

UNIVERSIDADE FEDERAL DE VIÇOSA

**Animal welfare and innovation: pursuing improvements in the dairy system
and new technologies**

Tássia Barrera de Paula e Silva
Doctor Scientiae

**VIÇOSA - MINAS GERAIS
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Thesis submitted to the Animal Science
Graduate Program of the Universidade
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the requirements for the degree of *Doctor
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Adviser: Polyana Pizzi Rotta

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To my parents, with all my love.

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“Você nunca poderá amar uma pessoa tanto quanto pode sentir a falta dela.”
(John Green)

ABSTRACT

SILVA, Tássia Barrera de Paula e, D.Sc., Universidade Federal de Viçosa, January, 2025. **Animal welfare and innovation: pursuing improvements in the dairy system and new technologies.** Adviser: Polyana Pizzi Rotta.

The dairy production system faces the challenge of meeting the growing demand for animal-derived foods while balancing production efficiency, animal welfare, and sustainability. In this context, innovative practices and the use of emerging technologies have stood out as promising strategies to improve herd management, animal health, and milk quality. The first study in this thesis explores the use of polycresulene as an alternative method for dehorning dairy calves, compared to the traditional hot-iron method. Although the polycresulene treatment caused less immediate discomfort, most calves exhibited horn regrowth in the long term. Additionally, a consumer survey revealed that individuals without experience in agribusiness tend to value animal welfare practices and are willing to pay more for products from such farms, whereas those with agricultural experience prefer conventional methods. The second study investigated the association between teat hyperkeratosis (HY), teat size, and age in Holstein cows. Larger teats and older animals showed a higher risk of developing HY, possibly due to structural changes after multiple lactations. Proper milking management and correct equipment settings were highlighted as essential strategies to prevent this condition and preserve animal welfare. The third study focused on measuring milk volume in bulk tanks, comparing manual readings using a graduated stick with an automated monitoring system based on the Internet of Things (IoT). While both approaches demonstrated good accuracy, the IoT system showed a high degree of reliability and potential to reduce human error, promoting greater efficiency and traceability in the production process. Taken together, these studies highlight the importance of technological innovation, animal welfare, and milk quality as key pillars for the sustainable advancement of dairy farming.

Keywords: animal welfare; dehorning; dairy production; iot system

RESUMO

SILVA, Tássia Barrera de Paula e, D.Sc., Universidade Federal de Viçosa, janeiro de 2025. **BEM-ESTAR ANIMAL E INOVAÇÃO: BUSCANDO MELHORIAS NO SISTEMA DE PRODUÇÃO DE LEITE E NOVAS TECNOLOGIAS..** Orientadora: Polyana Pizzi Rotta.

O sistema de produção leiteira enfrenta o desafio de atender à crescente demanda por alimentos de origem animal, conciliando eficiência produtiva, bem-estar animal e sustentabilidade. Nesse cenário, práticas inovadoras e o uso de tecnologias emergentes vêm se destacando como estratégias promissoras para promover melhorias no manejo, na saúde dos animais e na qualidade do leite. O primeiro estudo desta tese aborda o uso de policresuleno como método alternativo de descorna em bezerras leiteiras, em comparação ao método tradicional com ferro quente. Embora o tratamento com policresuleno tenha causado menos desconforto imediato, a maioria das bezerras apresentou crescimento dos chifres em longo prazo. Além disso, uma pesquisa com consumidores mostrou que pessoas sem experiência no agronegócio tendem a valorizar práticas de bem-estar animal e estão dispostas a pagar mais por produtos provenientes dessas fazendas, enquanto indivíduos com experiência agrícola preferem métodos convencionais. O segundo estudo investigou a associação entre hiperqueratose (HY) no teto, tamanho do teto e idade de vacas Holandesas. Tetos maiores e animais mais velhos apresentaram maior risco de desenvolver HY, possivelmente devido a mudanças estruturais após várias lactações. O manejo adequado da ordenha e a correta regulação dos equipamentos foram destacados como estratégias essenciais para prevenir essa condição e preservar o bem-estar animal. O terceiro estudo abordou a medição do volume de leite em tanques a granel, comparando a leitura manual por régua graduada com um sistema automatizado de monitoramento por Internet das Coisas (IoT). Embora ambas as abordagens tenham demonstrado boa precisão, o sistema IoT mostrou alto grau de confiabilidade e potencial para reduzir erros humanos, promovendo maior eficiência e rastreabilidade no processo produtivo. Em conjunto, esses estudos destacam a importância da inovação tecnológica, do bem-estar animal e da qualidade do leite como pilares para o avanço sustentável da pecuária leiteira.

Palavras-chave: bem estar animal; descorna; produção de leite; sistema de internet das coisas

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1. INTRODUCTION

General concept of animal welfare

Animal welfare has become a prominent topic over the past decade, receiving extensive attention in media outlets, industrial sectors, and scientific research. This growing interest is driven not only by consumer demands for ethically produced animal-derived products but also by the regulatory requirements of importing countries and the ongoing pursuit of less invasive and more efficient management practices by professionals in animal production systems.

The modern discussion of animal welfare began in 1964 when journalist Ruth Harrison, in the United Kingdom, raised concerns about the treatment of production animals. Harrison argued that animals were often treated as machines rather than sentient beings, leading to the publication of her influential book, *Animal Machines* (Van de Weerd and Sandilands, 2008). Her critiques captured the attention of British authorities and prompted the establishment of the Brambell Committee in 1965.

The Brambell Committee was tasked with evaluating animal husbandry practices and the conditions within intensive production systems, ultimately proposing recommendations to improve animal welfare. From this work emerged a document defining fundamental freedoms for animals, including the ability to stand, lie down, turn around, stretch their limbs, and express natural behaviors. These principles, later termed the "Five Freedoms of Brambell" established the minimum standards for the ethical treatment of production animals (Broom, 2011).

Building on these principles, Hughes (1976) defined animal welfare as a state of balance between the animal and its environment, emphasizing complete physical and mental health. However, this definition primarily focuses on positive conditions, excluding scenarios where animals face challenges. To address this limitation, Broom (1986) expanded the concept to encompass both positive and negative experiences, defining welfare as the state of an individual in relation to its ability to adapt to the environment in which it lives (Broom, 2011).

In 1979, the British Farm Animal Welfare Council (FAWC) was established and subsequently reformulated Brambell's principles into the widely recognized "Five Freedoms of Animal Welfare". These freedoms stipulate that animals should be: (1)

Free from hunger, thirst, and malnutrition; (2) Free from pain, injury, and disease; (3) Free from discomfort; (4) Free from fear and distress; (5) Free to express normal behaviors.

These guidelines became the foundation for global animal welfare policies and practices (FAWC, 2009). While the Five Freedoms serve as a general reference and establish a minimum ethical standard for animal care, they focus exclusively on mitigating negative experiences and do not account for elements of positive welfare. Positive welfare aims to provide animals with enriching and stimulating experiences, which are increasingly recognized as vital components of overall well-being (FAWC, 2009).

The Five Domains of Animal Welfare

Building on the foundations established by the Brambell Committee and Broom's definition of animal welfare, Mellor and Reid (1994) developed an animal welfare assessment model initially intended for animals used in research, education, and testing in New Zealand. Over time, this concept expanded to include companion animals, farm animals, wild animals (both in captivity and in the wild), and even animals classified as pests (Mellor et al., 2012).

To address the growing demand for practical welfare assessments, the model underwent significant evolution, culminating in the development of the Five Domains model by Mellor (2009). This framework considers four interactive physical-functional domains—Nutrition, Environment, Health, and Behavior—and a fifth domain that focuses on the animal's mental state.

The first three domains evaluate physiological and pathophysiological disturbances, assessing whether the animal can maintain homeostasis and identifying potential disruptions caused by deficiencies in nutrition, environmental challenges, or health impairments. The fourth domain examines the animal's capacity to express natural behaviors, including physical, social, and biotic interactions. The fifth domain integrates the outcomes of the preceding domains, emphasizing how these factors interact to influence the animal's overall mental state, incorporating both positive and negative affective experiences (Mellor et al., 2012).

Examples of these interactions include deprivation of feed and water, which triggers neural responses linked to hunger and thirst; injuries that generate pain

through nociceptive pathways; and external threats that induce fear via sensory inputs such as sight, sound, or smell (Mellor and Beausoleil, 2015). Initially, the model emphasized negative affective states, such as hunger, pain, fear, and distress, as proposed by Mellor and Reid (1994). However, subsequent revisions expanded the framework to include specific affective states such as nausea, weakness, frustration, boredom, and loneliness, enhancing the depth of welfare evaluations (Mellor et al., 2012).

Despite its utility, the original Five Domains model predominantly focused on mitigating welfare impairments, highlighting the absence of suffering rather than the presence of positive welfare states. Recognizing this limitation, Mellor and Beausoleil (2015) proposed updates that emphasized the importance of positive affective experiences. These included the enjoyment derived from quenching thirst, satisfying hunger, and engaging with enriching environments. Additionally, they highlighted pleasurable experiences such as energy, social interactions, comfort, and security. These positive stimuli can shift an animal's welfare state from neutral or negative to predominantly positive, reinforcing the importance of environmental enrichment in promoting sustained welfare improvements (Edgar et al., 2013; Boissy and Lee, 2014).

The updated Five Domains model integrates the foundational principles of the Five Freedoms—Nutrition, Environment, Health, Behavior—and extends them by emphasizing emotional welfare through the inclusion of positive mental states. This holistic approach reflects the growing consensus that welfare encompasses more than the absence of suffering and requires proactive efforts to enhance the quality of life for animals. Over time, these concepts have been adopted by international organizations such as the World Organization for Animal Health and have influenced the development of national welfare legislation, certification protocols, and assessment methodologies worldwide, ensuring not only physical and emotional health but also a high quality of life for animals (Broom, 2017; Spigarelli et al., 2021).

Animal Welfare and Consumer Perception of Dairy Products

Significant transformations in public interest issues have intensified over the past few decades, including concerns about the inefficient use of natural resources, negative human health impacts, environmental degradation, and low animal welfare standards. Additionally, the misapplication of sustainable practices in livestock

systems and by suppliers of animal-derived products remains a pressing issue. These factors have contributed to shifts in consumer eating habits, with greater demand for products certified to meet animal welfare standards (Alonso et al., 2020).

For the food industry, understanding consumer perception is crucial, as it directly influences product selection, purchase behavior, marketing strategies, and market positioning. Industries approach animal welfare not only from an ethical standpoint but also as a commercial and economic objective (Verbeke, 2009).

Consumers typically associate animal welfare with free-living conditions, non-confinement, and access to open pastures. In a systematic review of public attitudes and behaviors toward farm animal welfare, Clark et al. (2016) reported that consumers appreciate the safety, consistency, and accessibility of food products facilitated by modern agriculture. However, they also express concerns about the intensification of production systems. A subset of consumers with stronger ethical concerns about animal welfare has reduced their consumption of animal-derived products and demonstrated a willingness to pay a premium for products certified as meeting welfare standards.

Conversely, according to Alonso et al. (2020), consumers perceive "animal-friendly" products as safer, more acceptable, and environmentally sustainable. However, these products are often considered "premium" and come at higher prices. As a result, despite a general interest in animal welfare, consumers tend to prioritize other product attributes, such as price, when making purchasing decisions. Vanhonacker et al. (2010) reported similar findings, suggesting that high prices limit consumer demand for welfare-certified products.

The situation is no different for dairy products, particularly milk. Consumers express concerns about farm management practices and distinguish between products based on production system characteristics, including the type of housing and confinement used for dairy animals (Graaf et al., 2016). In a study on consumer intentions to buy welfare-certified milk, Graaf et al. (2016) found that consumers considered both price and animal welfare equally important in their purchasing decisions. However, price remained the primary determinant of purchase behavior, despite growing concerns for animal welfare. The authors suggest that this concern may lead to an overestimation of welfare importance and an underestimation of price sensitivity.

In general, products certified for sustainability and animal welfare add value but also increase costs, which can hinder their acceptance by consumers. This issue is exacerbated by limited public knowledge about animal welfare and a lack of industry and government initiatives to disseminate information (Verbeke, 2009).

Kitano et al. (2022) investigated the impact of information asymmetry on consumer behavior in the milk market in Japan. They found that accurate information about production systems significantly influenced consumer behavior and increased hypothetical demand for milk produced under welfare-certified conditions. Similarly, Vargas-Bello-Pérez et al. (2015) reported that most consumers had limited knowledge of animal welfare practices during production and processing. Among their study participants, only 12.4% associated welfare with production animals, while 73% expressed interest in more information and were willing to pay higher prices for certified products.

Social media plays a critical role in shaping public perceptions of animal welfare, frequently pressuring production systems to adopt better handling practices. However, misinformation disseminated through social media often misguides consumers, further distancing them from the realities of the production chain (Alonso et al., 2020). Clear, scientifically accurate information, whether shared through product labeling or social media, could enhance both consumer understanding and product value, benefiting both industries and consumers alike (Graaf et al., 2016; Zuliani et al., 2018).

Animal Welfare in Dairy Systems

Evaluating the welfare of dairy cattle presents unique challenges, as animals are often housed in groups and confined, particularly outside research settings. Confinement introduces numerous stress-inducing factors, including dietary changes, limited space per animal, reliance on humans for feeding, social regrouping, increased pathogen exposure, and extreme weather conditions. These stressors can trigger adaptive mechanisms to maintain homeostasis, resulting in behavioral, metabolic, and endocrine changes that negatively impact weight gain, feed intake, feed efficiency, and, in the context of dairy production, milk yield (Veissier & Boissy, 2007).

Despite the recognized importance of animal welfare, Wilson et al. (2024) reported reluctance among some dairy farms to adopt good welfare practices. This resistance is often justified by the misconception that such practices do not yield

immediate financial benefits or by hesitation to embrace new ideas. However, evidence contradicts this notion, as seen in heavier weaning weights and increased milk production during the first lactation, both linked to improved early-life management and welfare practices.

A variety of strategies can enhance dairy cattle welfare, ranging from environmental enrichment during the prepartum period (Da Silva et al., 2021) to music during milking (Kochewad et al., 2022), grooming during nursing (Da Silva et al., 2022), and the provision of enrichment objects such as brushes and ropes (Strappini et al., 2021).

One critical aspect of animal welfare is ensuring comfort and minimizing pain and discomfort. The disbudding technique in dairy calves, for example, can cause pain and discomfort when performed incorrectly and without anesthesia (Adcock et al., 2018). Hyperkeratosis, a condition that affects the teats of dairy cows, is a prevalent concern in dairy herds, especially in animals subjected to mechanical milking. (Cerqueira et al., 2018).

Hyperkeratosis, the most common teat condition in dairy cows, is characterized by keratinization at the teat's tip due to chronic stimulation. The teat canal, lined with stratified squamous epithelial cells embedded with keratin, serves as a physical barrier. When this keratin layer is disrupted, excessive keratin accumulates at the teat end, potentially impairing teat canal closure and creating a reservoir for pathogens (Sterrett et al., 2013). This condition is associated with elevated somatic cell counts, increased mastitis incidence, and contamination of milking equipment and milk (Guarín et al., 2017).

Factors influencing hyperkeratosis include age, genetics, lactation stage, milk yield, and parity (Neijenhuis et al., 2000). However, external factors, such as improper milking practices, poorly adjusted vacuum and compression settings, malfunctioning pulsation systems, and prolonged milking, are the primary triggers (Sterrett et al., 2013). These external factors highlight the need for regular equipment maintenance and staff training to minimize discomfort and injury.

Dehorning, in turn, is a common management practice in dairy calves, typically performed around eight weeks of age, to prevent injuries to handlers and herd mates as social hierarchies form (Stock et al., 2013). The procedure involves removing the germinal buttons on the calf's head to prevent horn growth. Traditionally, this was performed using a hot iron without anesthesia (Cozzi et al., 2015). However, advances

in welfare practices have led to the widespread adoption of cornual nerve blocks with synthetic anesthetics, reducing pain during the procedure (Adcock et al., 2018).

An alternative method is the use of caustic paste, which has gained popularity due to its reduced need for animal restraint. The paste causes gradual corrosion of the horn bud, preventing horn development. However, it is often applied without anesthetics, causing pain and leading to healing times twice as long as those observed with hot iron dehorning (Drwencke et al., 2023; Sheedy et al., 2024).

In the search for less painful and invasive methods, policresulene has emerged as a potential alternative for dehorning through the Alta Cria Program (Azevedo et al., 2022). Policresulene, derived from acids used in human and animal wound cauterization and hoof treatments (Kim et al., 2015; Janssen et al., 2016), is under investigation for its effectiveness in calf dehorning, though further testing is required to validate its efficacy and welfare implications.

Technologies and innovation in the dairy system

The global intensification of technology has driven transformative changes in agriculture and livestock systems, with technological innovations playing a pivotal role in reshaping animal management practices. According to Weary and Von Keyserlingk (2023), emerging technologies that enable early detection of health issues are categorized under "precision livestock farming." This approach involves the integrated use of individual or multiple technologies to provide real-time, individualized monitoring of animals within a herd (Aquilani et al., 2022).

Precision livestock farming technologies are diverse and adaptable. Aquilani et al. (2022) identified tools specifically developed for pasture-based systems, including radio frequency identification tags, weighing scales at gates for individual and herd weight measurements, remote body temperature monitoring devices, and global positioning systems (GPS) for locating animals. These technologies not only help mitigate issues such as theft but also facilitate the assessment of feeding behaviors and reproductive activities.

Behavioral monitoring technologies have also shown potential in enhancing animal welfare assessments. For instance, Adcock et al. (2023) evaluated behavioral changes in calves dehorned with a hot iron using neck accelerometers. Over a 21-day

monitoring period, the technology identified inactivity and restlessness, providing valuable insights into behavioral adaptations post-dehorning.

The integration of the Internet of Things (IoT) into modern livestock farming has further revolutionized management practices. IoT technologies enable real-time monitoring, automation, and advanced data analysis, driving precision, sustainability, and quality in livestock systems. Akhigbe et al. (2021) highlighted that IoT facilitates data-driven decision-making and process optimization, paving the way for proactive approaches in animal management.

In dairy farming, IoT-powered robotic milking systems exemplify the application of precision livestock farming. These systems allow individual monitoring of cows while collecting data on behavior, health, productivity, and milk quality, contributing to improved management and animal welfare (Ji et al., 2022).

The incorporation of modern technologies into the dairy industry has revolutionized operations, enhancing precision, efficiency, and sustainability. IoT, artificial intelligence, and data analysis have enabled real-time herd health monitoring, resource optimization, and proactive decision-making. These advancements have reduced costs, improved milk quality, and fostered animal welfare, positioning technology as an indispensable ally in addressing the challenges faced by the dairy sector. By embracing innovation, the industry ensures a competitive and sustainable future, meeting the growing demand for efficiency and ethical production practices.

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2- CHAPTER 1 - Policresulen as an alternative method for dehorning dairy calves.

Interpretative summary: Policresulen as an alternative method for dehorning dairy calves.

By Silva et al. This study compared two dehorning methods in dairy calves: the conventional hot-iron technique and a novel approach using policresulen, a medicinal compound. Policresulen was effective in inhibiting horn bud growth during the rearing phase and offered moderate pain mitigation. However, partial horn regrowth was observed during the heifer phase, with regrowth reaching approximately one-third the size of horns in non-dehorned calves. A consumer perception survey revealed that individuals without agribusiness experience viewed the policresulen method as less invasive and less labor-intensive than traditional techniques. Furthermore, consumers expressed a willingness to pay a premium for dairy products sourced from farms utilizing medicinal dehorning methods.

Running Head: Policresulen application for dehorning dairy calves

Policresulen as an alternative method for dehorning dairy calves

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ABSTRACT

Dehorning is a routine practice on dairy farms, with the hot-iron method remaining widely used despite its association with considerable pain and distress in calves. This study comprised two experiments aimed at evaluating the potential of policlesulen as an alternative dehorning method for dairy calves and exploring consumer perceptions of dehorning techniques. In Experiment 1, 24 Holstein calves were randomly assigned to one of two treatments: policlesulen dehorning (POD) or hot-iron dehorning (HID). All calves were dehorned at 21 ± 2 d of age. The POD group received 0.2 mL of a 36% policlesulen solution applied to each horn bud, while calves in the HID group underwent complete horn bud cauterization. Outcomes evaluated included behavioral responses, horn bud regression, feed intake, serum cortisol concentrations, average daily gain, and weaning body weight. Calves in the POD group exhibited fewer scratching and head-shaking behaviors and showed more rapid regression in horn bud diameter compared to those in the HID group. However, by 12 mo of age, 9 out of 12 POD-treated calves exhibited substantial horn regrowth. These results indicate that further research is necessary to validate the efficacy and long-term effectiveness of policlesulen under practical field conditions. Experiment 2 assessed consumer perceptions of the POD and HID methods. A video demonstrating both techniques was disseminated via social media, accompanied by a questionnaire sent to 1,000 randomly selected individuals; 236 respondents completed the survey. Participants with agribusiness experience were more familiar with dehorning procedures and more likely to perceive HID as a simpler and less invasive method. In contrast, individuals without an agricultural background were 2.17 times more likely to express willingness to pay a premium for dairy products from farms implementing animal welfare practices, with 76% favoring products from POD-treated farms. Conversely, respondents with agribusiness experience were 2.76 times more likely to prefer products from

farms using the HID method. These findings underscore the growing consumer awareness of animal welfare practices in dairy production. Preferences for dehorning methods appear to be influenced by familiarity with agricultural systems: individuals with agribusiness experience tend to prioritize technical knowledge and practical feasibility, while those without such experience are more inclined to support and pay premium prices for products from farms that adopt animal welfare-focused practices.

Keywords: dairy calves, dehorning, policresulen.

INTRODUCTION

With the intensification of dairy farming, cattle are increasingly raised in confined environments with high stocking densities, where the presence of horns can hinder management and compromise safety. Horns pose risks to both handlers and animals, particularly as cattle establish social hierarchies through aggressive interactions (Stock et al., 2013).

Horns are bony projections of the frontal bone covered by a keratinized sheath. Horn buds typically appear within the first few days of life and remain unattached to the skull until approximately 2 mo of age. During this period, the corium—the tissue that supports horn development—begins to fuse the horn bud to the skull. Thus, the optimal window for preventing horn growth is within the first two months of life by destroying the corium through dehorning. Beyond this developmental stage, the horn becomes fully attached to the skull, requiring more invasive surgical removal, which increases pain and stress for the animal (Yu et al., 2014).

Hot-iron cauterization remains the most widely used method for dehorning on dairy farms and is generally effective when applied correctly (Sheedy et al., 2024). However, it is known to cause significant pain and results in open wounds that may take 6 to 13 wk to heal, leading to prolonged discomfort (Casoni et al., 2019).

Policresulen has recently attracted interest as a potential alternative dehorning method for calves, particularly within the Alta Cria Program (Azevedo et al., 2022)—one of the largest calf and heifer data collection programs globally, encompassing over 207,000 animals. Policresulen, a compound derived from sulfonic and formaldehyde acids, is widely used in human medicine for wound cauterization (Kim et al., 2015), wart removal (Simamora et al., 2012), and treatment of genitourinary conditions (Ivarsson et al., 2019). In veterinary applications, it has been used to treat reproductive tract disorders (Sancler-Silva et al., 2018) and to promote wound and hoof healing (Janssen et al., 2016).

As public concern over animal welfare in dairy production grows, there is increasing demand for dehorning methods that minimize pain and other negative impacts on calf well-being. We hypothesized that policlesulen would be an effective, less painful alternative to hot-iron dehorning and that consumers would demonstrate more favorable perceptions toward its use. Therefore, the objective of this study was to evaluate the efficacy of policlesulen as an alternative to the conventional hot-iron dehorning method in dairy calves and to assess whether consumer purchasing decisions are influenced by on-farm welfare practices.

MATERIAL AND METHODS

Study 1 – Dehorning Techniques

This study was approved by the Research Ethics Committee in accordance with institutional ethical guidelines (protocol no. 0132/2023). The experiment was conducted at the University of Viçosa, Viçosa, Minas Gerais, Brazil.

Twenty-four Holstein dairy calves were blocked by sex and randomly assigned to one of two dehorning treatments: policlesulen dehorning (**POD**) or hot-iron dehorning (**HID**), with 12 calves per group. At birth, calves were weighed and housed individually. Colostrum was administered at 10% of BW, with a Brix score $>25^{\circ}$, within the first 2 h of life, followed by a second feeding (5% of BW) with colostrum of similar quality at 8 h postpartum. Water was provided ad libitum. Calves were fed milk twice daily according to the following schedule: 3 L of transition milk per feeding until 5 d of age; 3 L of raw milk per feeding from 6 to 30 d; 4 L per feeding from 31 to 60 d; and 3 L per feeding until weaning at 90 d. A starter concentrate composed of soybean meal, ground corn, mineral mix, and a palatability enhancer was offered

ad libitum from birth until weaning. From 40 d of age onward, Coast Cross hay was provided ad libitum.

Dehorning was performed at 21 ± 2 d of age. Calves were restrained in right lateral recumbency, and the cornual region was clipped. A cornual nerve block was administered using 5 mL of 2% lidocaine hydrochloride. In POD calves, 0.2 mL of Albocresil® (36% polycresulen) was injected into the center of each horn bud using a 30×0.8 mm needle. In HID calves, the horn bud was cauterized with a gas-flame-heated iron following local anesthesia. All calves received meloxicam (0.5 mg/kg BW, intramuscularly) for three consecutive days post-procedure.

Calf behavior was monitored from 12 h before dehorning to 48 h after, using an ethogram adapted from Adcock et al. (2018). Behaviors recorded included standing, lying, head shaking, scratching the horn area with a limb, rubbing the horn on objects, vocalization, and feeding. Wound healing was evaluated via standardized photographs taken twice weekly for 10 wk post-dehorning. Images were captured 15 cm from the calf's head using a Nikon® semi-professional camera and used for visual comparisons over time. Horn bud regression was measured with a caliper on days 0, 10, 20, 30, and 60 post-dehorning.

Blood samples were collected at 0 (pre-dehorning), 15 min, 1, 12, 24 h, and at 3 and 7 d post-dehorning to quantify serum cortisol concentrations. Samples were drawn into non-anti-coagulant vacuum tubes with gel, centrifuged at $1,500 \times g$ for 15 min, and stored at -20°C . Cortisol concentrations were measured using chemiluminescence and reported in nanomoles per liter of serum. Heart rate and respiratory rate were recorded at 0, 0.5, 1, 1.5, 2, 4, 6, and 24 h post-dehorning.

Productive performance was assessed using ADG and total average weight gain (TAG). Average daily gain was calculated from monthly BW measurements by dividing the change in weight by the number of days since dehorning. Total average weight gain represented the total weight gain from the start of the study to 90 d post-dehorning, divided by the elapsed time.

Calves were weighed at -1, 30, 60, and 90 d relative to the dehorning procedure using an electronic scale. Weighings were conducted on three consecutive days, and the average was used to minimize discrepancies due to gut fill. After weaning, calves were moved to group pens and fed a total mixed ration consisting of corn silage and a concentrate of soybean meal, ground corn, and minerals once daily.

Data were analyzed using the PROC GLIMMIX procedure of SAS (University Edition). For each outcome variable, the d -1 measurement was tested as a covariate and removed from the model if not significant ($P > 0.05$). The main and interaction effects of dehorning method (POD vs. HID) and time were included, with sex as a random blocking factor. Time was treated as a repeated measure in the model according to the following structure:

$$Y_{ijkl} = \mu + M_i + \delta_{ij} + T_k + (M \times T)_{ik} + S_l + \varepsilon_{ijkl}$$

Where: Y_{ijkl} = observation ijk ; μ = the overall mean; M_i = fixed effect of Method i ; δ_{ij} = random error with mean 0 and variance σ_{δ}^2 , the variance between animals within the method and it is equal to the covariance between repeated measurements within animals; T_k = fixed effect of time k ; $M \times T_{ik}$ = fixed effect of interaction between the method i and Time k ; S_l is the random effect of calf sex l , and ε_{ijkl} = random error with the mean 0 and variance σ^2 , the variance between measurements within animals. Fifteen variance-covariance structures were tested for each response variable. Then, we used the variance-covariance structure that provided the best fit based on the lowest Akaike information criterion. The observations with externally studentized residuals greater than $|2.5|$ were first checked to make sure they were not a recording error. After checking and evaluating that the information was not a recording error, the observations were considered outliers and consequently excluded from the dataset. The analysis of possible outliers was performed only once for each outcome variable to avoid erroneous looping outliers. The least-square means were considered different when $P < 0.05$.

Study 2 – Dairy Consumer Perceptions of Calf Dehorning Methods

This study was approved by the Research Ethics Committee in accordance with institutional ethical guidelines (protocol no. 79349324.2.0000.5153).

A video was produced to demonstrate the two dehorning techniques evaluated in this study. Brief descriptions of each procedure accompanied the video, which was uploaded to YouTube (<https://www.youtube.com/watch?v=fRtyvn69nR0&t=12s>). A seven-item questionnaire was developed to assess participants' perceptions of dehorning, covering the following topics: (1) profession, (2) prior knowledge of dehorning, (3) agreement with the practice of dehorning, (4) perceived ease of performing each technique, (5) perceived invasiveness, (6) willingness to pay a premium for dairy products from farms implementing welfare-friendly dehorning practices, and (7) preference regarding which dehorning method justifies paying a higher price.

The questionnaire and video link were distributed via social media to approximately 1,000 randomly individuals. A total of 236 respondents completed the survey. Based on participants' professions, individuals were categorized into two groups: those with agribusiness experience and those without. This classification allowed for broader interpretation of participants' familiarity with and perspectives on the dehorning methods presented.

Descriptive analyses were conducted using logistic regression, with odds ratios estimated relative to the HID method as the reference category. Statistical analyses were performed using the PROC GLIMMIX procedure in SAS (SAS Institute Inc., Cary, NC), and significance was declared at $P < 0.05$.

RESULTS

Study 1

Behavioral Observations and Wound Healing

There were no significant differences ($P > 0.05$) between treatments in the frequency of vocalization, lying, standing, or feeding behaviors (Figure 1). However, the dehorning method significantly influenced ($P < 0.05$) the frequency of scratching behaviors (using a limb or against objects), vocalization, and head shaking, with calves in the HID group displaying these behaviors more frequently ($P < 0.05$) than those in the POD group. Complete epithelialization of the horn region was observed in both groups by 60 d post-dehorning (Figure 2). Additionally, calves in the POD group showed a significantly greater reduction ($P < 0.05$; Figure 3) in horn bud circumference compared with those in the HID group.

Physiological Responses

Serum cortisol concentrations increased in both treatment groups, peaking between 15 min and 1 h post-dehorning, and subsequently returning to baseline within 1 h ($P > 0.05$; Figure 4). No differences were observed between treatments for serum cortisol concentrations ($P = 0.85$). Similarly, heart rate and respiratory rate did not differ between treatments ($P > 0.05$); however, both parameters fluctuated significantly over time during the initial hours following the procedure ($P < 0.05$; Figure 5).

Feed Intake and Growth Performance

No differences were observed between POD and HID treatments in concentrate DMI (Figure 6). Similarly, ADG, TAG (Figure 7), weaning weight, and post-weaning weight (Figure

8) did not differ between groups ($P > 0.05$). However, by 12 mo of age, no horn regrowth was observed in calves treated with HID, whereas 9 out of 12 calves in the POD group exhibited horn regrowth. The regrown horn structures in POD calves measured 2.76 ± 0.91 cm in diameter and 3.40 ± 0.68 cm in height.

Study 2

Of the 236 participants surveyed, 69% reported having experience or a background in the agricultural sector. Individuals with agribusiness exposure were more likely ($P < 0.001$; Table 1) to be familiar with dehorning practices, exhibiting a 23.9-fold increased likelihood of prior knowledge compared with those without such exposure. They were also 13.7 times more likely ($P < 0.001$) to agree with the practice of dehorning.

When comparing the two techniques, 73% of agribusiness-affiliated participants considered HID easier to perform ($P < 0.001$), corresponding to a 0.263 relative likelihood of perceiving POD as the easier method. Regarding perceived invasiveness, individuals with agribusiness experience were 2.76 times more likely to view HID as less invasive, with 64% selecting HID over POD ($P < 0.001$). By comparison, this subgroup had a 0.362 likelihood of considering POD the less invasive method.

Only two respondents reported non-consuming dairy products, both of whom were from non-agribusiness backgrounds. Thus, 100% of participants with agribusiness exposure reported dairy consumption.

Among dairy consumers, those without agribusiness experience were 2.17 times more likely ($P < 0.001$) to express willingness to pay a premium for dairy products from farms practicing welfare-conscious dehorning, with 76% indicating a preference for products from farms using

the POD method. In contrast, participants with agribusiness backgrounds were 2.76 times more likely to prefer and pay a premium for products from farms utilizing HID.

DISCUSSION

Study 1

Only head shaking and scratching of the horn area (with a limb or against objects) differed between treatments. The combined use of local anesthetics and analgesics during dehorning has been shown to markedly reduce or even eliminate pain (Stock et al., 2013), which may explain the limited behavioral differences observed between groups in the present study. Both analgesia and cornual nerve blocks were employed to minimize pain, likely contributing to the absence of differences in most behavioral and physiological parameters. Despite their importance, the use of anesthesia and analgesia during calf dehorning remains uncommon on many dairy farms (Sheedy et al., 2024).

The higher frequency of head shaking and horn scratching in calves subjected to HID indicates greater discomfort compared with those treated with POD. This difference may be attributed to the less invasive nature of the POD method, which avoids the large open wounds associated with HID. By 60 d post-procedure, complete epithelialization of the horn region was observed in both groups; however, POD calves exhibited a greater reduction in horn bud circumference, suggesting more rapid healing.

Although both treatments induced increases in serum cortisol concentrations—an indicator of acute stress—levels returned to baseline within 1 h following dehorning. This rapid normalization may be attributed to the administration of local anesthetics and analgesics, which have been shown to reduce prolonged stress and pain responses (Stilwell et al., 2012). The

transient rise in cortisol during the first hour, along with elevated heart and respiratory rates observed in the first 30 min, may be more reflective of handling and physical restraint rather than the dehorning procedure itself. Stewart et al. (2008) reported similar heart rate patterns in disbudded calves with and without anesthesia, noting that anesthetized calves returned to baseline values more rapidly than untreated calves. Therefore, the immediate physiological changes observed in the current study are likely associated with handling stress rather than treatment-specific pain.

Feed intake increased over time in both groups, and no differences were observed in ADG or TAG before weaning. These findings suggest that neither dehorning method negatively affected calf growth performance during the rearing period, likely due to the effective pain management and proper procedural execution implemented in this study.

However, by 12 mo of age, 9 out of 12 POD-treated calves exhibited horn regrowth, with horn structures measuring 2.76 ± 0.91 cm in diameter and 3.40 ± 0.68 cm in height, while no regrowth was observed in the HID group. It is hypothesized that the age at which dehorning was performed (21 ± 2 d) may have contributed to this outcome. Future research should investigate whether the application of policlesulen at an earlier age (< 21 d) could more effectively prevent horn regrowth. Although earlier dehorning may trigger more pronounced pain responses (Adcock et al., 2018), optimizing the timing of policlesulen application may enhance its effectiveness as a non-surgical dehorning alternative. Given the novelty of policlesulen use for this purpose, further studies are warranted to refine the protocol and evaluate its long-term efficacy in fully inhibiting horn bud development and eliminating the need for subsequent surgical intervention.

Study 2

It was observed that individuals without agribusiness experience possess limited or no knowledge of animal welfare practices, suggesting inadequate dissemination of this information to consumers. Although animal welfare and the conditions under which farm animals are raised have become increasingly important to consumers of animal-derived products, research indicates that the public receives limited information on welfare practices at both the production and processing stages (Vargas-Bello-Pérez et al., 2015).

A significant disconnect exists between producers and consumers, whereby concerns about animal welfare and willingness to pay for welfare-certified products coexist with limited understanding of production systems. This gap is largely driven by the physical and informational distance between urban consumers and agricultural operations, as well as the influence of media and animal advocacy organizations, which often emphasize negative aspects of livestock farming without offering a balanced view (Alonso et al., 2020). Consequently, consumer opinions are frequently shaped more by perceptions and misconceptions than by evidence-based knowledge. Anthropomorphism further affects public attitudes toward livestock production, underscoring the need for effective scientific communication about animal welfare and the realities of agricultural systems (Zuliani et al., 2018).

In addition to agricultural background, demographic factors such as age, income, and education also influence concern for animal welfare. Younger individuals, particularly those frequently exposed to social media, tend to show greater awareness of welfare issues. While concern may be expressed across income brackets, individuals with higher income levels are generally more willing to pay premiums for welfare-certified products. Similarly, increased education levels are associated with a better understanding of and concern for agricultural systems (Clark et al., 2016).

In the present study, dairy consumers without agribusiness experience were more likely to express willingness to pay a premium for products from farms implementing welfare-friendly

dehorning methods. Clark et al. (2016) attributed such behavior primarily to urban populations, who typically have limited direct contact with agricultural production. In contrast, participants with agribusiness backgrounds were more inclined to perceive HID as less invasive and easier to perform, reflecting a greater emphasis on technical feasibility and economic efficiency. The HID remains the predominant dehorning method on dairy farms (Sheedy et al., 2024), and producers may be reluctant to adopt novel alternatives in the absence of clear cost–benefit evidence.

These factors collectively shape the profile of consumers concerned with animal welfare. Although studies such as Comin et al. (2022) have explored both producer and consumer perspectives, limited research has focused specifically on the knowledge gap between agribusiness and non-agribusiness stakeholders. Differences in technical training, practical experience, and reduced susceptibility to external influences likely contribute to the contrasting perspectives. Agricultural professionals, who work directly with animals, tend to prioritize health, productivity, and economic considerations, and are generally less influenced by media narratives. Conversely, individuals outside the agricultural sector often rely on secondary information sources and may base opinions on idealized representations or selective reporting.

In this study, non-agribusiness participants demonstrated a greater willingness to pay a premium for products originating from farms with animal welfare-focused dehorning practices. This growing consumer interest in animal welfare represents an opportunity for the dairy sector to enhance productivity and create value-added dairy products (Comin et al., 2022). However, animal welfare is a complex, multidimensional construct that is challenging to measure. Recent research has focused on developing science-based welfare indicators and incorporating them into certification systems (Spigarelli et al., 2021).

The increasing demand for welfare-compliant dairy production presents both challenges and opportunities. While agribusiness professionals often adopt a technical and production-

oriented perspective, urban consumers tend to form opinions based on emotional responses, media coverage, and societal expectations. Implementing transparent, scientifically grounded welfare practices may help reconcile these differing viewpoints, enhance competitiveness, and improve the public perception of the dairy industry. Therefore, disseminating clear, accessible, and evidence-based information is essential to bridge the knowledge gap and foster informed consumer decisions aligned with sustainable industry practices.

CONCLUSION

Policresulen-based dehorning resulted in smaller wounds and faster healing compared with the conventional hot-iron method. However, horn regrowth was observed in nine out of twelve calves treated with policresulen, necessitating surgical dehorning at 12 mo of age. These findings suggest that while policresulen may offer short-term welfare benefits, further research is needed to optimize its effectiveness. Specifically, studies should aim to determine the ideal age for application and the appropriate dosage required to completely inhibit horn bud development and eliminate the need for subsequent surgical intervention.

Consumers without agribusiness backgrounds exhibited greater concern for animal welfare and demonstrated a higher willingness to pay for dairy products originating from farms that adopt welfare-oriented practices. In contrast, individuals with agribusiness experience were more likely to prioritize technical knowledge and practical feasibility in evaluating dehorning methods.

NOTES

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Ethics committee: All procedures were meticulously approved by the Animal Use Ethics Committee of the Department of Animal Science at the Federal University of Viçosa, Minas Gerais, Brazil (protocol no. 0132/2023 and protocol no. 79349324.2.0000.5153).

Conflicts of interest: The authors declare no conflicts of interest.

Nonstandard abbreviations used: POD = policlesulen dehorning; HID = hot-iron dehorning; TAG = total average gain.

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Table 1. Analysis in relation to agribusiness-affiliated individuals in consumer perception research on dairy products.

Questions	Answer	Odds ^a	SE	<i>P</i> -value
Do you know the dehorning procedure?	Yes	23.98	0.4339	< 0.001
Do you agree with the dehorning procedure?	Yes	13.74	0.4969	< 0.001
Which procedure do you consider the easiest to do?	HID ^b	3.8	0.3017	< 0.001
Which procedure do you consider less invasive?	HID	2.76	0.3582	< 0.001
Are you a consumer of dairy products?	Yes	355.28	>999.99	< 0.001
Would you be willing to pay a higher price for dairy products from brands that adopt good practices related to dehorning?	Yes	0.46	0.3379	< 0.001
Which practice would you be willing to pay a higher price for?	HID	2.76	0.3582	< 0.001

^aOdds = Odds ratio.

^bHID = dehorning with hot iron.

Figures

Figure 1. Behavioral analysis of the animals before and after the dehorning procedure. (A) Scratching behavior with the limb; (B) Behavior of scratching against an object; (C) Vocalizing behavior; (D) Head shaking behavior.

Figure 2. Weekly photographic monitoring of the polycresulen treatment (POD) and hot iron treatment (HID).

Figure 3. Evaluation of the regression of the left and right corneal button diameter in different treatments. (A) Right horn; (B) Left horn.

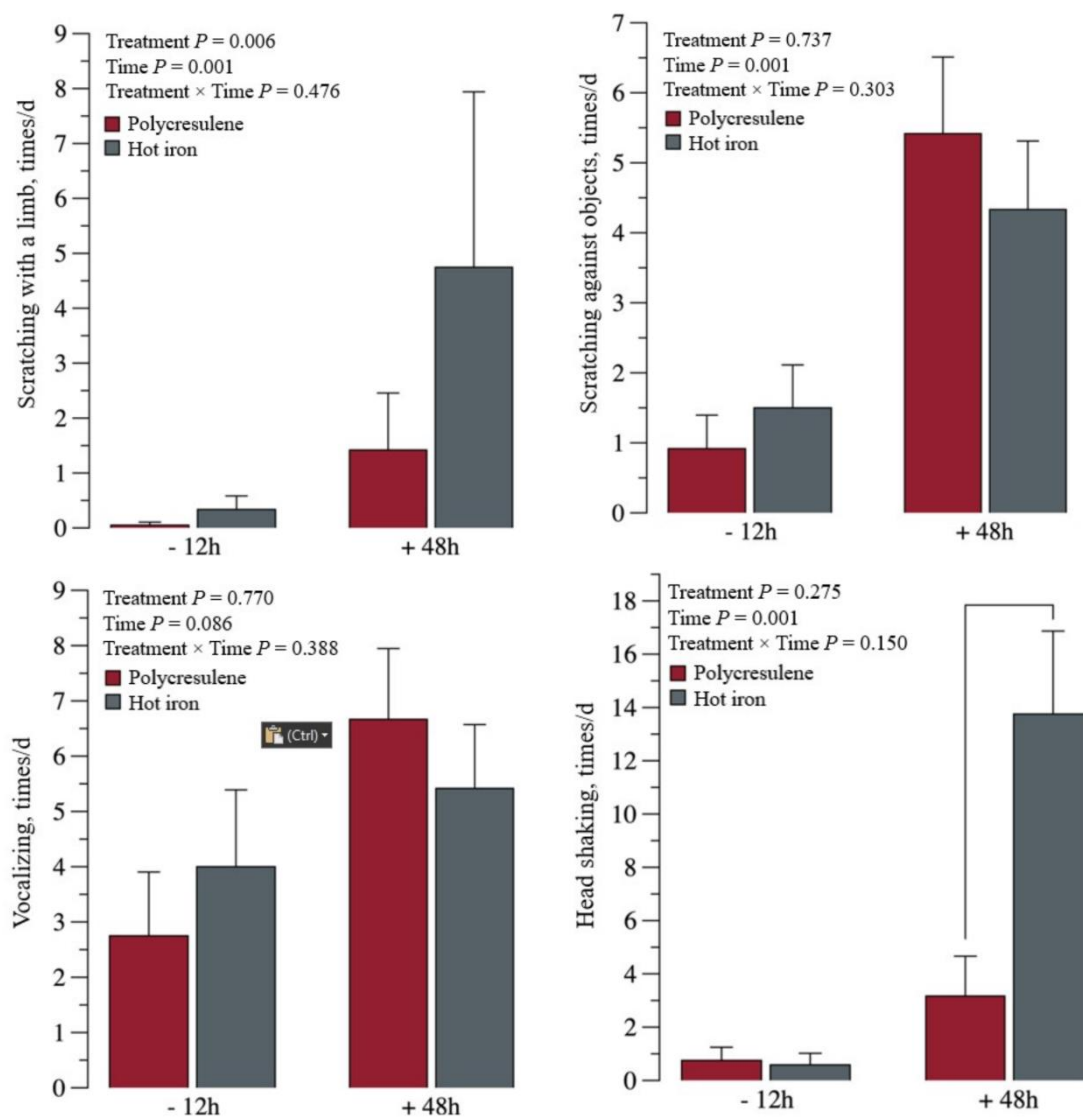
Figure 4. Serum cortisol concentration in animals subjected to the dehorning procedures.

Figure 5. Heart rate and respiratory rate of the animals before and 24 h after the procedure. (A) Heart rate of the animals; (B) Respiratory rate of the animals.

Figure 6. Dry matter intake of concentrate by calves before and after the dehorning procedure.

Figure 7. (A) Average daily weight gain of calves before 30, 60 and 90 d after the dehorning procedure; (B) Total average weight gain from the day of the procedure until weaning.

Figure 8. Weaning weight of the animals at 90 d of age.

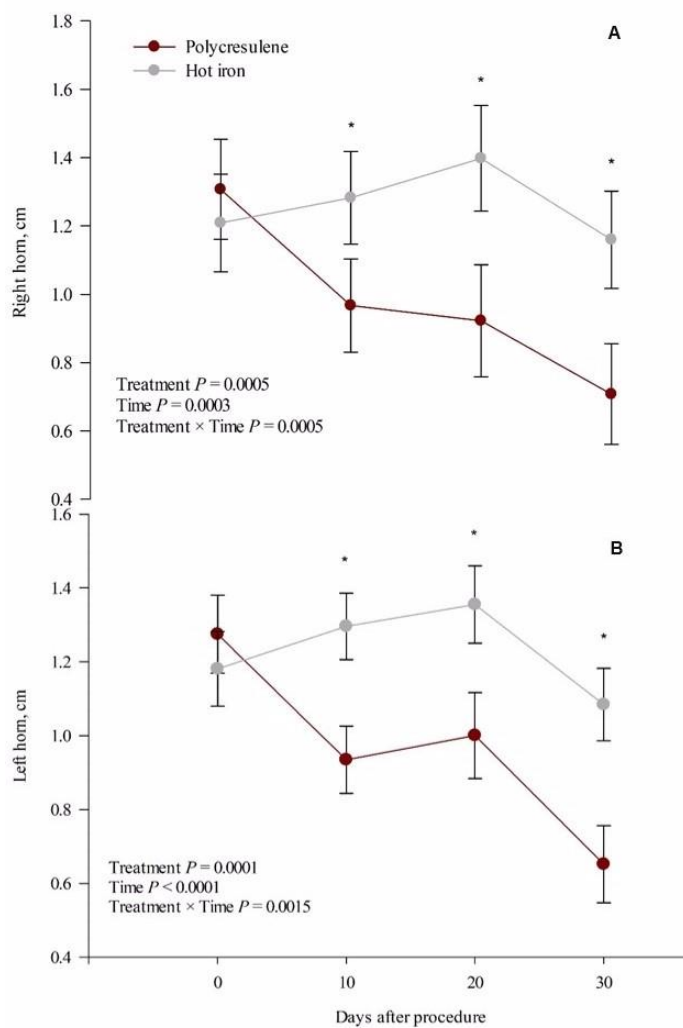


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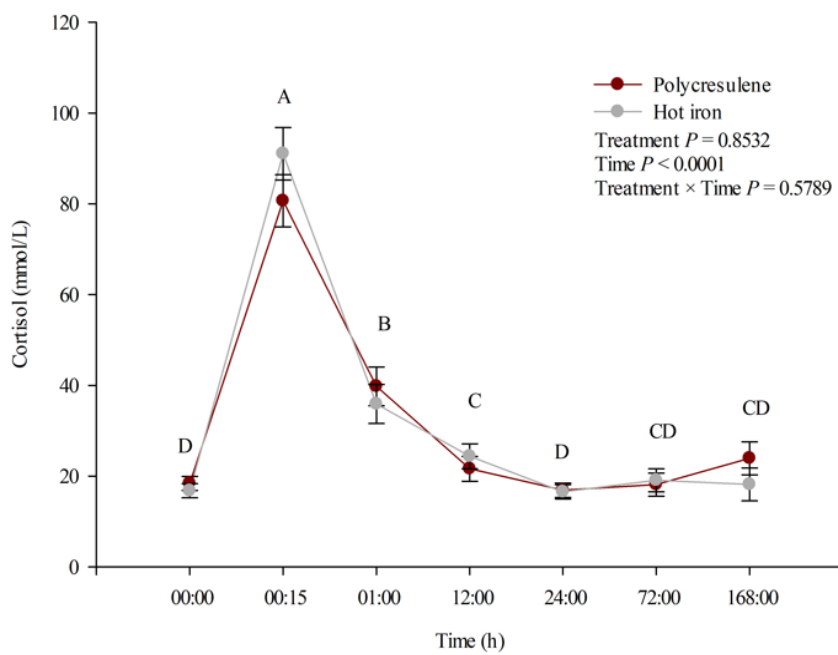


*S = Week

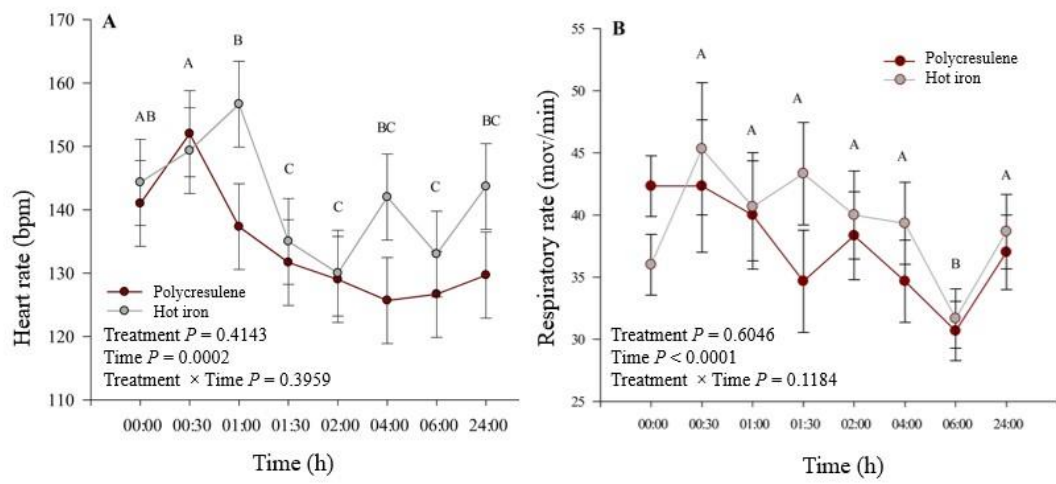
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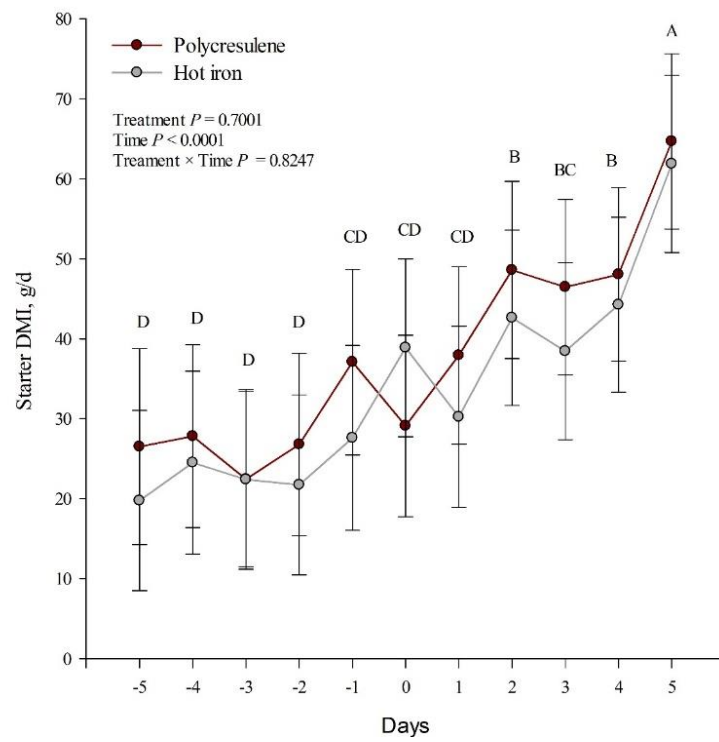
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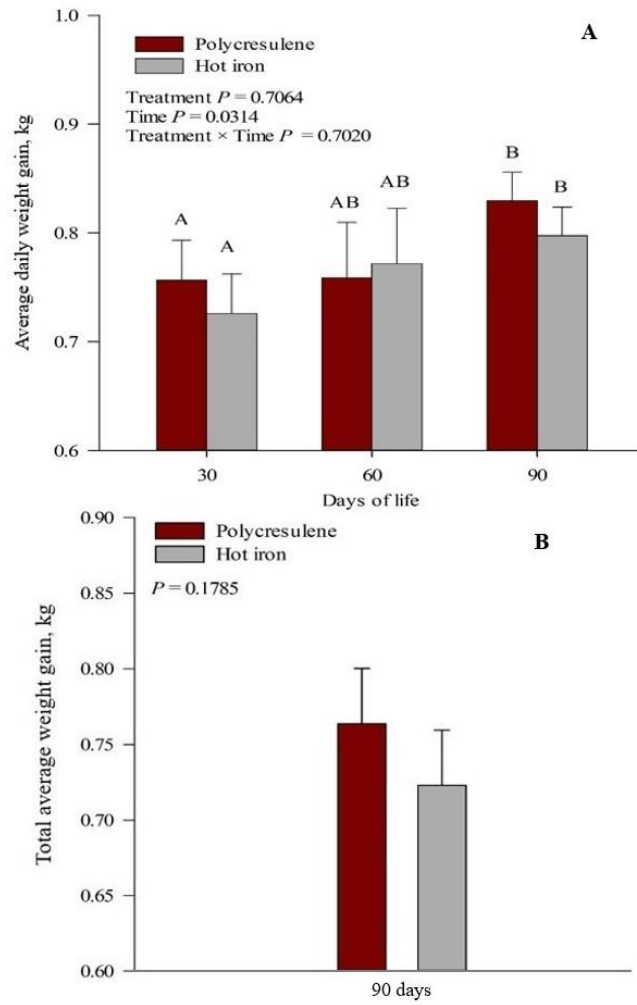
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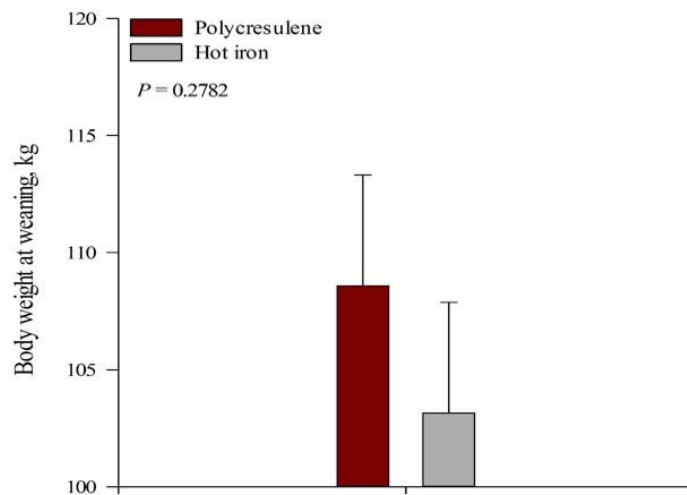
Silva, Figure 5.



Silva, Figure 6.



Silva, Figure 7.



Silva, Figure 8.

3 - Chapter 2 - Short Communication: Influence of Teat Size and Age on Teat-End Hyperkeratosis in Dairy Cows.

Short Communication: Influence of Teat Size and Age on Teat-End Hyperkeratosis in Dairy Cows

Running title: Predisposing factors for hyperkeratosis in dairy cows

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ABSTRACT

Mammary gland health and milk quality are critical for successful dairy production. This study assessed the association between teat-end hyperkeratosis (HY), teat size, and age in Holstein cows. Thirty-seven Holstein cows with an average milk yield of 32.8 ± 7.61 kg/d, BW of 582 ± 71.8 kg, and 153 ± 99.0 DIM were evaluated. Cows were milked three times per day using an automatic milking system, and HY was scored post-milking. The HY data were organized in a binary format, dividing the animals into two groups: with or without the presence of HY. The presence or absence of HY was analyzed using analysis of variance (ANOVA). The influence of teat size and animal age on the presence of HY was analyzed using binomial odds ratios (OR), employing the glm function in R. A significance level of 0.05 was considered for all analyses. Among the 152 teats examined, 40.8% were classified as score 1, 46.7% as score 2, 9.87% as score 3, and 2.63% as score 4. The average teat size was 4.55 ± 1 cm. The anterior teats showed an increased OR for HY as teat size increased (OR = 1.23 and 1.21 for the left and right anterior teats, respectively). Age was associated with HY in the left posterior and anterior teats ($P = 0.048$), suggesting that older cows, likely due to a higher number of lactations and changes in mammary structures, are more susceptible to HY. Larger teat size also increased the risk of HY, potentially impacting udder health. Proper milking management and correct adjustment of milking machines can prevent the occurrence or exacerbation of HY, promoting greater animal welfare and preventing disease development.

Key words: hyperkeratosis, mechanical milking, milk yield.

INTRODUCTION

Hyperkeratosis (HY) is one of the most common conditions affecting the teats of dairy cows, characterized by keratinization of the teat end due to chronic stimuli (Pantoja et al., 2020). Milking management and equipment settings, such as the mechanical force exerted by vacuum and liner compression, pulsation systems, and overmilking, are chronic factors associated with the development of HY (Neijenhuis et al., 2000; Sterrett et al., 2013).

The teat end consists of smooth muscles, which aid in closing the teat canal sphincter between milkings, and the epithelial layer, the primary defense of the mammary gland against infectious agents. This layer comprises stratified squamous cells filled with keratin, which acts as a physical barrier plug, with fatty acids in its composition providing bacteriostatic activity (Guarín et al., 2017; Pantoja et al., 2020).

The HY occurs when the biological keratin layer is lost due to chronic stimuli, leading the animal's body to respond by producing and excessively accumulating keratin in the epithelial layer of the teat end (Sterrett et al., 2013). This new defense layer, consisting of an increased amount of keratin, may compromise the proper closure of the teat canal and serve as a reservoir for pathogens and an ideal environment for bacterial colonization. This can result in higher SCC and increased cases of mastitis, as well as contamination of the milking machine liners and extracted milk (Guarín et al., 2017). Animals with high levels of HY may experience pain and discomfort, exhibiting behaviors such as kicking and movement during milking (Cerqueira et al., 2018).

Intrinsic predisposing conditions of the animal that contribute to the development of HY in teats should also be considered, especially when combined with external factors over time. Breed, genetics, age, stage of lactation, milk production, and parity are some of these factors (Neijenhuis et al., 2000). Teat shape and length are conformation characteristics that may be associated with a predisposition to HY (Cerqueira et al., 2018).

Issues related to the individual characteristics of the animal, milking management, and the interaction between these factors are essential requirements for preventing HY and managing existing cases. The objective of this study was to highlight factors that may cause or exacerbate HY in the teats of dairy cows subjected to mechanical milking. The hypothesis proposed that the longer the teat length, the higher the likelihood of HY occurrence, and that older cows might present more issues related to HY.

MATERIAL AND METHODS

The experiment was conducted at the Dairy Cattle Teaching, Research, and Extension Unit of the Universidade Federal de Viçosa. Research on animals was conducted according to the Ethics Committee on animal use of the Universidade Federal de Viçosa (protocol no. 054/2023).

Thirty-seven Holstein cows, both primiparous and multiparous, with a milk yield of 32.8 ± 7.61 kg/d, 153 ± 99 DIM, an age of 4 ± 1.2 years, and 582 ± 71.8 kg of BW were used. The animals were housed in a freestall system and fed a TMR formulated according to National Research Council (2021) requirements.

The animals were milked three times a day, 06:00, 14:00 and 20:00, using automatic mechanical milking, with a teat cup extraction flow of 750 g/min, frequency of pulsation of 62 beats/min, and vacuum level of 46 kPa.

The animals were evaluated for their teat score after the second milking of the day, immediately after the automatic extraction of the teat cups, carried out by the same evaluators, according to methodology suggested by Mein et al. (2001). The measurement the size of the animal's teats was done with the aid of a caliper, with measurements given in centimeters. The analyses were conducted on 2 different days of the same month, the last being used as a repeat of the previous collection.

Data regarding HY were organized in binary form, dividing the animals into two groups, with or without the presence of HY. The presence or absence of HY was analyzed by analysis of variance (ANOVA). The influence of the teats size and the age of the animals on the presence of HY was analyzed by binomial odds ratios (OR), using the glm package function of R. For all analyses, significance of 0.05 was considered.

RESULTS

Of the 152 teats evaluated, 62 teats were classified with score 1 (40.8%), 71 teats with score 2 (46.7%), 15 teats with score 3 (9.87%), and 4 teats with score 4 (2.63%). Anterior teats show increased susceptibility to OR for HY as teat size increases (OR = 1.23 and 1.21, for left and right anterior teats, respectively), as described in Figure 1. The average size of the animal teats was 4.55 ± 1 cm, as shown in Figure 1. There was an effect between the age of the animal and the presence of HY for the left rear teat and the front teats ($P = 0.048$; Figure 2).

DISCUSSION

Anatomical differences, such as teat size and end structure, can influence tissue integrity and the variability of changes induced by mechanical milking, such as HY (Asadpour et al., 2015). The vacuum pressure of the milking machine is associated with congestion and edema in the teat tissue. Depending on the machine's compression and teat length, there may be insufficient relief of congestion at the teat end, increasing the likelihood of HY (Penry et al., 2017). Teat length was one of the factors observed in this study as susceptible to HY occurrence, with front teats being more prone.

According to Neijenhuis et al. (2000), the higher susceptibility of front quarters to HY may be related to their lower milk production compared to rear quarters. Both front and

rear quarters remain attached to the automatic milking units for the same duration, leading to overmilking of the front quarters. The overmilking time for front teats can be twice that of rear quarters due to little or no milk flow while milk is still flowing from the rear quarters (Paulrud et al., 2005). Although impractical for many milking systems, Sterret et al. (2013) demonstrated that teat cup liners programmed with individually adjusted vacuum levels for each udder quarter could reduce overmilking and long-term HY at the teat ends.

In addition to the increased susceptibility of front teats to HY, it was also observed that teat length, measured in centimeters, can be another predisposing factor. The greater the teat length, the greater the chances of developing HY. Haverkamp et al. (2017) linked teat length to HY scores for both front and rear teats, finding that teats with higher HY scores were, on average, longer. This contrasts with Penry et al. (2017), who found a higher likelihood of HY in shorter teats. According to Wendt et al. (2007), excessively long or short teats are exposed to continuous vacuum pressure because they do not receive adequate massage during the milking process, resulting in greater mechanical stress on the teat ends.

Front teats were also associated with greater susceptibility to HY as animal age increased. Parity, which is inherently linked to age, is a factor to consider, as changes in udder and teat size are observed with increasing age and lactation cycles (Sterrett et al., 2013). Asadpour et al., (2015) reported increases of up to 10% in teat length and diameter from the first to the fourth lactation, along with a 20% increase in udder depth. Similarly, Haverkamp et al. (2017) and Cerqueira et al. (2018) observed higher HY scores with increasing parity. This loss of mammary tissue integrity is not confined to front teats, which are more affected by overmilking due to lower milk production, but extends to the entire mammary gland. It results from a combination of factors throughout the animal's

life, including age, parity, stage of lactation, DIM, cases of mastitis, and milking management (Pantoja et al., 2020).

Additionally, HY is recognized as a condition that can impact mammary gland health, as it serves as both a reservoir and an entry point for bacteria. Bacteria can infiltrate the teat canal, disrupt the mammary gland's homeostasis, and lead to clinical mastitis (Ndiokubwayo and Koç, 2021). In a meta-analysis conducted by Pantoja et al. (2020), HY was identified as a risk factor for various pathogens responsible for clinical and subclinical mastitis, contributing to increased SCC. Furthermore, it is detrimental to udder health and compromises animal welfare.

Taking HY into account, external factors that can affect animal health, such as equipment regulation and milking management, can help preserve and reduce harm to lactating animals. Intrinsic factors of the animal, as they increase, such as age, parity, and udder conformation, may be considered undesirable traits to maintain in the herd, as they predispose to the pathology.

CONCLUSION

Teat size can influence the occurrence of HY, especially in the front quarters. The front quarters are more susceptible to HY due to lower milk production compared to the rear quarters and the fact that both types of quarters remain in the milking units for the same amount of time, leading to overmilking. The age of the animal can also affect the development of HY as the animal ages. The predisposition to HY is a combination of intrinsic and extrinsic factors of the animal; however, proper milking management and the correct adjustment of milking machines can prevent the onset or exacerbation of HY, promoting greater animal welfare and preventing disease development. Additionally,

older animals or those with a high HY score may be subject to culling from the herd, as they are more likely to experience incomplete milking and milk contamination.

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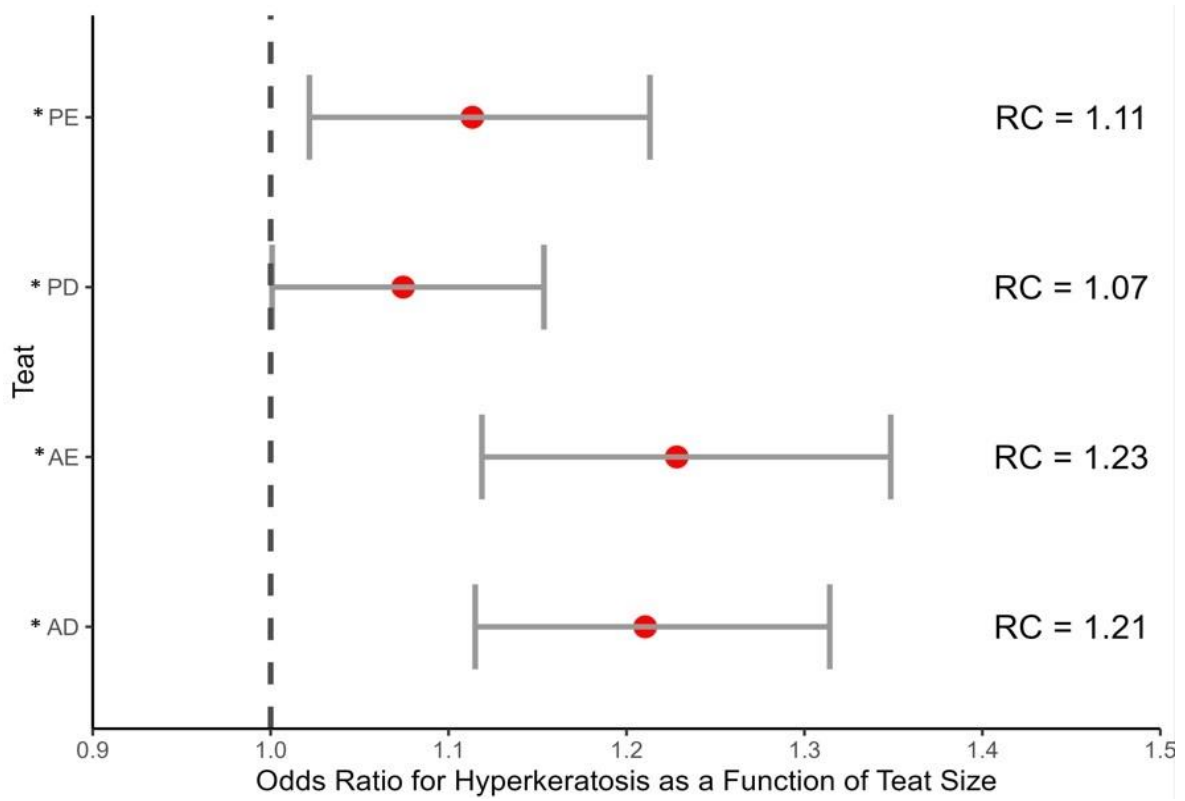
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NOTES

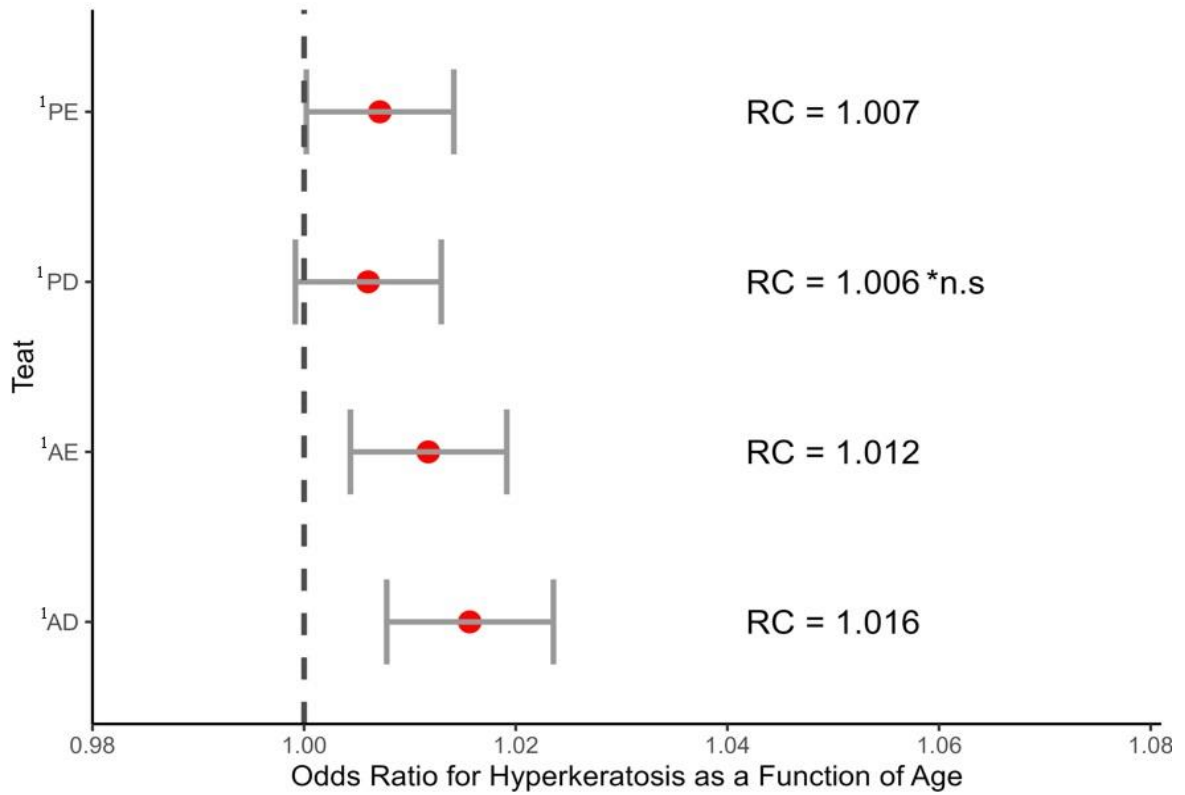
The entire dataset supporting the results of this study was published in the article itself.

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*PE: Left posterior teat; PD: Right posterior teat; AE: Left anterior teat; AD: Right anterior teat. ($P = 0.048$).

Figure 1. Odds ratio for hyperkeratosis as a function of teat size.



¹PE: Left posterior teat; PD: Right posterior teat; AE: Left anterior teat; AD: Right anterior teat.

*Non-significant result.

Figure 2. Odds ration for hyperkeratosis as a function of age.

4. Chapter 3 - Evaluation of real-time milk tank monitoring using IoT system and error analysis in manual measurements

Evaluation of real-time milk tank monitoring using IoT system and error analysis in manual measurements

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Structured Abstract

Accurate measurement of milk volume in bulk tanks is essential to ensure its traceability and to provide safety for both producers and industries in the purchase and sale of the product. However, human errors in reading the graduated ruler and technical failures can compromise the accuracy of this measurement. With the advancement of digital technologies, there is a growing opportunity to apply smart solutions in agriculture, promoting greater efficiency and reliability in processes. The objective of this study was to quantify the potential errors in interpreting the graduated ruler used to measure milk tank volume (Study I) and to assess the effectiveness of a real-time monitoring system using Internet of Things (IoT) technology for measuring volumes in bulk milk tanks (Study II). Six evaluators participated in a visual perception test using the graduated ruler at different milk volumes (Study I). In Study II, an IoT monitoring system was installed in a 1000-L tank for automated volume measurement. Statistical analyses were performed using mixed-effects linear regressions with the `lmer` function from the `lme4` package in R. The analysis evaluated whether the intercept was significantly different from 0 and whether the slope was different from 1. In the graduated ruler model, the variation attributed to the evaluator accounted for 7.86% of the total variance. Both measurement approaches showed good precision and accuracy in the Bland-Altman analyses. The IoT system demonstrated high agreement with actual volumes and stable performance in real-time measurement. This study proposes more reliable methods for milk volume measurement, directly contributing to a more efficient and a process free from human error. Additionally, it promotes the digitalization of agriculture by encouraging modern and technological practices with the potential to drive development in rural areas and the agricultural sector.

Keywords: dairy, graduated ruler, IoT system, milk tank.

INTRODUCTION

The dairy farming sector has undergone significant transformations in response to shifting demands, particularly with a heightened emphasis on the quality and the traceability of raw milk (Ribeiro Júnior *et al.* 2020). This evolving landscape has pressured dairies to comply with new milk collection and storage standards regulations. Consequently, producers must integrate technology and enhance control measures to align with market demands (Harwood and Drake 2018).

The milk volume measurement can be done in various ways, depending on the type of tank and the type of meter available on the farm. Large-scale dairy farms may employ electronic rulers inserted inside the bulk tanks or scales to directly measure tank weight for milk volume determination. However, the high cost of electronic rulers makes this use unfeasible for small- and medium-scale dairy producers. In these farms, milk volume is typically manually measured by the truck driver using a graduated ruler and a conversion table provided by the tank manufacturer (Papadopoulos *et al.* 2016).

This critical measurement of raw milk volume in bulk tanks is crucial for both the producer and the dairy. Still, it is prone to variability or discrepancies arising from technical issues with measuring devices and, more prominently, human errors during the collection process. Common problems include tank imbalances or misaligned graduated rulers, potentially resulting in unquantified losses for dairy farm operations. Furthermore, fraudulent activities, such as manipulating conversion tables to inflate the milk volume falsely, can also occur.

In addition to these challenges, the geographical distribution of milk collection points presents a logistical problem for the dairy. Effective milk collection management necessitates integrated planning with the farm to determine the volume of milk to be collected to ensure alignment with the capacity of the collection truck (Darchuk *et al.* 2015). This collaborative

approach ensures efficient operations and effective milk collection management, addressing technical and logistical complexities in the dairy's daily workflow.

In similar situations, monitoring technologies incorporating sensors with IoT systems (Jan *et al.* 2022) have been employed for controlling large volumes of stored items, such as water tanks and underground gas tanks (Hidayanti 2020). These sensors aim for precise measurement without the need for manual handling. These sensors hold the potential to contribute to dairy production systems, offering rapid and precise real-time information essential for effective farm management (Cabrera *et al.* 2020).

Thus, the study hypothesizes that varying interpretations of both the manual ruler measurement and the conversion table can result in human errors during milk collection. Additionally, automated milk bulk tank monitoring systems enhance accuracy, precision, and sensitivity, reducing errors in milk storage, and collection for sale. The objectives of the study were to identify potential interpretative errors associated with the use of manual graduated rulers for volume measurement of milk bulk tanks conducted by different individuals and evaluate the accuracy, precision, and sensitivity of one real-time milk tank volume monitoring system.

MATERIALS AND METHODS

Study I - Perception Test

This study was approved by the Research Ethics Committee (protocol 79349424.3.0000.5153) to conduct the research in accordance with ethical standards.

For the graduated ruler perception test conducted by different individuals, the same procedure of filling the tank with a capacity of 1,000 L was repeated. Six evaluators with varying educational levels and no prior training participated in interpreting the milk tank

volume using a graduated scale provided by the tank manufacturer, marked in 40-L intervals, until the tank reached its full capacity. The tank was filled in increments of 40 L using a volumetric calibrator considered the gold standard in this study and certified by the National Institute of Metrology, Quality, and Technology (INMETRO), the Brazilian regulatory and certification authority for measurements, volumetry, and technical standards. The participants were blinded to the amount of milk added to the tank at each measurement. The procedure was repeated over three different days, with the same individuals performing the interpretations on each occasion.

Each individual made three interpretations for each measurement taken every 40-L interval. To reach the tank's total capacity, 25 additions of 40 L of milk were required. Therefore, each evaluator interpreted 75 measurements per day, resulting in 450 interpretations per day. Therefore, considering the six evaluators, 1,350 different interpretations from the graduated ruler were recorded.

Study II -Real-Time Monitoring Sensor with IoT

For real-time milk tank volume and temperature monitoring it was used the sensor Volutech 3.0® (Volutech®, version 3.0, Viçosa, Minas Gerais, Brazil; Fig 1A). This device can be installed in any tank from any brand or model, as long as it adheres to maximum dimension limits. Additionally, the equipment monitors the tank's leveling, the operation of the cooling system, and the tank agitator.

Four Volutech 3.0® sensors were used in a vertical cylindrical bulk tank with a 1,000 L capacity (DMBrasil®, Perdizes, Minas Gerais, Brazil). These sensors, for which a patent application is deposited with the Federal University of Viçosa under number BR 10201902310009, were utilized to monitor the volume of the milk within the tank. The sensors were positioned in the center of the refrigeration tank (Fig 1B). To measure the height of the

milk column and enable volume calculation, optical time-of-flight light detection and ranging technology sensors were used. These sensors, found in small-sized modules, were designed to measure the distance of white objects in dark environments. The employed sensor was the VL53L0x, capable of accurately measuring distances up to 2000 mm. It operated through acrylic protection, ensuring isolation from the external environment.

Volume calculation was based on the value measured by the sensor and the tank dimensions provided during equipment calibration. The volume measurement conducted by the sensors utilizes time-of-flight laser technology, where light was emitted towards the milk and subsequently returned to the sensor. The time it takes for the light to return to the sensor was used to calculate the distance between the sensor and the milk. This distance measurement was then employed to accurately determine the volume of milk in the tank.

The volume calibration procedure involved filling the tank using a 20 L volumetric calibrator. Values from the measuring monitoring sensors were recorded in 40 L interval increments. Twenty-five calibration points were collected until the total capacity of the tank reached 1,000 L. This complete tank-filling process was repeated five times on different days, totaling 500 analyzed measurements. To prevent errors, the equipment was designed to identify the activation status of the agitator, ensuring that measurements were taken only when the milk was in a rest state.

Statistical Analyses

In the statistical analyses, model validation involved a mixed-effects linear regression, where the observed values, corresponding to the liters of milk added through the validated gauge, were compared to the estimated values measured by the sensors. The model incorporated sensor variation as a random effect. The lmer function from the lme4 package in R was used to test hypotheses, examining whether the intercept significantly differs from 0 and whether the slope

significantly differs from 1. Differences considered significant when the P -value < 0.05 . For evaluation, the following equation model used for validation was:

$$Y_{ij} = \beta_0 + B_1 + \gamma_i + \varepsilon_{ij}$$

Where: Y_{ij} = individual observation; γ_i = random effect of sensor or evaluator; ε_{ij} = random error and β_0 and β_1 are the equation parameters.

The response variable predicted by the model was estimated, and the fixed predictor variable of the model was observed, testing if β_0 was equal to zero and β_1 was equal to one.

To understand the effect of the evaluator or the sensor in the total variation obtained in the models, it was computed the participation of the random variance in the total variance of the adjusted model.

The Bland-Altman test was also applied to validate the precision and accuracy of both the sensors and the measurements taken by the gauge, in order to assess the agreement between the two distinct methods evaluating the same quantitative dependent variable. The mean squared error was used to estimate the model's precision and accuracy, subdivided into mean error (indicating the central tendency of the deviation), systematic bias (indicating the deviation of the slope from 1), and random error (not explained by the regression), as suggested by Tedeschi (2006) and tested by Silva et al. (2019). The concordance correlation coefficient (CCC) was also used to test the model's precision and accuracy, subdivided into the estimated correlation coefficient (ρ), which shows the model's precision, and the bias correction factor (C_B), where these values range from 0 to 1, with values closer to 1 indicating higher accuracy. The coefficient of determination (R^2) and correlation coefficient (r) were also described, indicating that the closer the estimated and observed values are to 1, the more correlated they are (Tedeschi, 2006).

RESULTS

Study I - Perception Test

The model used for the estimated values, placed inside the tank with a volumetric calibrator, and the observed values, measured by the evaluators, are shown in Table 1.

The observed values within the milk tank showed correlation with the values estimated by the graduated ruler evaluators ($P = 0.347$; Fig 2A). The intercept value (-0.3652) was not significantly different from 0 ($P = 0.847$), and the slope of the line (0.9987) was not significantly different from 1. The distribution of the residuals was evenly distributed around 0 (Fig 2B). Of all the random variation observed in the model, the effect of the evaluator represented 7.89% of the total variance.

In the Bland-Altman analysis, a bias was found between the estimated and observed values by the evaluators. The random distributions of the mean deviations were close to 0 but diverged at one specific point in the entire analysis ($P < 0.05$; Fig 3).

Study 2 - Real-Time Monitoring Sensor with IoT

The model used for the estimated values, placed inside the tank with a volumetric calibrator, and the observed values, measured by the sensor, are shown in Table 2.

The volume of milk estimated by the real-time monitoring sensors showed strong agreement compared to the volume of milk observed within the tank ($P = 0.176$; Fig 4A). The intercept value (-0.348) was not significantly different from 0 ($P = 0.31$), and the slope of the line (1.001) was not significantly different from 1. The dispersion of the residuals was evenly distributed around 0, suggesting that there is no systematic error associated with the measured milk volume (Fig 4B). Of all the random variations in the model, the sensor variation was insignificant, representing less than 0.0001%.

In the Bland-Altman analysis, no bias was found between the estimated values and the values observed by the real-time monitoring sensors. The random distributions of the data means were close to 0 ($P = 0.155$; Fig 5).

The adequacy of the models for both the sensor and the ruler is demonstrated in Table 3. Both models showed mean square error (MSE) below 1% (0.14 and 0.21%, respectively), indicating a small error associated with the models used. However, the ruler measurement demonstrated a mean error of 0.09%, while the sensor measurement had a mean error of 1%. The analyses revealed CCC, p , and C_B values close to 1, R close to 0, and R^2 close to 1, reinforcing the accuracy and precision of both models.

DISCUSSION

Manual measurements using graduated rulers showed a 7.86% variation between evaluators, representing a significant portion of the total random variance when compared to the real-time monitoring system. This observed variance indicated that milk volume measurements conducted by different evaluators could vary significantly, potentially leading to substantial economic losses for milk producers. Therefore, although the graduated ruler can provide accurate measurements of milk volume, there may be variation when different individuals read the ruler. The main problem is that the truck drivers, who collect the milk, are not always the same, leading to discrepancies in tank volume interpretation and, consequently, in the payment for the milk sold.

This issue highlights that the volume monitoring system measurement can be more consistent and reliable, with significantly lower variations and, consequently, more effective. The evaluated system demonstrated a lower mean error for a 1,000 L tank, underscoring its high accuracy.

Even in light of these data, it is important to emphasize that the difference between the monitoring system and the ruler was significantly different in terms of its mean error due to only one collection point, which could be considered an outlier and removed from the data. However, since it resulted from human error, it can be considered valid to highlight that these types of errors are likely to occur when different people measure the milk volume with the graduated ruler, evidencing potential biases in this type of measurement.

Furthermore, measuring milk tank volume using a ruler is a precise and accurate method, and it is commonly used on dairy farms. However, modernization and the use of technologies have become increasingly frequent in dairy systems. The combination of technology and artificial intelligence can effectively bring economic benefits, save labor, and promote efficient management, in addition to ensuring the quality and safety of the milk (Zhang *et al.* 2021).

Similar approaches, including the development of algorithms that analyze field photos captured with portable photo studios, the use of 3D models generated with portable optical photometric scanners (Papadopoulo *et al.* 2016), and comparative investigations involving milk meters and rulers (Ubbels *et al.* 1974), have been studied to develop more accessible and modern milk tank volume measurements than the commonly used graduated ruler. However, researchers face a substantial challenge due to the high implementation costs of these measurement systems. This challenge is similar to the issue faced with electronic scales and rulers commonly found in large-volume milk cooling tanks, which tend to be higher-cost equipment (Papadopoulo *et al.* 2016).

An inherent advantage of the monitoring system in this study is its operation independent of the operator, facilitated by a digital display integrated into the monitoring unit for volume indication. Additionally, since the measurement is performed using a laser, there is no physical contact with the milk, unlike the graduated ruler, which potentially reduces the risk of bacterial contamination during collection.

The results obtained from the milk volume sensors are promising, indicating a high degree of correspondence between the estimated volumes and the actual volumes of the milk tanks. Although manual measurement is also effective and accurate, the adoption of advanced technologies and the implementation of effective management practices can substantially improve the reliability and efficiency of milk volume monitoring. This feature positions the technology as a valuable tool for the industry, providing real-time indications of the milk volume in the tank. This capability is crucial for optimizing transportation logistics, simplifying the collection process carried out by tanker trucks, and reducing the level of responsibility assigned to the truck driver. Additionally, the system monitors the milk temperature, tank level, and the operation of the cooling system and agitator blades. However, for the purposes of this study, only the volume was assessed.

CONCLUSION

Measuring milk volume in bulk tanks using a graduated ruler is a precise and accurate method, but it is not free from potential human errors when operated by different evaluators. Integrating advanced milk volume monitoring systems can offer a more automated and accurate approach, reducing reliance on manual measurements and mitigating errors and discrepancies. The real-time monitoring system demonstrated high reliability, accuracy, and precision in the data, confirming its potential suitability for use in dairy farms.

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AUTHOR CONTRIBUTIONS

Tassia Barrera de Paula Silva: Investigation; writing – original draft; investigation; formal analysis; validation; writing – review and editing. **Luis Henrique Rodrigues Silva:** Investigation; formal analysis. **Gustavo Ramos Duque Estrada:** Investigation; formal analysis. **Lucas Capucho Sandres:** Investigation; formal analysis. **Alex Lopes da Silva:** Formal analysis; investigation; validation. **Marcos Veiga Santos:** Formal analysis; investigation; validation. **Marcos Inácio Marcondes:** Validation; writing – review and editing. **João Henrique Cardoso Costa:** Writing – review and editing; validation. **Polyana Pizzi Rotta:** Supervision; validation; writing – review and editing.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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Table 1 Values from the model used for the estimated values inside the tank and the observed values measured by the evaluators.

Item	Parameter	P-value
Intercept	-0.3652 ± 1.8920	0.847
Slope	0.9987 ± 0.0013	0.347

Table 2 Values from the model used for the estimated values inside the tank and the observed values measured by the sensor.

Item	Parameter	P-value
Intercept	-0.3480 ± 0.3422	0.310
Slope	1.001 ± 0.0008	0.176

Table 3 Adequacy parameters of the equations to measuring milk tank volume

Item	Sensor	Ruler
MSE	0.14	0.21
Partition of MSE (%)		
Mean bias	1.00	0.09
Systematic bias	0.40	0.51
Random error	98.6	99.4
CCC (ranging from 0 to 1)		
p	0.99	0.99
C_b	0.99	0.99
R	0.99	0.99
R^2	0.99	0.99

MSE, mean square error; CCC = concordance correlation coefficient; P = correlation coefficient estimate; C_b = bias correction factor.

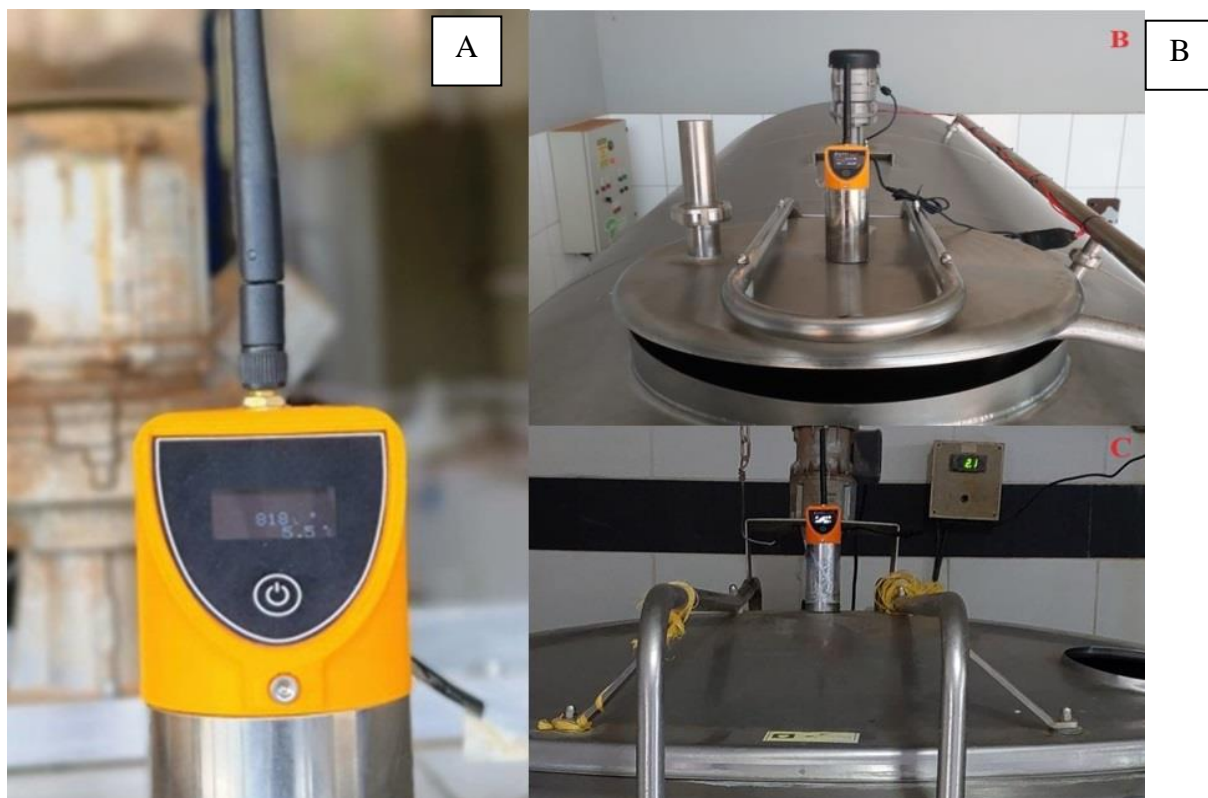


FIGURE 1. A: Real-time volume monitoring system. B: Demonstration of the monitoring system attached to a horizontal refrigeration tank.

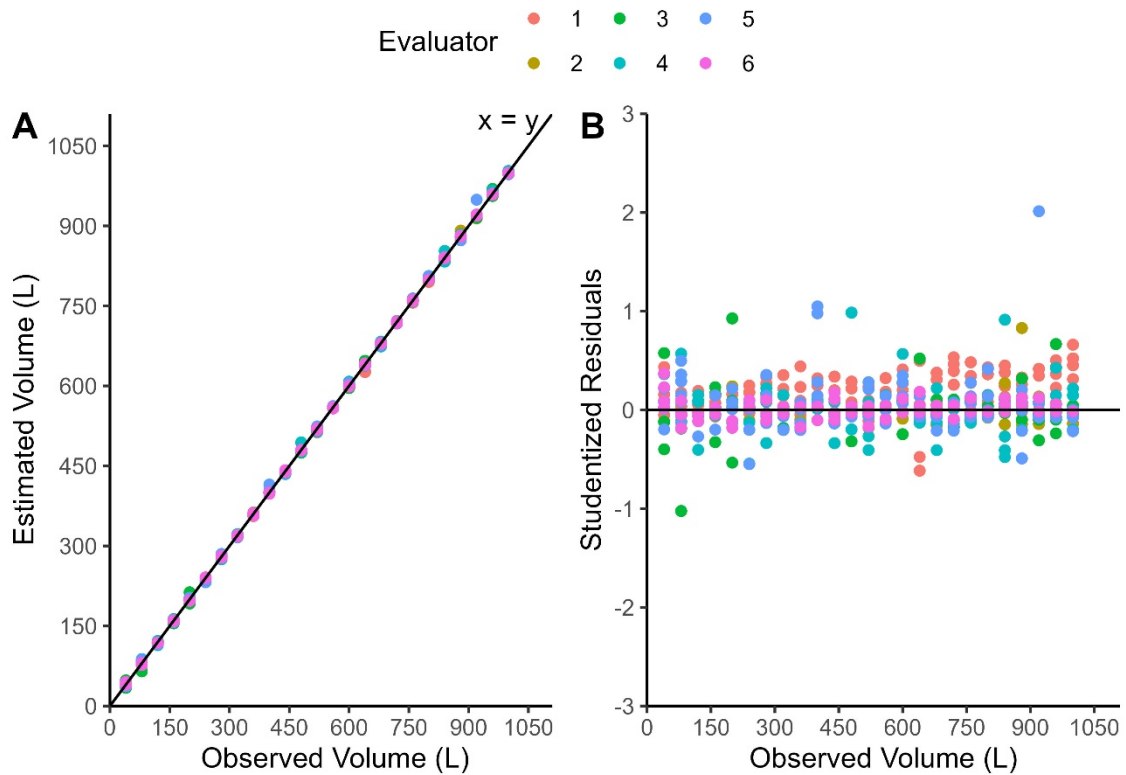


FIGURE 2 Observed and estimated values and residual dispersion of the graduated ruler. (A) Relationship between observed and estimated milk tank volume values on the graduated ruler, highlighting the slope of the line (P – value = 0.347). (B) Dispersion of the studentized residuals according to the observed milk tank volume values remains consistent regardless of the measured volume of milk.

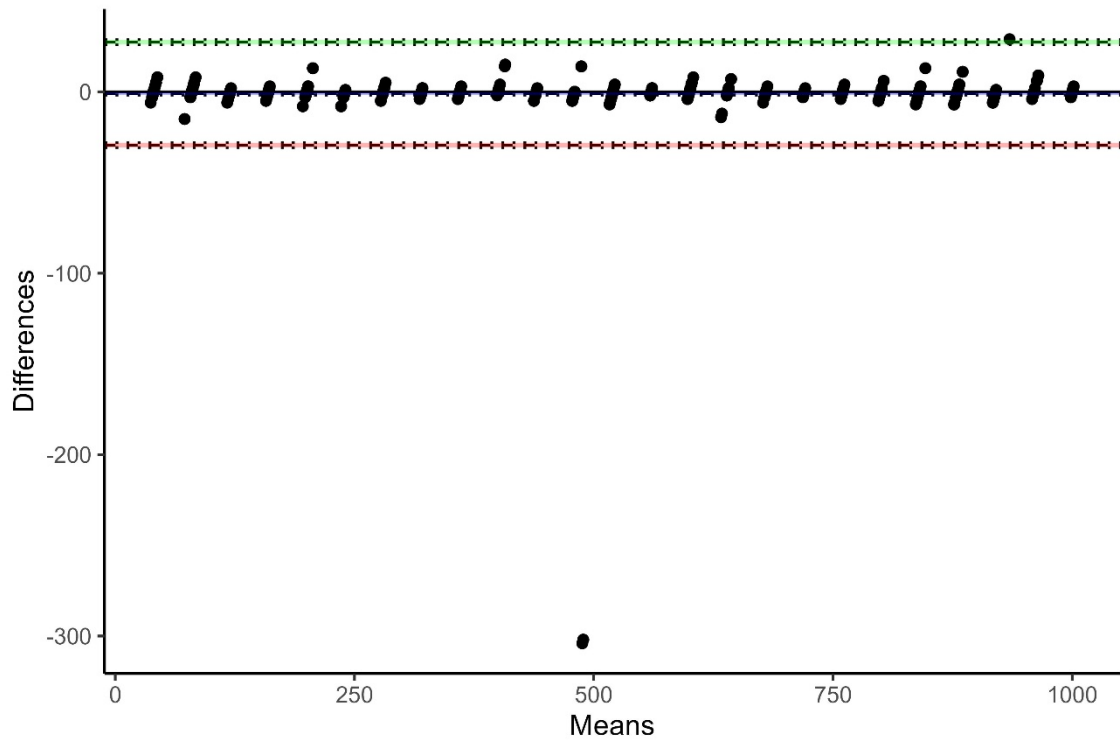


FIGURE 3 Bland-Altman analysis of the evaluators using the graduated ruler. Means of the observed and estimated values from the Bland-Altman test ($P < 0.05$).

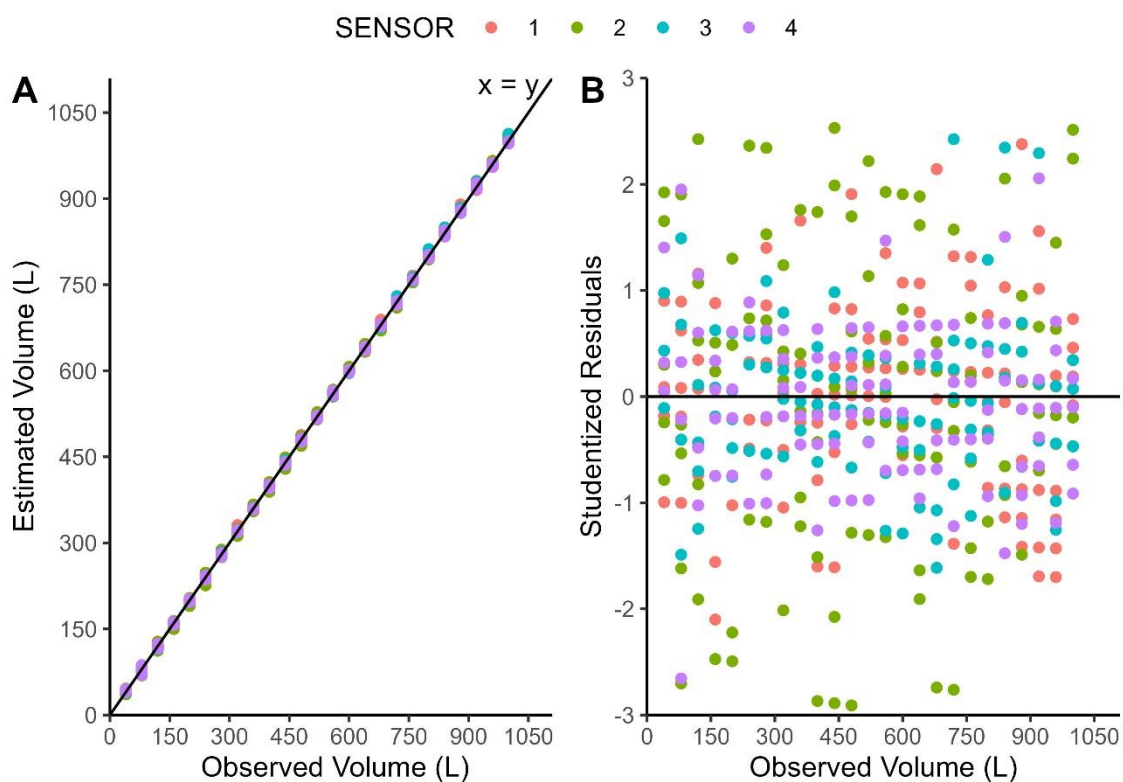


FIGURE 4 Observed and estimated values and residual dispersion of the real-time monitoring sensors. (A) Relationship between milk tank volume estimated by sensors and actual volume across all measurements ($P = 0.176$). (B) Dispersion of the studentized residuals according to the milk tank volume values observed by the sensors highlights consistent error distribution and homogeneous estimates, irrespective of the measured.

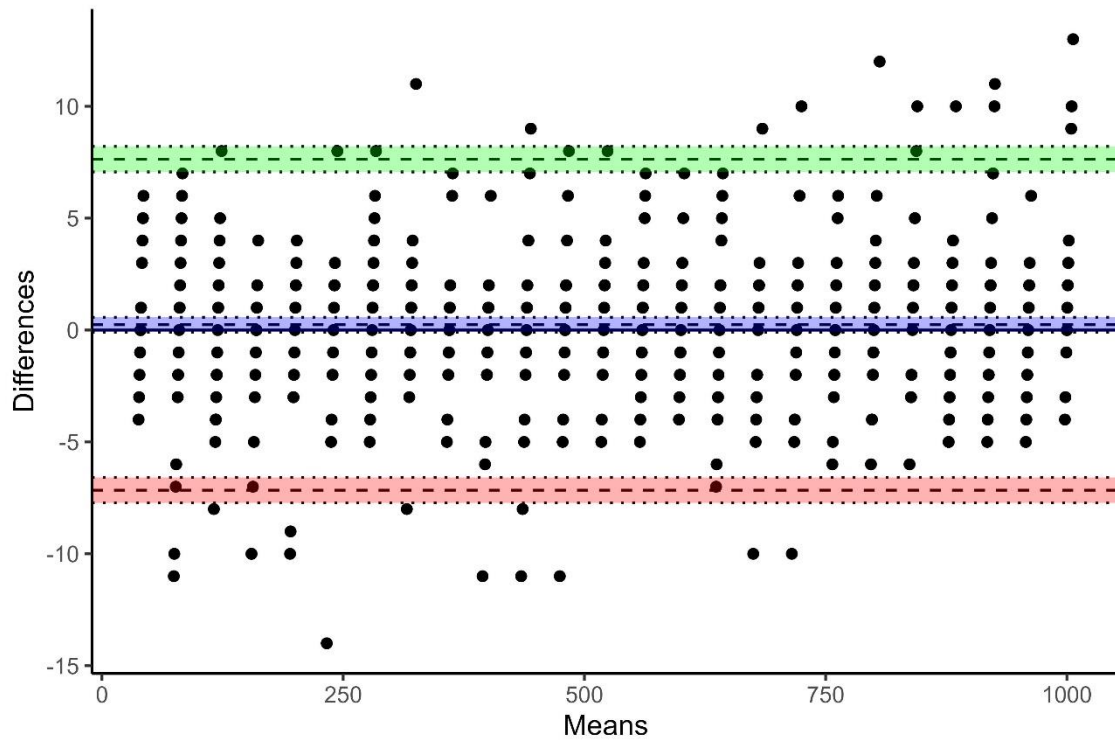


FIGURE 5 Bland-Altman analysis of the evaluators using the real-time monitoring sensors. Means of the observed and estimated values from the Bland-Altman test ($P = 0.155$).