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

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SUPPLEMENTARY FILE

Supplementary Table S1: Descriptive information of the studies, farms and animals enrolled

Variables	Frequency	%
Year of publication		
2010-2015	7	22.5
2016-2022	24	77.5
Study designs		
RCT and Quasi-experimental	4	12.9
Cross-sectional (observational)	17	54.8
Prospective (Observational)	6	19.3
Number of animals enrolled (observational studies)	6-6292	
Number of animals enrolled (experimental)	10-25	
Number of herds enrolled		
1 -10	25	80.6
10-20	4	12.9
More than 20	3	9.6
Basis for enrollment		
Already lame cows	16	51.6
From non-lame to lame status	4	12.9
Claw lesions	11	35.4
Claw lesions information		
Cows with claw lesions (no lameness status)	7	22.5
Both claw lesions and lameness status	5	16.1
Predominant claw lesions		
Non-infectious	10	32.2
Infectious	3	9.6

Supplementary Table S2: Categories of potential biomarkers of lameness in cattle investigated in the included studies

Biomarkers	Components/specific substances	Number of studies	References
Acute phase proteins	Haptoglobin, SAA, C-reactive protein, fibrinogen, lipopolysaccharide-binding protein	**9**	Pirkkalainen et al., 2022; Abuelo et al., 2016; Ilievska et al., 2019; Kuala et al., 2010; Kontturi et al., 2020; Nazifi et al., 2011; Bagga et al., 2016; O'Driscoll et al., 2017; Zhang et al., 2015
Cortisol (strss hormones)	Serum/Plasma cortisol	**8**	Kleinhenz et al., 2019, Bustamante et al., 2015, Warner et al., 2021, O'Driscoll et al., 2014, 2017; Juozaitiene et al., 2021; Janßen et al., 2018; Martin et al., 2022
	Hair cortisol	**4**	Martin et al., 2022, Fischer-Tenhagen et al., 2018, Sharma et al., 2019, Van Eerdenburg et al., 2021
	Salivary cortisol	**1**	Martin et al., 2022
Nociceptive neuropeptides	Substance P, β-endorphin, and nor-epinephrine	**5**	Kleinhenz et al., 2019; Bustamante et al., 2015; Warner et al., 2021; Rodriguez et al., 2018; Martin et al., 2022;
Metabolites (serum, salivary and urinary)	Lactate, glucose, non-esterified fatty acids, amino acids, β-hydroxybutyrate (ketone bodies)	**5**	Janßen et al., 2018; Zhang et al., 2015; O'Driscoll et al., 2014; Konturri et al., 2020; Dervishi et al., 2020
	provitamins (serum β-carotene, retinol, and α-tocopherol)	**1**	Strickland et al., 2021
	Saliva analytes **(**lipase and total esterase)	**1**	Contreras-Aguilar et al., 2020

	Urinary metabolites	**1**	Zhang et al., 2020
Proteomes	HMG-CoA reductase (HMGCR) transmembrane glycoprotein, apolipoprotein, haptoglobin, and conglutinin	**2**	Dong et al., 2015; Herzberg et al., 2020b
	Chaperone and stress proteins (Hsp70, Hsc70, Hsp90, and interacting proteins)		Herzberg et al., 2020a
Milk constituents	Milk protein (Lactoferrin), DMI, milk fat (phosphatidylglycerol) and fat-to-protein ratio, cortisol	**7**	Altena et al., 2016; Zhang et al., 2015; Juozaitiene et al., 2021; He et al., 2022; Gellerich et al., 2015; Zwierzchowski et al., 2020; Eckel et al., 2020
Proinflammatory Cytokines and gene expression	IL2, IL4, IL-10, IL-8, IL-13, IL-17, IL-21, IL-36ra, IFN-λ, IFN-α, IFN-γ, TNF-α, IL-1α, IL-1β, CXCL8, MMP-9, MMP-13, GR-α, Fas, Hp, and CD62L, CXCL10/IP-10, CXCL9/MIG, IFN-α, IFN-γ, and MIP-1β, A2ML1, SCGB, MGC	**4**	Zhang et al., 2015; Herberg et al., 2020b; Driscoll et al., 2015; Vermeersch et al., 2022

Supplementary Table S3: Studies assessing the alterations in acute phase proteins and their potential use as biomarkers of lameness in cattle

	Reference	Biomarkers	Study design and sample size	Country	Specific claw lesions/lameness status	Main findings
1	O'Driscoll et al. (2015)	Plasma Hp	Cross-sectional (n = 24 cows).	Ireland	Cows with sole ulcers only	Serum DHEA and cortisol concentrations were higher ($p < 0.05$) in lame compared to sound cows. The former also tended to reflect higher haptoglobin levels and mRNA expression than those without claw lesions.
2	O'Driscoll et al. (2017)	Plasma Hp	Cross-sectional, (mild = 17, moderate = 18, and severe = 12)	Ireland	Cows with sole haemorrhage	Cows with severe sole haemorrhage recorded higher ($p < 0.05$) plasma Hp concentrations relative to those with moderate and mild cases
3	Zhang et al. (2015)	Hp, SAA, lipopolysaccharide-binding protein (LBP)	Cross-sectional, (n = 100 multiparous cows). Data from healthy (n = 6) and lame (n = 6) cows were analysed.	Canada	Lame vs non-lame cows	The concentrations of SAA were higher in lame cows than in the control group. Cows that became lame had higher SAA at 8 and 4 weeks before parturition.
4	Pirkkalainen et al. (2022)	SAA and Hp	Prospective longitudinal (Heifers and cows with claw lesions and 29 healthy cows)	Estonia	Sole ulcers, white line disease and digital dermatitis	Cows with sole ulcers exhibited significantly higher SAA levels compared to the control group, as well as decreased IL-6 and RT from day 0 to day 7. No significant difference in the APPs between cows with either white line disease or digital dermatitis relative to healthy cows.
6	Abuelo et al. (2016)	Hp and SAA	Longitudinal study, (n = 30)	USA	Non-specific (non-lame cows)	Oxidant status and APP concentrations did not differ between cows with claw lesions and the healthy group.
7	Kujala et al. (2010)	SAA and Hp	Cross-sectional (Lame cows with sole ulcer (n = 8) and white line abscess (n = 6), both hoof lesions (n = 2) and healthy cows (n = 15)	Finland	Sole ulcer and white line abscess	Both APPs were significantly higher at day 0, 4, 7, and 14 in lame cows with SU compared to those with white line abscess and control groups.
8	Kontturi et al. (2020)	SAA, Hp, albumin	Cross-sectional (n = 21 farms, 203 sampled cows and 60 with acute IP).	Finland	Interdigital phlegmon	Acute IP cows recorded significantly higher levels of Hp and SAA and lower albumin levels, particularly in farms with high morbidity compared to those with moderate morbidity.

9	Nazifi et al. (2011)	Hp and SAA	Cross-sectional, (n = 8 farms, n = 15 cows).	Iran	Digital dermatitis (lame and those positive for F. necrophorum)	Groups that were lame and F. necrophorum-positive + lame recorded higher ($p<0.01$) SAA and Hp levels compared to the healthy group. Lame and F. necrophorum-positive + lame groups had 4.6 and 8.0 times higher mean SAA levels respectively relative to the control group. Lame group had 3.3 times higher Hp than the control group.
10	Bagga et al. (2016)	C-reactive protein (CRP), Hp, SAA and fibrinogen	Cross-sectional, (n = 30 lame cows and 10 non-lame cows).	India	Sole ulcers, white line disease and sole haemorrhage	SAA, Hp, fibrinogen and c-reactive protein levels were 3, 20, 4, and 4 times higher ($p<0.01$) in lame cows compared to non-lame cows
11	Ilievska et al. (2019)	SAA and Hp	Cross-sectional (n = 50 cows claw lesions and 14 clinically healthy heifers).	Macedonia	Heel horn erosion (HE), Acute laminitis (AL), Sole ulcer (SU), Digital dermatitis (DD) and White line separation (WLS).	SAA and HP were higher ($p<0.01$) in the SU group relative to the DD, WLS, and HE groups. Among the latter three groups, both APPs were higher ($p< 0,05$) in the cows with DD.

Supplementary Table S4 Characteristics and main findings from studies reporting changes in nociceptive neuropeptides in lame dairy cows

	Reference	Biomarkers	Study design and sample size	Country	Specific claw lesions/lameness status	Main findings
1	Kleinhenz et al. (2019)	Substance P	RCT, (3 groups, n = 10 cows /group)	United States	Locomotion scores	No evidence of significant alterations in Substance P among treatment groups and control.
2	Bustamante et al. (2015)	Substance P, Haptoglobin, nor-epinephrine, beta-endorphin	Quasi-experimental (n = 12 non-pregnant heifers)	Chile	NA	Plasma concentrations of substance P, norepinephrine, and beta-endorphin increased significantly in treated cows starting after 6 hours and attaining peak levels at 12 hr, 6 hr, and 24 hr, respectively
3	Rodriguez et al. (2018)	Norepinephrine, substance P, beta-endorphin	Cross-sectional, (n = 100 cows in 4 groups on locomotion scores (0-3))	Chile	Locomotion scores	Cows with higher locomotion scores (LS 2 and 3) recorded higher BE and SP levels which were more than 90.0% higher compared to those with LS1
4	Warner et al. (2021)	Substance P	Double-blinded RCT (4 groups, n = 12 cows/group)	United States	Locomotion scores	The quantification of substance P did not yield any significant or biologically relevant differences between the treatment groups
5	Martin et al. (2022)	Substance P	Cross-sectional	Canada	Lame vs non-lame	Results comparing analgesic effects to uncontrolled pain consistently yielded AUC values <0.7 (95% CI: 0.28 to 0.90) for substance P at different periods after lameness diagnosis

Supplementary Table S5: Characteristics and main findings from studies reporting changes in blood, hair, and salivary cortisol levels as stress biomarkers in lame cows

	Reference	Biomarkers	Research objective and study design	Country	Specific claw lesions/lameness status	Main findings
1	Fischer-Tenhagen et al. (2018)	Hair cortisol	Cross-sectional, (n = 21 chronically lame and 21 non-lame cows)	Germany	Lame vs non-lame cows	No difference was observed in the cortisol level between lame cows (2.38 ± 0.95) and non-lame cows (2.26 ± 1.35 pg/mg) during the study period.
2	Van Eerdenburg et al. (2021)	Hair cortisol	Cross-sectional (n = 10 cows from each of 58 dairy farms)	Netherland	Lame cows	Most of the welfare protocols including those with lameness and hoof health data did not depict any significant correlations with hair cortisol concentrations.
3	Martin et al. (2022)	Plasma, salivary cortisol and hair cortisol	Cross-sectional	Canada	Lameness cases	Consistent AUC values greater than 0.7% were recorded for findings comparing analgesic effects to uncontrolled pain for plasma cortisol, hair cortisol, and salivary cortisol
4	Sharma et al. (2019)	Hair cortisol	Cross-sectional (n = 10 cows from 54 shelters)	India	Lameness and claw overgrowth	At the cow level, no significant association was found between cortisol levels and welfare measures relating to lameness and claw overgrowth
5	Kleinhenz et al. (2019)	Serum Cortisol	Quasi-experimental (3 groups, n = 10 cows/group)	United States	Lame vs non-lame cows	The Lame + flunixin group recorded significantly lower cortisol concentrations starting at 1.5 hrs post-drug administration.
7	Bustamante et al. (2015)	Plasma Cortisol	Quasi-experimental (n = 12 non-pregnant heifers)	Chile	Lame vs non-lame cows	Plasma cortisol increased after lameness induction starting at 6hr and reaching maximum concentrations at 24 hr

8	Janßen et al. (2018)	Serum and faecal cortisol	Quasi-experimental (n = 21 lame and 21 non-lame cows)	Germany	Lame cows with sole ulcers or white-line disease	Blood and faecal cortisol levels increased significantly in lame cows compared to control cows 15 mins after treatment.
9	Warner et al. (2021)	Plasma Cortisol	Double-blinded RCT (4 groups, n = 12 cows/group)	United States	Lame vs non-lame cows	Lower cortisol levels were observed in cows treated with flunixin meglumine compared to those administered meloxicam but both were more effective compared to the control groups ($p < 0.001$).
10	O'Driscoll et al. (2014)	Serum cortisol	Same information as in Table 1	Ireland	Lame cows with only sole ulcers	Lame cows recorded significantly higher concentrations of serum cortisol than sound cows.
11	O'Driscoll et al. (2017)	Plasma cortisol	Same information as in Table 1	Ireland	Cows with sole haemorrhages	Cortisol levels did not differ significantly between cows with various severity of sole haemorrhages
12	Juozaitiene et al. (2021)	Serum cortisol	Cross-sectional study (n = 73 non-lame and 55 lame cows)	Lithuania	Lame vs non-lame cows	Lameness risk was indicated by increased blood cortisol levels. The odds of identifying a lame cow increased by 4.9 times as the blood cortisol level exceeded 1 µg/dL

Supplementary Table S6: Characteristics and main findings from studies employing proteomics and investigating alterations in serum, plasma, urine and milk metabolites as biomarkers of lameness in dairy cows

	Reference	Biomarkers	Study design and sample population	Country	Specific claw lesions/lameness status	Main findings
	Proteomes (Proteomic analysis and profiling)					
1	Dong et al. (2015)	Serum proteins	Cross-sectional, (n = 4680 were screened, followed by selecting 36 cows with acute laminitis and 15 healthy cows)	China	Claw horn lesions	A total of 16 protein spots (upregulated = 12, downregulated = 4) were expressed differently in the lame group relative to the control. The proteins were involved in lipid metabolism, carbohydrate metabolism, immune regulation, molecular transport, oxidative stress, and inflammatory response.
2	Herzberg et al. (2020b)	Proteomes from spinal cord samples	Cross-sectional, (12 cows from one farm, samples were collected from 5 lame cows and 7 non-lame cows)	Chile	Infectious and non-infectious claw lesions	LC-MS/MS was able to identify 177 proteins and 129 of them were quantified. Interacting proteins with chaperone and stress functions (i.e., Hsp70, Hsc70, Hsp90and interacting proteins involved in glycolysis) were strongly upregulated in lame cows. The most down regulated protein in lame cows was myelin basic protein.
	Metabolites (Serum, plasma, and urinary)					
1	Zhang et al. (2020)	Urinary metabolites	Nested case-control (lame (n =6) and non-lame (n= 20).	Canada	Lame vs non-lame	Seven metabolites were excreted at lower concentrations in lame cows during diagnosis. The five most significant metabolites were Tyr, adipate, glycerate, 3-hydroxy-3-menthyl glutarate, and uracil. The concentrations of N-acetyl aspartate, glutamine, imidazole, pantothenate, beta-alanine and trimethylamine also differed significantly at +4 weeks after calving, as well as pantothenate 1,3-dihydroxyacetone, galactose, and Tyr at 8 weeks after calving.

2	Eckel et al. (2020)	Urinary metabolites	Nested case-control (Lame cows (n = 6) and non-lame (n = 20)	Canada	Lame vs non-lame	The group of metabolites with the strongest variation in pre-lame and lame cows' urine were ACs and glycerophospholipids, especially PCs. LysoPCs were also altered to a lesser degree at all time points. Urinary amino acids; arginine, tyrosine, and aspartate were elevated at -8 to -4 weeks before calving, while glutamate was decreased at both time points. One BA and numerous sphingomyelins were altered lame and pre-lame cows.
3	Dervishi et al. (2020)	Serum metabolites	Nested case-control (6 pregnant multiparous lame cows and 20 healthy control cows)	Canada	Lame vs non-lame	Pre-lame and control cows were differentiated by 18 and 15 serum metabolites at 8 and 4 weeks before calving. Ten metabolites were altered during the week of lameness detection. Higher concentrations of Gly, Leu, Phe, Ser, Val, D-mannose, Myo-inositol, and phosphoric acid (PA) were recorded in pre-lame cows. Six metabolites showed a high accuracy at 8 weeks before calving and 9-11 weeks before lameness diagnosis: Glu, Orn, Phe, Ser, Val, and PA. Leu, Orn, Phe, Ser, and D-mannose were also increased at 4 weeks before calving and at 5-7 weeks before lameness diagnosis
4	Strickland et al. (2021)	serum β-carotene, retinol, and α-tocopherol concentrations	Cross-sectional	United States	Lame vs non-lame	No significant association was found between any of the vitamin biomarkers and the odds of lameness occurrence post-calving
5	Zheng et al. (2020)	Serum metabolites	Cross-sectional (healthy cows (n = 10) and those with foot rot (n = 10)	China	Footrot	A total of 21 metabolites were expressed differently in both groups, whereby 4 and 17 metabolites were higher and lower respectively in cows with foot rot compared to healthy cows. The top five associated pathways were the metabolism of glycine, serine, and threonine, synthesis and degradation of ketone bodies, methane metabolism, biosynthesis of valine, leucine and isoleucine, and pyruvate metabolism

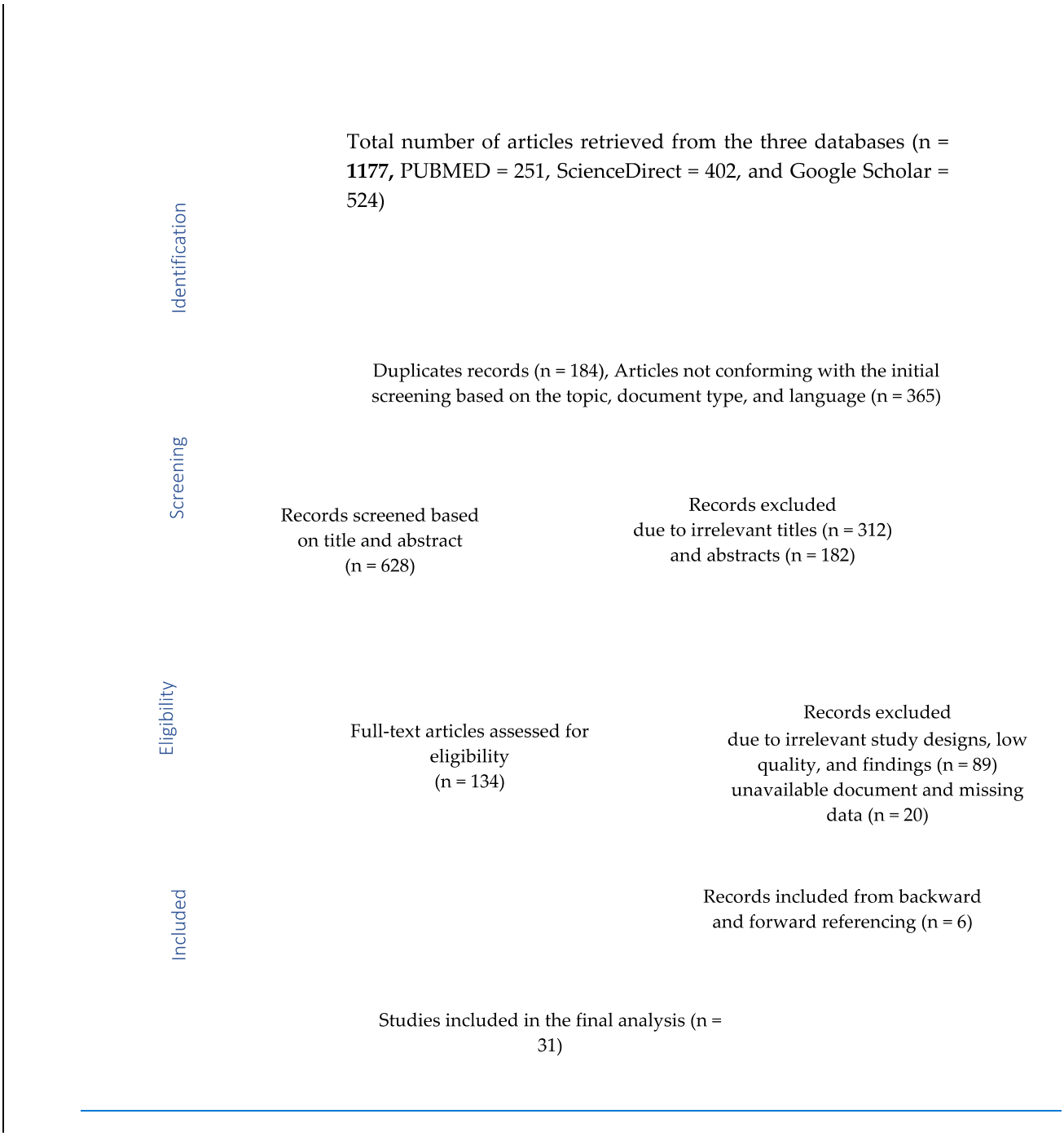
6	Janßen et al. (2018)	Fatty acids, lactate, and glucose	Fatty acids, lactate, and glucose	Germany	Lame cows with sole ulcers or white-line disease	Concentrations of blood fatty acids and glucose were higher in lame than in non-lame cows after treatment.
7	Kontturi et al. (2020)	Albumin	Cross-sectional (n = 21 farms, 203 sampled cows and 60 with acute IP).	Finland	Cows with acute IP and without hoof lesions	A lower concentration of albumin was evident in acute IP cows.
	Milk metabolites					
1	Juozaitiene et al. (2021)	Milk yield	Cross-sectional study (n = 73 non-lame and 55 lame cows)	Lithuania	Lame vs non-lame	Milk yield in the first minute was 1.77 kg higher in non-lame than lame cows. Electrical conductivity during the initial milking time was 0.24 mS/cm lower in non-lame than the lame cows.
2	Zhang et al. (2015)	Milk production, DMI, milk fat and fat-to-protein ratio.	Nested case-control (lame (n =6) and non-lame (n= 20).	Canada	Lame vs non-lame	Lame cows recorded lower overall milk production, DMI, milk fat and fat-to-protein ratio.
3	Zwierzchowski et al. (2020)	Milk metabolites	Nested case-control (20 healthy cows vs 6 lame cows)	Canada	Lame vs non-lame	A total of 168 metabolites were identified of which decreased levels of 35 assisted in differentiating lame vs non-lame cows at 2 weeks postpartum. Lame cows' milk recorded a significant increase in two metabolites: phosphatidylethanolamine ae C42:1) and sn-glycero-3-phosphocholine
4	Mineur et al. (2020)	Milk mid-infrared predicted biomarkers	Prospective longitudinal (n= 6,292 cows on 161 farms)	Austria	Locomotion scores	Significant but low correlations were detected between biomarkers and high locomotion scores.
5	Altena et al. (2016)	Milk protein composition	Cross-sectional	Netherlands	locomotion scores	Low-resistant cows expressed 13 specific proteins more significantly relative to high-resistant cows. LF level in milk was significantly associated with the risk of lameness.
6	He et al. (2022)	Dried milk spots	Cross-sectional	United Kingdom		Phosphatidylglycerol (PG 35:4) was identified as the strongest and most sensitive indicator of lameness among the discriminative metabolites based on stability selection
7	Gellrich et al. (2015)	Skimmed milk cortisol	Prospective longitudinal (n = 24 multiparous cows)	Germany	Lame cows with specific claw lesions postpartum vs non-lame cows	Lame cows had significantly higher cortisol levels (6.05 ± 0.38 nmol/L) in morning milk compared to that of the sound cows (3.69 ± 0.16, $P < 0.01$).

PCs = Phosphatidylcholines, LysoPCs = Lysophosphatidylcholines, ACs = acylcarnitines, BA = biogenic amines, IP = interdigital phlegmon

Supplementary Table S7: Articles reporting pro-inflammatory cytokines and expression of genes associated with lameness in dairy cows

	Reference	Biomarkers	Research objective and study design	Country	Specific claw lesions/lameness status	Main findings
1	Vermeersch et al. (2022)	IL-17, interleukin-8 IL-8, SKALP, A2ML1	Prospective longitudinal (n = 22 skin samples from cows in six dairy farms).	Belgium	Digital dermatitis	A clustering of the acute M1, M2 and M4.1 stages vs the chronic stages (M3 and M4) were observed. Most of the up and down-regulated genes in both stages are those involved in inflammation, such as PI3, A2ML1, CCL11 and elafin-like proteins. Keratins and anti-inflammatory molecules (MGC and SCGB1D) were the most downregulated genes. The IL-17 signalling pathway was activated via the upregulation of IL-17F in all the M stages.
2	O'Driscoll et al. (2014)	IL-1α, IL-1β, IL-10, IL4, IFN-λ, or TNF-α CXCL8, and MMP-9, MMP-13, GR-α, Fas, Hp, and CD62L	Cross-sectional	Ireland	Sole ulcers	Significant differences were recorded for MMP-13 ($P \leq 0.01$) and CD62L ($P \leq 0.01$). The expression of the gene coding for colony-stimulating factor 2 tended to be higher in sound cows than in lame cows. Lame cows tended to have higher expression of IL- 1α, IL-1β, CXCL8, IL-10, Fas and haptoglobin than sound cows.
3	Herzberg et al. (2020a)	TNF-α IL-1α, IL-13, CXCL10/IP-10, CXCL9/MIG, IFN-α, IFN-γ, IL-21, IL-36ra, MIP-1β	Cross-sectional, 14 dairy cows (lame = 7 and non-lame = 7)	Chile	Lame vs non-lame	Lame cows recorded significantly higher concentrations of IL-1-α, IL-13, IFN-α, IFN-γ, TNF-α, CXCL10, and CXCL9 in their dorsal horn compared to non-lame cows. Meanwhile, the concentration of IL-21 was reduced.
4	Zhang et al. (2015)	IL-1, IL-6 and TNF	Nested case-control (lame (n =6) and non-lame (n= 20).	Canada	Lame vs non-lame	Lame cows had higher concentrations of IL-6 and tended to have higher TNF compared to non-lame cows. Enhanced serum concentrations of IL-6 at ́8 and ́4 weeks before parturition were different in lame cows relative to the control group.

Supplementary Figure S1: Literature search process according to the PRISMA guidelines



Supplementary Figure S2: Distribution of the studies based on countries