on the poultry industry, as well as closures and reduced offerings from food service establishments, which account for a significant portion of chicken consumption in Canada. Canada also imports chicken, and imports are regulated by tariff-rate quotas (TRQ). In 2021, imports amounted to 164.7 million kilograms in carcass weight, primarily from the USA, followed by Brazil, Thailand, and Hungary. In the same year, 111.8 million kg of meat and meat products (fresh, chilled, frozen) valued at 554.8 million USD were exported to over 50 countries worldwide, with the USA, Philippines, and Taiwan being the largest importers (Canada's chicken industry, 2024).

In April 2023, Canadian Poultry Magazine (Ontario investing in new poultry research centre, 2023) reported that the Ontario government is investing 13.5 million USD in a new poultry research centre in Elora. The Ontario government, the Agricultural Research Institute of Ontario (ARIO), and the province's four poultry councils have signed a Memorandum of Understanding to invest in the new Ontario Poultry Research Centre to advance research and innovation in the poultry sector. "As part of the Grow Ontario Strategy, our government is focused on enhancing our research and innovation capabilities across the agri-food sector," says Lisa Thompson, Minister of Agriculture, Food and Rural Affairs.

N. Berkhout (2021) noted that imports account for about 18% of total domestic chicken meat consumption in Mexico, with chilled leg quarters and mechanically deboned chicken meat from the USA making up almost half of all imports. Frozen breasts, wings, and drumsticks are also imported from Brazil. Companies that further process poultry for use in products such as sausages, ham, and meat assortments are the main source of demand for imports.

The COVID-19 pandemic and economic recession led to a decrease in chicken meat consumption in 2020 and resulted in a decline in imports. A persistent risk to the highly concentrated poultry industry is avian influenza. Despite vaccination of breeding and laying flocks, the virus is endemic in some states. The prolonged COVID-19 pandemic impacted the poultry industry, especially chicken meat production, in 2021 (Mexico's role in the global poultry industry, 2022).

L. Estévez-Moreno & G. Miranda-de la Lama (2022) indicate that meat is a significant component of contemporary Mexican culture. Its consumption is linked to ancestral traditions as well as agri-food globalisation. Mexicans consume three types of meat the most: chicken (35 kg/person/year), pork (20 kg/person/year), and beef (15 kg/person/year). The authors also note that Mexico has a significant population of people adhering to plant-based diets: 19% are vegetarians, 15% are flexitarians, and 9% are vegans. Overall, throughout the 21st century, Mexican society has become increasingly concerned about the negative health impacts of meat, animal welfare, and the environmental effects of livestock farming. Given that Cobb and Ross crosses are the most common in North America, the authors analysed the performance indicators of Cobb broiler crosses – a leading American breeding company (Table 3).

An analysis of the presented data indicates that the Cobb-500 and Cobb-700 crosses exhibit differences in both live weight and feed conversion. Cobb-500 broilers have an advantage in terms of live weight. At 42 days of age, they reach a live weight of 3278 g, surpassing the Cobb-700 cross (2,847 g) by 431 g. At 56 days, this difference increases to 528 g, and the feed conversion ratio is also lower in Cobb-500 broilers. However, over a 42-day growing period, they require 5,100 g of feed per bird, compared to 4547 g for the Cobb-700 cross. Overall, Cobb-700 broilers grow more slowly and can be raised to 63 days of age. Cobb-700 broilers achieve a live weight similar to Cobb-500 broilers one week later. Therefore, the producer chooses a cross depending on the final goal of production in a particular farm.

Table 3. *Live weight and feed conversion in Cobb broilers*

Age, days	Cobb-500			Cobb-700		
	Live weight, g	Feed intake, kg/kg gain	Cumulative Feed Consumption, g	Live weight, g	Feed intake, kg/kg gain	Cumulative Feed Consumption, g
0	42	-	-	42	-	-
7	202	0.891	180	183	0.883	162
14	570	1.029	588	468	1.028	481
21	1,116	1.018	1,320	916	1.174	1,076
28	1,783	1.322	2,359	1,500	1.319	1,978
35	2,521	1.441	3,635	2,163	1.460	3,158
42	3,278	1.555	5,100	2,847	1.597	4,547
49	4,001	1.686	6,749	3,509	1.727	6,058
56	4,641	1.842	8,549	4,113	1.849	7,605
63	-	-	-	4,643	1.964	9,117

Source: adapted by the authors from Cobb 500 Broiler Performance & Nutrition Supplement (2022); Cobb 700 Broiler Performance & Nutrition Supplement (2020)

In the poultry processing industry, North American broiler plants are increasingly shifting from water chilling to air chilling carcasses. Several large processors in the USA are already extremely satisfied users of air chilling tunnels (Air chilling growth in North America, 2021). Canadian broiler processors have also been effectively using air chilling systems for some time. Marel is a leading company in carcass processing and offers a product portfolio ideally suited for this market. Immersing products in a chill chiller, which has been the most popular cooling method in the USA, leads to additional water absorption. A representative of the American processor Bell & Evans comments on this: "With conventional chilling systems, chickens absorb up to 12% of their body weight in added chlorinated water. This water "weeps" out of the meat and is trapped in the "diaper" you will find in most fresh chicken packaging". In North America, sanitising chemicals are added to the water tank. Air chilling does not require chemicals, while still maintaining a super-hygienic process. Air chilling fully supports the process with less labour. This also enables better tracking of the entire process. End-to-end traceability combined with food safety is a top priority for customers and consumers in North America (Air chilling growth in North America, 2021).

S. Barbut (2019) indicates that in recent years, there has been an increasing incidence of myopathies in young broilers worldwide, such as white striping (WS), woody breast (WB), and spaghetti meat (SP) in breast muscle tissue. According to some industry reports, the prevalence of WB in fast-growing heavy broilers (~4.0 kg) can reach 20%, which is estimated to cost the US poultry industry over 500 million USD annually, although actual losses may be significantly higher. The severity and frequency of these anomalies depend on the specific flock and are associated with factors such as genetics, nutrition (particularly protein levels in the bird's diet during rapid growth), growth rate, chick activity at an early age, sudden physical exertion, the number of embryonic stem cells, and litter management in the poultry house. These three myopathies can occur separately or together, but there is some interrelationship between them. During meat processing, they create quality issues (denser meat structure and/or reduced water-holding capacity, poor appearance), but do not affect food safety. The poultry industry is now focused on finding ways to reduce or eliminate these anomalies. Recent progress has been made due to a better understanding of the interaction between environmental conditions and management practices, such as nutrition, and some producers have already begun to apply new approaches.

Thus, broiler production in countries such as the USA, Canada, and Mexico exhibits certain differences, as evidenced primarily by the analysis of statistical data on poultry populations and meat production. The Cobb and Ross crosses are the most common in North America, characterised by high meat production performance. Broilers are predominantly kept in industrial conditions on litter floors. A distinctive feature of poultry processing in North American countries is the widespread use of air chilling for carcasses.

Characterisation of leading North American meat poultry producers and processed product suppliers

Tyson Foods is a leading meat production company in the USA, founded in the 1930s in Arkansas (Our history, n.d.). Before characterising the activities of Tyson Foods, it is worth quoting its chairman, John Tyson: "From the beginning, our company has been built on faith, family, and hard work. That tradition, our Core Values, and "doing what's right" are deeply embedded in our culture" (Our core values, n.d.).

K. Christensen (2020), a PhD and Senior Director of Animal Welfare at Tyson Foods, discussed the creation of a new broiler research farm at Tyson Foods. This farm represents a new initiative focused on protecting and promoting the welfare of poultry. Tyson Foods' approach to animal welfare is rooted in a commitment to becoming an industry leader, relying on sound scientific evidence. The company openly shares key animal welfare metrics and results of independent audits in its annual sustainability report. The new Tyson Foods research farm with a focus on broiler welfare is unique in the industry. It is a four-house farm that allows for the study of the impact of social enrichment methods on bird behaviour in production settings. Observation rooms on the farm enable researchers and customers to observe the birds without disturbing them. This allows for the discovery of entirely new behavioural patterns when birds do not perceive a human presence. The facility is also used to test new technologies aimed at improving welfare assessment methods and continuously enhancing welfare standards. The company representative further elaborated on the research projects conducted at the farm, highlighting the scientists' focus on lighting and environmental enrichment to stimulate the birds. The farm's staff concentrated on enrichment that was both appealing to the birds and safe for the producer. They explored enrichment made from recycled materials that could be easily cleaned and installed. To carry out this research, the company's scientists received a US poultry industry grant to study the effects of various lighting and environmental enrichment schemes on the welfare and leg health of broilers. The lighting research involved refining the existing system, providing bright light over feeders and optimal lighting in other areas of the house. The company employed various methods to analyse bird preferences and assess welfare indicators to confirm that behaviour, performance, and physiological parameters indicated the best outcomes. It was emphasised that chickens have a very different perception of their environment, and this must be considered when making technological decisions. For instance, their response to light differs significantly from humans, so it is crucial to consider these characteristics when developing lighting programs. What may seem comfortable for humans can cause stress or discomfort in poultry.

Cargill is the largest privately held corporation in the United States, headquartered in Minneapolis, Minnesota. Founded by the Cargill and McMillan families, Cargill is a family-owned company. In 1865, William Wallace Cargill purchased a small grain elevator in Conover, Iowa, marking the beginning of the company's history. In the late 1890s, the family merged with another family involved in banking and the grain business, D.D. McMillan & Sons, forming W.W. Cargill. In 1898, a son of the elder McMillan married a daughter of the elder Cargill, further strengthening the business alliance through family ties. By 1935, Cargill had acquired its own fleet. During the war, the company lost most of its branches in occupied European countries but compensated for this through shipping and shipbuilding. Concurrently, Cargill developed the industrial processing of soybeans and later ventured into livestock, poultry, and other agricultural sectors. Today, Cargill is a large international corporation with a presence in 70 countries and over 150,000 employees, supplying a quarter of US grain exports and one-fifth of the domestic meat market. Cargill employees also work in the energy, steel, textile, oil, and chemical industries, as well as providing financial services. Eighty-three per cent of the company's shares are owned by descendants of the founders, representing the fourth and fifth generations of the Cargill-McMillan family clan. Eighty per cent of profits are reinvested in business growth (Cargill, 2024).

It has been reported (Cargill timeline, 2022) that the company's annual revenue in 2021 was 134.4 billion USD. Notably, in 2019, Cargill and Heifer International joined forces to launch the global Hatching Hope initiative, aimed at improving nutrition and boosting the economic wellbeing of 100 million people by 2030 through the development of poultry production, promotion, and consumption.

Michelle Grogg, Vice President of Corporate Responsibility at Cargill, noted that the conflict between Ukraine and Russia has been a significant challenge for the world and the company (Cargill annual report, 2022). While ensuring the safety of Cargill employees was the primary priority, the company also worked to support its colleagues and others in the region in need of humanitarian aid. One of the key humanitarian aid centres in Ukraine is the Kyiv Food Bank, established with the help of Cargill in 2012. Cargill employees in Ukraine provided essential logistical support and warehouse space, serving over 3,000 people daily. The company allocated an initial 40 million USD for humanitarian aid in Ukraine, neighbouring countries, and across Europe. This includes support for global humanitarian organisations such as Save the Children, CARE, the Red Cross, and the European Food Banks Federation. Additionally, the company is collaborating with local humanitarian organisations, assisting Ukrainian employees who are members of Cargill Cares Councils. Their activities encompass donating food and supplies to support families and pets, as well as organising and delivering welcome packages to Ukrainian refugees upon arrival at their destinations.

Cargill noted in its 2022 annual report that with 157 years of experience, it is no stranger to challenging times. However, the extreme events of the 2022 financial year, including the ongoing COVID-19 pandemic, severe weather conditions, trade disruptions, and the Russian invasion of Ukraine, forced the company to focus on what matters most: providing the world with essential food. The company believes that food is a basic human right and is committed to doing everything possible to help farmers bring their products to market. Cargill supplies its customers with the ingredients needed to produce food. Wherever the company operates, it acts as a global partner, focused on creating a more sustainable and efficient food system.

Founded in 1894, Bell & Evans is the oldest branded poultry company in the USA (Bell & Evans timeline, n.d.). In 1894, Howard G. Bell of Bellmore, New Jersey, started a poultry business on the family farm. In 1910, Bell partnered with another local farmer, Carlton Evans, to form the Bell & Evans company. In 1926, Bell & Evans opened its first poultry processing plant in Camden, New Jersey, and in 1927 the company was officially incorporated. In 1958, the company, headed by CF Manbeck, Inc./Farmers Pride, began branding products under the strict Bell & Evans standards. In 1984, Clarence Manbeck retired and sold the business to Scott Sechler. The following year, Sechler initiated the use of cement floors in poultry houses and mandatory disinfection between flocks. In 1986, Sechler acquired the Bell & Evans brand from the Bell family and merged it with Farmers Pride. In 1998, the company introduced all-natural feeds and began raising poultry without antibiotics. In 2000, a 10 million USD production expansion was completed, doubling the plant's capacity, increasing the number of employees to over 700, and the number of farming families to over 90. In 2001, Bell & Evans launched chicken nuggets, in 2005 gluten-free nuggets, and 2009 introduced a line of organic products.

In 2010, Bell & Evans introduced innovative, eco-friendly packaging suitable for freezing and made from recycled materials. In 2011, the company implemented the Slow Induction Anaesthesia (SSA) method and introduced the Bell & Evans Humane Animal Welfare standard, which received high praise from the Humane Society of the United States (HSUS) and People for the Ethical Treatment of Animals (PETA). In 2015, Bell & Evans opened a state-of-the-art further processing, packaging, and frying facility, with a total cost of 110 million USD and an area of 160,000 square feet. In 2016, the company launched a premium line of organic, gluten-free, ready-to-cook products. In 2017, Bell & Evans opened the world's first certified organic chicken hatchery, focused on animal welfare. In the same year, the company introduced a new chicken breed (cross) called Das Klassenbester™, designed for high animal welfare standards. In December 2021, Bell & Evans opened a new Organic-Certified Chicken Harvesting Facility (Bell & Evans timeline, n.d.).

The company closely manages every stage of chicken production to ensure the health of the birds and the quality of the product. The breeder farm is where male and female chickens produce hatching eggs, which are transported to an organic, certified incubator focused on animal welfare and then transferred for rearing. The company uses its own developed breed (cross) called Das Klassenbester, which is adapted to housing conditions that meet poultry welfare requirements (Our farms, n.d.). Thus, American companies such as Tyson Foods, Bell & Evans, and Cargill are leaders not only in North American production but also have a global reputation.

Scientific research by scientists from the USA, Canada, and Mexico on broiler meat production technology

There is a substantial body of literature characterising changes in broiler chicken productivity and highlighting the challenges faced by these birds due to increased growth rates as a result of long-term selective breeding. For example, scientists from the University of Guelph in Canada (Widowski & Rentsch, 2022), in characterising the broiler industry, indicate that broiler chickens are primarily selected for rapid growth rates, improved feed conversion efficiency, high productivity, and a high breast meat yield. Broilers currently reach a market live weight of 2.1 kg at just 35 days of age. A comparison of two commercial broiler crosses from 1950 and 2005 shows a 400% increase in growth rate, a 50% improvement in feed conversion, and an 80% increase in breast meat yield.

Scientists from Auburn University, a public research university located in Auburn, Alabama, USA, L. Orellana *et al.* (2023) investigated the impact of eggshell transparency and intensity of colouration on the hatchability of eggs, weight loss, and hatchling weight of Ross 708 broiler chicks. The researchers' results indicated that translucency affected initial egg weight and egg weight on the 18th day of incubation, where low-translucent eggs had a higher weight (68.12 g and 62.21 g) than high-translucent eggs (67.50 and 61.08 g). Regarding chick weight, those from lowtranslucent eggs were 1.44 g heavier than those from high-translucent eggs. The percentage of weight loss at hatch ($P < 0.0001$), in contrast to the above, was higher in high-translucent eggs (9.5%) than in low-translucent eggs (8.9%). Eggshell thickness ($P < 0.0001$) was found to be greater in high-translucent eggs (468.6 μm) compared to low-translucent eggs (432.2 μm). It was found that eggshell colour affected egg weight. Darker eggs had a higher weight (68.10 g, 62.07 g) than lighter eggs (67.45 g, 61.07 g). Chicks hatched from dark-coloured eggs were 0.55 g heavier than those from light-coloured eggs. Weight loss ($P < 0.0001$) was higher in light-coloured eggs (9.9%) than in dark-coloured eggs (8.8%). Thus, low-translucent and dark-coloured eggs had better egg quality based on the studied parameters. The greatest decrease was observed for weight loss and eggshell thickness in eggs that were highly translucent or light-coloured.

Canadian scientists A. Tsementzis *et al.* (2023) conducted an analysis of the impact of adding specialised protein feed and antibiotic growth promoters to the starter diet of broiler chickens on growth performance up to 49 days of age. Specialised highly digestible protein feeds and antibiotic growth promoters are often used to partially replace traditional soybean meal in the early stages of broiler rearing. However, the number of comparative studies evaluating the effects of different feed components on the productivity and development of poultry throughout their entire life cycle remains limited. Bacitracin methylene disalicylate and narasin were used as antibiotic additives in the period from 0 to 10 days of age. At 49 days of age, broiler chickens of the Ross 708 cross in this group had a live weight of 3,479 kg.

Results of research by scientists from Purdue University (Indiana, USA) A. Aderibigbe *et al.* (2020) indicate improved growth rate and nutrient utilisation in broiler chickens fed diets supplemented with α-amylase. Although starch and energy digestibility varied depending on the intestinal segment, the effectiveness of the α-amylase supplement was greater in the small intestine compared to other intestinal sections.

At the Department of Poultry Science, Auburn University, D. Ventura *et al.* (2023) investigated the interactive effects of dietary isoleucine-to-valine ratios and leucine-to-lysine ratios on broiler growth and intestinal gene expression. The researchers found that different amino acid ratios may alter gene expression in the small intestine, which could help explain the mechanisms affecting broiler growth. S. Fathima *et al.* (2023) note that poultry producers are interested in using alternatives to feed antibiotics that can enhance bird performance and reduce disease incidence. For instance, yeasts and their fermentation products are commonly used in animal feed. Yeast cell walls are composed of polysaccharides, including chitin, mannans, 1,6-glucans, and β-1,3-glucans. These biologically active substances enhance the bird's immune response, reducing the burden of pathogens, and influencing digestive enzymes. W. Al Hakeem *et al.* (2022) highlight that *C. jejuni* is a primary cause of gastrointestinal illnesses in humans consuming poultry, beef, and pork. This pathogen is prevalent in poultry farms. Broilers are typically infected at 2-4 weeks of age. In the USA, where chickens are raised without the use of antibiotics, additional measures are necessary to reduce the prevalence of *C. jejuni* on broiler farms. Vaccination and the addition of prebiotics, synbiotics, probiotics, organic acids, bacteriophages, and bacteriocins to broiler feed can improve gut health during the rearing period. B. Dixon *et al.* (2022) emphasise that the meat productivity of broilers and feed efficiency are influenced by the bird's gut health, which is determined by the intestinal microbial balance. Furthermore, the authors note that probiotic preparations improve the microbial balance of the gastrointestinal tract and have a positive impact on the bird's organism.

T. Thornton *et al.* (2023) from the University of Tennessee and the University of TennesseeKnoxville (USA) highlight that Precision Livestock Farming (PLF) technologies offer the opportunity to increase production efficiency and labour productivity while addressing consumer concerns about poultry production conditions. In the US poultry science community, there is a significant knowledge gap regarding current perceptions and needs for PLF. American researchers conducted a study aimed at developing a survey to examine attitudes and needs for PLF among scientists. The survey was conducted online using the Qualtrics platform and included 25 questions. The survey was sent to 276 poultry scientists in the USA, identified through university websites, and lasted for a month. A total of 68 responses were received. Data was exported from Qualtrics and analysed using SPSS. Respondents were classified by their role (consultant, researcher) and specialisation (animal scientist, engineer, veterinarian). Analysis was conducted using the chi-square test, frequency analysis, and the Monte Carlo method to account for the small sample size. Results showed that 92% of scientists agreed that the cost of production influences a farmer's decision to implement PLF. Additionally, 92% of researchers were familiar with the term PLF, but only 55% of them had or used these technologies.

G. Li *et al.* (2020) developed and tested image analysis algorithms for the automated monitoring of feeding and drinking behaviour in group-housed broiler chickens to improve farm management. The study used 60 Ross 708 broiler chickens aged 26-28 days, housed in a 2.9×1.4 m pen with a tubular feeder and five nipple drinkers. The developed algorithms demonstrated a high level of accuracy (on average over 90%) for analysing broiler behaviour, making them promising tools for automated image-based poultry behaviour monitoring systems. I. Chan *et al.* (2022) note that with the increasing demand for poultry meat, concerns about chicken welfare and the harms caused by the current technology of raising fast-growing broilers are also growing. In this regard, the scientists considered the option of raising chickens on pasture and using slower-growing crosses and breeds. However, they noted that this requires a significant additional 43.8-60.1% land use.

J. Smith (2019) notes that the US Food and Drug Administration's restrictions on antibiotic use in broiler production should not significantly impact broiler performance and health. Instead, marketing programs that significantly restrict or completely prohibit the use of antibiotics, including ionophores, in broiler production are becoming increasingly important. The main consequences of such programs include: difficulties in controlling necrotic enteritis and coccidiosis, problems with litter moisture management and associated diseases, and an increase in neonatal infections due to the removal of antibiotics from Marek's disease vaccines. To address these issues, significant changes in poultry feeding and treatment are required, as well as the application of alternative nonantibiotic agents, such as chemically synthesised coccidiostats. However, such changes can only partially compensate for the negative impact of antibiotic-free programs.

E. Ornelas-Eusebio *et al.* (2020) identified two key challenges facing the Mexican poultry industry: the responsible and effective use of antimicrobial drugs and the risk of infectious disease outbreaks. For instance, since the first detection of the highly pathogenic avian influenza H7N3 subtype in Mexico in 2012, outbreaks have repeatedly occurred among domestic poultry. Both of these threats can be minimised through the implementation of good management practices and enhanced biosecurity on farms. The study aimed to analyse the biosecurity measures applied to different types of poultry farms in Mexico and to obtain data on the use of antimicrobial drugs. Using agglomerative hierarchical cluster analysis, three clusters of farms were identified. Each cluster was dominated by a specific type of farm.

The key biosecurity measures that significantly differentiated the farms and formed the basis for their clustering were: the use of personal protective equipment (including masks, hairnets, and safety goggles), adherence to hygiene protocols before entering and after leaving the farm, the use of dedicated workwear by staff and visitors, the presence of disinfectant foot baths at the entrance to the premises, and methods for disposing of dead birds. The stricter the biosecurity measures within a cluster, the less frequently farms use antimicrobial drugs. On farms with less stringent biosecurity, antimicrobial drugs critical for public health were used more often. The researchers also provided recommendations for improving biosecurity on farms. Overall, scientists from South American universities are conducting numerous studies that are highly relevant to the current state of broiler production.

Conclusions

A comprehensive analysis of broiler production in North America reveals certain distinctions between countries such as the USA, Canada, and Mexico, as evidenced primarily by an analysis of statistical data on stocks chickens and meat production. American companies such as Tyson Foods, Bell & Evans, and Cargill are leaders not only in North American production but are also renowned worldwide. An analysis of sources regarding the use of chicken crosses for broiler production indicates that Cobb and Ross crosses are the most common in North America. The Cobb-500 and Cobb-700 crosses differ in both live weight and feed conversion. Broilers of the Cobb-500 cross have a higher live weight, reaching 3,278 g at 42 days of age, exceeding the Cobb700 cross (2,847 g) by 431 g. Overall, Cobb-700 broilers grow more slowly and can be raised to 9 weeks of age. The Cobb-700 broilers reach a live weight similar to Cobb-500 broilers a week later. Therefore, depending on the end goal, poultry farmers in North America choose the appropriate cross.

Broilers are predominantly housed on litter floors in industrial conditions. In North American poultry farms, air chilling of carcasses is a common practice at slaughter. Additionally, an analysis of the reviewed literature indicates that scientists from North American universities are conducting a variety of studies that are highly relevant to the current state of the broiler meat industry. The majority of research focuses on solving problems related to broiler nutrition. Future research prospects include an analysis of the state of egg production in EU countries.

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Conflict of Interest

None.

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Сучасні тенденції виробництва м’яса бройлерів у Північній Америці: огляд літератури

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Анотація. Світове виробництво м'яса бройлерів зростає швидше, ніж будь-якого іншого м'яса. Сполучені Штати є найбільшим виробником бройлерів у світі. У 2022 р. США разом з Канадою й Мексикою забезпечили 20 % загального виробництва м’яса бройлерів. Мета дослідження – проаналізувати та узагальнити літературні джерела щодо сучасних тенденцій технології виробництва м'яса курчат-бройлерів у США, Канаді та Мексиці. У дослідженні використовували методи аналізу й синтезу, контент-аналізу. Встановлено, що серед країн Північної Америки США посідають перше місце як за поголів’ям курей, так і за виробництвом м’яса бройлерів. Загалом у світі у 2022 році поголів’я курей становило 26,561,634,0 тис. голів. При цьому перше місце належить Китаю (5,185,477,0 тис. голів), США посіли п’яте місце (1,528,000,0 тис. голів), Мексика – восьме (611,202,0 тис. голів), а Канада – 29-е (173,942,0 тис. голів). У світовому рейтингу серед виробників м’яса бройлерів у 2022 р. на першому місці – США, Мексика – на сьомому, Канада – на 22-му. У динаміці виробництва м’яса бройлерів у країнах Північної Америки упродовж 2018-2022 рр. спостерігалася тенденція до збільшення даного показника. У 2022 р. у США було вироблено 20,652,970,91 т м’яса бройлерів, у Канаді – 1,332,967,57 т та у Мексиці – 3,668,552,28 т. Провідними виробниками м’ясної продукції у США, а також у всій Північній Америці є «Tyson Foods», «Cargill» та «Bell & Evans». Найпоширенішими кросами у птахогосподарствах Північної Америки є «Кобб» і «Росс». Порівняльний аналіз продуктивності кросів «Кобб-500» та «Кобб-700» засвідчив, що бройлери кросу «Кобб-500» мають перевагу за живою масою. Загалом бройлери кросу «Кобб-700» ростуть повільніше, і їх можна вирощувати до 63-добового віку. Найбільш поширеним є метод утримання бройлерів на підлозі з використанням підстилки. Вчені з університетів Південної Америки проводять різноманітні дослідження, котрі є актуальними на сучасному етапі розвитку м’ясного птахівництва. При цьому науковці розв’язують проблеми щодо утримання, годівлі, благополуччя, здоров’я бройлерів, якості тушок тощо

Ключові слова: США; Канада; Мексика; м'ясо птиці; крос; жива маса; годівля

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Application of brewer's spent grain in the technology of protein texturates for the meat processing industry

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Abstract. The topic's relevance lies in addressing the rational utilisation of brewing by-products, specifically brewer's spent grain. Containing approximately 20% protein, it can enhance the compositional properties of textured soy protein. The study aimed to develop a formulation for grain-protein texturates within the framework of waste-free technologies and environmentally sustainable production. To achieve this, modern and promising methods for obtaining protein concentrates, isolates, and texturates from plant-based raw materials were reviewed. A technology was developed for producing texturates based on soy flour, soy flakes, gluten, and brewer's spent grain flour. The quality of the resulting texturate was assessed using organoleptic, microbiological, and physicochemical parameters. According to sensory evaluation, a porous textured protein with a fibrous macrostructure was obtained. Moisture content was determined by the drying method to a constant weight at a temperature of 105°C±2°C; protein content was measured using the Kjeldahl method; fat content was assessed by the Soxhlet method; and fibre content was analysed using the FIBERBAG method. Microbiological indicators were assessed as follows: *Escherichia coli* was detected through inoculation on selective diagnostic media; the count of mesophilic aerobic and facultative microorganisms was determined via inoculation in solid media; yeasts and mould

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fungi were identified by cultivation on nutrient media, followed by classification based on the characteristic growth features and cell morphology of the isolated microorganisms. The study established that the produced texturate complies with the requirements of DSTU 4538:2006. A comparison with the existing standard revealed a 1% increase in protein content and a 46% reduction in fat content. The organoleptic evaluation confirmed that the product is free of foreign odours and tastes, featuring a highly fibrous structure identical to the texture of natural meat. Microbiological analysis confirmed the safety of the product, as no pathogenic microorganisms were detected, and the total microorganism count remained within permissible limits. The practical significance of this research lies in determining the quality of the grain-protein texturate for its subsequent application in the meat processing industry

Keywords: utilisation; gluten; soy flour; protein; organoleptic evaluation; microbiological indicators

Introduction

Recently, according to L. Bal-Prylypko *et al.* (2022), there has been a significant demand for sausages among the Ukrainian population. This has prompted the development of new recipes that combine animal and plant-based ingredients. To achieve a denser sausage filling, M. Nazarenko & I. Ustymenko (2023) proposed combining animal and plant proteins by partially replacing the meat component with spelt flour, a type wild wheat of spelt. The addition of plant-based ingredients to meat products not only expands the product range but also results in a new, high-quality product with functional properties. Studies on the effects of plant proteins on diseases such as diabetes and cardiovascular disease have shown positive results. Patients who regularly consumed products enriched with plant proteins experienced significant reductions in cholesterol and stabilised blood glucose levels (Sharma & Thakur, 2022). Therefore, the question of alternative plant-based protein sources is highly relevant. As noted by M. Zainol *et al.* (2020), legumes are considered the most common, offering a high nutritional profile. Their addition to meat products has improved the sensory qualities and nutritional value of the final products, while also reducing production costs. However, it is not only the lower cost that has encouraged consumers to reduce their consumption of animal products. A key factor is the balance between taste, texture, price, and nutritional value (Boukid, 2021). Soybeans are the primary ingredient as they are considered a complete protein source, meaning they contain all the essential amino acids and are nutritionally comparable to animal proteins (Azman *et al.*, 2023). The human body can synthesise only some amino acids through carbohydrate or lipid metabolism, and these are called non-essential amino acids. The rest must be obtained from the diet and are called essential amino acids. Human health depends on a balanced diet that provides all the essential amino acids.

Ya. Skorobogatyi *et al.* (2020) indicate that the human diet must provide a mixture containing eight essential and two conditionally essential amino acids. The absence of even one essential amino acid leads to a negative nitrogen balance, impaired central nervous system function, and vitamin deficiency. After analysing the amino acid composition of soy and its products, the authors determined a deficiency of tryptophan and methionine. Tryptophan is an α-amino-β-indolepropionic acid and belongs to the aromatic amino acids (Moroz *et al.*, 2022). It normalises the nervous system and digestion, improves immunity, and its deficiency causes skin and hair problems, anaemia, and insomnia. Methionine and cysteine in grain proteins are similar to those in animal proteins, but they are generally lower in legume proteins. Methionine is involved in the synthesis of cysteine through the transsulfuration pathway, which limits the rate of synthesis of the key antioxidant molecule, glutathione. Methionine is also a primary methyl donor in the body, involved in the synthesis of several key metabolites, including creatine and phosphatidylcholine, during periods of high demand. Soy proteins are high in lysine, making them a valuable addition to grains, which are typically low in this amino acid. Therefore, when developing a soy-based protein product, it is essential to carefully combine ingredients to achieve a complete amino acid profile. For example, barley flour contains tryptophan and a significant amount of methionine, so adding brewer's spent grain, which is primarily composed of barley malt and flour, to a soy product can help balance the amino acid profile (Moroz *et al.*, 2022).

To meet the physiological needs of the body, an "ideal" protein is required, but it does not exist in nature. This problem can be solved by combining and rationally utilising protein resources through selection and calculation. Therefore, the main goal of the research was not only to address environmental issues but also to obtain a protein concentrate with functional properties.

Literature Review

The growing interest of scientists in plant-based products has led to an increase in the production of soy protein concentrates due to their functional and nutritional properties (Baune *et al.*, 2022). There are two main methods of obtaining concentrates: dry and liquid. The dry method is based on aspiration and is rarely used due to significant protein losses. The liquidextraction method can be divided into water, alcohol, and acid-based processes. The alcohol method is the most common, as the resulting concentrate has high organoleptic properties and is free of contaminants. In a scientific article by R. Bou *et al.* (2022), the latest technologies for obtaining high-purity protein isolates and their impact on the technological and functional properties of raw materials and human health are considered. The most modern and promising are ultrafiltration technologies, such as ultrafiltration, reverse osmosis, and electrodialysis. The application of these methods promotes protein dissolution and cell wall disruption, increasing protein yield and reducing the need for solvents, processing time, and waste generation.

Technologies are being developed to modify protein structure to improve digestibility, reduce allergenicity, and tailor technological properties, making soy a sustainable protein source with a promising future as an alternative to meat and meat products. One such technology is texturisation, which creates a product with fibres similar to those found in many meat products. Various methods have been proposed to mimic the fibrous texture of meat; however, extrusion is the only industrially viable option for functionalising and structuring plant materials into fibrous products (Stelick *et al.*, 2021). In this process, proteins are plasticised/melted through a combination of heating, hydration, and mechanical deformation. Depending on the moisture content, high-moisture extrusion (50-80%), which produces textured proteins with a fibrous structure more similar to meat, and low-moisture extrusion (25-35%) can be distinguished. In this study, high-moisture extrusion was used to obtain a product with a fibrous structure that resembles muscle tissue fibres, for use in meat analogue and blended product technology. The primary raw materials for producing textured plant proteins are soy protein, pea protein, and wheat gluten, according to researchers B. Zhang *et al.* (2022). Previous studies have shown that different raw materials have varying effects on the textured material. For instance, pea protein can be extruded at a moisture content of 55% as high temperatures and rapid heating deteriorate its gel-forming properties. Wheat protein, due to its high water-absorption capacity, can form gluten which is shaped into a plastic structure through high-moisture extrusion. Unlike pea protein, wheat gluten has significantly better gelforming properties, hence it is often mixed with other plant proteins such as soybeans and peas, as indicated in the research by X. Zhang *et al.* (2022). During extrusion, various physical and chemical changes occur, leading to the formation of a unique fibrous structure that affects the functional properties of the protein, enhancing its digestibility (Zhang *et al.*, 2020).

Textured plant proteins are used as partial meat substitutes to enhance colour stability, moisture retention, and juiciness in sausages. Improving the ability of plant-based proteins to mimic meat flavour has gained significant interest among both researchers and manufacturers (Yuliarti *et al.*, 2021; Cornet *et al.*, 2022). Experiments have been conducted to replace meat in sausages with textured proteins at 20%, 50%, and 75%. Results showed a 25% increase in water content due to the high water-holding capacity of texturised proteins (Revilla *et al.*, 2022), and when 20% of pork was replaced, sufficient essential amino acids were retained (Broucke *et al.*, 2022). Particular attention is paid to various protein blends, using different products such as defatted pumpkin and sunflower flour, peanut protein, and others. This article proposes a partial replacement of soy flour with brewers' spent grain flour, a byproduct of the filtration process during beer wort.

The primary raw material in brewing is barley malt. To prepare the mash, the grains are crushed and mixed with water. During the mashing process, enzymatic changes occur, resulting in approximately 60-70% of the dry matter being transferred to the wort, while the remainder remains in the brewer's spent grain. After filtration, a wet grain is obtained, consisting of insoluble particles of malt, primarily from the husks of the malt grains, mixed with endosperm, unfermented starch, and other substances. As noted by researchers M. Parchami *et al.* (2021), spent grain has a very valuable chemical composition, with protein and fibre being particularly noteworthy, accounting for approximately 20% and 70% of its composition, respectively. About 30% of the total protein content consists of essential amino acids, the primary one being lysine, which is considered the limiting amino acid in cereal products. In turn, dietary fibre is primarily composed of cellulose and hemicellulose fibres, which include particles of xylose, arabinose, glucose, and lignin. Spent grain is also rich in minerals, the most common of which are silicon, phosphorus, and calcium.

Due to its valuable composition, research is being conducted into the use of spent grain in various sectors of the food industry, including baking, confectionery, and meat processing (Chetrariu & Dabija, 2023). A known technology involves producing bread with sourdough, with the addition of 25%, 50%, 75%, and 100% spent grain. Samples of bread made with 25% and 50% sourdough exhibited higher porosity, acidity, and appropriate moisture content compared to samples made with 75% and 100% sourdough, which had lower porosity and acidity. Authors O. Saitan *et al.* (2022) also found that the shelf life of the bread increased by 24-48 hours. However, there are known negative consequences of partially adding brewer's spent grain flour during mixing, namely a decrease in gluten yield and a deterioration in its quality, leading to a softer dough. This is due to the prolonged mixing time and the high content of fibre and protein. In terms of appearance, crust, and crumb properties, bread with 10% spent grain did not differ significantly from bread made with wheat flour; however, the bread's colour changed from light cream to brown as the amount of spent grain was increased (Czubaszek *et al.*, 2022). Researchers Y. Shih *et al.* (2020) demonstrated not only the feasibility of using brewer's spent grain in the confectionery industry but also the functional properties of the resulting products (muffins). A study involving 18 participants who consumed muffins with 30% brewer's spent grain daily for 8 weeks showed positive effects related to reduced blood pressure and insulin levels compared to the control group. This is attributed to the muffins' high content of protein, fibre, and antioxidants, as a serving size contains more than 10% of the recommended daily intake of each nutrient (Combest & Warren, 2022). Additionally, a group of scientists, including M. Nazarenko & I. Ustymenko (2023), investigated the use of brewer's spent grain in the production of meat products, specifically sausages. The authors used fractionated flour and determined that the finished products showed significant improvements in both organoleptic and physicochemical properties.

Materials and Methods

The protein texturate was produced using a traditional technology according to DSTU 4538:2006 (2007). Research was conducted at the innovation and production complex of LLC "KR Ingredients" during the first half of 2024. The subject of the study was soy flour and brewer's spent grain flour (the main raw materials). All ingredients, according to the recipe (Table 1), were mixed with water, intensively mixed at high speeds for 2-5 minutes, and loaded into an extruder, where they were heated to a temperature of 160-210°C using an external ceramic heater. As a result of the high temperature, the raw material is converted into a melt, and the proteins denature. When the melt exits the extruder, there is a sharp decrease in temperature and intensive evaporation of moisture, forming a porous textured protein with a fibrous macrostructure. The texturate recipe is presented in Table 1.

Table 1. *Recipe for grain-legume protein texturate*

No.	Ingredients	Mass fraction of crude protein (on a dry matter basis), %	Raw material weight per 100 kg of product
1	Defatted soy flour	52	29
2	Soybean meal	46	25
3	Brewer's spent grain flour	22	7.0
4	Gluten	90	7.0
5	Sodium bicarbonate	-	0.5
6	Sodium chloride	-	0.5
7	Water	-	31

Source: developed by the authors

A similar method for producing a textured soy product was patented by S. Glushchenko (2022), which proposed using soybean meal "Bila Peliustka", soy protein isolate, soy flour, wheat gluten, sodium bicarbonate, and sodium chloride as raw materials. The raw materials were mixed with water to a moisture content of 18-30% and the resulting mass was extruded at a temperature range of 110 to 210°C.

In the recipe for the grain-legume protein texturate, soy flour was partially replaced with brewer's spent grain flour to reduce production costs and address the issue of waste from the brewing industry. The finished product was analysed for organoleptic, physicochemical, and microbiological parameters. The organoleptic evaluation was conducted on a 5-point scale according to DSTU 4538:2006 (2007). Physicochemical and microbiological parameters were obtained in an accredited testing laboratory for food and light industry products in Cherkasy, Ukraine. Moisture content was determined according to DSTU ISO 6496:2005 (2006); protein content – DSTU 7169:2010 (2011); fat content –

DSTU ISO 1443:2005 (2008); and fibre content – DSTU 8844:2019 (2020).

Microbiological analysis was conducted for the following indicators. It is generally considered that if a product is produced without the involvement of microorganisms that cause fermentation processes, the number of mesophilic aerobic and facultatively anaerobic microorganisms (MAFAM) according to DSTU 8446:2015 (2017) does not exceed 10^5, and if it increases to 10^6 per 1 g (cm^3) or more, it becomes potentially dangerous for consumers, even if there are no organoleptic signs of product spoilage (Caprelyants, 2020). Coliform bacteria – 1.0 g according to DSTU 30726-2002 (2003); yeasts, colony-forming units (CFU) – 1 g, DSTU 8447:2015 (2017); pathogenic microorganisms, including *Salmonella* – 25 g (DSTU EN 12824:2004, 2005); moulds – CFU per 1.0 g (DSTU 8447:2015, 2017).

Results and Discussion

Organoleptic properties are subjective and primarily descriptive in nature. Sensory evaluation allows for a quick and objective overall assessment of a food product's quality. The results of the sample were compared to the requirements of the standard DSTU 4538:2006 (2007) and were found to have high sensory characteristics, which were assessed on a five-point scale. In terms of appearance, colour, and taste, the sample received five points. Regarding the odour, a slight malt note was detected, indicating the presence of brewer's spent grain. The organoleptic properties are presented in Table 2.

Table 2. *Organoleptic evaluation of the grain-legume protein texturate*

No.	Parameter	Points	Characteristics
1	Appearance	5	Fibrous structure resembling muscle tissue fibres
2	Colour	5	Light brown
3	Taste	5	No specific bean taste, bitterness, sourness, or other off-flavours
4	Odour	4	Typical of soy protein texturate, with subtle malty notes

Source: developed by the authors

Textured vegetable protein has a fibrous structure formed by oriented protein macromolecules, resembling muscle fibres (Fig. 1), allowing its use as a protein source in the production of sausages, minced semi-finished products, and meat and meat-plant-based canned goods.

Figure 1. Grain-legume protein texturate
Source: authors' photo

The physicochemical properties of texturised vegetable protein are crucial in meat product manufacturing. The primary property affecting the quality of meat products is the water-binding capacity of proteins. In meat, water is a natural component and is bound to its constituents in various ways, forming stable structural elements. These bonds can be capillary, adsorbed, or osmotic, determining the meat's ability to retain varying amounts of moisture. The amount of bound water and its distribution according to the type and strength of the bond influence the meat's properties, including its texture. In animal proteins, water is more strongly bound to collagen and elastin, which are primarily found in bones, muscles, and animal skin. For the production of sausages, minced meat is used, which typically has a lower protein content than pieces of muscle meat. In this category of products, animal protein can be replaced with plant-based protein with high functional properties (Day *et al.*, 2022). In the study of M. Nikolaenko (2021), it was shown that adding 15% plant-based protein to minced meat increased its water-binding capacity. The physicochemical properties based on the results of this research are presented in Table 3.

Table 3. *Physicochemical properties of the grain-legume protein texturate*

No.	Parameter, units	Reference RD for the test method	Result of testing	Norms according to DSTU 4538:2006 (2007)
1	Mass fraction of raw fibre, recalculated to dry matter, %	DSTU 8844:2019 (2020)	6.4	-
2	Mass fraction of raw fibre, %	DSTU 8844:2019 (2020)	5.9	-
3	Mass fraction of raw fat, recalculated to absolutely dry matter, %	DSTU ISO 1443:2005 (2008)	6.9	15.0
4	Mass fraction of raw fat, %	DSTU ISO 1443:2005 (2008)	6.3	-
5	Mass fraction of raw protein, recalculated to absolutely dry matter, %	DSTU 7169:2010 (2011), p.7	45.4	45.0
6	Mass fraction of raw protein, %	DSTU 7169:2010 (2011), p.7	41.5	-
7	Moisture, %	DSTU ISO 6496:2005 (2006)	8.8	9.0

Source: developed by the authors

According to the standard, the crude protein content on a completely dry matter basis for texturate made from soy flour and meal should be at least 45%. In the obtained sample, it was 45.4%. Regarding fat, compared to the standard, a decrease of 46% was observed. This is due to the fact that both brewer's spent grain flour and soy flour were defatted to increase the hydrophilicity of proteins.

Table 3 also shows that the obtained texturate contains a high amount of fibre, a plant polysaccharide that is only obtained from food. As noted by authors I. Bernyk *et al.* (2022), enriching meat products with fibre contributes to the stabilisation of the rheological properties of the mince. Due to its high water-holding capacity, it improves product formation, increases the yield of the finished product, and enhances organoleptic properties. Additionally, it enriches food products with dietary fibre and reduces their calorie content. Fibre is essential for health, lowering cholesterol levels and stabilising blood sugar.

Microbiological control of food products allows for the rapid detection of pathogenic microflora and eliminates the risk of food poisoning, as well as preventing the growth of saprophytes, which are the primary causative agents of spoilage in finished products. To assess the quality of raw materials, semi-finished products, and finished products according to DSTU 8446:2015 (2017), there is an indicator of the sanitary-hygienic condition, namely the total microbial count. Mesophilic aerobic and facultatively anaerobic microorganisms are microorganisms with an optimal growth temperature of 25-40°C in the presence or absence of oxygen. These primarily include putrefactive spore-forming and non-spore-forming bacteria, *cocci* (*staphylococci*, *micrococci*, *sarcinae*), and some conditionally pathogenic bacteria. The higher this indicator, the greater the likelihood of pathogens of infectious diseases and food poisoning entering the test object (Solomon *et al.*, 2020).

The primary indicator microorganisms are coliform bacteria, which are divided into three genera: *Escherichia*, *Citrobacter*, and *Enterobacter*, all belonging to the *Enterobacteriaceae* family. *Escherichia* is the most common. As noted in their research by A. Solomon *et al.* (2020), coliforms are highly variable and can lose many of their characteristic features when exposed to the external environment. Therefore, all types of coliform bacteria are considered as indicator microorganisms. The presence of coliforms in a product indicates a violation of the technological process. Significant contamination of objects with coliforms is an indicator of the possible presence of their companions: cholera and dysentery pathogens. *Salmonella*, the causative agent of typhoid fever and paratyphoid A and B, are resistant to both high and low temperatures.

Microbiological tests on the sample found no pathogenic microorganisms, including *Salmonella*, and no coliform bacteria, which fully complies with the requirements of the national standard DSTU 4538:2006 (2007). Regarding mesophilic aerobic and facultatively anaerobic microorganisms (MAFAM), with a maximum permissible level of 1.0×10^5 microorganisms per gram of texturate, 5.1×10^2 were found, indicating the safety of the product.

Moulds and yeasts are indicators of the sanitary condition of food products: moulds produce carcinogenic mycotoxins while yeasts shorten the shelf life of finished products. The results of the coliform test were within the acceptable range, and no yeasts were detected. The obtained microbiological indicators of the protein texturate are presented in Table 4.

***Table 4.** Microbiological indicators of grain-legume protein texturate*

No.	Parameter	Norms according to DSTU 4538:2006 (2007)	Test results	Reference RD for the test method
1	Number of mesophilic aerobic and facultative anaerobic microorganisms, CFU per 1 g, not exceeding	$1.0 \cdot 10^5$	$5.1 \cdot 10^2$	DSTU 8446:2015 (2017)
2	Coliform bacteria (coliforms)	Not allowed	Not detected	DSTU 30726-2002. (2003)
3	Pathogenic microorganisms, including *Salmonella* species, in 25 g	Not allowed	Not detected	DSTU EN 12824:2004 (2005)
4	Mould fungi, CFU per 1 g, not exceeding	$1.0 \cdot 10^2$	Not detected (less than 10*)	DSTU 8447:2015 (2017)
5	Yeasts, CFU per 1 g, not exceeding	$1.0 \cdot 10^2$	Not detected (less than 10*)	DSTU 8447:2015 (2017)

Source: developed by the authors

The obtained results prove the feasibility of producing textured soy protein with a partial replacement of defatted soy flour with brewer's spent grain flour, which will reduce the cost of the product (the cost of dry spent grain is 2.5 times less than the cost of soy flour) and also solve the problem of utilising brewery waste. The developed technology allows for obtaining a product that is safe in terms of microbiological indicators, with a high protein content (41.5%) and a low fat content (6.1%).

Conclusions

Given the global decline in livestock farming and the increasing demand for food, scientists are seeking alternative sources of high-quality food. The utilisation of brewer's spent grain, a byproduct of the brewing industry, has emerged as a promising approach. It has been established that this material is a rich source of proteins, minerals, fatty acids, amino acids, fibre, and bioactive compounds, making it a suitable raw material for protein texturate.

This not only reduces the amount of waste in beer production but also enhances the nutritional value of the final product.

Based on experimental characteristics, the produced grain-legume texturate exhibits excellent organoleptic and physicochemical properties. Although the odour had slightly sweet notes, this did not significantly impact the overall sensory evaluation. Analysis of the product's chemical composition revealed that its high protein content and fibre presence enhance the water-binding and water-holding capacity of meat fillings, resulting in increased yields of finished sausages. Additionally, the developed texturate is distinguished by its fibre content, which positively impacts gut health.

The grain-legume texturate produced using the developed technology is almost indistinguishable in terms of consumer qualities from the texturate produced using traditional technology. Therefore, grain-legume texturate can be used both for the preparation of vegan products and in the meat processing industry for the production of sausages and semi-finished products. Consequently, there is an urgent need to utilise this byproduct and develop sustainable methods for its use. Prospective directions for further research include adding grain-legume texturate to the formulation of cookedsmoked sausages. The positive results of the research indicate that this technology requires further study and development. Therefore, this study will continue to determine the organoleptic and physicochemical characteristics of finished products depending on the concentration of texturate in the products.

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Conflict of Interest

None.

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Застосування пивної дробини у технології білкових текстуратів для м'ясопереробної промисловості

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Анотація. Актуальність теми полягає у вирішенні питання щодо раціонального способу утилізації побічних продуктів пивоваріння, а саме пивної дробини. До її складу входить близько 20 % білка, що дозволяє поліпшити композиційні характеристики соєвих текстуратів. Метою роботи було розробити рецептуру зерно-білкового текстурату на основі створення безвідходних технологій та екологічно чистих виробництв. Для її реалізації було розглянуто сучасні та перспективні способи отримання білкових концентратів, ізолятів та текстуратів з рослинної сировини та розроблено технологію отримання текстурату на основі соєвого борошна, соєвої «пелюстки», глютену та борошна пивної дробини. Якість отриманого текстурату визначали за органолептичним, мікробіологічними та фізико-хімічними показниками. Відповідно до сенсорної оцінки, отримали пористий текстурований білок з волокнистою макроструктурою. Вологу визначали методом висушування до постійної маси за температури 105°C ± 2°C; вміст білка – методом К'єндаля; вміст жиру – методом Сокслета; клітковину – методом «FIBERBAG». Щодо мікробіологічних показників визначали: бактерії *Escherichia coli* шляхом посіву на селективно-діагностичні середовища; кількість мезофільних аеробних та факультативних мікроорганізмів – посівом у тверді середовища; дріжджі та плісеневі гриби – шляхом культивуванням на поживних середовищах, з подальшим визначенням належності виділених мікроорганізмів до плісеневих грибів і дріжджів за характерними ознаками росту і морфологією клітин. Встановили, що отриманий продукт відповідає ДСТУ 4538:2006. Порівнюючи отриманий текстурат з існуючим стандартом, визначили, що вміст білка збільшився на 1 %, натомість вміст жиру зменшився на 46 %. За органолептичними показниками отримали продукт без сторонніх запахів і присмаків, який має висоководокнисту структуру, ідентичну текстурі натурального м'яса. Мікробіологічний аналіз підтвердив показники безпечності продукту, оскільки патогенна мікрофлора відсутня, а кількість мікроорганізмів знаходилась в межах норми. Практичне значення проведених досліджень полягає у визначенні якості зернобілкового текстурату з подальшим використанням його в м'ясопереробній промисловості

Ключові слова: утилізація; глютен; соєве борошно; білок; органолептична оцінка; мікробіологічні показники

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Development of technology for extended-shelf-life meat products

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Abstract. The development of new technologies to preserve the quality of meat products is of significant relevance in light of the growing global demand for safe, durable, and functional food products that meet modern healthy eating standards. This is particularly important given the challenges associated with ensuring global food security. This study aimed to design an innovative production technology for meat products that ensures an extended shelf life without

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compromising their organoleptic properties. The research employed contemporary methods of physicochemical and microbiological analysis, along with biotechnological approaches, particularly the use of starter cultures of lactic acid bacteria and bifidobacteria. The findings confirmed the positive influence of probiotic cultures on the biochemical processes occurring in meat raw materials. Specifically, these cultures facilitated fermentation, maturation, and the development of stable organoleptic characteristics. Furthermore, it was established that the use of probiotics reduced water activity and pH levels, thereby significantly enhancing the microbiological stability of meat products. The analysis of technological parameters, such as salting, maturation, and drying periods, demonstrated their critical role in shaping the flavour and aroma profiles, texture, and colour of the final product. As a result of this study, innovative production technology for meat products was developed, enabling the creation of items with high protein content, low fat content, and stable microbiological safety, with an extended shelf life of up to 45 days. The practical value of this research lies in the potential for wide-scale implementation of the developed innovative technology in the food industry for the production of high-quality dry-cured and fermented meat products. These products meet contemporary standards of safety and nutritional value. The use of probiotic cultures contributes to the creation of products with enhanced organoleptic properties and additional functional benefits, aligning with healthy eating trends and addressing consumer demand for natural and environmentally friendly products

Keywords: biotechnology; dry-cured meat products; safety; expiration date; quality

Introduction

The most pressing global challenges (energy, environment, food) are particularly relevant to the food industry, and their solutions require fundamentally new approaches to raw material processing. The development and implementation of highly efficient equipment and technologies, as well as the creation of new-generation products, are priorities for the food industry. The current state and growth of specialised enterprises in Ukraine necessitate the optimisation of relationships between farms and the agro-industrial processing sector, as well as the improvement of the pricing mechanism for the formation of raw material and finished product markets. The rapid growth of the global population has led to food shortages, stimulating the development of technologies for growing agricultural products and raising livestock, and consequently, the production of food (Cherednichenko & Bal-Prylypko, 2019).

According to K. Pushparaj *et al.* (2024), cured meat products have a low tendency to lose fat during processing, which positively affects their appearance and visual appeal. A significant advantage of such products is their high storage stability due to the reduced rate of oxidation of bound fats, preventing rancidity. Their flavour profile is distinctive and intense, characteristic of mature meat products. An additional benefit of cured meat, used in the production of dry-cured meat products, is the preservation of all the nutritional and beneficial properties inherent in fresh meat. Moreover, a long shelf life (approximately 6 months) increases the convenience of its use. Such products become an optimal food option in conditions of limited access to traditional food, particularly during business trips, travel, or at work, providing a complete and nutritious diet.

O. Cherednichenko *et al.* (2021) noted a growing interest in organic food and healthy lifestyles in Ukraine. The development of functional food product formulations should be based on modern principles of balanced and nutritious diets. In this context, the production of dry-cured meat products holds significant potential, as this product category meets the requirements of healthy eating, characterised by high protein content and low fat levels. Moreover, due to specific processing technologies, such products contain virtually no flavour enhancers, trans fats, colourants, flavourings, or other harmful chemical components, further enhancing their nutritional value and safety.

J. Wang *et al*. (2020) assert that starter cultures play a pivotal role in the production of drycured and fermented sausages, providing controlled fermentation to achieve the desired texture, flavour, and colour of the final product. They also reduce the risk of undesirable microbial growth during ripening and storage. Globally, the primary criterion for selecting microorganisms for starter cultures is their impact on the sensory characteristics of the product under conditions of intensified and modernised production processes. The use of such cultures is crucial for ensuring the high quality and safety of meat products. B. Łaszkiewicz *et al*. (2021) described a series of experiments on colour stabilisation of meat products using sodium nitrite (E250). This substance plays a significant role in the formation of colour, flavour, and aroma, and partially influences microbial stability during storage. However, sodium nitrite is a toxic compound, and excessive consumption can lead to serious adverse health effects. For instance, the pharmacological toxic dose of sodium nitrite is 0.3 g. Even small amounts of free nitrite can cause the formation of methaemoglobin in the blood, leading to anaemia and serving as a precursor for carcinogenic nitrosamines. This highlights the need for strict control over the use of nitrites in the food industry to minimise risks to consumer health.

M. Shakil *et al*. (2022) investigated the challenges of reducing sodium nitrite content in meat product technology, highlighting its significance for public health. The current lack of full substitutes for sodium nitrite complicates its complete removal from formulations, necessitating the search for alternatives that can provide similar functionality while reducing the concentration of this compound in the final product. One promising solution is the use of beetroot extract (BE) in combination with denitrifying bacteria. Beetroot, due to its rich chemical composition, contains natural bioactive substances that not only bind and eliminate toxic compounds but also contribute to stimulating the body's immune system (Jha & Sit, 2022). This approach allows for the simultaneous preservation of product quality, reduced health risks for consumers, and increased functional value.

Therefore, the integration of plant-based additives into meat product manufacturing, combined with modern biotechnological methods, can serve as an effective alternative to chemically synthesised components. This paves the way for the creation of a new generation of products that meet modern requirements for safety, taste, and nutritional value. The development of such approaches requires further technological advancements and the implementation of innovative solutions in industrial practice. This research aimed to provide a scientific rationale and develop a comprehensive technology for producing meat products using probiotic cultures, ensuring their microbiological stability, extended shelf life, and high sensory quality, following modern food safety and functionality requirements.

Literature Review

Starter cultures have gained significant attention in the production of meat products. They are used in traditional products worldwide, including Turkey, Croatia, Romania, Greece, Italy, Spain, Portugal, Thailand, and China (Väkeväinen *et al*., 2020; Munekata *et al*., 2023; Liu *et al*., 2023). The application of these cultures is a crucial and sustainable method for preserving certain foods, offering recognised technological advantages. According to research by Y. Liu *et al*. (2023), various types and strains of microorganisms are used as starter cultures to inhibit the growth of pathogenic microorganisms and suppress the development of spoilage organisms. Depending on technological requirements and consumer preferences, different strains are used in various products. According to M. Fuka *et al*. (2020), the most widely used starter cultures in meat products are LAB (Gram-positive, catalase-negative cocci or bacilli), Gram-positive, catalase-positive cocci, mainly CNS, and *Micrococcaceae*, moulds, or yeasts, whose metabolism produces several compounds with antimicrobial activity. These microbial cultures can be used as individual or mixed cultures.

According to S. Smaoui *et al*. (2024), LAB used as starter cultures in fermented meat products are typically facultative anaerobes and primarily belong to the genera *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus,* and *Enterococcus*. Among CNS species most frequently used for meat fermentation, facultative anaerobes *Staphylococcus carnosus* and *S. xylosus* are commonly employed (Stavropoulou *et al*., 2018). In the *Micrococcaceae* family, *Kocuria spp*., which are aerobes, are primarily used for sausage fermentation (Ding *et al*., 2021). Research by M. Fuka *et al*. (2020) has shown that the most common yeasts used as meat starter cultures are *Debaryomyces spp*. and *Candida spp*., which can exhibit aerobic or facultatively anaerobic metabolism. Both bacterial and yeast starter cultures were inoculated into the meat batter by researchers.

Mould starters, being strict aerobes, are inoculated on the surface and primarily belong to the species *Penicillium nalgiovense* and *P. gladioli* (Fuka *et al*., 2020). Reuterin (3hydroxypropionaldehyde) is a well-known broad-spectrum antimicrobial compound produced by *Lactobacillus reuteri* during anaerobic fermentation (Mu *et al*., 2018). According to S. Soltani *et al*. (2021), reuterin can be converted into various compounds, making it challenging to pinpoint the exact mechanism behind its antimicrobial activity. Reuterin spontaneously converts to acrolein, a cytotoxic electrophile, but it is reuterin, not acrolein, that is responsible for the antimicrobial effect. S. Bennett *et al*. (2021) and H. Yehia *et al*. (2022) investigated the potential use of reuterin as a food additive to prevent food spoilage and the growth of pathogens in various food matrices. Reuterin also demonstrated its effectiveness in reducing viable cells of *E. coli* O157:H7 and *L. monocytogenes* in pork over a storage period of 1 week.

Finally, it has been demonstrated that certain LAB possess a nitrite reductase enzyme system, which under anaerobic conditions reduces nitrite, a preservative used in some meat products, suggesting that LAB contribute to nitrite depletion in many foods (Liu *et al*., 2023). This is a significant finding for food safety, considering the EFSA recommendations to reduce the use of nitrates and nitrites in food preservation. The presence of nitrite reductase and heme-dependent nitrite reductase, capable of converting nitrite to NO, NO_2, and N_2O, has also been described in *Lb. sakei* (Liu *et al*., 2023). In fact, nitrite concentrations were significantly lower in fermented sausages inoculated with *Lb. sakei* compared to control sausages, likely due to its nitrite reductase, which is responsible for nitrite depletion. In the scientific manuscript by M. Daneshniya *et al*. (2023), it is established that several factors have a significant impact on combating *L. monocytogenes* and *C. botulinum*, and require low concentrations of enzymes for rapid nitrite metabolism. Therefore, developing technology for long-shelf-life meat products, such as cured meats, using bacterial preparations and reducing sodium nitrite content is relevant for food safety.

Materials and Methods

Experimental research was conducted in the laboratories of the Department of Technologies of Meat, Fish and Marine Products at the National University of Life and Environmental Sciences of Ukraine (NULES) and the Institute of Food Resources of the National Academy of Agrarian

Sciences of Ukraine (NAAS) during the period from 2019 to 2022. The developed technology was tested under the conditions of the production and research laboratory of NULES Ukraine.

The development of dry-cured meat product technology involved three main stages. The first stage focused on investigating the impact of starter cultures on biochemical processes in meat raw materials during salting, as well as justifying the use of beetroot extract (BE) as a substitute for reducing the sodium nitrite content in the formulation of extended-shelf-life meat products. The second stage examined the quality and safety indicators of the meat raw materials, including the structural-mechanical and physicochemical properties of beef used for experimental samples. The third stage involved the industrial testing of the developed biotechnology. Comprehensive quality and safety indicators of the final products were assessed, including physicochemical, microbiological, and organoleptic parameters.

Standard analytical methods were employed to ensure the accuracy and objectivity of the research. Moisture content was determined by drying the sample to constant mass at a temperature of 103 ± 2°C (DSTU ISO 1442:2005). The protein content was measured using the Kjeldahl method (DSTU ISO 5983:2003), fat content was determined with a Soxhlet extraction apparatus (DSTU ISO 1443:2005), and table salt content was assessed using the Mohr method (DSTU ISO 9297:2007). The energy value of the product was calculated according to DSTU 4590:2006. Active acidity (pH) was measured following DSTU ISO 2917:2001.

The oxidation-reduction potential (ORP) was measured using the multifunctional "Combo" device. Water activity (a_w) was assessed using the *HygroPalm* HP23-AW device (UK) based on the "dew point" principle (DSTU ISO 21807:2007). Sensory evaluation was conducted according to DSTU 4823.2:2007. Water-holding capacity (WHC) and meat plasticity were determined using the Grau and Hamm method modified by Volovinska (DSTU ISO 1442:2005). The ultimate shear stress was measured using an *Ulab* 3-31 penetrometer. Drying loss histograms were calculated using *Multitab* software. The yield of the final product was determined by weighing the item on analytical scales with a precision of 0.001 g, both before and after thermal processing. This approach allowed for the assessment of weight changes during production (Mushtruk *et al.*, 2023).

The quantitative evaluation of colour characteristics was conducted using the RGB system (Standard Practice for Calculating Yellowness and Whiteness Indices from Instrumentally Measured Colour Coordinates: E 313-20, 2020) and a multifunctional *Epson Stylus* TX400 device. Colour codes were identified with the *Adobe Photoshop CS6* software package. The obtained RGB colour coordinates were used to calculate L°, a°, and b° coordinates (colour intensity, redness, and colour saturation) within the international CIE colourimetric system. Based on these values, the colour hue (H (*Redness index*)) and chroma (C (the level of colour of the sample relative to the brightness of an absolutely white object)) were calculated to determine the redness index and the sample's colour saturation relative to the brightness of a purely white object (Standard Practice for Calculating Yellowness and Whiteness Indices from Instrumentally Measured Colour Coordinates: E 313-20, 2020) using the following formulas (1), (2):

$$H = \frac{a}{b}, \quad (1)$$

$$C = a^2 + b^2, \quad (2)$$

where *a* is the redness coordinate, *b* is the colour saturation coordinate.

Microbiological safety indicators were determined following the DSTU EN ISO 15189:2015 methodology. The formulations of the experimental samples incorporated sea salt, which possesses unique properties due to its enrichment with macro- and microelements of oceanic origin. Compared to rock salt, sea salt contains less sodium chloride, offers a milder flavour, and includes approximately 80 additional components such as calcium, magnesium, potassium, iodine, and other elements.

One of the objectives of the study was to eliminate sodium nitrite from the formulation of extended-shelf-life meat products. To achieve this, beetroot extract (BE) was employed as an organic substitute for sodium nitrite, in combination with denitrifying microorganisms and a starter culture. To develop the desired product properties and establish an effective barrier against pathogenic microflora during salting, the preparation B-LC-78, containing *Pediococcus acidilactici* and *Staphylococcus carnosus*, was utilised. Optimal conditions for their growth included oxygen availability, a minimum temperature of +6°C, and a limited salt concentration (up to 10% in water).

The salting process was conducted using a dry method at a temperature of 4-6°C with unrestricted oxygen access. To assess the effectiveness of this approach, three samples were developed and studied: control sample – beef sticks without the addition of starter cultures to the salting mixture; experimental sample 1 – beef sticks with the addition of the starter cultures B-LC-78 and CS-300, along with sea salt; experimental sample 2 – beef sticks with the addition of the starter cultures B-LC-78, CS-300, sea salt, and beetroot extract.

Results and Discussion

The detailed composition of the curing mixtures for each sample is presented in Table 1. This allowed for a comparison of the classical and experimental production technologies, assessing their impact on product quality and safety.

Table 1. *Composition of the curing mixture for the control sample per 100 kg of raw material*

Ingredients	Amount, kg
for the control sample	
NaCl *(sodium chloride)*	3.5
E 250 *(sodium nitrite)*	0.015
Glucose *(dextrose)*	1
Spices	1.2
Sodium isoascorbate (E 316)	0.07
for experimental sample No. 1	
Sea salt	3.3
Sodium nitrite (E 250)	0.015
Glucose (*dextrose*)	1
Spices	1.2
Sodium isoascorbate (E 316)	0.07
Preparation B-LC-78 (*bacterial*)	0.025
Preparation CS 300 (*bacterial*)	0.025
Sea salt	3.3
for experimental sample No. 2	
Sea sodium salt	3.3
Beetroot extract	2
Glucose (*dextrose*)	1
Spices	1.2
Sodium isoascorbate (E 316)	0.07
Preparation B-LC-78 (*bacterial*)	0.025
Preparation CS 300 (*bacterial*)	0.025

Source: authors' development

Lactic acid bacteria, which are a component of the typical microflora of meat raw materials, are mostly capable of developing during salting. Therefore, in this study, the amount of "extraneous" lactic acid microflora in the control samples was also determined.

The results of the experiment (Fig. 1) demonstrated a rapid growth of the viable cell population in the experimental samples.

Thus, in experimental sample No. 1, the number of viable lactic acid bacteria (LAB) increased from 6.11 to 7.02 log CFU/g within 24 hours. In a similar sample No. 2, an increase from 6.09 to 7.01 log CFU/g was observed. The control sample showed a slower growth rate of the microflora – from 2.03 to 2.9 log CFU/g. These results are consistent with previous studies, which confirm the accelerated growth of beneficial microflora under conditions of adding starter cultures, changing the level of acidity and redox potential (ORP). Together, these factors help assess the stability of *Pediococcus acidilactici* under the conditions of the raw cured product manufacturing process and support its potential use for fermenting meat raw materials.

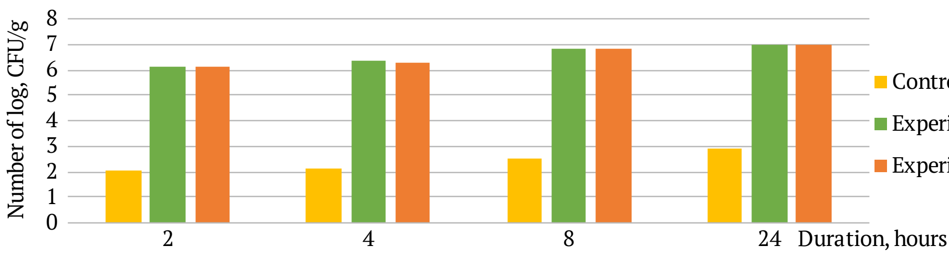

Figure 1. Dynamics of the number of lactobacilli in control and experimental meat samples during salting

Source: authors' development

Analysis of the dynamics of changes in pH and redox potential (ORP) of meat samples (Fig. 2) indicated the influence of the activity of the microflora that was introduced into the raw material. The vital activity of this microflora contributes to the fermentation process of dextrose (added to the curing mixture) and meat carbohydrates, which causes the formation of organic acids and, as a result, a decrease in the pH of the medium. In the control samples, a tendency towards a decrease in pH was also observed, but this process developed more slowly than in the experimental samples.

In the experimental samples, the pH decreased from 5.67 to 5.53 within 24 hours, which is a result of the enzymatic activity of lactic acid bacteria, which process dextrose and carbohydrates, forming organic acids (including lactic acid), thus lowering the pH level. It is known that at this stage, when the pH of the meat reaches the range of 5.5-5.7, tenderisation of muscle fibres, partial denaturation of proteins, and the formation of flavour compounds occur, affecting the organoleptic characteristics of the product. The decrease in ORP in the experimental samples is explained by the production of antioxidants by microorganisms. As shown in Figure 2, the ORP in the control samples increases from 150 to 239 mV after 24 hours of incubation, which may be due to oxidative processes and the development of indicator microflora.

The salting process, along with the redistribution of salt components, also causes changes in the moisture content of meat, accompanied by changes in WHC. These changes are of significant technological importance as they affect the product yield and its quality, including juiciness, texture, colour, taste, and aroma. The

WHC of meat products was investigated to assess the ability of the meat raw material to bind and retain moisture during processing and to ensure the yield of the finished product. The dynamics of WHC changes were studied after the introduction of the curing mixture with starter cultures into the meat raw material over the first 24 hours (Fig. 3).

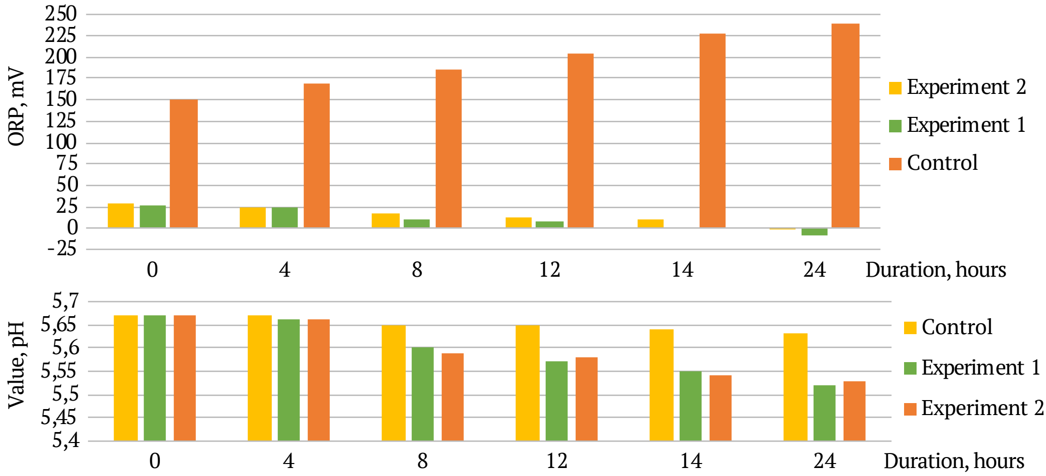

Figure 2. Variation in the oximetric (ORP) and pH values of control and experimental meat samples throughout the salting process

Source: authors' development

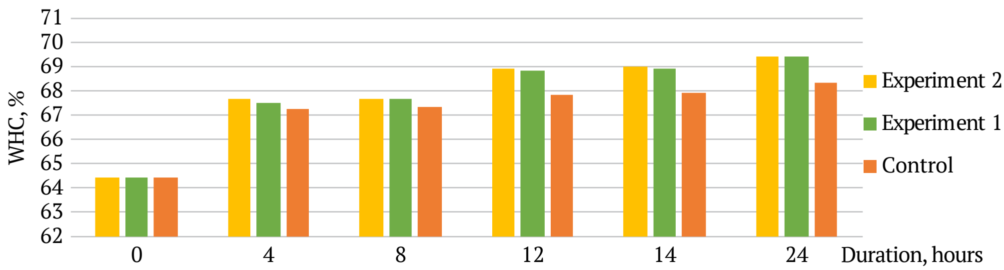

Figure 3. Dynamics of the change in water-holding capacity

Source: authors' development

During the salting of meat, an increase in WHC was observed in all samples. This is due to the fact that sodium chloride promotes the formation of a tissue fluid concentration that is close to the optimum for the solubility of the actomyosin fraction of proteins. As a result, the number of hydrophilic centres of myofibrillar proteins increases, which in turn leads to an increase in the amount of adsorption-bound water and an increase in the WHC of meat. It should be noted that the level of WHC is also influenced by factors such as the pH and redox potential of the meat system. The vital activity of *Pediococcus acidilactici* and *Staphylococcus carnosus* leads to the formation of organic compounds that affect meat proteins, in particular through a decrease in pH. As shown in Figure 3, the experimental samples, in which a bacterial preparation was used during salting, are characterised by a higher WHC value compared to the control sample. In experimental sample No. 1, the

WHC increased from 64.41% to 69.42% within 24 hours, while in the control sample, a slower increase in this indicator was observed – from 64.41% to 68.32%.

Reducing water activity in food products is an effective method of preventing microbial spoilage and several chemical reactions that can degrade product quality during storage. To investigate the effect of a bacterial preparation on the amount of water activity in meat raw materials, changes in water activity and moisture content dynamics in meat were studied over 24 hours from the beginning of salting. The results of this study are presented in Figure 4.

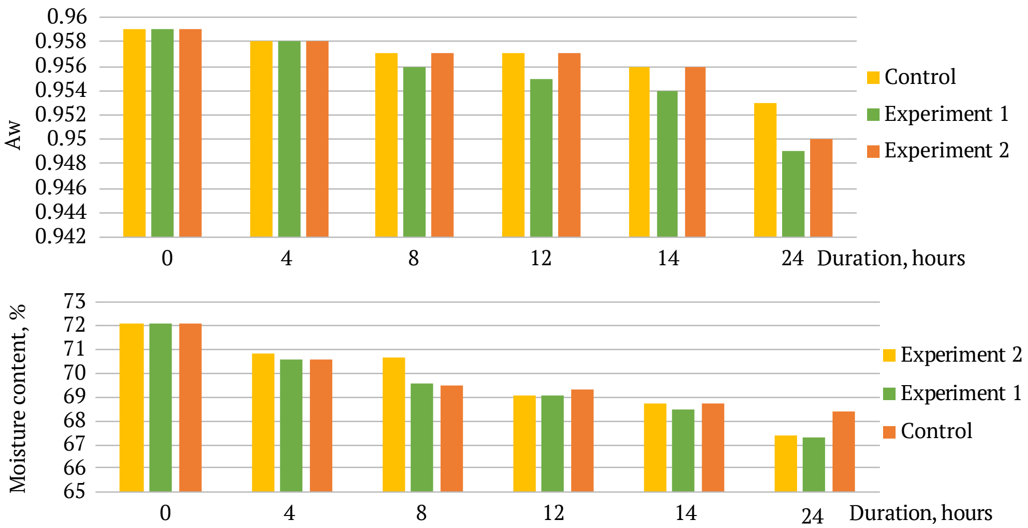

Figure 4. Change in moisture content and water activity (Aw) during the salting process
Source: authors' development

In traditional salting methods, regardless of the brine concentration, including dry salting, at the initial stage, the osmotic pressure of the brine is significantly higher than the osmotic pressure of the tissue fluid of the meat. This causes tissue dehydration. However, as salts and products of the breakdown of meat components accumulate by diffusion, the osmotic pressure in the meat increases, while in the brine it decreases. Such dynamics of processes lead to an increase in the water-holding capacity of meat and a transition from the dehydration phase to the hydration phase (Mushtruk *et al.*, 2023).

Meat texture is a crucial parameter for assessing product quality and determining the duration of processing. It reflects structural-mechanical characteristics such as plasticity, elasticity, and strength. Research has shown that the presence of organic acids during salting enhances proteolysis, positively affecting the meat's plasticity (Sun *et al.*, 2022). The influence of acidic compounds also leads to the swelling of collagen bundles and the weakening of intermolecular cross-links, resulting in a softer and more tender texture. The change in meat plasticity during salting is depicted in Figure 5.

According to the obtained data (Fig. 5), the plasticity of the samples increases proportionally with ripening time in both experimental and control samples, which is due to changes in the colloidal-chemical state of proteins and the activation of tissue enzymes during salting. The highest plasticity was observed in the experimental samples, reaching a value of 2.95×10^{-4} m^2 after 24 hours of salting. For the control sample, this value was 2.69×10^{-4} m^2 after the same period. These changes can be explained by biochemical processes, in particular the proteolytic activity of lactic acid bacteria and the accumulation of their metabolites, which contribute to changes in the surface tension of the meat raw material and affect soluble meat proteins.

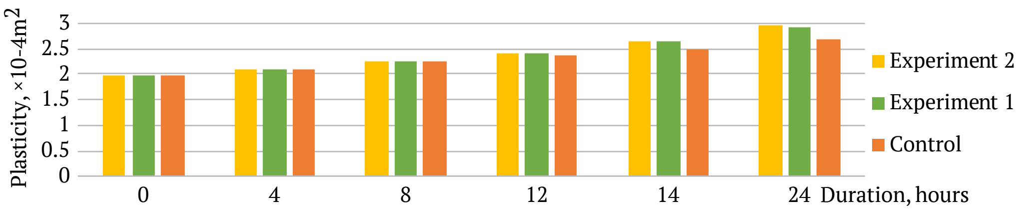

Figure 5. Alterations in the plasticity of meat raw materials during the salting process
Source: authors' development

The ultimate shear stress in both experimental and control samples exhibited a decreasing trend. Throughout the salting process, the values for the experimental samples remained lower than those of the control samples. The dynamics of changes in ultimate shear stress during meat salting are depicted in Figure 6. After 24 hours, the experimental samples reached an ultimate shear stress of 161 kPa, while the control samples had a value of 177 kPa. This confirms that the proteolytic activity of bacteria and the accumulation of their metabolites affect the soluble proteins in the meat and alter the surface tension of the meat raw material.

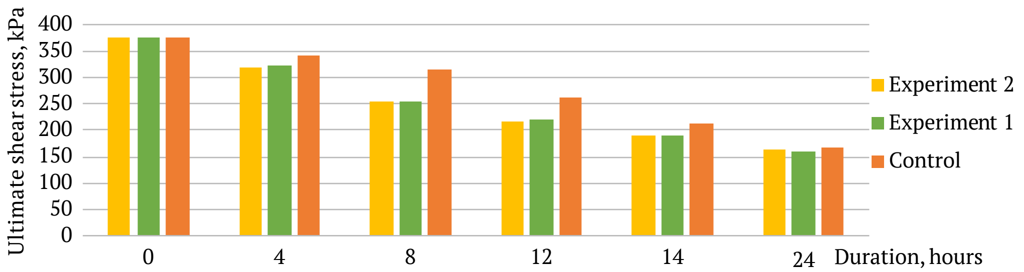

Figure 6. Change in ultimate shear stress of meat raw materials during the salting process
Source: authors' development

The results of the conducted research confirm the positive impact of adding bacterial preparations to meat raw materials during salting. This allows for improving the structural and mechanical properties of the product, reducing losses during heat treatment, and increasing water-holding capacity, which collectively positively affects the quality of the finished product and contributes to increasing the microbiological stability of the process. M. Shakil *et al*. (2022) stated, that sodium nitrite, due to its versatility and effectiveness, is indispensable in the meat industry, but its use is associated with certain risks, as it can be a precursor to carcinogenic nitrosamines. Given these risks, modern meat processing technologies involve the use of beetroot extract (BE) together with denitrifying bacteria to reduce the nitrite content in the product and also to improve colour formation.

Beetroot contains betaine and betanin, which have healing properties. It is known that these compounds contribute to strengthening blood vessel walls, and are effective means of preventing colds, overloads of the body and immunodeficiency states, in particular in connection with carcinogenesis and the effects of radiation. Betanin and betaine also belong to lipotropic substances that are involved in fat metabolism, due to their chemical similarity to choline and lecithin (Zheplinska *et al*., 2020). Nitrates, which are naturally found in beetroot, can only be reduced to nitrites by a microbiological pathway, thanks to the actions of nitrate-reducing bacteria. Thus, the use of vegetable extracts can be a key step in preserving the high-quality characteristics of the product, ensuring its safety for the consumer.

The starter culture Bactoferm CS-300, containing *Staphylococcus carnosus* bacteria capable of nitrate reduction, and SafePro B-LC-78, which includes both *Staphylococcus carnosus* and *Pediococcus acidilactici*, actively influence the nitrate reduction process. The efficiency of this reaction depends on the pH of the environment, with reduction reactions occurring more intensely at lower pH values (Guimarães *et al*., 2022). The optimal pH range for colour formation is between 5.0 and 6.0. Studies have shown that the addition of starter cultures containing lactic acid bacteria significantly accelerates the decrease in pH. For a more accurate determination of the colour characteristics of the samples, a sensory analysis was conducted, supplemented by additional studies, the results of which are presented in Table 2.

Table 2. *Colour characteristics of control and experimental samples of long-term stored beef products ($n = 3$, $p \geq 0.95$)*

Indicator	**Studied samples**		
	Beef		
	Control sample	**Experiment 1**	**Experiment 2**
$^*L$ Colour Intensity	157.22 ± 0.540	157.19 ± 0.424	157.23 ± 0.375
$^*a$ Redness	8.5 ± 0.22	8.4 ± 0.25	8.4 ± 0.64
Redness index (H)	0.868 ± 0.342	0.877 ± 0.541	0.876 ± 0.223
$^*C$ Colour Level relative to Brightness of Absolute White Object	169 ± 0.45	171 ± 0.38	172 ± 0.11

Source: authors' development

Analysing the data presented in Table 4, it can be concluded that the use of starter cultures in combination with beetroot extract (BE) during the colour formation of long-term stored meat products has a positive impact on their colour characteristics. Using beef products as an example, it can be seen that experimental sample No. 2, treated with BE, has colour indicators that are not inferior to either the control sample or experimental sample No. 1, where sodium nitrite was used. This confirms the assumption that beetroot contains enough nitrates to ensure the colour formation process of the meat product.

In addition, experimental sample No. 2 demonstrates the best results in terms of colour intensity, redness index, and chroma, indicating the high efficiency of using BE. This proves that reducing the level of residual sodium nitrite in the product contributes to a more active formation of nitrosopigments, which in turn improves the colour indicators of the finished meat product. Starter cultures are an important tool for ensuring the safety of fermented meat products, as they contribute to reducing the growth of microorganisms that can cause spoilage or be pathogenic. This process occurs through the production of metabolites or the competitive exclusion of other microbes. Thanks to starter cultures, the need to use chemical additives such as nitrites and nitrates is reduced. Moreover, starter cultures are able to metabolise these compounds, which reduces their residual content in finished products.

Starter cultures not only positively impact product safety but also improve batch-to-batch consistency, reduce production time, and enhance sensory properties. Due to these effects, starter cultures play a multifunctional role in the technology of fermented meat product production. Experimental studies have shown that the addition of the bacterial preparation B-LC-78 and the additive CS-300 in combination with beetroot extract (BE) to the curing mixture significantly improves the structural-mechanical, physicochemical, and sensory characteristics of the finished meat product. This has allowed for the improvement of the technology for producing long-term stored dry-cured meat products, increasing their biological and nutritional value.

The improved technology involves the additional introduction of a mixture of bacterial preparations B-LC-78 and CS-300, as well as BE, in doses that meet the requirements of the technological process. This allows for a reduction in production time, improvement of the product's sensory properties, and an increase in its shelf life. As a result of the experiment, dry-cured products were produced using the improved technology, applying a technological scheme that was implemented in the research laboratory of the National University of Life and Environmental Sciences of Ukraine. The improved technological scheme for the production of dried meat products with an extended shelf life is presented in Figure 7.

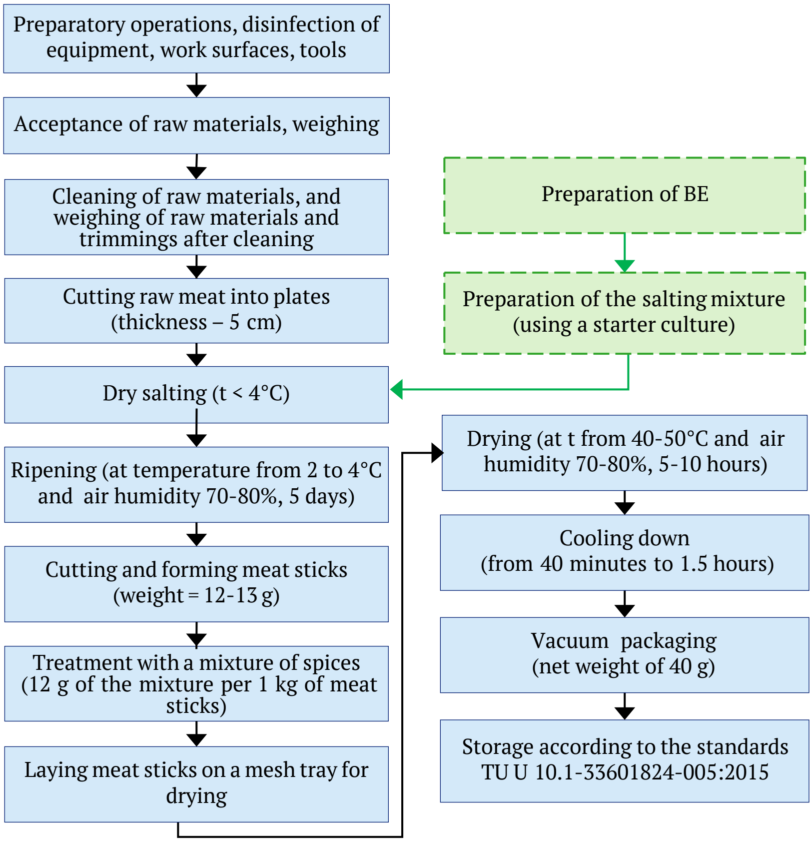

Figure 7. An improved technological scheme for the production of cured meat products with an extended shelf life

Source: authors' development

The production process involved preparing the meat (receiving, weighing, cleaning), mincing, salting, ripening, and drying. High-quality beef (longissimus dorsi muscle) was used and sliced into 5 cm thick plates. The meat was dry-salted using specialised curing mixtures. After salting, the raw material was aged for 3-4 days at a temperature of 2-4°C, after which the plates were cut into sticks (12-13 g) and treated with spices. Drying was carried out at a temperature of 40-50°C for 5-10 hours, depending on the type of raw material. After drying, the product was cooled, which took from 40 minutes to 1.5 hours depending on the microclimate in the production area. An innovation in the technology development was the introduction of bacterial preparations B-LC-78 and CS-300 directly into the curing mixture, which contributed not only to improving the quality of the product but also to maintaining its safety and increasing its shelf life.

The advanced biotechnology employed for producing extended-shelf-life meat products has resulted in a product of superior quality, particularly in terms of nutritional value, microbiological stability, and safety. The substitution of sodium nitrite with extracts from biotechnological starter cultures has significantly reduced the use of chemical preservatives while preserving all organoleptic characteristics. The introduction of the bacterial preparations B-LC-78 and CS-300, along with beetroot extract, has not only enhanced the final product's quality but also ensured adequate microbiological stability. The use of sea salt, another component of the technological scheme, has contributed not only to preservation but also to increasing the product's nutritional value. Compared to traditional technologies that relied on nitrites and nitrates, these innovations have achieved a reduction in residual chemical compounds, positively impacting product safety.

The implementation of new technologies in production laboratories is particularly significant, allowing for controlled conditions of ripening, drying, and storage, as well as vacuum packaging, which ensures a long shelf life without compromising quality. Considering all these factors, it is possible to significantly improve the final quality indicators of the product and make it more appealing to consumers. These results support the feasibility of introducing bacterial cultures and environmentally friendly technologies into the production of extended-shelf-life meat products, aligning with contemporary demands for healthy eating and environmental safety. They also open up prospects for further research and expanding production capabilities in this field.

Conclusions

Through a study of the chemical composition, production technology, and bioprotective and colour-regulating properties of bacterial preparations, their significant impact on the physicochemical and microbiological characteristics of meat raw materials has been established. Specifically, the introduction of bacterial preparations at a rate of 25 g per 100 kg of raw material provided a complex effect, including increased water-holding capacity, plasticity of the raw material, and a rapid decrease in pH and redox potential (ORP). Experimental studies have shown that 24 hours after the introduction of the preparations, the concentration of viable lactic acid bacteria increased 2.4 times compared to the control. As a result of the activity of these bacteria, a decrease in the pH of the medium from 5.67 to 5.53 was observed, which is due to the fermentation of dextrose and carbohydrates with the formation of carboxylic acids, in particular lactic acid. This process is effective for controlling the pH of meat raw materials, which positively affects the stability of the product.

In experimental samples containing bacterial preparations, water-holding capacity increased by 1.01 times compared to control samples, while shear stress decreased 2.3 times after 24 hours. This indicates an improvement in the structural and mechanical properties of the meat, reduced cooking losses, and an enhancement in the quality of the final product. Additional data confirms the importance of using natural beetroot extract in combination with denitrifying bacteria as an effective alternative to sodium nitrite. This combination allows for the complete elimination of sodium nitrite in the production of dry-cured meat products while maintaining colour stability, which is critical for producing high-quality meat products. Thus, the results of the study highlight the significant potential of bacterial preparations for improving the quality of meat products, stabilising their colour characteristics, and reducing the use of chemical preservatives. These innovations contribute to the development of safe and efficient technology for producing extended-shelf-life meat products.

Future research in this area should focus on a detailed study of the impact of various types of probiotic bacteria on fermentation processes in meat raw materials, aiming to optimise technological parameters such as salting time, ripening, and drying. Further attention should be paid to the development of new compositions of natural extracts with increased antioxidant and bioprotective activity, which can provide additional protection against oxidation and microbial spoilage. A significant direction is the scaling up of proposed technologies for industrial use and the evaluation of their economic efficiency. It is also advisable to expand research to other types of meat products to verify the universality of the proposed approaches, as well as to study their impact on the organoleptic properties, safety, and functionality of the final products in the context of modern consumer demands.

Acknowledgements

None.

Conflict of Interest

The authors declare no conflict of interest in the publication of this article.

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Розроблення технології м’ясних продуктів подовженого терміну зберігання

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Анотація. Розроблення нових технологій для збереження якості м’ясних продуктів є надзвичайно актуальним у контексті зростаючого глобального попиту на безпечні, довговічні та функціональні харчові продукти, що відповідають сучасним стандартам здорового харчування, особливо з урахуванням викликів, пов’язаних із забезпеченням продовольчої безпеки у світі. Метою цього дослідження було створення інноваційної технології виробництва м’ясних продуктів, яка забезпечує їх подовжений термін зберігання без втрати органолептичних характеристик. У роботі застосовувалися сучасні методи фізико-хімічного та мікробіологічного аналізу, а також біотехнологічні підходи, зокрема використання стартових культур молочнокислих бактерій і біфідобактерій. Проведені дослідження підтвердили позитивний вплив пробіотичних культур на біохімічні процеси, які відбуваються у м’ясній сировині. Зокрема, вони сприяли ферментації, дозріванню та формуванню стійких органолептичних характеристик. Окрім цього, було встановлено, що використання пробіотиків дозволяє знизити активність води та рівень pH, що суттєво покращує мікробіологічну стабільність м’ясних продуктів. Аналіз впливу технологічних параметрів, таких як періоди засолу, дозрівання та сушки, показав, що вони мають ключове значення для формування смакових і ароматичних властивостей, текстури та кольору кінцевого продукту. У результаті роботи було розроблено інноваційну технологію виробництва м’ясних продуктів, яка дозволяє отримати вироби з високим вмістом білка, низьким вмістом жиру та стабільною мікробіологічною безпечністю, із подовженим терміном зберігання до 45 діб. Практична цінність проведеного дослідження полягає у можливості широкого впровадження розробленої інноваційної технології на підприємствах

харчової промисловості для виробництва високоякісних сирокопчених і сиров’ялених м’ясних виробів, які відповідають сучасним стандартам безпечності та корисності харчових продуктів. Використання пробіотичних культур сприятиме створенню продуктів із покращеними органолептичними властивостями та додатковими функціональними перевагами, що відповідають тенденціям здорового харчування та задовольняють попит споживачів на натуральні та екологічно чисті продукти

Ключові слова: біотехнологія; в'ялена м'ясна продукція; безпечність; термін придатності; якість

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