FIMEK

Journal of Agronomy, Technology and Engineering Management
ISSN 2620-1755

Review

Beneficial Effects of Glycerol Usage in Dairy Cows Nutrition

Nikola Puvača [1,*] and Dragan Glamočić [2]

[1] Faculty of Economics and Engineering Management in Novi Sad, University Business Academy in Novi Sad, Cvećarska 2, 21107 Novi Sad, Serbia.
[2] Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia.
* Correspondence: nikola.puvaca@fimek.edu.rs

Received: 28 April 2024; Accepted: 02 August 2024

Abstract: This review explores the multifaceted benefits of glycerol usage in dairy cow nutrition, focusing on its impact on metabolic health, milk production, physiological and biochemical mechanisms, and broader implications for animal health, welfare, and environmental sustainability. Glycerol, a byproduct of biodiesel production, serves as an effective energy source for dairy cows, particularly during periods of negative energy balance, reducing the risk of metabolic disorders such as ketosis. It has been shown to enhance milk production by improving glucose metabolism and supporting lactation. The review also delves into the physiological and biochemical mechanisms by which glycerol influences rumen fermentation, energy partitioning, and overall cow health. Furthermore, the broader implications of glycerol supplementation, including its potential to improve animal welfare and reduce the environmental impact of dairy farming, are examined. The integration of glycerol into dairy nutrition represents a promising approach to optimizing cow health, productivity, and sustainability in the dairy industry.

Keywords: Milk; dairy; cows; nutrition; feed additives; glycerol.

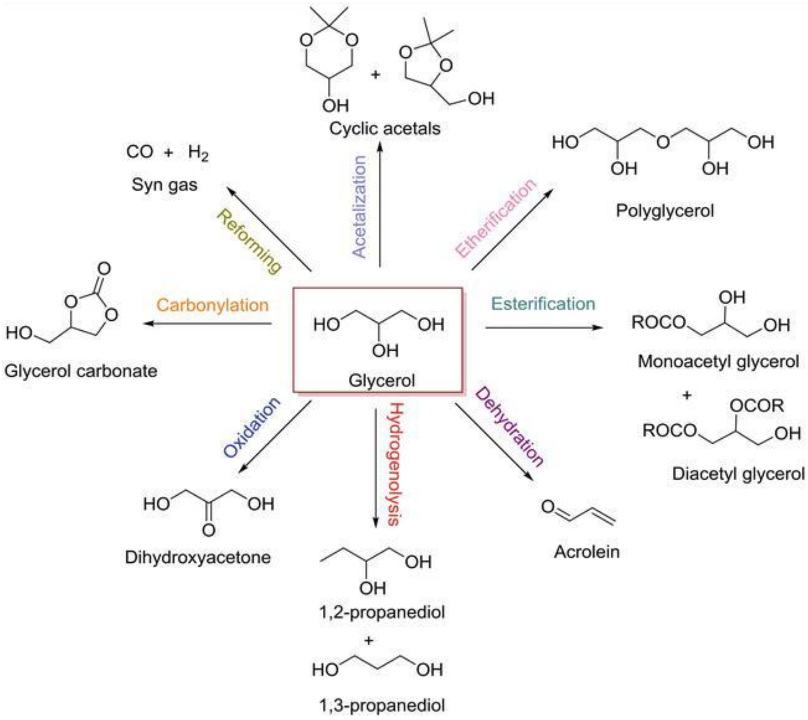
---

1. Introduction

The field of dairy cow nutrition has evolved significantly over recent decades, driven by the need to enhance milk production efficiency, improve animal health, and address environmental sustainability concerns [1,2]. One promising area of research within this domain is the incorporation of alternative feed additives that can optimize energy utilization and improve overall health outcomes for dairy cows [2,3]. Among these additives, glycerol has garnered considerable attention due to its potential multifaceted benefits [4].

Glycerol, also known as glycerin or 1,2,3-propanetriol, is a simple polyol compound that has traditionally been used in various industrial and pharmaceutical applications [5] (Picture 1). However, with the rise of biodiesel production, glycerol has become more readily available as a byproduct, presenting an opportunity for its use in animal feed. The integration of glycerol into dairy cow diets is particularly appealing due to its properties as an energy-dense and gluconeogenic substrate. Glycerol can serve as an efficient energy source that can be rapidly metabolized into glucose, thereby supporting the high energy demands of lactating cows [6].

The physiological and biochemical mechanisms by which glycerol exerts its beneficial effects in dairy cows are of particular interest [7]. Upon ingestion, glycerol is absorbed in the rumen and can be quickly converted into volatile fatty acids (VFAs), such as propionate, which are precursors for gluconeogenesis in the liver. This conversion process can help maintain glucose homeostasis, particularly during periods of negative energy balance (NEB) commonly experienced during early lactation [8]. The ability to support glucose levels is critical for preventing metabolic disorders such as ketosis, which is characterized by elevated ketone bodies in the blood due to excessive fat mobilization.



Picture 1. Glycerol transformation to value-added 1,3-propanediol production [9].

Furthermore, the inclusion of glycerol in dairy cow diets has been associated with improved milk yield and composition [10]. Studies have shown that glycerol supplementation can lead to higher milk production and an increase in milk fat content, potentially due to the enhanced availability of glucose and the positive energy balance it helps maintain [11]. This aspect is particularly valuable in the dairy industry, where milk yield and quality are paramount for economic viability.

In addition to direct metabolic benefits, glycerol usage in dairy nutrition may have broader implications for animal health and welfare [12]. By supporting energy balance and reducing the incidence of metabolic disorders, glycerol can contribute to overall cow health, leading to better reproductive performance and longevity. This aspect aligns with the growing emphasis on animal welfare in agricultural practices, where maintaining the health and well-being of livestock is a critical component of sustainable farming systems [13].

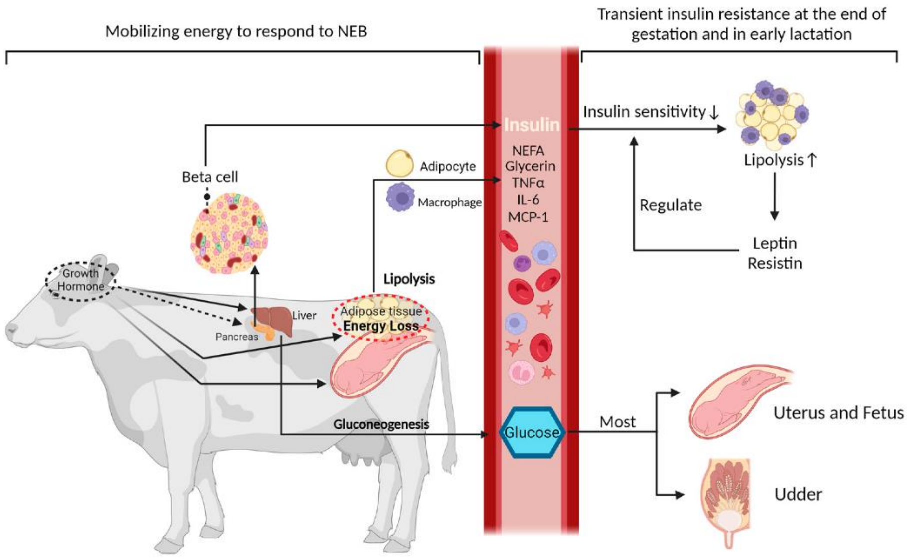
Moreover, the environmental impact of glycerol usage in dairy nutrition is an area of emerging interest. The repurposing of glycerol from the biodiesel industry into animal feed aligns with principles of circular economy and resource efficiency [14]. By utilizing this byproduct, the dairy industry can reduce waste and improve the sustainability of both biodiesel and dairy production systems [15]. This synergy between industries highlights the potential for integrated approaches to agricultural sustainability, where waste products from one process can be effectively utilized as inputs for another.

Despite the promising benefits, the incorporation of glycerol into dairy cow diets is not without challenges [16]. There are considerations regarding the optimal dosage, potential impacts on rumen fermentation dynamics, and long-term effects on animal health that need to be thoroughly evaluated. Additionally, economic feasibility and supply chain logistics for glycerol must be addressed to ensure its practical application in dairy farming [17].

To provide a comprehensive understanding of glycerol's beneficial effects, this paper will review the current scientific literature on its usage in dairy cow nutrition.

2. Impact of glycerol on cows metabolic health

Glycerol is a three-carbon molecule that serves as the backbone for triglycerides, the primary form of fat storage in animals. In recent years, the production of biodiesel has increased the availability of glycerol as a byproduct, making it an economically viable feed additive [18]. Glycerol is readily absorbed in the rumen and can be rapidly metabolized to produce energy [19], making it an attractive supplement for dairy cows, particularly during the transition period when cows are at high risk of developing metabolic disorders [20]. The transition period, which includes the weeks before and after calving, is critical for dairy cows. During this time, cows often experience NEB due to the high energy demands of lactation coupled with reduced feed intake (Picture 2). This energy deficit can lead to the mobilization of body fat reserves, resulting in increased plasma levels of non-esterified fatty acids (NEFAs) and the development of ketosis [20].



Picture 2. Metabolic dynamics and hormonal interplay during the peripartum period in dairy cows [21].

Glycerol has been shown to help mitigate the effects of NEB by serving as a glucogenic precursor. Once absorbed, glycerol is converted into glucose via gluconeogenesis in the liver, providing an immediate source of energy. This helps to reduce the mobilization of body fat reserves, thereby lowering NEFA concentrations and the risk of ketosis. Studies have demonstrated that cows supplemented with glycerol exhibit improved energy balance, reduced incidence of ketosis, and better overall metabolic health [22].

Ketosis is a metabolic disorder characterized by elevated levels of ketone bodies in the blood, such as beta-hydroxybutyrate (BHB), due to excessive fat mobilization and insufficient glucose availability [23]. It commonly occurs during the early lactation period when the energy demand for milk production is high.

Glycerol supplementation has been widely studied as a preventive measure against ketosis. By providing an additional source of glucose, glycerol reduces the need for the cow to mobilize fat reserves, thereby decreasing the production of ketone bodies. Several studies have reported that cows receiving glycerol supplementation show lower BHB levels and a reduced incidence of clinical and subclinical ketosis. This not only improves the metabolic health of the cow but also enhances milk production and reproductive performance.

The rumen is the primary site of fermentation in cows, where microbial populations break down feedstuffs to produce VFAs, which serve as the main energy source for the animal. The introduction of glycerol into the diet can influence rumen fermentation patterns [24].

Glycerol is rapidly fermented by rumen microbes, leading to an increase in the production of propionate, one of the key VFAs [25]. Propionate is a crucial precursor for gluconeogenesis in the liver, which contributes to maintaining blood glucose levels. The increased production of propionate, therefore, complements the direct glucose supply from glycerol metabolism, further supporting energy balance [18].

However, the impact of glycerol on rumen fermentation is dose-dependent [26]. While moderate supplementation can enhance propionate production and support metabolic health, excessive glycerol can lead to rumen acidosis, a condition characterized by a drop in rumen pH due to the accumulation of VFAs. This underscores the importance of careful dosing when incorporating glycerol into dairy cow diets, as well in other animals such as rabbits [27] for example.

While the benefits of glycerol supplementation are well-documented, several factors must be considered to optimize its use in dairy nutrition. The appropriate dosage is critical to achieving the desired metabolic effects without causing adverse outcomes like rumen acidosis. Most studies recommend glycerol supplementation at levels ranging from 100 to 500 grams per day, depending on the cow's stage of lactation and overall diet composition. Moreover, the timing of glycerol administration is important. Supplementing glycerol during the transition period, particularly in the weeks leading up to and following calving, is most effective in preventing NEB and ketosis. Additionally, the form of glycerol (e.g., liquid or powder) and its purity can influence its effectiveness and palatability [28].

Despite the promising results, there are some challenges associated with glycerol supplementation. Impurities in crude glycerol can affect its palatability and safety, necessitating careful selection and processing before use in animal feed [28]. Another challenge is the risk of rumen acidosis when glycerol is overfed or improperly balanced with other dietary components [29].

3. Influence of dietary glycerol on milk production

The inclusion of dietary glycerol in dairy cow diets has attracted significant interest in recent years due to its potential to enhance milk production and overall metabolic health. Glycerol, a byproduct of biodiesel production, is an energy-dense, easily fermentable carbohydrate that can serve as a glucogenic precursor. Its role in ruminant nutrition, particularly for lactating dairy cows, is multifaceted, influencing energy metabolism, rumen fermentation, and ultimately, milk yield and composition. Glycerol is readily absorbed in the rumen and metabolized into glucose in the liver via gluconeogenesis [10]. This characteristic makes glycerol an attractive feed additive for dairy cows, particularly during periods of NEB, such as the transition period around calving. During this time, cows often experience a significant energy deficit due to the high demands of milk production and reduced feed intake, leading to the mobilization of body fat and increased risk of metabolic disorders like ketosis. The supplementation of glycerol in the diet provides an immediate source of glucose, which can help mitigate the effects of NEB, support lactation [6], and improve overall cow health. The following sections will explore how glycerol influences milk production through its effects on energy metabolism and rumen fermentation.

The energy status of a dairy cow is a critical determinant of milk production. During early lactation, when energy demands are highest, cows often struggle to consume sufficient feed to meet their energy requirements. This leads to the mobilization of body fat reserves, resulting in elevated levels of NEFAs in the blood, which can predispose cows to ketosis and other metabolic disorders. Glycerol plays a crucial role in alleviating this energy deficit by serving as a glucogenic precursor [30]. Once absorbed, glycerol is converted into glucose in the liver, providing an immediate source of energy that can be used to support milk synthesis. Several studies have demonstrated that glycerol supplementation can improve energy balance in lactating cows, leading to increased milk yield [31–33]. For instance, cows supplemented with glycerol during early lactation exhibit higher milk production compared to those not receiving glycerol [32]. This is largely due to the enhanced energy status of the cow, which allows for greater allocation of nutrients towards milk synthesis rather than fat mobilization. Additionally, glycerol's role in reducing NEFA concentrations and preventing ketosis further supports milk production by maintaining overall cow health.

The rumen is the primary site of fermentation in cows, where microbial populations break down feedstuffs to produce VFAs, which serve as the main energy source for the animal. The introduction of glycerol into the diet can significantly influence rumen fermentation patterns, thereby affecting milk production and composition.

Moreover, glycerol supplementation has been reported to influence milk composition, particularly milk fat content [10]. Some studies have indicated that glycerol can increase milk fat percentage, likely due to the enhanced availability of glucose and propionate, which are used as substrates for de novo fatty acid synthesis in the mammary gland. This increase in milk fat content can be particularly beneficial for dairy producers, as it improves the overall quality of the milk. While moderate supplementation can enhance milk fat content, excessive glycerol can lead to rumen acidosis, a condition characterized by a drop in rumen pH due to the accumulation of VFAs. This underscores the importance of careful dosing when incorporating glycerol into dairy cow diets.

Ketosis is a metabolic disorder that commonly occurs during early lactation when the energy demands of milk production exceed the cow's energy intake. This condition is characterized by elevated levels of ketone bodies, such as beta-hydroxybutyrate (BHB), in the blood due to excessive fat mobilization and insufficient glucose availability [20].

Glycerol supplementation has been widely studied as a preventive measure against ketosis. By providing an additional source of glucose, glycerol reduces the need for the cow to mobilize fat reserves, thereby decreasing the production of ketone bodies. Several studies have reported that cows receiving glycerol supplementation show lower BHB levels and a reduced incidence of clinical and subclinical ketosis [34]. This not only improves the metabolic health of the cow but also enhances milk production. The prevention of ketosis through glycerol supplementation is particularly important for sustaining high milk yields. Cows that avoid ketosis are more likely to maintain consistent feed intake, energy balance, and milk production throughout the lactation period [35]. Additionally, the improved metabolic health associated with glycerol supplementation can positively impact reproductive performance, further supporting sustained milk production [8]. While the benefits of glycerol supplementation for milk production are well-documented, several factors must be considered to optimize its use in dairy diets. The appropriate dosage is critical to achieving the desired effects without causing adverse outcomes, such as rumen acidosis. Most studies recommend glycerol supplementation at levels ranging from 100 to 500 grams per day, depending on the cow's stage of lactation and overall diet composition [36]. Moreover, the timing of glycerol administration is important. Supplementing glycerol during the transition period, particularly in the weeks leading up to and following calving, is most effective in preventing NEB and supporting early lactation milk production. While glycerol is generally cost-effective due to its availability as a byproduct of biodiesel production, fluctuations in its price and supply can impact its feasibility as a long-term feed additive. Therefore, it is important to balance the cost of glycerol supplementation with its potential benefits for milk production and overall herd health [8].

4. Physiological and biochemical mechanisms of glycerol in dairy cows

Once ingested, glycerol is rapidly absorbed in the rumen, the first compartment of the cow's stomach, where it is either directly absorbed into the bloodstream or fermented by rumen microbes. The majority of glycerol is absorbed intact through the rumen wall, entering the portal circulation and subsequently the liver, where it undergoes further metabolism [37]. In the liver, glycerol is a substrate for gluconeogenesis, the metabolic pathway responsible for producing glucose from non-carbohydrate precursors [38]. Glycerol is first phosphorylated by glycerol kinase to form glycerol-3-phosphate, which is then oxidized to dihydroxyacetone phosphate (DHAP) [39]. DHAP is an intermediate in both glycolysis and gluconeogenesis, and it can be converted into glucose or enter other metabolic pathways depending on the cow's energy status and metabolic needs. This ability to serve as a glucogenic precursor is particularly valuable during periods of NEB, when the cow's energy requirements for lactation exceed its dietary intake [36]. By providing an additional source of glucose, glycerol helps to mitigate the effects of NEB [40], reduce the mobilization of body fat reserves, and prevent the onset of ketosis, a metabolic disorder characterized by elevated levels of ketone bodies in the blood [20].

Gluconeogenesis is the primary metabolic pathway through which glycerol exerts its effects in dairy cows. This process is crucial for maintaining blood glucose levels, particularly during times of high energy demand, such as early lactation. Glucose is the main energy source for many tissues, including the brain, and is also a key substrate for lactose synthesis in the mammary gland, which directly influences milk production [40]. Glycerol's role in gluconeogenesis begins with its conversion to glycerol-3-phosphate in the liver. This reaction is catalyzed by glycerol kinase, an enzyme that is relatively abundant in the liver and is key to the metabolism of glycerol. The glycerol-3-phosphate is then converted to DHAP by glycerol-3-phosphate dehydrogenase. DHAP can then enter the gluconeogenic pathway, where it is ultimately converted to glucose. The glucose produced via gluconeogenesis can be released into the bloodstream to maintain blood glucose levels or used within the liver for other metabolic processes. By supporting glucose production, glycerol helps to reduce the cow's reliance on body fat reserves for energy, thereby decreasing the production of NEFAs and ketone bodies [41]. This is particularly important during early lactation when the risk of ketosis is highest. Glycerol also exerts significant effects on rumen fermentation, a process crucial for the digestion and utilization of dietary nutrients in dairy cows. The rumen is home to a diverse population of microorganisms that ferment feedstuffs to produce VFAs, which serve as the main energy source for the cow. The primary VFAs produced in the rumen are acetate, propionate, and butyrate. When glycerol is introduced into the diet, it is rapidly fermented by rumen microbes, leading to an increase in the production of propionate, one of the key VFAs. Propionate is particularly important because it is a major precursor for gluconeogenesis in the liver [42]. The increased production of propionate from glycerol fermentation thus complements the direct conversion of glycerol to glucose, further supporting energy balance and milk production. In addition to enhancing propionate production, glycerol fermentation can also influence the overall rumen environment. The fermentation of glycerol produces less methane compared to other carbohydrates, which can have a positive impact on feed efficiency and environmental sustainability. However, it is important to note that excessive glycerol supplementation can lead to rumen acidosis, a condition characterized by a drop in rumen pH due to the accumulation of VFAs. This underscores the importance of careful dosing and monitoring when incorporating glycerol into dairy cow diets [42].

Glycerol's effects on glucose metabolism are closely linked to the regulation of insulin, a hormone that plays a central role in energy balance and nutrient partitioning. Insulin promotes the uptake of glucose by tissues such as muscle and adipose tissue and inhibits the mobilization of fat stores. During periods of NEB, cows typically experience insulin resistance, a condition in which the body's tissues become less responsive to insulin. This is a physiological adaptation that prioritizes glucose for vital organs and lactation. Glycerol supplementation can help to alleviate insulin resistance by providing a direct source of glucose [33], thereby reducing the need for gluconeogenesis from protein and fat stores. Additionally, glycerol has been shown to influence the secretion of other hormones involved in energy metabolism, such as glucagon and growth hormone [31]. These hormones work in concert with insulin to regulate blood glucose levels and support the metabolic adaptations required for high milk production.

5. Glycerol usage in dairy nutrition and its broader implications for animal health and welfare

As the dairy industry continuously seeks ways to optimize milk production and improve animal welfare [43], glycerol offers several advantages when used appropriately in dairy diets. This section delves into the various aspects of glycerol usage in dairy nutrition, examining its metabolic effects, impact on milk production, and broader implications for animal health and welfare [44]. Glycerol is a trihydroxy alcohol that, when ingested by dairy cows, is absorbed primarily through the rumen. Once absorbed, glycerol serves as an immediate source of energy, bypassing the more complex metabolic pathways required for the utilization of other energy sources such as fiber and starch. The metabolic effects of glycerol in dairy cows are primarily mediated through its conversion to glucose in the liver via gluconeogenesis [25]. During periods of NEB, which commonly occurs during early lactation, cows are unable to consume sufficient feed to meet the high energy demands of milk production. This energy deficit leads to the mobilization of body fat reserves, resulting in the production of NEFAs and ketone bodies such as BHB. Elevated levels of NEFAs and BHB are indicative of ketosis, a metabolic disorder that can severely impact milk yield, reproductive performance, and overall cow health [20]. Glycerol supplementation mitigates the effects of NEB by providing a readily available source of glucose. When cows consume glycerol, it is rapidly absorbed and transported to the liver, where it is phosphorylated by glycerol kinase to form glycerol-3-phosphate. This intermediate is then converted into DHAP, which enters the gluconeogenic pathway to produce glucose. The glucose generated through this pathway is crucial for maintaining blood glucose levels, supporting lactose synthesis in the mammary gland, and reducing the reliance on body fat mobilization [3]. By improving the energy status of dairy cows, glycerol supplementation can significantly reduce the incidence of ketosis. Studies have shown that cows supplemented with glycerol exhibit lower blood concentrations of NEFAs and BHB, indicating a reduced risk of both clinical and subclinical ketosis. This reduction in metabolic stress not only enhances milk production but also supports the overall health and welfare of the cow [45].

The rumen is the primary site of microbial fermentation in dairy cows, where complex carbohydrates are broken down into VFAs, gases, and microbial protein. These end products are crucial for the cow's energy metabolism and overall health. Glycerol, when introduced into the rumen, can significantly influence fermentation patterns and the composition of the rumen microbiota. Glycerol is rapidly fermented by rumen microbes, leading to an increase in the production of propionate, one of the key VFAs. Propionate is the primary glucogenic VFA, serving as a precursor for glucose synthesis in the liver. The increased production of propionate from glycerol fermentation supports energy metabolism and helps to maintain blood glucose levels, particularly during periods of NEB. In addition to propionate, glycerol fermentation also produces acetate and butyrate, although to a lesser extent. Acetate is primarily used for fatty acid synthesis, while butyrate serves as an energy source for the epithelial cells of the rumen [46]. The balance between these VFAs is crucial for maintaining rumen health and optimizing milk production. Glycerol's rapid fermentation also influences the rumen pH, which can have both positive and negative effects. On one hand, the production of propionate with relatively low methane production can improve feed efficiency and reduce the environmental impact of dairy farming. On the other hand, excessive glycerol supplementation can lead to an accumulation of VFAs, resulting in a drop in rumen pH and the potential development of rumen acidosis [46]. Rumen acidosis can disrupt microbial populations, leading to a decrease in fiber digestion and overall feed efficiency. To mitigate the risk of rumen acidosis, it is essential to balance glycerol supplementation with other dietary components, such as fiber, which helps to buffer rumen pH. Additionally, the form and purity of glycerol used in dairy diets can influence its fermentation characteristics and overall impact on rumen health [47].

The use of glycerol in dairy nutrition has broader implications for animal health and welfare, particularly in terms of metabolic health, reproductive performance, and disease prevention. By improving energy balance and reducing the incidence of metabolic disorders such as ketosis, glycerol supplementation can enhance the overall well-being of dairy cows, leading to improved productivity and longevity. Metabolic disorders are a significant concern in the dairy industry, as they can lead to a cascade of health issues, including reduced milk yield, impaired reproductive performance, and increased susceptibility to infectious diseases [48]. Ketosis, in particular, is associated with a higher risk of displaced abomasum, mastitis, and reduced fertility. By preventing ketosis through glycerol supplementation, dairy producers can improve the overall health and welfare of their herds, leading to better reproductive outcomes and reduced veterinary costs. In addition to its role in preventing metabolic disorders, glycerol supplementation can also support immune function and disease resistance [49]. The energy provided by glycerol is not only essential for milk production but also for the maintenance of immune function. During periods of NEB, cows are more susceptible to infections such as mastitis due to the diversion of energy away from immune responses and towards milk production. By improving energy balance, glycerol can help to sustain immune function and reduce the incidence of infectious diseases. Furthermore, glycerol's impact on reproductive performance is of particular importance in the dairy industry, where fertility is a key determinant of profitability [8]. Energy deficits during early lactation can lead to delayed ovulation, poor conception rates, and increased days open, all of which negatively impact reproductive efficiency. Glycerol supplementation has been shown to improve reproductive performance by supporting energy balance and reducing the metabolic stress associated with early lactation. This, in turn, leads to more timely and successful breeding, shorter calving intervals, and increased lifetime productivity of the cow [32].

Another aspect of animal welfare that is influenced by glycerol supplementation is the reduction of stress associated with NEB. Cows experiencing severe energy deficits are more likely to exhibit signs of distress, including decreased feed intake, weight loss, and changes in behavior. By alleviating the energy deficit through glycerol supplementation, cows are better able to maintain normal feeding behavior, body condition, and overall well-being [50,51]. It is also important to consider the environmental implications of glycerol usage in dairy nutrition. The inclusion of glycerol in diets has been associated with reduced methane production during rumen fermentation, which is beneficial from an environmental perspective [52]. Methane is a potent greenhouse gas, and reducing its production in the dairy industry is a key goal for improving sustainability. By promoting the production of propionate over acetate and methane, glycerol supplementation can contribute to a more environmentally friendly dairy industry [8].

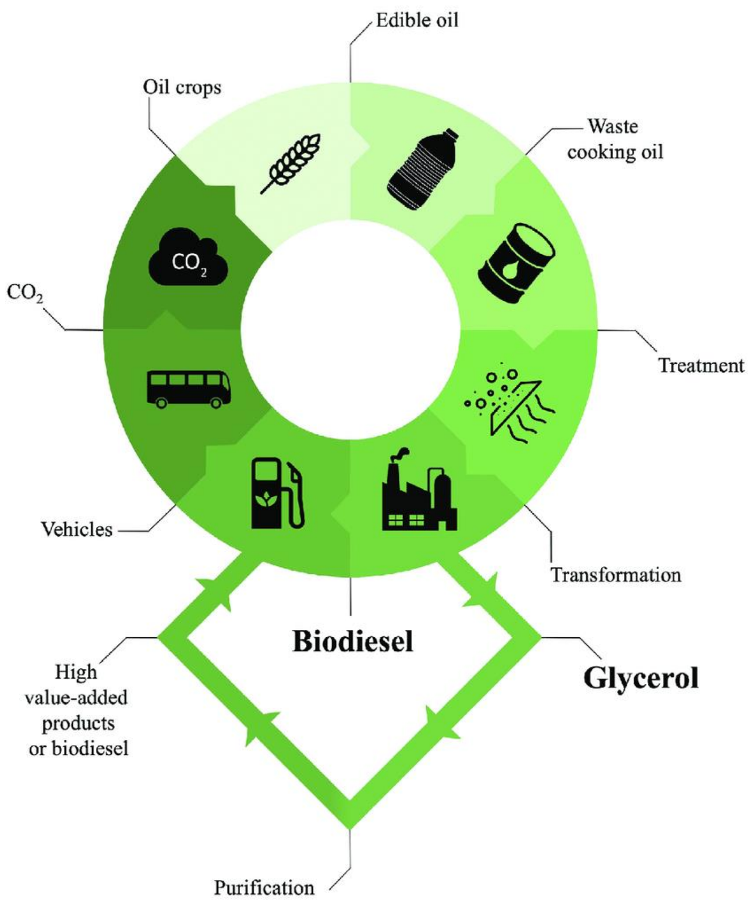
Economic considerations also play a role in the adoption of glycerol in dairy nutrition. While glycerol is often a cost-effective energy source, its price can fluctuate based on the biodiesel market. Producers must weigh the benefits of glycerol supplementation against its cost and consider alternative energy sources if glycerol becomes prohibitively expensive [8]. Lastly, while short-term studies have demonstrated the benefits of glycerol supplementation, there is a need for long-term research to fully understand its effects on cow health, productivity, and welfare over multiple lactations. This research will help to refine dosing strategies, identify potential interactions with other dietary ingredients, and ensure the sustainable use of glycerol in dairy nutrition.

6. The environmental impact of glycerol usage in dairy nutrition

Glycerol, a byproduct of biodiesel production, has gained attention in dairy nutrition due to its potential benefits as an energy source for cows, particularly during periods of negative energy balance. However, beyond its immediate benefits in dairy production, glycerol usage also carries significant environmental implications. These implications are multifaceted, involving the lifecycle of glycerol production (Picture 3), its influence on greenhouse gas emissions from dairy operations, and its role in waste management and resource efficiency [53].

This section delves into these environmental aspects, exploring how the use of glycerol in dairy nutrition can contribute to or mitigate environmental impacts associated with modern dairy farming.

Glycerol's environmental impact begins with its production, primarily as a byproduct of biodiesel manufacturing [55]. The biodiesel industry generates large quantities of crude glycerol, which must be refined before it can be used in animal nutrition. The production process of biodiesel itself has various environmental implications, depending on the feedstock used, the energy intensity of the production process, and the management of byproducts like glycerol. The environmental footprint of glycerol production is closely tied to the type of feedstock used in biodiesel production [56]. Biodiesel can be produced from a variety of feedstocks, including vegetable oils (such as soybean or canola), animal fats, and waste cooking oils. The choice of feedstock influences the overall carbon footprint of the glycerol produced. For instance, biodiesel from waste cooking oils typically has a lower carbon footprint compared to biodiesel from virgin vegetable oils, as it repurposes waste materials that would otherwise require disposal.



Picture 3. Lifecycle of glycerol during oils transformation into biodiesel [54].

The refining of crude glycerol to make it suitable for animal feed also carries environmental costs. The refining process involves removing impurities such as methanol, salts, and fatty acids, which can be energy-intensive and generate waste byproducts. However, the environmental impact of refining must be weighed against the benefits of using glycerol as an energy source in dairy nutrition, which can potentially reduce the reliance on other feed ingredients that may have a higher environmental footprint. Another aspect of glycerol's lifecycle impact is its transportation from production sites to dairy farms. Transportation contributes to the overall carbon footprint of glycerol, particularly if long distances are involved [57]. The environmental benefits of using glycerol in dairy nutrition must, therefore, be assessed in the context of its entire supply chain, from production and refining to transportation and final usage on farms.

One of the most significant environmental aspects of glycerol usage in dairy nutrition is its influence on greenhouse gas (GHG) emissions from dairy farming [58]. Methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O) are the primary greenhouse gases associated with dairy production, with methane being particularly important due to its high global warming potential. Methane emissions from dairy cows are largely a result of enteric fermentation in the rumen, where microbes break down feedstuffs and produce methane as a byproduct [59]. The composition of the diet plays a crucial role in determining the amount of methane produced. Diets high in fiber, for example, tend to produce more methane, while those with higher concentrations of easily fermentable carbohydrates, such as glycerol, can reduce methane production. Glycerol, when included in dairy diets, is rapidly fermented in the rumen, leading to an increase in the production of propionate, a VFA that serves as a glucogenic precursor [46]. The fermentation of glycerol results in less methane production compared to the fermentation of more fibrous feed components like cellulose. This is because propionate production is associated with a lower hydrogen yield, which in turn reduces the substrate available for methanogenesis (the production of methane by methanogenic archaea). Studies have shown that glycerol supplementation can lead to a reduction in enteric methane emissions from dairy cows [60]. This reduction is not only beneficial for the environment but also improves feed efficiency, as less energy is lost as methane. By diverting hydrogen away from methanogenesis and towards the production of more energy-efficient VFAs like propionate, glycerol enhances the overall energy utilization of the cow [46]. In addition to reducing methane emissions, glycerol can also influence the carbon footprint of dairy farming by potentially decreasing the need for other feed ingredients with higher environmental impacts. For example, if glycerol can replace a portion of high-energy feeds like corn or grain, which require significant land, water, and energy resources to produce, the overall carbon footprint of dairy farming could be reduced. This substitution effect is particularly relevant in regions where feed crops are associated with deforestation, habitat loss, or other environmental concerns. However, the extent to which glycerol can reduce the carbon footprint of dairy farming depends on several factors, including the level of supplementation, the specific diet formulation, and the overall management practices on the farm. Careful consideration must be given to balancing glycerol supplementation with other dietary components to optimize both environmental and production outcomes [61].

The use of glycerol in dairy nutrition also intersects with broader issues of waste management and resource efficiency, particularly in the context of the biodiesel industry [62]. As a byproduct of biodiesel production, glycerol presents a challenge in terms of disposal and utilization. Historically, the surplus of crude glycerol has led to concerns about its disposal, with options such as landfilling or incineration being environmentally undesirable. Incorporating glycerol into animal feed, including dairy nutrition, provides a valuable outlet for this byproduct, turning a potential waste stream into a resource. This practice aligns with the principles of circular economy, where waste products are repurposed and reintegrated into production systems, thereby reducing the overall environmental impact. By using glycerol as a feed ingredient, the dairy industry helps to absorb some of the surplus glycerol generated by the biodiesel industry, mitigating the need for environmentally harmful disposal methods [57]. This not only benefits the environment by reducing waste but also adds value to the biodiesel production process, making it more economically viable and sustainable. Moreover, the use of glycerol in dairy nutrition can contribute to more efficient resource utilization within the broader agricultural sector. For instance, if glycerol can replace a portion of other high-energy feed ingredients, it can potentially reduce the demand for these crops, which often require significant inputs of water, fertilizer, and land. This reduction in demand can alleviate some of the environmental pressures associated with intensive crop production, such as soil degradation, water pollution from agricultural runoff, and loss of biodiversity. However, it is important to consider the environmental trade-offs associated with glycerol use [63]. While it offers benefits in terms of waste management and resource efficiency, the environmental impact of its production and refining process must also be accounted for. Ensuring that glycerol is sourced from sustainable and environmentally responsible biodiesel production processes is key to maximizing its environmental benefits.

While glycerol has the potential to offer significant environmental benefits in dairy nutrition, its use is not without risks. One of the primary environmental concerns associated with glycerol supplementation is the risk of rumen acidosis, particularly when glycerol is included at high levels in the diet. Rumen acidosis occurs when the pH of the rumen drops due to the rapid fermentation of carbohydrates, leading to the accumulation of VFAs. This condition can negatively impact cow health, reduce feed efficiency, and increase the excretion of undigested nutrients, which can contribute to environmental pollution [63]. To mitigate the risk of rumen acidosis, it is essential to carefully manage the inclusion rate of glycerol in dairy diets. Research has shown that moderate levels of glycerol supplementation can be safely incorporated into dairy rations without adversely affecting rumen health. However, excessive levels can lead to a rapid drop in rumen pH, necessitating careful monitoring and adjustment of the diet to ensure that fiber intake remains adequate to buffer rumen acidity. Another potential environmental risk associated with glycerol usage is the impact of impurities in crude glycerol. Crude glycerol from biodiesel production can contain contaminants such as methanol, salts, and fatty acids, which may pose risks to both animal health and the environment if not properly managed [64]. Methanol, in particular, is toxic and can cause health issues in cows if present in significant quantities. To address this risk, it is crucial to ensure that glycerol used in dairy nutrition is of high quality and has been adequately refined to remove harmful impurities. The development of industry standards for glycerol purity can help to ensure that only safe and environmentally friendly glycerol is used in animal feed. Additionally, ongoing research into the effects of different impurities on both cow health and environmental outcomes will be important for optimizing the use of glycerol in dairy nutrition. The environmental impact of glycerol supplementation also extends to manure management. The composition of manure, including its nutrient content and the rate at which it is excreted, is influenced by the cow's diet. Diets high in fermentable carbohydrates, such as glycerol, can alter the nitrogen content and pH of manure, which in turn affects its potential for environmental pollution when applied as fertilizer. Managing the dietary balance and ensuring proper manure handling practices are essential for minimizing the environmental impact of manure application, particularly in terms of nitrogen runoff and greenhouse gas emissions [2].

The environmental impact of glycerol usage in dairy nutrition must be considered within the broader context of sustainable dairy farming practices. Sustainable dairy farming involves a holistic approach to managing resources, minimizing waste, and reducing environmental impacts while maintaining high levels of productivity and animal welfare [65]. Glycerol supplementation can be integrated into sustainable dairy farming practices in several ways. First, it can be part of a broader strategy to reduce the carbon footprint of dairy production by improving feed efficiency and reducing methane emissions. By optimizing the energy utilization of dairy cows and reducing methane production, glycerol contributes to more sustainable and environmentally friendly dairy operations. Second, glycerol usage aligns with sustainable waste management practices by repurposing a byproduct of the biodiesel industry that would otherwise require disposal. This integration of waste products into animal feed systems exemplifies the principles of circular economy and resource efficiency, key components of sustainable agriculture. Third, glycerol can be part of a diversified approach to dairy nutrition that includes the use of alternative feed ingredients, such as byproducts from other industries (e.g., distillers grains, beet pulp). By incorporating a variety of feed ingredients, dairy producers can reduce reliance on conventional feed crops, which are often associated with high environmental costs, and create more resilient and sustainable feeding systems.

Finally, the use of glycerol in dairy nutrition can support the development of more sustainable dairy farming practices by contributing to improved animal health and welfare [66]. Healthier cows are more productive and require fewer inputs in terms of veterinary care and supplemental feeding, which in turn reduces the environmental impact of dairy farming [20]. By preventing metabolic disorders such as ketosis, glycerol supplementation helps to maintain the overall health and productivity of the herd, supporting the long-term sustainability of dairy operations.

7. Conclusion

In conclusion, the exploration of glycerol usage in dairy cow nutrition presents a promising avenue for enhancing milk production efficiency, improving animal health, and contributing to agricultural sustainability. As the dairy industry continues to evolve, integrating alternative feed additives like glycerol can play a crucial role in meeting the growing demands for dairy products while adhering to principles of sustainability and animal welfare. This paper contributes to the ongoing discourse by providing a detailed analysis of the current knowledge and potential future directions for glycerol in dairy nutrition.

The inclusion of glycerol in dairy nutrition has demonstrated significant potential in improving energy balance, preventing metabolic disorders, and enhancing milk production in dairy cows. By supporting glucose metabolism, glycerol helps to mitigate the effects of negative energy balance, reduce the risk of ketosis, and support overall cow health and welfare. However, its usage must be carefully managed to avoid potential negative outcomes, and further research is needed to fully understand its long-term implications for dairy production. With the right strategies, glycerol can be a valuable tool in the ongoing effort to optimize dairy nutrition and improve the sustainability of the dairy industry.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Akintan, O.; Gebremedhin, K.G.; Uyeh, D.D. Animal Feed Formulation—Connecting Technologies to Build a Resilient and Sustainable System. *Animals* **2024**, *14*, 1497, doi:10.3390/ani14101497.
2. Džermeikaitė, K.; Krištolaitytė, J.; Antanaitis, R. Relationship between Dairy Cow Health and Intensity of Greenhouse Gas Emissions. *Animals* **2024**, *14*, 829, doi:10.3390/ani14060829.
3. Tufarelli, V.; Colonna, M.A.; Losacco, C.; Puvača, N. Biological Health Markers Associated with Oxidative Stress in Dairy Cows during Lactation Period. *Metabolites* **2023**, *13*, 405, doi:10.3390/metabo13030405.
4. Tedeschi, L.O.; Muir, J.P.; Naumann, H.D.; Norris, A.B.; Ramírez-Restrepo, C.A.; Mertens-Talcott, S.U. Nutritional Aspects of Ecologically Relevant Phytochemicals in Ruminant Production. *Front. Vet. Sci.* **2021**, *8*, doi:10.3389/fvets.2021.628445.
5. Negrón-Pérez, V.M.; Fausnacht, D.W.; Rhoads, M.L. *Invited Review:* Management Strategies Capable of Improving the Reproductive Performance of Heat-Stressed Dairy Cattle. *Journal of Dairy Science* **2019**, *102*, 10695–10710, doi:10.3168/jds.2019-16718.
6. Kholif, A.E. Glycerol Use in Dairy Diets: A Systemic Review. *Animal Nutrition* **2019**, *5*, 209–216, doi:10.1016/j.aninu.2019.06.002.
7. DeFrain, J.M.; Hippen, A.R.; Kalscheur, K.F.; Jardon, P.W. Feeding Glycerol to Transition Dairy Cows: Effects on Blood Metabolites and Lactation Performance. *Journal of Dairy Science* **2004**, *87*, 4195–4206, doi:10.3168/jds.S0022-0302(04)73564-X.
8. Brkić, I.; Puvača, N. Economic and Ecological Sustainability of Dairy Production. *J Agron Technol Eng Manag* **2024**, *7*, 1088–1104, doi:10.55817/TQXU2115.
9. Samudrala, S.P. Glycerol Transformation to Value-Added 1,3-Propanediol Production: A Paradigm for a Sustainable Biorefinery Process. In *Glycerine Production and Transformation - An Innovative Platform for Sustainable Biorefinery and Energy*; IntechOpen, 2019 ISBN 978-1-78984-691-1.
10. Kupczyński, R.; Szumny, A.; Wujcikowska, K.; Pachura, N. Metabolism, Ketosis Treatment and Milk Production after Using Glycerol in Dairy Cows: A Review. *Animals* **2020**, *10*, 1379, doi:10.3390/ani10081379.
11. Boyd, J.; West, J.W.; Bernard, J.K. Effects of the Addition of Direct-Fed Microbials and Glycerol to the Diet of Lactating Dairy Cows on Milk Yield and Apparent Efficiency of Yield. *Journal of Dairy Science* **2011**, *94*, 4616–4622, doi:10.3168/jds.2010-3984.
12. Sammad, A.; Wang, Y.J.; Umer, S.; Lirong, H.; Khan, I.; Khan, A.; Ahmad, B.; Wang, Y. Nutritional Physiology and Biochemistry of Dairy Cattle under the Influence of Heat Stress: Consequences and Opportunities. *Animals* **2020**, *10*, 793, doi:10.3390/ani10050793.
13. Piantoni, P.; Allen, M.S. Evaluation of Propylene Glycol and Glycerol Infusions as Treatments for Ketosis in Dairy Cows. *Journal of Dairy Science* **2015**, *98*, 5429–5439, doi:10.3168/jds.2015-9476.

14. Leoneti, A.B.; Aragão-Leoneti, V.; de Oliveira, S.V.W.B. Glycerol as a By-Product of Biodiesel Production in Brazil: Alternatives for the Use of Unrefined Glycerol. *Renewable Energy* **2012**, *45*, 138–145, doi:10.1016/j.renene.2012.02.032.
15. Vapa, T.J.; Puvača, N.; Giannenas, I.; Tufarelli, V.; Ignjatijević, S. Food Safety Policy in the European Union. *Journal of Agronomy, Technology and Engineering Management* 2022.
16. Donkin, S.S.; Koser, S.L.; White, H.M.; Doane, P.H.; Cecava, M.J. Feeding Value of Glycerol as a Replacement for Corn Grain in Rations Fed to Lactating Dairy Cows. *Journal of Dairy Science* **2009**, *92*, 5111–5119, doi:10.3168/jds.2009-2201.
17. He, Q. (Sophia); McNutt, J.; Yang, J. Utilization of the Residual Glycerol from Biodiesel Production for Renewable Energy Generation. *Renewable and Sustainable Energy Reviews* **2017**, *71*, 63–76, doi:10.1016/j.rser.2016.12.110.
18. Moklis, M.H.; Cheng, S.; Cross, J.S. Current and Future Trends for Crude Glycerol Upgrading to High Value-Added Products. *Sustainability* **2023**, *15*, 2979, doi:10.3390/su15042979.
19. Bezerra, H.F.C.; Santos, E.M.; de Carvalho, G.G.P.; de Oliveira, J.S.; da Silva, F.F.; Cassuce, M.R.; Guerra, R.R.; Pereira, D.M.; Ferreira, D. de J.; Nascimento, T.V.C.; et al. Metabolic Profile of Goats Fed Diets Containing Crude Glycerin from Biodiesel Production. *Front. Vet. Sci.* **2023**, *10*, doi:10.3389/fvets.2023.1236542.
20. Tufarelli, V.; Puvača, N.; Glamočić, D.; Pugliese, G.; Colonna, M.A. The Most Important Metabolic Diseases in Dairy Cattle during the Transition Period. *Animals* **2024**, *14*, 816, doi:10.3390/ani14050816.
21. Qiao, K.; Jiang, R.; Contreras, G.A.; Xie, L.; Pascottini, O.B.; Opsomer, G.; Dong, Q. The Complex Interplay of Insulin Resistance and Metabolic Inflammation in Transition Dairy Cows. *Animals* **2024**, *14*, 832, doi:10.3390/ani14060832.
22. Ye, G.; Liu, J.; Liu, Y.; Chen, X.; Liao, S.F.; Huang, D.; Huang, K. Feeding Glycerol-Enriched Yeast Culture Improves Lactation Performance, Energy Status, and Hepatic Gluconeogenic Enzyme Expression of Dairy Cows during the Transition Period1. *Journal of Animal Science* **2016**, *94*, 2441–2450, doi:10.2527/jas.2015-0021.
23. Akram, M. A Focused Review of the Role of Ketone Bodies in Health and Disease. *Journal of Medicinal Food* **2013**, *16*, 965–967, doi:10.1089/jmf.2012.2592.
24. Abo El-Nor, S.; AbuGhazaleh, A.A.; Potu, R.B.; Hastings, D.; Khattab, M.S.A. Effects of Differing Levels of Glycerol on Rumen Fermentation and Bacteria. *Animal Feed Science and Technology* **2010**, *162*, 99–105, doi:10.1016/j.anifeedsci.2010.09.012.
25. Trabue, S.; Scoggin, K.; Tjandrakusuma, S.; Rasmussen, M.A.; Reilly, P.J. Ruminal Fermentation of Propylene Glycol and Glycerol. *J. Agric. Food Chem.* **2007**, *55*, 7043–7051, doi:10.1021/jf071076i.
26. Wang, C.; Liu, Q.; Huo, W.J.; Yang, W.Z.; Dong, K.H.; Huang, Y.X.; Guo, G. Effects of Glycerol on Rumen Fermentation, Urinary Excretion of Purine Derivatives and Feed Digestibility in Steers. *Livestock Science* **2009**, *121*, 15–20, doi:10.1016/j.livsci.2008.05.010.
27. Paal, D.; Strejcek, F.; Slanina, T.; Kovacova, R.; Lukac, N.; Qoja, A.O.; Ondruska, L.; Formicki, G.; Tvrda, E.; Massanyi, P. Crude Glycerol Negatively Affects Rabbit Spermatozoa Motility in Vitro. *J Hellenic Vet Med Soc* **2018**, *67*, 223, doi:10.12681/jhvms.15642.
28. Tan, H.W.; Abdul Aziz, A.R.; Aroua, M.K. Glycerol Production and Its Applications as a Raw Material: A Review. *Renewable and Sustainable Energy Reviews* **2013**, *27*, 118–127, doi:10.1016/j.rser.2013.06.035.

29. Wu, G. Management of Metabolic Disorders (Including Metabolic Diseases) in Ruminant and Nonruminant Animals. In *Animal Agriculture*; Bazer, F.W., Lamb, G.C., Wu, G., Eds.; Academic Press, 2020; pp. 471–491 ISBN 978-0-12-817052-6.
30. Remesar, X.; Alemany, M. Dietary Energy Partition: The Central Role of Glucose. *International Journal of Molecular Sciences* **2020**, *21*, 7729, doi:10.3390/ijms21207729.
31. Osborne, V.R.; Odongo, N.E.; Cant, J.P.; Swanson, K.C.; McBride, B.W. Effects of Supplementing Glycerol and Soybean Oil in Drinking Water on Feed and Water Intake, Energy Balance, and Production Performance of Periparturient Dairy Cows. *Journal of Dairy Science* **2009**, *92*, 698–707, doi:10.3168/jds.2008-1554.
32. Van Soest, B.J.; Pereira, M.N.; Duffield, T.F.; Steele, M.A.; DeVries, T.J. Effect of Pre- and Postpartum Supplementation of a Pure Glycerol Product to Dairy Cows on Feed Intake, Metabolic Markers, and Milk Yield and Components. *Journal of Dairy Science* **2023**, *106*, 6798–6815, doi:10.3168/jds.2022-23173.
33. Lomander, H.; Frössling, J.; Ingvartsen, K.L.; Gustafsson, H.; Svensson, C. Supplemental Feeding with Glycerol or Propylene Glycol of Dairy Cows in Early Lactation—Effects on Metabolic Status, Body Condition, and Milk Yield. *Journal of Dairy Science* **2012**, *95*, 2397–2408, doi:10.3168/jds.2011-4535.
34. Lei, M.A.C.; Simões, J. Invited Review: Ketosis Diagnosis and Monitoring in High-Producing Dairy Cows. *Dairy* **2021**, *2*, 303–325, doi:10.3390/dairy2020025.
35. Mann, S.; Yepes, F.A.L.; Overton, T.R.; Wakshlag, J.J.; Lock, A.L.; Ryan, C.M.; Nydam, D.V. Dry Period Plane of Energy: Effects on Feed Intake, Energy Balance, Milk Production, and Composition in Transition Dairy Cows. *Journal of Dairy Science* **2015**, *98*, 3366–3382, doi:10.3168/jds.2014-9024.
36. Wang, C.; Liu, Q.; Yang, W.Z.; Huo, W.J.; Dong, K.H.; Huang, Y.X.; Yang, X.M.; He, D.C. Effects of Glycerol on Lactation Performance, Energy Balance and Metabolites in Early Lactation Holstein Dairy Cows. *Animal Feed Science and Technology* **2009**, *151*, 12–20, doi:10.1016/j.anifeedsci.2008.10.009.
37. Ferraro, S.M.; Mendoza, G.D.; Miranda, L.A.; Gutiérrez, C.G. *In Vitro* Gas Production and Ruminal Fermentation of Glycerol, Propylene Glycol and Molasses. *Animal Feed Science and Technology* **2009**, *154*, 112–118, doi:10.1016/j.anifeedsci.2009.07.009.
38. Kalemba, K.M.; Wang, Y.; Xu, H.; Chiles, E.; McMillin, S.M.; Kwon, H.; Su, X.; Wondisford, F.E. Glycerol Induces G6pc in Primary Mouse Hepatocytes and Is the Preferred Substrate for Gluconeogenesis Both in Vitro and in Vivo. *Journal of Biological Chemistry* **2019**, *294*, 18017–18028, doi:10.1074/jbc.RA119.011033.
39. Doi, Y. Glycerol Metabolism and Its Regulation in Lactic Acid Bacteria. *Appl Microbiol Biotechnol* **2019**, *103*, 5079–5093, doi:10.1007/s00253-019-09830-y.
40. Rotondo, F.; Ho-Palma, A.C.; Remesar, X.; Fernández-López, J.A.; Romero, M. del M.; Alemany, M. Glycerol Is Synthesized and Secreted by Adipocytes to Dispose of Excess Glucose, via Glycerogenesis and Increased Acyl-Glycerol Turnover. *Sci Rep* **2017**, *7*, 8983, doi:10.1038/s41598-017-09450-4.
41. Guo, J.; Peters, R.R.; Kohn, R.A. Effect of a Transition Diet on Production Performance and Metabolism in Periparturient Dairy Cows. *Journal of Dairy Science* **2007**, *90*, 5247–5258, doi:10.3168/jds.2007-0326.

42. Larsen, M.; Kristensen, N.B. Precursors for Liver Gluconeogenesis in Periparturient Dairy Cows. *animal* **2013**, *7*, 1640–1650, doi:10.1017/S1751731113001171.
43. Kostadinović, L.; Lević, J. Effects of Phytoadditives in Poultry and Pigs Diseases. *J Agron Technol Eng Manag* **2018**, *1*, 1–7.
44. Kostadinović, L. Hydroponic Feed and Quality in Sustainable Dairy Animal Production. *J Agron Technol Eng Manag* **2023**, *6*, 965–974, doi:10.55817/YSNF9052.
45. Carenzi, C.; Verga, M. Animal Welfare: Review of the Scientific Concept and Definition. *Italian Journal of Animal Science* **2009**, *8*, 21–30, doi:10.4081/ijas.2009.s1.21.
46. Wang, L.; Zhang, G.; Li, Y.; Zhang, Y. Effects of High Forage/Concentrate Diet on Volatile Fatty Acid Production and the Microorganisms Involved in VFA Production in Cow Rumen. *Animals* **2020**, *10*, 223, doi:10.3390/ani10020223.
47. Syahniar, T.M.; Andriani, M.; Ridla, M.; Laconi, E.B.; Nahrowi, N.; Jayanegara, A. Glycerine as a Feed Supplement for Beef and Dairy Cattle: A Meta-Analysis on Performance, Rumen Fermentation, Blood Metabolites and Product Characteristics. *Journal of Animal Physiology and Animal Nutrition* **2021**, *105*, 419–430, doi:10.1111/jpn.13468.
48. Bernabucci, U.; Ronchi, B.; Lacetera, N.; Nardone, A. Influence of Body Condition Score on Relationships Between Metabolic Status and Oxidative Stress in Periparturient Dairy Cows*. *Journal of Dairy Science* **2005**, *88*, 2017–2026, doi:10.3168/jds.S0022-0302(05)72878-2.
49. Obućinski, D.; Soleša, D.; Kučević, D.; Prodanović, R.; Simin, M.T.; Pelić, D.L.; Đuragić, O.; Puvača, N. Management of Blood Lipid Profile and Oxidative Status in Holstein and Simmental Dairy Cows during Lactation. *Mljekarstvo* **2019**, *69*, 116–124.
50. Kitkas (Γ.Χ. Κιτκας), G.C.; Panousis (Ν. Πανουσης), N.; Valergakis (Γ.Ε. Βαλεργακης), G.E.; Karatzias (Χ. Καρατζιας), Ch. Subacute Ruminai Acidosis in Dairy Cows. *J Hellenic Vet Med Soc* **2017**, *62*, 352, doi:10.12681/jhvms.14867.
51. Kitkas, G.C.; Valergakis, G.E.; Kritsepi-Konstantinou, M.; Gelasakis, A.I.; Arsenos, G.; Kalaitzakis, E.; Panousis, N. Effects of Ruminal pH and Subacute Ruminal Acidosis on Milk Yield and Composition of Holstein Cows in Different Stages of Lactation. *J Hellenic Vet Med Soc* **2019**, *70*, 1551, doi:10.12681/jhvms.20861.
52. Mladenovic, V.; Maric, M.; Könyves, T.; Stojsin, M.; Đordevic, S.; Puvaca, N.; Halász, A.; Papadopoulos, E. Comparison of Hematological and Biochemical Profile between Podolian Grey Steppe and Holstein Cows. *J Hellenic Vet Med Soc* **2021**, *71*, 2445, doi:10.12681/jhvms.25918.
53. Chowdhury, R.; Freire, F. Bioenergy Production from Algae Using Dairy Manure as a Nutrient Source: Life Cycle Energy and Greenhouse Gas Emission Analysis. *Applied Energy* **2015**, *154*, 1112–1121, doi:10.1016/j.apenergy.2015.05.045.
54. Decarpigny, C.; Aljawish, A.; His, C.; Fertin, B.; Bigan, M.; Dhulster, P.; Millares, M.; Froidevaux, R. Bioprocesses for the Biodiesel Production from Waste Oils and Valorization of Glycerol. *Energies* **2022**, *15*, 3381, doi:10.3390/en15093381.
55. Monteiro, M.R.; Kugelmeier, C.L.; Pinheiro, R.S.; Batalha, M.O.; da Silva César, A. Glycerol from Biodiesel Production: Technological Paths for Sustainability. *Renewable and Sustainable Energy Reviews* **2018**, *88*, 109–122, doi:10.1016/j.rser.2018.02.019.
56. Callegari, A.; Bolognesi, S.; Cecconet, D.; Capodaglio, A.G. Production Technologies, Current Role, and Future Prospects of Biofuels Feedstocks: A State-of-the-Art Review. *Critical Reviews in Environmental Science and Technology* **2020**, *50*, 384–436, doi:10.1080/10643389.2019.1629801.

57. Rodrigues, A.; Bordado, J.C.; Santos, R.G. dos Upgrading the Glycerol from Biodiesel Production as a Source of Energy Carriers and Chemicals—A Technological Review for Three Chemical Pathways. *Energies* **2017**, *10*, 1817, doi:10.3390/en10111817.
58. Karlsson, J.; Ramin, M.; Kass, M.; Lindberg, M.; Holtenius, K. Effects of Replacing Wheat Starch with Glycerol on Methane Emissions, Milk Production, and Feed Efficiency in Dairy Cows Fed Grass Silage-Based Diets. *Journal of Dairy Science* **2019**, *102*, 7927–7935, doi:10.3168/jds.2018-15629.
59. Stepanchenko, N.; Stefenoni, H.; Hennessy, M.; Nagaraju, I.; Wasson, D.E.; Cueva, S.F.; Räisänen, S.E.; Dechow, C.D.; Pitta, D.W.; Hristov, A.N. Microbial Composition, Rumen Fermentation Parameters, Enteric Methane Emissions, and Lactational Performance of Phenotypically High and Low Methane-Emitting Dairy Cows. *Journal of Dairy Science* **2023**, *106*, 6146–6170, doi:10.3168/jds.2022-23190.
60. Appuhamy, J.A.D.R.N.; France, J.; Kebreab, E. Models for Predicting Enteric Methane Emissions from Dairy Cows in North America, Europe, and Australia and New Zealand. *Global Change Biology* **2016**, *22*, 3039–3056, doi:10.1111/gcb.13339.
61. Silva, T.S.; Matos, E.; Cordeiro, O.D.; Colen, R.; Wulff, T.; Sampaio, E.; Sousa, V.; Valente, L.M.P.; Gonçalves, A.; Silva, J.M.G.; et al. Dietary Tools To Modulate Glycogen Storage in Gilthead Seabream Muscle: Glycerol Supplementation. *J. Agric. Food Chem.* **2012**, *60*, 10613–10624, doi:10.1021/jf3023244.
62. Venkata Mohan, S.; Dahiya, S.; Amulya, K.; Katakojwala, R.; Vanitha, T.K. Can Circular Bioeconomy Be Fueled by Waste Biorefineries — A Closer Look. *Bioresource Technology Reports* **2019**, *7*, 100277, doi:10.1016/j.biteb.2019.100277.
63. Strucko, T.; Zirngibl, K.; Pereira, F.; Kafkia, E.; Mohamed, E.T.; Rettel, M.; Stein, F.; Feist, A.M.; Jouhten, P.; Patil, K.R.; et al. Laboratory Evolution Reveals Regulatory and Metabolic Trade-Offs of Glycerol Utilization in *Saccharomyces Cerevisiae*. *Metabolic Engineering* **2018**, *47*, 73–82, doi:10.1016/j.ymben.2018.03.006.
64. Chilakamarry, C.R.; Sakinah, A.M.M.; Zularisam, A.W.; Pandey, A. Glycerol Waste to Value Added Products and Its Potential Applications. *Syst Microbiol and Biomanuf* **2021**, *1*, 378–396, doi:10.1007/s43393-021-00036-w.
65. Shamsuddoha, M.; Nasir, T.; Hossain, N.U.I. A Sustainable Supply Chain Framework for Dairy Farming Operations: A System Dynamics Approach. *Sustainability* **2023**, *15*, 8417, doi:10.3390/su15108417.
66. Makkar, H.P.S. Animal Nutrition in a 360-Degree View and a Framework for Future R&D Work: Towards Sustainable Livestock Production. *Anim. Prod. Sci.* **2016**, *56*, 1561–1568, doi:10.1071/AN15265.