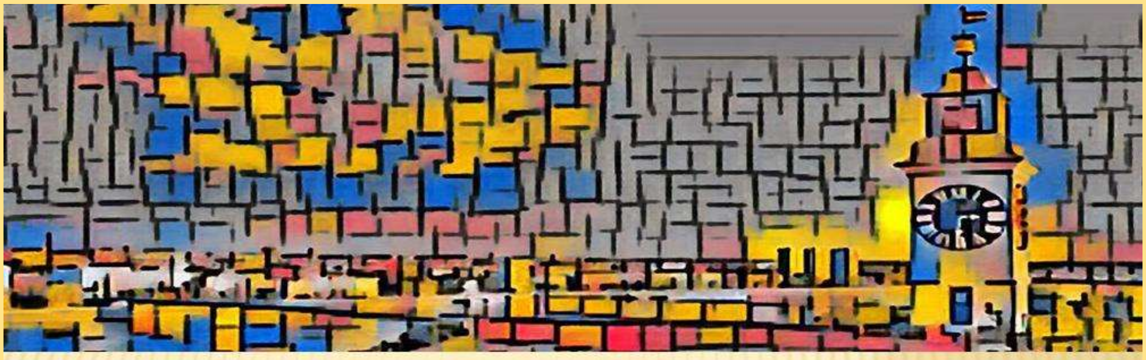

PROCEEDINGS

26TH INTERNATIONAL CONGRESS OF THE MEDITERRANEAN FEDERATION FOR HEALTH AND PRODUCTION OF RUMINANTS

FeMeSPRum

Novi Sad (Serbia), 20th – 23rd June, 2024

ZBORNIK RADOVA

26. MEĐUNARODNI KONGRES MEDITERANSKE FEDERACIJE ZA ZDRAVLJE I PRODUKCIJU PREŽIVARA

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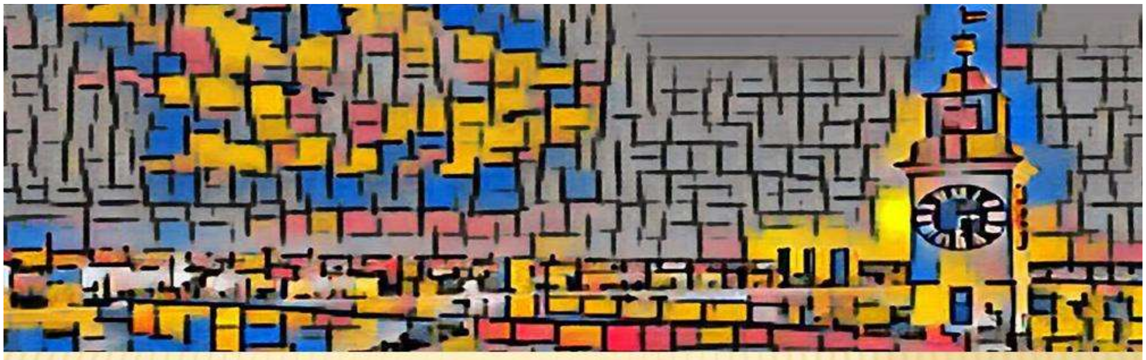

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Dear Participants of the FeMeSPRum Congress,

Welcome to the 26th Congress of the Mediterranean Federation for Health and Production of Ruminants (FeMeSPRum). It is an honor to gather with you esteemed veterinarians and animal scientists dedicated to advancing the health and productivity of ruminants in the Mediterranean region.
This year's Congress is in the beautiful city of Novi Sad, Serbia. Nestled on the banks of the Danube River, Novi Sad is renowned for its vibrant culture, rich history, and stunning architecture. Known as the "Serbian Athens," it is home to the majestic Petrovaradin Fortress, numerous museums, galleries, and the lively Danube Park. As the European Capital of Culture for 2022, Novi Sad offers a perfect blend of tradition and modernity, providing a picturesque and inspiring backdrop for our meeting.
This year's Congress will focus on critical topics that directly impact the health, production, and welfare of the animals we care for. Topics include Biosecurity and heat stress on ruminant farms, Parasite control in ruminants, and Clinical pathology and healthcare of ruminants. These scientific sessions will provide cutting-edge insights and innovative solutions, besides fostering collaboration and the exchange of expertise among leading professionals from the region.
The Mediterranean region has a unique climate, geography, and agricultural practices that present specific challenges and opportunities for ruminant health and production. Advancing the health of domestic ruminants in this region is crucial for ensuring sustainable agriculture, enhancing food security, and supporting the livelihoods of countless farmers and communities. Your work and dedication play a vital role in addressing these challenges and promoting the well-being of domestic ruminant populations.
The Mediterranean Federation for Health and Production of Ruminants (FeMeSPRum) is an organization with immense potential. Its core idea is to serve as a medium for fruitful collaboration among stakeholders in ruminant production. This platform is not only for exchanging information and good practices but also aims to provide a consortium that can cooperate in writing international project proposals and succeed in international project calls. By working together, we can be more innovative and have an impact in our field. With this in mind, I am sure this Congress will boost this idea and strengthen our Federation.
All this would not be possible without the dedicated organizing committee and especially Prof. Dr. Marko Cincović, president of the organizing committee, who have done their best to prepare everything for a smooth congress. Your hard work and dedication are deeply appreciated. Additionally, thank you to all our sponsors, whose generous support has made this event possible.
Your participation and contributions to the Congress are not only crucial to the success of this Congress but also to the existence of the Federation. Together, we will explore new strategies, share best practices, and pave the way for significant advancements in ruminant health and production.
Thank you for being here, and I look forward to a productive and inspiring congress in the charming city of Novi Sad.
With best wishes,

Prof. Dr. Jože Starič
President of the Mediterranean Federation for Health and Production of Ruminants (FeMeSPRum)

Drage koleginice i kolege,
Mediteranska federacija za zdravlje i proizvodnju preživara (FeMeSPRum) je međunarodno udruženje koje okuplja različite profesionalce iz akademske i istraživačke sfere (najčešće veterinare, ali i agronome, inženjere animalne proizvodnje i dr.) koji su posvećeni brizi o preživarama, proučavanju i prevenciji bolesti ovih životinja, kao i povećanju i poboljšaju njihove proizvodnje (meso, mleko, vuna, itd.), dobrobiti i svega onoga što će uticati na dobijanje kvalitetnog i zdravstveno bezbednog proizvoda za krajnjeg potrošača. FeMeSPRum promoviše organizovanje obuka, diskusija, seminara i konvencija, sa definisanom periodičnošću, i podržava sva dešavanja koja doprinose unapređenju ovog sektora i saradnji između zemalja članica, a njeni direktni korisnici su stručna lica iz oblasti veterinarske medicine ali i drugih srodnih oblasti. Kao što mu ime govori, sfera uticaja se proteže na nekoliko zemalja mediteranskog regiona, uključujući Italiju, Španiju, Grčku, Tursku, Sloveniju, Hrvatsku, Siriju, Egipat, Tunis, Maroko. Iako naziv federacije ukazuje na njenu geografsku pripadnost, u eri globalne razmene i unapređenog transfera znanja i pomeranja klimatskih pojaseva, FeMeSPRum je proširio svoje delovanje i na zemlje u okruženju, a posebno značajna zemlja za ovu organizaciju je Srbija. U Srbiji smo 2011.godine imali kongres u Beogradu, a ove 2024.godine kongres se održava u Novom Sadu koji, na naše zadovoljstvo, organizujemo zajedno sa dve partnerske respektabilne ustanove i to su Departman za veterinarsku medicinu Novi Sad i Udruženje veterinara praktičara Srbije.
Dobro došli!

Prof. dr Jože Starič
Predsednik Mediteranske federacije za zdravlje i produkciju preživara (FeMeSPRum)

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Periodičnost: Jedna sveska godišnje
Vrste radova: Originalni radovi, Pregledni radovi, Meta-analize, Radovi iz istorije veterinarske medicine, Radovi iz oblasti veterinarskog obrazovanja, Prikaz slučaja, Prikaz tehničkih rešenja, Prikazi knjiga, Naučna kritika, Pisma uredništvu, Izveštaji sa seminara i drugih stručnih događaja. Format: Časopis izlazi u štampanoj/elektronskoj formi i biće publikovan na sajtu Departmana za veterinarsku medicinu
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Papers: original papers, review articles, meta – analysis, papers from the history of veterinary medicine, papers in the field of veterinary education, case report, technical solutions, book, scientific polemic, letters to the Editor, reports from seminars and other professional events.
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Article
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DETERMINATION OF NEW WELFARE AND STRESS INDICATORS ON CATTLE AND PIG FARMS BASED ON PREVIOUSLY PUBLISHED STUDIES

UTVRĐIVANJE NOVIH POKAZATELJA DOBROBITI I STRESA NA GOVEDARSKIM I SVINJARSKIM FERAMAMA NA OSNOVU RANIJE OBJAVLJENIH STUDIJA

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ABSTRACT

In recent years, numerous papers have been published that consider indicators of the welfare and stress of the animals on cattle and pig farms to improve their health and productivity. These indicators are mostly determined in numerous international projects and published in indexed journals and proceedings from international symposia. Their usefulness in on-farm assessments of animal welfare and stress is generally well recognized. In the assessments of the welfare and stress of the animals in different systems of rearing and accommodation, the need to determine new welfare and stress indicators on cattle and pig farms was observed. The paper discusses the determination of new indicators of animal welfare and stress on cattle and pig farms based on analysis of previously published studies which include the most important papers in indexed journals and proceedings from international symposia that discussed the existing indicators. The analysis of the results of those studies will be used to determine the main characteristics of the existing new welfare and stress indicators on cattle and pig farms with a focus on their applicability. The results, discussions and conclusions in those papers will be used to generate ideas to define new welfare and stress indicators of the animals on cattle and pig farms.

Key words: cattle, pig, welfare indicators, stress indicators, analyses of literature

SAŽETAK

Poslednjih godina objavljeni su brojni radovi koji razmatraju pokazatelje dobrobiti i stresa životinja na farmama goveda i svinja za poboljšanje njihovog zdravlja i produktivnosti. Ovi pokazatelji se uglavnom utvrđuju u brojnim međunarodnim projektima i objavljuju u indeksiranim časopisima i zbornicima sa međunarodnih simpozijuma. Njihova korisnost u proceni dobrobiti životinja i stresa na farmi je generalno dobro poznata. U procenama dobrobiti i stresa životinja u različitim sistemima gajenja i smeštaja uočena je potreba za utvrđivanjem novih indikatora dobrobiti i stresa na farmama goveda i svinja. U radu se razmatra određivanje novih indikatora dobrobiti životinja i stresa na farmama goveda i svinja na osnovu analize ranije objavljenih studija koje obuhvataju najvažnije radove u indeksiranim časopisima i zbornike sa međunarodnih simpozijuma na kojima se razmatraju postojeći indikatori. Analiza rezultata ovih studija biće korišćena za utvrđivanje glavnih karakteristika postojećih novih indikatora dobrobiti i stresa na farmama goveda i svinja

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sa fokusom na njihovu primenljivost. Rezultati, diskusije i zaključci u tim radovima biće korišćeni za generisanje ideja za definisanje novih indikatora dobrobiti i stresa životinja na farmama goveda i svinja.

Ključne reči: krave, svinje, indikatori dobrobiti, indikatori stresa, analiza literature

INTRODUCTION

Protecting health, ensuring well-being and maintaining maximum production in cattle and pigs are influenced by numerous environmental factors as well as by the status of the animal organism itself. The organism of these animals is affected by many factors from the external and internal environment of their organism that can cause stress or even distress. That is why it is very important to monitor welfare and stress through relevant indicators that will serve to reduce the negative impact of stressors from the external and internal environment of the organism. When it comes to stress animals can experience three types: physical (due to fatigue, injury, etc.), physiological (due to hunger, thirst, temperature control, etc.) and behavioural (due to the environment, unfamiliar people, surroundings, etc.). It is important to emphasize that animals might be able to tolerate a single stressor for a short time, but multiple stressors over a long period may lead to distress and suffering. The ability of cattle and pigs to cope with stress will also depend on the genetic background of the species and the animal's past experiences. Some degree of stress is inevitable during the life of animals, the aim must be to keep it to a minimum (1,2).

Animal-based indicators were mostly determined in The Welfare Quality project (3), while management-based and resource-based were considered as the Animal Need Index (4) and in Bristol Welfare Assurance Program (5). An overview of existing methods related to the aggregation of measures to produce an overall assessment of animal welfare was given by Botreau et al. (6) as well as an analysis of constraints (7).

After 2009, numerous published studies in journals and proceedings of symposia followed, which resulted in the consideration of existing and determination of new welfare and stress indicators. These studies of welfare and stress are used as a basis for the creation of main concepts, principles, indicators, parameters and criteria that are incorporated into protocols for the assessment of these very important conditions. The studies take into account the ability of animals to cope physiologically, behaviourally, cognitively and emotionally with the physicochemical and social environment (8). It should be borne in mind that assessing animal welfare and stress on cattle and pig farms is time-consuming and costly (9). There are numerous indicators of animal welfare and stress used in on-farm assessment and science, such as resource-based indicators, management-based indicators and animal-based indicators. In the scientific research that is published in indexed journals and proceedings from international symposia, new relevant indicators are continuously determined that can be used for scientific purposes and purposes of on-farm assessment.

The paper presents results, discussions and conclusions selected in published papers after 2009, intending to be used to generate ideas to define new welfare and stress indicators of the animals on cattle and pig farms, for on-farm assessment as well as for scientific research.

METHODOLOGY OF SELECTION AND ANALYSIS OF PAPERS

The authors of the paper selected in total 137 of the most significant and cited original and review papers using the Google Scholar and ScienceDirect.com database from 2009 until now in which indicators of well-being and stress in cattle and pigs are considered. After that authors identified in selected papers the most significant new welfare and stress indicators concerning the indicators identified in the Animal Need Index and Welfare Quality project. Also, when choosing indicators of welfare and stress, authors took into account the characteristics of selected indicators in terms of the possibility of use for on-farm assessment or further scientific research. Some significant papers were taken into account before 2009 because of the connection with papers after that period.

NEW WELFARE AND STRESS INDICATORS ON DAIRY CATTLE FARMS

Farm animal welfare and stress are now considered to be well-established scientific disciplines. In these disciplines, numerous multidimensional concepts and indicators have been developed based on the

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influence of factors from the external and internal environment as well as their interaction. It is well known that a wide variety of indicators should be applied to assess well-being and stress (1). The results of many studies indicate that improved cow comfort and welfare and reduced influence of stress are associated with greater farm productivity and profitability margins calculated over the replacement costs. The numerous interactions found between the management-, animal-, and resource-based welfare indicators emphasize the complexity of the association between animal well-being and farm profitability, especially in connection with the farm's management and culling strategy (10,11).

Stress is constantly present in dairy cows, especially when they are lactating. It arises as a consequence of the action of external or internal factors, i.e. external and internal stressors (12). External stressors are environmental factors such as heat or farming factors, as well as hygiene factors. The main internal stressors cause metabolic stress characterized by pronounced lipolysis, ketogenesis, insulin resistance, inflammation and immunosuppression. It arose as a result of unilateral selection for milk production and redirection of glucose to the udder, while the body uses fat for energy needs. Some stressors arise from the interaction of the external and internal environment, and these primarily cause different diseases. Stress indicators in dairy cows can be measured non-invasively (adspection, thermography, sensors, etc.), semi-invasively (routine measurement from milk during daily milking) and invasively (blood parameters). In many cases, animal-based stress response requires more specific measurements and sampling. Table 1 provides some basic information about welfare and stress indicators on dairy cattle farms that were determined in publications after 2009. In the review by de Vries et al. (13) the 27 VRHD the main types of data that are collected in national herd databases of developed countries, and related to identification and registration, management, milk production, and reproduction of dairy herds were included. Moreover, 34 WI were based on the Welfare Quality Assessment Protocol for Cattle. Twenty-three VRHDs were associated with 16 WI. The VRHD related to milk yield, culling, and reproduction were associated with the largest number of WI. Few associations were found for WI that referred to behavioural aspects of animal welfare, nonspecific disease symptoms, or resource-based indicators. For 18 WI, associations with VRHD were not significant (n = 5 WI) or no studies were found that investigated associations with VRHD (n = 13 WI). It was concluded that many VRHDs have the potential to estimate the level of animal welfare on dairy farms. As strengths of associations were not considered in this review, however, the true value of these VRHDs should be further explored. Moreover, associations found at the animal level and in an experimental setting might not appear at the farm level and in common practice and should be investigated. Cross-sectional studies using integrated welfare scores at the farm level are needed to more accurately determine the potential of VRHD to estimate levels of animal welfare on dairy farms.

In the paper by Rushen et al. (14), the automatic monitoring of behavioural indicators of animal welfare is considered. Automated feeders and devices attached to animals (e.g. accelerometers or GPS devices) can help measure the activity levels of animals with a high degree of accuracy. Technological developments have provided everyone who monitors animal welfare with a variety of tools that can be used to monitor behaviour automatically, and these have great potential to improve the ability to monitor animal welfare indicators on farms.

The authors Vasseur et al. (15) believe that a training program is needed to perform BCS accurately. Assessors need to be provided with the scoring chart and need to be trained to use this chart with proper training material.

Authors Robichaud et al. (10) in their study provide indications to farmers using freestall housing that greater cow comfort and welfare on-farm can benefit them financially through improved production. To achieve this, every aspect of housing needs to provide the highest level of comfort for the animals. Also, farmers should aim to keep lameness and leg lesions prevalence to a minimum. In this sense, the importance of good stall management in terms of dryness and cleanliness for increased milk production and quality is highlighted. The paper by Robichaud et al. (11) points out that to maximize the welfare of cows kept on tie-stall farms, every aspect of the stall needs to provide the highest level of comfort for the animals. In addition, it is indicated that minimizing the number of cows with low BCS and managing hoof health to reduce lameness also improves animal longevity and farm profitability.

Cows are animals that live in a group with a clear hierarchy, so the socialization of cows is of great importance in assessing stress or the tendency to future stress. Gibson et al. (16) have developed suitable tests that could be used to measure the sociability of individual cows on commercial farms. A standardised runway test was used as a "gold standard" test of social motivation and was repeated three times on 46 focal cows. In the runway test, the average latency to reach 5 m and 2 m from the herd and the time spent in these areas were recorded and analysed for repeatability. The results indicate that these measures could be used to assess the sociability of individual dairy cows in on-farm studies.

Body temperature during heat stress in cows, eye temperature during cow manipulation and heart rate during lameness stress were used as indicators of this response. Martello et al (17) analysed surface body temperature as an indicator of heat stress. Rectal temperature (RT), respiratory frequency (RF), body surface (BST), internal base of tail (TT), vulva (VT) and auricular temperatures (AT) were collected, from 37 Black and White Holstein cows at 0700, 1300 and 1800 hours. The AT, TT, VT and BST presented similar patterns and followed the variations of DBT throughout the day. Temperatures measured at different anatomical sites of the animal body have the potential to be used as indicative of the thermal stress in lactating dairy cows. Herbut et al. (18) concluded that THI formulas that determine the environmental risk factors for cows are unfortunately still imperfect because they take into account only factors that shape the microclimate of the air. Other indicators of cow response do not include, for example, the role of the floor (ground) in animal cooling. Since cows spend 8–16 hours a day in a lying position, at which time 20–30% of their body surface comes into contact with the ground, it will be necessary to develop a THI of the surface on which the cow is lying. Gómez et al. (19) investigated if visible eye white and eye temperature measurements are feasible non-invasive physiological indicators of acute stress in cows when they are exposed to cattle crush treatment for claw trimming. The maximum eye temperature increased during and after both situations in Brown Swiss cows, whereas in Red Holstein cows, it increased after (but not during) both situations. Kovacs et al. (20) investigated heart rate (HR) and heart rate variability (HRV) as indicators of the autonomic nervous system activity and faecal glucocorticoid concentrations as the indicator of the thehypothalamic-pituitary–adrenal axis activity in lame (with locomotion scores 4 and 5; n = 51) and non-lame (with locomotion scores 1 and 2; n = 52) Holstein-Friesian cows. HRV indices were affected by lameness. Heart rate was lower in lame cows than in non-lame ones. Vagal tone parameters were higher in lame cows than in non-lame animals, while indices of the sympathovagal balance reflected a decreased sympathetic activity in lame cows. All geometric and non-linear HRV measures were lower in lame cows compared to non-lame ones suggesting that chronic stress influenced linear and non-linear characteristics of cardiac function. Results demonstrate that HRV analysis is a reliable method in the assessment of chronic stress.

There is an increasing trend towards non-invasive cortisol measurement in cows. In research from Sharma et al. (21) cows in 54 shelters across India were assessed for historic evidence of physiological stress, through the determination of hair cortisol in 540 samples from 10 cows in each shelter by enzyme immunoassay. At a cow level, high hair cortisol concentrations were associated with dirty flanks, hock joint ulceration, carpal joint injuries, body lesions, dehydration, an empty rumen, old age, and low levels of body hair loss. Hair cortisol level promises to be an effective biomarker of stress in cows. Ebinghaus et al. (22) explored associations of faecal cortisol metabolite concentrations (FCM) with farm factors including human–animal contact, cows' fear behaviours towards humans, and milk production and udder health, involving 25 dairy farms and repeated faecal samples (n = 2625) from 674 focal cows. Farm factors via interviews and observations, avoidance distance (AD) and qualitative behaviour assessment (QBA) during a human–animal interaction were recorded. Milk yield and somatic cell scores (SCS) were calculated from milk recordings. Levels of FCMs were in general relatively low. Correlations between FCMs, QBA and SCS were significant but on a low level.

Table 1. New welfare and stress indicators on dairy cattle farms

Year	Author(s)	Some basic information about indicators in the paper
2011	De Vries et al.	The paper aims to consider the strategy to monitor animal welfare more efficiently in such a way that the level of animal welfare on a farm based on routine herd data that are available in national databases would be assessed first. It was concluded that it is not currently known which variables of routine herd data (VRHD) are associated with dairy cattle welfare indicators (WI). It was determined that twenty-three

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		VRHDs were associated with 16 WI.
2012	Rushen et al.	The authors discuss some of the issues with using automated methods to measure animal behaviour within the context of assessing animal welfare.
2013	Vasseur et al.	The author's work aims to point out the need for training assessors for a body condition score (BCS) in dairy cattle.
2019a	Robichaud	The study aims to provide indications to farmers using free-stall housing that show that cow comfort and welfare on-farm can benefit them financially through improved production.
2019b	Robichaud	The objective of this study was to evaluate the associations between the on-farm prevalence of several animal-, management-, and resource-based welfare indicators and measures of farm productivity in the use of tie-stall housing for dairy cattle.
2010	Gibbons et al.	The paper considered latency to reach the 5 m line in runway test.
2010	Martello et al.	The body surface (BST), the internal base of the tail (TT), the vulva (VT) and auricular temperatures (AT) are useful indicators of thermal stress
2012	Cincović et al.	NEFA (>0.79mmol/L) and BHB (>1.05mmol/L) are important diagnostic indicators that allow the separation of cows with parameters out of the reference values from cows with normal values of parameters in the metabolic profile and blood count.
2012	Trevisi et al.	The paper considered the Liver Functionality Index (LFI) to identify cows at risk in the transition period toward improved farm management.
2012	Alvåsen et al.	Higher mortality was associated with larger herd size, longer calving intervals, and herds that had local Holstein as the predominant breed. Lower mortality was observed in herds with a higher herd average milk yield, during the fall and winter, and in organically managed herds.
2015	Konvičná et al.	Malondialdehyde (MDA) is an indicator of oxidative stress with inverse relation with antioxidant status (ferric reducing ability of plasma (FRAP); superoxide dismutase (SOD); glutathione peroxidase (GSH-Px); selenium (Se); vitamin E in dairy cows.
2015	Kovacs et al.	Heart rate was lower in lame cows than in non-lame ones. Vagal tone parameters were higher in lame cows than in non-lame animals.
2017	Des Roches et al.	Cows were less attentive toward their surroundings (score, 0.54), had high plasma cortisol (31.3 ng/mL) and SAA (100.3 µg/mL) concentrations, and rumen temperature was increased (40.3°C).
2017	Ježek et al.	The cut-off concentration of BHB in milk set at ≥0.080 mmol/L (AUC=0.91±0.03; p<0.001) is a significant indicator for subclinical ketosis in dairy cows.
2018	Herbut et al.	Proposal: Develop a THI of the surface on which the cow is lying.
2018	Gómez et al.	The maximum eye temperature increased during and after cattle manipulation
2018	Belić et al.	Poor metabolic adaptation of cows in early lactation (eight weeks after calving) was recognized by anabolic (insulin, IGF-I) and catabolic (NEFA) indicators in the first week after calving.
2019	Sharma et al.	At a cow level, high hair cortisol concentrations were associated with dirty flanks, hock joint ulceration, carpal joint injuries, body lesions, dehydration, an empty rumen, old age, and low levels of body hair loss.
2020	Rilanto et al.	Animal-level risk factors for culling were Holstein breed, older parity, lower milk yield breeding value, older age at first calving, longer previous calving interval, having assisted calving, stillbirth and the birth of twins/triplets. Lower milk yield, somatic cell count over 200,000 cells/ml and fat/protein ratio over 1.5 at first test-milking after calving was associated with greater culling hazard during the lactation. Cows from larger herds, herds with decreasing size and higher milk yields had a higher culling probability.
2020	Ebinghaus et al.	Faecal cortisol metabolite correlates with qualitative behaviour assessment (QBA)and somatic cell scores SCS.
2020	Jerram et al.	Production values alone do not equate to high welfare and the high levels of lameness on the farm combined with its effect on salivary cortisol suggest that cow stress continues to need consideration when changing systems on commercial dairy farms.
2022	Bahrami-Yekdangi et al.	Dry period length, calf birth weight, and parity were the most important cow-level risk factors for the incidence of dystocia. Calving year, calving season, parity, twin status, dry period length, calf birth weight, calf sex, and dystocia were significantly associated with the incidence of stillbirths.
2022	Krnjaić et al.	Cows milked 3X had higher levels of NEFA, BHB, AST, GGT, TBIL, and CORT and lower levels of GLU, Ca, INS, and T4.
2022	Grelet et al.	Blood fructosamine and hair cortisol are promising indicators of chronic stress. Milk loss may be an effective and easy way to detect general problems. This may enable to monitor and reduce chronic stress in dairy farms. Heart rate was lower in the stress group.
2023	Heirbaut et al.	Milk production data (from DHI) in combination with on-farm routine measured milk fatty acid (MFA) and ketone (BHB) determined by mid-infrared (MIR), gives model DHI + BHB (MIR] + MFA (MIR] allowed to automatically predict metabolic status during early lactation.
2023	Wang et al.	Daily rumination time, daily activity, parity, body condition score, season of calving and dystocia score are indicators for ketosis included in the web application.

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2023	Džermeikaitė et al.	Wearable sensors can monitor eating, rumination, rumen pH, rumen temperature, body temperature, laying behaviour, animal activity, and animal position or placement. A new farming method called “precision agriculture” and big data collection from all parts of cows' lives and production creates an opportunity for early prediction of disease.

Jerram et al. (23) investigated the long-term impact of the introduction of an automatic milking system (AMS), their study aimed to assess short-term and chronic stress associated with a change in the milking system by measuring salivary and hair cortisol levels and to assess the impact on health and production parameters. Cows from one farm changing their milking system were recruited to the study and sampled for saliva and hair before and after installation. Salivary cortisol showed no diurnal pattern but was affected by lameness and gestation. Non-lame cows showed a reduction in salivary cortisol after AMS introduction ($p < 0.001$). Hair cortisol levels increased after AMS, but it was unclear if this change was seasonal. Grelet et al. (24) compared and evaluated potential biomarkers for chronic stress after inducing stress over 4 weeks through severe overstocking, restricted access to feed and isolated unusual events. The heart rate was lower in the stress group and showed more heterogeneity at the end of the stress period. No differences were observed regarding salivary cortisol, blood glucose, β-endorphin, thyroxine and leucocyte profile. A higher level of hair cortisol and blood fructosamine was observed in the stress group at the end of the stress period. Regarding the practical use of the highlighted biomarkers, milk loss may be an effective and easy way to detect general problems, including stress. The blood fructosamine and the hair cortisol concentrations are promising indicators to assess chronic stress in commercial farms. Mortality, culling and dystocia are major stressors that most directly increase the level of stress on the farm, either through direct losses or through chronic and severe pain that the animals suffer. To predict these stressors, a large number of measurable indicators were determined to assess the occurrence of these stressors on the farm. Those factors can be classified as herd-based and cow-based and include farm size, quantity and quality of milk produced, age at first calving, period between two calvings, characteristics of calves and course of pregnancy, diseases, etc. Alvåsen et al. (25) evaluate time trends in on-farm dairy cow mortality in Sweden and identify potential herd-level risk factors. Rilanto et al. (26) identified the culling rates of Estonian dairy cows and identified the farmers’ stated reasons and risk factors for culling. This observational study used registry data of all cows from herds with ≥20 cow-years in 2013–2015. Cow lactation-level analyses included data from 86,373 primiparous cows from 409 herds and 177,561 lactations of 109,295 multiparous cows from 410 herds. The most common reasons farmers stated for culling were feet/claw disorders (26.4%), udder disorders (22.6%), metabolic and digestive disorders (18.1%) and fertility problems (12.5%). Bahrami Yekdangi et al. (27) have investigated cow-level risk factors associated with dystocia and stillbirth in a relatively large sample of dairy cows using multivariable linear regression models. The incidence of dystocia was associated with the interactions of twin status × calf birth weight and twin status × stillbirth. According to our analysis, the incidence of stillbirth is caused by interactions among several factors, such as twin status × length of dry period, twin status × calving season, and twin status × parity. The highest incidence of dystocia (21.3%) and stillbirths (5.4%) was observed in hypocalcemic cows.

A particularly significant stressor in dairy cows is mastitis. Mastitis is evaluated based on the number of somatic cells in individual or pooled milk samples. Because of their pain, toxicity and systemic effect, coli mastitis is very significant, and inflammatory response indicators are also included in the evaluation of this mastitis. Des Roches et al. (28) tested behavioural and pathophysiological responses as possible signs of pain experienced by cows after an experimental intramammary challenge (mastitis) with *Escherichia coli*. Cows were less attentive toward their surroundings (score, 0.54), had high plasma cortisol (31.3 ng/mL) and SAA (100.3 µg/mL) concentrations, and rumen temperature was increased (40.3°C). In phase 3 (32 to 80 h postinoculation), bacterial concentrations decreased concomitantly with high SCC levels. Cows had high levels of haptoglobin (0.57 mg/mL) and SAA (269 µg/mL) but showed no behavioural changes. Dairy cows displayed changes in behavioural, inflammatory, and stress parameters after E. coli mammary inoculation. Our results suggest that cows may have experienced discomfort in the preclinical phase (phase 1) and pain in the acute phase (phase 2)

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but neither discomfort nor pain in the remission phase (phase 3).
Oxidative stress occurs as a result of the action of external and internal stressors and is a significant stressor in the development of various diseases. Oxidative stress was defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defences. Negative energy balance and extensive lipolysis further lead to peroxidation of released fatty acids when oxidative stress occurs. In cows, both prooxidants and antioxidants must be taken into account as indicators to gain a full insight into the level of oxidative stress in early lactation. Konvičná et al. (29) evaluated the indicators of oxidative stress (malondialdehyde, MDA) and antioxidant status (ferric reducing ability of plasma, FRAP), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), selenium (Se) and vitamin E in dairy cows. Significant changes between MDA and indicators of oxidative stress (SOD, GSH-Px, vitamin E) confirm that during parturition and onset of lactation, oxidative stress occurs in dairy cows. Exposure of peripartal cows to oxidative stress may cause an increased incidence of metabolic diseases.
A large number of extremely important diseases are metabolic diseases, and the queen of all metabolic diseases in cows is ketosis (often with fatty liver), which can be both a cause of stress and a consequence of the action of stressors. Metabolic indicators are therefore related to all other indicators of stress and well-being in dairy cows. Indicators of metabolic stress have been developed in the past 15 years in the following way: 1. the limit value of NEFA and BHB was determined, which indicates that there is a metabolically burdened and poor adaptation in cows in early lactation (30), 2. it was determined liver functional status index (LFI) which is related to adaptation and inflammatory processes in the body in cows (31), 3. cows were classified based on the values of catabolic and anabolic indicators of metabolic stress in early lactation to assess the long-term prediction of metabolic stress in cows (32). Metabolic, endocrine and immunological parameters are mostly determined from blood and are slightly invasive methods that require additional involvement of veterinarians and support staff. Therefore, in parallel with indicators in blood, indicators in milk were also determined, which would indicate metabolic stress and the health of cows. Thus, it was found that metabolic stress and subclinical ketosis in cows can be predicted based on BHB concentration in milk (33), and the introduction of mid-infrared (MIR) technology enabled the routine monitoring of metabolites from milk during the milking process (34). An increase in milking frequency is a stressogen for animals, which is measured by an increase in milk production and an increased dependence of metabolites on NEFA and BHB, which indicates an increased stress adaptation in these cows (35).
In recent times, completely new indicators or new ways to measure already known indicators of health, welfare and stress in cows have been found. These indicators are measured automatically in real-time and completely non-invasively, using different sensors. Collecting a large amount of data also results in the formation of complex linear and polynomial models within the framework of machine learning. Once determined, the models become part of easily accessible applications, in which farm-based or cow-based data can be easily entered (manually or automatically from sensors) to obtain information about the risk of developing a stressful situation such as disease. Wang et al. (36) made the XGBoost model to predict the risk of ketosis in dairy cows using machine learning models based on noninvasive prenatal indicators of parity, body condition score, dystocia score, daily rumination time, daily activity, and season of calving. In the XGBoost model, daily rumination time (60.15%) and daily activity (16.73%) were identified with the highest percentage contribution to the model, followed by parity (10.41%), body condition score (6.42%), season of calving (4.23%), and dystocia score (2.06%). The probability of ketosis increased with decreasing daily rumination time and daily activity. Moreover, parity 3+ and summer may also increase the probability of ketosis. Džermeikaitė et al. (37) reviewed the importance of the use of sensors (tail, nose, ear, leg, reticulorumen) and big data collection within the new farming method called "precision agriculture" in the early recognition of disease development as the most important stressors on the cow farm.

NEW WELFARE AND STRESS INDICATORS ON PIG FARMS

The Welfare Quality project (38) has pioneered the development of standardized methodologies and scientific instruments as protocols for the evaluation of animal welfare, providing valuable insights into the well-being of farmed animals. Despite its widespread use, many researchers have raised concerns that these protocols as time-consuming followed by a lack of score transformation that reflects in welfare outcomes and therefore costly (39). These limitations hinder its practicality for farmers. Additionally, the method used to amalgamate measures into a single welfare assessment score lacks transparency, potentially leading to debates regarding the fairness and relevance of welfare assessments based on this tool. In this context, there is a need to develop more concise methods that can effectively summarize the key aspects of the Welfare Quality® protocol (WQ) or serve as initial screening tools to identify farms with compromised welfare before implementing the complete WQ protocol. Finding new feasible indicators would therefore further contribute to the field of assessing animal welfare. Therefore, the researchers seek innovative approaches that combine behavioural and physiological indicators to advance this endeavour and further enhance the assessment of animal welfare. Such developments would contribute significantly to the field of animal welfare assessment. Table 2 provides some basic information about welfare and stress indicators on pig farms that were determined in publications after 2009.

In the most recent Scientific Opinion on the welfare of pigs on farms published by the European Food Safety Authority (EFSA) in 2022 (40), 16 significant welfare consequences were identified as highly relevant. These include restriction of movement, resting problems, group stress, isolation stress, separation stress, inability to perform exploratory or foraging behaviour, inability to express maternal behaviour, inability to perform sucking behaviour, prolonged hunger, prolonged thirst, heat stress, cold stress, locomotory disorders (including lameness), soft tissue lesions and integument damage, respiratory disorders and gastro-enteric disorders.

The fact that animal welfare at the same time is a science-based and value-based issue releases different approaches to animal welfare specification. Vitali et al. (41) introduced a novel protocol for on-farm assessing the welfare of suckling piglets. This protocol drew upon a combination of welfare parameters from existing sources, including the Welfare Quality® (38), Classyfarm (42), and AssureWell (43) protocols, either in their original form or with slight modifications. Additionally, after conducting a thorough literature review, the researchers introduced a few parameters that were not present in these existing protocols. These parameters were categorized into four groups: Qualitative Behavior Assessment (QBA), behavioural measures, lesion evaluation, and health measures. This study has shown that negative social behaviour was more frequent than positive social behaviour.

Looking for a comprehensive assessment protocol that can be used in intensive pig farming systems, Renggaman et al. (44) developed a pig welfare assessment protocol comprising 17 criteria aligned with four main principles of welfare (good feeding, good housing, good health, and appropriate behaviour). They employed a 3-point scale (0 for good welfare, 1 for moderate welfare, and 2 for poor welfare) to evaluate feeding, housing, and health, while appropriate behaviour was assessed by assessment of positive and negative social behaviours relying on qualitative behaviour assessment and human-animal relationship tests. Aggregating 25 animal-based measurements with input on 38 experts' opinions on inter-measurement and inter-stage weights Brandt et al. (45) developed an animal welfare index (AWI). Developed AWI relies on the holistic approach that combines animal, environment-, and management-based factors.

Many authors look inside environmental- and resource-based measures to find non-invasive welfare indicators. Villarroel et al. (46) develop and compare temperature and enthalpy time derivatives related to behavioural data of pigs, like latency to drink, frequency of drinking, duration of drinking and duration of resting. They concluded that times derivatives of temperature or enthalpy could be used as non-invasive welfare indicators on-farm and during transport of pigs. Stocking density, space allowance and pen size and their connection with behaviours, lesions on the body and tail, lameness scores, bursitis, body temperature, manure on the body, concentration of salivary cortisol and performance of growing pigs were used as welfare indicators in some studies (47-50). Coherently concluded that high stocking density and movement limitation reduced the welfare and performance of growing pigs. In an environment of high stocking density, pigs exhibited several notable behavioural and physical differences compared to those in the middle or low stocking density groups. Specifically, pigs in high-density environments allocated more time towards drinking and engaging in negative social interactions. Moreover, they displayed more severe body lesions, had a greater dirtiness of manure on their bodies, and demonstrated reduced resting times and fewer instances of positive social interactions. Conversely, pigs in the middle stocking density group exhibited a higher frequency of positive social behaviours in contrast to both the high and low stocking density groups. Elevated values of cortisol can be indicative of stress and, therefore, poor welfare (51). Vermeer & Hopster (52) established threshold values for climate-related measurements as an indication of welfare risk.

Pierozan et al. (53) established a connection between the feed conversion ratio (FCR) and daily feed intake (DFI) of growing-finishing pigs in the context of animal welfare concluding that the conditions related to poor welfare were associated with an impairment in animal performance.

During the last ten years, other novel approaches were tested to establish animal welfare indicators on farms. VHAT (Voluntary Human Approach Test) was tested by Wegner et al. (54) and HRQL (Health-Related Quality of Life) was tested by Wiseman-Orr et al. (55). Although these methods are feasible for on-farm use, they are valid only in combination with behaviour and environmental welfare indicators. Many other authors were focused on novel feasible animal-based measures as screening tools for on-farm pig welfare. Telkanranta et al. (56) used tear staining or chromodacryorrhea as a promising new indicator for pig welfare assessments. Recently, there has been growing research indicating a link between tear staining in pigs and various environmental stressors, as well as physiological markers of stress. The authors found a correlation between tear staining tail and ear damage, and approach latency. Valros et al. (57) have promising results that tail-biting lesions can be a potential measure of on-farm pig welfare, as a large range of stressors increases the risk for tail-biting outbreaks. Additionally, their research illustrates the feasibility of conducting a comprehensive tail scoring to discern various lesion types within a slaughterhouse meat inspection. In a study by Diana et al. (58), an association was established between tail, ear, and skin lesions and the production flow, revealing that all production flows were linked to a heightened risk of lesions, consequently indicating a compromise in pig welfare.

The Welfare Quality Protocol for Pigs (38) uses the Qualitative behavioural assessment (QBA) as a measure of positive emotional states through indicators of social affiliative behaviours, exploratory behaviour and play behaviour. The protocol states that negative emotions such as distress or fear should be avoided whereas positive emotions such as contentment should be promoted. Moreover, the Five Freedoms framework primarily addresses the negative aspects of animal welfare, except for the freedom to express natural behaviour. Various alternative approaches have emerged to emphasize positive animal welfare. These include the "quality of life" perspective, the positive emotions approach, the positive affective states approach, and the happiness approach (59). The "quality of life" (QoL) approach, as proposed by Rowe & Mullan (60), seeks to enhance animals' lives by providing them with pleasures, comforts, and a balanced ratio of positive to negative experiences. The positive emotions concept lies in the fact that animals are not only capable of experiencing short-term emotions triggered by events in their environment, such as pleasure, but also long-term emotions like happiness (59). Furthermore, Mellor (61) introduced the concept of positive affective states within the realm of positive welfare. This term encompasses a broader range of experiences than emotions, including all subjective feelings and sensations that animals consciously perceive as pleasant or unpleasant. These affective states motivate animals to behave in particular ways, directing their actions toward achieving specific goals, whether those attempts result in success or failure. However, a multidimensional scientific approach is needed for feasible on-farm measurement and evaluation of positive animal welfare.

Table 2. New welfare and stress indicators on pig farms

Year	Author(s)	Basic information about indicators in the paper
2010a	Faucitano et al.	Anamnestic data of feeding or fasting time and resting in lairage
2011	Villarroel et al.	Temperature time derivatives and enthalpy time derivatives related to behavioural data: latency to drink (the time the pigs waited after unloading to drink), frequency of drinking (the number of times each pig engaged in a drinking bout), duration of drinking (total amount of time spent drinking) and duration of resting (the total amount of time sitting or lying)

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2011	Nielsen	Data warehouse for assessing animal health, welfare, risk management and-communication
2015	Knage-Rasmussen et al.	Routinely collecting data along or after the production process, such as the use of routine meat inspection
2011	Wiseman-Orr et al.	Health-related quality of life (HRQL)
2014	Dokmanović et al.	Time spent in the abattoir depot significantly affected blood lactate, carcass rigour mortis, skin damage, drip loss, colour and meat quality of slaughtered pigs
2014	Valros et al.	Higher abscesses and arthritis occurrence rate, pour carcass characteristics, rise in condemnations at slaughter and pourer meat quality
2014	Vermeer et al.	The effect of stocking density and space allowance on welfare (skin and tail lesions, lameness scores) and performance parameters
2015b	Mellor	Feeding behaviour, sexual and mating behaviour
2015	Renggaman et al.	Pig welfare assessment protocol that combines animal-, environment-, and management-based measures
2016	Telkanranta et al.	Tear staining or chromodacryorrhea
2016	Fu et al.	The effect of stocking densities on welfare indicators, such as behaviours, lesions on the body and tail, body temperature, manure on the body and concentration of salivary cortisol of growing pigs
2016	Meyer-Hamme et al.	The effect of group size on various animal-based measures of the Welfare Quality® protocol for growing pigs (body condition, bursitis, manure, wounds, tail biting, lameness, laboured breathing, scouring, skin condition (inflammation or discolouration), hernias, twisted snouts and rectal prolapse)
2017	Van Staaveren et al.	Carcass tail and skin lesions at meat inspection for the assessment of pig health and welfare on farms (poor body condition, bursitis and severe tail lesions)
2017	Brown et al.	Play behaviour
2017	Spinka	Exploratory and feeding behaviour
2017	Matthews et al.	Automated system with a single type of sensor–a depth video camera–to track 3D pig positions and measure multiple behaviours non-invasively
2017	Brandt et al.	Animal Welfare Index (AWI)
2018	Ahloy-Dallaire et al.	Play behaviour
2018a; 2018b	Marcet Rius et al.	Tail and ear movement
2018	Vermeer and Hopster	Climate-related measurements
2018	Amos et al.	The Business Benchmark on Farm Animal Welfare
2019	Keeling	Exploratory behaviour, play behaviour, social affiliative behaviours, synchronization
2019	Rault	Pro-social behaviours
2019	Vigors and Lawrence	Qualitative interviews to directly examine livestock farmers' perspectives of positive welfare
2019	van Staaveren et al.	The PIG WELFare INDicators (PIGWELFIND) project (Research Stimulus Fund 11/S/107) was developed to progress the development of ante and post-mortem MI (Meat inspection) as a pig health and welfare diagnostic tool
2020	Valros et al.	Validation of a scoring system sensitivity for properly differentiating farms with different levels of tail-biting
2020	Vullo et al.	Piglet Grimace Scale (PGS), a facial-expression-based pain coding system
2020	Blomke et al.	Development and evaluation of an automated system for the assessment of ear and tail lesions as welfare indicators in pigs at the abattoir
2020	Pierozan et al.	Animal welfare indicators (Welfare Quality® assessment protocol for pigs) and their possible associations with feed conversion ratio (FCR) and daily feed intake (DFI) of growing-finishing pigs
2020	Camerlink & Ursinus	Ear and tail postures
2020	Vitali et al.	Behavioural, lesion and health measures
2020	Wegner et al.	VHAT (Voluntary Human Approach Test)
2020	Courboulay et al.	BEEP: An advisory pig welfare assessment tool developed by farmers for farmers
2020	Pfeifer et al.	"Animal Welfare Indicators: Practical Guide—Pigs" developed by the German Association for Technology and Structures in Agriculture
2020	Statham et al.	The Rapid Defence Cascade (DC) response (startle, freeze): (i) sparse feature tracking computer vision image analysis of 200Hz video, (ii) load platform, (iii) Kinect depth camera, and (iv) Kinematic data.

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2020	Haigh et al.	Open Field (OF) and Novel Object (NO) tests
2020	World Animal Protection	Animal Protection Index
2020	Sandoe et al.	Welfare scores for 15 dimensions
2021	Laurijs et al.	Vocalizations
2021	Gomez et al.	Precision livestock farming (PLF) technologies: vision-based solutions, load-cells, accelerometers and microphones, thermal cameras, photoelectric sensors, radio-frequency identification (RFID) for tracking, infrared thermometers and pyrometers
2021	Hansen et al.	Grad-CAM for assessment of the Pig Grimace Scale
2021	Larsen et al.	Information technologies (sensors) for welfare monitoring
2022	Papageorgiou	The "quality of life" approach, the positive emotions approach, the positive affective states approach, and the happiness approach
2022	Rowe & Mullan	Good Life Frameworks for Pigs
2023	Andersen et al.	Associations of group size, floor space and type of feed with selected welfare indicators (proportion of pigs per pen with bite marks on ears, body and tail, hernia, and movement disorders, and proportion approaching vs. fleeing from an unfamiliar human) and performance indicators (daily weight gain, feed conversion ratio, and mean weight of the slaughtered pigs) in finishing pigs
2023	Michelsen et al.	Danish Animal Welfare Index (DAWIN)
2023	Plut et al.	Combination of Serum and Oral Fluid Cortisol Levels

Papageorgiou (62) in his master thesis makes a review of behavioural indicators for positive animal welfare. Based on literature data meta-analysis, he found out that play is the behaviour that most frequently has been studied as a positive welfare indicator. The content and neurobiological foundation of play suggests that an animal engaged in play is experiencing a positive state of well-being (63). Some studies deal with the experimental assessment of play behaviour (64-68), while others studies explain the concept of play behaviour theoretically (63, 69-71, 59). The motivation of animals to play is very strong because of the pleasure they experience leading to good welfare (69). Indeed, maternal care, social affiliative behaviours, social play, and synchronization have been discussed as potential positive welfare indicators for pigs. These behaviours are essential aspects of pigs' natural behaviour and social dynamics, making them valuable indicators when assessing the well-being of these animals. Mellor (63) directs attention toward feeding behaviour, highlighting the pleasure animals derive from exploring and savouring their food. In contrast, Keeling (70) concentrates on exploratory behaviour as a potential indicator of positive welfare, albeit with some reservations related to the possibility of fear-induced exploration. According to Keeling (70), exploration is tied to cognition and can be categorized into two types: inquisitive exploration, where animals seek change, and inspective exploration, where animals respond to changes in their environment. Keeling (70) also delved into the concept of synchronization as a potential indicator of positive welfare in pigs, recognizing their inherently social nature. Given that pigs possess a strong innate drive to explore and root, often dedicating a significant portion of their time to foraging in their natural habitats (72), both exploratory and feeding behaviours emerge as promising indicators of positive welfare for domestic pigs. Additionally, pigs form close social bonds not only with related individuals but also with unrelated ones, particularly when they have been raised together. Pro-social behaviours, for example sharing resources of space and food with other individuals in the group, can be considered a positive welfare indicator in pigs when they happen consistently (73). Based on the literature results, sexual behaviour is another animal-to-animal interactive behaviour which indicates that the individuals are experiencing positive affective states (63). Social affiliative behaviours, reflecting these strong social connections, hold the potential as positive welfare indicators. Rius et al. (74) and Marcet-Rius et al. (75) stated that tail and ear movements in pigs are promising positive welfare indicators for the on-farm welfare assessment. Nonetheless, implementing positive animal welfare measurements at the farm level can be a complex endeavour.

Vocalizations in pigs have indeed been the subject of extensive study. However, to establish vocalizations as reliable positive welfare indicators, more investigation and understanding of the nuances of pig vocalizations are required, including differentiating between types and contexts of

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vocalizations, It's worth noting that research has shown that certain vocalizations, such as screams, are generally indicative of negative emotions (76). These vocalizations are employed, for example, in the Welfare Quality protocol to measure fear through Qualitative Behavior Assessment (QBA) in slaughterhouse settings.

Pain assessment in pig farming faces practical limitations, due to the lack of reliable and feasible tools. Vullo et al. (77) and Hansen et al. (78) used the Piglet Grimace Scale (PGS) as a coding system for facial expression related to on-farm surgery interventions and making distinctions between stressed and unstressed pigs. The authors found that the PGS score increased after surgery and inter-observer reliability was excellent.

To perceive the on-farm feasibility of positive welfare indicators Vigors & Lawrence (79) developed questionnaires to investigate the beliefs and attitudes of farmers toward perspectives of positive welfare. Findings reveal that farmers describe elements of positive welfare which are broadly in line with indicators suggested in the positive welfare literature. Furthermore, this study reveals that farmers tend to prioritize the mitigation of negative welfare aspects as their foremost management concern. Positive welfare is often construed as a consequence of addressing these negative aspects, rather than being directly addressed as a separate and explicit goal in farm management.

Integrating indicators for pig health and welfare into meat inspection processes holds the potential to reduce the necessity for on-farm assessments. Notably, skin and tail lesions are significant welfare indicators in pigs, offering a promising avenue for data collection during meat inspections. These indicators may serve as indirect, "iceberg" markers of on-farm welfare conditions. As noted by Van Staaveren et al. (80), both carcass tail and skin lesions have demonstrated the capacity to account for the prevalence of various welfare issues on farms, underscoring their potential as valuable umbrella indicators in this context. These findings were results from the project PIG WELFare INDicators (PIGWELFIND) with the main aim of the development of ante- and post-mortem meat inspection as an on-farm pig health and welfare diagnostic tool.

Many authors have promising results in using Precise Livestock Farming technology for the assessment of pigs' welfare on-farm or in the abattoir. Matthews et al. (81) introduced an innovative automated system that utilizes a single type of sensor, specifically a depth video camera, for tracking 3D pig positions and non-invasively measuring various behaviours. These behaviours encompass standing, feeding, drinking, and locomotor activities. This automated system is suitable for use in commercial farms because it offers continuous monitoring of multiple behaviours, providing metrics that are not only more intuitive but also possess diagnostic validity. This technology holds promise for enhancing the welfare assessment and management of pigs in practical, real-world contexts. To enable cross-country comparisons of pig welfare, Benchmarking Farm Animal Welfare tools were defined, covering several welfare dimensions and features typically modified in legislative and market-driven welfare initiatives aimed at pig production (82-84).

Basically, on-farm welfare assessments are the main method for assessing pig welfare, but these approaches are hard to do and time-consuming (85), and they include an increased risk of biosecurity and disease transmission within and between farms (86). As is given in Table 2, it makes an increased interest in routinely collecting data along or after the production process (87,88).

The focus of several studies was to evaluate the welfare of dairy herds based on routinely collected data, including at meat inspection (89-91), but there is limited work on the use of routine meat inspection data for pig health and welfare assessment (87,88), such as incorporating welfare indicators during meat inspection at abattoirs as a surveillance tool for pig health and welfare (92-94). Friedrich et al. (95) suggested the use of so-called “iceberg” indicators to assess overall animal welfare and to provide a picture of the overall welfare of the animal and function as a warning signal for underlying problems. Tail and skin lesions are among the most frequently cited animal-based indicators of pig welfare and expert panels proposed to use them in finishing pigs (96). As is well noticed in the van Staaveren et al. (80) study, incorporating indicators for pig health and welfare at meat inspection could reduce the need for on-farm assessments. During meat inspection all body parts are available and it is very easy to notice body lesions, which leads to conclusions regarding slaughter animals' pre-mortem welfare. Van Staaveren et al. (80) used an adapted version of the Welfare Quality protocol inspecting pigs of different ages (4-8 wk, 8-13 wk and 13-23 wk). The average prevalence of welfare outcomes for each stage was calculated. One batch of pigs was observed at slaughter and skin and tail lesions were scored according to severity for each carcass. The average prevalence of carcass lesion outcomes was calculated for each farm, using linear regression models to predict the prevalence of each welfare outcome in each stage based on the prevalence of the different carcass lesions.

Van Staaveren et al. (97) made recommendations to further progress the development of meat inspection as a pig health and welfare diagnostic tool and address some of these barriers since obtained findings can act as a valuable source of information on pig health and welfare. The PIG WELFare INDicators (PIGWELFIND) project (Research Stimulus Fund 11/S/107) was developed to progress the development of *ante* and *post-mortem* meat inspection as a pig health and welfare diagnostic tool in Ireland. Investigators organized three multi-stakeholder focus groups to explore areas of conflict and agreement between stakeholders' vision for including pig health and welfare indicators in meat inspection and how to achieve this vision. Each focus group consisted of eight stakeholders: pig producers, Teagasc pig advisors, pig processors, veterinarians involved in meat inspection, private veterinary practitioners, and personnel with backgrounds in general animal health and welfare and food safety policy. In general, stakeholders expressed positive attitudes towards the use of meat inspection data to inform pig health and welfare when standardization of recording and feedback is improved, and the meat inspection system provides real-time benchmarking possibilities. Most emphasis was placed on health indicators as a priority, while it was felt that welfare-related indicators could be included after practical barriers had been addressed (i.e., line speed/feasibility, standardization and training of meat inspectors, data ownership).

The combination of physiological and behavioural indicators could provide useful information on the welfare state of an animal. Research performed by Candiani et al. (98) to identify pig welfare indicators that could help in recognizing on-farm stressful practices. The study evaluated behavioural and physiological indicators (cortisol and negative acute phase proteins) in 2 groups of 20 female pigs 4 months old after a 48-hr transport. The first group (A) was transported at the end of May, and the second (B) in June. Behavioural observations and blood collection occurred at arrival (D1) and 28 days later (D28). Compared with within-animal control samples obtained 28 days later, pigs of Group A had increased cortisol levels and decreased albumin concentrations after arrival. As demonstrated by lesion and behaviour observations, the effect on cortisol and albumin was higher in Group B pigs after a tail-biting episode occurred. The study has reported no evidence of Retinol Binding Protein (RBP) in pigs. A method developed for swine RBP quantification found RBP strongly reduced in D28 samples of Group B, confirming it to be a negative protein in pigs.

According to Valros et al. (99), tail biting is a common and serious welfare-reducing problem in pig production. The occurrence rate of slaughtered pigs in countries where tail docking is prohibited is 6-11.7% (100,101) and about 3% in countries where tail docking is allowed (101). Affected pigs are more prone to abscesses and arthritis (102,103), and have adverse effects on carcass characteristics, as it may reduce growth (103,104) and cause an increase in condemnations at slaughter (100,104). Reduced welfare increases the risk of tail biting (105,106) and can also have negative consequences on meat quality (107). Even though there are many reasons to suppose that tail biting is linked to underlying stress (108,109) and that being a victim is stressful (110), there is still scarce information available on the consequences of tail biting to the victim.

The evaluation of prolonged or repeated psychological stress is challenging, and to get a reliable picture several measures should be used (111). Cortisol is a traditional measure of stress in pigs, being elevated by acute stress (112), but the effects of chronic stress on cortisol concentrations are less straightforward (99). Studies on humans and laboratory animals show that chronic stress or pain appears to ultimately cause a reduction in daily overall cortisol secretion, as well as in cortisol reactivity to stressors (113), confirmed by similar results in pigs housed in barren environments or under repeated noise stress (114-116).

Premortem stress affects muscle *post-mortem* pH. DFD (Dark, Firm, Dry meat) occurrence is associated with long-lasting pre-slaughter stress, *e.g.* during handling, transport and slaughterhouse lairage as well as a long fasting time (117-119); the glycogen reserves are reduced already before slaughter, due to the stress-induced degradation of muscle glycogen, and the ultimate lactic acid is lower than normally resulting in a pH value higher than 6.0. In the PSE case, those pigs that still have a normal glycogen level at slaughter and that have experienced psychological and/or physical stress just before slaughter have a fastened muscle glycogen breakdown *perimortem*. Lactic acid accumulates in the muscle when the muscle temperature is still high, and this combination causes a partial denaturation of meat proteins and thus a light colour and softness as well as a decrease in water-holding capacity. In addition, heat shock proteins (HSPs) are a potential measure of chronic stress. Cells react to stress by synthesizing HSPs, which help them to maintain intracellular protein homeostasis. HSP-induction is caused by several different cell-level stressors (120). Among the stress-inducible HSPs, the response of HSP70 has been studied most extensively. Its synthesis peaks 8-10 hours after stress, and the concentration stays high for several days (120). Therefore, short to intermediate transport and pre-slaughter handling may not last long enough to affect the amount of HSP70 and it has been speculated to reflect stressors the pigs have encountered on the farm (121). The study by Valros et al. (99,100) also indicates that HSP70 is a promising measure for chronic stress, while the cortisol response during acute stress is not an unambiguous reflection of previously experienced stress levels. The study showed support for the fact that tail-bitten pigs might produce less lean meat per carcass.

Withholding food from pigs before transport to the abattoir from the farm and keeping pigs in lairage before slaughter is a common practice, often regulated by law (122). It varies, but recommended times are usually between 12 and 24 h (123), although some studies have described up to 36 h (124). The reasons for feed withdrawal include many factors, such as higher animal welfare during transport, transport losses and travel sickness reduction, and a possible reduction in the incidence of pale, soft and exudative (PSE) meat (123). Nevertheless, pigs for commercial purposes are subjected to times of total fast (on-farm fasting plus transport time plus lairage) for an average of 46,5 h and can remain in the lairage for less than 24 h without being fed (125). This is an aspect that can impact the quality of the meat and cause a high incidence of dark, firm, and dry (DFD) meat (126), demonstrating the usefulness of a previous fast on the farm, a short transport time with homogeneous groups and without social mixing. These aspects favour handling during loading, unloading, and positive social behaviour during lairage. Physiological indicators show that transport and pre-slaughter management represented a challenge for the animals, which was compensated with rest times in the abattoir of fewer than 8 hours, allowing the recovery of the pigs, and leading to physiological and behavioural changes induced by stress (127). Meat inspection *post-mortem* changes, such as PSE and DFD meat occurrence rate, combined with data regarding time spent in the abattoir depot and feed withdrawal times before slaughter, may be used in finished fattening pigs stress assessment.

Dokmanović et al. (128) noticed that time spent in an abattoir depot significantly affected blood lactate, carcass rigour mortis, skin damage, drip loss, colour and meat quality of slaughtered pigs. In addition, the handling procedure influenced blood lactate, pH and temperature 60 minutes after slaughter, and may be seen as potential stress indicators. Long lairage is more stressful, and is detrimental to carcass quality, but causes better meat quality compared to short lairage. Rough handling was related to higher lactate and lower meat quality.

CONCLUSSION

Based on the analysed literature data related to the determination of new welfare and stress indicators on cattle and pig farms presented in the paper from previously published studies covering the period from 2009 to today, the following can be concluded:

in the analysed period, completely new indicators or new methods to measure already known

indicators of health, welfare and stress in cows and pigs have been found. These indicators are measured automatically in real-time and completely non-invasively, using different sensors. Collecting a large amount of data also results in the formation of complex linear and polynomial models within the framework of machine learning;

cattle and pig welfare and stress could be assessed by observing or measuring many physical, physiological or behavioural features of the animals and qualities of the animal's environment;

welfare and stress are complex, so it is usually important to assess more than one indicator to reveal the extent to which welfare is good or bad, and stress is present, rather than assessing just one aspect of the animal's biology or environment; there are three main sources of welfare and stress indicators: 1. the animal in its current situation (e.g. frequencies or

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durations of abnormal behaviour, concentrations of hormones or body condition), 2. the animal in a decision-making test (e.g. preference tests and cognitive bias tests) and 3. the animal's environment or situation (e.g. quality and quantity of the diet, presence of a hiding place, exposure to weather, or details of husbandry routines, etc.);
welfare and stress indicators can be measured via a continuum between two main approaches: 1. objectively, e.g. quantifying rates, durations, frequencies, concentrations or intensities and subjectively, e.g. owner/keeper questionnaires, qualitative behaviour assessment, lameness or pain scoring systems, etc. and which welfare and stress indicators should be assessed depends partly on whether concepts of welfare and stress include the animal's feelings, physical functioning, and/or naturalness. Feelings can be crucial to some concepts of welfare and stress, e.g. even healthy animals living in a naturalistic habitat could have poor welfare if they are anxious, bored, or socially stressed. Despite feelings being private to each individual, it is possible to measure the behavioural and physical signs of those underlying experiences.

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