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Potential biomarkers for lameness and claw lesions in dairy cows: A scoping review

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Review Article

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Abstract

One of the major challenges in lameness management is prompt detection, especially before visible gait disturbance. This scoping review describes the potential biomarkers for lameness in dairy cows reported in the literature, their relevance in lameness diagnosis, identifying cows at risk of developing claw lesions and monitoring recovery after treatment. Using specific keywords, a comprehensive literature search was performed in three databases: PubMed, Google Scholar and ScienceDirect to retrieve relevant articles published between 2010 and 2022. A total of 31 articles fulfilling the inclusion criteria were analysed. The categories of potential markers for lameness reported in the literature included acute phase proteins (APPs), nociceptive neuropeptides, stress hormones, proteomes, inflammatory cytokines and metabolites in serum, urine and milk. Cortisol, APPs (serum amyloid A and haptoglobin) and serum, urinary and milk metabolites were the most studied biomarkers for lameness in dairy cows. While APPs, nociceptive neuropeptides and blood cortisol analyses assisted in elucidating the pain and stress experienced by lame cows during diagnosis and after treatment, evidence-based data are lacking to support their use in identifying susceptible animals. Meanwhile, metabolomic techniques revealed promising results in assessing metabolic alterations occurring before, during and after lameness onset. Several metabolites in serum, urinary and milk were reported that could be used to identify susceptible cows even before the onset of clinical signs. Nevertheless, further research is required employing metabolomic techniques to advance our knowledge of claw horn lesions and the discovery of novel biomarkers for identifying susceptible cows. The applicability of these biomarkers is challenging, particularly in the field, as they often require invasive procedures.

Introduction

Lameness affects the sustainability of dairy farms due to the impact on animal health and production, which culminates in ethical and economic implications (Charfeddine and Perez-Cabal, 2017; Warner *et al.*, 2021). Despite efforts to reduce lameness levels in the dairy industry, recent studies reflect that the average prevalence in the United Kingdom is as high as 30%, ranging from 14% to 36% in other developed countries (Griffiths *et al.*, 2018; Randall *et al.*, 2019). In Asia, cross-sectional studies conducted in Malaysia, India and China recorded lameness prevalence between 21 and 39% (Chapinal *et al.*, 2014; Sadiq *et al.*, 2017a, 2017b, 2020).

Early detection and timely treatment are crucial to mitigating the impact of lameness in dairy cows (Miguel-Pacheco *et al.*, 2017). While visual locomotion scoring by trained observers is the most widely used diagnostic technique for lameness (Schlageter-Tello *et al.*, 2014), lame cows experience pain that is usually masked by their instinctive stoicism as prey species, making it difficult to detect the disease before the onset of clinical signs (O'Callaghan *et al.*, 2003). Intra- and inter-observer variability also represents another limitation of locomotion scoring (Schlageter-Tello *et al.*, 2014). Thus, diverse efforts are presently being explored to develop more sensitive and objective assessments for lameness diagnosis. Several studies have focused on identifying biomarkers or sensitive diagnostic markers of lameness (Zhang *et al.*, 2020; Dervishi *et al.*, 2020).

Biomarkers are well-defined and specific physiological, anatomical or biochemical parameters or substances, which have been established to be useful in diagnosing and treating

various conditions (Califf, 2018). Examples of blood biomarkers used as a marker of stress or acute pain in dairy cows include cortisol, nociceptive neuropeptides (i.e., norepinephrine, beta-hydroxybutyrate, substance P and beta-endorphin) and acute-phase proteins (APPs) such as haptoglobin and serum amyloid A (SAA: Kujala *et al.*, 2010; Kontturi *et al.*, 2020). There are numerous articles reporting alterations in these markers and metabolites during and after lameness diagnosis, and for elucidating the pain experienced by lame cows (Bagga *et al.*, 2016; Kontturi *et al.*, 2020). Nevertheless, their relevance in detecting cows susceptible to lameness and specific claw lesions, and efficacy in assessing recovery after treatment, are not fully understood.

Biomarkers could be highly relevant in lameness management by enabling the identification of cows at risk of becoming lame and predicting the specific claw lesion that may arise from ongoing metabolic alterations (Dervishi *et al.*, 2020; Barden *et al.*, 2023). However, if biomarkers are unable to yield this vital information, their significance in lameness management combined with high cost may not be economically friendly to farmers and stakeholders (Barden *et al.*, 2023). The advantages of investigating the biomarker need to outweigh its disadvantages since invasive procedures may be required that may affect applicability in the field. Given the recent interest in biomarkers for the production diseases in dairy cows, it is pertinent to assess predictive biomarkers for bovine lameness. Despite the extensive research on potential biomarkers for lameness and claw lesions in dairy cattle, no in-depth review has been published and no study has attempted to summarise the findings of previous studies on the topic. Scoping reviews are conducted to map relevant studies and present evidence on specific research topics (Arksey and O'Malley, 2005). Thus, this study aims to review potential biomarkers for lameness in dairy cows reported in the literature, particularly their relevance in lameness diagnosis, identifying cows at risk of becoming lame, predicting specific claw lesions and monitoring recovery or lesion progression after treatment.

Methods

The protocol applied in this study was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) extension for scoping reviews (Tricco *et al.*, 2018) and the scoping review framework of Arksey and O'Malley (2005). Our research questions were as follows: Which metabolites or substances have been widely investigated as biomarkers of lameness in dairy cows? Are the biomarkers reported in the literature effective in identifying dairy cows susceptible to lameness, predicting the risk or presence of specific claw lesions, and monitoring the disease progression after treatment?

Study design

This study entails a scoping review to map the existing literature on the topic. We decided to perform a scoping review given the expected diversity of study designs employed in previous studies (Arksey and O'Malley, 2005): scoping reviews are more suited to broader aims. Moreover, there are several potential biomarkers for lameness in dairy cattle, therefore, a broader aim is required to achieve the research objective. Likewise, an equitable risk of bias assessment is difficult due to the wide range of study designs. This is supported by accumulated evidence suggesting that risk of bias assessments is not compulsory in scoping reviews (Grant and Booth, 2009). In order to perform a systematic and transparent review, PRISMA guidelines were followed (Tricco *et al.*, 2018; Page *et al.*, 2021). The guidelines offer researchers the opportunity to define research questions, identify exclusion and inclusion criteria and assess relevant and accessible scientific articles while conducting a scoping review (Shamseer *et al.*, 2015).

Search strategy and search terms

Three search engines (PubMed, Google Scholar and ScienceDirect) were employed in this study. The literature search was restricted to studies published between 2010 and 2022 considering that studies on biomarkers for bovine lameness have gained more ground in the last decade (Zhang *et al.*, 2020). Moreover, this duration will enable the reviewers to gather more recent and relevant information on the research topic. The search was performed during late March, 2023. A backward reference list checking was also conducted by screening the reference lists of the included studies on the search topic. Furthermore, a forward reference list checking was carried out using the 'cited by' function available in Google Scholar.

Aligning with the key elements and objectives of this review, the search terms were broadly categorised into five components: lameness, dairy cows, claw/hoof lesions/diseases, biomarkers and metabolites. Alternative keywords were permitted for each component as denoted by the Boolean operator 'OR'. The separator 'AND' was used in combining each component with other wordings. Boolean operators were not used for the Google Scholar database. Meanwhile, the database to be evaluated for words beginning with specific letters was denoted by an asterisk. The literature search string is presented in the Supplementary file.

Study selection

Specific inclusion and exclusion criteria were considered during the study selection process. Irrespective of study designs, all articles written in English and describing biomarkers of lameness in dairy cows with alternative wordings utilised according to the search terms were initially retrieved. Subsequently, only original research articles, short communications and research presented at conferences were considered for further review while reviews and book chapters were excluded. Articles that were inaccessible by any means were also excluded from further assessment.

In this scoping review, all the titles and abstracts of all the retrieved articles were independently screened by MBS and SZR. In addition, both authors read the full texts of articles included in the first step. The full texts of selected articles were only read after fulfilling the eligibility criteria. UK was responsible for the resolution of any disagreements between the search authors. Intercoder agreement for screening titles and abstracts was assessed using the Cohen Kappa, and the obtained values were reliable and consistent (>0.70: Orwin and Vevea, 2009).

Data extraction

The type of extracted information was specified initially by the second author (SZR), a veterinary epidemiologist. A data extraction form was used in this study and the extracted data included the author, year of publication, study location, study design and population, investigated biomarkers, treatment or intervention provided and main findings. Two authors (MBS and SZR) performed the data extraction independently and a third reviewer

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(UK) was called upon to resolve any discrepancies in decisions between the former. A high level of agreement (Cohen Kappa = 0.83) was obtained (Orwin and Vevea, 2009).

Assessment of risk of bias

Generally, scoping reviews comprise studies with different research designs, which makes it challenging to assess the risk of bias (Grant and Booth, 2009). This position aligns with the current review as the retrieved studies had broad aims and diverse methods were applied, ranging from experimental to observational studies. Therefore, a risk of bias assessment was not performed in this review.

Data synthesis

We applied a narrative approach to synthesise the data extracted from the retrieved studies. Themes were generated according to the findings of the included studies. Thematic analyses have been used in several scoping and systematic reviews on metritis, mastitis and several other diseases in dairy cows (Garzon *et al.*, 2022; Giagu *et al.*, 2022). Since this review is exploratory, an inductive approach was employed to directly synthesise themes from the data. Specifically, MBS examined and scrutinised the extracted data for more familiarity. This was then followed by systematic data coding, which was carried out by SZR. Themes were then generated based on the codes assigned. The generated themes were checked by all authors to ensure they aligned with the extracted data and assigned codes. Any discrepancy was resolved by discussion. All authors also participated in defining and naming the emerging themes. The analysis process was managed using Microsoft Excel.

Results

Search results

Online Supplementary Figure S1 depicts the total number of articles retrieved from the three databases ($n = 1177$). Upon removing 184 duplicates, 628 titles and abstracts were screened. The screening process led to the exclusion of 312 titles and 182 abstracts. The full text of 134 articles was read and 109 were excluded. The reasons for the exclusion are presented in the same Figure. Finally, 6 articles were retrieved from the backward and forward reference checking. Overall, 31 articles fulfilled the inclusion criteria and were reviewed in this study.

Descriptive analyses

Most of the studies entail cross-sectional ($n = 17$), prospective longitudinal ($n = 6$), randomised control trials (RCT) and quasi-experimental ($n = 4$) as well as nested case–control ($n = 4$) research designs (online Supplementary Table S1). A higher number of articles were published between 2017 and 2022 ($n = 24$) compared to those published from 2010 to 2016 ($n = 7$). Studies were conducted in North America (United States, $n = 5$ and Canada, $n = 5$), Europe ($n = 14$), Asia and Oceania ($n = 5$) and South America ($n = 2$: online Supplementary Figure S2). Convenience sampling was used in selecting herds in all the relevant studies, and 26 studies enrolled cows within the herd by convenience. Only 18 of the studies described the sample size calculation approach. Nevertheless, 3 of the 4 experimental studies reported sample size estimation method, randomisation and blinding of the personnel involved. Two-thirds of the studies enrolled 1–10 herds ($n = 25$), 10–20 herds ($n = 4$) or >20 herds ($n = 2$). The number of animals enrolled in the articles ranged from 10 to 25 per group and 6–6292 in the experimental and observational studies, respectively.

In terms of the studied population, most studies involved already lame cows or those transiting from non-lame to lame status without investigating the specific causes or claw lesions ($n = 20$). Meanwhile, 7 studies focused on cows with claw lesions of different severity without mentioning their lameness status and only 5 studies considered both lameness and the associated claw lesions. The most prevalent claw lesions investigated in this scoping review were non-infectious lesions such as sole ulcers and white line disease ($n = 10$), while only 3 studies involved cows affected with infectious claw lesions, namely interdigital phlegmon and digital dermatitis.

Synthesis of results

Based on the specific biomarkers investigated in the reviewed studies, 7 categories of potential biomarkers of lameness in dairy cows were identified: (1) acute phase proteins (2) cortisol (serum, plasma, salivary and hair) (3) serum and urinary metabolites (4) proteome (5) milk constituents and metabolites (6) nociceptive neuropeptides and (7) inflammatory cytokines and gene expression. Detailed information on these results is presented in online Supplementary Table S2.

Acute phase proteins, SAA and haptoglobin

Nine studies investigated the role and alterations of APPs in lame cows (detailed in online Supplementary Table S3). The findings can be broadly categorised into those reporting the changes in APPs between lame and non-lame cows (Zhang *et al.*, 2015), with and without claw lesions (Abuelo *et al.*, 2016; Ilievska *et al.*, 2019; Pirkkalainen *et al.*, 2022) and a combination of both (Bagga *et al.*, 2016; Kontturi *et al.*, 2020). Zhang *et al.* (2015) found that the concentrations of SAA were significantly increased at 8 weeks and 4 weeks before parturition in cows that later became lame postpartum. Pirkkalainen *et al.* (2022) reported a significant increase in SAA and Hp levels in cows with sole ulcers compared to the control group on day 7 after diagnosis, but the finding was not detected in those affected with white-line disease and digital dermatitis. Similar results were conveyed by O'Driscoll *et al.* (2017) and Ilievska *et al.* (2019). Nevertheless, elevated serum APP levels were recorded in cows with digital dermatitis relative to those with heel erosion, white line separation and the control group (Ilievska *et al.*, 2019). Nazifi *et al.* (2012) also found that both APPs increased significantly in cows with digital dermatitis compared to those without such lesions.

Three of the 9 articles considered both clinically lame cows and the specific claw lesion (O'Driscoll *et al.*, 2015; Bagga *et al.*, 2016; Kontturi *et al.*, 2020). Cows with severe sole haemorrhage recorded higher ($P < 0.05$) plasma Hp concentrations, which were associated with increasing locomotion scores relative to those with moderate and mild cases (O'Driscoll *et al.*, 2015). Given that Hp was the only parameter that differed consistently with increasing lameness score, it was considered a promising candidate and marker for painful claw horn lesions. Apart from SAA and Hp, only one study in the revised articles assessed changes in fibrinogen and C-reactive

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protein in lame cows (Bagga *et al.*, 2016). Lame cows with sole ulcers, white line disease and sole haemorrhage had 3, 20, 4, and 5 times higher concentrations of SAA, Hp, fibrinogen and C-reactive protein respectively compared to the non-lame cows. Kontturi *et al.* (2020) highlighted that acutely lame cows in farms with high morbidity and combined infection with *Fusobacterium necrophorum* and *Dichelobacter nodosus* (i.e., infectious causes of interdigital phlegmon) depicted the highest response in Hp and SAA, with significant reduction in albumin levels compared to farms with moderate morbidity.

Nociceptive neuropeptides

Substance P, β-endorphin, and norepinephrine were the most investigated nociceptive neuropeptides in bovine lameness-related studies. As presented in the online Supplementary Table S4, we found six articles in which these biomarkers were analysed in cows with induced lameness (Bustamante *et al.*, 2015; Kleinhenz *et al.*, 2019; Warner *et al.*, 2021), natural lameness (Herzberg *et al.*, 2020a) and those with higher locomotion scores (Rodriguez *et al.*, 2018; Martin *et al.*, 2022).

All the studies involving lameness induction reported a significant elevation in plasma concentrations of substance P, norepinephrine and beta-endorphin irrespective of the inducing agent used (Bustamante *et al.*, 2015; Kleinhenz *et al.*, 2019; Warner *et al.*, 2021). Likewise, Rodriguez *et al.* (2018) revealed higher concentrations of beta-endorphin and substance P in cows with increasing locomotion scores compared to those with sound locomotion. In contrast, no significant alterations in substance P were detected in lame cows after treatment with either flunixin meglumine or ketoprofen relative to the control groups (Kleinhenz *et al.*, 2019; Warner *et al.*, 2021).

Hair, blood and salivary cortisol

Hair cortisol concentrations were not affected by either acute or chronic lameness (Fischer-Tenhagen *et al.*, 2018) and were not associated with any of the welfare protocols involving lameness and hoof health data (Van Eerdenburg *et al.*, 2021). Sharma *et al.* (2019) also found no significant association between hair cortisol levels and stress-related disorders such as lameness and claw overgrowth in 54 cattle shelters. Similar findings were highlighted in studies involving salivary cortisol analyses (Martin *et al.*, 2022). These aforementioned studies concluded that hair cortisol may not reflect the ongoing stress experienced in lame cows. Meanwhile, serum and plasma cortisol were reported as stress indicators in lame cows, particularly those affected with severe sole lesions (O'Driscoll *et al.*, 2015, 2017). Juozaitiene *et al.* (2021) also found that the odds of identifying a lame cow increased by 4.9 times as the blood cortisol level exceeded 1 µg/dl, indicating the sensitivity of serum cortisol to the risk of lameness (Online Supplementary Table S5).

Some studies reported alterations in blood cortisol levels during the onset of induced lameness (Bustamante *et al.*, 2015; Kleinhenz *et al.*, 2019) or after treatment (Janßen *et al.*, 2016). Following lameness induction, plasma cortisol increased significantly starting at 6hr and reaching maximum concentrations at 24 h (Bustamante *et al.*, 2015). A randomised trial involving cows with amphotericin B-induced arthritis and synovitis which were later treated either with flunixin meglumine or left untreated revealed significant changes in serum cortisol 120 h after initial treatment (Kleinhenz *et al.*, 2019). Similar results were reported by Janßen *et al.* (2016) and Warner *et al.* (2021). In the latter, cows with amphotericin B-induced lameness demonstrated lower cortisol levels after treatment with flunixin meglumine relative to those treated with meloxicam and the control groups.

Serum/plasma, urinary and salivary metabolites

Numerous serum metabolites such as lactate, glucose (Janßen *et al.*, 2016), non-esterified fatty acids (NEFA), amino-acids (Dervishi *et al.*, 2020) and urinary metabolites (Zhang *et al.*, 2020) were reported as potential biomarkers of lameness in dairy cattle. Zheng *et al.* (2020) utilised the Nuclear Magnetic Resonance-based metabolomics and found that 21 metabolites were differently abundant between healthy dairy cows and those with acute foot rot, whereby the associated pathways were the synthesis and degradation of ketone bodies, biosynthesis of valine, leucine and isoleucine, and metabolism of methane, pyruvate, glycine, serine and threonine. Dervishi *et al.* (2020) also performed serum Gas Chromatography–Mass Spectrometry metabolomics analysis and found significant alterations in at least 10 at 4–8 weeks before calving and during the week of lameness diagnosis. In the latter, higher concentrations of glycine, leucine, phenylamine, serine, valine, D-mannose, myo-inositol, and phosphoric acid were recorded in pre-lame cows. In contrast, such alterations were not detected in the control cows either before or after calving.

For urinary metabolites, the concentrations of five metabolites (tyrosine, adipate, glycerate, 3-hydroxy-3-menthyl glutarate and uracil) decreased significantly in lame cows during diagnosis while those of N-acetyl aspartate, glutamine, imidazole, pantothenate, beta-alanine and trimethylamine differed significantly at 4 weeks post-calving (Zhang *et al.*, 2020). Similarly, Eckel *et al.* (2020) identified acylcarnitines and glycerophospholipids, especially phosphatidylcholines, as the group of urinary metabolites with the strongest variation in pre-lame and lame cows. As presented in the online Supplementary Table S6, other metabolites reported in the relevant studies were circulating ketone bodies (Zhang *et al.*, 2015), saliva analytes (Contreras-Aguilar *et al.*, 2020), vitamin-active compounds (Strickland *et al.*, 2021), lactate, glucose and fatty acids (Janßen *et al.*, 2016).

Proteome

Two articles employed proteomic analysis to identify specific proteins as potential candidates for detecting lame cows (Dong *et al.*, 2015; Herzberg *et al.*, 2020b). Dong *et al.* (2015) found that plasma proteins such as albumin and haptoglobin and those responsible for carbohydrates and lipid metabolism, such as 3-hydroxy-3-methylglutaryl-CoA, reductase transmembrane glycoprotein, apolipoprotein, haptoglobin and conglutinin, were differentially abundant in dairy cows with laminitis compared to the healthy control. Herzberg *et al.* (2020b) explored the proteomic profile of the spinal cord samples from lumbar segments (L2–L4) of chronic lame cows and observed a strong upregulation of interacting proteins with chaperone and stress proteins such as Hsp70, Hsc70, Hsp90, and interacting proteins related to the glycolytic pathway (online Supplementary Table S6).

Milk constituents and metabolites

Six studies in this review attempted to elucidate the alterations in milk protein, metabolites and overall yield as biomarkers of lameness in cattle (online Supplementary Table S6). The various milk-related

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parameters analysed include cortisol (Gellrich *et al.*, 2015), mid-infrared-based metabolites (Mineur *et al.*, 2020), electrical conductivity and milking time (Juozaitiene *et al.*, 2021), milk fat, fat-to-protein ratio (Zhang *et al.*, 2015), milk protein (Van Altena *et al.*, 2016; Zwierzchowski *et al.*, 2020), dried milk spots (He *et al.*, 2022) and milk yield (Zhang *et al.*, 2015; Juozaitiene *et al.*, 2021).

Three of the relevant studies employed metabolomics in identifying potential biomarkers of lameness risk by analysing milk protein composition and dried milk spots (Van Altena *et al.*, 2016; Zwierzchowski *et al.*, 2020; He *et al.*, 2022). Van Altena *et al.* (2016) found that lactoferrin levels in milk were significantly associated with the risk of lameness, particularly in low-resistant cows, and a higher probability of being culled within a year. Likewise, untargeted metabolomics was successfully used to identify phosphatidylglycerol as the strongest and most sensitive indicator of lameness among numerous discriminative metabolites in dried milk spots (He *et al.*, 2022). In the other reviewed studies, milk components such as milk fat and fat-to-protein were significantly lower in lame cows compared to their sound counterpart (Zhang *et al.*, 2015). Lame cows also demonstrated poor milk traits such as longer electrical conductivity and shorter milking time relative to non-lame cows (Juozaitiene *et al.*, 2021).

Pro-inflammatory cytokines and gene expression

Overall, four articles reported pro-inflammatory cytokines and expression of genes associated with lameness in dairy cows (O'Driscoll *et al.*, 2015; Zhang *et al.*, 2015; Herzberg *et al.*, 2020a; Vermeersch *et al.*, 2022). Two of the studies focused on cows with specific claw lesions, digital dermatitis and sole ulcers (O'Driscoll *et al.*, 2015; Vermeersch *et al.*, 2022) while the other involved acute and chronic lame cows (Zhang *et al.*, 2015; Herzberg *et al.*, 2020a). The most investigated cytokines were interleukins (IL), Tumour necrosis factor (TNF), interferon (IFN) and chemokine (C-X-C motif) ligand. Zhang *et al.* (2015) found that acutely lame cows had higher concentrations of IL-6 during diagnosis compared to non-lame cows. More importantly, the lame group recorded enhanced serum concentrations of IL-6 at 8 and 4 weeks before parturition. On the other hand, Herzberg *et al.* (2020a) investigated 10 cytokines in the spinal cord of chronically lame cows and found significantly higher concentrations of IL-1-α, IL-13, IFN-α, IFN-γ, TNF-α, CXCL10 and CXCL9 and a lower IL-21 concentration compared to those without perturbed gait (online Supplementary Table S7).

In a study that focused on specific claw lesions, higher expressions of tumour-derived matrix metalloproteinase-13 and l-selectin were observed in cows with sole ulcers relative to those without claw lesions (O'Driscoll *et al.*, 2015). In addition, the former tended to have higher expressions of IL- 1α, IL-1β, CXCL8, IL-10, Fas and haptoglobin. A recent study by Vermeersch *et al.* (2022) attempted to determine the inflammatory pathways and gene expression patterns of digital dermatitis lesions in dairy cows. The authors found distinct gene expression patterns between the acute (M1–M2, and M4.1) and chronic (M3 and M4) stages, and all stages demonstrated activation of the IL-17 signalling pathway through the upregulation of IL-17F. Meanwhile, keratins and anti-inflammatory molecules were significantly downregulated.

Discussion

This scoping review yielded a total of 31 articles fulfilling the inclusion criteria of studies on biomarkers for lameness in dairy cattle. Acute phase proteins and cortisol were the most studied biomarkers in the retrieved articles, whereas only a few studies focused on milk constituents or traits, inflammatory cytokines or metabolites of lipids, carbohydrates and protein. These findings might be linked to the relative relevance of the biomarkers in bovine lameness research, as well as the ease of performing the procedures. For instance, alterations in APPs and cortisol have consistently been reported in several other painful and stress-related procedures in cattle, such as dehorning and castration (Meléndez *et al.*, 2018; Park *et al.*, 2020), thereby supporting the attempts to investigate their role in bovine lameness.

APPs are blood proteins whose concentrations are altered in response to inflammatory conditions in animals. APPs are either negative (albumin and transferrin) or positive (C-reactive protein, Hp, SAA and fibrinogen), comprising those that decrease or increase in levels in response to tissue injury or bacterial toxins (Schneider, 2015). Thus, most of the reviewed articles investigated positive APPs while only two studies considered both positive and negative types (Bagga *et al.*, 2016; Kontturi *et al.*, 2020). Overall, SAA and Hp increased significantly in lame cows affected with non-infectious or infectious claw lesions compared to those without gait disturbance. These results were reported in cows assessed immediately after lameness diagnosis and a week later (Ilievska *et al.*, 2019; Pirkkalainen *et al.*, 2022). Claw horn lesions, particularly sole ulcers, often lead to lameness and are considered more painful relative to heel horn erosion and digital dermatitis (Sadiq *et al.*, 2017a, 2017b). The aetiopathogenesis which entails an ongoing systemic inflammation during the transition period, instability of the pedal bone, and pricking of the corium might explain the increased severity (Newsome *et al.*, 2016). Overall, the results reflect the sensitivity of SAA and Hp to the severity of claw lesions during diagnosis.

Cortisol has been widely employed as a stress biomarker in lame cattle based on the connection between acute pain and the activation of the hypothalamus–pituitary–adrenal (HPA) axis (Tadich *et al.*, 2013). As found in this review, cortisol was among the most investigated biomarkers in bovine lameness using different samples, including hair, saliva and plasma/serum. Nonetheless, there is little to no evidence to support the use of hair and salivary cortisol as potential markers of lame cows. None of the studies reviewed depicted significant differences in hair and salivary cortisol in lame cows or those affected with claw lesions compared to healthy cows, suggesting that both cortisol samples do not reflect the ongoing stress experienced in lame cows. Factors such as high inter-individual variability in baseline cortisol concentrations and the sensitivity of hair cortisol to environmental factors, nutrition, and cow-level factors may also contribute to these findings (Moya *et al.*, 2015; Ghassemi Nejad *et al.*, 2017). On the other hand, plasma or serum blood cortisol levels were significant markers of stress in lame cows from a few hours to days after treatment, as well as during recovery. However, there is a dearth of information on alterations in blood cortisol before lameness detection. It is also possible that samples taken over a period of time within individual animals could reveal changes and increased risk of deteriorating hoof condition. This research gap might be bridged by conducting longitudinal and repeated measures study designs in which blood cortisol or other potential biomarker is analysed from the pre-lame period to the onset and after lameness diagnosis.

Our review revealed extensive investigation of substance P, β-endorphin, and norepinephrine as predictive biomarkers of lameness. The assessment of these chemical messengers in most

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induced-lameness studies reflects their vital roles in inflammatory and painful conditions. Their plasma concentrations increased significantly irrespective of the lameness-inducing agent used (Bustamante *et al.*, 2015; Kleinhenz *et al.*, 2019). Substance P is among the first responders to most stressors in animals, especially those capable of compromising biological activity (such as lameness: Butler *et al.*, 2015; Kleinhenz *et al.*, 2019). On the contrary, no significant alterations in substance P were detected in lame cows after treatment with either flunixin meglumine or ketoprofen relative to the control groups (Kleinhenz *et al.*, 2019; Warner *et al.*, 2021). While the findings suggest that substance P may not be a reliable biomarker for monitoring recovery from lameness, both studies recruited cows with experimentally induced lameness rather than naturally occurring lameness. Further investigation into the association between lameness and neuropeptide concentrations, particularly in naturally lame cows, is necessary to elucidate their role in detecting lame cows and recovery after treatment.

A few studies in this review successfully used metabolomics analyses to investigate alterations in specific metabolites hypothesised as early predictive biomarkers of lameness (Dervishi *et al.*, 2020; Eckel *et al.*, 2020). Recent advances in metabolomics sciences have assisted in developing novel diagnostic monitoring technologies (Eckel *et al.*, 2020). The serum metabolomics analysis by Dervishi *et al.* (2020) highlighted convincing results as pre-lame cows were characterised by higher concentrations of numerous amino acids (glycine, valine, serine, leucine and phenylalanine) around 8 weeks before and during lameness episodes. Valine and leucine are branched chain amino acids that play active roles in the regulation of protein synthesis by immune cells and cytokine release (Li *et al.*, 2007; Rodriguez *et al.*, 2019). Serine, on the other hand, appears to be a requirement for the induction of IL-1β mRNA by LPS as its absence reduced LPS induction of the pro-inflammatory cytokine IL-1β (Stachlewitz *et al.*, 2000). Previous studies also demonstrated the immunosuppressive effects of glycine and phenylalanine, leading to the inhibition of T lymphocytes (Yang *et al.*, 2012). These amino acids, particularly the ketogenic types (leucine and phenylalanine), may be catabolised leading to the synthesis of acetyl CoA, which can be utilised to produce fatty acids or ketone bodies. This event might explain the tendency for pre-lame cows to have increased beta-hydroxyutyrate concentration at 4 weeks prepartum as reported by Zhang *et al.* (2020).

Apart from amino acids, carbohydrate metabolites such as lactate and monosaccharide/sugar isomers (myo-inositol and mannose) were significantly altered in lame cows (Dervishi *et al.*, 2020). Serum lactate concentrations were greater in lame cows relative to the control before and during lameness diagnosis, suggesting that pre-lame cows utilise phenylalanine and leucine to generate ketone bodies and lactate rather than gluconeogenesis. On the other hand, D-mannose is vital during inflammation by acting as a precursor of the omega-6 series and increases the synthesis of arachidonic acid, a vital component for potent inflammatory mediators such as thromboxane and prostaglandin (Dervishi *et al.*, 2020). Both linoleic and palmitic acids were found to be pro-inflammatory, triggering the release of IL-6 and TNF (Gupta *et al.*, 2012). Croze and Soulage (2013) equally highlighted the role of myo-inositol in phagocytosis. The increased concentration of these metabolites in lame and pre-lame cows reflects that D-mannose and myo-inositol might be promising biomarker candidates, since they assist cows in mounting an inflammatory response immediately after parturition.

In terms of milk constituents, promising results were reported by He *et al.* (2022) and Van Altena *et al.* (2016) in relation to lactoferrin and phosphatidylglycerol (PG 35:4), which were identified as lameness biomarkers in milk, with the capacity to identify cows at risk of becoming lame. These findings are consistent with a previous study in which glycerophospholipids and acylcarnitines were identified as urine metabolites that distinguished lame cows and controls (Eckel *et al.*, 2020). Alterations in these lipid species arise due to inflammation and immune response. Elevated lactoferrin concentrations in milk might be linked to the negative energy balance and increased fat mobilisation occurring around the calving period (Gross *et al.*, 2011). Likewise, lactoferrin plays an important role in inducing innate immunity by sequestering iron, thereby limiting the availability of iron, which is an important element for bacterial growth. Thus, increased lactoferrin levels in milk might be protective, which ought to reduce the risk of lameness. However, Van Altena *et al.* (2016) did not elucidate the specific lameness causes in their samples.

In this review, a few studies investigating pro- and anti-inflammatory cytokines and the expression of their related genes were identified in lame cows. Cytokines are either anti- or pro-inflammatory proteins that are synthesised and released by cells of the immune system and they affect the function or behaviour of other cells (Janeway *et al.*, 2005). Zhang *et al.* (2015) and Vermeersch *et al.* (2022) presented findings that could assist in predicting the risk of lameness based on alterations in pro-inflammatory and anti-inflammatory cytokines prior to the onset of clinical signs or lesion progression. Zhang *et al.* (2015) found that plasma concentrations of TNF-α and IL-6 were increased in transition cows before the onset of lameness postpartum, suggesting clinical inflammation during the period. Meanwhile, Vermeersch *et al.* (2022) reported the upregulation of some pro-inflammatory cytokines such as IL-6 and CXCL5 and the downregulation of keratins and anti-inflammatory molecules (MGC and SCGB1D) during the acute stages of DD. IL-6 is a primary cytokine that is produced by Th2 cells (Lin *et al.*, 2003) and it enhances early inflammatory response and wound healing. Previous research suggested the use of serum IL-6 as a prognostic biomarker for identifying cows with severe postpartum diseases such as endometritis, mastitis and retained placenta (Lin *et al.*, 2003). On the other hand, CXCL5 participates in the chemotaxis and homeostatic activities of neutrophils (O'Loughlin *et al.*, 2011).

The studies by O'Driscoll *et al.* (2015) and Herzberg *et al.* (2020a) only presented information on inflammatory cytokines associated with acute and chronic lameness during and after diagnosis. Lame cows affected with sole ulcer demonstrated higher expression of IL-1α, IL- 1β, CXCL8 mRNA and mRNA expression of IL-10, an anti-inflammatory cytokine (O'Driscoll *et al.*, 2015). Likewise, the spinal concentrations of TNF-α, IL-1α, IFN-α, IFN-γ, CXCL10 and CXCL9 were increased in chronically lame cows. TNF-α is among the most widely studied pro-inflammatory cytokine in pain research (Ji *et al.*, 2013) and IL-1α is known to trigger an early inflammatory response and is associated with the onset of an inflammatory loop during chronic inflammation. The inflammatory loop was reported to elicit subsequent expression of chemokines and other cytokines such as TNF-α and CXCL (Di Paolo and Shayakhmetov, 2016). CXCL chemokines are produced by immune cells in response to TNF-α and IFN-γ (Di Paolo and Shayakhmetov, 2016). IL-10 also participates in the regulation of keratinocytes, which could be vital in the onset of sole ulcers given that hoof horn is

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synthesised *via* the keratinisation of epidermal cells (Tomlinson *et al.*, 2004). Nevertheless, since changes in these inflammatory cytokines occur as part of the innate immune response to inflammation and infection, their specific role in lame cows and those suffering from claw disorders need to be further investigated. This gap in knowledge is pertinent to differentiate between cows that are likely to become lame and those susceptible to other metabolic disorders and diseases such as ketosis, metritis, mastitis and hypocalcaemia, particularly during the periparturient period.

The results from metabolomic studies conducted by Eckel *et al.* (2020), Dervishi *et al.* (2020) and Zhang *et al.* (2020) identified several metabolites that can be associated with lameness several weeks before and after the clinical manifestation. The metabolic markers can also be used in discriminating between sound and lame cows with a high level of accuracy in all accessed biofluids. However, the studies did not describe the causes of lameness, making it difficult to relate the findings to specific claw lesions. Since claw horn lesions such as sole ulcer and sole haemorrhage are the predominant affections occurring during the transition period (Griffiths *et al.*, 2018), there is potential for metabolomic protocols to identify the underlying metabolic pathways and elucidating the etiopathogenesis of these lesion-specific causes of lameness in dairy cows. Such novel information can be gleaned by conducting prospective and repeated measure study designs in which samples are collected within individual cows during the early lactation and monitored from the pre-lame period to the onset of clinical signs, specifically for claw horn lesions. Nevertheless, individual-specific responses during the period of high metabolic demands (Griffiths *et al.*, 2018) and several metabolic diseases such as subclinical ketosis and hypocalcaemia (Dervishi *et al.*, 2020) that may occur concurrently with these hoof lesions need to be considered.

Limitations

The limitations in this review are mainly related to variability in the methods and study designs used in the retrieved articles. Using three databases, only articles written in English and published between 2010 and 2022 were considered. Risk of bias assessment was also not performed given the diverse methods applied in the relevant studies. Descriptively, most of the studies were observational and only a few entailed randomised control or clinical trials. Thus, the findings from these observational studies only reflect potential associations between the investigated biomarkers and lameness occurrence. Given that only a few experimental studies considered claw health or actual causes of lameness, there is limited data to predict the risk of claw lesions based on the findings relating to the studied biomarkers. Most of the biomarkers investigated in the studies are basically pain mediators, nevertheless, metabolic analyses offer the opportunity to specifically delineate lameness and even lesion-specific biomarkers to facilitate accurate diagnosis and even predictive studies in future. The main limitation of these advanced techniques is their applicability and feasibility for field application given their cost implication and invasiveness.

Conclusions and future prospects

This scoping review revealed different categories of potential biomarkers for lameness in dairy cows, including acute phase proteins, nociceptive neuropeptides, stress hormones (cortisol), and metabolites in serum, urine, and milk. Most studies investigating the alterations in APPs and nociceptive neuropeptides demonstrated their relevance during lameness diagnosis and the pain and stress experienced by these animals before and after treatment. While the methods used in measuring the potential biomarkers are relatively easy and minimally invasive, there are less convincing results to support the use of these inflammatory markers in identifying susceptible cows, predicting specific claw lesions and understanding the metabolic status of pre-lame cows. On the other hand, metabolomic techniques have recently shown promising results in exploring metabolic alterations occurring a few weeks before and after lameness onset in periparturient cows. Numerous metabolites in serum, milk and urine were discovered that could be used to identify susceptible cows even before the onset of clinical signs. The metabolites in these biofluids also exhibited a high level of accuracy in discriminating between pre-lame and non-lame cows. Nonetheless, the causes of lameness and specific claw lesions are yet to be explored in metabolomics research. Given that all the studies employing metabolomic techniques focused on periparturient cows with a higher risk of claw horn lesions, these advanced approaches may assist in elucidating the metabolic pathways in lameness and its complicated aetiopathogenesis, as well as identifying cows that are susceptible to these claw diseases.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029924000487

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