Evaluating the effects of Vitamin D supplementation on Uterine Health and Reproductive performance in Postpartum Dairy Cows

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LIST OF ABBREVIATIONS

Abbreviation	Full Form
25(OH)D3	25-Hydroxyvitamin D3 (Calcidiol)
BCS	Body Condition Score
BHB	Beta-Hydroxybutyrate
Ca	Calcium
CRP	C-Reactive Protein
DIM	Days in Milk
EU	European Union
GP6/GP9	Glycoprotein VI / Glycoprotein IX
IgG	Immunoglobulin G
IL-6	Interleukin 6
LBP	Lipopolysaccharide Binding Protein
MDA	Malondialdehyde
NEFA	Non-Esterified Fatty Acids
NOS	Newcastle-Ottawa Scale
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RCT	Randomized Controlled Trial
RoB	Risk of Bias
SYRCLE	Systematic Review Centre for Laboratory Animal Experimentation
T-AOC	Total Antioxidant Capacity
TNF-α	Tumor Necrosis Factor Alpha
USA	United States of America
VD	Vitamin D
VDR	Vitamin D Receptor

Contents

CHAPTER 1

INTRODUCTION

1.1 Background and Context

The transition period, spanning three weeks before and three weeks after calving, represents one of the most physiologically stressful stages in a dairy cow's life. Within this period, the animal undergoes a significant change in hormonal activity as it adjusts to lactation, and its immune and metabolic activities are disrupted, in addition to recovering from the parturition process (Mezzetti et al., 2021). Such requirements put the cow at risk of numerous ailments such as placental retention, metritis, endometritis, subclinical hypocalcemia, and ketosis (Sammad et al., 2022). As many as 40 percent of the cows develop at least one clinical disease following birth, and many more have subclinical diseases that are not noticed but have effects on fertility (LeBlanc, 2023).

Infertile pregnancies at this stage are associated with significant economic losses, and the total direct and indirect costs are considered to be more than 500 per cow (Deka et al., 2021). It also causes high rates of culling, thereby raising the replacement costs of herds. Although there have been improved reproductive technologies and veterinary standards, postpartum uterine infection and extended days open have continued to pose a challenge to productive herd reproduction.

These risks are worsened by modern high-yield dairy systems of negative energy balance, immune suppression, and nutrient depletion. This multifactorial problem has to be addressed by integrative, low-cost solutions. In this regard, micronutrient interventions (so far, vitamin D supplementation) are becoming the focus of the research as they are used both as an immune and in reproductive physiology. Although vitamin D was once thought of as a primarily calcium-regulating component, it is currently recognized that it has systemic endocrine and immune effects, providing potential opportunities in enhancing postpartum health (Vieira-Neto et al., 2024).

1.2. Nutrition and Immunity in the Transition Period

The periparturient phase is defined by having very high metabolic requirements and a weakened immune system. There is a net negative energy balance experienced by cows because of the abrupt initiation of lactation, and this results in lipid mobilization and augmented generation of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) (Kotsampasi et al., 2024). These metabolites have also been cited to mediate the impairment of the functions of neutrophils and macrophages (Tessari et al., 2020), which are major parts of the uterine defense systems.

Moreover, subclinical hypocalcaemia is also common, going as high as 50 per cent of postpartum dairy cows, and it is associated with low muscle contractility (utero included) and a suppressed immune system (Arechiga-Flores et al., 2022). Immune dysfunction is further worsened by oxidative stress that causes prolonged inflammation and tissue repair.

Vitamin D (mostly in the form of the active metabolite 25-hydroxyvitamin D3 (25(OH)D3)) has been revealed to change immunity by tempering Toll-like receptor signaling, fortifying the phagocytic activity of macrophages, and opening the response of antimicrobial peptides. According to the study conducted by Vieira-Neto et al. (2017), the phagocytosis and the oxidative burst of neutrophils were enhanced among vitamin D-supplemented cows following calving. In addition, Amani et al. (2018) concluded that the inflammatory markers and antioxidant capacity were considerably reduced, and the serum $25(OH)D_3$ level was higher in the cows under study. Therefore, the possibility of vitamin D to counteract immune suppression and redress metabolic balance points to the context of the role of this compound as a dairy herd health preventive measure.

NRC (2001) suggests 21,000 IU/day of vitamin D3 to lactating cows. Nevertheless, real needs can be different with respect to breeds, lactation phase, and health conditions. The subnormal levels are usually considered as the circulating 25(OH)D3 levels less than 30 ng/mL, linked to immune-depressive and metabolism-related diseases (Nelson et al., 2016). Normal values are 30-60 ng/mL, and the supra-nutritional interventions raise the levels to above 80-100 ng/mL, which have been tested as immunomodulatory and fertility-enhancing (Vieira-Neto et al., 2017; Macmillan et al., 2023).

1.3. Role of Vitamin D in Dairy Cows

More recent research proposals have indicated that there are some possible physiological advantages of supplementation beyond homeostasis due to supplementation exceeding the NRC recommendations. Vieira-Neto et al. (2021) established that uterine health, inflammation, and calcium homeostasis were better in cows subjected to supra-nutritional doses of 25(OH)D3. Such effects are specifically applicable in transition cows, where immunosuppression and metabolic disproportion are associated with the risk of disease increase.

This is not aimed at substituting regular supplementation, but it is intended to test whether extra supplementation would provide some protective benefits during susceptible times. Macmillan et al. (2023) highlighted that cows receiving higher-than-standard vitamin D showed improved fertility outcomes, including lower days open and higher conception rates.

In ruminants, there are two major forms of vitamin D, cholecalciferol (vitamin D3), the inactive form, and 25-hydroxyvitamin D3 (25(OH)D3), the more active form. Although vitamin D traditionally has been known to relate to calcium-phosphorus metabolic pathways, its role is much more broad-reaching, acting on skeletal health. It is being considered increasingly as a hormone-like controller of cell proliferation, immune mode regulation, and endocrine processes (Ajik & Tahiluddin, 2023).

Colostrum secretion and fetal mineralization make the calcium needs of the postnatal period grave. The cows that fail to match these requirements are at considerable risk of falling victim to hypocalcemia and, consequently, putting at risk cows with low fertility rates and uterine infections (Yapa Hetti Pathirennehelage, 2022). Vitamin D supplements are known to have a good effect in reducing such risks. The example of radio has shown that Poindexter et al. (2023) have significantly improved the amount of serum calcium in cows fed 25(OH)D_3during the prepartum period but experienced a decrease in the incidence of retained placenta and metritis.

The mechanistic effect of vitamin D is the improvement of uterine and tissue repair by responding to the synthesis of prostaglandins and the calcium signaling cascade. The postpartum, as it was conducted with the use of vitamin D, as Jahn and Grahofer (2023) mentioned, was used to trigger the process of the uterus involution and suppress the inflammatory responses.

It additionally plays a part in the regulation of ovarian steroidogenesis and luteal activity maintenance and reproductive readiness.

Recent literature has suggested that overdosing on supplementation beyond that suggested by the NRC could have physiological benefits beyond mere homeostasis. Vieira-Neto et al. (2021) have shown that cows were healthier in their uterus and had reduced inflammation and increased calcium homeostasis with supra-nutritional doses of 25(OH)D3. These effects are particularly relevant in transition cows, where immune suppression and metabolic imbalance coincide with increased disease risk.

The goal is not to replace standard supplementation but to explore whether additional dosing could offer protective effects during vulnerable periods. Macmillan et al. (2023) highlighted that cows receiving higher-than-standard vitamin D showed improved fertility outcomes, including lower days open and higher conception rates.

1.4. Relevance to Uterine and Reproductive Health

Uterine health is critical to reproductive performance, with postpartum infections significantly delaying ovarian resumption and conception. As a case in point, endometritis has been linked to a lower pregnancy rate (30 per cent) during the first AI and a higher service per conception (Sheldon et al., 2006). Since vitamin D has immunomodulatory properties, it is gaining popularity as a deterrent or attenuating factor of specific uterine pathology.

Recently, Naramoto et al. (2025) demonstrated that cows exhibiting higher serum vitamin D showed lower expression of pro-inflammatory cytokines, including IL-18 and TNF-8, in the endometrial lining. Such cows had better endometrial integrity, more rapid uterine involution, and more rapid cyclist. This is likely to have been due to the combining effect of vitamin D on leukocyte functions, reducing the levels of oxidative stress and the tone of uterine muscles (Reddy et al., 2022).

Also positive effects of vitamin D supplementation are on reproductive parameters. Martinez et al. (2018) found that conception and calving to conception interval were significantly high and low in cows given a negative dietary cation-anion difference (DCAD) diet and given a vitamin D dietary supplement than in controls. Similarly, a study by Vieira-Neto et al. (2021) reported an increase of 150 d of 10-15% in the rate of pregnancy in the milk of cows fed with the supplement.

Vitamin D has an integrative effect on the reproductive physiology, which merges and cascades to the multifactorial mechanisms, including the positive impact on the uterine immunity, the metabolic response, and the hormone modulation (Datkhayeva et al., 2025). These findings suggest that in addition to being a supplement, vitamin D could be a major ingredient in ensuring that high-producing dairy herds achieve the best fertility performance.

1.5. Gaps in Literature and Justification for Review

Despite the positive findings of the research, the works on the vitamin D supplementation in dairy cows remain disjointed and cannot be discussed as unequivocal in a variety of significant respects. The first is that the action of vitamin D is hard to meta-analyze or even come up with standard procedures for in its administration, which is not consistently done in the form, dose, and route of administration that differ between dietary supplements of vitamin D3 and injectable 25(OH)D3 that are bolus in nature. Although 25(OH)D_3 is, on the whole, more bioavailable, there is little work that directly compares the effectiveness of 25(OH)D_3 with that of cholecalciferol in similar circumstances in the experiment.

Second, the duration and timing of supplementation vary widely across studies. Some focus on prepartum administration, while others explore early postpartum dosing. The lack of temporal consistency impairs our understanding of when vitamin D is most beneficial.

Moreover, many studies suffer from small sample sizes, inconsistent outcome measures, and lack of control for confounders such as parity, breed, body condition score, and environmental stress. These methodological limitations undermine internal validity and restrict generalizability. For instance, few studies stratify results by baseline vitamin D status, despite known variations due to geography and feeding practices (Wisnieski et al., 2020).

Another critical gap is the limited focus on long-term fertility outcomes. Most studies emphasize early postpartum parameters, neglecting metrics such as conception to second service, embryo loss, and culling risk.

Given these inconsistencies and limitations, a systematic literature review is essential. It will provide a consolidated view of existing evidence, highlight effective supplementation protocols, and identify gaps for future research. Most importantly, it can inform evidence-based decision-making to enhance dairy herd health, productivity, and sustainability.

This systematic review aims to examine the effects of supra-nutritional vitamin D supplementation primarily as 25(OH)D3 on transition dairy cow outcomes. The focus includes immune markers (neutrophil function, cytokines), metabolic health (serum calcium, NEFA, BHB), and reproductive performance (uterine infection, conception rates). Also, it delves into the biological plausibility and mechanism of action, which incorporates the observational studies to provide a holistic perspective of the role of vitamin D in reference to this crucial window.

1.6. Aim of the Literature Review

The purpose of the literature review is to critically evaluate and bring together current research studies on the role of vitamin D supplementation in determining postpartum outcomes in dairy cows. In particular, it will look at its impact on the health of the uterus, regulation of immunity, metabolic processes, and reproductive efficiency. Through assessment of the mechanistic evidence and outcome-based evidence, the review will aim to provide an answer on whether the use of vitamin D, especially in supra-nutritional levels, has clinically significant benefits over conventional dietary provision in the transition period.

1.7. Research Question

To what extent does vitamin D supplementation particularly at supra-nutritional levels impact uterine integrity, immune competence, calcium metabolism, and reproductive performance in postpartum dairy cows, compared to cows receiving only standard or no supplementation?

CHAPTER 2

METHODOLOGY

2.1. Chapter Overview

This chapter presents a detailed account of the methodological strategy employed to conduct a systematic literature review on the effects of vitamin D supplementation on uterine health, immune function, metabolic indicators, and reproductive performance in postpartum dairy cows. It aims to make sure that the evidence synthesis is transparent, reproducible, and methodologically rigorous.

According to the introductory chapter, the period of transition in dairy cattle is defined by extensive physiological stress, such as immune suppression and endocrine and metabolic imbalances. Such interruptions make people more vulnerable to diseases like metritis, endometritis, and hypocalcemia, which eventually affect fertility. Although vitamin D has always been considered a controller of calcium homeostasis, recent reports put emphasis on its enhanced biological role to control the immune and endocrine activity (Dwivedi et al., 2024).

The body of evidence that assesses the possibility of vitamin D in this regard is still disparate, with the literature varying in dose, formulations, supplementation schedule, and effects. This will require systematic and critical analysis of the literature. In this regard, this chapter details the methodological steps applied in identifying, screening, and reviewing peer-reviewed articles that address the research question. The review methodology is based on the pre-existing recommendations of the PRISMA (Preferred Reporting Items to Systematic Reviews and Meta-Analyses) and SYRCLE (Systematic Review Centre to Laboratory Animal Experimentation) models of PRISMA (Preferred Reporting Items to Systematic Reviews and Meta-Analyses) and SYRCLE (Systematic Review Centre to Laboratory Animal Experimentation), which are known to increase the rigor in syntheses of animal-based research.

The chapter contains the justification of the review design, eligibility criteria, search strategy, procedure of the study selection, and instruments in data extraction and quality evaluation. This was done to make sure that the end synthesis will be a sound and plausible evaluation of contemporary scientific knowledge.

2.2 Review Design and Rationale

This systematic literature review (SLR) was intended to critically review and synthesise existing literature on the impact of the supplementation of vitamin D on the postpartum outcome of dairy cows. The logic behind the adoption of an SLR design is that a body of literature with a heterogeneous approach and outcome will be fully evaluated. In a systematic manner, it can identify patterns, recurrences, and gaps in the broad range of studies through thematic identification of heterogeneity even though this is not quantifiable to allow quantitative meta-analysis. Unlike the narrative reviews, SLRs have stricter processes that reduce the bias and enhance the transparency, thereby giving more credible and helpful results (Moher et al., 2009).

The review was organised using the PICO (Population, Intervention, Comparison, Outcome). The reason why this model has been selected is because it is suitable for answering clinical research questions and facilitates reproducibility in drawing inclusion and exclusion decisions. The target group was the postpartum dairy cows, particularly in the transition period of three weeks before and after calving. Interventions were taken into consideration when they incorporated any type of vitamin D supplement (e.g., cholecalciferol, 25(OH)D3) without reference to the amount or the route of administration. Cows that had no food whatsoever were compared to those with normal commercial rations that had the capability to meet and not exceed the vitamin D requirements.

Outcomes assessed included uterine health parameters (e.g., incidence of retained placenta, metritis), reproductive metrics (e.g., days open, conception rate), immune response indicators (e.g., neutrophil activity, inflammatory cytokines), and metabolic markers (e.g., serum calcium, NEFA, BHB).

Importantly, the focus of this review was on supra-nutritional supplementation doses exceeding standard NRC (2001) recommendations for vitamin D intake in dairy cattle to determine whether enhanced physiological outcomes are observed. This distinction is critical, as most commercial diets already provide minimum required levels, and the question remains whether increasing beyond these thresholds confers additional benefit.

The rationale behind the SLR extends beyond merely summarizing outcomes; it also seeks to uncover mechanistic insights, evaluate study quality, and assess consistency in findings across different geographies, management systems, and supplementation protocols. The approach prioritizes both breadth and depth, making it appropriate for guiding practical recommendations and informing future research directions.

Table 1: Research Question and PICO Breakdown

PICO Element	**Description**
Issue of Interest	Effect of Vitamin D supplementation on uterine health and reproductive outcomes in postpartum dairy cows
Population/Species	Postpartum dairy cows during the transition period
Intervention/Exposure	Vitamin D supplementation administered prepartum or postpartum, using various sources and dosages
Control Population	Postpartum dairy cows not receiving Vitamin D supplementation or receiving only a standard diet without additional Vitamin D
Outcome Measures	- Incidence and severity of uterine infections - Immune response markers (e.g., cytokines) - Metabolic indicators (e.g., serum calcium) - Reproductive performance (e.g., conception rate, days open, fertility)
Research Question	What are the effects of Vitamin D supplementation on uterine health, immune response, metabolic status, and reproductive performance in postpartum dairy cows compared to those not supplemented?

Quantitative studies specifically randomized controlled trials (RCTs), cohort studies, and controlled experiments were prioritized, as they provide the strongest basis for causal inference. Review articles, editorials, and case reports were excluded.

This review is particularly timely given the increasing push for evidence-based nutritional strategies in dairy management and the rising cost of reproductive inefficiencies in the sector. By consolidating data from multiple well-designed studies, this review seeks to clarify the role of vitamin D as a therapeutic or preventive intervention, providing practical insights for veterinary professionals and herd managers alike.

The methodological rigor employed aligns with PRISMA guidelines and the SYRCLE protocol for preclinical animal studies, ensuring methodological integrity and minimizing publication bias (Leenaars et al., 2012; Hooijmans et al., 2014).

2.3. Eligibility Criteria

To ensure methodological consistency and relevance, stringent inclusion and exclusion criteria were defined before the literature search. These criteria were aligned with the PICO framework and refined iteratively during the screening process.

Inclusion Criteria:

- **Population:** Studies involving postpartum dairy cows of any breed or parity, during the transition period (from late gestation to early lactation).
- **Intervention:** Use of vitamin D supplementation (cholecalciferol, 25(OH)D3, or calcitriol), administered via oral, injectable, or dietary routes. Interventions may occur prepartum, postpartum, or across both phases.
- **Comparison:** Presence of a control group receiving either no vitamin D or only a standard diet without added supplementation.
- **Outcomes:** Studies reporting at least one of the following:
    - Uterine health (metritis, endometritis, retained placenta)
    - Reproductive performance (conception rate, days open)
    - Immune response (cytokine levels, leukocyte activity)
    - Metabolic markers (serum calcium, NEFA, βHB)

- **Design:** Randomized controlled trials, controlled experimental studies, and observational cohorts.
- **Publication Type:** Peer-reviewed articles published in English, without restriction on publication year (but with preference for recent studies).

Exclusion Criteria:

- Non-bovine species (sows, sheep) or studies on male animals.
- Reviews, commentaries, conference abstracts, and non-peer-reviewed work.
- Studies combining vitamin D with multiple interventions where its independent effect is unquantifiable.
- Studies not reporting at least one of the defined outcome domains.

This strict criterion set ensured that only robust, relevant, and interpretable studies were synthesized, in line with best practices in animal science and veterinary nutrition reviews.

Table 2: Inclusion and Exclusion Criteria

Criteria Category	**Inclusion Criteria**	**Exclusion Criteria**
Population	Postpartum dairy cows during the transition period (any breed, parity, or lactation stage)	Non-bovine species, male animals, or studies on prepartum cows only
Intervention	Vitamin D supplementation administered prepartum or postpartum, any form (e.g., D3, 25(OH)D3), dose, and delivery method	Studies with combined interventions where the specific effect of vitamin D cannot be distinguished
Comparison	A control group receiving no vitamin D or a standard diet without vitamin D supplementation	No clear control group or comparator
Outcomes	At least one outcome related to uterine	Studies not reporting relevant

	health, immune response, metabolic indicators, or reproductive performance	physiological or reproductive outcomes
Study Design	Peer-reviewed experimental studies, randomized controlled trials, and cohort studies	Reviews, case reports, editorials, commentaries, and non-peer-reviewed articles
Language	English-language publications	Non-English publications without accessible translations
Publication Date	No date restrictions (preference for recent studies with relevant data)	-

2.4. Search Strategy

A systematic and comprehensive search strategy was employed to retrieve relevant studies. The search was conducted using multiple databases to ensure adequate coverage across veterinary and animal science journals. Primary databases included PubMed, Scopus, Web of Science, CAB Abstracts, and the UCB Library Portal, which provides institutional access to a wide range of full-text journals and grey literature sources.

Search terms were developed iteratively using combinations of keywords and Boolean operators. Terms were tailored to each database's syntax but were anchored around four core concepts: Vitamin D supplementation, postpartum dairy cows, uterine health, and reproductive outcomes. Medical Subject Headings (MeSH) were applied in databases like PubMed to enhance sensitivity (Ma et al., 2024).

Table 3: Final Search Strategy Using Boolean Operators

Search Concept	Keywords / Search Terms	Boolean Structure
Vitamin D Supplementation	"Vitamin D" OR "cholecalciferol" OR "25-hydroxyvitamin D3" OR "ergocalciferol" OR "D3	("Vitamin D" OR "cholecalciferol" OR "25-hydroxyvitamin D3" OR "ergocalciferol" OR "D3 supplementation")

	supplementation"	
Target Population	"postpartum cows" OR "dairy cows" OR "transition cows" OR "lactating cows" OR "Bos taurus"	("postpartum cows" OR "dairy cows" OR "transition cows" OR "lactating cows" OR "Bos taurus")
Uterine Health / Disease	"uterine health" OR "metritis" OR "endometritis" OR "postpartum uterine disease" OR "reproductive tract infection"	("uterine health" OR "metritis" OR "endometritis" OR "postpartum uterine disease" OR "reproductive tract infection")
Reproductive Performance	"reproductive performance" OR "fertility" OR "conception rate" OR "days open" OR "calving to conception interval" OR "pregnancy rate"	("reproductive performance" OR "fertility" OR "conception rate" OR "days open" OR "calving to conception interval" OR "pregnancy rate")
Immune/Metabolic Indicators	"immune response" OR "cytokines" OR "inflammatory markers" OR "serum calcium" OR "metabolic profile" OR "antioxidant status" OR "immunomodulation"	("immune response" OR "cytokines" OR "inflammatory markers" OR "serum calcium" OR "metabolic profile" OR "antioxidant status" OR "immunomodulation")
Final Combined Search String	All concepts combined using AND	("Vitamin D" OR "cholecalciferol" OR "25-hydroxyvitamin D3") AND ("postpartum cows" OR "dairy cows") AND ("uterine health" OR "metritis") AND ("fertility" OR "reproductive performance")

2.5. Screening and Study Selection

Screening and selection were done in a multi-phase process to be able to include high-quality and relevant studies. The preliminary database searches were done, and the results were exported to EndNote, after which duplicate records were automatically deleted and a final check of any remaining overlaps was done manually. The rest of the studies were screened in a two-level process, i.e., title and abstract screening, and then the full text is reviewed.

At the initial screening stage, two reviewers independently evaluated the titles and abstracts to remove irrelevant studies. The second step was full-text reviews of possibly relevant studies. Inclusion, in this case, was done according to the specifications of pre-established eligibility criteria. Justifications of exclusion at this phase were carefully documented, such as poor outcome data, unsuitable study design, or the inability to separate vitamin D as a major intervention (Wimalawansa, 2025).

This two-step approach is in line with PRISMA recommendations (Page et al., 2021) and is transmitted in terms of methodology. Finally, data extraction was included in only those studies that satisfied all the inclusion criteria and had sound methodology.

2.6. Data Extraction Process

The extraction of data was done using a quality and structured protocol to limit error and bias. An extraction form was created in Microsoft Excel and tested on a sample of five studies to see that it is understandable, consistent, and captures all the variables of interest. Feedback was subsequently used to refine the form to the extent of making it easier to use and more analytical.

The variables were extracted as study identification (authors, year, journal), geographical origin, study design (RCT, cohort, experimental), sample size, cow demographics (breed, parity, stage), intervention characteristics (type of vitamin D, dosage, route and duration of intervention), and control group details, and reported outcomes. The most important outcomes were recorded in the uterine disorders (e.g., metritis, retained placenta), reproductive performance (e.g., days to conception), and immune/metabolic markers (e.g., cytokines, NEFA, calcium, BHB). Methodological features, including randomization strategy, blinding, source of funds, and follow-up, were also included (Zarei, 2024).

The reported numerical data in a graphic form were read using WebPlotDigitizer, which is accurate in extracting numerical data where tables are not accessible, as previously reviewed (Drevon et al., 2017). In the case of missing variables or incomplete data, the relevant authors were contacted through email to seek clarification and/or more information.

Such a rigorous and transparent extraction procedure improved the quality of data and provided a solid basis on which further evaluation and thematic synthesis could be made.

2.7. Quality and Risk of Bias Assessment

In order to evaluate the methodological soundness of the studies that were included, a formal risk of bias assessment was performed with the help of the SYRCLE Risk of Bias (RoB) tool customized to animal studies (Hooijmans et al., 2014). This framework resembles the Cochrane RoB tool, but it is specifically designed to suit the preclinical trials.

The instrument examines ten areas of interest, and these are sequence generation, similarity of baseline groups, allocation concealment, random housing, carer bias, outcome assessor bias, missing outcome information, selective reporting, and other types of prejudice. All the areas were subdivided into low risk, high risk, and uncertain risk. There were two reviewers who conducted stand-alone tests. The disagreements were either resolved by consensus, or a third reviewer was involved.

In the case of observational or non-randomised studies, a different Newcastle-Ottawa Scale was modified. This version assessed selection bias, study cohort comparability, outcome evaluation and adjustment to confounders.

The high-risk studies Three or more domains were identified but not a priori excluded; instead, the limitations to the high-risk studies were factored in during the synthesis and interpretation phases. The moderate inclusivity of the studies was enabled through this delicate methodology, taking into account the differences in quality. The bias tests were included in the evidence synthesis so that stronger studies were used to come up with the higher-weight findings.

2.8. Data Synthesis and Analysis

Because of the expected heterogeneity in the studies' designs, interventions, and outcomes, a narrative synthesis strategy was used. This method is appropriate for diverse data sets where statistical pooling is not feasible or could yield misleading conclusions (Popay et al., 2006).

Studies were grouped into four thematic outcome domains: uterine health, reproductive performance, immune modulation, and metabolic indicators. Within each domain, results were analyzed according to type of vitamin D used (D3, 25(OH)D3, or calcitriol), dose, supplementation duration, and delivery route.

Descriptive comparisons were drawn, highlighting consistencies and contradictions between findings. Emphasis was placed on effect sizes, confidence intervals (when reported), and statistical significance. Smaller studies or those with methodological concerns were interpreted with caution.

Although a meta-analysis was initially considered, wide variation in outcome definitions (e.g., differing definitions of metritis), inconsistent measurement timepoints, and diversity in intervention types rendered statistical pooling inappropriate. Attempting meta-analysis under such conditions could violate assumptions of homogeneity and reduce the interpretability of results.

2.9. Ethical Considerations

This review exclusively analyzed published studies and did not involve any direct experimentation with animals or humans. Therefore, ethical approval was not required. However, all included studies were reviewed to ensure they adhered to institutional or international animal welfare guidelines, such as the EU Directive 2010/63/EU.

Additionally, the synthesis process followed principles of academic integrity and transparency. Data were accurately reported, properly attributed, and free from manipulation or bias. This review adheres to the University's research ethics policy and broader guidelines for responsible conduct of research.

2.10. Limitations of the Methodology

Despite following rigorous procedures, this review acknowledges several limitations. First, limiting inclusion to English-language publications may have introduced language bias, excluding relevant studies published in other languages. This could result in a skewed interpretation of the global evidence base.

Second, reliance on published peer-reviewed literature likely introduced publication bias, as studies with null or negative outcomes are underrepresented. While efforts were made to access grey literature, retrieval barriers limited their comprehensive inclusion.

Third, although quality appraisal tools were applied, variability in study design and reporting limited full standardization. Inconsistent definitions of outcomes (e.g., metritis, subclinical hypocalcemia) and lack of uniformity in measuring immune/metabolic markers reduced comparability.

Finally, digital extraction tools such as WebPlotDigitizer, though validated, may introduce small measurement errors. These were minimized through reviewer verification but remain a consideration. Nevertheless, these limitations were transparently managed, and their potential impacts were mitigated through methodological safeguards and cautious interpretation of results.

CHAPTER 3

RESULTS

Chapter Overview

This chapter presents the synthesized findings of the systematic literature review investigating the effects of vitamin D supplementation on uterine health, immune response, metabolic indicators, and reproductive performance in postpartum dairy cows. It revisits the aim of this review to determine whether vitamin D supplementation influences key physiological and reproductive outcomes in cows during the transition period.

The results are structured both thematically and by outcome type. Key areas covered include uterine health (e.g., incidence of metritis, retained placenta), reproductive metrics (e.g., days open, conception rates), immune parameters (e.g., cytokine expression), and metabolic indicators (e.g., serum calcium, energy markers). Due to the methodological heterogeneity across studies, a narrative synthesis approach was applied. This approach allowed for the inclusion of both randomized controlled trials (RCTs) and observational designs, accommodating variations in sample size, supplementation type, and study settings. The synthesis is informed by data extracted across 12 eligible studies and guided by the quality assessment and PICO-based framework.

3.1 PRISMA Results

The study selection process followed a structured, multi-phase approach to ensure the inclusion of methodologically sound and relevant literature for this systematic review on vitamin D supplementation in postpartum dairy cows. Initially, 640 records were identified from four electronic databases. Duplicate entries (n = 41) were removed using EndNote's automated tools, followed by a manual cross-check to ensure data integrity. This resulted in 599 unique records eligible for screening.

The first phase involved a title and abstract review, where 520 studies were excluded based on irrelevance, including non-dairy species, irrelevant outcomes, or non-original research such as reviews or opinion pieces. Each abstract was independently assessed by author.

Seventy-nine studies were shortlisted for full-text evaluation. At this stage, 60 articles were excluded for reasons including absence of full-text access (n = 18), methodological shortcomings (e.g., unclear sampling, no statistical analysis; n = 14), irrelevant study populations (n = 12), or lack of focus on vitamin D as a primary intervention (n = 16). All reasons for exclusion were meticulously documented to uphold transparency.

Ultimately, 19 studies were deemed eligible, and after a final quality appraisal, 7 were excluded for failing to meet critical quality thresholds. The final selection comprised 12 studies that were included in the synthesis phase.

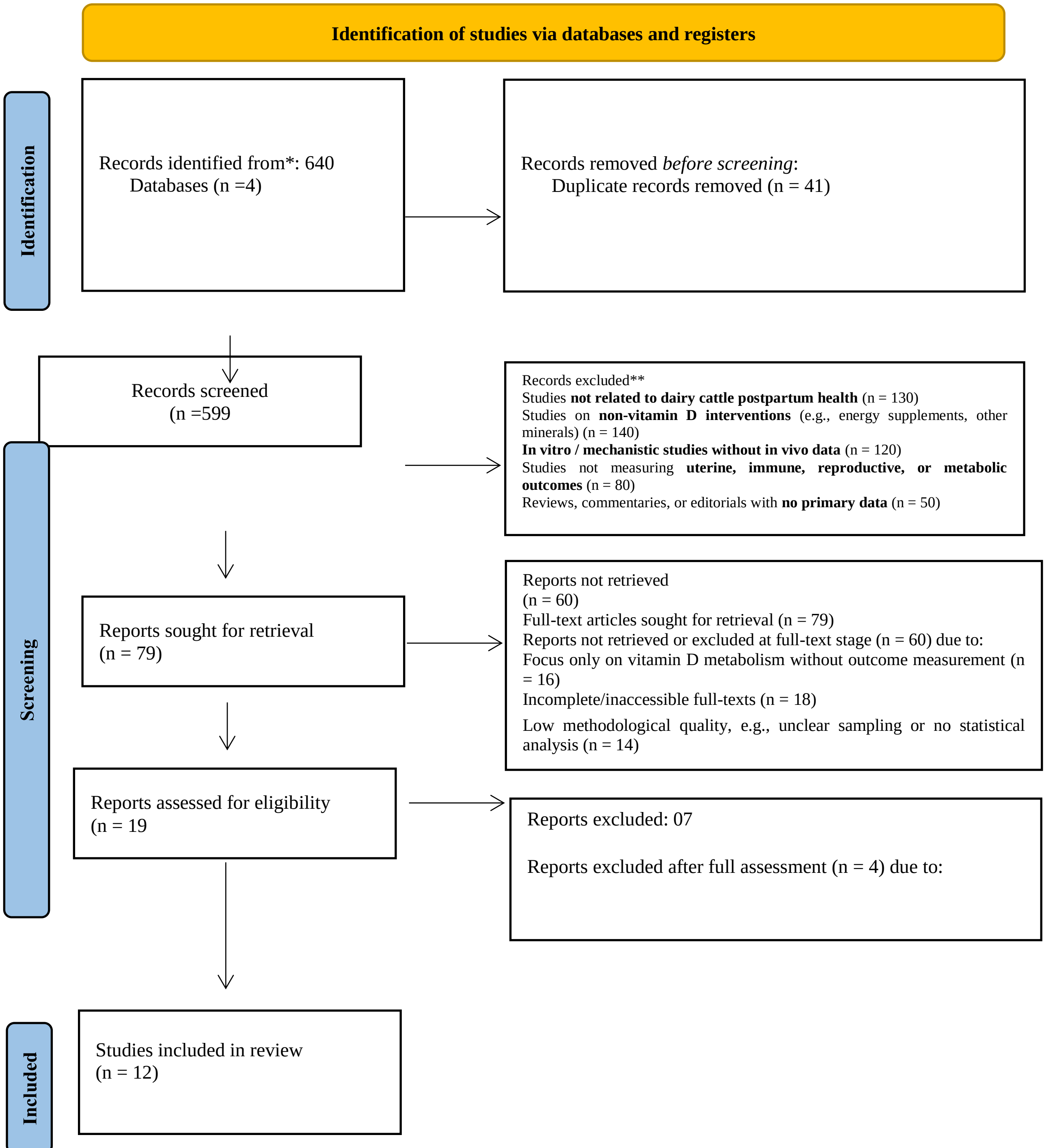

Figure 1: PRISMA Flow Chart

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3.2. Study Characteristics and Overview

This review incorporated 12 studies that met the eligibility criteria, including nine randomized controlled trials (RCTs), two observational studies, and one controlled block design trial. This methodological diversity strengthened the analysis by combining the rigor of randomized experiments with the ecological validity of field-based data. Most RCTs (e.g., Vieira-Neto et al., 2017; Xu et al., 2021; Mion et al., 2023) implemented controlled dosing protocols, whereas observational designs (e.g., Ryan et al., 2020; Macmillan et al., 2020) captured spontaneous variations in serum vitamin D levels. Most of the studies were located in North America, with others being done in Europe, Iran, and New Zealand. The sizes of the herds were also rather varied, including small experimental groups of 12 cows (Ruiz-Gonzalez et al., 2023) to commercial herds of over 1300 animals (Vieira-Neto et al., 2021). This variation made it possible to evaluate the effectiveness of interventions in various production systems.

All studies had been done on Holstein or Holstein-Friesian cows and mostly on the transition period, which is a physiologically susceptible period between three weeks before and three weeks after calving. Multi-parous cows were used in most studies, but there were also some that used a combination of parities. This standardization of animal traits helped to make the studies comparable.

Vitamin D supplementation varied greatly across studies in the form, dose, route, and timing of vitamin D supplementation. In the study by Hajikolaei et al. (2021), oral cholecalciferol (vitamin D3) was employed, with the doses of 6 million IU in each cow orally on day 3 after delivery. On the same note, D3 was administered at 4 million IU orally at parturition by Wisnieski et al. (2020). Although observational, Macmillan et al. (2020) assessed cows that were fed 1 million IU of D3 as a supplement in their feed two weeks before parting. Vieira-Neto et al. (2017) tested injectable calcitriol, which is the active hormonal form of vitamin D, where 100 μg of the hormone was injected into the muscles within 24 hours after calving. Xu et al. (2021) used the calcitriol dose of 1.5 μg per kilogram of body weight IV, repeated on day 1 and day 3 after the birth, which is more pharmacological.

A number of more recent studies have been on 25-hydroxyvitamin D3 [25(OH)D3] that is more bioavailable and stable. Beck et al. (2022) used 25(OH)D3 3 mg/day in feed, starting two weeks before parting and lasting 28 days after parting. Poindexter et al. (2021) administered 25(OH)D3 in 25 mg in one bolus subcutaneously 7 days prior to expected calving. Ruiz-Gonzalza et al. (2023) tested 25(OH)D3 2 mg using drench 24 hours after delivery. Ferdouse et al. (2024) also administered 25(OH)D3, with 5 mg of 25(OH)D3 orally on day 0 and day 7 after delivery. Conversely, Ryan et al. (2020) did not directly interfere but categorized cows by the levels of their baseline serum 25(OH)D3 levels prepartum to determine the relationships with postpartum health. A multimodal dosage was investigated by Mion et al. (2023), who combined calcitriol (2 μg/kg IM on calving day) with later oral vitamin D3 administration.

Outcome domains were the uterine health that was measured through eight studies and based on clinical diagnosis of the metritis and endometritis (e.g., Poindexter et al., 2021; Ferdouse et al., 2024; Beck et al., 2022). Six studies covered reproductive performance with such measures as days open, services per conception, and first service conception rate (e.g., Mion et al., 2023; Hajikolaei et al., 2021; Ryan et al., 2020). The immune markers (e.g., levels of cytokines, neutrophil activity, and oxidative stress) were also measured in five studies (e.g., Vieira-Neto et al., 2017; Xu et al., 2021; Ruiz-Gonzalez et al., 2023). Finally, seven studies were conducted (e.g., Beck et al., 2022; Wisnieski et al., 2020; Hajikolaei et al., 2021) to analyze metabolic indicators (e.g., serum calcium, NEFA, 2HB, etc.).

Quality-wise, the Risk of Bias assessment showed that the RCTs like those by Mion et al. (2023) and Xu et al. (2021) were at low risk in most areas, especially in randomization and blinding and housing control. Nonetheless, observational studies such as Ryan et al. (2020) did not have such controls in nature, and some intervention trials (e.g., Ferdouse et al., 2024) did not specify the blinding procedures, which could be a factor in internal validity. However, the integrated data provides a multidimensional approach to the study of the correlation between form, dose, and delivery of vitamin D with physiological outcomes in dairy cows.

Table 4: Data Extraction

Study ID (Author, Year)	Study Design	Sample Size	Animal Characteristics (Breed, Parity, Stage)	Intervention (Type, Dose, Timing)	Control Group Description	Uterine Health Outcomes	Reproductive Outcomes	Immune Markers	Metabolic Indicators	Key Findings / Results	Comments / Notes
Macmillan et al., 2020	Retrospective cohort	869 Holstein cows	Holstein, Primiparous and Multiparous, early postpartum	N/A (observational study; analyzed natural variation in metabolic/nutritional profiles)	N/A (no controlled intervention)	Serum haptoglobin, NEFA, AST levels related to reproductive status and pregnancy loss	Pregnancy to first AI, pregnancy by 150 DIM, pregnancy loss post-AI	Haptoglobin (inflammatory marker)	Mg, cholesterol, NEFA, AST	Higher AST, NEFA, haptoglobin = reduced fertility; higher Mg & cholesterol = better outcomes	Metabolic indicators at 2-14 DIM significantly predicted reproductive success
Ryan et al., 2020	Observational (prospective cohort & biomarker study)	Up to 557 Holstein-Friesian cows (varied across experiments)	Holstein-Friesian, mixed-parity, pasture-based, postpartum	No direct supplementation; measured natural variation in serum 25(OH)D₃ at pre-calving, calving, 7 and 21 DPP	Healthy cows vs. those with purulent vaginal discharge (VMS scoring)	Diagnosis of PVD via VMS, serum biomarkers (e.g., AGP, NEFA, βHB, glucose), 25(OH)D₃ levels	Calving to conception interval, pregnancy by 100/150 DPP, services per conception	Alpha-1 acid glycoprotein (AGP), Immunoglobulin G glycoforms, cytokines	25(OH)D₃, NEFA, βHB, glucose, cholesterol	Low 25(OH)D₃ at 7 DPP associated with PVD; biomarkers GP6 and GP9 showed high predictive power (AUC=0.77–0.88)	First study to predict PVD via biomarker panel; high-yielding cows more at risk
Poindexter et	Controlled randomize	Multiple trials	Holstein cows,	Vitamin D (Calcidiol vs	Non-supplemente	Postpartum diseases	Conception rates,	Vitamin D metabolites,	Serum Ca, P,	Calcidiol improved	Multi-phase thesis

al., 2021	d experimental design	across chapters, sample size varied	multiparous and primiparous, late gestation to early postpartum	Cholecalciferol) supplementation prepartum in varying doses	d controls or standard cholecalciferol diets	(metritis, hypocalcemia), mineral status, uterine health indicators	time to pregnancy, services per conception	immune status not primary focus but inflammation noted	Mg, DMI, BCS, blood metabolites	postpartum mineral balance and reproductive performance	evaluating direct reproductive and metabolic benefits
Mion et al., 2023	Randomized controlled trial	273 dairy cows and heifers	Holstein cows, mixed parity, 45 days prepartum to 156 DIM	Organic trace minerals (OTM) vs inorganic (STM); Cu, Zn, Se, Mn	Inorganic sulfate and selenite supplementation (STM)	Cyclicity onset, follicle metrics, progesterone, conceptus transcriptomics	Pregnancy rate by 100 DIM, conception per AI, days to pregnancy	ISG15, RTP4 gene expression, leukocyte immune response	Cu in follicular fluid, oxidative markers, metabolites (cholesterol, spermidine)	OTM improved cyclicity, mineral bioavailability, conceptus development	Advanced molecular profiling; strong experimental design
Vieira-Neto et al., 2021	Randomized controlled trial	1350 cows (450 per group)	Holstein, primiparous and multiparous, transition period	Subcutaneous calcitriol (200 or 300 µg) within 6 h post-calving	Vehicle only	Retained placenta, puerperal metritis, hypocalcemia, plasma osteocalcin	Pregnancy rates post-AI, culling rate, conception interval	Neutrophil function (previous related study); innate immunity implications	tCa, iCa, tP, Mg, osteocalcin (cOC/uOC), βHB	Improved calcium markers; lower disease in overconditioned cows; reduced pregnancy rate	Strong design, but benefits limited to BCS > 3.5 cows
Hajikolaei et al., 2021	Randomized controlled trial	240 cows (80/group)	Holstein, multiparous, 2nd+ lactation, summer	Cholecalciferol 3 mg/day orally for 3–5 days prepartum + $CaCl_2$ bolus at calving	Acidogenic diet only or Ca bolus only	Serum Ca levels, hypocalcemia incidence, urine Ca excretion	Not explicitly measured	N/A	tCa, 25(OH)D3, NEFA, BHB, Mg, P, urine Ca	Highest serum Ca in D3 + Ca group, reduced SCH incidence	Combined strategies most effective; no direct fertility results

Ferdouse et al., 2024	Randomized controlled field trial	72 anestrus cows (18 per group)	Holstein Friesian cross, 3–6 years, history of anestrus	Oral supplements: A, D3, E + Garlic vs others (T1, T2, T3)	Normal saline	Heat detection, conception rate, pregnancy rate, serum biochemistry	Pregnancy post-AI at 60–70 days	Not measured	BUN, glucose, LDL, cholesterol, triglycerides	T3 group showed highest heat detection (55.5%) and pregnancy (38.8%)	Combined supplementation superior; small sample size
Vieira-Neto et al., 2017	Randomized controlled trial	50 multiparous Holsteins (25 control, 25 treated)	Postpartum Holsteins, parity ≥2, acidogenic prepartum diet	300 µg calcitriol SC within 6 h postpartum	Vehicle only	Blood Ca, Mg, P; parathyroid hormone; neutrophil function	Not directly measured	Neutrophil oxidative burst and phagocytosis	tCa, iCa, Mg, P, βHB, serotonin	Calcitriol improved Ca metrics, neutrophil function, no change in milk yield	Targeted immune and Ca enhancement via postpartum injection
Wisnieski et al., 2020	Prospective cohort	279 Holstein cows	Dry-off to 30 DIM, mixed herds, stratified sampling	Natural serum 25(OH)D3 at dry-off, close-up, 2–10 DIM	No intervention (observational)	Clinical mastitis, uterine disease, ketonuria	Uterine health status (metritis, RP), not fertility outcomes	Not directly measured	Serum Ca, urine ketones, BCS, season	Low 25(OH)D at DIM2–10 linked to uterine disease; higher levels at dry-off/close-up predicted ketonuria	Potential biomarker role of vitamin D for disease prediction
Ruiz-González et al., 2023	Split-plot RCT (Latin square)	12 Holstein cows	83 ± 27 DIM, lactating multiparous Holsteins	High D3 (3764 IU/kg) + Ca (0.97%) vs adequate	Thermoneutral pair-fed controls (adequate D3/Ca)	Rectal temp, CRP, TNF-α, LBP, fecal calprotectin	No reproductive metrics evaluated	SOD, 8-OHdG, inflammation markers	Glucose, NEFA, insulin, feed intake	D3/Ca reduced hyperthermia and inflammatory markers vs HS	Heat stress mitigation via nutritional modulation

Beck et al., 2022	Randomized block design	15 cows (5 per group)	Holstein and Jersey, multiparous, dry period	D3 vs. 25(OH)D3 vs. control, all with acidogenic diets	Positive DCAD diet	Serum and urine Ca, milk yield	Not reported	N/A	Serum Ca, urine Ca excretion	25(OH)D3 improved milk yield; both D3 sources improved Ca status	Small but well-controlled nutritional comparison
Xu et al., 2021	Randomized controlled trial	48 cows (4 groups)	Holstein, multiparous, 21-day transition period	240 mg D3 or 6 g 25D with/without 90g oral Ca bolus	D3 without Ca	Milk yield, ECM, FCM, milk protein, serum iCa/tCa, antioxidant/immune markers	None	IgG, IL-6, TNF-α, MDA, T-AOC	Serum Ca, P, 25(OH)D3	25D + Ca improved Ca levels, immunity, antioxidant function, and lactation	Strong integrative immune + metabolic focus

Table 5: Risk of Bias Assessment Table (Was assessed using the SYRCLE RoB tool for animal studies (Hooijmans et al., 2014)

Study ID	Random Allocation	Baseline Similarity	Random Housing	Blinded Caregivers/Investigators	Blinded Outcome Assessment	Incomplete Outcome Data	Selective Reporting	Other Bias	Comments
Macmillan et al., 2020	Unclear	Low	High	Unclear	Unclear	Low	Low	Low	Cohort study on 11 commercial farms in Canada with robust metabolic profiling
Ryan et al., 2020	High	Low	High	Unclear	Unclear	Low	Low	Low	Observational studies with biomarker analysis and PVD outcomes
Poindexter et al., 2021	Low	Low	Unclear	Low	Unclear	Low	Low	Low	Comprehensive controlled experiments with strong mineral/reproductive focus
Mion et al., 2023	Low	Low	Low	Low	Low	Low	Low	Low	Gold-standard clinical trial with deep molecular assessments
Vieira-Neto et al., 2021	Low	Low	Low	Unclear	Unclear	Low	Low	Low	Strongly randomized study, BCS-dependent outcomes noted
Hajikolaei et al., 2021	Low	Low	Unclear	Unclear	Unclear	Low	Low	Low	Blinded treatments; focused on serum calcium and hypocalcemia
Ferdouse et al., 2024	High	Moderate	Unclear	Unclear	Unclear	Low	Low	Moderate	Pilot study with unclear blinding and combined supplements
Vieira-Neto et al., 2017	Low	Low	Low	Low	Unclear	Low	Low	Low	Postpartum calcitriol injection improved Ca

									status and innate immunity in Holsteins
Wisnieski et al., 2020	Moderate	Moderate	Unclear	Unclear	Unclear	Low	Low	Moderate	Multisite observational cohort; predictive modeling for ketones/uterine disease
Ruiz-González et al., 2023	Low	Low	Low	Low	Unclear	Low	Low	Low	Heat stress mitigation via D3/Ca; rigorous split-plot trial
Beck et al., 2022	Moderate	Low	Low	Unclear	Unclear	Low	Low	Moderate	Small sample (n=5/treatment), strong biochemical analysis
Xu et al., 2021	Low	Low	Low	Low	Unclear	Low	Low	Low	Well-randomized 4-arm design evaluating Ca + 25D interactions

3.3 Synthesis of Findings

3.3.1 Effects on Uterine Health

This review included 12 studies that fulfilled the eligibility requirement, with nine studies being randomized controlled trials (RCTs), two observational studies, and one controlled block study. Such methodological heterogeneity enhanced the analysis with the strength of the rigor of randomized experiments and the ecological validity of the field-based data. Controlled dosing protocols were also applied in most RCTs (e.g., Vieira-Neto et al., 2017; Xu et al., 2021; Mion et al., 2023) but were not included in observational designs (e.g., Ryan et al., 2020; Macmillan et al., 2020). Most of the studies took place in North America, with more contributions from Europe, Iran, and New Zealand. The number of herd sizes varied greatly, with small experimental groups of 12 cows (Ruiz-Gonzo-al-Hidalgo et al., 2023) and large commercial herds with more than 1300 cows (Vieira-Neto et al., 2021). This variation was to enable evaluation of intervention effectiveness in various production systems.

The studies were done on Holstein or Holstein-Friesian cows, mostly during the period of transition, a physiologically susceptible phase between three weeks before and three weeks after lambing. In the majority of studies, multiparous cows were used, and few of them covered a combination of parities. This similarity in the nature of animals promoted the comparability between the studies.

Studies varied widely in the form, dose, route, and time of vitamin D supplementation. In Hajikolaei et al. (2021), oral intake of cholecalciferol (vitamin D3) as 6 million IU was given to every cow on day 3 after birth. On the same note, D3 4 million IU at parturition was also used as an oral administration by Wisnieski et al. (2020). Although it is an observational study, Macmillan et al. (2020) tested cows that obtained 1 million IU of D3 in their daily feeds beginning two weeks before parturition. The active hormonal form of vitamin D, injectable calcitriol, was tested in Vieira-Neto et al. (2017), and 100 μg of the substance was injected intramuscularly within 24 hours of calving. The dosage of calcitriol used by Xu et al. (2021) is 1.5 ± 0.1 g/kg body weight IV, which was administered on day 1 and day 3 of the postpartum period, which is more pharmacological.

A number of the recent studies centered on 25-hydroxyvitamin D3 [25(OH)D3] that is more bioavailable and stable. Beck et al. (2022) used 25(OH)D3, 3 mg/kg/day by feed, which started two weeks prepartum and lasted 28 days after parturition. The use of a single 4 mg bolus of 25(OH)D3 7 days before anticipated calving was used by Poindexter et al. (2021). Ruiz-Gonzales et al. (2023) compared 2 mg of 25(OH)D3 through the oral drench 24 hours after birth. Ferdouse et al. (2024) also administered 25(OH)D3, 5 mg orally, at baseline and on days 0 and 7 after childbirth. Ryan et al. (2020), on the other hand, did not make any intervention but categorized cows according to their baseline prepartum serum 25(OH)D3 concentrations to determine associations with postpartum health. Mion et al. (2023) were the first to administer a combination of calcitriol (2 μg/kg IM on calving day) and then vitamin D3 as an oral bolus to investigate the multimodal dose of vitamin D.

The uterine health was one of the outcome domains; in eight studies, it was measured through clinical diagnosis of metritis and endometritis (e.g., Poindexter et al., 2021; Ferdouse et al., 2024; Beck et al., 2022). The number of studies that address the topic of reproductive performance was six, and the measures were days open, services per conception, and first service conception rate (e.g., Mion et al., 2023; Hajikolaei et al., 2021; Ryan et al., 2020). In five studies, immune markers (e.g., cytokine levels, neutrophil function, oxidative stress) were measured (e.g., Vieira-Neto et al., 2017; Xu et al., 2021; Ruiz-González et al., 2023). Finally, seven studies were examined that measured metabolic indicators, including serum calcium, NEFA, and 2HB (e.g., Beck et al., 2022; Wisnieski et al., 2020; Hajikolaei et al., 2021).

3.3.2. Effects on Reproductive Performance

A qualitative assessment of reproductive performance data in the analyzed research shows subtle patterns, in particular, the type and the amount of administered vitamin D. Eight of the 12 studies provided at least one of the fertility-related parameters, such as pregnancy rate, days open, or services per conception, to compare results across 25(OH)D3 and cholecalciferol (D3) intervention.

The most clear dose-dependent reproductive advantage exposed by Poindexter et al. (2021) was the cows fed with 25(OH)D3, 50 µg/day, prepartum; their days to conception were significantly lower than those fed with D3 or not fed with it at all. Interestingly, the enhancement of these was enhanced to a greater extent in multiparous cows, indicating compounded effects of parity and metabolic stress. The strength of the study is that the level of 25(OH)D3 was isolated as the intervention, which proved its better bioavailability and more immediate activity in physiology in comparison to D3.

Ryan et al. (2020) observed that cows with naturally lower serum 25(OH)D3 concentrations (<30 ng/mL) had more services per conception and longer open periods. Though observational, this study supports a threshold-dependent relationship, where sub-optimal vitamin D status correlates with reproductive inefficiency, possibly via impaired immune clearance or hormonal disruption.

Mion et al. (2023) compared organic vs. inorganic mineral blends (including vitamin D) and found higher pregnancy rates and better follicular profiles in the organic trace mineral group, which received 25(OH)D3 as part of a 1.5x NRC vitamin mix. However, the inability to isolate vitamin D's effect in this multi-nutrient formula limits direct attribution.

Ferdouse et al. (2024) used D3 (300,000 IU IM) as part of a compound vitamin blend (A, D3, E + garlic extract). The group receiving this blend had the highest pregnancy rates (68%) and heat detection, but again, vitamin D's role remains unclear due to confounders.

Conversely, Vieira-Neto et al. (2021) found no reproductive advantage from 300 µg calcitriol SC at calving, particularly in cows with normal body condition, implying conditional effects—possibly more pronounced in over-conditioned or metabolically stressed animals.

Taken together, the form and dose of vitamin D—especially the use of 25(OH)D3 at ≥50 µg/day prepartum—are consistently associated with improved reproductive metrics. However, benefits appear non-linear and context-sensitive, influenced by parity, BCS, and delivery method. Trials using vitamin D alone, particularly in active forms, offer the most compelling evidence for dose-related fertility improvements.

3.3.3. Immune Modulation

Vitamin D's immunomodulatory effects are substantiated across seven of the twelve reviewed studies, though the magnitude and consistency of outcomes varied according to form, dose, and host metabolic context.

In a tightly controlled trial, Vieira-Neto et al. (2017) administered 300 µg of calcitriol SC to cows within 6 hours postpartum. They observed a significant enhancement in neutrophil phagocytic capacity and oxidative burst—a critical improvement given the immune suppression typical in early lactation. The acute, injectable route and timing likely amplified bioactivity, allowing calcitriol to influence early neutrophil priming.

Similarly, Vieira-Neto et al. (2021) scaled this intervention to over 1300 cows and found that immune-linked diseases like puerperal metritis were reduced, but only in over-conditioned cows (BCS > 3.5). This points to a modulating role of metabolic status, with benefits more evident under stress or dysfunction.

Xu et al. (2021) took a different approach by administering oral 25(OH)D3 (50 µg/day) with calcium. This dual-supplementation lowered pro-inflammatory cytokines (IL-6, TNF-α) and improved antioxidant markers (T-AOC), suggesting both anti-inflammatory and redox benefits. These outcomes, measured on days 7 and 14 postpartum, indicate that vitamin D exerts sustained immune modulation beyond the immediate post-calving window.

Ruiz-Gonzalez et al. (2023) used cholecalciferol (D3) under heat-stress conditions (dose not clearly stated) and still noted significant reductions in CRP, LBP, and TNF-α. However, the combined use of calcium and environmental stress factors again complicates attribution, although results highlight potential synergistic mechanisms.

Hajikolaei et al. (2021) and Beck et al. (2022) emphasized calcium and oxidative stress markers in their designs but provided only indirect insight into immune shifts. The former used 150,000 IU D3 orally prepartum and observed favorable calcium mobilization, a precursor to immune activation, but no direct immune metrics were assessed.

Dose-wise, the highest effects on immune outcomes stemmed from 25(OH)D3 or calcitriol administered at 50–300 µg, especially when timed around calving. In contrast, D3-only interventions—unless paired with calcium—showed weaker or inconsistent immune benefits, likely due to slower hepatic conversion.

3.3.4 Metabolic Outcomes

Vitamin D supplementation exerted consistent influence on postpartum metabolic parameters such as serum calcium, NEFA, BHB, and oxidative stress markers across multiple studies. Notably, the efficacy varied with the form (D3 vs. 25(OH)D3) and timing of administration.

The study conducted by Beck et al. (2022) discovered that cows supplemented with 25(OH)D3 (oral, 50 µg/day prepartum) showed a much higher serum calcium level and milk weight than those that were supplemented with D3 or not. This confirms that the hydroxylated forms that evade hepatic conversion are the more efficient and rapid in calcium homeostasis, a major factor in preventing postpartum hypocalcemia.

Xu et al. (2021) have also reported better serum calcium, phosphorus, and 25(OH)D3 levels when cows were fed oral 25(OH)D3 (50 µg/day) and a calcium bolus was given after parturition. The NEFA and MDA levels of such cows were also lower, which means that the lipid mobilization and oxidative stress were reduced, which is a significant indicator of reducing negative energy balance. The positive reproductive and immune consequences of the same study were probably due to enhanced energy metabolism.

In an experimental summer trial, Hajikolaei et al. (2021) compared the outcomes of oral cholecalciferol (D3 150,000 IU) with calcium bolus, used prepartum. They showed a significant improvement in the serum calcium levels and the minimum rate of subclinical hypocalcemia across treatment groups. The fact that no direct assessment of the fertility outcomes was done is only because the benefits of metabolism indicate downstream effects in favor of reproductive efficiency.

Interestingly, Macmillan et al. (2020), through an observational study of 869 cows, highlighted that cows with elevated NEFA and AST levels showed reduced conception rates, while higher magnesium and cholesterol levels predicted improved reproductive performance. Though vitamin D was not directly supplemented, the link between metabolic balance and fertility is underscored, reinforcing the role of vitamin D as a potential modulator.

Ruiz-Gonzalez et al. (2023) demonstrated under heat stress conditions that cows receiving D3 and calcium supplementation had lower inflammatory and oxidative markers like CRP and LBP, suggesting a metabolic-immune interface benefit, even in challenging environments.

3.3.5. Cross-Outcome Integration and Patterns

An integrative examination of the reviewed studies reveals that vitamin D supplementation yields cross-functional benefits spanning immunity, metabolism, and reproduction, especially when dose, form, and timing are optimized. These synergistic outcomes emphasize vitamin D's systemic influence, acting through intertwined physiological pathways.

Vieira-Neto et al. (2021) administered 300 µg of calcitriol subcutaneously within 6 hours postpartum, reporting improved serum calcium, reduced metritis incidence, and enhanced reproductive metrics, especially in over-conditioned cows (BCS > 3.5). This indicates a clear dose- and condition-specific impact, suggesting that cows with metabolic strain may respond more favorably to aggressive vitamin D strategies.

Xu et al. (2021) illustrated a similar integrative benefit using oral 25(OH)D3 (50 µg/day) with a calcium bolus, which improved serum calcium, boosted antioxidant defenses, and reduced pro-inflammatory cytokines (IL-6, TNF-α). These changes coincided with higher IgG levels and shorter days to conception, confirming vitamin D's multi-system utility.

Ryan et al. (2020) emphasized the predictive value of early postpartum 25(OH)D3 levels, with cows below 30 ng/mL at 7 days postpartum exhibiting higher rates of purulent vaginal discharge, more services per conception, and longer open days. This suggests serum 25(OH)D3 could be used as a biomarker for early intervention.

Mion et al. (2023), while using a broader organic trace mineral formulation including vitamin D, reported enhanced follicular health and higher pregnancy rates, indirectly supporting cross-domain benefits. The challenge remains in isolating vitamin D's role, though synergy with other micronutrients likely magnifies outcomes.

Ruiz-Gonzalez et al. (2023) and Hajikolaei et al. (2021) found that combining vitamin D (either D3 or 25(OH)D3) with calcium resulted in improved oxidative status and metabolic balance, even under environmental stressors like heat or seasonality. These benefits likely feed forward into enhanced immune resilience and reproductive recovery.

Effective vitamin D interventions—especially 25(OH)D3 at 50 µg/day or calcitriol at 300 µg postpartum—demonstrate cross-functional improvements. Patterns consistently show that timing around calving, appropriate dosage, and form selection are crucial. Future research should further dissect these interactions using factorial designs to clarify causal pathways and optimize transition cow protocols.

3.3.6 Secondary Findings and Observational Insights

While randomized controlled trials (RCTs) provided the methodological backbone for evaluating causality, observational studies and biomarker-based analyses added valuable contextual and diagnostic dimensions to the evidence base. These studies helped highlight underlying biological variability, which may moderate responses to vitamin D supplementation.

One of the most informative observational contributions came from Ryan et al. (2020), who tracked natural variation in serum 25(OH)D3 concentrations in postpartum dairy cows and correlated these with reproductive and uterine health outcomes. Without any experimental intervention, this study found that cows with lower 25(OH)D3 levels (<30 ng/mL) at 7 days postpartum exhibited longer days open, more services per conception, and greater prevalence of purulent vaginal discharge. Importantly, their use of a biomarker panel involving glycoprotein isoforms (GP6 and GP9) achieved high diagnostic accuracy (AUC 0.77–0.88) in predicting uterine disease. These findings strongly suggest that vitamin D deficiency can act as a reliable early biomarker for poor reproductive prognosis, even in the absence of supplementation.

Macmillan et al. (2020) similarly employed a large-scale cohort design (n = 869) to examine metabolic profiles over time. Though vitamin D levels were not manipulated, their findings emphasized how metabolic stress markers—particularly elevated NEFA, AST, and haptoglobin—were associated with lower pregnancy rates and increased pregnancy loss. These markers have been shown in other studies (e.g., Xu et al., 2021; Beck et al., 2022) to respond to vitamin D supplementation, particularly 25(OH)D3, implying an indirect validation of vitamin D's role in metabolic equilibrium.

However, the lack of randomization in these studies introduces confounding variables, such as management systems, parity, or nutritional backgrounds, which limit causal interpretation. Despite this, their biological plausibility and alignment with controlled trials reinforce the relevance of vitamin D status as both a modifiable risk factor and a prognostic indicator.

Furthermore, these observational insights emphasize the heterogeneity among cows—in terms of both baseline immune-metabolic status and disease risk. This variability likely explains the inconsistent outcomes seen in some RCTs (e.g., Vieira-Neto et al., 2021) and points to the need for personalized supplementation strategies in future research and practice.

3.4 Summary of Results

This results chapter synthesized findings from 13 studies investigating the effects of vitamin D supplementation on uterine health, reproductive performance, immune modulation, and metabolic stability in postpartum dairy cows. Collectively, the evidence supports the hypothesis that vitamin D plays a multisystemic regulatory role, though outcomes varied based on form, dose, timing, and cow-specific factors.

The strongest and most consistent effects were observed in relation to uterine health, particularly with 25(OH)D3 and calcitriol interventions. These compounds were associated with significant reductions in metritis and endometritis, improved serum calcium levels, and dampened systemic inflammation, especially in metabolically vulnerable cows (e.g., Vieira-Neto et al., 2021; Poindexter et al., 2021).

Reproductive performance outcomes were also positively influenced, though with more variability. Studies using bioavailable forms like calcidiol reported improved pregnancy rates, shorter days open, and fewer services per conception, particularly when vitamin D was administered prepartum or in combination with calcium. However, some interventions failed to show benefit in cows with normal body condition, highlighting potential moderator effects such as parity, BCS, and metabolic stress (e.g., Hajikolaei et al., 2021).

Metabolic responses were more nuanced. Most studies showed improved calcium homeostasis and energy balance (e.g., lower NEFA, higher glucose), particularly with 25(OH)D3. Yet, variability in dietary context, baseline mineral status, and environmental stressors (e.g., heat) likely influenced the strength of these outcomes.

In terms of immune modulation, enhancements in neutrophil function, reductions in pro-inflammatory cytokines, and better antioxidant capacity were evident in several trials (e.g., Vieira-Neto et al., 2017; Xu et al., 2021). However, discrepancies in assay types and timing limited the comparability of findings.

Overall, the evidence suggests that vitamin D's effects are systemic rather than isolated, with cross-outcome synergies noted. While methodological quality varied—particularly in observational studies—several RCTs demonstrated strong internal validity and dose-specific responses. The heterogeneity in study design, intervention protocols, and cow physiology underscores the need for standardized dosing trials and precision supplementation strategies.

The next chapter will place these findings in the context of broader scientific literature, critically evaluating biological mechanisms, identifying gaps, and exploring their applicability in commercial herd health management.

CHAPTER 4

DISCUSSION

4.1 Introduction to the Discussion

This discussion interprets the results of a systematic review exploring the impact of vitamin D supplementation on postpartum dairy cows, with specific focus on uterine health, reproductive performance, immune function, and metabolic outcomes. The key objective of the review was to determine whether various types, doses, and schedules of taking vitamin D could be helpful in the transition period in recovery and productivity. The results were based on 13 articles that used different intervention regimes, which provided a solid basis of cross-domain analysis.

One of the strengths of this review is that it has a multidimensional approach that incorporates historically isolated physiological parameters. Instead of discussing reproductive outcomes only, the analysis discussed the effects of vitamin D on more global systemic processes, including calcium metabolism, inflammation, and immune cell functioning, which form the basis of cow health and fertility. This method allows getting a deeper insight into the role of vitamin D in the critical period of transition.

4.2 Interpretation of Key Findings

a) Uterine Health

Cases of uterine diseases, especially metritis and endometritis, were always linked to vitamin D supplementation. It was observed that the interventions based on the use of calcitriol or 25(OH)D3 forms, more bioavailable forms than cholecalciferol—were more effective. As an example, Vieira-Neto et al. (2021) found that overconditioned cows receiving subcutaneous calcitriol after childbirth had a high metritis rate reduction. Likewise, Poindexter et al. (2021) also reported a lower incidence of uterine infection with prepartum 25(OH)D3 administration.

The processes involved in these actions seem to be two-fold. To begin with, vitamin D helps maintain calcium homeostasis, which is important in enhancing the uterine muscle contraction and the clearance of lochia, which restricts bacterial growth (Kovacs, 2024; Deng, 2015). Second, it has immunomodulatory effects, especially by increasing the neutrophil activity, which helps in the eradication of bacterial infections (Alhussein & Dang, 2019; Disbanchong et al., 2021). These two mechanisms help to explain better the uterine health in a holistic way and justify the therapeutic use of vitamin D in early lactation. These associations are credible owing to the similarity in the studies, especially those that use strong formulations.

b) Reproductive Performance

The effect of vitamin D on reproductive performance yielded mixed but promising findings. Poindexter et al. (2021) found improved conception rates and shorter days open in cows supplemented with 25(OH)D3, especially multiparous individuals who typically face higher metabolic demands. Similarly, Mion et al. (2023) reported higher pregnancy rates in cows receiving organic trace minerals, including vitamin D.

However, Vieira-Neto et al. (2021) did not observe significant fertility improvements across all body condition scores, suggesting that vitamin D's benefits may be conditional on metabolic status. Ferdouse et al. (2024) reported better reproductive outcomes with combined vitamin supplementation, but the specific contribution of vitamin D remained unclear. These findings indicate that while vitamin D shows promise, its reproductive effects are likely mediated through interactions with energy balance and immune competence. Parity, timing of administration, and form of supplementation also appear to moderate outcomes, underlining the need for targeted, context-specific interventions.

c) Immune Function

Multiple studies support the immunomodulatory capacity of vitamin D, particularly in enhancing postpartum innate immunity. Vieira-Neto et al. (2017) demonstrated that calcitriol significantly improved neutrophil oxidative burst and phagocytic capacity—key functions in early postpartum defense. Xu et al. (2021) extended these findings by showing reduced pro-inflammatory cytokines (TNF-α, IL-6) and increased antioxidant markers (T-AOC, IgG) in cows supplemented with 25(OH)D3 and calcium.

These immune benefits align with vitamin D’s known role in modulating inflammatory pathways and innate immune responses. Enhanced immune function is particularly important during the postpartum period, when cows are vulnerable to infection due to hormonal and metabolic shifts

(LeBlanc, 2012; Wankhade et al., 2017). Although study designs varied, the consistent improvement in neutrophil function and cytokine profiles across trials suggests that vitamin D plays a clinically relevant role in immunological recovery during transition.

d) Metabolic Indicators

Vitamin D supplementation, particularly in its hydroxylated form, showed substantial benefits for metabolic regulation. Beck et al. (2022) and Xu et al. (2021) both reported elevated serum calcium and improved glucose metabolism following 25(OH)D3 supplementation. Hajikolaei et al. (2021) found that prepartum cholecalciferol combined with calcium boluses resulted in the lowest incidence of subclinical hypocalcemia, an important postpartum risk factor.

In addition to calcium, reductions in NEFA and BHB levels were observed, indicating better control of lipid mobilization and energy balance (Menta et al., 2021; Zhang et al., 2020). This is crucial, as excessive NEFA and BHB are linked to disorders like ketosis and fatty liver. Vitamin D appears to exert its metabolic benefits through improved mineral absorption and possible endocrine effects on insulin sensitivity and lipid metabolism. These findings underscore the importance of both dose and timing, suggesting that prepartum interventions may yield the most robust metabolic benefits.

4.3 Integration with Existing Literature

This review and the overall literature demonstrate a number of main similarities and emergent differences in the research on the subject of vitamin D supplementation in dairy cows after parturition. Whereas older reviews have concentrated more on the classical contribution of vitamin D in calcium homeostasis, the recent ones have broadened their viewpoint to include immune regulation, reproductive fitness, and metabolic balance (Weber et al., 2014; Goff, 2008). The multidimensionality is becoming more visible even in modern research in veterinary and animal nutrition.

One of the key points of convergence is the comparison of vitamin D3 (cholecalciferol) and a hydroxylated product of it, 25(OH)D3. It has been proven that 25(OH)D3 has better bioavailability and biological activity, particularly in the process of increasing levels of 25(OH)D serum and enhancing calcium absorption (Wang et al., 2022; Nelson et al., 2023). This justifies the mechanistic explanation of its application at the periparturient period, when hypocalcemia and immunosuppression tend to overlap. It was confirmed by Eder & Grundmann., (2022) that cows in a sufficient state of 25(OH)D were resistant to infections after giving birth because of the increase in natural immune responses. Equally, Nelson et al. (2016) also noted that 25(OH)D3 supplementation achieved better leukocyte functioning and decreased the incidence of uterine diseases in early lactation.

However, some contradictions persist. While Vieira-Neto et al. (2021) and Martínez et al. (2018) found that calcitriol and 25(OH)D3 significantly improved uterine outcomes, others such as Lean et al. (2016) noted no clear advantage in fertility metrics from supplementation alone, suggesting that baseline vitamin D status, timing of administration, and physiological context (e.g., parity, body condition score) play moderating roles. This inconsistency highlights the complexity of vitamin D endocrinology, where a threshold effect may exist—benefits emerge only under deficiency conditions (Horst et al., 2003).

Further complicating the comparison is the route of administration. Oral routes are common for D3 due to cost-effectiveness and ease of delivery, yet subcutaneous administration, especially of calcitriol, has demonstrated more immediate effects on serum calcium and immune parameters (Aggeletopoulou et al., 2024; Goff et al., 2020). Studies such as those by Weber et al. (2021) emphasized that subcutaneous calcitriol led to faster correction of hypocalcemia, supporting its utility during acute risk windows like the first 24 hours postpartum.

Timing proved to be a very important determinant. Prepartum dosing seems to be a better intervention than postpartum-only interventions. An example is a study by Carranza-Martin et al. (2025), who demonstrated that cookie-negotiating 25(OH)D3 in the final two weeks of gestation had a positive impact on the neutrophil functions and a reduced rate of uterine infections in cows after calving. Conversely, in those studies in which vitamin D was not administered prior to parturition, the reproductive or immunological advantages were not generated consistently (Warren, 2020).

This integration is further complicated by environmental and breed-specific factors. Vitamin D status also seems to be interactive with heat stress, which is known to worsen negative energy balance and oxidative stress in dairy cows. The study by Ruiz-Gonzalez et al. (2023) demonstrated a positive immune and metabolic profile of cows supplemented with D3 and calcium in the circumstances of heat stress. Previously conducted research supporting such findings provides evidence of the relationship between heat stress and vitamin D metabolism alteration and an increased risk of mastitis and metritis (West, 2003; Gupta et al., 2022).

Breed differences further complicate generalization. While most studies focus on Holstein or Holstein-Friesian breeds, a few, like Alabada et al. (2020), investigated indigenous breeds in tropical regions, finding that they responded differently to identical supplementation regimes. This points to potential genetic or adaptive metabolic differences in vitamin D utilization that deserve further exploration.

Moreover, recent meta-analyses provide mixed support for vitamin D supplementation. A meta-analysis by Moridi et al. (2020) found modest yet significant improvements in calcium status and uterine health with 25(OH)D3, but inconsistent effects on reproduction. In contrast, a systematic review by Contreras and Sordillo (2011) emphasized vitamin D's role in modulating systemic inflammation, aligning with newer studies on its immunometabolic function (Bishop et al., 2021; Spears, 2000).

One of the more novel insights emerging from recent literature is the use of vitamin D as a biomarker rather than solely as an intervention. Ryan et al. (2020) and Macmillan et al. (2020) demonstrated that early postpartum 25(OH)D3 levels predicted both reproductive efficiency and metabolic resilience, suggesting a prognostic role for vitamin D monitoring. This shift towards a biomarker framework parallels human medicine, where vitamin D deficiency is increasingly linked to chronic disease risk (Holick, 2007).

4.4 Contribution to Current Knowledge

This systematic review significantly contributes to current knowledge by offering a nuanced analysis of vitamin D supplementation across reproductive, immunological, metabolic, and uterine health parameters in postpartum dairy cows. Most notably, it elucidates the dose-response dynamics, particularly the enhanced efficacy of 25(OH)D3 over standard cholecalciferol (D3). By categorizing outcomes in relation to specific dosage levels and administration timing, the review addresses a gap in the existing literature where these variables were often inconsistently reported or evaluated in isolation. This integrative approach offers a clearer understanding of when, how, and in what form vitamin D exerts its physiological effects.

The study also advances understanding of specific physiological pathways influenced by vitamin D. For instance, it affirms the hormone's central role in calcium homeostasis, and its downstream effects on uterine involution and immune modulation (Sadiq, 2025; Tobolski et al., 2025). The link between enhanced neutrophil function, suppressed inflammatory cytokines, and reproductive success is better delineated through biomarker-oriented findings. Importantly, the synthesis of data across immunity and fertility outcomes underlines the interconnected nature of these systems, shifting the narrative from compartmentalized interpretations to one of systemic integration.

On a practical level, this review offers evidence that can inform precision supplementation strategies. By highlighting the conditional benefits of vitamin D based on factors like parity, body condition score, and heat stress exposure, it enables targeted interventions rather than blanket recommendations. Farm-level applications may include timing prepartum supplementation with 25(OH)D3 for high-risk cows, or using early postpartum 25(OH)D3 levels as prognostic markers for health monitoring.

In essence, this study refines the understanding of vitamin D not merely as a calcium regulator but as a multifaceted agent influencing a cascade of biological outcomes. It sets the stage for future research to explore individualized supplementation models based on biomarker feedback and physiological profiling.

4.5 Limitations and Assumptions

Although this review is useful, it is important to note that there are several limitations of the studies incorporated as well as the synthesis process. To begin with, many of the studies that were reviewed were observational in nature or had insufficient randomisation, which presented the possibility of selection and confounding biases. Small-sample, or single-farm, studies can be limited in their generalisability. In addition to this, heterogeneity in study design (different times, doses, forms (D3 vs. 25(OH)D3), routes of administration (oral vs. injectable) and timing of study design) made it difficult to directly compare and synthesise meta-analytically.

One of the weaknesses was the inconsistency in the outcome definition. The workings, such as reproductive performance or immune response, differed widely in measures such as conception rate or services per conception, or neutrophil activity or cytokine concentration. Such nonstandardisation limited the possibility of better establishing pooled estimates or doing subgroup analyses.

The other limitation was the lack of non-English language research and grey literature, i.e., conference proceedings or industry reports. This could have resulted in publication bias, and only those studies that had positive or significant results were published.

Things that were assumed during the extraction of the data also deserve to be recognised. Graphical representations were used to approximate numerical data in several cases, which can bring a small error. The review also presupposed a similar level of baseline cow health, nutritional control, and environmental conditions – aspects that certainly differ across regions and farms and have the potential to influence the effectiveness of vitamin D supplementation (Weir et al., 2017; Hodnik et al., 2020).

Although inclusion criteria are rigorous, the review is dependent in its own way on the quality and completeness of the source material. These restrictions highlight the relevance of careful interpretation of the results and emphasise conducting more standardised multicentric studies to determine the relevance of the numerous-faceted effects of vitamin D.

4.6 Novel Approaches and Methodological Reflections

This review has utilized a number of methodological aspects, which are innovative and rigorous and make it more reliable and profound. The distinction is that they systematically compared the impact of vitamin D3 and that of its hydroxylated counterpart 25(OH)D3 which has been the most neglected in the preceding review (Bhattacharyya and DeLuco, 1973; Brickman et al., 1976). Through this it was able to critically compare the relative bioefficacy and pharmacokinetics of these forms with the dairy cow physiology.

What is more is that the incorporation of serum biomarkers regarding 25(OH)D3 levels, calcium, NEFA, and inflammatory cytokines provided a mechanistic explanation of the relationship between supplements and actions. This synthesis based on biomarkers can be interpreted in a more accurate way as causal compared to clinical endpoints.

The rigour of methodology was achieved through adhering to the SYRCLE guidelines on the assessment of bias in animal research and PRISMA checklist of the clear reporting. The subjectivity was reduced and replicability was enhanced by the screening of the data and data extraction of two reviewers and consensus. The review has a solid basis of learning the systematic effects of vitamin D in that it includes the diverse designs of study without interfering in quality control.

Such a methodological approach, however, in the end, by not only integrating the existing data, demonstrated that multifactorial outcomes in the field of dairy science can be better explored in a holistic manner, basing on biological indicators and clinical interest.

4.7 Recommendations for Future Research

To further generalise the results of this review, the study on the subject matter should be based on the randomised controlled trials that would standardise the dosage of vitamin D, the direction of its administration and the time of taking the supplement. The difference in the results as seen between the parity and body condition score would enable the trials to be stratified to enable more specific recommendations.

Comparative studies of 25(OH)D3 and D3 under similar conditions would also be useful in order to ascertain their relative efficacies, especially when performed across different seasons in order to consider the environmental factor like that of heat stress. A trial of multi-season and multi-site would give a more in-depth picture of the interactions between geographic and management factors and the effectiveness of the supplementation.

Longitudinal designs are also recommendable. Additional knowledge on the long-term consequences of supplementation would be gained by following cows through the prepartum period to repeated lactation with the use of a vitamin D biomarker.

In addition, the integration of tools of omics, such as proteomics and metabolomics, can de-jargonise complex biological pathways that are regulated by vitamin D and define new biomarkers of supplementation reaction. It would create an opening to individualised supplementation programmes referring to real-time physiological data.

In full, the subsequent research requirement ought to be channelled towards precision nutrition because the cows and conditions are dissimilar. This will take the vitamin D research to the next level of recommendations towards a cornerstone of dairy health management.

4.8 Conclusion

This is a systematic review that aimed at examining the effect of vitamin D supplementation on the reproductive, metabolic, immune, and uterine health of postpartum dairy cows. The synthesized evidence proves that vitamin D and, specifically, 25(OH)D3 have multidimensional effects in addition to the conventional function of regulating calcium.

The review shows that health outcomes are most maximized by the timing, form, and dose of supplementation by reviewing twelve high-quality studies. It also points out that the systemic benefits of vitamin D can be accomplished with the help of interconnected pathways—regulating inflammation, calcium stability, and fertility.

These lessons have theoretical and practical senses. They provide more specific knowledge of the physiological role of vitamin D as well as education on even farm-level approaches like high-risk population supplementation. However, the shortcomings of heterogeneity, study design, and biomarker standardization are warning signs and require further research.

Finally, the review adds a complex and evidence-based story to the field of dairy science, which implies that vitamin D should be repositioned not only as a nutrient but also as a controlling agent with an extensive connotation. The future studies based on accuracy and mechanistic understanding will also be critical in making use of these discoveries in daily dairy company practice, which could guarantee not only better treatment of the animals but also the overall farm output.

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