

**(04-028) - Design thinking and experimental designing for precision nutrition application in dairy cattle's methane mitigation**

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The livestock industry is responsible for approximately 14% of the world's total greenhouse gas emissions (Aguilar-Marin et al., 2020). Within the ruminant organism enteric fermentation is the main methane creation process, producing around 86-116 million metric tons of methane per year (Chang et al., 2019; Palangi & Lackner, 2022). The diet and the ruminant microbial community are critical factors in reducing this production, especially in dairy cows. Therefore, integrating precision nutrition approaches, supported by nutrigenomics, chronobiology and microbiota of the same organism, with design thinking and design of experiments, trigger understanding and determine the changes needed in the ruminant's diet to mitigate carbon emissions, while maintaining a flexible outlook for unexpected changes, and generating creativity and innovation in solving the problems involved in methane production within ruminant organisms. The present contribution describes the solution to the defined problem.

Keywords: Design thinking; Design of Experiments; Nutrigenomics; Ruminants; Precision Nutrition; Gut Microbiome;

**Design thinking y diseño experimental para la aplicación de nutrición de precisión en la mitigación de metano en ganado lechero**

La industria ganadera es responsable de aproximadamente 14% de la emisión total de gases de efecto invernadero en el mundo (Aguilar-Marin et al., 2020). Dentro del organismo rumiante la fermentación entérica es el proceso principal de creación de metano, produciendo alrededor de 86-116 millones de toneladas métricas de metano por año (Chang et al., 2019; Palangi & Lackner, 2022). El régimen de alimentación y la comunidad microbiana rumiante son factores críticos para reducir esta producción, especialmente en las vacas productoras de leche. Por lo tanto, integrando los enfoques de nutrición de precisión, que se apoya de la nutrigenómica, cronobiología y la microbiota del mismo organismo, con las técnicas de design thinking y diseño de experimentos (DOE), detonan la comprensión y determinan los cambios necesarios en la dieta del rumiante para mitigar la emisión de carbono, manteniendo una perspectiva flexible ante cambios inesperados, y generando creatividad e innovación en la resolución de los problemas que intervienen en la producción de metano dentro de organismos rumiantes. La presente contribución, describe la solución al problema definido.

Palabras clave: Design thinking; Diseño de Experimentos; Nutrigenómica; Rumiantes; Nutrición de precisión; Microbiota intestinal

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## 1. Introduction

Due to the rising world population, which is estimated to rise from 8.1 billion people (2024) to 8.5 billion people (2030) (FAOa, 2024), there is a need to increase production of animal-source foods (de Ondarza *et al.*, 2023). Agriculture and livestock practices are among the top industries that contribute to the source of methane (CH<sub>4</sub>) emissions; they are responsible for approximately 14% of the world's total greenhouse gas emissions (Aguilar-Marin *et al.*, 2020; Rivera & Chará, 2021; Pitta *et al.*, 2022). However, it is one of the most sensitive industries to climate change (Wu *et al.*, 2023).

The sectors that have the largest methane emissions from the livestock industry are enteric fermentation and manure management (Wu *et al.*, 2023). Enteric fermentation, which is the main source of methane emission, was estimated in two evaluations, 2000-2009 and 2008-2017, to produce around 86-116 million metric tons of methane per year (Chang *et al.*, 2019; Palangi & Lackner, 2022). On the other hand, ruminant manure storage can produce approximately 109 million tons of methane per year, wherein 86% originates from cattle (Rivera & Chará, 2021). And according to the Food and Agriculture Organization of the United Nations these quantities will only rise (Table 1) (FAOb, 2024).

**Table 1: Total world emissions of methane (CH<sub>4</sub>) of ruminant livestock for the years 2021, 2030 (Forecast value), and 2050 (Forecast value) (FAOb, 2024).**

Ruminant organism	Value (Tg)	Year
Buffalo	12.08	2021
Cattle, dairy	20.69	
Cattle, non-dairy	56.89	
Goats	5.75	
Sheep	7.29	
Total estimated CH <sub>4</sub> emission value from ruminant livestock		102.70
Buffalo	12.24	2030
Cattle, dairy	21.80	
Cattle, non-dairy	61.43	
Goats	5.64	
Sheep	7.93	
Total forecast CH <sub>4</sub> emission value from ruminant livestock		109.05
Buffalo	13.89	2050
Cattle, dairy	23.84	
Cattle, non-dairy	67.87	
Goats	6.92	
Sheep	8.92	
Total forecast CH <sub>4</sub> emission value from ruminant livestock		121.44

Cattle are the top contributor of methane emission from all the livestock animals, including buffalo, chickens, ducks, goats, swine, among others; it is responsible for 70% of the total methane emissions (FAOb, 2024). Several factors contribute to methane emissions from this livestock animal, such as cattle breed, lactation status, geographic region, feed chemistry, and animal characteristics (Yan *et al.*, 2006; Shibata & Terada, 2010; Liu *et al.*, 2017; Zhang *et al.*, 2020; Wu *et al.*, 2023). Multiple efforts have been studied to reduce methane production, such as dietary manipulation, rumen manipulation, genetical selection, and in recent years anti-methanogenic vaccines (Beauchemin *et al.*, 2022; Smith *et al.*, 2022).

Precision nutrition refers to the science of tailoring dietary regimens to meet the specific nutritional needs of individual animals or groups, optimising their health, and performance (Zuidhof, 2020). When applied to dairy cattle, precision nutrition involves formulating diets to provide the optimal balance of nutrients, such as carbohydrates, proteins, fats, and minerals; to support milk production, reproduction, and overall well-being (González *et al.*, 2018; Zuidhof, 2020). Considering an environmental impact, the objective of precision animal nutrition is to formulate low-cost diets for more responsible production where animal performance is high and waste is as minimal as possible, without compromising health and well-being. This may involve adjusting diet composition and optimising nutrient utilisation to minimise excess fermentation in the rumen, and enhance digestive efficiency (Wu *et al.*, 2023).

Feeding practices play a crucial role in methane mitigation in dairy cattle, as methane is a byproduct of the digestion process in ruminants (Beauchemin *et al.*, 2022; Wu *et al.*, 2023). Ruminants have a digestive system that relies on microbial fermentation. The rumen, which is a compartment from the digestive system is where microbes break down fibrous plant material (e.g. grasses, and grains) into volatile fatty acids and gases, including methane (Cammack *et al.*, 2018). Therefore, the composition of the diet and feeding practices directly influence methane emissions from dairy cattle. Diets high in fibrous materials, such as forages, can result in elevated methane emissions compared to diets rich in easily digestible carbohydrates (Yan *et al.*, 2006; Haque, 2018; Króliczewska *et al.*, 2023).

Addressing methane emissions from dairy cattle is crucial for sustainable development, particularly in line with the objective 13 of the Sustainable Development Goals (SDGs), which focuses on climate action (United Nations, 2024). Methane is a greenhouse gas with a significantly high global warming potential over a relatively short time frame (Chang *et al.*, 2019). By developing sustainable solutions to reduce methane emissions from cattle, such as optimising feeding strategies, improving dietary efficiency, and implementing methane reduction technologies; we can contribute to mitigating climate change while ensuring the long-term viability of dairy farming systems (Beauchemin *et al.*, 2022; Tseten *et al.*, 2022).

Design thinking allows the researcher to take a more user-orientated approach as it questions the user for its needs and addresses complex issues to solve problems (Buhl *et al.*, 2019; Cai, Lin, & Zhang, 2023). Depending on the author, design thinking can have multiple innovation phases: problem framing, user focus, diversity, visualisation, experimentation, and iteration (Buhl *et al.*, 2019); discovery, ideation, experimentation, and implementation (Cai, Lin, & Zhang, 2023); or empathise, define, ideate, prototype, test and assess (Hurst & Spiegel, 2023). Moreover, incorporating different project methodologies, such as soft systems, within each stage can potentialize design thinking to ideate more powerful creative ideas. Design of experiments is useful to perform an accurate systematic evaluation of the variables involved in the process. It provides information on the main effects and interactions over a response variable (Bhadani *et al.*, 2024; Ebri & Klaus, 2024). The combination of both design thinking and design of experiments produces creative solutions that take into consideration the influence of the factors, variables, or parameters involved in the project's systems (Gonzalez-Almaguer *et al.*, 2021).

Involving, design thinking and design of experiments with dynamic systems and soft

methodologies, will help explain all the parts that are involved and how they can be modified to reach the final goal by simulating the design processes. Furthermore, the application of this blending into solutions to mitigate methane emissions from Holstein dairy cattle will enable a comprehensive approach. By understanding user needs, we can ideate innovative strategies and systematically evaluate their effectiveness to lead with practical and effective solutions for reducing methane emissions while ensuring the sustainability of milk farming. Moreover, it constructs creative ecosystems to understand the problem and enable a diverse generation of solutions, while also maintaining flexibility to modify solutions, their implementation and their validation if required, and an adaptability for their continuous improvement in the future.

## 2. Objectives

As mentioned before, the livestock sector is responsible for a significant part in the production of global greenhouse gases, especially methane. The diet and the ruminant microbial community, which is responsible for the fermentation process, are critical factors in reducing this production. The present contribution aims to answer *how to reduce methane emissions from Holstein dairy cows with biotechnological and nutritional feeding tools without affecting milk production?* with the help of design thinking, design of experiments, and soft system methodologies to understand, describe and evaluate solutions to the dairy cattle methane emissions problem.

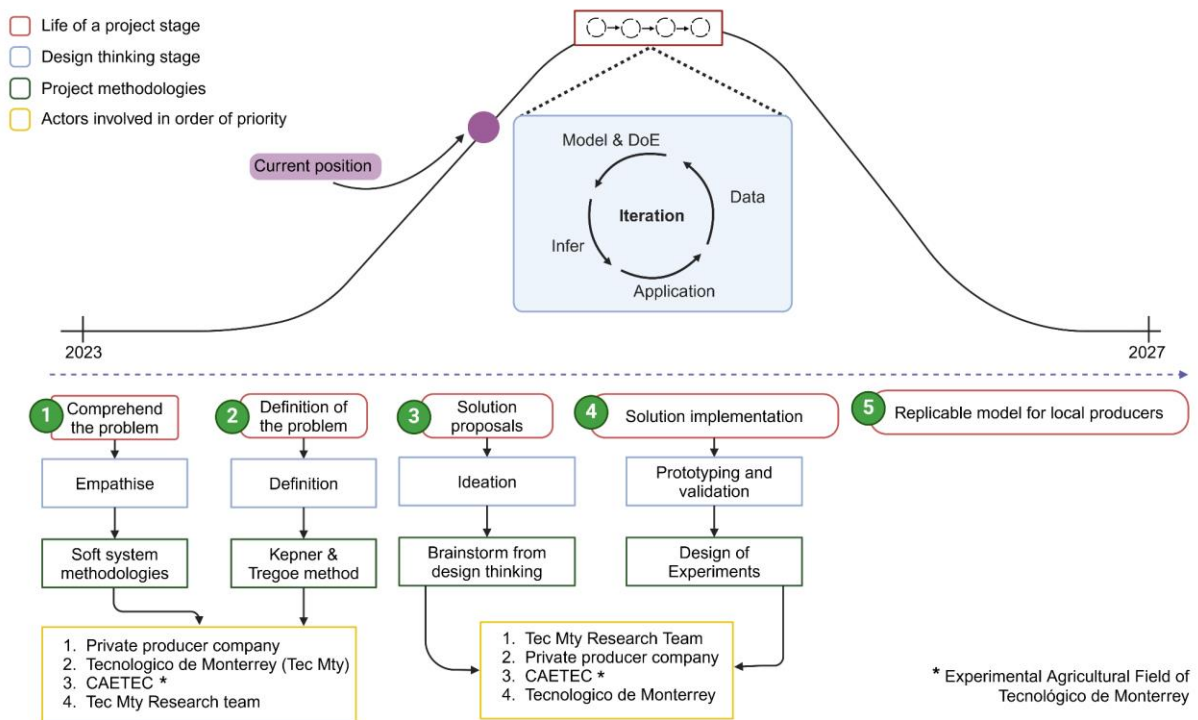
## 3. Methodology

As a first step to understand the problem, a dialogue was held with the involved parties, initially including the food company interested in providing solutions to the dairy farmers that supply it with milk, the University Tecnológico de Monterrey, Querétaro campus, which handles the initial administrative management, and the Experimental Agricultural Field of Tecnológico de Monterrey (CAETEC, Spanish acronym), where the experimental part is and will be conducted. In this step, the soft systems methodology is applied to visualise the problem as a process involving different factors, considering the needs of the food company and the end user, who in this case are the dairy farmers who will implement the sought-out solution. For the problem definition stage, the Kepner and Tregoe methodology is applied to provide a better structure.

Subsequently, in the ideation stage, the involved parties are not only the company, the university, and the experimental field, but also a team of scientific researchers. To suggest solutions the scientific research team used brainstorming, which is a tool from Design Thinking, and a deep and thorough literature search to generate ideas. The proposals must be approved by all the involved parties before proceeding to the next step. Currently, the project is in this transition; and is moving towards prototyping and validation. Moreover, the university is no longer involved as a main actor because the administrative processes have been completed, making the research team the leading actor. Nonetheless, the food company will continue to be consulted for decision-making, and CAETEC will serve as the experimental space.

This stage is crucial due to the application of design of experiments as a tool to evaluate the proposed ideas. Moreover, during this phase, data is managed according to the model and the DoE, allowing inferences to be made and data to be re-applied to conduct more tests until a strategy, that shows the most favourable results for significantly reducing methane emissions per kg of milk produced and has an accessible cost, has been achieved. Validation is ongoing as long as there is continuous improvement in the proposed solutions. This step is always aiming for better replicable and accessible solutions for the region's dairy farmers to be found, without neglecting animal and consumer welfare (Figure 1).

Figure 1: Projects methodology, Created with BioRender.com.



#### 4. Development

Design thinking can help scientific researchers understand the needs and challenges of dairy farmers and food companies by using their user-focus approach through collaborative ideation and prototyping (Buhl *et al.*, 2019) and generating innovative solutions to be applied in dairy barns. It helps researchers identify novel approaches to reduce methane emissions while considering factors such as cost-effectiveness, practicality, and scalability. On the other hand, the design of experiments, a statistical tool to validate results by researchers, can be used to systematically evaluate the effects of various dietary interventions and environmental factors on methane emissions, changes in ruminal microbiota, digestibility, and milk production.

By following the five-stage methodology of design thinking: Empathise, Definition, Ideation, Prototyping, and Validation, as mentioned by Hurst & Spiegel (2023), and integrating design of experiments in the last two steps (Figure 1) as noted by Gonzalez-Almaguer and collaborators (2021), and using soft system methodologies in the first two steps; the research team, consisting of experts in animal nutrition, biotechnology, microbiology, and data analysis, was able to propose solutions and strategies based on the needs of the users, who in this case are the dairy farmers and a private food company, to reduce methane emissions while maintaining milk productivity and ensuring animal health.

These methodologies will be implemented in a study which will be conducted at CAETEC, a semi closed farm system that ensures traceability and control over forage production and livestock management. For the initial steps, several administrative meetings were held to understand the needs of the dairy farms and the interests of the private food company. Subsequently, work sessions were planned for the scientific team and several questions were generated (Table 2) to trigger ideas and define solutions following the established criteria provided by the users:

1. Wellbeing of the dairy cattle.

2. Safety in measurement practices for the dairy cattle.
3. Safety for consumers.
4. Soil safety.
5. Economic viability.

**Table 2: Generated queries for producing nutrition feed strategies to mitigate dairy cattle methane emissions.**

Innovation stage	Questions generated
Empathise	<ul style="list-style-type: none"> <li>● <i>What is the issue?</i></li> <li>● <i>What can be the target of change?</i></li> <li>● <i>Which is the user?</i></li> <li>● <i>Who is affected by the problem?</i></li> <li>● <i>What are the limitations of the challenge?</i></li> <li>● <i>What is it essential to consider?</i></li> </ul>
Definition	<ul style="list-style-type: none"> <li>● <i>How to delimit the problem?</i></li> <li>● <i>Which is the problem statement?</i></li> <li>● <i>What other problems can be generated by proposed solutions?</i></li> <li>● <i>What are the objectives?</i></li> <li>● <i>What is the hypothesis?</i></li> <li>● <i>Which is the origin of the issue?</i></li> </ul>
Ideation	<ul style="list-style-type: none"> <li>● <i>What has been done before?</i></li> <li>● <i>What still can be done?</i></li> <li>● <i>What resources are needed for the solution?</i></li> <li>● <i>What impact can the solutions have in the involved parts of the issue?</i></li> </ul>
Prototyping	<ul style="list-style-type: none"> <li>● <i>How many solutions can be experimented?</i></li> <li>● <i>What is the user's feedback?</i></li> <li>● <i>What information do we need to generate to see a successful solution?</i></li> <li>● <i>Is the user in agreement with the selected solution proposal?</i></li> <li>● <i>What are the controllable variables for the solutions implementation?</i></li> <li>● <i>Is all necessary information for the solutions implementation available?</i></li> <li>● <i>Is it needed a previous step or adjustments before implementing the solutions?</i></li> <li>● <i>What permits, safety procedures/methodologies and ethical</i></li> </ul>

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	<p><i>approvals are needed for the solutions implementation?</i></p> <ul style="list-style-type: none"><li>• <i>For the control and experimental group, what information and characteristics are needed for the solutions implementation?</i></li><li>• <i>Are there risks and challenges to implement the solutions?</i></li><li>• <i>How to evaluate the impact between existing solutions and the proposed solution?</i></li></ul>
Validation	<hr/> <ul style="list-style-type: none"><li>• <i>How to measure the progress of the implemented solutions?</i></li><li>• <i>How to measure the success of the implemented solutions?</i></li><li>• <i>How to compare the resulting solutions to existing or alternative solutions?</i></li><li>• <i>What statistical analysis can be implemented?</i></li><li>• <i>What is the user's feedback?</i></li><li>• <i>What is the scalability of the implemented solution?</i></li><li>• <i>What is the long-term impact of the solution?</i></li><li>• <i>What is the economic viability of the implemented solutions?</i></li><li>• <i>Are the results in-line with the objective and hypothesis?</i></li><li>• <i>Are there other added-value effects?</i></li></ul> <hr/>

By answering the questions, it was possible to identify the focus point, finish defining the problem, know the area that will be impacted/treated, as well as point out the necessary tools and variables to be considered during the experimental design. Moreover, addressing these questions enabled the research team to generate creative ideas and devise solutions that align with user expectations, including those of private companies and dairy farm producers. This approach allowed for flexibility in the ideation and prototyping process, guided by the collected data. Additionally, it helped us identify validation techniques tailored to user needs and expectations.

## 5. Results

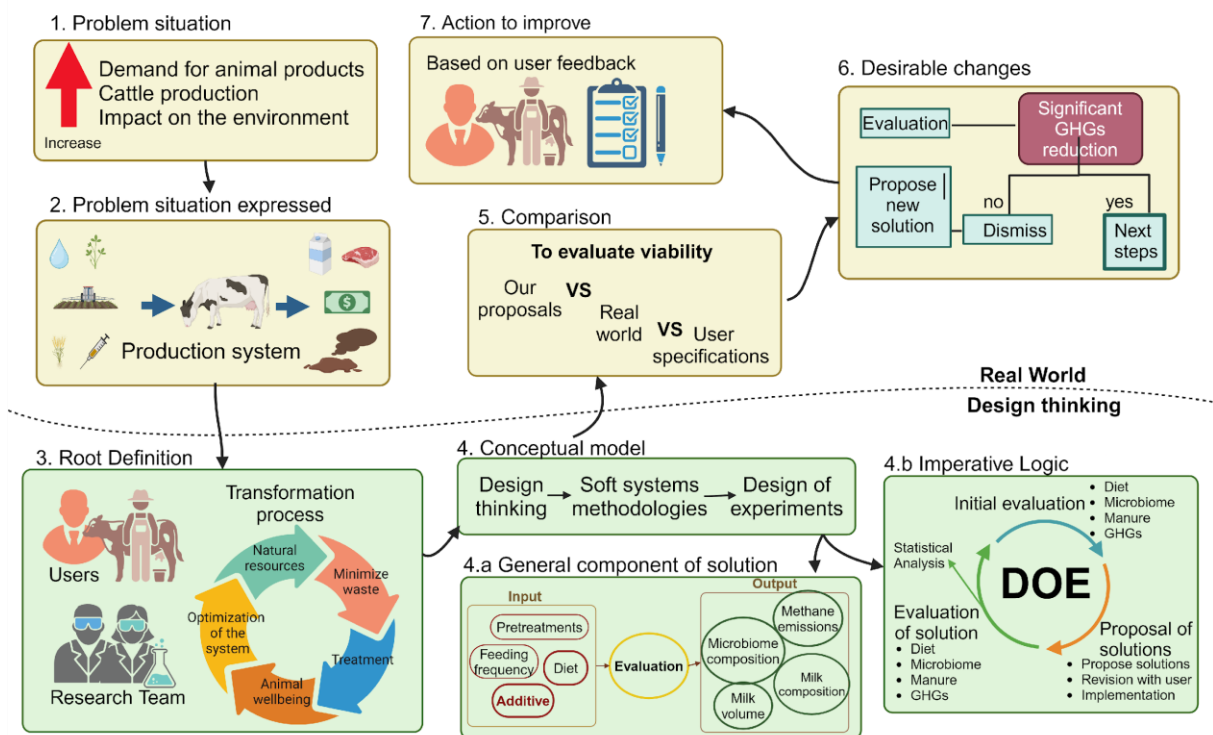
During several work sessions and meetings with the stakeholders' the research team was able to answer the generated questions (Table 2). This enabled establishing a robust foundation for implementing experiments aimed to solve the present issue and create an integrated conceptual framework with design thinking, design of experiments, soft system methodologies, and the most vital information.

### 5.1 Empathise

For this stage, the scientific research team answered the questions that were generated for this step (Table 2): What is the issue? What can be the target of change? Which is the user? Who is affected by the problem? What are the limitations of the challenge? What is it essential to consider? By answering these questions, the group was able to create a rich picture of the problem (Figure 2). In addition, it was found that dairy cattle are the second highest ruminal livestock animal that produces methane, following non-dairy cattle. Because of this, it was

thought that cattle are a potential target to reduce the industries carbon footprint. By researching the organism of focus, the solution was decided to be: 1) Change the enteric fermentation of the dairy cow by changing the feed by adding a supplement; or 2) Modify the residual manure management. Moreover, to further understand the challenge, several factors were needed to take into consideration: Quality/Quantity (Q/Q) of milk, symbiosis of ruminal microbiome and dairy cattle, enteric fermentation, digestibility, source origins, supply chain of resources, management of wellbeing of the cattle and of the cattle's waste, and economic viability. Ultimately, it was decided to go through both targets: changing the diet and implementing a way to reduce the emissions from manure. However, the present paper will only focus on dietary interventions to the feed of the Holstein cattle breed found at CAETEC.

**Figure 2: Rich picture of the problem; an application of soft system methodologies in addition to design thinking and design of experiments to ideate solutions, Created with BioRender.com.**



## 5.2 Definition

To delimit the problem and answer the generated questions for this stage, a new question was discussed: *How to reduce methane emissions from Holstein dairy cattle within our own field of expertise?* When answering with biotechnology, microbiology, and animal nutrition, another question came out with the purpose of defining the project's objective: *How to reduce methane emissions from Holstein dairy cattle with biotechnological and nutritional feeding tools without affecting milk production and animal health?* This was answered with: 1) Change the enteric fermentation via microbiome manipulation; 2) Change the diet composition to modify microbiota composition and thus enteric fermentation; 3) Not change but add to the diet to change microbiome and fermentation; and 4) Intervene in early stages to modify the rumen composition. With the ideas generated to answer the question, which the most viable were putting additives to the feed and evaluate the change they produce via the microbiome, a hypothesis was formulated: *The implementation of successful modifications to the diet of dairy cattle can produce changes to the ruminal and faecal microbiome, which can change the methanogenesis route within the enteric fermentation and reduce methane emissions.* With



this statement, the problem's definition was elucidated. It's worth mentioning that all the generated questions follow the basis of the Kepner-Tregoe method, which as mentioned before can help frame a problem better.

### 5.3 Ideation

This stage seeks to open minds and produce as many ideas as possible; it was carried out with the help of work sessions of the scientific team where multiple proposals were formulated based on scientific articles. 487 scientific papers and books were found to have significance in the topic. Previous efforts to reduce methane emissions from Holstein dairy cows have involved various approaches, including dietary modifications, supplementation with additives or probiotics, and management practices aimed at improving feed efficiency and reducing enteric fermentation (Tseten *et al.*, 2022; Palangi & Lackner, 2022; Haque, 2018; Beauchemin *et al.*, 2022).

Despite advancements in methane mitigation strategies, there is still room for further research and innovation. Future efforts could involve novel biotechnological interventions, such as genetic selection for low-methane-producing cattle strains, ruminal microbiome manipulation, development of specialised feed additives with enhanced efficacy and sustainability, integrating management practices, and optimising feeding regimes based on precision nutrition principles. In this step, incorporating brainstorming from design thinking and information from previous scientific studies helped to identify several opportunities and alternative solutions, which are proposed to the food company to assist in decision-making according to their needs. Once authorised by all involved parties and an ethics committee, the next step can be taken with the *in vivo* tests.

### 5.4 Prototyping & Validation

Precision nutrition entails customising diets to address the unique requirements of individual animals (Zuidhof, 2020). This project aims to formulate diets that reduce methane emissions without compromising milk production. This may involve adjusting the composition and feeding practices to optimise nutrient utilisation, changing fermentation products in the rumen, and enhancing digestive efficiency (Wu *et al.*, 2023).

After analysing the obtained data from the ideation stage, the current steps involved using the design of experiments to conduct a first evaluation or baseline analysis. This analysis will examine the current nutritional profile of the Holsteins dairy cattle's diet. It must take into consideration uncontrollable variables, such as quality/availability of food, genetic factors, and individuality variability; as well as controllable variables like diet composition and quantity, feeding schedules, environmental conditions, and milking time protocols. Moreover, to understand the changes that the additive will make to the enteric fermentation, different protocols will be used to characterise the ruminal and faecal microbiome; the research team will assess their efficiency. Other measurements that will be carried out include methane, ammonia, and nitrous oxide (N<sub>2</sub>O) emissions, as well as milk yield and composition.

Furthermore, currently there are discussions with the users (private company and head-management of CAETEC) to determine if they are agreeable with the proposed solutions, as well as confirming the sample size and the measurement techniques to be used. Other current discussion involves selecting the design of experiments method, such as Latin square, Random block, and/or factorial arrangement, that will be used for the dietary experimental part, and deciding which statistical analysis will be used to validate the data and evaluate how the variables are affected; presently a regression analysis, which will allow to analyse both categorical and numerical variables, is being considered.

## 6. Future steps and areas of opportunity

A positive control test is being planned to be implemented during the months of July-November 2024; this validation will be done using Bovaer (DSM, Bright Science Brighter Living™, Heerlen, Netherlands) the go-to compound to just reduce methane emissions in cattle. Further into the future, there will be the experimental implementation of the selected feed additives to reduce methane emissions; it is expected to start testing them late 2024 or early 2025. The uncontrollable and controllable variables will stay the same as the baseline analysis to prevent unwanted bias. Similarly, an assessment of the changes that the ruminal and faecal composition had will be performed, and likewise a comparison analysis will be made from the data obtained from the preliminary evaluation and the future evaluation to compare methane emissions and identify if there has been a reduction or an improvement in milk yield parameters.

The dietary interventions will be done on a group of 10 cows, which will be randomly selected within a group of the same number of lactations and milk yield, another 10 cows will be used as the control group (without the additive feeding). The methane emission measurement will be done with the help of three GreenFeed systems (C-Lock Inc., Rapid City, South Dakota, USA). Both feedings will be provided to the cows with a BouMatic feeding system (BouMatic, Madison, USA). Milk will be collected using a Voluntary Milking System (DeLaval, Tetra Laval Group, Pully, Switzerland) and it will be sent to an external laboratory for characterization. Rumination monitoring will be registered using the Sense Hub™ monitoring collars (Merck & Co., New Jersey, USA).

## 7. Conclusions

The application of methodologies, such as design thinking, design of experiments, and soft systems, in the study of methane emissions in dairy cattle has allowed us to understand this complex problem more deeply and in detail. Through the formulation of key questions, it has been possible to identify solutions and propose an organised project. The clear definition of the project's range and the focus on biotechnology and animal nutrition have generated a clear path for research. The results obtained so far lay the groundwork for future experimental phases in animals, which will allow the assessment, validation, and approval by stakeholders of the solutions proposed by the team.

Crucially, the success of this project hinges on the active collaboration between the academy, comprising the research team and the university, and the industry. This partnership ensures that the research is grounded in practical application and benefits from the academic rigour and innovative thinking, while also addressing real-world industry needs and constraints. The continuous involvement of both sectors facilitates the development of solutions that are not only scientifically robust but also practically viable and ready for implementation by dairy farmers. This integrated approach is essential for achieving sustainable and impactful outcomes in methane emission reduction.

In summary, the application of various methodologies has generated ideas and fundamental decisions to the successful development of the research project on methane emissions in dairy cattle. This convergence represents a powerful paradigm to face various issues, promoting synergy between user-oriented creativity and scientific rigour, thus offering global and effective responses to complex challenges. Furthermore, highlighting the value of this methodology as a research design tool, it provides a structured yet flexible framework that enhances problem-solving capabilities while ensuring innovative approaches to complex environmental challenges.

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