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Trends in Measurement Techniques in Laying Hen Farm Welfare: A Review

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ABSTRACT

Animal welfare is a crucial issue in animal production, and researchers are seeking optimal methods to evaluate animal welfare in the field. In poultry farming, laying hen health and welfare are critical to consumer perception of product quality. The aim of the review was to examine traditional and advanced measurement trends of animal welfare in laying hens' farms. Emerging technologies have facilitated a more profound comprehension of animal responses to diverse scenarios encountered in livestock production systems. Currently, conventional methods, such as behavioral observations, are time-consuming and highly dependent on the experienced observer's expertise; likewise, other valuable indicators, including physiological parameters, hormonal levels, thermographic changes in the body, and hematological parameters, are widely used but are being re-evaluated. Currently, technological advances are developing comparatively non-invasive methods for multiple and long-term monitoring, such as machine vision and deep learning algorithms to track bird behavior. In addition, molecular techniques have emerged as promising tools to understand the cellular responses under internal or external stressful conditions and improve farm animal welfare. However, several challenges exist in terms of standardization and implementation of the new technologies, especially in developing countries. These challenges include limited access to advanced tools, costs, among others, and hinder implementation. In this review, we conclude that welfare research requires a holistic and interdisciplinary approach, utilizing both conventional measurements and new technologies to enable a more comprehensive assessment of animal welfare.

Keywords: behavior; omics; physiology; production systems; welfare

INTRODUCTION

The fast growth of the population has raised the demand for food and required the development of more efficient food production systems (Hemathilake & Gunathilake, 2022). The poultry sector is a leader in ensuring global food security in the livestock industry. Poultry production substantially contributes to providing high-quality and affordable protein sources, such as eggs and meat. Likewise, with intensive farming techniques, the poultry industry has been able to respond to the growing demand for these proteins (Attia *et al*., 2022; Gržinić *et al*., 2023; Mottet & Tempio, 2017). However, although intensive farming methods have improved productivity, public concerns have arisen regarding the welfare of production animals, particularly laying hens, and consumers demand higher animal welfare standards in all animal production systems (Clark *et al*., 2016; Sadeghi *et al*., 2023). Laying hen´s welfare constitutes an essential issue in the poultry industry and influences birds' health and productivity (Ferrante, 2009).

Several studies highlight the beneficial effects of enriched environments on the welfare and egg quality of laying hens across different production systems (Barnett & Hemsworth, 2003; El-Sabrout *et al*., 2022; Herrera-Sánchez *et al*., 2024; Tainika & Şekeroğlu, 2021). Likewise, hens raised in poor welfare conditions, such as overcrowded and suboptimal housing, experience increased stress, reduced egg production, and higher mortality rates (Lay Jr *et al*., 2011; Tahamtani *et al*., 2014).

Assessing and quantifying welfare in laying hens is a task that demands a holistic approach. It requires a thorough comprehension of cognition, behavior, physiology, responses to species-specific stressors, and molecular processes (Main *et al*., 2012). Several biomarkers and indicators have been employed to measure laying hens' welfare (EFSA AHAW Panel *et al*., 2023; Bhanja & Bhadauria, 2018; Li *et al*., 2020; van Veen *et al*., 2023). However, conventional methods, such as behavioral observations, are time-consuming and highly dependent on the experienced observer's expertise and accuracy, which are variable (Fujinami *et al*., 2023). Other valuable indicators utilized to evaluate the stress status and welfare of laying hens include physiological parameters (Barnett *et al*., 1994; Kim *et al*., 2021a), hormonal levels, especially corticosterone (Downing & Bryden, 2008; Lee *et al*., 2022; Scanes, 2016; Zaytsoff *et al*., 2019), thermographic changes in the body of hens (Cai *et al*., 2023; Ouyang *et al*., 2021), hematological parameters as heterophil to lymphocyte ratio (Kim *et al*., 2021a; Lee *et al*., 2022; Nwaigwe *et al*., 2020; Scanes, 2016), and the comprehensive oxidative stress status (Herrera-Sánchez *et al*., 2024; Oke *et al*., 2024; Temple *et al*., 2020; Tilbrook & Fisher, 2020; van den Heuvel *et al*., 2022a).

Currently, technological advances have enhanced our understanding of animal welfare and behavior, developing comparatively non-invasive methods for multiple and long-term monitoring, such as machine vision and deep learning algorithms to track bird behavior (Li *et al*., 2020; Okinda *et al*., 2020; Paneru *et al*., 2024; Sozzi *et al*., 2023; Subedi *et al*., 2023; Zaninelli *et al*., 2018). Likewise, among the innovative methodologies, molecular techniques have emerged as promising tools to understand the cellular responses under internal or external stressful conditions and improve farm animal welfare by providing insights into genetic structures, disease detection, and phenotypic outcomes at a molecular level (Demir *et al*., 2021; Fabrile *et al*., 2023; Herrera-Sánchez *et al*., 2024; Rodríguez-Hernández *et al*., 2021). The use of molecular tools such as transcriptomics, proteomics, and metabolomics can provide valuable information on the changes in physiological and underlying molecular mechanisms in the animal's production welfare (Carvalho *et al*., 2022; Herrera-Sánchez *et al*., 2024; Taborda-Charris *et al*., 2023). These approaches provide potential instruments for monitoring and improving farm laying hen welfare while increasing economic efficiency and overall animal well-being. Thus, this review aims to describe technologies with potential applications for evaluating and enhancing the welfare of laying hens.

Traditional Measures

Behavioral observation. Animal behavior refers to the actions, reactions, and activities exhibited by animals in response to internal or external stimulation (Broom & Johnson, 1993; Kokocińska & Kaleta, 2016; Malott & Kohler, 2021). The EFSA guidance on animal welfare risk assessment defines "response of an animal or an effect on an animal" as an animal-based measure. It may be taken directly or indirectly from the animal and includes animal records. However, evaluation of some behaviors may vary according to available resources, and behaviors should be assessed together (EFSA AHAW Panel *et al*., 2023)

Understanding animal behavior is crucial in assessing their welfare. Behavioral observations can provide insight into animals' physical and mental states by identifying patterns and deviations of natural behavior (Broom, 2010; Harikrishnan, 2021; Pisula, 1999). The natural behavior that animals exhibit is a result of their developed cognitive and emotional systems that enable them to interact with the environment, including performing certain pleasurable behaviors and promoting biological functioning (Hemsworth & Edwards, 2020; Khullar & Jena, 2021)

Animals experiencing positive welfare are more likely to exhibit natural behavior. Deprivation of natural behaviors can lead to physiological distress, reduced production, and increased mortality. Consequently, behavioral observations are crucial in identifying animal stress and discomfort, allowing timely intervention to enhance animal welfare and reduce stress levels. The classical methods used to identify and measure behaviors can be complex in a large group of animals, but their importance cannot be overstated. It is urgent that we address animal stress and discomfort, and behavioral observations are a key tool in this endeavor.

In the case of laying hens, multiple housing systems have been developed accommodating large groups of hens that exceed 25,000 birds, making it unfeasible to observe individual animals through conventional methods (Siegford *et al*., 2016). Therefore, the use of new technologies in observing the behavior of laying hens is crucial due to the limitations of traditional methods (Yang *et al*., 2023b; Yang *et al*., 2024). New technologies have been reported to improve behavioral assessment through continuous automated monitoring, offering more accurate and objective insight (Daigle, 2013; Leroy *et al*., 2006; Watters *et al*., 2021). For example, studies conducted in laying hens demonstrated that thermal imaging cameras could accurately detect plumage damage with differences between body regions (Pichová & Bilčík, 2017; Schreiter & Freick, 2022). Wearable sensors have been successfully utilized to monitor laying hen behaviors, providing real-time data on behavior and physiological responses (Fujinami *et al*., 2023). The sensors can be attached to hens and recognize various hen behaviors, categorizing them into different intensity levels for optimal management of modern poultry systems (Shahbazi *et al*., 2023). For instance, wearable inertia sensor technology and a machine learning model (ML) can analyze laying-hen behaviors with an accuracy of 90%, allowing early detection of stress or distress by identifying and analyzing changes in vocalization patterns, such as frequency, duration, and intensity (Derakhshani *et al*., 2022; van den Heuvel *et al*., 2022b). Currently, different technologies have been developed to evaluate changes in laying hen´s behavior as listed in Table 1.

In summary, knowledge concerning animal behavior is essential for improving animal production because it allows the design of production systems that meet animals' needs and promote positive welfare, which can lead to reducing stress and improving animal health in general, generating greater productivity and profitability for farmers (Madzingira, 2018; Orihuela,

Table 1. Technologies for measuring behavioral changes related to the welfare of laying hens

Behavioral changes	Application	Methodology	References
Feeding behavior	Monitoring feed intake.	Audio technology to collect feed intake audio using a voice recorder.	Ji *et al.* (2018)
Drinking behavior	Monitoring locomotion, perching, feeding, drinking, and nesting behaviors.	3D Computer Vision and Radio Frequency Identification.	Nakarmi *et al.* (2014)
Social behavior	Determination of feather pecking conditions.	Audio technology collects feed intake audio using a voice recorder.	Aydin & Berckmans (2016)
	Assessing feather damage.	Optical flow sensor and Markov models.	Lee *et al.* (2011)
	Analyzing the behaviors of laying hens to support farmers in managing hens in loose housing systems.	Wearable inertia sensor technology and machine learning (ML) models.	Derakhshani *et al.* (2022)
Reproductive behavior	Tracking movement and nesting behaviors in real-time.	Radio Frequency Identification (RFID).	Li *et al.* (2020b)
	Tracking overall movement	Sensors, often combined with ML algorithms.	
Resting and sleeping behavior	Classifying resting and sleeping behaviors of laying hens.	Inertia Sensor and ML Technologies	Derakhshani *et al*. (2022)
Locomotion and activity levels	Analyze laying-hen behaviors, such as jumps and flight trajectories.	Wearable inertia sensor technology and ML.	Banerjee *et al.* (2014)
	Supporting farmers in the management of laying hens in loose housing systems through behavioral analysis	Wearable inertia sensor technology and ML model.	Derakhshani *et al.* (2022)
	Evaluating space use and diverse behaviors.	Geographic Information Systems (GIS).	Daigle *et al.* (2014)
Stress-related behavior	Detecting stress.	Audio technology and bird vocalizations were analyzed using software to extract vocalization acoustic parameters.	Pereira *et al*. (2014)
Health-related behavior	Identifying early deviations in health and welfare to reduce the subjectivity of assessments.	Cameras and microphones.	van Veen *et al.* (2023)
	Measuring activity behaviors to provide early warning of disease.	Image processing technology.	Li *et al.* (2020b)
Thermoregulatory behavior	Evaluating thermoregulatory features and metabolic changes.	Infrared technologies.	Ben Sassi *et al*. (2016)

2021). However, behavior observation technologies have limitations, in the case of traditional technologies, subjectivity, difficulty in quantifying behaviors, influence of environmental factors, sampling bias, and lack of standardization (Bateson & Martin, 2021; Dawkins, 2004; Decina *et al*., 2019; Fraser & Matthews, 1997; Jones, 1996; Weeks & Nicol, 2006). In the case of new technologies, despite the generation of objective data without disturbing the animals, they have limitations in terms of implementation at a commercial scale (Ben Sassi *et al*., 2016).

Physiological measures. Physiological measures refer to quantitative assessments of biological processes within an organism, providing insights into its internal state and functioning (Serra *et al*., 2018). Physiological markers offer advantages, such as objectivity, comparability between species, and the ability to reflect past well-being states with different temporal resolutions, allowing a dynamic view of well-being over time (Beaulieu, 2024; Filazzola & Cahill Jr, 2021). Some examples of physiological markers include endocrine and hormonal parameters, metabolic and biochemical indicators, oxidative stress markers, cardiovascular and respiratory indicators, behavioral and physical health observations, immune function, and body temperature (Guevara *et al*., 2022; James *et al*., 2023).

Endocrine biomarkers. Endocrine indicators refer to hormones that regulate various body functions (Hiller-Sturmhöfel & Bartke, 1998). For example, hormone levels in biological samples such as blood, plasma, feathers, eggs, droppings, or urine provide insight into the physiological state of the individual (Carbajal *et al*., 2014; Downing & Bryden, 2008; Häffelin *et al*., 2020; Rettenbacher *et al*., 2004; Steckl & Ray, 2018). Thus, several hormonal stress biomarkers in birds have been described (Table 2).

Methodologies used to measure hormone biomarkers are listed in Tables 3 and 4. This includes Enzyme-Linked Immunosorbent Assay (ELISA), radioimmunoassay (RIA), Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), and high-performance liquid chromatography (HPLC) *(Tian et al., 2018)*. Also, among these methods, immunoassays such as RIA and ELISA are the most used for quantifying hormones in biological samples (Nouri *et al*., 2020). Both methods use similar principles for quantifying hormones and bioanalytical methods, in which the

Table 2. Hormonal biomarkers for assessing welfare in laying hens

Hormone biomarkers	General responses	Measure methods	Samples
Glucocorticoids (*e.g.*, corticosterone)	Corticosterone levels increase in response to severe physiological or psychological stressors but return to baseline or decrease with prolonged exposure to these stressors (Babington *et al.*, 2024)	Commercially available ELISA (Enzo Life Sciences Inc).	Feathers (Häffelin *et al.*, 2020)
		Radioimmunoassay technique kit (AA-13F1, Biotech-IgG, Copenhagen, Denmark).	Egg white and yolk (Royo *et al.*, 2008)
		Immunoassays.	Droppings (Alm *et al.*, 2014)
Prolactin	Prolactin levels may decrease in response to acute stressors (Schmid *et al.*, 2011).	Radioimmunoassay technique.	Plasma and pituitary tissues (Talbot & Sharp, 1994)
Estrogen	Estrogen levels decline in response to stressors (Wang *et al.*, 2017).	Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS).	Blood or plasma (Prokai-Tatrai *et al.*, 2010)
Progesterone	Progesterone levels decrease under heat-stress conditions (Anjum *et al.*, 2016).	ELISA Quantitative Diagnostic Kit for estradiol or progesterone (North Institute of Biological Technology, Beijing, China).	Follicular granulosa cells (Yan *et al.*, 2022)
Luteinizing hormone (LH)	Luteinizing hormone (LH) is downregulated in response to stressful circumstances; it may initially increase due to immediate exposure to stress-inducing stimuli but decline with prolonged exposure (Babington *et al.*, 2024)	Enzyme-Linked Immunosorbent Assay (ELISA)	Egg (Prastiya *et al.*, 2022)

Table 3. Technologies for measuring hormonal biomarkers in laying hens

Methodologies	Principles	Hormones measured	Advantages	Disadvantages	References
ELISA	Antibody-antigen binding detected by enzyme-substrate reaction	Corticosterone, Estradiol, Progesterone	• Exhibits high sensitivity and specificity, user-friendly, cost-effective, and delivers rapid results. • Provides a non-invasive indicator of physiological status. • Suitable for analyzing feather puddles from laying hens.	• Possesses a limited dynamic range, potential for cross-reactivity, and requires calibration. • Hormonal values vary between different types of feathers and processing methods.	Häffelin *et al.* (2020); Häffelin *et al.* (2021)
RIA	Radioactive labeling of antigen or antibody	Corticosterone, Estradiol, Progesterone	• Demonstrates high sensitivity and specificity, established methodology, and quick results. • Facilitates accurate and precise measurement of hormonal levels in feathers.	• Limited dynamic range involves handling of radioisotopes and is costly. • Lack of standardized procedures for feather analysis. • Requires species-specific validation before application.	Alm *et al.* (2014); Häffelin *et al.* (2020)
GC-MS	Separation of compounds by gas chromatography followed by mass spectrometry	Estradiol, Testosterone, Progesterone	• Offers high sensitivity and specificity, high-throughput analysis, and precise quantification of hormonal metabolites. • Provides measurements of hormone levels in eggs from laying hens, a possible indicator of stress in laying hens. • Enables accurate assessment of corticosterone content in eggs, indicating a possible stress level in laying hens.	• Requires specialized equipment and expertise, incurs high costs, and has potential for false positives/negatives. • GC-MS sensitivity to sample handling, such as freeze-thaw cycles. • Requires meticulous sample handling to ensure precision and repeatability of measurements.	Sas *et al.* (2006)
LC-MS/MS	Separation of compounds by liquid chromatography followed by mass	Corticosterone, Testosterone	• High specificity, capable of simultaneously measuring multiple hormones. • Identifies and quantifies cortisol and its metabolites in various samples. • Allows evaluation of the effects of dietary supplementation on hormonal levels.	• Expensive and requires technical expertise. • Affordability for smaller laboratories varies.	Field (2013); Stanczyk & Clarke (2010)

Table 4. Omics for measuring welfare in laying hens.

Omics techniques	Principles	Biomarkers measured	Advantages	Disadvantages	References
Genomics (Whole Genome Sequencing (WGS), Genotyping Arrays)	Study of the complete set of DNA, including all of its genes.	Genetic variants, SNPs, CNVs	Comprehensive genetic information, identification of genetic predispositions	High cost, extensive data sets requiring complex analysis.	
Epigenomics (Bisulfite Sequencing, Chromatin Immunoprecipitation (ChIP)	Analysis of DNA methylation patterns.	DNA methylation status of stress and immune-related genes	• Provides insights into genetic regulation and can elucidate the long-term effects of stress. • Serves as a potential predictive tool for stress and contributes to the enhancement of animal welfare.	• Interpreting complex and expensive data necessitates high-quality DNA. • Presents potential challenges in elucidating the functional implications of DNA methylation changes about general well-being.	Bird (2002); Nery da Silva *et al.* (2021); Zhang *et al.* (2017)
Transcriptomics (RNA-Seq, Microarrays)	Analysis of the complete set of RNA transcripts produced by the genome.	Whole transcriptome analysis, stress-related gene expression.	• Exhibits comprehensive coverage, high performance, and exceptional sensitivity. • Facilitates a detailed understanding of the biological processes and pathways of stress response and well-being regulation. • Enables the identification of potential biomarkers associated with well-being.	• Incur significant costs and necessitates considerable expertise in data analysis. • Transcriptomic analysis produces extensive datasets that demand advanced bioinformatics proficiency. • RNA-Seq is highly sensitive to variations in sample handling and processing. • The financial burden associated with RNA-Seq experiments is substantial.	Li *et al.* (2015); Wang & Ma (2019); Wang *et al.* (2009)
Proteomics (Mass Spectrometry (MS), Protein Microarrays)	Identification and quantification of proteins.	Stress proteins, cytokines, and other stress-related proteins.	• Comprehensive in detecting post-translational modifications, with high performance. • Enables the identification and quantification of numerous proteins and the detection of their modifications.	• Significant expenses are incurred, and experience in data analysis and intricate sample preparation is required. • Requires specialized equipment and expertise.	Campbell *et al.* (2022); Mann & Jensen (2003)
Metabolomics (Nuclear Magnetic Resonance (NMR), Mass Spectrometry)	Analysis of metabolites in a biological system.	Metabolic changes associated with stress.	• Delivers functional information with high performance. • Provides a comprehensive overview of the physiological state of laying hens, facilitating the identification of well-being biomarkers. • Contributes to a more holistic understanding of laying hen welfare by complementing other omics approaches, including transcriptomics, DNA methylation analysis, proteomics, and miRNAs.	• Involves substantial costs and demands significant expertise in data analysis and complex sample preparation. • Requires advanced analytical techniques and experience for accurate interpretation.	Alm *et al.* (2014)

reaction of an antigen (analyte) and an antibody is employed to detect and quantify the analyte (Aydin, 2015; Darwish, 2006). However, detecting the antibody-antigen complex differs: ELISA uses enzymes, whereas RIA uses radioisotopes (Hackney, 2018; Klee, 2003). Therefore, because of radioactive isotopes, RIA has been replaced by ELISA kits that allow the quantification of hormones without radioactivity (Kinn Rød *et al*., 2017). For example, RIA has been used to measure corticosterone in hens housed in cages, floor, and organic systems (Pia Franciosini *et al*., 2005), and used ELISA to measure the effect of chronic exposure to high temperatures and ammonia concentrations on reproductive hormones in birds (Li *et al*., 2020a).

However, these types of immunoassays have disadvantages, such as providing data for only one hormone per run and substantial cross-reactivity (Abdel-Khalik *et al*., 2013). Freeze-thaw cycles of samples can significantly decrease corticosterone concentrations from their initial values (Häffelin *et al*., 2020), and differences in sensitivity between kits and techniques can alter the results (Bekhbat *et al*., 2018).

Nevertheless, other methods could be more accurate in measuring hormones. Chromatography is a method characterized by the separation of different molecules in a mixture by the distribution between two phases, called a stationary phase (SP) and a mobile phase (MP) (Coskun, 2016). Gas chromatography-mass spectrometry (GC-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and high-performance liquid chromatography (HPLC) provide good separation, sensitivity, and limit of detection for hormones superior to immunoassays (Chafi & Ballesteros, 2022; McDonald *et al*., 2011a). The HPLC separates analytes according to their distribution between a mobile liquid phase and a stationary solid phase (Hell *et al*., 2014).

Gas chromatography (GC) coupled with mass spectrometry (MS) is commonly used for the identification of potential steroids and metabolites because of its high chromatographic resolution capacity and reproducible ionization efficiency (Niessen, 2001; Stan, 2005). Although GC/MS has better chromatographic resolution than LC-MS/MS, it must overcome problems related to derivatization (Bowden *et al*., 2009). Derivatization is the process of chemically altering an analyte or analytes. Chromatography has been used to determine stress-related hormone levels in broilers, hens, and ducks from serum, feather, egg albumen, and yolk samples under different conditions (Afrouziyeh & Zuidhof, 2022; Caulfield & Padula, 2020; Oluwagbenga *et al*., 2022). The results of LC-MS/MS and ELISA methods for measuring stress-related hormone (corticosterone) concentrations in plasma were highly correlated in broiler breeders (Afrouziyeh & Zuidhof, 2022). Also, GC-MS has been used to detect steroid hormones in eggs despite being involved in a tedious derivatization process (Fritsche *et al*., 1999; Hartmann *et al*., 1998). In contrast, without derivatization, LC-MS/MS has been employed to assess synthetic steroid hormones in egg samples derived from eight standard commercial poultry layer breeds (Li *et al*., 2019). Therefore, despite its capacity for high throughput and potential, LC-MS/MS exhibits several constraints, including sensitivity, specificity, and performance (Adaway *et al*., 2015; Grebe & Singh, 2011; McDonald *et al*., 2011b).

Physiological parameters. On the other hand, heart rate has been used to indicate animal welfare to allow understanding of some responses to the environment and challenges in their environment. It is of interest for research on social behavior, animal cognition, and individual differences (Wascher, 2021). Heart rate and heart rate variability (HR/HRV) are non-invasive techniques that can assess welfare, with potential applications in real-time monitoring of welfare (Kim *et al*., 2021b; Von Borell *et al*., 2007; Wascher, 2021). Wearable bioelectric recording systems have been used successfully to monitor the heart rate and its variability through electrocardiography signals in chickens. The backpack electrocardiography system used in this study may be best suited for application in freely moving poultry (Ahmmed *et al*., 2023), but heart rate is strongly affected by social interactions in a wide range of species and used to mark and quantify individual levels of stress in response to anthropogenic disturbances or environmental challenge (Wascher, 2021) which could generate individual variations in the measurements.

Likewise, respiratory rate has been used to indicate avian stress and health status. However, it is pivotal to detect respiratory rates that are contactless and stress-free in poultry to avoid alterations due to manipulation or external factors. Moreover, with many birds in production systems in commercial conditions, it is unfeasible to detect a reliable respiratory rate truth evaluation with manual measures. Wang *et al*. (2022) compared respiratory rate estimation techniques without the video magnification algorithm (RR-D) and with the video magnification algorithm (RR-D-EVMGS) to improve the detection accuracy of the broiler respiration rates. This technique and the algorithm require further optimization, but it is a promising prospect to bring support for respiratory diseases and stress monitoring.

Animal body temperature, such as respiratory and heart rates, is closely related to the physiological, metabolic, emotional, and welfare status (Giloh *et al*., 2012). Body temperatures respond to external and internal factors and may reflect responses to the environment or some internal challenge of the animals. Therefore, it is an essential indicator for measuring the state of the animal. The body temperature of laying hens can be monitored using different technologies, among them thermal imaging, as the non-invasive method is capable of evaluating the temperature through the energy emitted by the animal's skin surface captured by an image visible to the human eye (Morgado *et al*., 2022). Giloh *et al*. (2012) used infrared thermographic measurement by infrared thermal imaging of skin surface temperature in monitoring the thermal status of chickens in a commercial flock. They concluded that this methodology requires the selection of specific surface sites and correlating their body temperature under various environmental conditions, and found that facial surface temperature is strongly correlated with body temperature, which can provide valuable information regarding their thermal comfort and potential heat stress (Morgado *et al*., 2022). In addition, infrared measurements have shown acclimation to persistent high temperatures, and acclimated birds did not display high concentrations of corticosterone, which highlights their lower stress level (Giloh *et al*., 2012). Assessing welfare is difficult with a single parameter; doing so only by measuring physiological parameters is challenging. It is challenging due to the absence of well-defined physiological standards for each condition. Different rearing conditions, feeds, environments, breeds, densities, genetic lines, and immunity status can interact and cause response variations depending on the conditions.

Environmental parameters. Environmental conditions significantly impact animal welfare, providing the necessary conditions for animals to exhibit their natural behaviors in their natural habitat (Koknaroglu & Akunal, 2013). Critical environmental factors that ensure animal welfare include temperature, relative humidity, air quality, illumination, and noise (González-Salcedo *et al*., 2020; Li *et al*., 2023a). High temperature and humidity generate heat stress in laying hens, affecting their reproductive performance, eggshell quality, and immune function (Mashaly *et al*., 2004; Nardone *et al*., 2010).

Thermographic imaging through infrared thermography (IR) can indirectly assess physiological activity that occurs when animals react to different environmental situations and emotional stimuli by measuring the surface temperatures of specific regions (comb, beak, eye, and head) that are influenced by blood perfusion, tissue thermal conductivity, and metabolic heat generation (Tattersall, 2016; Travain & Valsecchi, 2021; van den Heuvel *et al*., 2022a).

In addition, the detrimental effects of poor air quality, regarding dust and ammonia, on laying hen welfare have been reported (David *et al*., 2015). Equipment to measure multiple parameters of air quality has been created. The portable monitoring unit (PMU) allows the measurement of ammonia (NH_3) and carbon dioxide (CO_2). The iPMU (Intelligent Portable Monitoring Unit) was created and has undergone significant upgrades, including a new data acquisition and control system, wireless data transfer capability, and a new commercial NH_3 electrochemical sensor (Ji *et al*., 2016).

Other potential environmental stressors that cause stress and distress should be routinely monitored. This includes ambient light and noise levels (National Research Council (US) Committee on Recognition and Alleviation of Distress in Laboratory Animals, 2008). Exposing laying hens to levels of continuous noise measured as 80 dBA and 100 dBA caused reductions in their egg-laying rates and caused changes in the rates of abnormal eggs. Continuous noise, increased stress hormone cortisol (Lee *et al*., 2003), and 75 dB sound stimulus caused stress and fear in laying hens. Noise negatively influences their fearfulness, showing increments in the tonic immobility duration (Campo *et al*., 2005). In broilers, noise stimuli of both 80 dB and 100 dB intensities for 10 min significantly elevated plasma corticosterone levels (Chloupek *et al*., 2009). Some noise-related technologies include sound meters and loggers to measure and record decibel levels in hen housing. Then, sensor technologies can obtain objective, continuous, and contactless measures of animal behavioral and physiological welfare indicators.

Health status. Animal health assessment is non-invasive and can be performed through cage-side or pen-side visual observation and/or physical examination of an animal (Cohen & Ho, 2023). Nonetheless, it is crucial to recognize that animal health encompasses more than merely the absence of illnesses and injuries. The Swiss Animal Welfare Act not only focuses on the health of animals but also seeks to safeguard their dignity and overall well-being (Thomann *et al*., 2023). Assessing the health of laying hens for welfare purposes involves the evaluation of health indicators, such as infectious and parasitic diseases, production diseases, physical damage, and mortality (Erensoy *et al*., 2021). New technology, such as computer vision or deep learning models, allows monitoring of the spatial distribution of cage-free hens and some behaviors to indicate a flock's health and welfare (Yang, 2023a; Yang *et al*., 2022a).

Radio Frequency Identification (RFID) is a technology employed to monitor the movement behavior of hens and predict individual health status, such as infections (Welch *et al*., 2023). Likewise, respiratory diseases can be detected by changes in vocalizations and ground throat vocalizations or sneeze detection (Banakar *et al*., 2016; Carpentier *et al*., 2019; Mahdavian *et al*., 2021). The onset of specific viral diseases like Newcastle (Cuan *et al*., 2022), avian influenza (Astill *et al*., 2018; Cuan *et al*., 2020), and infectious bronchitis can be detected by vocalizations. The reactions to vaccines in hens can also be differentiated by acoustic technology (Ginovart-Panisello *et al*., 2024).

Physical damage, such as plumage condition, feather pecking, cannibalism, and injuries, is the most critical factor affecting feather conditions in laying hens (Erensoy *et al*., 2021). Using machine vision (RGB and RGB-D cameras), Lamping *et al*. (2022) assessed plumage conditions on commercial white-laying hen farms from a deep convolutional neural network called ChickenNet (Lamping *et al*., 2022). This system provides a holistic assessment of the plumage by computing a plumage condition score for each hen detected. The best result obtained among all tested configurations was a mean average precision of 98.02% for hen detection. In comparison, 91.83% of the plumage condition scores provide a sufficient basis for automated monitoring of plumage conditions in commercial laying hen farms.

Another technology widely used to measure feather cover quality is IR. This useful tool is not biased by the subjective component and provides higher precision than feather damage scoring (Pichová & Bilčík, 2017). The IR is a tool that can evaluate the changes in the surface temperature, derived from an inflammatory process that would make it possible to objectively determine the depth of the damage to the dermis Zhang *et al*. (2023) demonstrated that the proposed RGB-D-T model based in the deep learning was more efficient than the other three traditional stereo matching algorithms in the detect the region of feather damage and assess the depth of feather damage (Zhang *et al*., 2023). In addition, automated image processing and statistical analysis using optical flows and Markov models for predicting feather damage in laying hens allow the identification of flocks with the probable prevalence of damage and injury later in the lay (Lee *et al*., 2011). Some behaviors of birds reveal health problems in the flock, which are related to diseases such as lameness (de Alencar Nääs *et al*., 2021). Yang *et al*. (2024) used multiple chicken trackers developed using six convolutional neural networks to monitor activity in cage-free chickens, and the results indicate that the average accuracy is between 80% and 94% (Yang *et al*., 2024). This tracker can detect piling and smothering behaviors and footpad problems in cage-free chicken environments. It can be a valuable tool for detecting early problems in the flock in real-time and a handy tool for evaluating multiple welfare parameters.

Finally, evaluating mortality rates and pathological changes in laying hens has been widely used to assess welfare in flocks (Erensoy *et al*., 2021). The flock's health status can be assessed using management-based measures, which are based on records. In this case, the total mortality of the flock is a pivotal indicator at the end of production and allows the welfare of the farm and the production system to be assessed (EFSA AHAW Panel *et al*., 2023).

Preference tests. Preference tests have been used as a tool in the study of animal welfare by establishing animals' preferences for shared resources and enrichments (Fraser & Matthews, 1997) A behavioral preference indicates the outcome when a bird chooses, *e.g.*, between different foraging, nesting, or dustbathing substrates or for perches of different characteristics (EFSA AHAW Panel *et al.*, 2023) under different situations and used as a welfare indicator. Several studies have evaluated animal preferences through choice tests that involve repeated measurements of stimulus choices, such as food items, to understand captive animals' preferences (Lewis *et al.*, 2022; Turner *et al.*, 2023). In laying hens, preference tests remain a valuable tool in welfare assessments, establishing preferences for resources and enrichment environments (Nicol, 2023). However, obtaining a feasible measure of these tests without making inferences about what animals prefer is complex. Moreover, early chick environments, such as the provision of litter and perches, can predict laying hen welfare. In the study conducted by Skånberg *et al.* (2021), Leghorn classic chicks were presented with six different types of litter (crushed straw pellets, hemp shavings, peat, sand, straw, wood shavings) and six different types of perches (narrow or wide forms of rope, flat or round wood) (Skånberg *et al.*, 2021). The study showed that different litter types were preferred for different chicks' behaviors. Dust bathing occurred on sand and peat, but chicks foraged more on wood shavings, hemp shavings, and sand than peat and pellets. The study also found that perch width and shape affected perch use and balance, measured as the likelihood of successful or problematic landings, and suggested that presenting several litter types could better fulfill laying hens' chicks' behavioral needs. Additionally, other preference studies showed a hen's preferences for sunlight-filtering shade cloth shelters about different sunlight wavelengths on the range of commercial free-range laying hens. They showed hens prefer shelters that block more sunlight, especially with high sunlight intensity (Rana *et al.*, 2022). Therefore, preference studies are essential to determine birds' comfort based on their perception and to adjust situations or infrastructure that improve the flock's welfare.

Omics Technologies To Measure Animal Welfare

Utilizing omics methodologies provides a comprehensive strategy for thoroughly examining biological systems by analyzing and assessing enormous amounts of data representing a specific biological system's composition and operational mechanisms within a particular context or level (Dai & Shen, 2022). In animal welfare, omics technologies have the potential to provide novel insights into the general biological understanding of the interactions between various physiological systems that participate in stress resilience, behavior, and production (Kasper *et al.*, 2020). Among the emerging technologies for animal welfare assessment, genomics, epigenomics, transcriptomics, proteomics, and metabolomics have been included because of their ability to comprehensively study biological systems (Suravajhala *et al.*, 2016).

Genomics involves the study of whole genomes, including coding and non-coding components (Nguyen, 2024). Approaches used for genomic research include whole-genome sequencing, whole-exome sequencing, and targeted sequencing to acquire detailed data, as well as the use of bioinformatics tools for genome assembly, annotation, detection of structural variations, and comparative analysis between species (Cammen *et al.*, 2016; Satam *et al.*, 2023). This offers the potential to understand the host genetic factors that influence susceptibility, resistance, and immune responses to infectious diseases, creating an excellent opportunity to enhance the genetic well-being of animals by improving the precision of breeding values for the selection of candidates or related individuals, even in the absence of additional stressors (Brito *et al.*, 2020; Nguyen, 2024).

Genomics has the potential to address a variety of welfare concerns by improving the fitness of the animal for the given environment, which might lead to increased contentment and decreased stress of birds in those

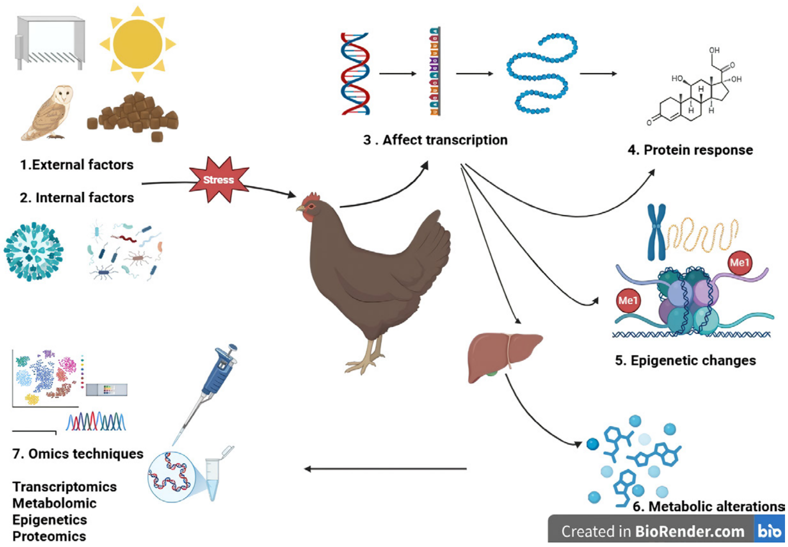

Figure 1. Omics to evaluate the hen´s responses to external and internal factors as a tool to assess animal welfare (Created with BioRender.com)

production environments (Muir *et al*., 2014). Genomic selection is an emerging tool that can be used for effective and rapid selection under different environmental conditions (Budhlakoti *et al*., 2022). In breeding programs for layers, genomic selection can increase the efficiency of breeding programs regarding genetic progress and economic gain by enhancing selection accuracy or shortening the generation interval (Sitzenstock *et al*., 2013). Similarly, Alemu *et al* (2016) indicated that genomic selection for socially affected traits is a promising tool for improving survival time in laying hens with intact beaks (Bahrndorff *et al*., 2016).

Genome-wide association studies (GWAS) are an approach used in genomics that allows the identification of genomic regions associated with groups of individuals with a particular phenotype (*e.g*., diseases, traits, behavioral outcomes) across a population to understand the genetic architecture of the phenotype better (Sitzenstock *et al*., 2013; Uffelmann *et al*., 2021). By GWAS, animal breeding programs can improve animal welfare by contributing to better health care and management by identifying genetic markers associated with desirable traits such as disease resistance, temperament, and physical characteristics, thus allowing selective breeding programs to improve (Baker *et al*., 2019). Lutz *et al*. (2017) used GWAS to identify genetic factors associated with feather pecking and aggressive pecking, discovering that numerous genes with minor effects were responsible for controlling these behaviors; however, no single nucleotide polymorphism (SNP) had a significant impact that justified its use in marker-assisted selection (Lutz *et al*., 2017).

Epigenetics is the study of changes in gene function that are mitotically and/or meiotically heritable, yet potentially reversible, molecular modifications to DNA and chromatin without altering the underlying DNA sequence (Wu & Morris, 2001). Epigenetic mechanisms include but are not limited to DNA methylation/demethylation and hydroxymethylation, histone acetylation/deacetylation, histone phosphorylation/dephosphorylation, noncoding RNA, microRNAs, and transcriptome actions, which play essential roles in modulating genomic function and stability (Ibeagha-Awemu & Yu, 2021; Steiger & Thaler, 2016). These mechanisms function as intermediates between the genome and the environment, regulating various cellular processes and expressing the phenotype (Ibeagha-Awemu & Yu, 2021).

Among the techniques employed in the examination of epigenomics are DNA methylation profiling, chromatin accessibility mapping, histone modification analysis, chromatin conformation analysis, and the merging of DNA methylation profiles with RNA-seq data (Satam *et al*., 2023). Research is being conducted in epigenetics to identify epigenetic markers of long-term stress in production animals (Nery da Silva *et al*., 2021). Epigenetic biomarkers are particularly promising for analyzing animal welfare and other attributes of interest in the animal agriculture industry because they integrate multidimensional context-dependent information. They could be applied to animal health and environmental exposure monitoring, two critical aspects of animal welfare assessments (Whelan *et al*., 2023).

Several studies showed epigenetic changes in hens in different conditions. For example, Pértille *et al*. (2020) used a study of the epigenome through methylome (Pértille *et al*., 2020). They identified stress-associated DNA methylation profiles from male White Leghorn chickens subjected to social isolation compared to controls across different biomes to detect whether a standard stress-related epigenetic profile is a potential, and obtained some candidate genes for stress diagnosis across layer populations of chickens reared in different conditions. Likewise, Guerrero-Bosagna *et al*. (2020) concluded that relative DNA methylation differences in the nidopallium are responsible for the non-genetic factors involved in the emergence of differential behavioral patterns in hens (Guerrero-Bosagna *et al*., 2020).

Transcriptomics refers to the study of the structure, function, and evolution of the 'transcriptome,' *i.e*., the complete set of all the ribonucleic acid (RNA) molecules (called transcripts) expressed in some given entity, such as a cell, tissue, or organism (Skerrett-Byrne Anthony *et al*., 2023). Some of the goals of transcriptomics include cataloging the entirety of transcriptome components, such as mRNAs, ncRNAs, and small RNAs (excluding rRNAs), investigating post-transcriptional modifications, and quantifying fluctuations in transcript expression during developmental stages and diverse conditions (Skerrett-Byrne Anthony *et al*., 2023).

Currently, transcriptome research studies have become a popular methodology due to technological advances and high sensitivity, throughput, and accuracy techniques used to quantify mRNA (Long, 2020; Rodríguez-Hernández *et al*., 2021). Transcriptome research has been studying stress and stress factors due to their capacity to elucidate stress mechanisms and their influence on the production based on a genetic level (Herrera-Sánchez *et al*., 2023; Li *et al*., 2011), which could help to improve animal welfare evaluation (Wang & Ma, 2019). The production systems and stress factors during poultry production can evoke changes in gene transcription related to productivity and metabolism, among others (Chen *et al*., 2021; Herrera-Sánchez *et al*., 2024), and could affect protein synthesis, producing changes in internal and external egg quality (Rodríguez-Hernández *et al*., 2024).

For example, through transcriptome analysis of heat-treated and control layers, it is possible to identify the differentially expressed genes (DEGs) related to the layer's response to stressors and may serve as targets for genetic selection to improve heat tolerance in layers (Wang *et al*., 2021). Another example of transcriptome use includes using the brain transcriptome study using RNA-seq to identify genes and biological pathways responsible for feather pecking (Falker-Gieske *et al*., 2020). In our studies, we have evaluated the transcriptome of caged and cage-free hens, finding statistically significant differences in the hypothalamus (138 DEGs), in liver tissues (209 DEGs) and spleen (19 DEGs), between hens from both egg production systems, in the liver transcriptome of hens housed in the conventional cage versus cage free production system, genes such as *TENM2*, *GRIN2C*, *ACACB*, and *SH3RF2* were identified, which can modulate fat synthesis in the liver, indicating that the production system would produce changes in triglyceride production in birds, demonstrates the influence of the production system and production conditions on genetic regulation under these conditions (Herrera-Sánchez *et al*., 2025).

Proteomics refers to the study of the proteome, *i.e.*, the entire complement of proteins, including different posttranslational modifications (PTMs) expressed by cells or homogeneous tissues at a specific time (Conti & Alessio, 2015). The main proteomic approaches encompass the study of a specific proteome in a cell type or tissue, including information on protein abundance, their variations, and modifications, together with the analysis of protein-protein interactions with partners and networks to understand gene function (Liang *et al*., 2002). Proteomics using biomarkers is a very suitable method in animal breeding for understanding physiological processes and adaptation to environmental conditions, including stress and welfare (Adnane *et al*., 2024).

Some studies have used proteomics to measure the influence of feed components, additives, or environmental or microbial challenges in hens. Ding *et al* (2020) identified by proteomics analysis the effects of tea polyphenol supplementation on the mechanism of albumen quality by regulating the antioxidant activity of proteins that affect egg weight, Haugh Units, albumen height, strength, hardness, gumminess, and chewiness of albumen. Likewise, (Liang *et al*., 2024) found that HSP90, XDH, and POSTN proteins in chicken serum may be optimal biomarkers for detecting heat stress levels in chickens. Also, Shen *et al.* (2021) identified critical proteins in chicken serum that may play a role in follicle development during reproductive phase transitions.

In addition, Kang & Shim (2020) carried out a proteomic analysis of chronic and early heat exposure in one-day-old chicks. They found that acute heat stress caused significant changes in the expression of 97 filtered proteins compared with the control. Early exposure to heat improved the expression of 62 proteins after chickens were subjected to acute heat stress. Zheng *et al*. (2021) challenged broiler chickens with *Escherichia coli* lipopolysaccharide (LPS) and determined that 111 proteins were differentially expressed in the liver of broiler chickens, which triggered alterations in their hepatic proteome. This study provided new insights into the mechanisms by which immune challenge impairs bird growth or productivity.

Metabolomics has emerged as a powerful tool to elucidate biochemical processes and principles in organisms. This technique studies all low molecular weight molecules (metabolites) within a biological sample (cell/tissue/organelle) following a specific cellular process (Færgestad *et al*., 2009; Lee *et al*., 2024). Unlike other "omics" technologies, metabolomics serves as a direct biomarker of biological systems by investigating the changes of metabolites over time after stimulation or perturbation of biological systems, such as mutation of a particular gene or environmental change (Patti *et al*., 2012). Metabolomics, a relatively new field that emerged in response to genetics and proteomics, can illustrate the physiological state of an organism by monitoring changes in endogenous metabolites (Huang *et al*., 2022).

Techniques used in metabolomics, such as Nuclear Magnetic Resonance spectroscopy (NMR), Fourier transform-infrared spectroscopy (FT-IR), and MS coupled with liquid chromatographic separation techniques, including GC-MS, LC-MS, FT-MS, and UPLC-MS, can be used for large-scale metabolomics analysis (Tolani et al., 2021). Among the analytical platforms in metabolomics, GC–MS and LC-MS techniques are the most used (Sun & Xia, 2023). Metabolomics studies have been used to measure health status and hen welfare. Metabolomics identifies metabolic changes in hosts in response to disease. Lee *et al*. (2024) employed a metabolomics approach to explore differentially expressed amino acids and rewired metabolic networks under multiple *Eimeria* species challenges in laying hens.

It has also been shown that restrictive and non-restrictive production systems can affect the metabolism of birds. Yang *et al*. (2022b) showed that restrictive and non-restrictive production systems can affect the metabolism of birds using Jianghan hens reared in caged and cage-free groups, resulting in differences in glycolipid and lipid metabolism and altered levels of intramuscular fat content and other flavor precursors. Pathways such as glycerolipid metabolism, adipocytokine signaling, and metabonomic pathways such as linoleic acid, glycerophospholipid, arginine, proline, and β-alanine metabolism may be responsible for the meat quality and flavor change, and the cage-free system showed a positive effect on the improvement of chicken-muscle-eating quality.

Likewise, animal husbandry can be improved by identifying how metabolic pathways change due to diet, environmental stress, health, and mental state. This will define management strategies to improve animal welfare in food-producing animals (Fabrile *et al*., 2023). Lee *et al*. (2022) investigated the effect of an animal-friendly raising environment on chicken thighs' quality, storage stability, and metabolomic profiles in two different environment-raising systems. They resulted in the differential regulation of metabolic pathways and physicochemical quality, especially in Glycolysis-related products. The results indicated that the animal welfare environment could influence the metabolomic properties of breast thigh meat in broilers, which may affect the sensory quality of meat. Another example is the use of metabolomics to examine the influence of rearing methods (floor and cage) on bone quality parameters in chickens using metabolomics analysis using LC-MS/MS. Li *et al*. (2023b) identified 257 differential metabolites and 15 metabolic pathways responsible for bone quality parameters; these results suggest that the cage-rearing system deteriorates bone quality parameters.

In laying hens, for example, (Huang *et al*., 2022) combined analysis of transcriptomics and metabolomics to identify differential metabolites and genes potentially regulating egg production with correlations and integrated gene-metabolite between two groups of laying hens with high and low egg production, the Ninghai indigenous chicken and Wuliangshan black-boned chicken. Analyses of metabolomics and transcriptomics found the genes that potentially regulate egg production processes, including *P2RX1*, *INHBB*, *VIPR2*, and *FABP3*, as well as the essential ovarian metabolites 17α-hydroxyprogesterone, iloprost, spermidine, and adenosine. They identified two essential metabolite pairs through gene and metabolite association analysis, namely, VIPR2–Spermidine and P2RX1–Spermidine during egg production.

The use of omic sciences allows for a closer approach to biological processes in birds. New studies that involve correlations between transcriptomic and metabolomic data are valuable data that, together with productive parameters and other indicators of welfare, can give a more holistic view of poultry welfare status.

Currently, omics methods are routinely used to identify genes involved in host-pathogen interactions, assess environmental resistance and fitness traits, and pinpoint animals with disease resistance. However, several challenges remain in implementing these technologies, particularly in developing countries, including limited access to advanced tools, high costs of laboratory tests, and the need for continued research on animal welfare in specific contexts to discover new biomarkers. Moreover, research using these technologies requires a holistic and interdisciplinary approach integrating ethology, neuroscience, data analysis, and evolutionary biology to enable a more comprehensive evaluation of animal welfare (Choudhary *et al*., 2024; Neethirajan, 2025).

CONCLUSION

Consumer concern for animal welfare in production worldwide requires establishing precise animal welfare parameters through reliable methodologies and criteria adjusted to the type of production, animal breed/line, short/long-term exposure to a stressor, and environmental conditions. The measurement of animal welfare in the case of production should be as constant as possible since the welfare state is dynamic according to internal or external situations or challenges in production, mainly when environments are not controlled. Although the science of animal welfare is relatively new, it is important to include new technologies used in biomedical sciences, especially omics, which allow a better approach to the evaluation at the molecular and cellular level of the responses of organisms to different environments, internal or external situations, and which, together with other traditional welfare indicators in birds and production parameters, will allow us to understand the physical, physiological and behavioral response of animals and in this case of hens and possibly their feelings.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the author(s) used [PAPERPAL] in order to correct English grammar. After using this tool/service, the author(s) reviewed and edited the content as needed and take full responsibility for the publication’s content.

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