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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 rumenprotected 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 low-density 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