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TAG 'N' TRACK

ENHANCING POULTRY RESEARCH THROUGH RFID TECHNOLOGY: IMPLEMENTATION AND COMPUTER-ASSISTED VALIDATION

Cumulative Dissertation

to obtain the academic degree Doctor of Agricultural Sciences (Dr.-agr.)

at the Faculty of Agricultural and Environmental Sciences of the University of Rostock

by

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À mon père, **Denis Flavien**,

à la mémoire de ma mère, **Christine**,

et à toi, **Josiane**, mon épouse bien-aimée.

Abstract

Radio Frequency Identification (RFID) technology has become an indispensable tool in poultry research, enabling automated, individual-based tracking. However, despite its widespread adoption, a comprehensive, species-specific framework accounting for the unique behavioural and environmental contexts of chickens is lacking. This gap has led to methodological inconsistencies in system deployment, validation, and behavioural inference, ultimately limiting the reliability and comparability of findings. This thesis addresses these shortcomings by establishing standardised protocols, validation tools, and a conceptual framework to advance RFID applications in poultry science through four interconnected papers. Paper I lays the foundation by systematically reviewing RFID applications (2003–2023), highlighting major shortcomings such as frequent misinterpretation of sensor detections as specific behaviours, lack of system validation in 43% of studies, and omission of technical details in 26%. It proposes a multi-phase roadmap for system deployment, validation, and reporting. Building upon these insights, Paper II tackles the interpretational gap, demonstrating that mere presence detection by RFID cannot reliably infer actual resource use, thus highlighting the need for refined data interpretation protocols. Extending this line of inquiry, Paper III builds on the functional zone concept introduced in Paper II and develops a computer vision–assisted validation tool using 3D ArUco markers, achieving over a 95% F1-score and reducing manual annotation time by 82.7%, thereby offering a scalable, reliable alternative to manual validation. Finally, Paper IV integrates methodologies and recommendations from the preceding papers in a practical deployment within a mobile poultry barn. While high tracking performance is achieved for wintergardens, perches, and nest boxes (over 78% F1-score), lower performance for feeders and drinkers (around 64%) highlights the ongoing need for complementary technologies such as computer vision. By linking technical innovation to biological relevance, this thesis enhances RFID methodology for poultry behaviour research and offers a transferable model for broader animal research applications.

Zusammenfassung

Radio Frequency Identification (RFID) hat sich als unverzichtbares Instrument in der Geflügelforschung etabliert und ermöglicht die automatisierte, individuelle Verfolgung von Tieren. Trotz der weiten Verbreitung fehlt jedoch ein umfassendes, artspezifisches Rahmenkonzept, das den besonderen Verhaltensweisen und Umweltanforderungen von Hühnern gerecht wird. Diese Lücke hat zu methodischen Inkonsistenzen in der Systemimplementierung, Validierung und Verhaltensinterpretation geführt, was die Zuverlässigkeit und Vergleichbarkeit der Ergebnisse einschränkt. Diese Dissertation adressiert diese Defizite durch die Entwicklung standardisierter Protokolle, Validierungswerkzeuge und eines konzeptionellen Rahmens zur Weiterentwicklung von RFID-Anwendungen in der Geflügelforschung auf der Grundlage von vier miteinander verbundenen Publikationen. Die erste Publikation bildet die Grundlage durch eine systematische Analyse von RFID-Anwendungen (2003–2023) und identifiziert zentrale Schwächen, wie die häufige Fehlinterpretation von Sensordaten als spezifische Verhaltensweisen, das Fehlen einer Systemvalidierung in 43 % der Studien sowie unzureichende technische Dokumentation in 26 %. Ein mehrstufiger Leitfaden für Systemimplementierung, Validierung und Berichterstattung wird vorgeschlagen. Aufbauend auf diesen Erkenntnissen zeigt die zweite Publikation, dass reine Anwesenheitsdaten mittels RFID nicht zuverlässig auf tatsächliche Ressourcennutzung schließen lassen, und unterstreicht die Notwendigkeit präziserer Interpretationsprotokolle. Die dritte Publikation erweitert dieses Konzept und entwickelt ein computerbasiertes Validierungswerkzeug mit 3D-ArUco-Markern, das eine F1-Genauigkeit von über 95 % erreicht und den manuellen Aufwand um 82,7 % reduziert. Die vierte Publikation überträgt die entwickelten Methoden und Empfehlungen auf einen Praxiseinsatz in einem mobilen Hühnerstall. Während hohe Erkennungsraten für Wintergärten, Sitzstangen und Legenester (über 78 % F1) erzielt werden, zeigten Futter- und Tränkesysteme geringere Werte (ca. 64 %), was den weiteren Bedarf an ergänzenden Technologien wie Computer Vision verdeutlicht. Diese Arbeit verbindet technische Innovation mit biologischer Relevanz und liefert ein übertragbares Modell für die Verhaltensforschung auch anderer Tierarten.

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I. General Introduction

1.1. Introduction to the Thesis

Individual-level tracking of poultry behaviour provides critical insights for welfare assessment, production optimisation, and fundamental research. Monitoring interactions with key resources (e.g. feeders, drinkers, perches) enables early illness detection through behavioural changes (Winckler, 2019; van der Eijk et al., 2022), improves resource management (Zhao et al., 2012; Schrader and Malchow, 2020), and reveals flock dynamics (Gómez et al., 2022).

Traditionally, behavioural monitoring has relied on direct human observation or manual video labelling. While foundational for behavioural science, these manual approaches are time-consuming, labour-intensive, and subject to observer bias and fatigue (Altmann, 1974; Girard and Cohn, 2016; Alarcón-Nieto et al., 2018). Their lack of scalability limits their application in modern, high-density production systems.

Automated tracking technologies, particularly Radio Frequency Identification (RFID), have emerged as practical solutions. RFID's affordability and capacity for non-invasive continuous monitoring have made it a dominant wearable technology in poultry research (Siegford et al., 2016). However, significant limitations persist: the lack of species-specific implementation guidelines, inconsistent validation protocols, and uncritical data interpretation undermine RFID's full potential.

The overall aim of this thesis is to establish a comprehensive framework for RFID-based poultry behaviour research through four research papers: (1) a diagnostic review of current RFID use in poultry research; (2) quantification of data interpretation errors; (3) development of a computer-assisted validation tool; and (4) a practical implementation case study. Together, these papers are intended to provide standardised methodologies for reliable, reproducible RFID applications in poultry science.

The thesis follows a cumulative format. It begins with a general introduction that expands on the background, defines the research scope, details the objectives, and explains how the four papers contribute to achieving the overall aim. It ends with a general discussion, which integrates the findings and reflects on their implications, and an outlook section that proposes directions for future research.

1.2. Background and Context

1.2.1. Key Concepts and Definitions

Animal behaviour. This can be broadly understood as the dynamic interaction between an organism and its environment, ultimately directed toward enhancing survival, welfare, and reproductive fitness. While conceptualisations found in the literature differ slightly in emphasis, they remain complementary and collectively enrich our understanding of behavioural processes.

Tembrock (1992) placed emphasis on the functional role of behaviour, describing it as the organism's continual exchange of information with its environment in order to access essential resources, thereby supporting ecological and reproductive fitness. Levitis et al. (2009) expanded the scope by focusing on the coordination of internal processes, defining behaviour as the internally regulated responses—both active and passive—of whole organisms to internal and external stimuli. Kappeler (2010) brought attention to the behavioural mechanisms themselves, highlighting the control and expression of movements and signals that facilitate interaction with conspecifics and with the physical and social environment.

In applied animal behaviour science, particularly within farm systems, the emphasis shifts toward the functional and welfare-related significance of behaviour. In this context, behaviour is regarded as the observable actions, reactions, and social interactions of animals in response to their environment and human handlers, serving as a practical indicator of their emotional state, health, and ability to adapt to housing, management, and handling conditions (Duncan, 1993; Fraser, 2008; Hemsworth & Coleman, 2010; Grandin, 2014)

Animal welfare concept. Animal welfare is a complex and evolving concept that has been defined in multiple ways across disciplines. This diversity of definitions stems primarily from two key factors. First, its multidimensional nature: animal welfare encompasses physical health, mental well-being, and the ability to express natural behaviours. Different stakeholders—scientists, veterinarians, farmers, policymakers, and the public—may prioritise these dimensions differently, leading to varied interpretations. Second, diverse ethical perspectives also play a significant role. Cultural, societal, and individual beliefs shape how people think animals should be treated, further contributing to the variety of definitions.

Despite this diversity, most frameworks agree on the importance of considering both the physical and mental well-being of animals, ensuring they can engage in natural behaviours and are free from suffering. Moreover, animal behaviour is recognised across all major concepts as a fundamental component for assessing welfare, highlighting the importance of monitoring behaviour objectively and reliably.

One of the earliest and most influential welfare frameworks is the Five Freedoms, originally rooted in the Brambell Report in 1965 and later formalised by the UK's Farm Animal Welfare Council (FAWC) in 1979 (Dawkins, 2021). This framework outlines five basic provisions animals should enjoy:

1. Freedom from Hunger and Thirst – access to fresh water and a nutritious diet.
2. Freedom from Discomfort – appropriate shelter and resting areas.
3. Freedom from Pain, Injury, or Disease – through prevention or timely treatment.
4. Freedom to Express Normal Behaviour – sufficient space and social interactions.
5. Freedom from Fear and Distress – conditions that avoid mental suffering.

These freedoms have been widely adopted as guiding principles in welfare assessment.

Building on these early frameworks, Fraser et al. (1997) proposed a more integrative model that incorporates three core elements:

1. Basic Health and Functioning – animals should be healthy and free from disease or injury.
2. Affective States – the emotional experiences of animals, including pain, distress, and pleasure, must be considered.
3. Natural Living – animals should be able to perform behaviours typical of their species in environments suited to their biological needs.

Broom (1996) defines animal welfare as the state of an animal in its attempt to cope with the environment. This includes both the success or failure of those coping efforts and the feelings—positive or negative—associated with them.

Puppe et al. (2012), while echoing previous definitions, emphasise that beyond defining animal welfare as the state of physical and mental health resulting from behavioural and physiological

adaptation to environmental challenges, it is also essential to consider the individual's cognitive needs, abilities, and experiences.

The World Organisation for Animal Health offers a definition that aligns with many international standards: animal welfare refers to “the physical and mental state of an animal in relation to the conditions in which it lives and dies” (WOAH, 2024).

Finally, Arndt et al. (2022) propose the Dynamic Animal Welfare Concept (DAWCon). According to this view, welfare is achieved when an individual can respond appropriately to its living environment and attain a state it experiences as positive. This definition emphasises adaptability, internal experience, and the importance of promoting positive welfare states—not merely avoiding negative ones.

1.2.2. Key Welfare-Related Behaviours in Poultry

In poultry, certain behaviours can be recognised as particularly relevant for ensuring welfare due to their clear functional significance, internal or external regulatory control mechanisms, and potential consequential effects upon deprivation, which can manifest through frustration, stress responses, rebound effects, or redirected injurious behaviours.

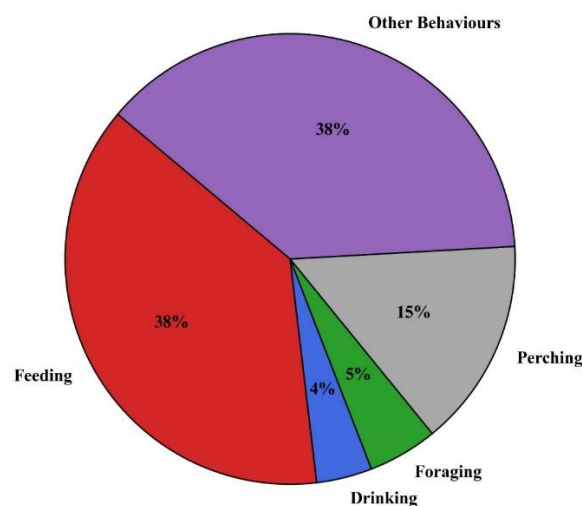


Figure 1: Proportion of Time Spent in Different Activities in a Barn Featuring a Raised, Slatted Area (Adapted from Appleby et al., 2004)

Feeding Behaviour. Feeding is classified as a maintenance behaviour, crucial for sustaining physiological equilibrium and survival. It provides the chicken with essential nutrients and

energy necessary for growth, immune system maintenance, and egg production. For instance, adequate calcium intake contributes significantly to robust eggshell formation and prevents bone disorders, highlighting feeding's physiological importance (An et al., 2016; Alfonso-Carrillo et al., 2021). An optimal diet rich in vitamins, minerals, and amino acids promotes feather development, bone strength, and reproductive success. Feeding is internally regulated, displaying a clear diurnal rhythm controlled by neural and hormonal pathways (Savory, 1980). It represents a major component of the daily time budget in laying hens (Figure 1).

Drinking Behaviour. Drinking behaviour, another maintenance behaviour, directly influences survival by supporting digestion, thermoregulation, and nutrient absorption, thus ensuring overall poultry welfare (Oviedo-Rondón, 2019; Wasti et al., 2020). Drinking behaviour closely aligns with feeding patterns and occupies around 4% of layers's daily activity in open drinking systems (Figure 1). Proper hydration through clean and abundant water sources is therefore fundamental to poultry welfare.

Nesting Behaviour. Nesting is a significant welfare-relevant behaviour, critical due to negative welfare consequences if restricted. Hens display a strong internal motivation to locate suitable nesting sites prior to oviposition, actively performing nesting behaviours up to one hour before laying an egg. The inability to express nesting behaviour leads to clear signs of frustration, aggression, and increased physiological stress markers. Alm et al. (2016) demonstrated this stress response by reporting significant increases in faecal corticosterone metabolites (rising from approximately 550 ng/g to 750 ng/g dry matter) and in heterophil-to-lymphocyte ratios (from 0.11 to 0.15) in hens that were denied access to nests.

Foraging Behaviour. Foraging, which includes litter scratching and scavenging, is a highly motivated natural behaviour predominantly expressed in (semi-)natural environments, where it can occupy 60% of a chicken's active time budget (Dawkins, 1989). In non-cage systems such as straw yards or deep litter setups—hens devote a significant proportion of time (5–25%) to foraging activities (Appleby et al., 2004). Hens have a strong intrinsic motivation to forage. They exhibit contra-free-loading behaviour, actively seeking foraging opportunities even when feed is freely available (Lindqvist and Jensen, 2008; 2009). Gunnarsson et al. (2000) provided strong evidence of this motivation, showing that hens were willing to work—performing between 5 and 200 pecks per access—to obtain access to a straw substrate. Restricting foraging opportunities can redirect hens' motivation toward injurious pecking, resulting in adverse welfare consequences (Wen et al., 2025).

Dustbathing Behaviour. Dustbathing is considered a comfort behaviour playing roles in plumage maintenance, ectoparasite control, feather lipid removal, and plumage insulation (Olsson and Keeling, 2005; Martin and Mullens, 2012; Murillo and Mullens, 2016). It is externally cued, intermittent, and performed opportunistically (Widowski and Duncan, 2000). However, it has lower priority compared to other behaviours (Petherick et al., 1993; Keeling, 1994).

Perching Behaviour. Perching is a key comfort behaviour in chickens with important functional purposes. It originally allowed birds to rest safely off the ground, reducing their vulnerability to predators. This ancestral survival strategy persists as a behavioural vestige, despite generations of domestication and selective breeding. Perching is also important for physical health, contributing to bone strength and overall well-being (Anderson et al., 2024). Additionally, it plays a crucial social role by providing subordinate birds with refuge from dominant conspecifics (Cordiner and Savory, 2001; Wang et al., 2022). Chickens show a strong internal motivation to perch at night, as evidenced by the frustration and stress they experience when access is denied (Olsson and Keeling, 2000), and by their willingness to exert up to 75% of their maximum physical effort to reach elevated perches (Olsson and Keeling, 2002).

1.2.3. Importance of Tracking Welfare-Relevant Behaviours

Understanding and monitoring welfare-relevant behaviours in poultry is essential for informing evaluators, guiding scientific recommendations, and supporting farmers in improving both welfare and productivity. Firstly, observing individual behaviours enables the development of production environments and equipment that align with animals' preferences and needs, thereby enhancing welfare outcomes and profitability. For example, tracking behavioural preferences for perch materials has led to the design of more suitable housing equipment and layout configurations (Zhao et al., 2012; Schrader and Malchow, 2020). Furthermore, such data have directly informed scientific opinions and regulatory guidelines that promote sustainable and welfare-oriented farming practices (e.g., AHAW, 2025; 2023). Secondly, systematic tracking of collective behavioural patterns—particularly around key resources—supports timely environmental adjustments that help prevent productivity losses and protect animal welfare. For instance, spatial tracking of chicken distribution, such as their activity around feeders or drinkers, provides valuable data for optimising resource placement and identifying early signs of welfare risks (Guo et al., 2020; van der Eijk et al., 2022).

1.2.4. Traditional Methods of Behaviour Tracking and Associated Challenges

Traditional methods for tracking poultry behaviour—such as direct observation and video annotation—are foundational to animal welfare research but face critical limitations in reliability, scalability, and precision. One limitation is observer fatigue and subjectivity, which pose significant challenges: manual methods require prolonged attention to video reviews or real-time monitoring, which can lead to mental exhaustion and lapses in focus. Fatigued observers may overlook subtle behaviours (e.g., feather pecking) or inconsistently interpret ambiguous actions, thus introducing bias into datasets (Altmann, 1974; Girard and Cohn, 2016; Alarcón-Nieto et al., 2018). Another limitation is that these approaches lack the capacity for continuous, longitudinal tracking, as analysis typically occurs in short, predefined intervals. This intermittent sampling risks missing gradual behavioural adaptations to environmental changes (e.g., fluctuations in feeder or drinker visits, changes in perch use patterns) or rare events like infrequent access to winter gardens or outdoor ranges, limiting insights into long-term welfare trends (Brereton et al., 2022). A further limitation is that traditional methods often prioritise group-level analysis over individual-level tracking, obscuring variations critical for precise welfare assessments. In practice, resource competition or health issues in particular birds may go undetected, leading to skewed interventions (Winckler, 2019).

1.2.5. From Manual to Automated: Current Trends in Tracking Individual Poultry Location and Movement

As poultry farming systems become increasingly complex and large-scale, there is a growing need for researchers to monitor animal behaviour more accurately and efficiently. Automated tracking technologies enable in-depth analysis of both individual and group behaviours through behavioural sensors and video surveillance systems (Neethirajan et al., 2022). Several such technologies exist, each with its own advantages and limitations (Table 1).

Table 1: Overview of wearable and computer vision technologies for tracking behaviour, location, and movement in avian research

Technology	Category	Description	Applications	Advantages	Limitations	References
YOLO (You Only Look Once)	Computer Vision	Using deep learning to detect multiple objects simultaneously in images or videos, placing bounding boxes around each object to indicate their location and type	Detecting, localising, and counting chickens within crowded environments in images or videos.	Fast and efficient for detecting multiple objects simultaneously, pre-trained models freely available online, capable of real-time detection and tracking, ideal for continuous monitoring, good performance even in crowded environments	Line-of-sight requirement, cannot assign unique IDs when used alone, accuracy depends on image quality, lighting conditions and camera angle, performance may degrade with highly similar-looking animals.	(Neethirajan, 2022; Siriani et al., 2022)
Multi-Object Tracking algorithms (e.g., SORT, BoT-SORT, DeepSORT)	Computer Vision	Predicts positions of tracked objects in each new frame, matches predictions to new detections using spatial proximity and, in some cases, appearance features, and assigns temporary tracking IDs to maintain object continuity throughout the video	Counting, assigning individual IDs, tracking movement, quantifying activity levels within groups in videos	Non-invasive and does not require physical tags, enables continuous, automated behaviour monitoring, allows real-time, continuous monitoring, can be integrated with YOLO or other approaches	No true individual identification, identity switching during occlusion or crowding, requires high-quality, stable video, struggles with visually similar individuals, computationally demanding for real-time use.	(Siriani et al., 2022), Siriani et al., 2023; Yang et al., 2024; Triyanto et al., 2024)
Animal Pose estimation	Computer Vision	Detects and tracks, using machine learning (e.g., Deeplabcut), key points (e.g., joints) and captures motion on an animal's body to estimate its posture, position, and movement.	Gait scoring and lameness detection, estimating posture to assess ability to stand and maintain balance, classifying postures such as standing, sitting, walking, or lying, categorising behaviours like feeding, resting, and preening, monitoring individual activity levels and early signs of health issues	Non-invasive and markerless, detailed behaviour analysis, early detection of welfare issues, high precision and spatial resolution, works in real time or on recorded videos	Requires high-quality video input, computationally intensive, challenging with occlusion or overlapping birds, needs training data for each setup, limited generalization across environments	(Fang et al., 2021; Fodor et al., 2023)
Pixel-Based Tracking	Computer Vision	Monitors pixel changes across video frames by tracking pixel	Monitoring and quantifying general chicken activity as	Non-invasive and markerless, suitable for group-level activity analysis,	Does not track individuals but general flock movement patterns, sensitive to	(De Montis et al., 2023; van

		patterns to estimate motion vectors (direction and speed), detect movement, and provide measures of individual tracking or overall flock activity.	affected by enrichment, and tracking presence around functional areas such as feeders and drinkers	effective for detecting changes in overall movement patterns, useful in real-time monitoring systems, simple setup with standard video cameras	lighting and camera stability, and accuracy may drop in crowded environments.	Leuffen et al., 2023; van der Eijk et al., 2023)
Marker-Based Tracking	Computer Vision	Tracking individuals via visual markers (e.g., ArUco/QR Codes, colored leg bands) attached to birds	Identifying individual hens within group settings, tracking position and movement over time, measuring behavioural phenotypes (e.g., distance moved, proximity to others)	Enables individual identification across time, high accuracy, compatible with standard cameras and software, low-cost and easy to implement, no advanced programming skills needed, open-source markers and algorithms freely available, suitable for longitudinal tracking of specific birds	Requires clear line of sight and stable lighting, sensitive to marker visibility (dirt, damage, detachment), labor-intensive attachment may stress birds, risk of false detection in patterned environments, detects location but not specific behaviours	(Alarcón-Nieto et al., 2018, van Putten et al., 2025)
Thermal Imaging (or Infra Red thermography)	Computer Vision	Detects heat signatures based on temperature differences by capturing infrared radiation emitted from the animal's body.	Assessing behaviour, movement, and physiological responses linked to thermal stress; identifying abnormal temperature patterns associated with illness; counting number of birds in an area (nest boxes); and monitoring nighttime activity and rest patterns in low-light conditions.	Non-invasive and contact-free, works in both light and dark environments (suitable for nighttime monitoring), provides real-time temperature data, useful for early detection of heat stress and illness, can monitor large groups simultaneously	Cannot identify individual animals without additional tools, affected by environmental factors (e.g., dust, humidity, reflections), limited behavioural detail, requires calibration and proper positioning, high-quality thermal cameras can be expensive.	(McCafferty, 2013; Zaninelli et al., 2016; Noh et al., 2021; Cai et al., 2023;)
Passive RFID (Radio-Frequency Identification) and Passive Integrated Transponders (PIT)	Wearable Sensor	Uses electromagnetic fields to wirelessly transmit data between a reader and a tagged object, enabling automatic identification and tracking	Passive tags for logging individual's locations (e.g., feeder area, perch use)	No battery needed (passive tags), individual-based tracking, enables longitudinal monitoring, lightweight and non-invasive for birds, no need for line-of-sight, real-time data collection, effective in in-situ (natural or farm) conditions, durable for long-term use, automated data logging.	Reading distance limited by tag type and frequency, signal interference from metal, water, or dense enclosures, high tag-reader density can cause data collisions, fixed reader placement required for coverage, initial setup cost, potential tag loss or damage in active birds, accuracy depends on tag orientation and positioning	(Turner and Wendl, 2008; Gebhardt-Henrich et al., 2014; Li et al., 2019; Wang et al., 2019)

Ultra-Wideband (UWB)	Wearable Sensor	Sends short radio pulses across a wide frequency range. It measures the time it takes for signals to travel from a tag to multiple anchors. Using this time-of-flight data, the system triangulates the tag's position with centimetre-level accuracy.	Recording individual activity levels, monitoring movement of individuals, determining location within a given area	High-precision location tracking, fast data transmission, low interference with other signals, suitable for dense environments, real-time monitoring, effective indoors and in cluttered spaces, well-suited for dynamic movement tracking, scalable for multiple individuals, and minimally affected by surrounding metal.	Higher power consumption compared to passive systems, requires batteries on tags, more expensive infrastructure, limited range compared to some RFID systems, occasional signal degradation through walls or metal barriers, and more complex setup and calibration.	(Van Der Sluis et al., 2019; Baxter and O'Connell et al., 2023)
Accelerometers	Wearable Sensor	Measures acceleration forces along three axes—horizontal, vertical, and longitudinal. It detects changes in velocity and direction, allowing it to sense movement and orientation in space	Measuring activity levels (frequency, duration, intensity), classifying behaviours (e.g., steps, wing flaps), distinguishing active from inactive periods, estimating distance traveled, detecting changes linked to specific factors (e.g., parasitic mite infestations) and other applications (e.g., Jump detection, landing force)	Profiling activity reliably, collecting data automatically, monitoring in real time, tracking at the individual level, and operating independently of lighting and visibility conditions.	Requiring complex data interpretation, providing limited contextual information (e.g., no location or environmental cues), relying on battery power with limited lifespan, potentially affected by tag placement and bird posture	(Dawson, et al, 2007; Banerjee et al, 2014; Murillo et al., 2020; Yang et al., 2021)
Infrared (IR) Beam Transition Systems	Wearable Sensor	Infrared emitters placed in each zone transmit unique coded signals. Chickens wearing a receiver log the zone-specific signal when they enter the area, recording each transition or presence event with a timestamp.	Measuring zone transitions, duration spent in each zone, movement patterns across zones, individual location over time	Enabling individual-level monitoring, being non-invasive and lightweight, providing reliable zone identification, supporting multi-tier system tracking, allowing automated and continuous data collection, and performing well in low-light or visually obstructed environments	Providing limited spatial resolution, failing to capture detailed behaviours (e.g., interactions, posture, or environmental context), requiring birds to wear battery-powered receivers with limited lifespan and maintenance needs, lacking real-time data transmission (as data is stored locally and retrieved post-study), detecting movement only along a fixed line, unlike RFID systems which	(Rufener et al., 2018)

					can monitor presence across two- or three-dimensional space	
Wireless Sensor Network System	Wearable Sensor	Targets wore battery-powered radio tags that exchanged signals with fixed nodes in the environment. Each tag's response identified its nearest node. Data, collected at a defined rate, was transmitted to a base station, where researchers used GIS software to map locations and movement patterns.	Tracking individual's movement over time, identifying preferred and avoided areas, comparing space use among chickens, measuring spatial behaviour variation, mapping movement patterns using GIS (Geographic Information System)	Providing individual-specific tracking data, allowing automated and continuous data collection, operating wirelessly without line-of-sight or lighting requirements, enabling spatial analysis using GIS (e.g., heatmaps, zone use), offering higher spatial resolution than standard RFID, being less affected by crowding or occlusion compared to computer vision, functioning reliably in real farm environments without cameras	Requiring battery-powered devices with limited lifespan, lacking true real-time tracking due to low sampling rate, involving complex setup with multiple nodes and base stations, requiring technical expertise for data processing and GIS analysis, offering limited spatial accuracy compared to UWB systems, providing no visual or behavioural data like posture or pecking, being more complex and costly than passive RFID	(Daigle et al., 2014)

1.3. Research Problem and Thesis Objectives

Among the various wearable technologies available for tracking behaviour and movement in poultry research, RFID (Radio Frequency Identification) stands out as the most widely adopted in the scientific community (Pereira et al., 2023). This popularity is due not only to advantages such as individualised identification and lightweight, non-invasive devices, but also to the system's adaptability—through adjustable frequencies and sensitivity levels—to diverse experimental and commercial contexts (Table 1). Its rapid development and growing relevance in poultry farming and Precision Livestock Farming (PLF) further reinforce its scientific appeal.

However, significant gap exists in current literature, as researchers are currently left without a clear roadmap for RFID system development in poultry research; no comprehensive framework considers the unique behavioural and environmental particularities of poultry rearing, leaving researchers without clear guidelines on system limitations, deployment, validation, interpretation, and reporting for effective RFID implementation in poultry research.

The overall aim of this study is to develop a comprehensive and standardised framework for RFID-based poultry behaviour research by assessing current practices and designing methodological tools and validation protocols for accurate and reproducible behavioural monitoring.

Specifically, our study seeks to:

- Systematically review the current use of RFID technology in poultry behaviour research, identifying methodological gaps—particularly in deployment, validation, and reporting practices; synthesising existing validation strategies drawn from poultry studies as well as other areas of animal science (e.g., cattle and pigs); and proposing a multi-phase roadmap for improved RFID implementation (Addressed in Paper 1: Alindekon et al., 2023);
- Investigate the interpretational limits of proximity data as a behavioural proxy, by defining functional zones within the poultry barn and by empirically assessing the relationship between the detected presence of individual chickens within these zones and their actual resource use (Addressed in Paper 2: Alindekon et al., 2024);
- Develop and validate an automated, camera-based tool for RFID system verification using 3D ArUco markers to improve the efficiency and replicability of behavioural

validation; and to operationalise the functional zone concept introduced in earlier work (Addressed in Paper 3: Alindekon et al., 2025);

- Evaluate the practical application of the RFID framework and validation tool in a real-world poultry barn by deploying a full RFID setup across key resources, validating system performance using the automated method, and identifying both strengths and remaining limitations across different resource types (Addressed in Paper 4: Alindekon et al., 2025a).

1.4. List of Publications

- Paper 1:** Alindekon, S., T. B. Rodenburg, J. Langbein, B. Puppe, O. Wilmsmeier, and H. Louton. 2023. Setting the stage to tag “n” track: a guideline for implementing, validating and reporting a radio frequency identification system for monitoring resource visit behavior in poultry. *Poultry Science* 102:102799.
- Paper 2:** Alindekon, S., J. Deutsch, J. Langbein, T. B. Rodenburg, B. Puppe, T. Homeier-Bachmann, and H. Louton. 2024. Inferring resource use from functional area presence in a small, single-flock of chickens in a mobile barn. *Poultry Science* 103:104123.
- Paper 3:** Alindekon, S., J. Deutsch, T. B. Rodenburg, J. Langbein, B. Puppe, and H. Louton. 2025. Tag “n” Track: Tackling the validation challenge in animal behaviour studies through automated referencing with ArUco markers. *Computers and Electronics in Agriculture* 229:109812.
- Paper 4:** Alindekon, T. B. Rodenburg, J. Langbein, B. Puppe, B., O. Wilmsmeier, O., Wille, and H. Louton. 2025. Development and Computer-Assisted Validation of a Radio Frequency Identification System for Tracking Individual Chicken Visits to Functional Areas. *Poultry Science* 104:105627.

1.5. Publications Overview & Their Interconnections

This cumulative thesis includes four interconnected papers that collectively aim to provide a comprehensive roadmap for advancing RFID technology in poultry behaviour research (Figure 2).

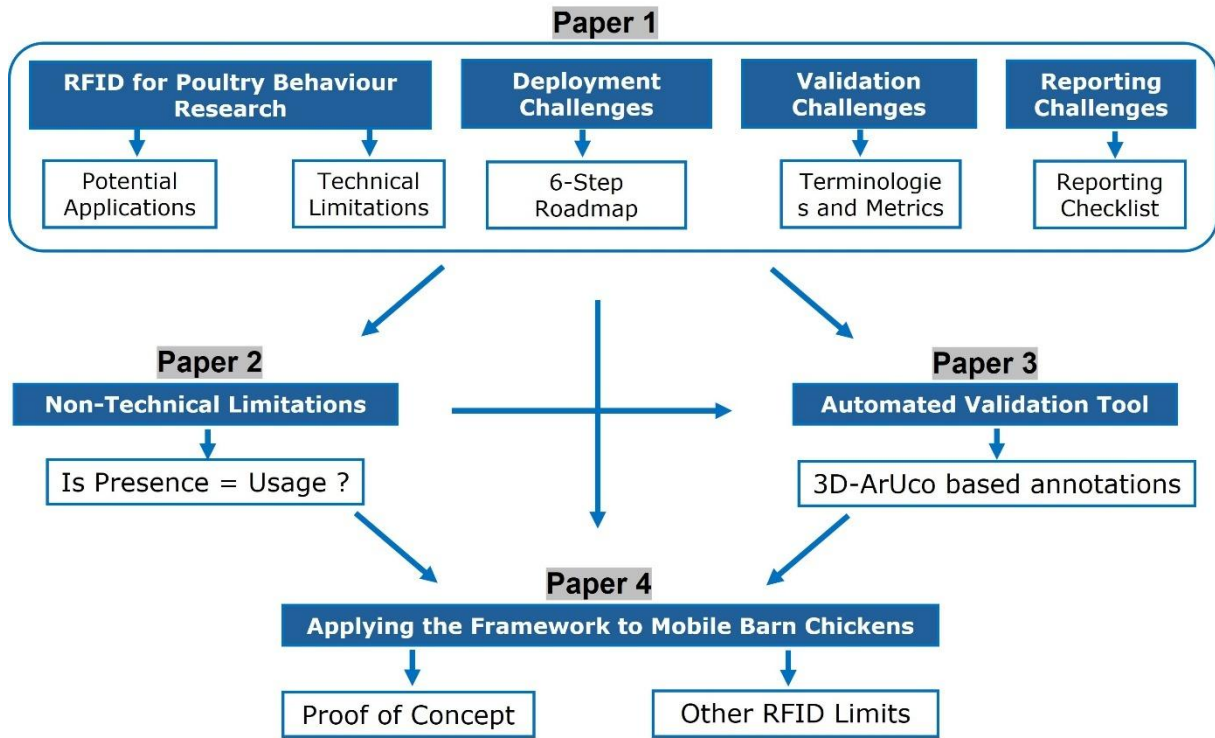


Figure 2: Interlink between thesis papers

Paper 1 presents a systematic review of the literature, revealing the major gaps in the current understanding of RFID deployment for poultry research. It highlights the need for guidelines on deployment, validation, and reporting, and outlines potential technical applications and limitations.

Paper 2 builds on this foundation by addressing non-technical limitations regarding the extent to which RFID captures true behavioural events, given that its fundamental operating principle measures only proximity. This raises significant questions regarding interpretations, as many studies claim to use RFID to measure actual behaviour. Accordingly, the paper empirically identifies and defines functional zones within a mobile poultry barn and investigates the relationship between mere proximity within functional areas and actual resource use for each key resource in a chicken barn.

Paper 3 addresses a major validation challenge underscored in Paper 1, particularly the fact that many studies deploy RFID without proper validation, often due to the labour-intensive nature of the process. To make RFID validation more practical and scalable, Paper 3 introduces an automated camera-based tool designed to support the verification of RFID tracking in areas around key resources. Building on the functional zone concept introduced in Paper 2, the tool uses 3D ArUco markers to accurately detect and annotate the presence of individual animals in these zones, enabling efficient and reliable validation of RFID-based presence data.

Paper 4 serves as a proof of concept by deploying an RFID-based system in a mobile barn setup. It tracks individual laying hens' visits to key resources (e.g., feeders, drinkers, nest boxes, metal perches, and wintergardens) and validates the system using the automated method from Paper 3, demonstrating both the practical utility of RFID in real-world poultry environments and the feasibility of automated validation.

II. Paper 1: Setting the stage to tag “n” track: a guideline for implementing, validating and reporting a radio frequency identification system for monitoring resource visit behavior in poultry

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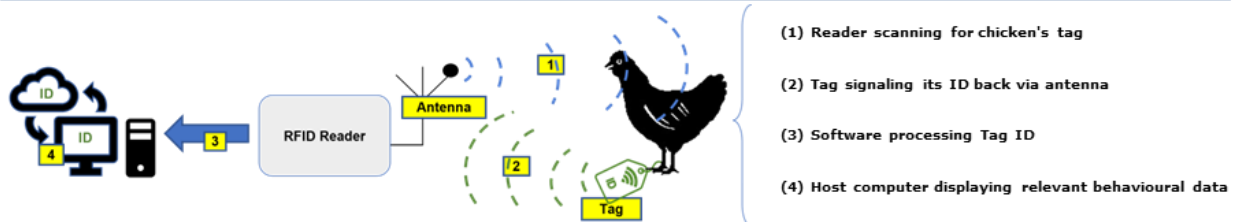
DOI: <https://doi.org/10.1016/j.psj.2023.102799>

Submitted February 11, 2023 / Accepted May 17, 2023 / Published: May 22, 2023

Graphical Abstract Paper 1

RFID SYSTEM FOR POULTRY BEHAVIOUR RESEARCH *How to set the scene to "Tag 'n' Track"?*

RFID Functioning



RFID Capabilities

Tracking Features

- Automated
- Individual-focused
- Longitudinal
- *in situ*
- Non-invasive

Investigation

- Behaviour patterns
- Functional area visit
- Mobility and Activity
- Social association
- Welfare

Behavioural Estimates

- Timestamps
- Duration
- Frequency
- Nbr. of visits
- Nbr. of visiting animals

Trackable Behaviours

- Drinking
- Feeding
- Nesting
- Perching
- Outdoor ranging

➤ However, there are no clear guidelines for applying RFID for poultry behaviour research, despite its capabilities

Literature Search and Evaluation

- Identification of Relevant RFID Systems and Reports
- Evaluation of Identified Systems

Development of a Guideline

- Integration and Consolidation of Identified Reports
- Development of a Guideline



➤ Validated?



➤ Reported Tech Aspects?



➤ Inconsistencies!



Conclusion:

- The proposed roadmap is a much-needed reference for animal behaviour scientists, RFID system integrators and equipment manufacturers.
- It will enable researchers to fully exploit the capabilities of RFID in poultry behaviour science.

Setting the stage to tag “n” track: a guideline for implementing, validating and reporting a radio frequency identification system for monitoring resource visit behavior in poultry

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ABSTRACT Passive radio frequency identification (RFID) can advance poultry behavior research by enabling automated, individualized, longitudinal, in situ, and noninvasive monitoring; these features can usefully extend traditional approaches to animal behavior monitoring. Furthermore, since the technology can provide insight into the visiting patterns of tagged animals at functional resources (e.g., feeders), it can be used to investigate individuals’ welfare, social position, and decision-making. However, the lack of guidelines that would facilitate implementing an RFID system for such investigations, describing it, and establishing its validity undermines this technology’s potential for advancing poultry science. This paper aims to fill this gap by 1) providing a nontechnical overview of how RFID functions; 2) providing an overview of the practical applications of RFID technology in poultry

sciences; 3) suggesting a roadmap for implementing an RFID system in poultry behavior research; 4) reviewing how validation studies of RFID systems have been done in farm animal behavior research, with a focus on terminologies and procedures for quantifying reliability and validity; and 5) suggesting a way to report on an RFID system deployed for animal behavior monitoring. This guideline is aimed mainly at animal scientists, RFID component manufacturers, and system integrators who wish to deploy RFID system as an automated tool for monitoring poultry behavior for research purposes. For such a particular application, it can complement indications in classic general standards (e.g., ISO/IEC 18000-63) and provide ideas for setting up, testing, and validating an RFID system and a standard for reporting on its adequacy and technical aspects.

Key words: poultry behavior, automated tracking, RFID installation, validation

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INTRODUCTION

Radio frequency identification (RFID) refers to an automatic identification technology and its technical infrastructure. Well-known examples of Auto-ID technologies include the barcode system and smart cards, but unlike these, RFID does not require human intervention, line-of-sight or direct physical contact with the target item to sense, identify, and track it; RFID uses electromagnetic waves to function (Bolic et al., 2010;

Finkenzeller, 2010). There are 2 main categories of RFID: active and passive. For some practical reasons (e.g., smaller size, no embedded batteries), passive RFID has mainly found increased applications in animal research. In animal behavior studies, targets for which passive RFIDs have been used as a tracking tool range from large animals, such as cows (Adrion et al., 2020), to tiny insects, such as bees (Streit et al., 2003).

Passive RFID can contribute to advancing poultry behavior studies in various ways. One is the automated and individual-based monitoring of how animals visit resources. For example, each bird can be equipped with a wearable RFID tag that automatically records the timestamp of each visit to the resource of interest with 92.5% accuracy (Li et al., 2019). Another contribution is the possibility of remote, noninvasive, in situ behavioral

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observation. Without being in sight and manipulating the birds or their environment, the behavior scientist can track them with RFID. Also, the wearable RFID device is typically lightweight (e.g., less than a gram) and as small as a grain of rice (Finkenzeller, 2010; Bleemel et al., 2019); as a result, it does not interfere with the behavior or physical health of the birds (Buijs et al., 2018; Stadig et al., 2018). In addition, RFID can be deployed on poultry farms (e.g., Gebhardt-Henrich et al., 2014b), allowing the study of bird behavior under commercially relevant conditions. A final contribution of this technology is that it makes continuous monitoring of animals practicable over an extended period. While assessing behavior based on video recordings can be time-consuming, and the human eye can only provide a snapshot (Pluym et al., 2013), RFID technology allows for automated, continuous, and longitudinal recording of resource visit behavior. For example, RFID technology continuously monitored outdoor range visits in poultry over 13 d (Hartcher et al., 2016) or over 53 wk (Kolakshyapati et al., 2020).

However, one can note 2 major bottlenecks that undermine the potential and how RFID technology can advance poultry science. The first is insufficiencies in reports on the validity and technical aspects of the RFID systems deployed in some poultry research studies. The second is inconsistencies in how the validity of deployed RFID systems has been assessed, interpreted, and reported among studies. These inconsistencies, notably about the terminologies and formulas used to address the adequacy and performance indicators of the RFID systems, may lead to confusion. In addition, the insufficiencies and inconsistencies in reporting details about implemented RFIDs' design, performance, and validity make it challenging to compare and replicate RFID systems from different studies. These bottlenecks are consequences of the lack of a guideline for implementing and reporting RFID developed for poultry behavior research.

Exclusive reliance on existing conventional technical documents, such as ISO/IEC 15693, ISO/IEC 18000-63, or GS1 Class1Gen2, to implement RFID technology in poultry behavior monitoring will not be sufficient. Indeed, these documents mainly address communication protocols and parameters for RFID tags and readers at specific operating frequencies (GS1, 2009; ISO/IEC, 2013, 2017), without considering the specific challenges and requirements related to the application of this technology in animal behavior studies. These reference documents do not address, for example, the potential impact of RFID tags on animal well-being, such as tag weight; or interference from the animal body, which is fluid-filled. They also do not consider issues related to unpredictable omnidirectional animal postures, high tag density in confined spaces, or the possibility of animals blocking or obscuring each other's signals. Likewise, these references do not address the potential damage to RFID installations caused by animal activity with beaks, claws or teeth, nor the inherent complexities of the farming environment, including metal structures, 3-

dimensional structures, and water. Furthermore, these technical documents are aimed more at RFID technology specialists than at animal behavior scientists, due to their technicality, jargon, and content. A guidance document that complements the existing technical documents is essential for the effective application of RFID technology to poultry behavior monitoring. Such an additional document should address the challenges and requirements of this application, taking into account the specifics of the animals' behavior, welfare, and environment. In this way, it will provide a more comprehensive framework for animal scientists and RFID developers to ensure the effective application of the technology in the study of poultry behavior.

In the present paper, we propose a guideline that includes the important considerations for installing, testing, validating, and reporting on a passive RFID system for monitoring poultry behavior. The paper is structured into 5 main parts. The first intends to provide nonspecialists with an understanding of the passive RFID technology's classes, functioning, and components. The second highlights the potential of this technology to advance the study of poultry behavior. The third describes a process for designing and implementing a passive RFID system for monitoring poultry behavior; this process is organized in phases, each broken down into successive steps to develop, install, test, and deploy the system. The fourth part reviews validation studies on the RFID system for monitoring animal behavior. The last part addresses essential points to report on the technical aspects and validity of a deployed RFID system.

A 4-STEP METHODOLOGY TO REVIEW EXISTING KNOWLEDGE ON RFID SYSTEMS AND DEVELOP A GUIDELINE

This paper intends to provide a broad nonspecialist audience with a comprehensive guideline for installing, testing, validating, and reporting passive RFID systems for monitoring poultry behavior. The literature-reviewing approach adopted to develop the guideline is integrative. The reviewing process was not intended to be critical of existing publications on the topic, nor to provide measurements or in-depth analyses of individual studies; instead, it served to distill important findings and synthesize best practices and valuable information from each of the relevant publications in order to draw relevant conclusions to inform the development of the guideline. The methodology adopted to write this guideline and review paper involved 4 steps: 1) Literature search and filtering; 2) Identification of the relevant RFID systems, 3) Retrieval of relevant reports on the identified RFID systems, 4) Integration and consolidation of extracted information (Figure 1).

Step 1: Literature Search and Filtering. The first step in our methodology was to identify relevant literature on RFID in poultry science. This step allowed us to understand the current state of RFID adoption in the

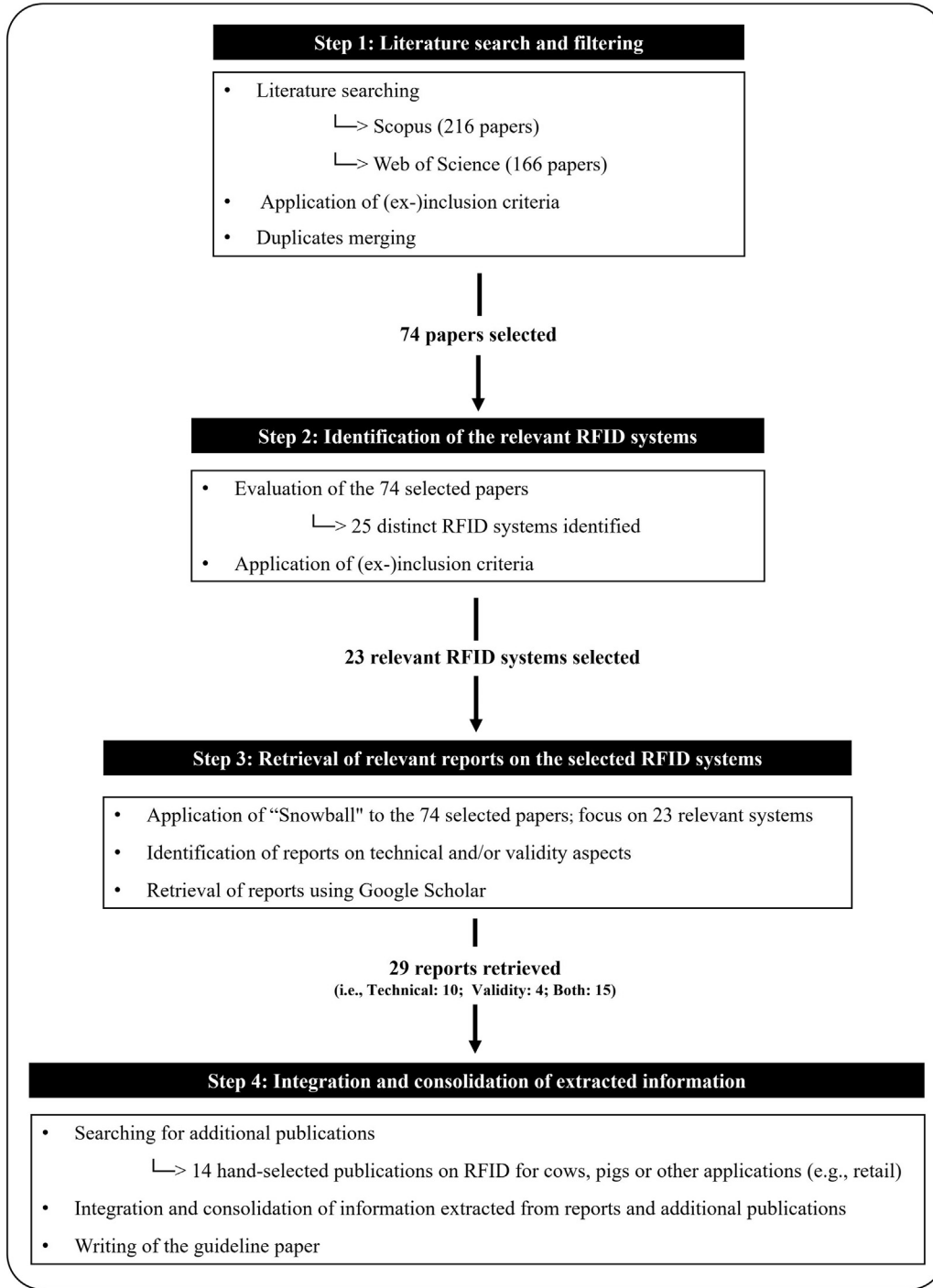


Figure 1. Flowchart of the methodology adopted for the development and writing of a guideline to facilitate the implementation, validation, and reporting of an RFID system deployed for monitoring resource visit behavior in poultry.

field and to identify a set of relevant papers that will provide a foundation and inform the subsequent steps of the methodology.

To this end, we performed a systematic search in Web of Science and Scopus on 19.11.2022; the search query was built as follows: Subject = (RFID OR "radio frequency identification") AND (poultry OR chick* OR bird OR hen OR broiler); search = "All fields." The search resulted in 166 papers in Web of Science and 216 in Scopus. Of these, only selected original papers were derived from research that used RFID for:

- recording activity in poultry;
- measuring behaviors relevant to physiological functioning, production, and health in poultry. Examples of such welfare-relevant behavior are: drinking, feeding, perching, nesting, and ranging;
- investigating the factors influencing the use pattern of resources relevant to welfare in poultry, and
- complementing other technologies for monitoring the behavior of farm birds for research purposes.

On the other hand, we excluded papers derived from research in which RFID was used on poultry carcasses,

for slaughter and transport operations, as a mere identification tool for routine animal record keeping, and on wild birds. After filtering, the results were merged; duplicates were removed, and 74 relevant papers were ultimately selected.

Step 2: Identification of the Relevant RFID Systems. The second step in our methodology consisted of cataloging the popular distinct RFID systems installed across various poultry research units worldwide. This step was crucial to ensure the guideline would be based on the most relevant systems from the existing literature.

To this end, we first evaluated the 74 selected papers to identify the different RFID systems they mentioned. It is important to highlight that several of these evaluated 74 papers mentioned the same RFID system. Initially, a total of 25 distinct RFID systems were identified. However, upon further scrutiny, 2 of these systems were disregarded because their validity or technical aspects were addressed by referencing publications about RFID systems deployed in a different environment and for a different purpose. A final count of 23 relevant RFID systems was obtained.

Step 3: Retrieval of Relevant Reports on the Identified RFID Systems. The third step of our methodology was to identify and retrieve relevant reports to evaluate the technical and validity aspects of the 23 RFID systems identified in Step 2. It should be noted that the process of evaluating these reports was a key component of our methodological approach: it provided us with the insight to 1) understand how the authors addressed the main technical and validation aspects of the RFID systems, 2) assess the consistency and clarity of the terminologies and calculation formulas used for the assessment of the performance of the RFID systems deployed, 3) decide which relevant elements should be included in the guideline document, and 4) inform how to structure the document.

Identifying and retrieving relevant reports were done in an atypical way. We used the "snowball" approach, as suggested by Wohlin (2014), which we applied to the 74 papers selected in Step 1; the approach consisted of following the citations and references in those papers along the thread to identify reports of interest related to different RFID systems. Since many of these reports were conference contributions absent in Scopus and Web of Science, we used Google Scholar to retrieve them. To be included in our selection, they had to address either (or both) the technical aspects or validation information of one of the 23 RFID systems selected in Step 2. We considered that the technical aspects of RFID were addressed by a report when it dealt with the system operating parameters, architecture, infrastructure and outcomes of the optimization tests. Similarly, we considered that information on the validation of RFID is provided by a report when it addressed the measurement of performance indicators such as precision, accuracy, reliability, specificity, validity, sensitivity, concordance or agreement, percentage of exact matches, or any other means of validation. In total, 29 reports addressing the technical aspects and validity of the 23 selected RFID systems were

identified and retrieved: 4 dealt with validity only, 10 with technical aspects, and 15 with both.

Step 4: Integration and Consolidation of Extracted Information. The fourth step of our methodology was to integrate the variously collected reports on the technical and validation aspects of RFID systems in poultry science and consolidate them with additional relevant resources.

In this step, we analyzed the 29 reports identified in Step 3. From these, we extracted valuable insights to inform our recommendations, thus providing readers with a detailed guideline specifically designed to facilitate RFID adoption in poultry science. For example, tables in our proposed guideline present several ways tags have been fitted to chicken bodies and provide some recommendable examples of how to present technical aspects, such as measures taken to ensure the proper functioning of the deployed system, data management system, and schematics of the adopted RFID architecture.

In addition to the reports on poultry science, we considered 14 publications that addressed RFID systems in other animal species (e.g., pigs and cows) or were developed for other contexts and applications, such as logistics in the retail industry. The additional resources allowed us to consolidate the literature identified on poultry. Indeed, they help us break down and simplify the description of how the RFID system works, develop a comprehensive roadmap for the implementation of the technology, obtain information to develop a roadmap for facilitating RFID implementation in poultry science, and provide a checklist for describing its aspects technical appropriately, and elaborate on how to conduct a validation study on RFID deployed for poultry science. The additional resources were suggested to us by animal scientists with experience in RFID for animal tracking, and RFID specialists; moreover, some of those resources were identified by us; and others originated from the reference lists of the 74 papers selected in Step 1. Here is an example of how these additional resources help consolidate the information extracted from the evaluated reports on RFID deployed in poultry: this guideline paper presents in a table an overview of 17 hand-selected papers on validation studies of RFID systems deployed to monitor animal behavior. These 17 papers, which are not limited to poultry science but also cover RFID in cows and pigs, provide readers with a broader perspective on potentially trackable behaviors, sample sizes, performance indicators, recording durations, and means to consider when planning a validation study of RFID for monitoring animal behavior.

A PROPOSED GUIDELINE FOR THE USE OF RFID SYSTEMS IN POULTRY BEHAVIOR RESEARCH

Understanding the RFID Technology

Classes of Passive RFID. Passive RFID technologies can be classified according to radio frequencies. The

most recognizable ones, used for identification and tracking of animals, are low frequency (**LF**; 125–134 kHz), high frequency (**HF**; 13.56 MHz), and ultra-high frequency (**UHF**; 868–868 or 902–928 MHz, depending on the country). [Brown-Brandl et al. \(2019\)](#) mentioned details on the differences between the passive RFID classes that are particularly relevant for livestock production and research.

Overview of How the Passive RFID System Works. An RFID system consists essentially of readers, antennas, tags, and data processing software. The system’s functioning is orchestrated by the reader, which relies on the antennas. Depending on the settings, the reader will regularly send power to the antennas. The antennas will convert this power into a radio frequency (**RF**) field around the resource, thereby covering the reading zone. As a tagged item enters this reading zone, it activates and modulates the RF (in the case of LF and HF tags) or backscatters a signal (in the case of a UHF tag) with its unique signature. The antenna will pick up that tag-specific digital signature and retransmit it to the reader. Finally, the reader will decode and pass this signature to the software for further processing ([Figure 2](#)). Ultimately, the information in the relevant format (e.g., tag ID, timestamp of resource visit) will be available and stored on the hosting computer ([Bolic et al., 2010](#); [Ebrahimi-Asl et al., 2016](#)).

RFID Components and Their Functionalities. Each component of the RFID system has specific characteristics and tasks complementing those of the other components.

- **Reader.** Also known as the interrogator or transceiver ([van der Togt et al., 2011](#); [Nikitin et al., 2012](#)), the reader is the RFID component through which communications begin and end. It has 4 main tasks. One is to frequently attempt the reading of tags in the area covered by the antenna-generated RF field: it functions as an interrogator. A second task is to supply power through cables to the antennas, thus allowing the passive tags to be inductively powered. A third task is to decode (e.g., analog-to-digital conversion) the tag signal communicated to it via the antennas. Lastly, it transmits the decoded digital information to the host/server or data management system for further processing ([Bolic et al., 2010](#)). The

reader usually has a built-in computer and a graphical user interface. In addition, it has configurable settings that allow the user to:

- configure the operating parameters of the network and antennas,
- define the read range, controllable for each target resource,
- define the transmit power (i.e., the amount of energy required to activate the tags in the desired read range), and
- specify how the tags should be interrogated. The specifications include the recording rate (i.e., the frequency at which an RFID reader can scan tags and record data from them). That rate may be slower if a large number of tags are in the reading zone. Other influencing factors of the recording rate include the tags’ size, their orientation relative to the antenna’s reading direction, the specific RFID protocol being used, and the system’s hardware and software capabilities ([Li et al., 2017, 2019](#); [Adrion et al., 2020](#)).

In addition, a reader can centralize data from several antennas ([Maselyne et al., 2014](#)). It can be fixed or have an integrated antenna and thus be hand-held ([Wadhwa and Lin, 2008](#); [Richards et al., 2011](#)).

- **Antenna.** The main task of the antenna is to enable communication between the reader and the tags that enter the reading zone. To enable this, the antenna first draws energy from the reader to generate an AC voltage RF field across the defined reading zone around the resources. This field allows passive tags, approaching the resources, to receive power and communicate their ID. Finally, the antenna retransmits the signal communicated by the tags to the reader ([Bolic et al., 2010](#)).
- **Tag.** Also known as “transponder” ([van der Togt et al., 2011](#)), the RFID tag is the component that carries the digital information (e.g., the ID number) of each tagged item. Its main task is to communicate this information to the reader as soon as that tagged item enters the reading zone and establishes communication with the reader. In its most basic form, the RFID tag consists of 2 parts: a tiny built-in antenna to

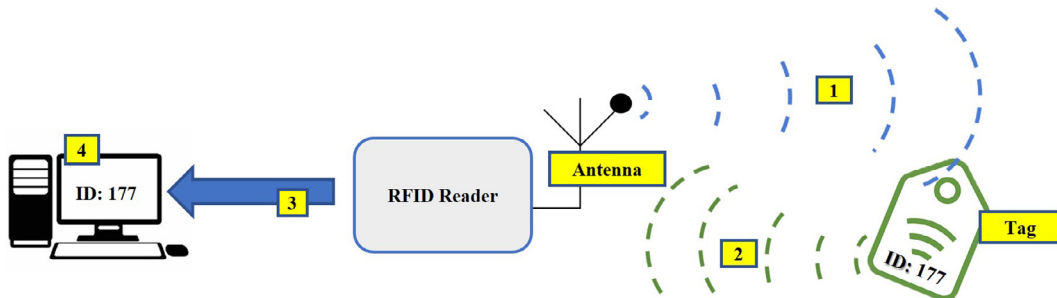


Figure 2. Schematic presentation of the simplified functioning of a passive RFID system (adapted from [Wadhwa and Lin, 2008](#)). In 1) a signal from the reader is transmitted by the antenna in an attempt to register the tags; in 2) a signal from the tag is backscattered as it enters the antenna-generated field in order to communicate its ID; 3) shows the interface with a hosting computer, and in 4) the ID that is decoded by the reader and communicated to the host/server or data management system is shown.

transmit and receive signals and an integrated circuit that stores the tag's unique ID (Bolic et al., 2010).

- **Software.** RFID software is the generic term for the various intelligent components of the system that manage and process data. Depending on the complexity and applications of the system, RFID software can include application software and middleware.
 - RFID application software is the program that uses the data collected by the RFID system for specific purposes, such as animal identification and behavior monitoring. Its functionality can include processing raw RFID data and providing behavioral estimates (Li et al., 2017; Wang et al., 2019).
 - RFID middleware acts as a bridge between the RFID hardware and the application software. Its primary function is to facilitate communication and data exchange between these 2 elements. It facilitates the management and maintenance of the RFID system as it can be used to configure RFID readers, update RFID tags, and monitor system performance (Bolic et al., 2010). It also improves the readability of RFID tags by means of algorithms that can filter out signal noise and mitigate read loss (Jeffery et al., 2006). The middleware's functionality can also be integrated into the reader, at least partly, thanks to increasingly powerful hardware.

Especially for animal behavior monitoring, a software package that includes these 2 components should be considered because of the complexity of the reader's communication system, data management, and the expected behavioral information.

RFID System Deliverable. When a tag enters the reading zone, it signals its presence to the reader at a predefined rate. At each signaling, the reader generates a row of raw data in a database system until the tag moves away. Based on an adaptation from Alfian et al. (2019), Anu (2014), and Wadhwa and Lin (2008), these data would typically include:

- Tag ID: the unique ID of the RFID tag. It is usually a hexa numerical code;
- Antenna ID: the specific ID of each antenna, which covers the reading zone surrounding a given resource;
- Recorded date-time: Timestamp corresponding to the date and time the reader detected the tag. This timestamp can be in UTC format: [YYYY-MM-DD HH:MM:SS];
- RSSI: defined as received signal strength indicator, representing a measure of the strength of the signal backscattered by the tag once in the reading zone. RSSI is high when the tag is very close to the reader's antennas, thus informative about the quality of the signal; it can be used to filter out false reads.

It is common for RFID data on animal presence at a particular resource to contain reading errors (both false and missed readings) or regular discontinuities.

Misreading and discontinuity may be due to interference, noise, and other distortions in the signal that the system could not counter, to some system-specific limitations (e.g., a tag sensitive to rapid and omnidirectional movement, or to the presence of other tags), or to settings (e.g., an interval between readings set to more than 1 s; Maselyne et al., 2016b; Li et al., 2017; Adrion et al., 2020).

To counter these misreading problems, a software-based technique called "tag smoothing" is commonly used. This technique involves making the reader's middleware operate with an algorithm that uses a "threshold." The "thresholds" are specific values determined by the RFID middleware, using the slope of a best-fit line based on a sample of observed data and adaptive window sizes, to best approximate the raw readings to reality. The middleware uses this threshold as a sliding-window to adjust the time frames of the raw RFID readings by interpolating missed readings and filtering out false readings. With this built-in data-cleaning technique, an accurate representation of reality is ensured. The width of the window should be large enough to aggregate lost readings belonging to the same read event, and small enough to segregate readings belonging to different read events (Jeffery et al., 2006). Although the enforcement of this RFID data stream cleaning technique can be integrated into the reader middleware and thus applied at the time of tag reading, many authors deploying RFID for animal behavior monitoring filtered their raw data afterward, for example, at the time of data analysis. These authors have generally referred to that "threshold" for gap-filling, reconstruction and correction of RFID dataset as the "bout criterion"—the maximum duration between 2 registrations considered when analyzing RFID data; it represents the threshold for including 2 successive RFID readings in the same resource visit event (Maselyne et al., 2016b; Li et al., 2017; Adrion et al., 2020). The various methods for calculating the "bout criterion" are reviewed in the session "*Validating an RFID System Deployed to Monitor Poultry Behavior*."

RFID measures "resource visit" but not directly "resource use" unless the technology is combined with a video recording system. This is because RFID can only indicate the presence of a tagged animal in the defined reading zone around a resource; it cannot directly indicate whether the identified animal uses the resource. As Campbell et al. (2017b) noted when tracking pophole use, some hens had jumped onto the pophole without crossing it, yet the RFID system would have identified them as users of the outdoor range unless further filtering had been applied to the data. One must differentiate between "resource visit" and actual "resource use" to understand what RFID can do for animal behavior research.

Estimates of resource visit behavior are one type of behavioral data that can be extracted from raw RFID data. Such extraction is done in several steps: after initial filtering, including the aggregation of successive readings using the defined bout criteria, the corrected raw RFID data can be summarized, on an hourly or daily basis, into numbers of animals using the resource

of interest, into estimates of resource visit such as duration, frequency, and detailed time series (Brown-Brandl et al., 2019). Further behavioral data, such as proxies for movement patterns (Gómez et al., 2022), can also be extracted from the raw RFID data.

Applications of RFID in Poultry Sciences

In poultry sciences, passive RFID systems have been used for various purposes. RFID as a tool for recording general activity levels was demonstrated by van der Sluis et al. (2020). These authors recorded activity levels using RFID technology by placing several antennas under the litter. Each of these antennas, covering part of the floor, detected the tag at a specific location in the barn. Then, the spatial travel distance was calculated using an algorithm based on the temporal sequence of tag registrations by adjacent antennas. As such, RFID can support genetic selection in a variety of ways. For example, Kjaer (2017) used it for the initial phenotyping of 2 chicken lines diverging in activity levels; similarly, van der Sluis et al. (2022) used it to examine the relationship between 2 traits of particular interest for genetic selection in broilers: increased early life activity and body weight change. Ellen et al. (2019) described how RFID could be used for selective breeding against feather pecking in laying hens.

Moreover, RFID has been proposed and proven to measure certain functional behaviors at the individual chicken level. Examples of measurements include resting time, order of arrival at the feeding area, and speed between functional locations (Zhang et al., 2016); frequencies and durations of visits around drinking and feeding areas (Li et al., 2019), in nest boxes (Li et al., 2017), on perches (Wang et al., 2019), and in ranging areas (Gebhardt-Henrich et al., 2014b). For all these applications, the system deployment principle is similar: the tag, attached to or implanted into a body part, is detected by an antenna that covers the surroundings of the visited facility with its magnetic field.

RFID has also been tested as a tool for indicating welfare in poultry. Numerous studies used it to examine how subpopulations with opposing outdoor range visit patterns differed on traditional animal welfare indicators. These studies found that chickens that consistently went out earlier, longer or further from the shed differed from their counterparts in terms of health and performance parameters such as body weight, parasite load, laying rate, scores for keel bone, plumage, comb, and beak (Richards et al., 2012; Hartcher et al., 2016; Bari et al., 2020; Sibanda et al., 2020b,c); or in terms of affective state—as revealed by attentional bias, open field, or tonic immobility tests (Hartcher et al., 2016; Campbell et al., 2019); or regarding physiological parameters such as albumen corticosterone levels after a stressful event (Campbell et al., 2020).

Another use of RFID systems is for investigating factors influencing resource visits in individual chickens. For example, previous studies examined how outdoor

range visits are influenced by animal-related factors like cecal microbiota composition (Bari et al., 2022) and personality traits such as fearfulness (Hartcher et al., 2016), curiosity (Kolakshyapati et al., 2020), or tendency to visit other components of the aviary (Sibanda et al., 2020d). In addition, by studying the range use, the behavioral and social organizations across individuals were explored using RFID. The technology revealed intra- and interindividual behavior patterns in chickens housed at high commercial stocking densities (Larsen et al., 2017; Campbell et al., 2018b). It also showed that chickens form social associations under commercial farming conditions (Gómez et al., 2022). Further, previous studies focused on the influence of husbandry practices such as enrichment during the rearing phase (Campbell et al., 2018a), indoor or outdoor stocking densities (Gebhardt-Henrich et al., 2014b; Campbell et al., 2017a), or provision of insects as supplementary feed (Ruhnke et al., 2018) on range use. Some other studies focused on factors unrelated to animal or farm management. For instance, Taylor et al. (2017) used RFID technology to investigate the influence of the year's season (winter, summer) and daytime (morning, evening) on outdoor range visits. Apart from the outdoor range visit, the influencing factors on the visit to other poultry facilities were also investigated with RFID. For example, Oliveira et al. (2019b) used the technology to examine the influence of feeder space on feeding behavior in laying hens.

Lastly, RFID has been used as a mere identification support tool for other animal behavior or performance monitoring systems. For example, RFID was used in conjunction with an automated system to record real-time feed intake and body weight in turkeys (Tu et al., 2011). Similarly, it was an added component in a machine vision system to identify multiple nest occupations in laying hens (Zaninelli et al., 2018) and other behaviors such as perching, moving, drinking, and feeding (Nakarmi et al., 2014).

To sum up, RFID has been used to investigate temporal components, motor patterns, underlying physiological mechanisms, and affective states associated with many animal behaviors. It has proven to be valuable for poultry science: it allows an in-depth understanding of various functional behaviors and their influencing factors. This understanding is essential to ensure chicken welfare. It can also help to optimize day-to-day farm management and investment (e.g., resources and labor). In addition, it may prompt, where necessary, updates to resource designs and legislation in poultry farming.

Developing and Implementing an RFID System for Poultry Behavior Monitoring

Developing and implementing an operational RFID system for automated monitoring of poultry behavior will involve 4 main phases (Figure 3). The essential tasks to be carried out in each of these phases are described below.

Defining the Tracking Objectives. The definition of tracking objectives, in the early stages of RFID development, is crucial to ensure the relevance and effectiveness of the technology; it guides the development and deployment of an RFID system to meet the study's specific needs effectively (van der Togt et al., 2011). By establishing clear and relevant objectives, researchers can optimize the system's design, select appropriate parameters, and ensure that the data collected is relevant to the analysis of animal behavior. This crucial phase may also help avoid errors or inaccuracies in the results and saves time and resources by avoiding subsequent adjustments. An example of an objective could be measuring the frequency and duration of animal visits to key resources, such as drinkers, feeders, and perches, to study the impact of a given environmental factor on the animals' resource use behavior and welfare. In this case, the RFID system should be designed to record relevant data, such as animal ID and time stamps of visits to the resources of focus. By defining such specific objectives, researchers can ensure that the RFID system is tailored to their needs and can provide valuable information for studying the animals involved.

Analyzing the Tracking Requirements and Conditions. There are mainly standardized RFID packages on the market, and no "one-size-fits-all" tag, reader or antenna works best in every situation (Reyes and Jaska, 2007). Deploying RFID in an animal facility would entail identifying the tracking conditions and requirements, and adjusting the system accordingly. Failing to perform such an analysis might lead to an ineffective RFID system and a waste of resources (van der Togt et al., 2011). Identifying the tracking conditions and requirements will necessitate 2 complementary efforts: on-site assessment and capitalization on the experience of previous studies.

The on-site assessment is the process by which the physical environment is thoroughly investigated. It aims at identifying factors that may influence the performance of the envisioned RFID system. The process involves an on-site assessment, preferably to be carried out by an RFID system specialist (van der Togt et al., 2011). The main concerns are usually materials containing water, metal, or other dielectric surfaces in the vicinity of the antennas and the tags. RFID systems, especially of UHF and HF frequencies, are susceptible to significant malfunctions caused by these materials. In fact, the proximity of water or metal can reduce the read range of a UHF RFID system by up to a third (Bolic et al., 2010). While water molecules absorb microwaves, thus preventing tags from having sufficient energy to be

activated, metals, and dielectric surfaces reflect them in all directions. These reflections can block, distort, multi-path, or attenuate the original RF field. As a result, blind or fading areas are created in the reading zone, causing the signal to be insufficient to power up the tag (Bolic et al., 2010; Zhou et al., 2015). Other potential sources of RFID signal disruptions include fluorescent lighting (Ibrahim and Plytage, 2010), materials containing carbon fibers (Veigt et al., 2020), and devices generating competing frequencies (Bolic et al., 2010); such items can be present in livestock environments.

Practical arrangements can be made to counter some of these concerns. For example, Adrion et al. (2020) used plastic to keep UHF RFID antennas away from the concrete wall. This arrangement allowed for an excellent reading performance of their system. In addition, taking advantage of recent developments in the field of RFID can help control these factors. Most recently, special tags and antennas capable of withstanding the influence of metal and water have been proposed (e.g., Ma et al., 2020).

The outcomes of the on-site analysis may consist of a comprehensive list of physical components incompatible with RFID, and a floor plan that locates the components along with the RFID infrastructure and the flow of tagged items. These outcomes may suggest modifying the environment as they highlight physical constraints to installing the RFID equipment (van der Togt et al., 2011). In addition, this analysis allows important purchasing decisions, such as equipment selection and understanding how to install the equipment considering the identified influencing factors and the tracking needs (Reyes and Jaska, 2007).

Capitalizing on previous studies' experience can also help identify some general requirements for implementing an effective RFID system for tracking poultry. Previous studies have reported some difficulties and ways to address them. For example, using orientation-sensitive tags to monitor poultry can be problematic. In the experiment of van der Sluis et al. (2020), where the antennas were embedded under the litter, and the tag attached along the vertical axis of the tarsus, the RFID system worked best when the birds were standing (i.e., tags were perpendicular to the antennas), but when the birds were lying down, the tags were often not detected. Tags oriented perpendicular to the antennas also offered the best performance in Li et al. (2017) and Wang et al. (2019). However, this is not always the case: in Sales et al. (2015), for example, the RFID worked best when tags were parallel to the antennas. Using circularly polarized UHF antennas could help prevent this

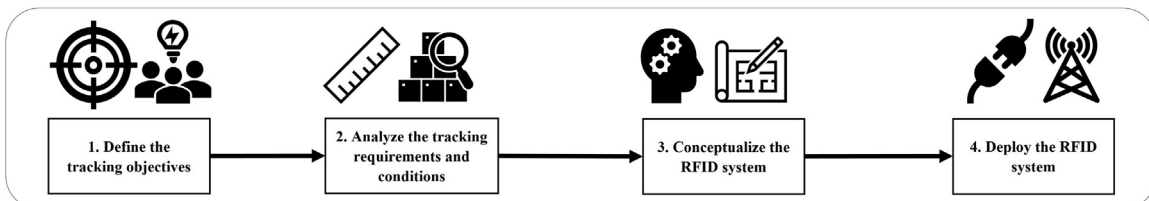


Figure 3. A 4-phase roadmap for developing and implementing an RFID system for poultry behavior monitoring.

problem, as these antennas can pick up the signal emitted by the tags in any direction (Parthiban, 2019). With such measures, the UHF RFID system deployed by Adrion et al. (2018) captured the signal despite the pigs' omnidirectional movements and body postures.

Second, the velocity of tagged animals can be a major limiting factor. For example, in Gebhardt-Henrich et al. (2014a) and Campbell et al. (2017b), where LF RFID systems were deployed to monitor the access to an outdoor range, the detection rate dropped below 10% when the tag speed exceeded 3.0 m/s and became zero above 9.3 m/s, respectively. This limitation could be overcome by using higher frequencies since UHF can detect tagged items moving at high speed, such as 69 m/s (Bolic et al., 2010).

Third, intertag interference or collisions can affect the performance of RFID systems (Sales et al., 2015). Intertag interference can occur in 2 ways. One is observed in tags that are densely colocated in the reading zone. Under these conditions, the backscattered signal from a given tag can overshadow that of the others. This shadowing effect degrades the read rate, which worsens as the intertag distance decreases (Zhou et al., 2015). The second case is observed when several tags attempt to communicate simultaneously with the reader using the same backscatter channel. In this case, the reader may fail to decipher the tag signals; as a consequence, those tags may go undetected within the reading zone (Bolic et al., 2010). Higher frequencies, such as UHF, readily admit an anticollision protocol (e.g., EPC Gen 2 in dense mode) for mass reading. Adrion et al. (2020) implemented such protocols that optimize communication between readers and tags to avoid collisions between and within these components.

Lastly, the long interval between readings, intrinsic to the LF RFID system, makes them unsuitable for continuous readings. Around resources such as feeders, continuous readings are essential to estimate the visit durations (Adrion et al., 2020). Prolonged intervals between readings with low frequencies would lead to nondetection of animal presence for some time, thus underestimating the resource visit behaviors (Sales et al., 2015). Therefore, when continuous identification is required, HF and UHF RFIDs might be more appropriate as they allow multiple readings in less than a second (Brown-Brandl et al., 2019). On the other hand, when just one registration at the resource is needed to estimate the number of resource visits, any RFID class can be used. In many studies, such an application has been made with LF RFID to identify visits to an outdoor range. For a visit to the outdoor range, only entry and exit readings are of interest (e.g., Gebhardt-Henrich et al., 2014b; Campbell et al., 2017b).

In summary, the on-site assessment can help identify potential influencing factors specific to the environment in which the system will be deployed. These factors may include metal-containing materials and water. The assessment can give an insight into the challenges that will be faced when installing RFID. Capitalizing on previous studies' experience may help identify some general requirements of an RFID system for poultry tracking.

For example, the deployment of this technology to track individual animals housed in groups would require orientation-insensitive tags capable of reading fast-moving items and unaffected by the proximity of other tags. A well-designed RFID system operating at higher frequencies, such as UHF or HF, can provide most of these capabilities. However, the major limitation of these higher frequencies is their vulnerability to interferences from metal and water (Brown-Brandl et al., 2019). Some ways to address this are mentioned above. The requirements analysis will provide the basis for designing the architecture and selecting the components of the envisaged RFID system.

Conceptualizing the System. This crucial phase in implementing RFID technology involves selecting the system infrastructure and designing its architecture. The equipment used in some prominent animal behavior studies deploying RFID has been supplied by a wide variety of manufacturers and distributors, such as Impinj Inc located in Seattle, Washington (Li et al., 2019; Adrion et al., 2020); Alien Technology based in San Jose, California (Toaff-Rosenstein et al., 2017); Gantner Pigeon Systems GmbH located in Schruns, Austria (Gebhardt-Henrich et al., 2014a); Feig Electronic GmbH based in Weilburg, Germany (Thurner et al., 2010; Maselyne et al., 2014); Guangzhou D-Think Technologies Inc located in Guangzhou, China (Wang et al., 2019); TransTech Systems Inc based in Latham, New York (Li et al., 2017, 2019; Oliveira et al., 2019a); Texas Instruments located in Dallas, Texas (Sales et al., 2015); Freaquent Froschelectronics GmbH based in Graz, Styria, Austria and Dorset ID based in Aalten, Gelderland, The Netherlands (van der Sluis et al., 2020). To make an appropriate choice, one can consider each system component's critical technical aspects (Table 1).

Apart from Adrion et al. (2020), hardly any studies have used commercially available software. Specially designed programs for ethological studies that take into account the specifics of animal behavior are not yet available on the market. As a result, most animal behavior monitoring studies with RFID have used customized or self-programmed data collection software. The programming languages or environment mentioned include C# (C Sharp) based on API (Li et al., 2017; Oliveira et al., 2019a), Java (Toaff-Rosenstein et al., 2017), Python (Li et al., 2018; Wang et al., 2019), HT Basic (Sales et al., 2015), and NI Lab-VIEW (Zaninelli et al., 2016). For data management, including processing, programs such as SQL, Excel VBA, or MATLAB were used (Maselyne et al., 2014; Sales et al., 2015; Li et al., 2017, 2018; Oliveira et al., 2019a; Wang et al., 2019). Designing an RFID system for animal behavior research would require transdisciplinary collaboration among the data management program developer, RFID system integrator and animal scientists.

The system architecture defines how the components and subsystems will be networking with each other and where the components will be installed to meet the application needs and tracking conditions. A specialist

Table 1. Relevant key technical characteristics to consider when selecting the main RFID component to develop an RFID system for monitoring poultry behavior.

Components	Important technical aspects	Definition and relevance
Reader	Operating radio frequencies and bandwidth	Operating radio frequencies refer to the specific range of radio frequencies that an RFID system uses for communication between tags and readers. Typically, these are low frequencies (LF), high frequencies (HF), or ultra-high frequencies (UHF). Knowing the operating frequencies is important to ensure compatibility among system components, regarding the intended application, and with (inter-)national regulations (Parthiban, 2019). The bandwidth is the range of frequencies—the difference between the highest and the lowest frequencies—within which the signals will be transmitted. It depends on (inter-)national regulatory directives (Bolic et al., 2010; Parthiban, 2019). For example, the bandwidth of an RFID operating in the European UHF frequencies (865–868 MHz) is 3 MHz, while that deployed in the United States (902–928 MHz) is 26 MHz. The greater the bandwidth, the greater the capacity of transmitting data and switching channels, thus the lower the risk of collision (Bolic et al., 2010).
	Read range max	The hypothetical maximal distance at which a tag can still be powered in an optimal environment. This detail is crucial for choosing a reader that fits the envisaged application and anticipating unwanted reads (Bolic et al., 2010).
	Reader sensitivity	The weakest signal threshold at which a reader can detect a tag (Nikitin et al., 2012). Knowing this beforehand is crucial as it determines the choice of other system components.
	Transmit power max	Represents the maximum power a reader can send out to the antenna. It would be essential to have these details from the manufacturer to anticipate, for example, how to define the read range and compensate for the loss, mainly due to the cables (Bolic et al., 2010; Buffi et al., 2017).
Antenna	Operating radio frequencies	Specific radio frequencies for which the RFID antennas are designed. If not tuned to those frequencies, the antennas cannot retransmit or receive information from the reader or tag. In addition, the antenna frequency must be compatible with the (inter-)national regulations, the application and other system components (Parthiban, 2019).
	Optimal operational conditions	Conditions external to the system, such as ambient temperature and humidity and quality of power supply. Temperature changes, for example, are known to affect the antenna's radiation parameters (Parthiban, 2019).
	Shape and size	The physical appearance of the antenna. Typically, the size correlates positively with the read range, hence critical to the choice of equipment. Physical appearance is notably of practical importance as it determines how to deploy the antenna across the barn and along the locations of interest (Parthiban, 2019). Examples of RFID antenna shapes include free-form cable-like antennas attachable along any support and standard patch antennas (Adrion et al., 2018).
	Antenna gain	Indication of how strong the signal sent or received by the antenna in a given direction would be. As it is correlated with the width and length of the reading area, knowing the gain helps to choose the suitable antenna (Balanis, 2016).
	Mode of operation	Indicates the length and beamwidth of the radiation field generated by the antenna. This characteristic of the antenna is important because it determines the reading range. Typically, the length of the radio field is described as "far-field" or "near-field" (Parthiban, 2019), and the beamwidth is referred to as "narrow," "intermediate," or "wide" or expressed in degrees (Balanis, 2016). A near-field antenna would be appropriate for monitoring the proximity of a given resource (Bolic et al., 2010).
	Directionality	Defines the focusing ability of the antenna's radiation, that is, the direction in which the antenna should transmit and receive the signal (Parthiban, 2019). In standard patch antennas, 2 types of directionalities are common: unidirectional and omnidirectional. While unidirectional antennas concentrate beams and allow reading in one direction (i.e., across a given plan), omnidirectional antennas radiate spherically and thus can be used for reading in any direction (Balanis, 2016). In contrast, cable-like antennas propagate a localized electromagnetic field cylindrically along the cable axis and contour (Buffi et al., 2017). The directionality characteristic is essential for choosing a suitable antenna. For example, a unidirectional antenna would be inadequate for animal tracking, as animals move in every direction; reading failures might occur.
	Polarization options	Polarization applies to the wave pattern generated by the antenna. It defines the geometric structure that characterizes waves' oscillation. In standard patch antennas, 2 polarization options are common: linear and circular. Linearly polarized antennas spread their waves in a single plane, either vertically or horizontally, while circular antennas spread their waves in a circular pattern. There is a further subclassification of circularly polarized antennas depending on whether their waves propagate clockwise or counter-clockwise: right-hand circular polarized and left-hand circular polarized antennas (Parthiban, 2019). These polarization options do not apply to cable-like antennas; in those antennas, the polarization effects can be optimized; for example, by installing them in U-shaped meander ways (Harting, 2015). Polarization is important as it determines how the signal will be affected by structures or objects in its pathway (Parthiban, 2019).

(continued)

Table 1 (*Continued*)

Components	Important technical aspects	Definition and relevance
Tag	Size	Is about the physical dimensions of the tag. The size is important because it correlates with the reading range. Typically, the larger the tag, the longer the reading range (Bolic et al., 2010).
	Weight	Passive RFID tags are generally small and lightweight, often weighing only a few grams or less (Blemel et al., 2019). Using a lightweight tag is crucial for animal tracking as it allows for attaching the tag easily to the animal's body, avoiding harm to the animal's well-being while providing the necessary data on resource visits. Lightweight tags are, for example, convenient for monitoring the activity of broiler chicks throughout the fattening period (van der Sluis et al., 2020).
	Ways of fitting the tag to the animal's body	The tag is fitted to birds in various ways: attached to the ankle (Wang et al., 2019; van der Sluis et al., 2020; Gómez et al., 2022), wing (Tu et al., 2011), neck (Li et al., 2017, 2019a), back (Daigle et al., 2012); or implanted into the feet (Zaninelli et al., 2016), wing, neck or abdominal cavity (Toth et al., 2013). It is important to consider this before purchasing the tag, as how it is fitted to the animal can significantly influence the tracking performance (Zaninelli et al., 2016).
	Operating radio frequencies	Operating radio frequencies are the frequencies (in hertz; Hz) at which a passive RFID tag will operate. Usually, these are LF, HF or UHF. It is important to know the operating frequencies to ensure compatibility with other system components, intended application, and (inter-)national regulations (Bolic et al., 2010).
	Tag range max	Hypothetical maximal range or distance at which a tag can be read in the ideal direction and environment. This detail is crucial for choosing the tag that fits the envisaged application or for anticipating unwanted reads (Bolic et al., 2010; Nikitin et al., 2012).
	Tag sensitivity	Indicates the minimum backscatter signal strength the tag would emit to be identified by a reader (Nikitin et al., 2012). Knowing this detail is important to set the reader's transmit power and reading distance.
	Orientation	Orientation refers to the physical positioning of an antenna, that is, the direction in which it is pointing in order to pick up signals. Here, it applies to the small antenna inside the tag (tag-integrated antenna is not described in the paper). All indications above about antennas also apply here. Some tags can be orientation insensitive, others not. Orientation-insensitive tags pick up the signal in any orientation, making them very suitable for tracking animal behavior, as animal postures and movements can be in any direction.
	Ideal operating conditions	The conditions to be met for the tag to function adequately. These conditions include particularities of the deployment environment such as ambient temperature and humidity ranges, dust levels, frequency of exposure to UV light, presence of metal, water or other tags, and the amount of power supplied by the antenna. Knowing these conditions is necessary to set up a system that meets the user's needs (Parthiban, 2019).
RFID cables	Length and attenuation factor	The length indicates how far the energy sent out by the reader has to travel through the cables to reach the antenna. The attenuation factor is an appreciation of the energy lost per unit of cable measurement; it helps to rate the insulation (Buffi et al., 2017). In particular, the series to which UHF coaxial cables belong, that is, 195, 240, or 400, indicates their insulation ratings. By knowing these 2 characteristics, Adrion et al. (2018, 2020) anticipated how much of the transmit power would be lost to the cables and how much antenna input would ultimately remain.

should carry out the system conceptualization; this guides the deployment of the infrastructure.

Deploying an RFID System. This phase involves 2 steps: installing the equipment and setting up the system.

The installation of an RFID system consists of mounting the equipment, for example, the antenna, reader, and auxiliaries, onto structures across the animal house as planned during the system conceptualization phase. While installing the components, practical arrangements and modifications to the physical environment can be made in order to counteract some potential sources of influence on the performance of the RFID system. Such measures are described in the literature. For example, plastic panels or empty carton boxes have been used to prevent the RFID system from registering animals out of the resources' functional areas (Maselyne et al., 2016a; Li et al., 2019). Likewise, changes can be made to the standard resource dimensions and format to reduce

unwanted registrations (Thurner et al., 2009). In addition, a blocking structure can be introduced to prevent animals using the resource from positioning themselves at points where the electromagnetic signal is weakest within the RFID reading zone (Wang et al., 2019). Plastic pipes (e.g., PVC) can be used to protect antennas from dirt, water, or chewing by animals (Adrion et al., 2018), or to distance the antennas from a wall containing metal (Adrion et al., 2020). Also, advantages can be taken from steel's shielding effect to constrain the antenna's magnetic field, thus restricting the reading range to the functional areas of the resource (Li et al., 2019). Most of the remaining adjustments will be in the component settings if appropriate practical arrangements and environmental changes are made during installation.

Setting up the RFID system entails identifying the ideal operational parameters of each component, fine-tuning and operating the system, under reserve of validation with animals, as intended. It is a meticulous

process as it includes a lot of trial and error, testing, and comparing combinations of different settings on the reader and antennas. The optimal setting gives the best result from the tests and comparisons. Best results are obtained when the read rate is 100%; the read rate being the fraction of the number of times a tag is read over the number of seconds during which the readings are performed (Ramakrishnan and Deavours, 2006) or over the number of requests sent to the tag (Bolic et al., 2010). Some authors, however, are more flexible; for example, in Wang et al. (2019), 3 reads out of 5 were sufficient.

The range map—a map of the read rate across the area of interest—is the ultimate metric for comparing the various combinations of parameters under test. As presented in Wang et al. (2019), this map is used to assess how detections vary across the area of interest. Sales et al. (2015) used a paper reference of over thirty grid nodes spaced 6 cm apart to map the range. First, the reference grid was placed in the desired reading zone, and the tags, attached to plastic support at a distance of 1 or 2 cm from the ground, were oriented perpendicular to the antennas. Then, the tags in such a position were tested from one node to another. At last, the read rates observed at each node were used to map the range. Range mapping can be used to optimize system performance, as it helps to identify possible dead spots in the zone of interest, destructive interference from the surrounding environment and limits of the applied settings.

Parameters that can be adjusted to achieve a desirable reading zone include “transmit power.” This is the amount of power the reader transmits to the antennas, and it correlates with the reading distance. Therefore, changing the transmit power allows the system’s configurator to define the read range of an RFID system around a given resource (Bolic et al., 2010). The optimal transmit power can be identified by testing several transmit powers starting from 0, as the set point, with increments of 0.5 units (Ramakrishnan and Deavours, 2006; Li et al., 2017). An aid to deciding on the best transmit power can be the “power mapper,” an instrument that measures the RF power at various points of the reading zone. This instrument can be informative about the quality and pattern of the RF signal across the reading zone (Xie et al., 2020). Besides the transmit power, the system configurators have control over other parameters, such as digital modulation and bit error rate, to achieve the desired operating range and best results (Bolic et al., 2010). Setting these latter operational parameters might require an RFID specialist’s assistance and the use of technical documents, such as ISO/IEC 15693 and ISO/IEC 18000-63.

Validating an RFID System Deployed to Monitor Poultry Behavior

The system validation involves testing it with animals to examine its deliverables and report on its fitness-for-

purpose: it establishes the reliability and suitability of the RFID system for behavioral monitoring. In other words, the validation study aims to establish the extent to which RFID technology can be accepted as an alternative to the traditional approaches of animal behavior monitoring, such as direct visual observation. Therefore, it would provide valuable evidence and support for adopting the technology.

Before fully adopting the RFID system, the validation study will always be important because each system is unique (Gebhardt-Henrich et al., 2014a). In fact, the read range is highly dependent on the environment and the system components, and there are no standard setting parameters that work for every application. The validation study will be the ultimate step to fine-tune the system setup. For example, in Li et al. (2017) and Adrion et al. (2020), the validation study enabled setting relevant operating standards as it required these researchers to identify technical anomalies and biases in the system outputs, unanticipated system limitations, and critical system elements or operational procedures using animals in the RFID deployment environment.

Approaches of Validating RFID. Various approaches have been used to validate RFID systems for monitoring animal behavior. These approaches may require a comparison of RFID system outputs with external or internal references.

The external reference is often called the “gold standard” or “benchmark.” It should indicate reliably and consistently how the animals use the resource. In behavioral sciences, the most common reference is the human vision which, for practical convenience, involves digital video recordings instead of live observations (reviewed in Table 2). Another external reference may be other automated devices or systems, used in conjunction with the cameras or not, which may indicate how animals use a given resource. For example, in addition to the camera recordings as a reference, Maselyne et al. (2016a) benchmarked their RFID system outputs against water flow meter data for pig drinking behavior; Adrion et al. (2020) used noseband pressure sensor data; van der Sluis et al. (2020) used ultra-wide band system measurements.

Internal reference applies when only information from the same system is used to assess its validity; for example, using the number of entries against the number of exits from the outdoor range (Gebhardt-Henrich et al., 2014a) or using the registrations obtained from a tag attached to 1 leg against those from a tag attached to the second leg of the same chicken (Sibanda et al., 2020a). This approach would be ineffective when 1 or both tags fail to operate. In this respect, the internal 2-tag validation approach might not be as convenient as video validation. Nevertheless, it has the merit of enabling comparison of performance between tags. Moreover, this 2-tag approach can add value to a video-based validation, as shown in Maselyne et al. (2016a).

Indicators of Reliability. The reliability of an instrument is a major indicator of its adequacy. It indicates how repeatable and consistent the measurements

Table 2. Overview of some aspects in selected validation studies of RFID systems deployed for animal behavior monitoring.

Validation study reports	Species	Behavior(s) of interest	Approaches	Duration of registrations and sample size for study or analyses	Bout criteria	Variable (s) used	Indicators used: authors' terminology and formulas
Thurner et al. (2008)	Laying hen	Nesting	RFID outputs vs. Camera recordings (external reference)	374 visits were extracted from the video recordings, and data were collected over 74 d. 699 hens, divided into 2 flocks, were used.	30 s	□ Timestamps of nest visits □ Number of nest visits	□ "Identification reliability" = (number of visits suggested by RFID $\times$ 100)/number of visits indicated by the cameras □ "Accuracy" (also referred to as "time discrepancies" and "time differences") = difference (in seconds) in timestamps between videos and RFID
Zaninelli et al. (2016)	Laying hen	Nesting	RFID outputs vs. Camera recordings (external reference)	1,120 nest visits extracted from the videos were analyzed. 20 individuals, equally divided into 2 groups, were involved.	-	Timestamps of nest visits	□ "Identification speed" = difference (in seconds) timestamps videos vs. RFID. It represents the time it takes to identify individual hens entering the nest.
Thurner et al. (2009)	Laying hen	Outdoor ranging	RFID outputs vs. Camera recordings (external reference)	16 d of video recording, at least 8 h/d; 181 individuals involved. Depending on the pophole width, 582, 606 and 3113 passages were used for the analyses.	-	Number of passages through the popholes	□ "Identification reliability" = (number of passages suggested by RFID $\times$ 100)/number of passages indicated by the cameras
Thurner et al. (2010)	Laying hen	Outdoor ranging	RFID outputs vs. Camera recordings (external reference)	12,195 passages through the pophole were extracted from a 5-day recording period on 225 hens and a 4-day recording period on 328 hens.	-	Number of passages through the popholes	□ "Identification reliability" = (number of passages suggested by RFID $\times$ 100)/number of passages indicated by the cameras
Gebhardt-Henrich et al. (2014a)	Laying hen	Outdoor ranging	Number of detected entries vs. Number of detected exits (internal reference)	Data were collected over 12 d. 100–900 focal individuals were tagged, representing 5% of the size of various flocks. 12 commercial flocks were involved.	-	Number of hens passing through barn and veranda popholes	□ "Probability of registration" calculated using binary variables: "1" if all hens exiting the barn or veranda return effectively, and "0" otherwise.
Li et al. (2017)	Laying hen	Feeding and nesting	RFID outputs vs. Camera recordings (external reference); 2 tags on one of the animals: 1 on the neck for monitoring feeding behavior and 1 on the left leg for nesting behavior	38 one-hour and 78 half-hour episodes of video observations were sampled for feeding and nesting, respectively. Episodes were extracted from a 3-day recording period, and only lighted hours were considered: from 5:00 am to 6:00 pm. A total of 60 individuals were monitored.	30 s for each of the 2 behaviors	Number of animals visiting the resources simultaneously	□ "Accuracy" = (number of hens detected at the resource according to RFID $\times$ 100)/number on videos □ Correlation between the number of hens at the videos vs. RFID
Wang et al. (2019)	Laying hen	Perching	RFID outputs vs. Camera recordings (external reference); a load cell module was used to enhance the overall performance of the monitoring system; 2 tags per bird, 1 attached to each leg	Recordings were over 7 d. 15 individuals, evenly distributed across 3 pens, were involved.	15 s or 3 s, depending on the considered variables	□ Number of visits □ Duration of visits	□ "Sensitivity" = $TP \times /P$ □ "Specificity" = $TN \times /N$ □ "Precision" = $TP \times / (TP + N - TN)$ □ "Accuracy" = $(TP + TN) / (P + N)$

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Table 2 (*Continued*)

Validation study reports	Species	Behavior(s) of interest	Approaches	Duration of registrations and sample size for study or analyses	Bout criteria	Variable (s) used	Indicators used: authors' terminology and formulas
Sibanda et al. (2020a)	Laying hen	Feeding, nesting, movement	Recordings from right leg tag vs. Recordings from left leg tag (internal reference)	Data collected over 30 d, 18 hens involved	-	- Number of visits - Duration of resource visits	- Coefficient of determination (r^2) for the number of visits suggested by right leg tag vs. left leg tag on each laying hen; linear regression was used - Coefficient of determination (r^2) for the duration of visits suggested by right leg tag vs. left leg tag on each laying hen; linear regression was used
Sales et al. (2015)	Laying hen	Preference and transition across functional locations	RFID outputs vs. Camera recordings (external reference)	256 h of recordings were collected over 24 h. 40 individuals were involved: 32 (divided into groups of 4) were monitored as a group, and 8 monitored individually.	-	- Timestamps of entries - Duration of visits - Number Frequency of visits	"Delayed detection times" (i.e., lag time-stamps of entries) video vs. RFID "Detection success rates" based on entries = (number of entries suggested by RFID $\times$ 100)/number of actual entries indicated by the cameras) "Detection success rates" based on duration = (duration of visits according to RFID $\times$ 100)/ duration of visits indicated RFID by videos) - Statistical comparisons of total visit duration, number of entries and average single visit duration between data from cameras and RFID
van der Sluis et al. (2020)	Broiler	Activity	RFID outputs vs. Recordings from cameras and ultra-wideband (UWB) tracking system (2 external references)	4 videos, each 7 min long, were selected at different time points over 34 d of recording; videos were converted into frames per second, and the bird's location was annotated. A total of 1,629 frames were analyzed. 40 broilers were involved.	-	- Locations visited - Distances walked (in meters)	"Percentage of exact matches" or "percentage of (dis)agreement" of identified location = registrations of locations according to RFID $\times$ 100/registrations of locations according to videos - Correlation between walk distances calculated from RFID outputs vs. Video recordings - Correlation between walk distances calculated from RFID data vs. UWB data
Li et al. (2019)	Broiler	Feeding and drinking	RFID outputs vs. Camera recordings (external reference); a tag attached to the neck used to monitor both behaviors	55 two-minute video recordings were obtained over 7 d. The videos were sampled every 2 h, and only the lighted hours of the day were considered. The videos were converted into frames on a 1-s basis: a total of 13200 frames were analyzed. A total of 120 tagged individuals equally distributed in 2 experimental rooms were used.	20 s for each of the 2 behaviors	- Instantaneous number of birds at each of the resources - Duration of visits	- For the instantaneous number of birds, "Accuracy" = (number of seconds for which the number of birds observed in each frame matches the number suggested by the RFID $\times$ 100)/ number of seconds in a 2-min video, i.e., 120 s) - For time spent at resources, "Accuracy" = (total number of birds at resources as suggested by RFID $\times$ 100)/ total number of birds at resources as indicated by the frames

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Table 2 (*Continued*)

Validation study reports	Species	Behavior(s) of interest	Approaches	Duration of registrations and sample size for study or analyses	Bout criteria	Variable (s) used	Indicators used: authors' terminology and formulas
Adrion et al. (2018)	Pig	Feeding	RFID outputs vs. Camera recordings (external reference)	13 d of observations, 10 h of recording per day, and 25 individuals involved	20 or 30 s, depending on the type of antenna	□ Duration of visits □ Number of feeding events (meals)	□ "Sensitivity" = TP/P □ "Specificity" = TN/N □ "Precision" = TP/(TP + FP) □ "Accuracy" = (TP + TN)/(P + N) □ Correlation of total daily duration visit based on data from RFID vs. Cameras □ Correlation of total daily number of meals based on data from RFID vs. Cameras
Maselyne et al. (2014)	Pig	Feeding	RFID outputs vs. Camera recordings (external reference); 2 ear tags used	11.5 h of video recording were analyzed. A total of 20 focal pigs were considered.	9 s	□ Duration of resource visits □ Number of resource visits	□ Correlation of visit durations based on data from RFID vs. Cameras □ "Sensitivity" = TP/P □ "Specificity" = TN/N □ "Accuracy" = (TP + TN)/(P + N) □ "Precision" = TP/(TP + N-TN) □ "Percentage of correct RFID registrations" on a 10-s basis □ "Percentage of correctly identified visits" □ "Agreement between video and RFID" on a 20-s basis
Maselyne et al. (2016b)	Pig	Feeding	RFID outputs vs. Camera recordings (external reference); 2 ear tags used	Two experiments: One with 20 pigs and 11.5 h of video recording obtained 1 d; the other with 6 pigs with 14 h of video recording obtained over 3 d.	20 s when 1 tag was considered, 10 s for 2 tags	□ Duration of resource visits □ Number of visits □ Inter-visit duration	□ "Sensitivity" = TP/P □ "Specificity" = TN/N □ "Accuracy" = (TP + TN)/(P + N) □ "Precision" = TP/(TP + FP) □ Correlation of visit durations based on data from RFID vs. cameras
Maselyne et al. (2016a)	Pig	Drinking	RFID outputs vs. Recordings from cameras and Water flow meters (2 external references); 2 ear tags used	393 visits were extracted from the camera recordings. The videos were collected over 2 d, with 12 h of observation per day. In total, 53 tagged animals were involved.	20 s	□ Duration visits □ Average duration □ Intervisit duration □ Number of visits	□ "Sensitivity" = TP/(TP + FN) □ "Specificity" = TN/(FP + TN) □ "Precision" = TP/(TP + FP) □ "Accuracy" = (TP + TN) / (TP + FN + FP + TN) □ Correlation RFID vs. Video recordings □ Correlation RFID vs. Water flow meter recordings □ Comparison test (paired <i>t</i> test) of recordings from RFID vs. Videos □ Comparison test (paired <i>t</i> test) of recordings from RFID vs. Water flow meters
Toaff-Rosenstein et al. (2017)	Cow	Grooming	RFID outputs vs. Camera recordings (external reference); 2 ear tags used	6 h of continuous video recording per group were obtained, and 2 groups of 8 cows each were used. The recordings were made over 2 d.	16 s	Duration of visits	□ Regression of visit duration based on data from RFID vs. Videos □ "Sensitivity" = TP/P □ "Specificity" = TN/N

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Table 2 (Continued)

Validation study reports	Species	Behavior(s) of interest	Approaches	Duration of registrations and sample size for study or analyses	Bout criteria	Variable (s) used	Indicators used: authors' terminology and formulas
Adrion et al. (2020)	Cow	Feeding	RFID outputs vs. Camera and Noseband pressure sensor recordings (2 external references).	48 h of video recordings from a 3-day observation period were analyzed. 10 cows were involved in the validation study	120 or 180 s, depending on the type of tag.	Duration of visits	□ "Sensitivity" = TP/P □ "Specificity" = TN/N □ "Precision" = $TP/(TP + FP)$ □ "Accuracy" = $(TP + TN)/(P + N)$ □ "Mean of the differences" in visit durations based on data from RFID vs. Cameras □ "Mean of the differences" in visit durations based on data from RFID vs. Noseband pressure sensor □ Correlation of hourly time spent at resource based on data from RFID vs. Videos □ Correlation of hourly time spent at resource based on data from RFID vs. Noseband pressure sensor

taken from the instrument are (Bateson and Martin, 2021). The body of literature on RFID in animal behavior research shows that reliability has been assessed using the following indicators: precision, sensitivity, correlation coefficient, and the difference in measurements between the reference and the RFID.

Precision refers to the degree to which measurements provided by an instrument are free of random error (Bateson and Martin, 2021). For example, in the context of RFID deployment, a 50% precision would indicate that about half of the registrations that RFID suggests are not real; these are false positives (FP, Figure 4), identified as such after comparisons with video registrations (Adrion et al., 2018). Low precision is observed when the antennas or the reader settings are not carefully tuned to ensure that the read range adequately covers the functional area of the resource of interest. As a result, the read range exceeds the functional area of the resource of interest, and FP (i.e., unwanted registrations) become significant (Brown-Brandl et al., 2019). Such a definition of precision was adopted by Adrion et al. (2020) and Maselyne et al. (2016a). The calculation formula used by these authors is: Precision = $TP/(TP + FP)$, where TP are true positives and FP are false positives. Figure 4 illustrates each of the terms in the formula. For the calculations, these authors used the number of seconds each visit sequence lasted or the number of visits. The precision calculated in this way is also called the "positive predictive value" (Adrion et al., 2020). The minimum accuracy recommended by Brown-Brandl et al. (2019) before adopting RFID in livestock tracking is 70%.

The sensitivity of an instrument indicates how many instances or figures measured by it are true (i.e., actual occurrences); sensitivity is the proportion of instances correctly identified by the instrument being evaluated relative to the total number of actual occurrences indicated by an external reference (Caguci, 2003). A quite common way of calculating sensitivity is to divide the correctly identified instances of the instrument by the "true" instances, which are marked as "gold standard" or "reference": Sensitivity = TP/P ; where TP are true positives and P are positives. For example, if there are 10 actual occurrences of a chicken passing through a pop-hole, as confirmed by video recordings, while only 7 of these are detected by the RFID system, the sensitivity of the RFID system would be 70% (7/10). In such a case, the 10 actual occurrences are referred to as "positives"; the 7 successfully detected occurrences by the RFID are termed "true positives"; and the 3 missing instances are regarded as "false negatives." According to that commonly used formula, the higher the fraction, the more sensitive RFID is. Sensitivity was defined and calculated in this way by Adrion et al. (2020), Maselyne et al. (2014), and Toaff-Rosenstein et al. (2017). However, other authors have used this formula but named it in different ways: percentage of "exact matches" or "(dis)agreement" (van der Sluis et al., 2020) and "identification reliability" (Turner et al., 2010). It is important to note that, like sensitivity, these metrics do not

	Positives (P)						Negatives (N)					
Videos	✓	✓	✓	✓	✓	✓	✗	✗	✗	✗	✗	✗
RFID	✓	✓	✓	✗	✗	✗	✗	✗	✗	✓	✓	✓
	Correct readings (True Positives; TP)			Reading failures (False Negatives; FN)			Correct readings (True Negatives; TN)			False readings (False Positives; FP)		

Figure 4. An illustration of the terms included in the formulas for quantifying the adequacy of RFID systems deployed for monitoring animal behavior. Positives (P) are seconds of visit events according to the videos. The negatives (N) are seconds of nonvisit events, according to the videos. True positives (TP) are seconds of visit events indicated by the RFID and which agree with the video recordings. True negatives (TN) are seconds of nonvisit events suggested by the RFID and confirmed by the video recordings. False negatives (FN) are seconds of visit indicated by the videos, but the RFID suggests otherwise. False positives (FP) are seconds of nonvisit events indicated by the videos, but the RFID suggests otherwise. Whereas TP and TN are correct readings, FN and FP are misreadings compromising the validity of the RFID system.

consider "false positives" and focus solely on correctly identified actual occurrences—"true positives." For example, in [Van der Sluis et al. \(2020\)](#), the "percentage exact matches" represented the proportion of correct location records made by the RFID system compared to video observations. Accordingly, the authors calculated that percentage without taking into account FP locations of tracked birds; "false positives" being the mismatches resulting from intermittent tag registrations between the antennas. "Sensitivity" would be calculated in the same way. Low sensitivity will generally be observed for an RFID whose recordings do not occur continuously but with an irregular interval between recordings; irregular reading is quite common in RFIDs deployed for animal tracking ([Adrion et al., 2018](#)). Nevertheless, [Brown-Brandl et al. \(2019\)](#) recommend a minimum sensitivity of 80% before fully adopting an RFID system for monitoring the behaviors of livestock, including poultry.

The correlation between the instances recorded by the behavior-measuring instrument and the reference (or gold standard) can also be an indicator of reliability ([Martin and Bateson, 2007](#)). It is quite common in reports of RFID validation for animal behavior monitoring ([Table 2](#)). The correlation between RFID and camera outputs expresses statistically the extent to which the 2 behavioral monitoring means agree. For example, with a correlation coefficient of $r = 0.7$, the coefficient of determination is $r^2 = 0.49$, meaning that the video recordings would statistically explain about 50% of the variance in the RFID data. In the body of literature on RFID in poultry behavior research, correlation as an indicator of reliability was calculated using variables such as the duration of resource visits, the number of visits ([Sibanda et al., 2020a](#)), or the number of animals in the read range ([Li et al., 2017](#)). [Martin and Bateson \(2007\)](#) recommended a minimum correlation coefficient of 0.7 to attest to the reliability of an animal behavior monitoring instrument.

Last, the difference in measurements between the reference and RFID was used as an indicator of adequacy. This approach, estimating variability using the mean or standard deviation, implies that the closer the

measurement of the 2 recording means, the more adequate the instrument is ([Salkind, 2010](#)). The formula is as follows: difference = video recordings – RFID recordings (reviewed in [Table 2](#)). However, the terminology used to refer to this formula varies among authors: "delayed RFID detection time" ([Sales et al., 2015](#)), "identification speed" ([Zaninelli et al., 2016](#)), "average differences" ([Adrion et al., 2020](#)), "accuracy," "time differences," and "time discrepancies" ([Thurner et al., 2008](#)). These inconsistencies may lead to confusion.

Indicators of Validity. Validity is a second major indicator of the adequacy of a newly developed instrument. It expresses the extent to which the instrument measures what it is supposed to measure ([Bateson and Martin, 2021](#)). Based on the literature, 2 indicators are commonly used to quantify the validity of the RFID systems deployed to monitor animal behavior: accuracy and specificity. The classic formulas used are "Accuracy" = $(TP + TN)/(P + N)$ and "Specificity" = $TN/(FP + TN)$, with the camera recordings as reference ([Table 2](#)).

However, using these formulas to quantify the validity of RFID deployed for monitoring indoor resource visit behaviors in group-housed chickens can be misleading. The main reason for this is that specificity and accuracy tend to be high and may therefore lead to overestimating the system's performance. Overestimations occur because, in the formulas, the numerators comprise "true negatives" (i.e., when the animal does not visit the resource), which are usually high. Chickens spend most of the day away from indoor resources; they usually use them only briefly and only at certain times of the day. For example, cage-free and colony-caged laying hens spend around 6.2 and 4.4% of their daily time budget in the nest boxes, respectively ([Oliveira et al., 2019a](#); [Sibanda et al., 2020d](#)). Broilers spend only about 3% of their day at the drinker and 13% at the feeder ([Li et al., 2020](#)). In addition to their tendency to inflate the performance indicators, true negatives are inefficient to record. Recording video only during the time windows when the animals mostly visit resources would be more practical and cost-effective than recording the lengthy periods of nonvisit.

Some authors introduced alternatives to the classic formula for calculating accuracy. For example, [Li et al. \(2019\)](#) used the instantaneous number of animals. The authors obtained this using frames extracted on a 1-s second basis from 2-min video samples. Accuracy was then calculated as the percentage of birds detected by the RFID system relative to the number of hens observed in the video images. [Li et al. \(2017\)](#) also used a similar approach.

Overall, we recommend that whenever an author evaluates and reports on the validity of RFID for monitoring poultry behavior, care should be taken to use the most common terminologies and calculation methodologies possible and, above all, to describe these. As for the interpretation of validity indicators, there is no black-and-white threshold above or below which the acceptability of the RFID system would be unequivocal. However, suggestions in the literature are provided in the present study.

Methods for Calculating the Bout Criterion. Bout criterion is the maximum inter-registration duration considered during RFID data processing; it is the threshold for the inclusion of 2 consecutive RFID registrations in the same resource visit event ([Maselyne et al., 2016b](#); [Li et al., 2017](#); [Adrion et al., 2018](#); [Wang et al., 2019](#)). For example, if a 10-s bout criterion is used, and the interval between 2 successive RFID registrations is shorter than 10 s, then the 2 registrations will belong to the same resource visit event. But, if that interval was larger than 10 s, these 2 registrations would belong to different visit events.

The methods for calculating the bout criterion vary according to the authors. [Brown-Brandl and Eigenberg \(2015\)](#) introduced a method consisting of 3 steps. In the first step, the authors plotted the average number of daily visits, and the total and average duration of daily visits for all animals studied against different bout values, potential optimal minimum intervals between visits. These examined potential bout values, for which the rationale for selection was not mentioned, were: 1, 2, 3, 5, 10, 15, 22.5, 30 and 60 s. Of these graphs of visit estimates, those that changed as uniformly as possible with a single inflection point were selected. For example, the plot of the number of meals per day was selected over others because it showed a steady decrease at the beginning, followed by a plateau. Next, the authors investigated the rate of change of this selected resource visit estimate. This investigation required the plotting of the first and second derivatives of estimates. Last, the authors identified the point where the first derivative is at its minimum and the second is equal to zero. The corresponding bout value was thus considered optimal. The reason behind this procedure is that, at that point, the rate of change of the resource visit estimates was also minimal. Following this method, [Adrion et al. \(2018\)](#) identified 20 or 30 s, depending on the type of antennas, as the optimal bout criteria applicable to their RFID data to determine the daily number of visits to the feed trough in pigs.

[Maselyne et al. \(2016b\)](#) introduced a second method based on the mean deviation between data obtained from

RFID technology and video recordings in a study on pig feeding behavior. This method was subsequently adopted by [Wang et al. \(2019\)](#), who referred to it as the "average error rate" to investigate the perching behavior of laying hens monitored with RFID. The approach involves 3 steps. First, different potential bout values were considered: 0, 1, 2, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 s in [Maselyne et al. \(2016b\)](#); and 1, 3, 5, 10, 15, 20, 25, 30 s in [Wang et al. \(2019\)](#). The rationale for choosing these specific potential bout values was not specified in both papers. Second, the measurements suggested by the RFID system for each animal involved were adjusted using the different bout values. In parallel, the corresponding "true" measurements were extracted from the video recordings. Third, from both types of measurements, the average error rates were calculated as follows: $\text{average error rate} = \text{AVERAGE}[(\text{bout-corrected measurements} - \text{video-based measurements}) \times 100 / \text{video-based measurements}]$; the absolute value was used. An illustrative example of this third step is as follows: if, after using a given bout value, the average bout-corrected number of visits according to the RFID is 401 while that indicated by the video recording is 443, then the average error rate will be: $\lceil (401 - 443) \times 100 / 443 \rceil = 9.48\%$. Finally, the bout value resulting in the lowest average error rate was adopted. [Maselyne et al. \(2016b\)](#) thus identified 20 s as an appropriate bout criterion applicable to their RFID registrations about feeding behavior in pigs tagged at 1 ear. Similarly, [Wang et al. \(2019\)](#) identified 15 s as suitable for their RFID data on the number of perch visits in laying hens.

A third method [Li et al. \(2017\)](#) proposed involves plotting the differences in duration between adjacent RFID recordings from a number of tags to obtain a histogram. These duration differences were then considered as potential bout values. The time difference for which 95% of the RFID recordings match those of the videos was then considered as the optimal bout criterion. Using this method, the authors identified 30 s as the optimal bout criterion for reconstructing out of their RFID data the visits to drinkers and feeders in broilers.

The last method is a 2-step method introduced by [Adrion et al. \(2020\)](#). In the first step, the authors considered different potential bout values. Then, they selected the bout value that gave the maximum average accuracy, the minimum FP and the minimum false negatives. Using this method, the authors identified 120 or 180 s, depending on the type of tags, as the optimal bout criteria applicable to their RFID data to reconstruct the duration of the cow visits to the feed trough.

To sum up, various methods of calculating the bout criterion have been developed, and all of them have merits. However, even if the use of the bout criterion allows to compensate for the missing data to a certain extent, it does not apply to false readings. Therefore, precautions are essential when designing and implementing the system since they help minimize false readings and missing data.

Data Requirements for RFID Validation in Poultry. Despite the importance of a validation study before the full adoption of RFID technology for

monitoring animal behavior, very little is known about the amount of data needed to conduct such a study; there is no golden rule in this respect. Here, we attempt to address this gap.

Based on 17 hand-selected papers on RFID validation in animal science (Table 2), the determination of the number of use events ("positives") and nonuse events ("negatives"), major outcome variables used as references, can be seen as a good starting point for assessing the amount of data required for a validation study. Although we did not find, in the context of RFID, any established reference mentioning the use of sensitivity and specificity to estimate the number of sequences corresponding to these outcome variables to be extracted from videos, we believe, following Bujang and Adnan (2016), Akoglu (2022), and Bujang (2023), this approach is relevant if complemented by other assumptions and statistical considerations, such as the prevalence of the targeted behavior and the desired level of statistical power. Then, several other factors can be taken into account to approximate the amount of data needed to validate RFID for poultry behavioral monitoring.

Conventional sample size estimation formulas (e.g., Akoglu, 2022) can be used to approximate the total number of use events ("positives") and nonuse events ("negatives") from video recordings to be included in the analyses. These formulas can be supplied with desired sensitivity and specificity values.

With a desired sensitivity, the approximation will be as follows:

$$Nr_seq = \frac{(Z_{\alpha/2})^2 \times Sensitivity \times (1 - Sensitivity)}{E^2}$$

where sensitivity, estimated from previous or pilot studies and desired by researchers, is the proportion of true positives (**TP**) among the positives (**P**): it measures the ability of RFID to identify use events correctly. The term $Sensitivity \times (1 - Sensitivity)$ represents the variance of the sensitivity. $Z_{\alpha/2}$ is the Z-score associated with the probability $\alpha/2$, and E is the tolerable margin of error for the sample estimate.

Similarly, with a desired specificity considered, the approximation will be as follows:

$$Nr_seq = \frac{(Z_{\alpha/2})^2 \times Specificity \times (1 - Specificity)}{E^2}$$

where specificity, estimated from previous or pilot studies and desired by researchers, is the proportion of true negatives (**TN**) among negatives (**N**): it measures the ability of the RFID to identify nonuse events correctly. In this formula, the term $Specificity \times (1 - Specificity)$ represents the variance of specificity; $Z_{\alpha/2}$ is the Z-score associated with the probability $\alpha/2$; E is the tolerable margin of error for the sample estimate.

Applying the 2 formulas results in 2 different sample sizes. In order to determine the relevant one, the larger of the 2 sample sizes can be selected, as this approach ensures that the sample is large enough to achieve the desired sensitivity and specificity. Regarding RFID for

monitoring animal behavior, sensitivity, and specificity should be considered important adequacy indicators (Brown-Brandl et al., 2019).

The Nr_seq calculated and selected in this way includes events and nonuse events, but these need to be proportional to each other to reflect how a given behavior is naturally expressed in the animal population. The ratio of the use events to nonuse events can be determined in advance by observing a sample of videos. Finally, this ratio will be applied to the considered Nr_seq in order to distinguish the number of use and nonuse events that should be randomly extracted from the videos and focused on for the analyses. By randomly extracting from the video recording sequences according to the considered ratio, one can ensure that the sample is representative of the animal population to be monitored with the RFID.

To illustrate, let us assume that we would like to validate an RFID monitoring system deployed to record chickens as they pass through a pophole to access an outdoor range. Let us also assume that the desired sensitivity is 80%; the desired specificity is 95%; and the assumed ratio of passages to nonpassages is 6:4. Finally, let us assume a 95% confidence level and a 5% margin of error are desired. The Z-score corresponding to the confidence level would be 1.96. Using the sample size estimation formulae, we can calculate the required sample sizes as follows:

$$\begin{aligned} \text{With the desired sensitivity value : } Nr_seq \\ = (1.96^2 \times 0.80 \times (1 - 0.80)) / 0.05^2; \\ Nr_seq \approx 246 \end{aligned}$$

$$\begin{aligned} \text{With the desired specificity value : } Nr_seq \\ = (1.96^2 \times 0.95 \times (1 - 0.95)) / 0.05^2; \\ Nr_seq \approx 73 \end{aligned}$$

Of the 2 obtained Nr_seq 's, the one calculated with sensitivity is the higher; it will be retained for further steps of the approximation. For the sample to be representative of the behavior that would be observed in the total population of birds studied, it would be appropriate to include approximately 60% of use events and 40% of nonuse events in the video sequences to be extracted for analysis—the assumed ratio of passages to nonpassages was 6:4. Thus, in this case, where 246 (i.e., the Nr_seq calculated using sensitivity) is retained, the required number of passages (i.e., use events or positives; P) to be extracted from the videos will be: $P \approx 148$ (i.e., 246×0.60); and that of the required number of nonpassages (i.e., nonuse events or negatives; N) will be $N \approx 98$ (i.e., 246×0.40).

To sum up, we would need for analysis a sample size of approximately 246 sequences (i.e., passages: 148; nonpassages: 98) to extract from the video recordings in order to achieve an 80% sensitivity and 95% specificity while maintaining the 6:4 ratio of use to nonuse events. It is important to note that this illustrated approach

cannot be generalized to all poultry behaviors; for simplicity, we chose the pophole usage because it is likely to be one of the least complex behaviors to monitor in poultry: monitoring it with RFID requires just entry/exit registrations as opposed to other more complex behaviors (e.g., feeding, drinking), whose monitoring would involve duration calculations or determination of bout criteria (Brown-Brandl et al., 2019).

Once the number of use events (P) and nonuse events (N) required are known, consideration can be given to the number of animals, pens, and facilities to focus on, the recording durations, and the sampling method necessary for the validation study. In order to obtain a representative data sample, several factors need to be taken into account. These factors include environmental variability, intra- and interbehavioral variability, intra- and interanimal variability, time of day, age, genetics, type of animal, and practicability of the validation study.

Environmental variability is an important factor to consider when deciding on the type and amount of data required to validate an RFID system in poultry behavior science. Environmental factors, such as position, accessibility, design, and other specifics of a given housing facility, and microclimatic conditions within the barn, can lead to variations in the use of resources by the birds. For example, chickens prefer less exposed nests located at the end of the row or in corners (Clausen and Riber, 2012), and perch as high as possible (Brendler and Schrader, 2016). In addition, a poultry barn may contain different pens or compartments, each with distinct characteristics, requiring data collection in each of these to capture behavioral variation fully. Therefore, it is essential to consider environmental variability when designing validation studies for poultry RFID systems.

Intrabehavioral variability is another important factor to consider when estimating the amount of data required to validate an RFID system in poultry behavior science. This variability may be reflected in differences in the frequency with which the behavior is performed at different times of the day or under different environmental conditions. For example, dustbathing behavior is more intense at the warmest or brightest times of the day. In addition, this behavior has several distinct aspects, such as scratching, crouching on the substrate, lateral laying and rubbing, and usually ends after about 20 min—if undisturbed—with body parts' straightening and shaking to remove the dust (Olsson and Keeling, 2005). It is, therefore, essential to consider these variations when recording videos and extracting relevant sequences to obtain a representative sample of the behavior expression. Also, it would be inappropriate to validate the use of RFID based on only one part of the behavior sequences or aspects. Therefore, researchers must be informed of and include all aspects of behavior when recording videos and selecting sequences for analysis.

Interbehavioral variability is another crucial factor to consider when estimating the amount of data needed to validate an RFID system for monitoring poultry behaviors. This is because the various types of behavior differ considerably from each other. For example, under

typical climate conditions, a white-strain laying hen in an enriched colony housing may spend, on average, 310 min at the feeder but only 56 min in the nest to meet its daily needs (Li et al., 2017). Therefore, the amount of data, the recording duration, the number of video footages, and animals required in the RFID validation study are behavior-specific. Moreover, it will be important to consider the age, genetic line, or type of animal involved.

Furthermore, animals may differ considerably from each other in the display or pattern of a specific behavior. For example, some birds may show consistent behavioral patterns across days, while others do not (Rufener et al., 2018; Gómez et al., 2022). This suggests that it would be inappropriate to validate the use of RFID based on the behavior of a single bird, as the behavior of an individual bird may not be representative and generalizable. Therefore, including as many individuals as possible in the study sample is essential to capture these variabilities.

A final important aspect to consider is the practicality of the validation approach to be used. Indeed, researchers need to strive for a balance between the representativeness of the data to be collected and the feasibility of its management, which includes, for instance, the time and resources needed to evaluate the videos. Although the use of videos to validate animal behavior is a very useful and recommendable approach, it can also present difficulties: identifying different animals on a video can be challenging for human observers. Some methods, such as plumage coloration or distinctive markings on the back of the birds, can help to differentiate them (Wang et al., 2019; van der Sluis et al., 2020). Nevertheless, it is conceivable that difficulties in differentiating individuals may arise when the sample size is large; the difficulty would be more pronounced in the case of a shared resource to be visited simultaneously by many animals. Therefore, determining an approach that minimizes constraints and allows for efficient collection, storage, and analysis of video and RFID recordings is crucial.

In summary, there is no golden rule for determining the amount of data needed to validate RFID systems for monitoring poultry behavior. However, considering several factors intrinsic to the environment, animals, and behaviors of interest, as well as the material resources available, researchers can assess the amount of data on which validations can be based. For such an assessment, the number of use events (i.e., positives; P) and nonuse events (i.e., negatives; N) to be extracted from the videos, considering previous studies, can be a reasonable starting point. A statistical approach to approximating these numbers was explained and illustrated with a hypothetical case of RFID deployed to monitor the pophole use behavior in chickens.

Reporting on Technical Aspects and Validity of a Deployed RFID System

A detailed report describing the technical aspects and presenting the results of a validation study of a deployed

Table 3. A checklist and examples of how to present the relevant technical aspects.

Relevant technical aspects	How to present them	Checkboxes
Indicate the motive for the RFID deployment	Indicate the tracking objectives, including estimates of behaviors to be computed, that motivate the deployment of the RFID system.	☐
Describe tracking conditions and requirements	Describe the particularities of the behaviors of interest and characteristics of the deployment environment, with a particular focus on how these behavioral and environmental specifics can be potential sources of influence on the RFID system's performance.	☐
Specify the measures taken to ensure the proper functioning of the deployed system	Describe and justify the optimization measures, be they environment-related or regarding the technical settings of the system components, or considering the behavioral specificities of the animal species, which are implemented to ensure the proper functioning of RFID. The way in which Li et al. (2019) presented these system optimization measures can serve as an example.	☐
Specify the technical characteristics of each of the main components of RFID	Specify each main RFID component's technical characteristics and why these characteristics have been used as criteria for selecting the components. If the components are commercially available, referencing them with at least the commercial name of the model and the name of the manufacturer would be sufficient. Sales et al. (2015) provide a good example. If these components are not commercially available (cases of customized or self-built components), giving information about each main RFID component's manufacturer and technical aspects will be necessary.	☐
Present a system architecture diagram	Provide a conceptual diagram that shows schematically how the components and the subsystem interwork. This structural diagram is meant to give an idea of the system's behavior and data flow. For example, Zhang et al. (2016) , Li et al. (2017) , and Sibanda et al. (2020a) present such conceptual diagrams for RFID deployed for animal behavior tracking.	☐
Present the data management system	Indicate the system for acquiring data, the procedure for processing them, and how the resource visit is estimated to answer the research questions. If the data management system is commercially available, the name of the program and the firm providing it should be specified. If it is custom-made, then specifying at least the programming environment and language and the database management tools will be crucial. Li et al. (2017) can inspire how to present the data management system.	☐
Describe measures taken to control potential influencing factors of system performance	Describe practical arrangements and modifications made to the physical environment to counteract the potential sources of influence on the performance of the RFID system. Examples of such descriptions are presented in Maselyne et al. (2016a) and Li et al. (2019) .	☐
Describe testing and fine-tuning processes	Describe the trial-and-error made with various combinations of configuration and operating settings of system components to achieve the best possible performance.	☐
Indicate the placement of the antennas	Describe the antennas' placement, including where their location and how they are physically mounted and protected in the deployment environment. This description also includes the configuration, for example, polarization. In the literature, antenna placement is described and presented in images by Maselyne et al. (2016a) and Adrion et al. (2020) . Their ways of describing the antennas' placement can be recommended.	☐
Indicate the operating parameters of the system finally adopted	Indicate the configuration and operating settings adopted finally. Such indications may include the adopted transmit or output power and mode of communication between tags and readers (Adrion et al., 2020).	☐
Characterize the reading range	Schematically present the tags' detectability across the reading area. For example, one could be inspired by Wang et al. (2019) and Adrion et al. (2020) .	☐

RFID system for observing animal behavior is essential to ensure the reproducibility of research involving this technology and the reliability of the data collected. The report should first address the technical aspects, such as the infrastructure, architecture, optimization tests, and their results, and the operating parameters adopted. It is also appropriate to mention technical anomalies, identified biases, unforeseen limitations of the system, and critical elements of the deployment environment. Next, the report should present the results of the system validation, examining the deliverables and determining its fitness-for-purpose. Details of external references used, such as video recordings or other forms of behavioral

measurement, and performance indicators, such as reliability, accuracy, validity, sensitivity, or agreement, should be included. To facilitate the writing of such a report, we propose a checklist and examples of presenting the relevant technical aspects ([Table 3](#)), and a review of validity and reliability indicators ([Table 2](#)).

CONCLUSIONS

This guideline paper explains, for a nontechnical audience, how RFID works and its potential to improve the study of poultry behavior. It provides a 4-phase

roadmap to facilitate the development and implementation of an RFID system for monitoring poultry behavior. It also summarizes validation approaches in the literature and highlights relevant aspects to be reported on the technical aspects and validity of a deployed RFID system. The guideline is intended for animal behavior scientists, RFID system integrators and equipment manufacturers. It provides a reference for effectively deploying an RFID system in poultry behavior research. By following this guideline, researchers will be able to exploit RFID technology better to advance the study of poultry behavior, thereby improving our understanding of these animals and ultimately contributing to animal welfare and sustainable production.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the present study.

SUPPLEMENTARY MATERIALS

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III. Paper 2: Inferring Resource Use from Functional Area Presence in a Small, Single-Flock of Chickens in a Mobile Barn

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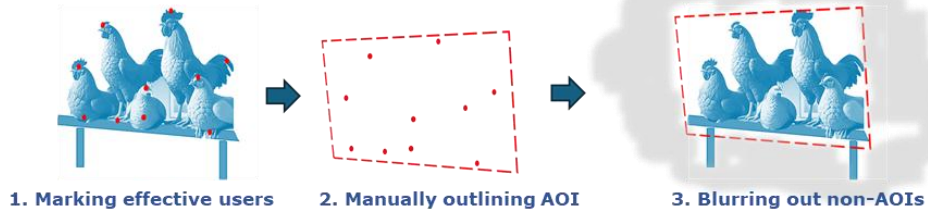
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Graphical Abstract Paper 2

Inferring Resource Use from Functional Area Presence

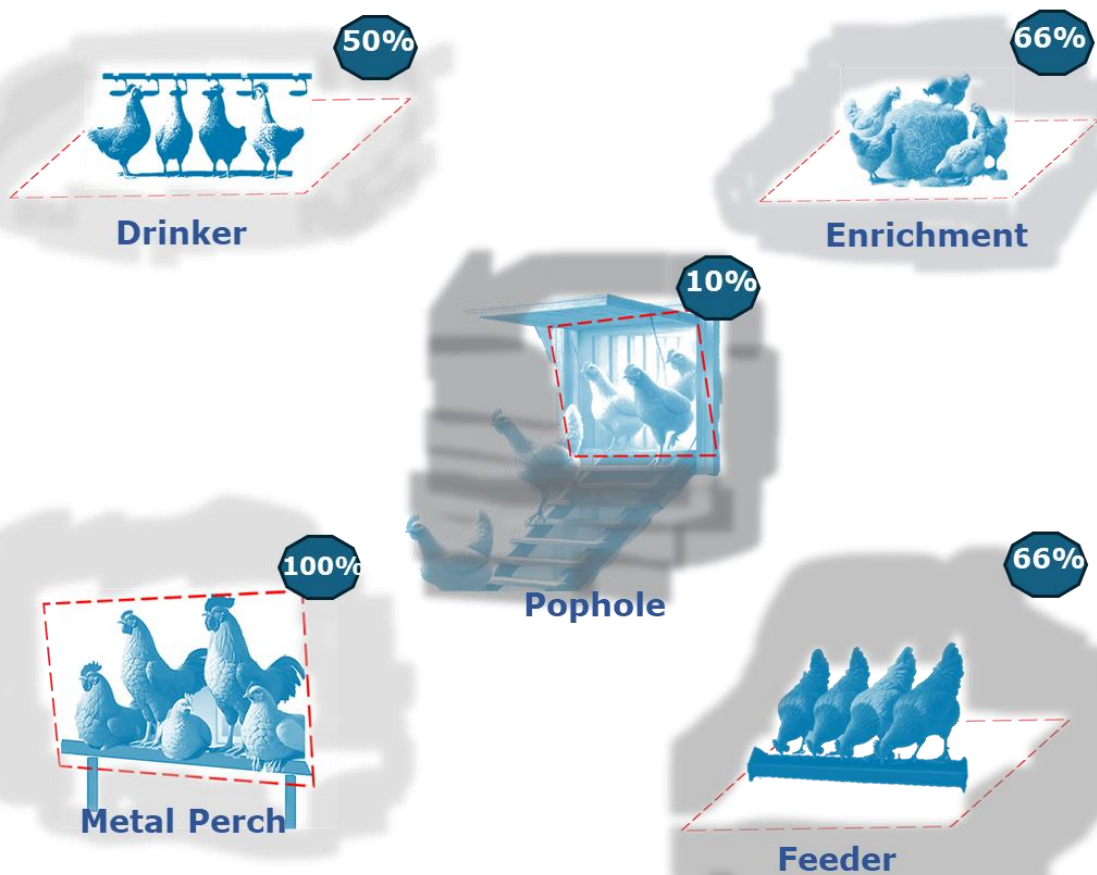
Approach

Defining the Areas of Interest (AOIs)



Results

Ratio Usage-to-Presence



Conclusion:

- Proximity does not necessarily equate to actual resource use.
- Overestimation common for feeders, enrichments, drinkers, and popholes.
- Data from tracking technologies (e.g., RFID) require careful interpretation.

Inferring resource use from functional area presence in a small, single-flock of chickens in a mobile barn

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ABSTRACT In poultry behavior research, the reliance on presence data to estimate actual resource usage has substantially increased with the advent of tracking technologies such as radio frequency identification (RFID) and image-based systems. Although such widely used technologies are fundamentally designed for presence tracking, many studies claim to use them to investigate actual resource usage. This study investigates whether the duration of chickens' presence near key resources accurately reflects their actual usage. To this end, we analyzed 210 ten-min video sequences from 5 days of recordings of 21 chickens, focusing on their proximity to and use of 6 key resources in a mobile poultry barn. Human observers manually assessed the durations of

proximity—presence in defined functional areas of interest—and resource use for each individual in the video sequences. Significant correlations (Spearman's coefficient 0.83–1) were found for most resources, except the pophole ($Rho = -0.30$). Usage-to-presence ratios varied: perches exceeded 87%, feeder and enrichments around 66%, drinker 50%, and pophole 10%. Our findings highlight that mere proximity to resources does not always guarantee their effective use. We emphasize the need for careful interpretation of data from tracking technologies, acknowledging the distinction between mere proximity and actual resource use. Future studies should include larger sample sizes and varied conditions to ensure broader applicability.

Key words: resource use, poultry behavior, tracking technology, precision livestock farming

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INTRODUCTION

Understanding chicken behavior, particularly their interactions with key resources such as drinkers, feeders, and perches, helps to develop housing and management practices that meet their needs, reduce stress, and promote positive human-animal relationships, ultimately optimizing costs and farm profitability.

Recent advancements in tracking technologies have significantly enhanced the study of chicken behavior, providing valuable spatiotemporal data on resource use and spatial distribution (Neves et al., 2015; Alindekon et al., 2023). However, technologies like RFID and basic image-based tracking, which are fundamentally designed

for presence tracking, may often fail to differentiate mere proximity from actual effective resource use (Doornweerd et al., 2023). When used alone, RFID detects an animal's presence by sensing changes in electromagnetic fields, while image-based systems, relying on pixel changes, can only indicate presence within an area of interest (AOI). Neither method can accurately depict specific animal actions without additional technologies or measures. Despite this limitation, many studies (reviewed by Alindekon et al., 2023, in the case of RFID) claim to measure complex behaviors, including feeding and drinking, based solely on proximity data. Whether such inferences are always valid remains underexplored.

This study investigates whether the duration of chickens' presence near key resources in a mobile barn accurately reflects their actual usage. Specifically, we (1) delineate and highlight functional areas—where chickens can stay and interact with the resources—around 6 key facilities and (2) compare the manually collected

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durations of mere presence within these areas to actual resource use.

It is crucial to ensure that resource use proxies from modern tracking technologies are reliable. If these proxies do not accurately reflect actual resource use, data interpretation must be refined to maintain biological relevance.

ANIMALS, MATERIALS AND METHODS

Ethical Considerations

Our research complied with the German Animal Welfare Act, and necessary approvals were obtained from the competent authorities (authorization number AZ 7221.3-18196_23).

Animals, Housing, and Husbandry

The study involved 21 chickens, a number chosen for practical reasons to enable efficient and feasible manual on-screen monitoring and focused observation. Furthermore, the low stocking density was intended to allow the chickens to perform their natural behaviors without hindrance from other chickens. The group included various lineages and sexes: 16 females and 1 male from the Vorwerk breed, all 68 wk old; 1 white Lohmann Selected Leghorn; and 3 Lohmann Brown chickens, all females and 45 wk old. The chickens had previously been housed together as a stable group for at least 12 wk. They were kept at a similar stocking density in a barn comparable to the one used in this study.

For this study, the chickens were housed in a ROWA 200 v4.0 mobile chicken barn at the “Friedrich harms” animal experimental station of the University of Rostock in Dummerstorf, Germany. The mobile barn was divided into 2 interconnected compartments: a 16-square-meter compartment with 6 Europa wooden nests, a 2.25-meter-long cylindrical metal perch, a 2-level L-shaped wooden perch, a 20 cm × 200 cm linear plastic trough feeder, and a drinker line with 3 bowls and 12 nipples. The other compartment, a 14-square-meter winter garden, offered a semi-open space with natural light, fresh air, and enrichment items like an alfalfa bale, mineral blocks, gravel, oyster shells, and a dust bath box. LED lighting provided 16 hours of light from 5:30 a.m. onwards.

We kept the chickens’ management consistent with their prestudy conditions, providing the same commercial feed (PANTO LMK Legemehlkorn, Germany) and routine daily checks. No keel bone damage or severe feather pecking was observed during the study.

Camera Configuration

Axis Communications cameras (M1135-E and M1135-E MK II), operating at 25 and 30 frames per second respectively, and equipped with 2-megapixel lenses (1920 × 1080), were used. The cameras were positioned

on the ceiling of the chicken barn, with angles ranging from 29 to 90 degrees and a distance of up to 2.5 meters from the resources. The resources of interest included a drinker, nutritional enrichments in the winter garden, a feeder, a pophole, and metal and wooden perches.

Video Recording and Segmentation

The recording period spanned 5 consecutive days (September 19–23, 2023). Video recordings for each resource were initially segmented into 10-min sequences, specifically capturing instances of animals interacting with resources throughout the day until the end of lighted hours. The number of manually segmented videos per resource were as follows: drinker (72), feeder (80), enrichment (80), wooden perch (72), metal perch (45), and pophole (45).

Defining the Areas of Interest

Defining the AOI, representing the functional areas of the resources where the chickens have the option to stay and interact with the resources, consisted of applying overlays to the segmented video sequences using Python’s OpenCV library (v4.8.0). The process involved 3 steps (Figure 1).

First, effective resource users were identified. This step involved extracting video frames showing chickens actively using the resources and manually marking and collecting coordinates of key points on these chickens. Key points included the middle of the back and extremities, such as the tail tips, flanks, and heads. The points were marked for the following number of effective resource users: drinker (41), feeder (157), enrichment (40), wooden perch (129), metal perch (89), and pophole (21). The coordinates were then superimposed onto a reference image, forming a cloud of points that represented the main activity area of the chickens interacting with the resources.

Next, the area of interest was outlined by drawing a polygon that encompassed most of the marked points. It is important to note that the size of the AOIs varied between resources due to the inherent specifics of the targeted behaviors, as well as the position and placement of chickens effectively using the resource in the marked frames.

Finally, noninterest areas were blurred out, and the overlay was placed on the videos. This process ensured that only the chickens within the AOI were distinctly visible for annotations of proximity and effective resource use.

Manual Annotations

Manual annotations were performed using the Behavioral Observation Research Interactive Software (BORIS v8.21.5) to accurately code the start and end timestamps for each individual’s effective resource use and presence within the functional area around the

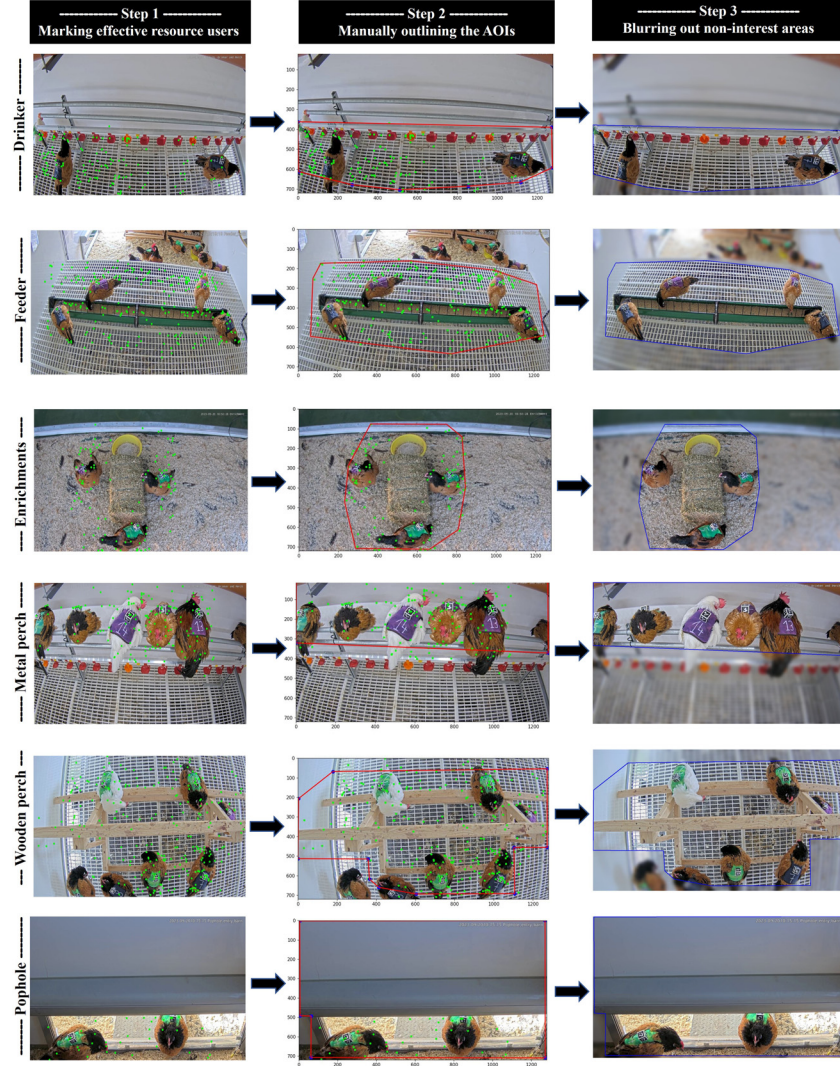


Figure 1. Images illustrating the 3 steps of defining areas of interest (AOIs) for each of the 6 key resources analyzed.

resources (AOI). Since the chickens were equipped with colored vests (Omlet, UK), each bearing unique handwritten IDs visible on screen, it was possible to differentiate between them within each 10-min video sequence.

For annotation, it was essential to follow each individual throughout each sequence, focusing on the ID. This approach necessitated re-watching and annotating the same video sequence for as many different individuals as appeared during the 10-min intervals. Although differentiation of individuals within a 10-min sequence was possible, continuous tracking of individuals between different 10-min sequences was not feasible because the vests were redistributed each new day. Two ethograms were utilized: one to code mere presence, and another to code effective resource use.

Ethogram for mere presence. Mere presence was defined as the simple presence of a chicken within the AOI, irrespective of its activities and purposes for being there. Annotation began as soon as at least half of the chicken's body was within the AOI or if both feet were located in the defined AOI.

Ethogram for effective resource use. Effective resource use was recorded when chickens interacted with the resource based on specific criteria for each type of resource:

- **Drinking:** observed standing in front of the drinking line, the chicken might have dipped its beak into a cup, come into contact with a nipple drinker, or lifted its head to drink water by gravity. If after such active engagement with the water resource, the chicken paused—indicated by immobility without any other activity—and then resumed drinking, the same drinker use event continued. However, if during this time, the chicken engaged in other behaviors such as preening, social interaction, or environmental exploration, or if it left the defined AOI, the previous drinker use event was considered to have ended from the moment the animal ceased interacting with the drinker.
- **Feeding:** observed with its head tilted downwards, the chicken may actively peck at the feed either

through a series of coordinated movements or with isolated pecks, or simply focus on it while in this position. Similarly to the drinking behavior described earlier, brief pauses can also be part of the same feeding event, and the criteria for initiating an effective feeder use event follow the same principles as described for drinking behavior.

- Perching: effective use of perches was recorded when chickens were either sitting or standing on the metal or wooden perch.
- Passing through the Pophole: effective use of the pophole was defined as chickens passing through the access hatch, transitioning from the main compartment to the covered winter garden, or in the opposite direction.
- Interactions with Nutritional Enrichments: effective use was recorded when chickens were observed pecking at enrichment items such as pecking stones, oyster shells, or an alfalfa bale, or showing interest in the fragments of these items on the ground nearby. Concerning the criteria for initiating an effective enrichment use event, the principles established for drinking behavior also apply here.

Human annotators. Two previously trained annotators coded the video sequences. Postannotation interrater reliabilities were calculated for the 2 utilized ethograms, with Cohen's kappa coefficients at 0.94 for resource use and 0.95 for mere presence within AOIs.

Data and Statistical Analyses

Variables and data handling. For each of the 6 key resources, 35 sequences were randomly selected from the total segmented sequences obtained, ensuring representativeness and complete coverage of the 5-day recording period conducted on all 21 chickens. We made sure to include sequences from various times of the day—early morning, midday, and evening. Each sequence was treated as a separate observation unit for subsequent statistical analyses.

Each sequence was broken down into individual seconds to determine, for each tracked individual, the number of time points where mere presence matched or mismatched effective resource use. This allowed for the calculation of usage-to-presence ratios and the investigation of correlations between these 2 measurements.

Comparing presence and effective use. Statistical analyses were performed using “scipy.stats” (v1.11.1) in Python (v3.10.9). Spearman's correlation analysis was employed to examine how the duration of presence in AOIs (visit event) relates to the aggregate duration of effective resource use during each visit event. Kruskal-Wallis and Dunn's tests were employed to examine significant differences in usage-to-presence ratios among resources.

RESULTS AND DISCUSSION

Examining Presence vs. Effective Use Across Various Resources

This study investigated the relationship between chickens' proximity to key resources in a mobile poultry barn and their actual usage, assessing whether proximity serves as a reliable indicator of resource use. We found particularly strong Spearman's correlations (ranging from 0.83 to 1, $P < 0.001$) between chicken presence and resource use for most resources analyzed, except for the pophole, which displayed a much lower correlation ($Rho = -0.30$, $P = 0.01$; [Figure 2](#)).

Further analysis showed that the ratio of effective use to mere presence varied significantly across resources ($H\text{-test} = 162.23$, $N = 210$; $P < 0.01$), with metal and wooden perches resulting in the highest usage-to-presence ratios, exceeding 87%. Enrichments and feeder exhibited intermediate values around 66%, while drinker, statistically similar to the latter, had a usage-to-presence ratio of 50%. The pophole distinctly showed the lowest ratio, about 10%.

These findings underscore that proximity to a resource does not always translate into effective use in a poultry barn. This discrepancy highlights the need to interpret proximity data to infer effective resource use with caution, particularly for certain types of resources. While presence data can provide a reliable estimate of perch use, inferring feeding and drinking behaviors solely based on proximity data requires more caution, as it can lead to a significant overestimation of the actual use of feeders, nutritional enrichments, and drinkers. This caution is even more critical for popholes, where the ratios of usage relative to presence were notably low, indicating that proximity data alone—especially from one side of the pophole—is insufficient for accurate assessment.

The strong relationship between presence and usage of perches can likely be attributed to the elevated nature of the perches and the more focused coverage of their AOIs, which concentrate on the perch line with less overlap with other noninterest areas. The usage-to-presence ratio is even stronger if the perch is a single, high, isolated line, ensuring that only perching animals are captured, as the AOI can exclude all chickens underneath. This implies that technologies capturing presence, such as RFID, would be reliable if they are properly configured to ensure the reading range exclusively covers the perch line in height, as done by [Wang et al. \(2019\)](#).

The less strong relationship between proximity and usage for drinker, feeder, and nutritional enrichments can be explained by the fact that chickens approaching these resources often interrupt usage episodes with other activities, or engage exclusively in other activities not directly related to the resource itself, such as remaining still, exploring the environment, resting, crossing the area, preening, and social interactions. This suggests caution when using presence data to infer effective use of those resources. Should presence-tracking technology be used, it would need to be complemented with additional

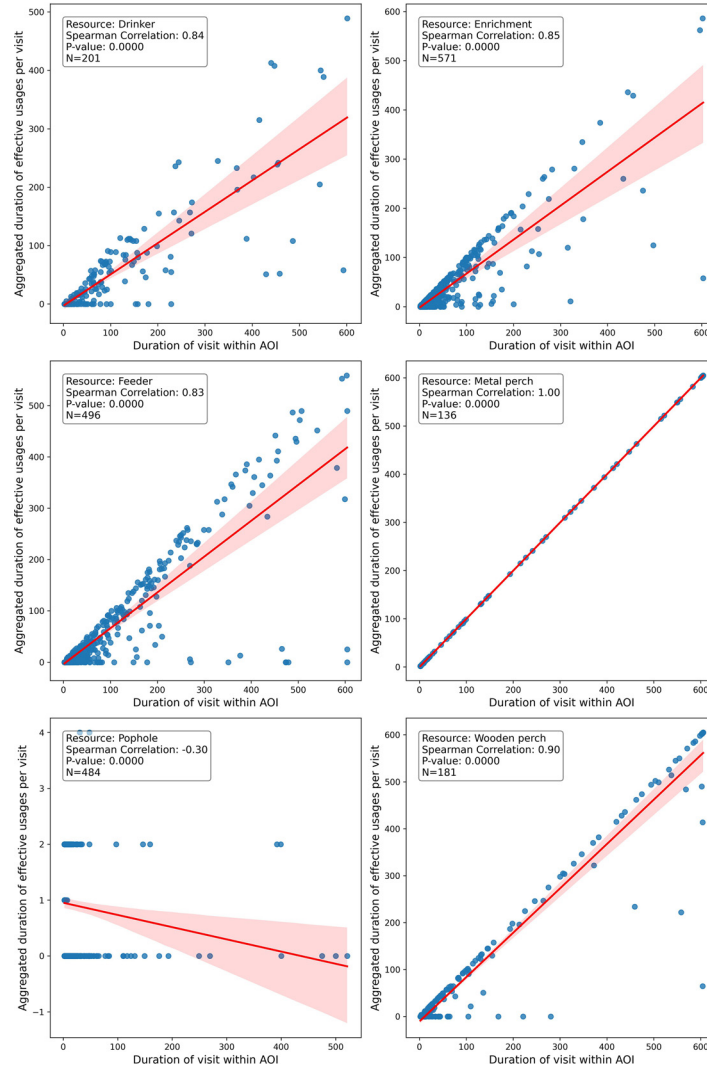


Figure 2. Correlation between the duration of visits in functional areas (i.e., Areas of Interest; AOIs) around key resources in the mobile barn and the aggregated duration of effective use within those visits. Each blue point represents a variable pair. The red line depicts the trend via a fitted regression line, while the pink band marks the 95% confidence interval.

methods to reliably provide information on usage. These complementary measures could include water level sensors and flow meters for water intake, as well as environmental modifications to better track resource use (Tang et al., 2021). Additionally, machine learning algorithms, such as You Only Look Once (YOLO) demonstrated by Nasiri et al. (2023), could significantly enhance the accuracy of feeding behavior measurements.

The weak relationship between presence and usage of the pophole is likely due to chickens quickly passing through it for usage, while their presence generally consists of spending long periods sitting in the passageway, as observed by Richards et al. (2011) using RFID. This finding shows that focusing exclusively on presence data, particularly from one side of the pophole, may not be reliable. To reliably capture pophole usage, a more thorough approach, such as monitoring both sides of the passageway, is needed. With RFID, this would necessitate antennas positioned on both sides of the pophole—one to log entries and the other to register exits—prior to confirming their passage, whether directly (Gebhardt-Henrich et al., 2014) or through a modified

pophole transformed into a tunnel equipped with RFID antennas at both the entrance and exit (Thurner et al., 2010). Alternatively, rather than solely monitoring pophole usage through tracking entries and exits, technologies could be employed to directly monitor animal presence in outdoor areas (e.g., winter garden).

Limitations and Future Research

This study has notable limitations that need to be addressed for broader applicability. We focused on a single small flock housed in a mobile barn, limiting the generalizability of the findings. Future research is needed to expand the scope to include larger sample sizes and multiple flocks. Additionally, it is essential to consider conditions representative of commercial husbandry systems, where higher density and limited space per animal prevail. In such systems, the relationship between mere presence and effective resource use may be even weaker due to mobility limitations and competition for space, leading birds to occupy any available areas

regardless of resource availability (Leone and Estévez, 2008).

Moreover, there was no exploration of how bird characteristics affected usage-to-presence ratios. The flock was idiosyncratic, with mixed breeds, ages, and sexes, which could have influenced individual and group behaviors. Understanding the dynamics within the flock, including social interactions, health status, and behavioral traits like aggression or fear levels, could provide deeper insights. Future studies should consider these factors to better interpret the relationship between presence and resource use.

Additionally, the way functional areas of interest are defined may impact the usage-to-presence ratios. In this study, functional areas were defined by marking areas where effective users were observed. However, other methods can also be considered. Future studies should statistically examine how different approaches, including variations in the size or shape of these areas, might affect the usage-to-presence ratios.

Lastly, while this research note discussed points particularly relevant for RFID and image-based tracking systems, similar investigations should be considered using other tracking technologies like accelerometers and thermal imaging.

Overall, this research note examined the relationship between the presence of chickens near key resources in a mobile poultry barn and their actual use. We showed that while presence detection can provide information about resource use, relying solely on presence data can overestimate actual resource use, particularly for certain resources such as feeders, enrichments, drinkers, and popholes. Our study highlights the need to systematically evaluate data collected from modern tracking technologies to ensure their validity and reliability in studying animal behavior.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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IV. Paper 3: Tag 'n' Track: Tackling the Validation Challenge in Animal Behaviour Studies Through Automated Referencing with ArUco Markers

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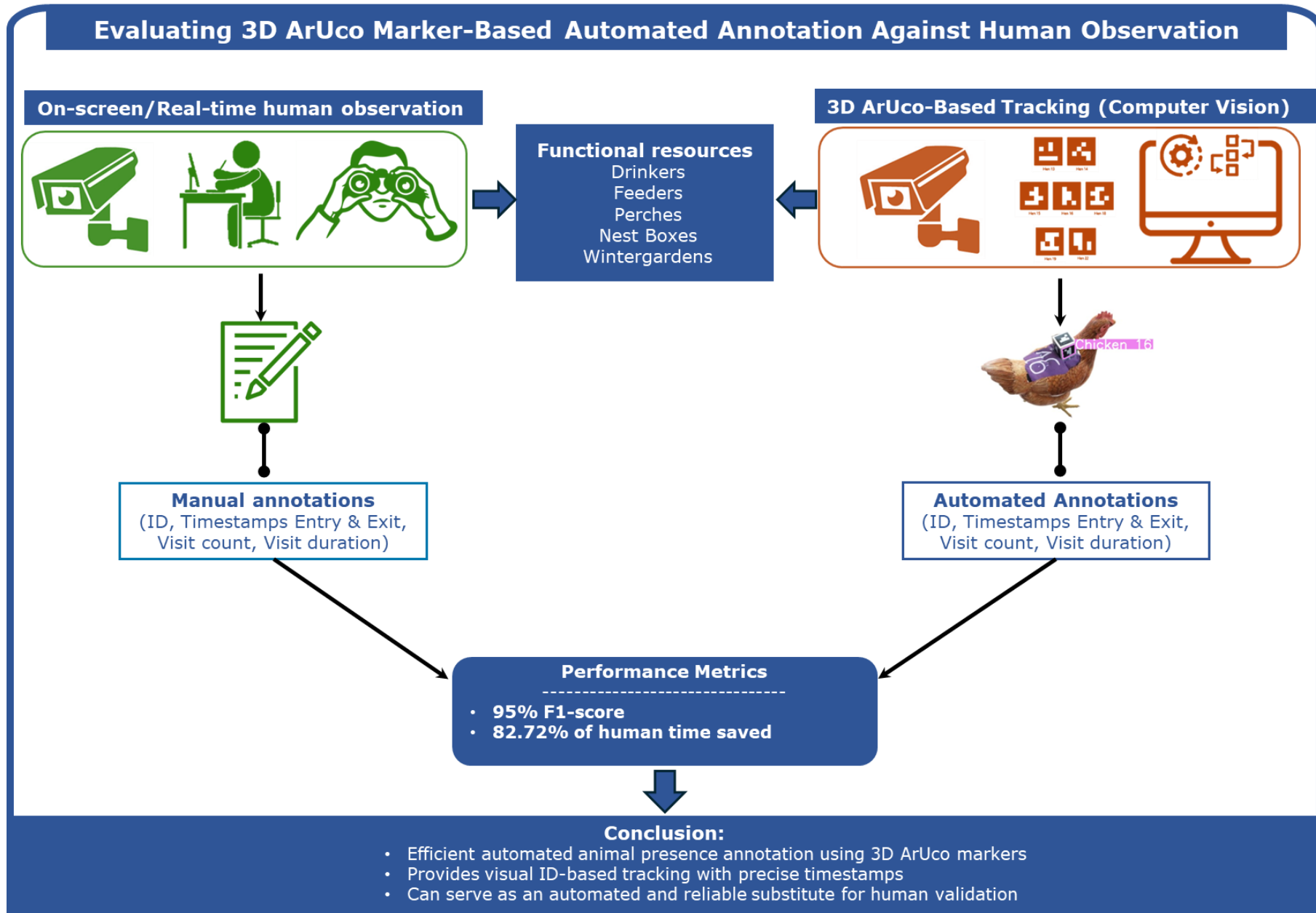
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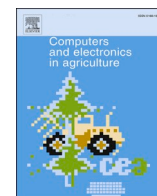
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Graphical Abstract Paper 3

Evaluating 3D ArUco Marker-Based Automated Annotation Against Human Observation





Original papers

Tag 'n' Track: Tackling the validation challenge in animal behaviour studies through automated referencing with ArUco markers

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ABSTRACT

Technological advances promise to greatly assist the study of animal behaviour, but the validation of these technologies is often neglected due to its tedious and labour-intensive nature. This paper addresses the challenges of manual annotation in validating technological tools for animal behaviour research. We detail the implementation and effectiveness of a computer vision method that automatically annotates animals within various regions of interest (ROIs). This method uses ArUco markers, open-source visual markers with a grid pattern, fitted onto vests worn by the animals. To validate this method, we used 245 10-minute videos capturing animals' visits to key resources, using a mobile barn housing twenty-one chickens. Our method generates annotated videos, revealing unique IDs of individuals and timestamps marking their presence in ROIs. Compared with traditional human observation, our method performed excellently: Spearman's correlation ($\rho = 0.96$, $p < 0.01$), 92.83 % sensitivity, 99.93 % specificity, 99.08 % accuracy, 98.77 % precision, and a 95.28 % F1-score. All recordings were annotated automatically in 40.96 h, saving 82.72 % of the time compared to the 222.84 h required for manual annotation. The proposed ArUco marker-based tracking method is easy to set up, based on open-source technology, and accessible to researchers without advanced programming skills. This method has the potential to replace or complement manual annotation, simplifying the validation of new technologies for automated individual tracking.

1. Introduction

How much can we rely on the emerging technologies that offer innovative approaches to monitoring animal behaviour? Given the proliferation of technological tools recently introduced to study animal behaviour, it becomes crucial to question their reliability and validity. The need for validation of such tools prior to an observational or experimental study, as Siegford et al. (2023) emphasised, should not be underestimated.

The study of animal behaviour, defined as the systematic observation of the coordinated responses of animals to various stimuli, whether internal or external (Levitis et al., 2009), is essential. Not only does it enable us to better understand animals' sensitivity and reactivity to their

physical and social environment, but it also aids the identification of essential factors that influence their preferences, welfare and productivity. In the study conducted by AHAW (2015) on perch design, a comprehensive analysis of individuals' behaviour revealed that various factors, including the material, shape, length, and width of the perch, exert a significant influence on hens' preferences and choices, emphasising the crucial role animal behaviour studies play in understanding their needs and optimising environmental conditions. This is an example of applied animal behaviour research informing policy and supporting the livestock industry. In fundamental research, careful observation of animal behaviour offers valuable insights into aspects such as social interactions (Collias and Collias, 1996; Gómez et al., 2022).

As more and more technologies are used in such essential studies, a

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problem emerges: some tools may provide incorrect information because their validity has not been adequately verified. Many researchers neglect this crucial step before adopting new tracking tools. For example, almost half of the studies using RFID to track poultry have not tested the tool's validity (Alindekon et al., 2023). This highlights significant concerns about the reliability of the data collected using these tracking technologies. It is difficult to ensure an accurate interpretation of animal behaviours without adequate validation — an assessment of whether a monitoring tool actually measures the behaviours it is supposed to measure in a reproducible and reliable manner.

The lack of thorough validation of animal behaviour monitoring tools can be attributed to the inherent labour-intensive nature of manual annotation by humans, either in real-time or on-screen, to evaluate the effectiveness of these tools. Manual annotation can be exhausting, requiring considerable labour, resources and time. Sales et al. (2015) manually analysed 256 h of video to validate an RFID technology, describing the experience as “time-intensive”.

Although manual annotation undeniably remains the benchmark for validation, its lack of practicality, sometimes rudimentary nature, and challenges have led us to propose an alternative method. This method is intended to substitute or complement manual annotation when constraints make it difficult or impossible. Our overall aim in this paper is to present an automated tracking technology based on visual markers that is open-source, reliable, and adaptable to many animal species with appropriate adjustments, requires no prior programming skills, and is intended to serve as a tool for validating other tracking technologies.

Among the tracking methods based on pattern recognition, ArUco markers are a popular option. They are widely used in various fields, such as video editing, augmented reality in cinematography, camera calibration (Čepón et al., 2023), robot navigation and localisation, autonomous vehicles (Blachut et al., 2022) and drones (Siki and Takács, 2021). ArUco markers are a simplified version of QR codes, characterised by their streamlined appearance (Fig. 1).

These markers have been specially designed to be easily detected and tracked, among other visual elements (Garrido-Jurado et al., 2014, 2016). As soon as a marker is identified during image analysis, the

algorithm provides the identifier, which can be associated with the identification timestamp or location (e.g., Eagan et al., 2022), enabling precise tracking and identification of the marked object (Wubben et al., 2019).

ArUco markers could encourage the validation of other animal-tracking technologies for a number of reasons. Firstly, their simplicity and accessibility are major advantages. Adopting ArUco markers does not require advanced programming skills; they are based on existing computer vision techniques (Garrido-Jurado et al., 2014, 2016). In addition, these markers are characterised by their accuracy, robustness and versatility. They can detect fine movements ranging from 0.1 to 0.01 mm (Siki and Takács, 2021). They remain effective even when the marker is tilted by up to 70 degrees (Ferrão et al., 2018; Koeda et al., 2018), or for fast-moving objects of up to 70 km/h (Blachut et al., 2022). Their size is not a constraint as long as they remain visible in the videos. They have been used successfully to track insects as varied as ants (Sclocco et al., 2021) and cockroaches (Othayoth et al., 2022).

Our paper has two specific objectives:

- (1) To detail the implementation of a 3D ArUco marker system for reliable automated annotation of animal presence within a region of interest; this can then be used to validate other animal tracking technologies that are typically used for tracking animal presence, as long as the 3D-marker does not interfere with their functionality. We assigned a unique ID to each animal by exploiting a pre-existing pattern identification system. Using chickens housed in a mobile barn as a model, we demonstrate the effectiveness of this method, particularly for analysing the use of key resources required to meet physiological and behavioural needs and to provide opportunities for improving welfare in poultry.
- (2) To validate the proposed 3D ArUco marker by comparing it with human annotation, widely recognised as a standard reference. Quantitative assessments were conducted to evaluate the performance of our proposed technique compared to human reference. These assessments included calculating key metrics such as sensitivity, specificity, accuracy, and precision. This second objective aims to establish a trustworthy reference for future studies and ensure the validity of our method. It is also meant to serve as a model or case study for other research, including validation efforts, reinforcing that the primary intention of this paper is to provide a reliable validation tool rather than a comprehensive behavioural study.

2. Materials and methods

2.1. Ethical considerations

The University of Rostock's internal animal welfare committee reviewed and approved the research protocol. The study commenced upon receiving the necessary approvals (No. AZ 7221.3-18196_23) from the ethics committee and the veterinary office in Mecklenburg-Pomerania, Germany.

2.2. Experimental setup

2.2.1. Animals, housing and management

Twenty-one chickens of mixed lines and sexes were included in this study. Four of them were of the Lohmann breed (1 white Lohmann Selected Leghorn and 3 of Lohmann Brown) and were 45 weeks old; the remaining consisted of 16 females and one male of the Vorwerk breed, a traditional dual-purpose breed of chicken, all of which were 68 weeks old. This diversity is essential to reflect lineage and sex variability in resource visits.

The study was carried out at the “Friedrich Harms” Animal Experiment Station of Rostock University in Dummerstorf, Germany, using a customised version of the mobile poultry barn ROWA 200 v4.0 (M&Z



Fig. 1. This shows what an ArUco marker looks like. This is a marker from a set to which ID = 1 has been pre-assigned. These patterns are made freely available from one of the many online ArUco marker generators to create markers with fixed IDs.

Manufaktur GmbH, Germany). The mobile barn has two areas: a main compartment and a winter garden.

The main compartment (6.04 m × 2.80 m × 2.00 m) was equipped with the wooden Europa Nest®, with six nests in two rows. These nests measured 28 cm × 38 cm × 25 cm × 50 cm (length × width × height1 × height2). The animals had access to two types of perches. One was a cylindrical metal rod, 2.25 m long, placed above the drinking line. The other was a two-tiered L-shaped perch made of pine wood and inclined at 38 degrees. Each level of this perch was 1.95 m long. The upper level was positioned 60 cm above the floor, while the lower level was 18 cm from it. The feeder was linear, made of plastic, measuring 20 cm × 200 cm, and accessible from both sides for the chickens. The drinker line comprised three bowls and 12 nipples. LED lighting has also been installed, programmed to provide 16 h of light daily, starting at 5:30 am.

The winter garden (6.04 m × 2.48 m × 2.00 m) was a semi-open compartment shielded by a partially transparent tarpaulin with netting. It was an indoor walking area with an outdoor climate that allowed the animals to perform natural behaviours like scratching while benefiting from natural light and fresh air and still being protected. The floor was covered with wood shavings. The winter garden was accessible through a 120 cm × 35 cm pophole with automatic opening and closing. The winter garden also featured an alfalfa bale, a tray with mineral blocks, scattered grit and oyster shells as supplements, and a box filled with a mix of zeolite rock powder and wood shavings for the chickens' dust baths. We occasionally scattered grains in the winter garden throughout the day to promote foraging behaviour.

The hens had access to 3 kg of feed (PANTO® LMK Legemehlkorn, Germany), distributed each morning manually. Water was available *ad libitum*. The animals had been vaccinated against Newcastle disease, and their well-being was visually checked every day. Poultry lice traps were set, and hen droppings were sampled regularly for parasite control. Production, health and environmental data were regularly monitored and amended as required. The winter garden was accessible to the chickens from 9:00 am to 8:15 pm. The animals were gradually acclimatised for 15 days to the main compartment, the winter garden and body-worn equipment.

Throughout this paper, we refer to seven resources collectively as “key resources”. These are: drinker, enrichments within the winter garden, feeder, indoor litter area, pophole allowing access to the winter garden, and both metal and wooden perches. These are considered as such because they are important for the chickens to meet their physiological and behavioural needs; they also offer them behavioural opportunities, enhancing their welfare. During data collection, the animals preferred laying eggs in the indoor litter area rather than in the provided nests. Consequently, we considered that area as a “key resource” over the actual nest. The listed key resources served as an effective model for our study because they are generally the focus of research where technologies are used to investigate poultry behaviour.

2.2.2. Animal-worn Equipment: 3D-ArUco marker and vest

Selection of ArUco Markers for Detection in Poultry Barn. We selected the ArUco DICT_4X4_1000 dictionary because it stood out compared to others (DICT_5X5, DICT_6X6, DICT_7X7) that we had tested beforehand with chickens. This dictionary offers 1000 distinct patterns, minimizing the risk of overlap in marker identification. The markers were easily detectable across a range of image resolutions, even when tracking fast-moving chickens running from the barn to the winter garden. Each ArUco marker consists of a grid of 4x4 squares, resulting in 16 small squares, or bits, which form a unique binary pattern. Additionally, it is open-source through OpenCV and accessible at <https://ch.ev.me/arucogen/>. For our study, we used the first 22 markers from this dictionary, excluding marker number 17 (see Appendix A) due to its tendency to cause frequent false detections during preliminary tests in our poultry barn. Marker 17 was too simplistic, and at times, the poultry barn features shared visual similarities with that marker's pattern, creating visual noise for the detection algorithm and leading to false

detections. This was particularly the case with the plastic slats with hollow white edges, which were sometimes mistaken for the pattern of marker 17.

Dimensions of ArUco Markers. We maintained a white buffer zone around the markers. This zone, measuring 0.2 cm on all sides, framed the unique pattern of the marker, making it easier to distinguish for automated detection. A sufficient buffer space ensured that the ArUco pattern stood out clearly and was readily detectable, minimising environmental interference or potential stains that could mislead the algorithm. Our markers, measuring 3.4 cm square (1:1 square aspect ratio), were printed on white paper before being laminated to ensure durability. With the buffer zone included, each marker covered a total of 3.8 cm per side. As for the size chosen, the challenge was to find a balance: large enough to be detected by the video processing algorithm but small enough not to disrupt the animal's natural behaviours. The size was also determined by the available space on the chicken's dorsal region—the area between the neck and the tail—where the vest could hold the marker securely without impairing movement. Several tests were carried out beforehand with various sizes to arrive at this effective compromise, ensuring detection while considering the anatomical constraints of the bird.

3D-optimised Design of the ArUco Markers. Although the flat 2D markers could be practical, they may have limitations in terms of the angle of detection. Therefore, we innovated with a 3D design, a cube-like marker (Fig. 2). This 3D-marker, with each face measuring 3.8 cm per side and the entire marker weighing 4.5 g, enhances detection by featuring the same ArUco pattern on each face. The cubic design would ensure that at least one marker was visible, regardless of the animal's orientation, covering almost every position and direction. It was made from durable laminated paper glued to a lightweight foam support. The effectiveness of this design is backed up by previous research from Eagan et al. (2022) and Vagvolgyi et al. (2022), suggesting that placing several copies of the same ArUco pattern on the target can maximise visibility and detection, thus ensuring continuous identification of the animal, regardless of its orientation.

Chicken Vests: Supports for both Automated Detection and Manual Identification. Specially designed chicken vests, High-Vis Chicken Jacket® (Omlet, UK), were used for two main functions. The first was the carriage of the ArUco 3D marker, strategically positioned in the centre of the vest, between the wings and at a roughly equal distance from the chicken's neck and tail. After preliminary testing, this location ensured optimum marker visibility during recording. The second use of these vests was as the primary means of identification, with a unique number written manually on each vest. This identifier, written with indelible ink, could be easily seen by the annotators when viewing the videos, allowing independent validation of the results of the automatic tracking system (Fig. 2). For the 21 hens, vests in three different colours — green, blue, and purple — were used. This colour diversity facilitated annotation, with each animal easily distinguishable, particularly when the vest's colour was considered in addition to the number written on the marker (Appendix A).

During data collection, the vests were worn discontinuously, removed after a maximum of 24 h, and then put back on the following day. Each hen was quickly fitted with a vest held in place by Velcro fasteners, ensuring a comfortable fit. The vests were mainly worn on days when the temperature was below 20 degrees Celsius. When the animals were fitted with the vests, IDs were randomly assigned to individuals, ensuring that the same subjects did not wear the same ID throughout the study; this approach ensured that the vest reading performance was not affected by the animal-specific routine.

2.2.3. Camera configuration and installation

Seven Axis network cameras, 4 of the M1135-E model and 3 of the M1135-E MK II model (Axis Communications, Sweden), were used for this study. With high-definition resolution, these cameras were equipped with a 2-megapixel lens (1920 × 1080) with a horizontal field of

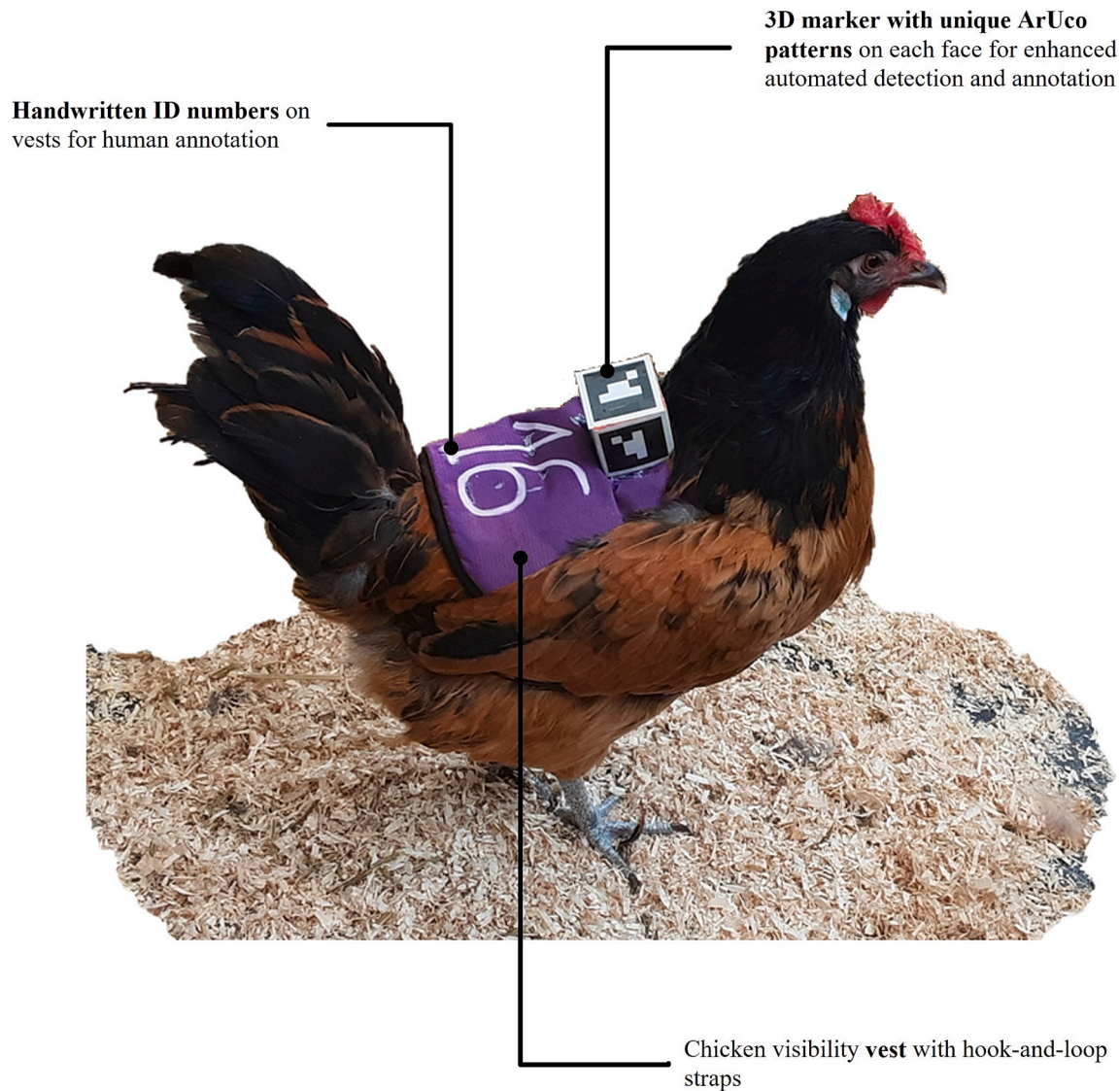


Fig. 2. A chicken from our validation study outfitted with a visibility vest with “16” as its handwritten ID number. On its vest, there is also a cube fitted with the pattern associated with ID 16 from the ArUco_4x4_1000 library, with that pattern displayed on all five visible sides of the cube.

view of 92 degrees. The lens’s focal length varied from 3.0 mm to 10.5 mm, ensuring wide coverage and vision of the area of interest. Both camera models had a light sensitivity of 0.1 lx. AXIS Camera Station software was used to set up the cameras.

All settings, from camera position to focal length, were adjusted before recording to ensure optimum image quality. To optimise the view and minimise blind spots, we fixed the cameras to the barn ceiling using suitable clamps, ensuring easy rotation thanks to an adjustable mount with a pivot range of up to 180 degrees. Due to the physical constraints of the environment, overhead positioning was not always possible; most of the cameras were tilted slightly to adjust the viewing angle. Each

camera was positioned at a specific distance from its target area, at a determined height above the slatted floor or litter, and oriented at a particular angle relative to the vertical axis. Details of the camera model and position measurements are presented in [Table 1](#).

The video was recorded using the H.264 codec in the MP4 format. We opted for high frame rates to capture the chickens’ rapid movements precisely. To put this into perspective, 30 frames per second video means we capture 30 successive pictures of a chicken moving through space, all within one second.

The camera system utilised Power over Ethernet (PoE) technology, which means they are powered directly through the ethernet cable. All

Table 1
Characteristics and positional measurements of cameras at key resources.

No.	Location of camera	Model	Distance	Angle	Frame rate
1	Drinker	M1135	1.18 m	35.86°	25f/s
2	Enrichment facilities	M1135	2.09 m	32.41°	30f/s
3	Feeder	M1135-E MK II	1.61 m	34.06°	30f/s
4	Indoor litter area	M1135-E MK II	1.95–2.50 m	19.34°	30f/s
5	Metal perch	M1135	1 m	49.46°	25f/s
6	Pophole	M1135-E MK II	2.277 m	31.57°	30f/s
7	Wooden perch	M1135	0.77–1.35 m	Overhead	30f/s

the cameras were interconnected via an ethernet network and linked to a central workstation computer.

2.2.4. Workstation computer configuration

For our study, we used a Lenovo ThinkStation P360 Tower computer. This is equipped with a 12th generation Intel® Core™ i7-12700 processor running at 2.10 GHz, a 16.0 GB DDR5 UDIMM memory (15.7 GB usable), an NVIDIA T600 video card with 4 GB video memory, a 512 GB M.2 SSD, and two hard disks with a capacity of 4 TB each.

2.3. Data acquisition and video sequence preparation

2.3.1. Video recording and sequence export

The recordings spanned five days and were conducted only when the animals were fitted with the body-worn equipment (Fig. 2). The video recordings were set to capture automatically, covering only the illuminated periods in the barn, typically from 5:30 am to 9:30 pm. All raw videos collected pertained to the seven resources of interest, capturing both the presence and absence of animals near these resources.

After collecting the raw videos, we split them post-recording into 10-minute sequences using Axis software. This splitting was done to simplify manual annotations and reduce the need to process long video stretches for automated analyses. The total number of manually segmented videos per resource was as follows: metal perch (45), pophole (45), drinker (72), wooden perch (72), feeder (80), enrichment (80), and indoor litter area (80). For each of the seven key resources, 35 sequences were randomly selected from the total segmented sequences, ensuring representativeness across the five-day recording period involving all 21 chickens. The selection process ensured that sequences were distributed across different times of the day—early morning, midday, and evening—based on their timestamps to account for varying lighting conditions.

2.3.2. Pre-processing of video sequences

Our video sequence pre-processing primarily involved delineating a Region of Interest (ROI), marked on each video to identify where most interactions between animals and a specific resource occurred. This ROI,

essential for accurate manual and automated annotations, encompassed the resource’s main functional area. We used Python’s OpenCV library (v4.8.0) to define these ROIs, drawing boundary lines around the resource’s functional zones. Defining the ROIs involved three steps (Fig. 3; Appendix B).

Starting with a static camera view focused on the resource, we used this consistent visual reference to extract images from our recordings, showing actual animal interactions with the resource. One randomly selected image served as a baseline for outlining the resource’s functional area. This process involved manually marking specific points on each image, such as the tip of a chicken’s tail or the position of a 3D-marker on chickens genuinely using the resource. These markings, capturing the primary area where animals interacted with each resource, considered behaviour-specific traits (Table 2). We then converted these points into coordinates, overlaying them onto our baseline image to sketch the ROI.

In the final step, the defined ROI from the baseline image was highlighted while deliberately blurring the peripheral regions. The delineated ROI was then superimposed onto each frame of the videos, producing videos where only the animals within the ROI were distinctly visible.

2.4. Manual annotation using BORIS: Reference or gold standard for validation

We employed human observation as a benchmark to evaluate the effectiveness of the proposed automated annotation technique in accurately identifying animal presence within regions of interest (ROIs). Two observers manually annotated all the pre-processed video sequences using BORIS (v.8.21.5).

Following a pre-established annotation protocol, the annotations were performed on pre-processed video sequences in which only the ROI was visible. The manual annotation of video sequences was based on the mere appearance within the ROI. It commenced when the midsection of the animal’s back crossed into or out of the ROI’s limits. In cases where the first criterion is not applicable, it started or ceased when both feet were fully positioned inside or outside the ROI, respectively. Here, the

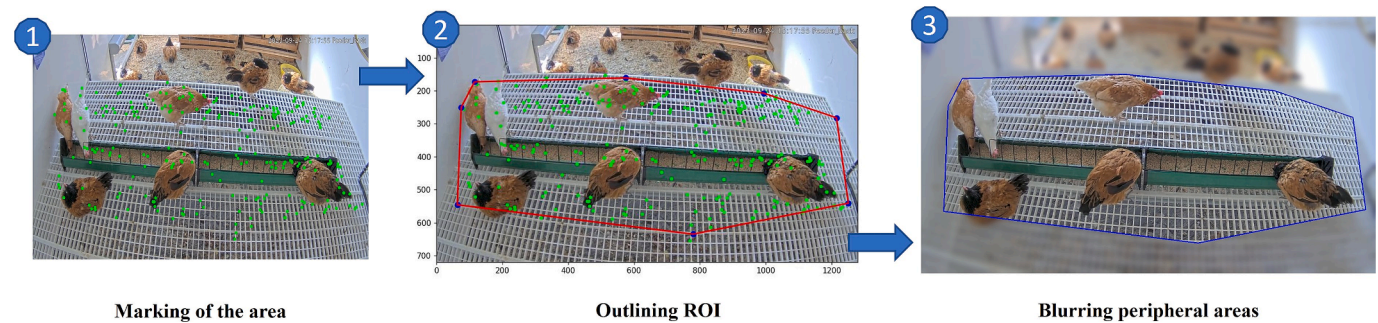


Fig. 3. Definition of Regions of Interest (ROIs), using the feeder as an example. In Step 1, the areas primarily occupied by animals using the resources are marked. In Step 2, an ROI that encompasses most of the marked area is manually outlined. In Step 3, the defined ROI is highlighted, and the peripheral regions are intentionally blurred.

Table 2
Behaviour-specific characteristics used for defining the various regions of interest.

Behaviours	Indicators of effective resource use
Feeding	The chicken is in a lowered head position with the beak in the feeder, pecking at the feed.
Drinking	The chicken is observed standing in front of the drinking line, either with its beak inside a drinking cup, at a nipple, or with its head raised, utilising gravity to ingest the water.
Perching	The chicken is observed sitting or standing on a perch line, using both feet.
Use of the indoor litter area	The chicken is observed standing, walking, scratching the ground, or lying down in the litter area of the barn.
Use of barn-to-winter garden pophole	The chicken passed through the access pophole, leaving the main barn compartment for the winter garden or returning to the barn.
Use of enrichment facilities	The chicken is observed near enrichment elements like the pecking stone, oyster shells, and alfalfa bales, either pecking directly at them or showing interest in nearby fragments on the floor.

specific behaviour of the animal (such as whether it was effectively using the resource or not) was not a determining factor. This ethogram is applied similarly across all resources. The annotators used predefined keys to start and stop the annotations. Handwritten IDs, visible on the videos, were used to track the subjects. Where necessary, the colour of the vest worn by the chickens was also considered to ensure correct manual annotations.

Measures were taken to guarantee reliability and inter-rater agreement. Before starting the actual annotation, a preliminary training phase was conducted, during which the two annotators were trained by an experienced researcher. This training involved coding sample video sequences and discussing the annotation protocol in detail. A sample of 27 video sequences, comprising 3 to 4 clips for each of the seven focus resources, was used for this preliminary phase. The aim was threefold: (1) to identify behavioural coding ambiguities using BORIS, (2) to practise scoring, and (3) to ensure excellent inter-rater reliability between the two annotators. The annotators agreed on the rules for annotation and practised coding before beginning the actual 245 video sequences. Data recorded via BORIS included the subjects' ID, start time, end time, and duration of presence within the ROIs.

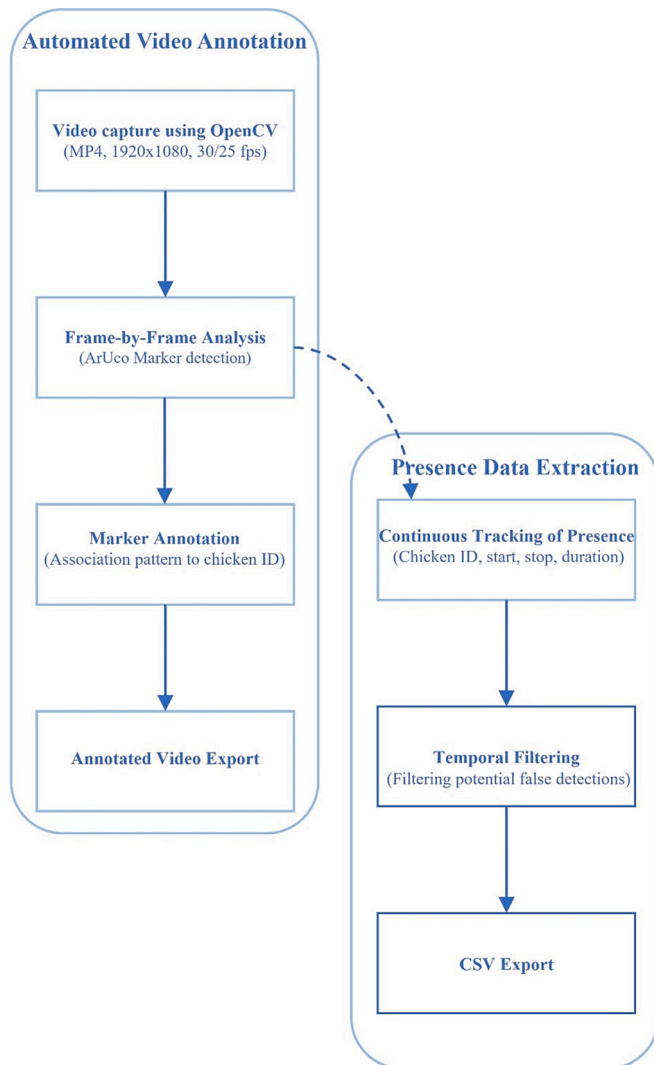


Fig. 4. Flowchart outlining the computational framework used for automated annotation of animal presence within Regions of Interest. Outputs included annotated videos displaying individuals annotated with their respective IDs and the CSV containing data on the presence of the detected individuals.

2.5. Marker detection and automated annotation

The “cv2.ArUco” module of the OpenCV (v4.8.0), a Python library for automated video processing and computer vision (OpenCV Developers, 2023), was employed. This library detected the ArUco markers. Pandas v2.0.3 (The pandas development team, 2023) was used to organise and export the data. Our computational framework primarily consisted of two steps: (1) video processing, which produced a video annotated with chicken ID, and (2) data extraction, resulting in a CSV file that listed the appearances of the various IDs (i.e., individual chickens) over time (Fig. 4).

The first step was to open the pre-processed video sequences with OpenCV’s “VideoCapture”. These videos, all in MP4 format with a 1920 × 1080 resolution and frame rates of 30 or 25 frames per second, and already pre-processed to highlight only the regions of interest, were our input. Each video was then analysed frame-by-frame. At each frame, the presence of ArUco markers was checked, and once detected, each marker pattern was associated with its predefined unique ID. We adapted the algorithm to filter relevant markers: only markers between the first 22 of the ArUco.DICT_4X4_1000 dictionary, except 17, should be read. Each detected marker was colour-framed to highlight it, and an ID, such as “Chicken 16”, was displayed alongside it. These annotated images were then compiled together to create the annotated video.

The second step, data extraction, was also based on frame-by-frame analysis. The extracted data involved the duration of each chicken visit. We inferred the presence of specific chickens at each instant using the known frame rate of the input videos and the number of successive video frames in which individuals appeared. For example, in a video at 30 frames per second, detecting an ID in one frame signifies a presence duration of 1/30th of a second, i.e., approximately 33.33 ms. Therefore, continuous presence in 100 successive frames equate to a total duration of presence of 3.33 s. The visit duration was determined by accumulating moments of presence across successive video frames. A visit was considered ongoing until there was an interruption—loss of continuous detection, either due to the chicken leaving the ROI or the marker becoming obstructed or undetected—exceeding one second without detection. At that point, we considered the visit event as interrupted and recorded a new visit event if the same chicken appeared again in the video sequence.

The data extracted was filtered before being exported as a final CSV output. ArUco marker detection can lead to false readings, sometimes detecting markers from random objects in the environment (Hurník et al., 2021). To address these issues, we employed temporal filtering, particularly because initial tests revealed frequent false readings in certain barn sections. Most errors occurred in specific frames 1, 2, and 32, regardless of frame rate (in fps). Therefore, filters were applied to these specific durations. For example, with a 30-fps camera, we excluded detections of exactly 0.033 s, 0.067 s, and 1.067 s; for 25 fps, 0.04 s, 0.08 s, and 1.28 s were omitted. The cause was likely due to instability in the video encoding process at the time of recording, where suboptimal buffering and encoding settings, particularly in compression and bitrate control, led to detection errors in specific frames. The data, including chicken IDs, start and end times, and duration, were saved in a CSV files for analysis.

2.6. Validation metrics for comparison of automated annotation vs. Human reference

Validation of a tool is the process whereby its measurements are compared with an established reference. This validation usually takes place over a short period and involves calculating various metrics to assess the tool’s performance. We employed two distinct approaches to evaluate the performance and validate the proposed automated annotation tool.

The first approach involved a correlation analysis focused on the aggregated presence duration per ID in each video sequence, as detected

by automated annotation, and the corresponding duration observed by human annotators. This approach, which only considered presence data, provides an insight into the agreement between the two annotation methods. We used Spearman's correlation for this analysis, as the aggregated durations were not normally distributed.

The second approach, involving the calculation of classic performance metrics, was inspired by the principles of validation as explained and illustrated by [Adrion et al. \(2020\)](#), [Alindekon et al. \(2023\)](#), and [Siegford et al. \(2023\)](#). In this approach to validation, where both presence and absence could be taken into account, several steps were necessary ([Fig. 5](#)). The first step involved chronologically aligning data from the automated annotation with the manually annotated reference, followed by a second-by-second comparison of both methods to determine classification outcomes (also known as confusion matrix elements):

- True Positive (TP): second when automated and manual annotations agreed on the presence of an individual within a specific region of interest.
- True Negative (TN): second when both methods agreed on the absence of an individual within a specific region of interest.
- False Negative (FN): second when the automated system missed an individual that the manual annotation had identified within a specific region of interest.
- False Positive (FP): second when the automated system reported an individual that the manual annotation had not identified.

Next, we standardised TP, FP, TN, and FN sums at the video sequence level, adjusting for varying chicken presence across resources by dividing these counts by the total presence duration per sequence. This standardisation was crucial to account for longer presence yielding more detection instances and to offset the tendency of animals to spend more time away from resources, as [Adrion et al. \(2020\)](#) and [Alindekon et al. \(2023\)](#) noted. Standardisation minimised bias, ensuring a fair comparison of classification outcomes across resources.

Then, for each video sequence, we computed classic validation metrics to evaluate the effectiveness of the proposed automated annotation tool. Metrics were calculated individually for each video sequence using the standardised TP, FP, TN, and FN counts. We considered 35 video sequences per resource, treating each sequence as an individual evaluation unit and as a separate replicate to assess the performance of the corresponding resource. Ultimately, the average of these metrics across units was used to characterise the resources. The performance metrics used were:

- Sensitivity, obtained by the formula: $TP_{std} / (TP_{std} + FN_{std})$. Sensitivity refers to the proportion of seconds when an ID was present within a given ROI and the system correctly identified it as such.
- Specificity, with the formula: $TN_{std} / (TN_{std} + FP_{std})$. Specificity assesses the ability of our automated approach to correctly identify seconds when a targeted ID is absent in the ROI.
- Accuracy, which was calculated as: $(TP_{std} + TN_{std}) / (TP_{std} + TN_{std} + FP_{std} + FN_{std})$. Accuracy determines the overall ability of the

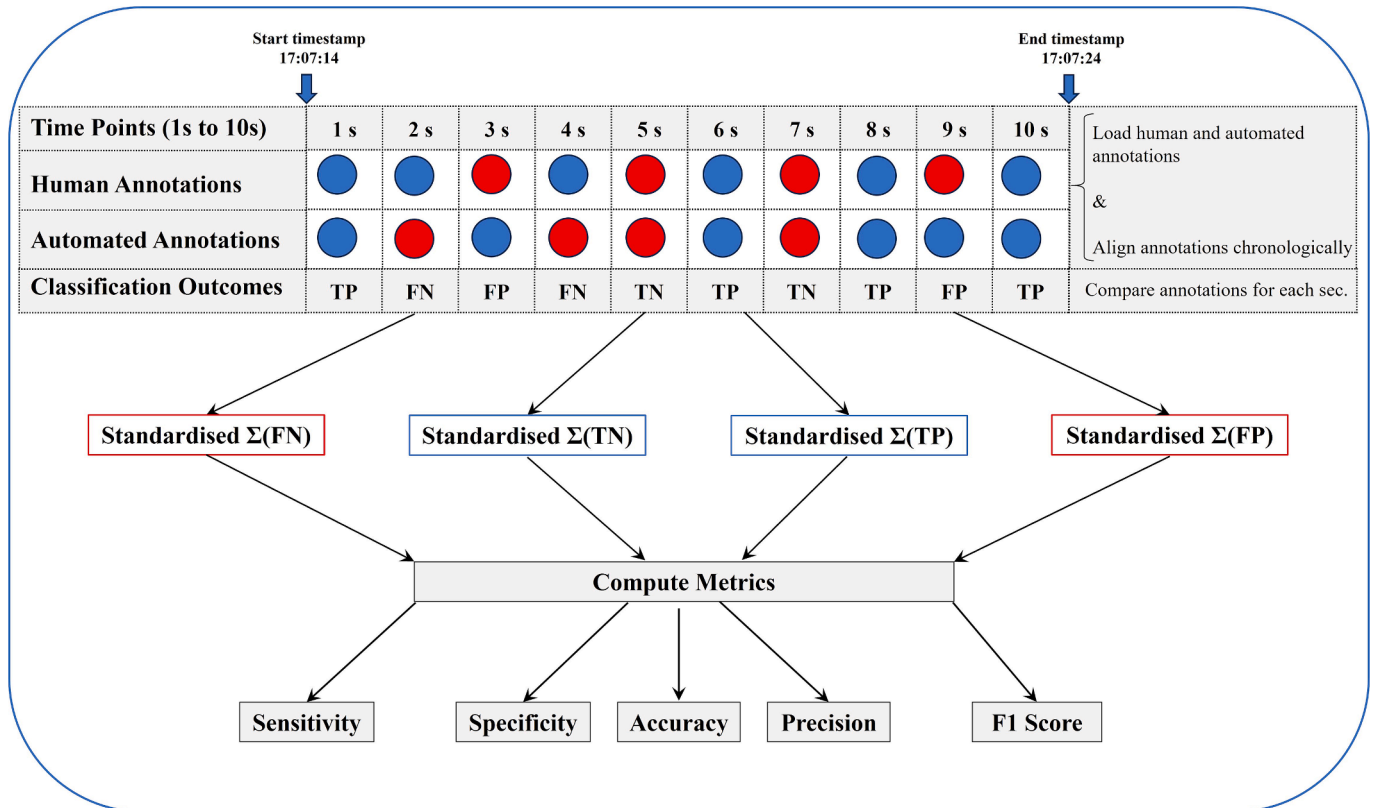


Fig. 5. Diagram summarising the procedure used to calculate the classic performance metrics employed to validate automated annotation against a manual reference over the time points that constitute a video sequence. Here, a 10-second-long video sequence is considered for illustration purposes. The comparison process begins with loading both datasets, aligning them chronologically, and comparing their annotations for each video second. Each circle represents the annotation for a specific second, coloured based on a given individual's presence (blue) or absence (red). Each comparison results in one of four outcomes (or confusion matrix elements): True Positives (TP), True Negatives (TN), False Positives (FP), or False Negatives (FN). Next, the standardised sums of TP, FP, TN, and FN that would account for the total duration of the chickens' presence in each video were calculated. In this illustration, standardised sums of TP, FP, TN, and FN can be respectively obtained as follows: $TP_{std} = 4/6$, $FP_{std} = 2/6$, $TN_{std} = 2/6$, $FN_{std} = 2/6$ (with the total duration of presence in the video, obtained from human annotation data, as the denominator). Based on these results, classic validation metrics—such as Sensitivity, Specificity, Accuracy, Precision, and F1-Score (balanced average of precision and sensitivity)—are computed to assess the performance of the automated annotation.

Table 3
Comparison of video annotation durations between our automated system and human annotators for each key resource.

Key Resources	Total Video Length (in hours)	Automated Annotation Duration (in hours)	Manual Annotation Duration (in hours)	Time Savings (%)
Drinker	5.86	5.69	19.17	70.32
Enrichment	5.85	4.69	32.50	85.57
Feeder	5.85	6.46	42.33	84.74
Indoor litter area	5.85	6.64	62.67	89.40
Metal perch	5.85	4.12	18.17	77.33
Pophole	5.85	4.49	32.00	85.97
Wooden perch	5.85	6.41	16.00	59.94

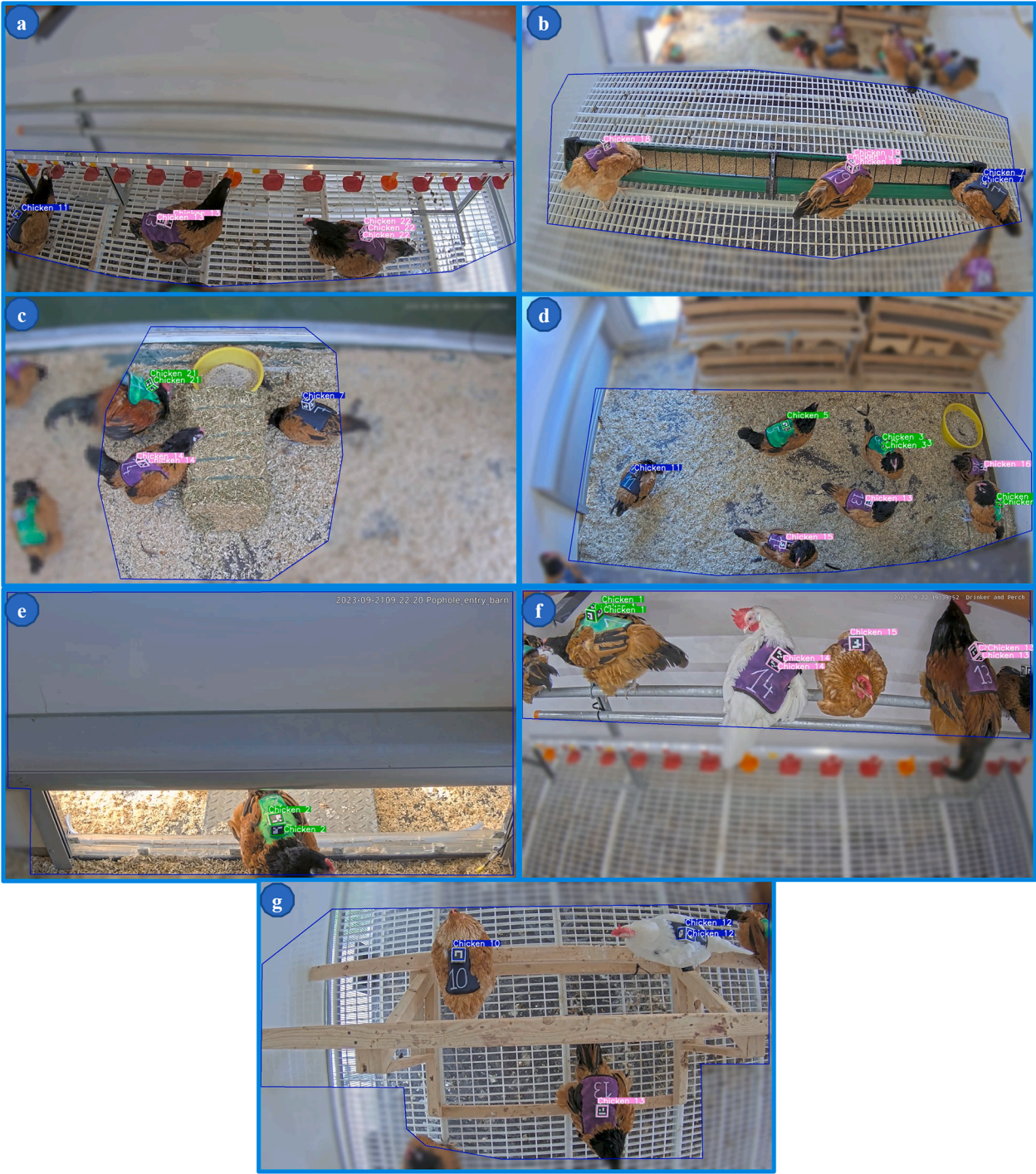


Fig. 6. Selected snapshots from video outputs after automated annotation of ArUco markers on animals present in resource-specific areas of interest—clearly defined, non-blurred zones delineated by thin blue lines. a = region of interest (ROI) around the drinker, b = ROI around the feeder, c = ROI around the enrichment material, d = ROI around the indoor litter area, e = ROI around the pophole, f = ROI around the metal perch, g = ROI around the wooden perch.

automated annotation tool to correctly identify seconds, whether the targeted ID is present or absent.

- Precision, calculated as: $TP_{std} / (TP_{std} + FP_{std})$. Precision quantifies the proportion of seconds identified as positive (i.e., ID present within ROI) that were actually correct.
- F1-Score, with the formula: $2 \times (\text{Precision} \times \text{Sensitivity}) / (\text{Precision} + \text{Sensitivity})$. This metric serves as a harmonic mean that balances Precision and Sensitivity, providing a single score to represent the reliability of the automated annotation tool in identifying chicken presence within the ROI.

Lastly, using Python libraries such as SciPy (v1.11.1) and Scikit-posthocs (v0.8.0), we conducted statistical analyses to explore the influence of various resources on standardised confusion matrix elements (TP_{std} ; FP_{std} ; TN_{std} ; FN_{std}) and derived performance metrics. The Kruskal-Wallis test was employed for these comparisons, as all data distributions exhibited either right or left skewness.

3. Results

We conducted this study to propose an automated method, based on 3D-ArUco markers, to automatically annotate animal presence within regions of interest, namely within functional areas of key resources in poultry farming. A total of 245 10-minute-long sequences of videos of chickens interacting with seven key resources were used. The automatic annotation made it possible to annotate all these video sequences in 40.96 h, an 82.72 % time saving compared to 222.84 h of continuous manual annotation by human observers in front of computer screens (Table 3). Validation of the automated annotation, carried out by two human reference annotators, with an index of inter-rater reliability—Cohen's Kappa, $k = 0.95$ (based on seven 10-min videos)—led to the establishment of performance metrics.

3.1. Output from the developed program

The developed program enabled automated annotation of individuals, generating videos in which individuals' IDs were displayed

alongside markers for all visible sides of the cube as long as they were within the region of interest. It offered behavioural data that quantified presence, indicating start and end timestamps, as well as the duration in seconds of each ID during its presence in the region of interest, and compiled these measurements into a dataset. To illustrate and demonstrate these stated capabilities, we present snapshots of some annotated videos in Fig. 6; short annotated video clips are provided in the Supplementary material (Clip 1 to 7), and Fig. 7 offers a summary of the duration data capturing the presence of markers in the region of interest in selected videos.

3.2. Performance evaluation of the automated system

3.2.1. Correlation automated vs human annotations based on aggregated presence duration

Spearman's correlation analysis on all resources revealed an overall correlation coefficient of $\rho = 0.96$ ($p < 0.01$, $N = 1457$). This was calculated with the aggregated duration of the presence of each ID in the videos, comparing automated annotations with human ones (Fig. 8).

When further broken down by resource, the lowest correlation was observed with videos recorded on metal perch ($\rho = 0.56$), while those for all others ranged from 0.92 to 0.99 (Table 4).

3.2.2. Standardised classification outcomes (or confusion matrix elements) across resources

The standardised classification outcomes varied according to resources (H-test, $p < 0.01$; Table 5). Feeder visit recordings showed the highest true positives, contrasting with enrichment, indoor litter, metal perch and pophole. True negatives peaked in pophole recordings and were lowest in the indoor litter area.

While false positives were generally low, they were highest in videos of wooden perch, drinker, and feeder—resources for which plastic slats appeared in the regions of interest. Conversely, indoor litter, metal perch, and enrichment videos had the fewest. Pophole videos presented a mix of these trends. False negatives were most frequent in metal perch, indoor litter, enrichment, and pophole videos, with metal perch notably higher in raw values. Feeder videos recorded the fewest false negatives.

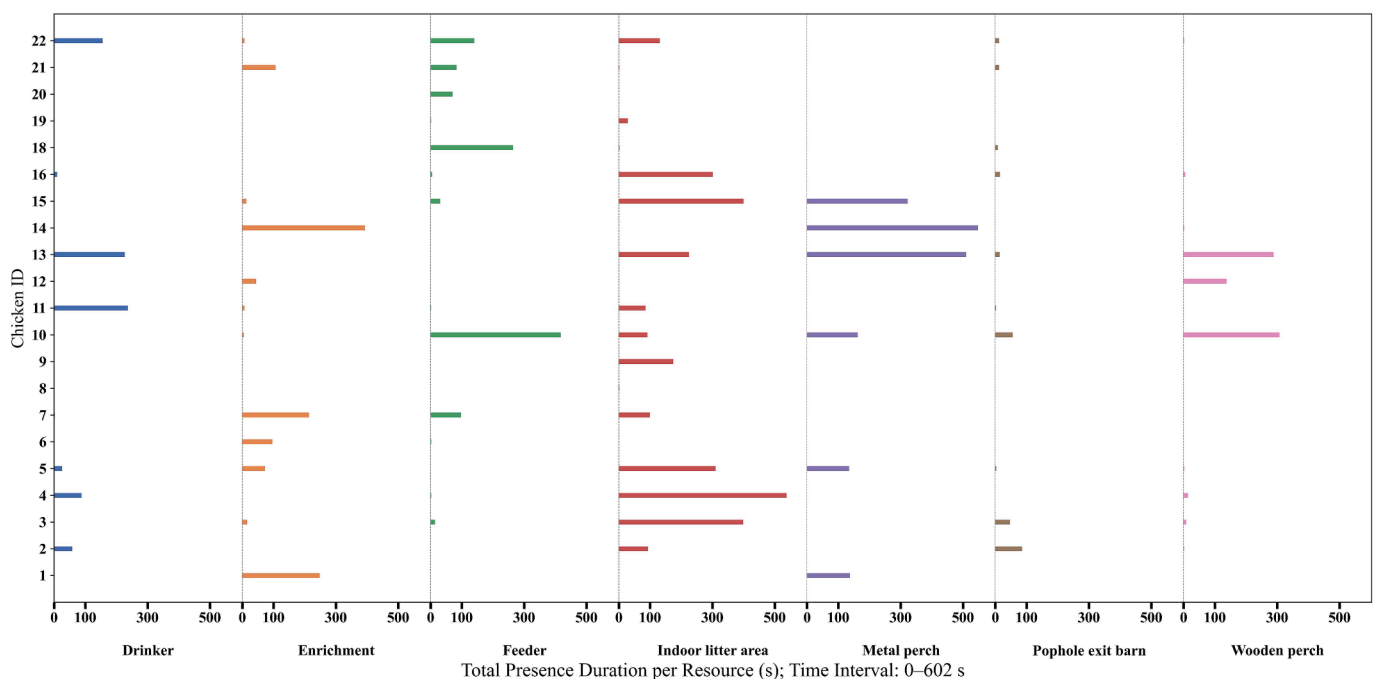


Fig. 7. An illustration of possible behavioural data output (aggregated visit duration for each ID at various resources of interest for a specific timespan). The data were generated along the annotated videos. Video sequences featuring the maximum number of observed individuals per resource were selected for optimal representation.

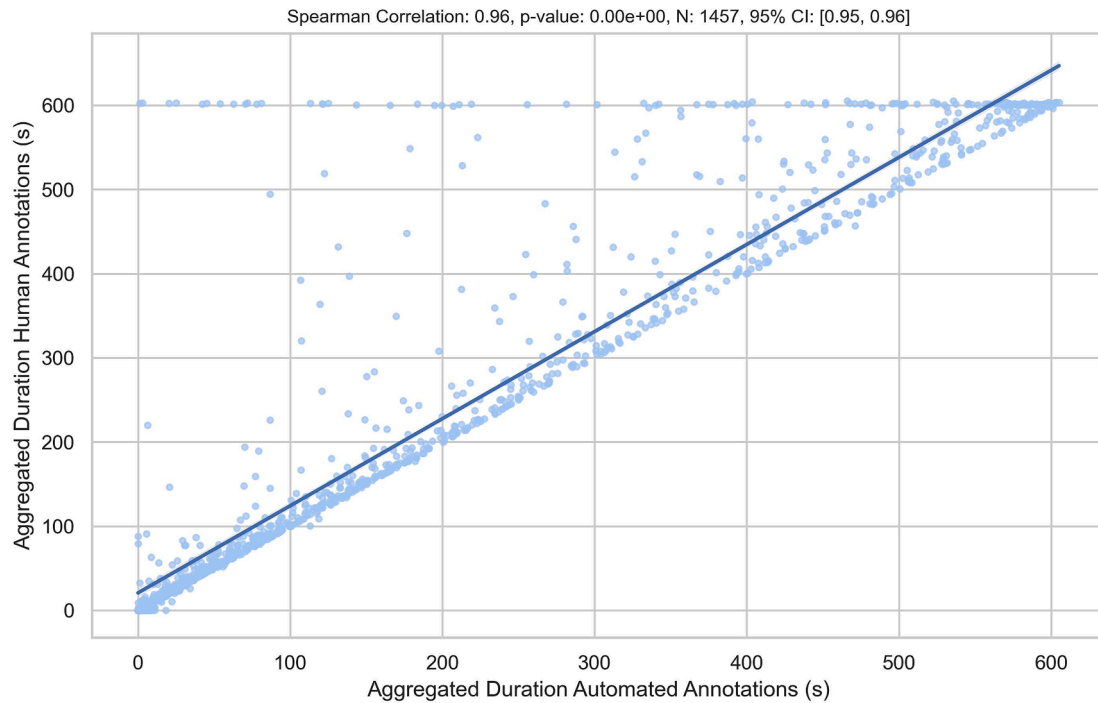


Fig. 8. Scatter plot illustrating the Spearman correlation between automated and human annotations. Light blue data points represent aggregated presence duration for each ID per video.

Table 4

Spearman correlation coefficients by resource based on aggregated presence duration per ID for comparing automated and human annotations.

Key Resources	Spearman's rho (r)	95 % confidence interval	p-value	N
Drinker	0.97	[0.96, 0.98]	< 0.01	141
Enrichment	0.98	[0.98, 0.99]	< 0.01	202
Feeder	0.98	[0.98, 0.99]	< 0.01	254
Indoor litter area	0.92	[0.90, 0.93]	< 0.01	380
Metal perch	0.56	[0.44, 0.69]	< 0.01	117
Pophole	0.99	[0.98, 0.99]	< 0.01	195
Wooden perch	0.94	[0.92, 0.96]	< 0.01	168

Examples of false positive and false negative cases are provided in [Appendices C and D](#), respectively.

3.2.3. Metrics-Based performance overview

The automated annotation method showed overall performance metrics of 92.83 % sensitivity, 99.93 % specificity, 99.08 % accuracy, 98.77 % precision, and a 95.28 % F1-Score, with significant variations

Table 5

Comparison of standardised values of the classification outcomes: True Positives (TP), True Negatives (TN), False Positives (FP), and False Negatives (FN), accounting for total durations of chicken presence in each video.

Key Resources	TP _{std}	TN _{std}	FP _{std}	FN _{std}
Drinker	0.95 ^{ab}	27.15 ^b	0.02 ^a	0.05 ^{ab}
Enrichment	0.95 ^b	24.84 ^b	0.00 ^b	0.05 ^a
Feeder	0.98 ^a	10.37 ^b	0.01 ^a	0.02 ^c
Indoor litter area	0.93 ^b	2.70 ^c	0.00 ^b	0.07 ^a
Metal perch	0.81 ^b	10.30 ^b	0.00 ^b	0.19 ^a
Pophole	0.93 ^b	108.11 ^b	0.02 ^{ab}	0.07 ^a
Wooden perch	0.96 ^{ab}	15.96 ^b	0.02 ^a	0.04 ^{ab}

In the table, 'a', 'b', and 'c' categorise groups by statistical significance, with 'a' indicating the highest group. Across columns, values with different superscripts are statistically different ($p < 0.05$).

Table 6

Performance metrics for evaluation of validity across resources.

Key resources	Sensitivity (%)	Specificity (%)	Accuracy (%)	Precision (%)	F1-Score (%)
Drinker	94.80 ^{ab}	99.92 ^{ab}	99.75 ^a	97.98 ^b	95.96 ^{ab}
Enrichment	94.55 ^b	99.97 ^a	99.60 ^{ab}	99.59 ^a	96.96 ^b
Feeder	97.63 ^a	99.89 ^b	99.70 ^{ab}	98.82 ^b	98.09 ^a
Indoor litter area	92.78 ^b	99.93 ^{ab}	97.76 ^c	99.82 ^a	96.10 ^b
Metal perch	81.29 ^b	99.97 ^{ab}	97.36 ^{bc}	99.62 ^a	88.32 ^b
Pophole	92.79 ^b	99.97 ^{ab}	99.71 ^{ab}	97.78 ^b	94.72 ^b
Wooden perch	95.95 ^{ab}	99.87 ^b	99.65 ^{ab}	97.77 ^b	96.79 ^{ab}

In the table, 'a', 'b', and 'c' categorise groups by statistical significance, with 'a' indicating the highest group. Across columns, values with different superscripts are statistically different ($p < 0.05$).

across resources (H-test, $p < 0.01$; [Table 6](#)). Feeder visit recordings had the highest sensitivity, in contrast to the enrichment, indoor litter area, metal perch and pophole, with the metal perch showing notably lower sensitivity at 81.29 %. A similar trend was seen in F1-scores.

Specificity was generally high, exceeding 99 % in all resources, with the lowest figures at the wooden perch and feeder; the differences stemmed from the decimals. The lowest accuracies, around 98 %, were recorded in metal perch and indoor litter area, while other resources exceeded 99 %. Precision values were lowest for wooden perch, pophole, drinker and feeder, ranging from 98 % to 99 %, compared to above 99 % for other resources.

4. Discussion

With the aim of promoting automation in the annotation of animal presence within a region of interest, which is crucial for validating and adopting new technologies in ethology, our paper proposed an automated annotation method based on 3D-ArUco markers attached to the

animals' backs. This method has been detailed and can be used to acquire annotated videos showing individuals' IDs as they move through a delimited region of interest (ROI), thought to represent the functional area of the resources used. It can also collect individual-focused behavioural data, including the start and end timestamps of each presence within the ROIs. The validation performance of this automatic behavioural annotation tool, compared with human observations, the limitations of the tool, and suggestions for its optimal deployment are discussed.

4.1. An interpretation of the system performance through multiple indicators

In our study, we used six key indicators to evaluate the quality and suitability of the proposed automated annotation method. This multi-dimensional evaluation is essential as it provides a more holistic and reliable assessment of the method's validity. Comparing the outcomes of our study with those of previous studies was challenging, as many authors do not consistently apply classic performance validation indicators. Nevertheless, we have attempted to draw analogies, which may be debatable, based on the calculation approach or aim of the metrics, between our performance measurements and those of other studies.

4.1.1. Spearman correlation

Comparison of the automated annotation against that carried out by human observers revealed a Spearman correlation of 0.96 for the total duration of presence per individual from each video. This value indicates a robust correlation (Martin and Bateson, 2007). Spearman's correlation measures the strength and direction of the relationship between two variables; it provides insights into the overall agreement between the two annotation methods. In practical terms, the observed correlation suggests that for each minute of video, the estimation of presence by the automated system closely matched the human annotations for at least 57 s, underlining its potential as a reliable tracker.

In comparison with the existing literature, our overall correlation analysis, aimed to assess the general agreement between two annotation methods, is in line with the approach, at least in terms of its purpose, of Intraclass Correlation Coefficients (ICC) used by Eagan et al. (2022). Our results corroborate with theirs (ICC of 80 % and 96 %) for validating ArUco markers attached to cats for tracking within regions of interest. Such corroboration is not surprising given our similar methodologies; we both used 4x4-bit markers and placed several unique markers on the same subject to improve detectability. Our method involved multiple markers on the sides of a cube, whereas theirs used multiple unique markers along necklaces. In addition, both studies operated with a high frame rate for video recording, approximately 30 frames per second. The importance of achieving high detection performance becomes evident when considering the use of a substantial number of frames and multiple unique markers on a subject.

4.1.2. Sensitivity

In our study, a sensitivity of 92.83 % was obtained, indicating high performance (Brown-Brandl et al., 2019). This metric evaluates the system's ability to identify actual presence correctly. Such a value implies that, during a one-minute period when tagged animals are within a region of interest, the automated tool would correctly detect them for at least 55 s, demonstrating its strong effectiveness in capturing actual presences within the region of interest.

Compared to previous literature, our sensitivity is higher than the 64.58 % detection rate reported by Alarcón-Nieto et al. (2018) after interpolation for ArUco-like markers attached to domesticated zebra finches. Aside from their subjects being smaller than ours, the discrepancies between our results may be due to our different approaches to mounting the markers. We used a 3D-marker design featuring multiple unique IDs with low potential for physical interaction, minimising marker damage and obscuration, thus improving the chances of

detection. The larger size of our markers (38 mm versus 10 mm for theirs) and perhaps the technical capabilities of the specific algorithms and marker dictionaries used, which these authors have not documented, could be other factors contributing to the divergence of results.

4.1.3. Specificity

Our validation study revealed a specificity of 99.93 %, reflecting a robust identification of true negatives: the automatic annotation method showed a significant ability to identify exactly what it is supposed to, without going beyond this objective (Martin and Bateson, 2007; Brown-Brandl et al., 2019). Specificity assesses the validity of a tool in recognising actual absences, which translates into approximately 59 s of accurate non-detection per minute. We have not been able to establish a link with previous literature related to ArUco-like tracking validation using specificity.

4.1.4. Accuracy

An accuracy of 99.08 % was achieved, a value considered excellent (Martin and Bateson, 2007). Accuracy indicates correct classifications of presence and absence, demonstrating the extent to which the system resists systematic error. In practice, the value obtained is equivalent to a margin of error of less than one second per minute, suggesting an exceptional confidence level for annotating tagged animals within a region of interest. We have also been unable to draw parallels with previous literature on ArUco-like tracking for this metric.

4.1.5. Precision

The proposed annotation method had a precision of 98.77 %, indicative of the system's high reliability in true positive detections (Martin and Bateson, 2007; Brown-Brandl et al., 2019). Such a value suggests only 1 s of false detection per minute of a tagged animal within a region of interest.

Compared to the literature, our overall precision is higher than that of Sclocco et al. (2021), who reported a precision of 38.53 % for ArUco tracking in ants. Precision reflects the proportion of true results among all cases examined. Technical differences at the hardware level might explain this variance. They used a lower frame rate than ours (10 versus 25/30 frames per second that we used). A higher frame rate could reduce motion blur, allowing a smoother and more continuous capture of subject movement and adapting more quickly to changes in speed and direction (Sclocco et al., 2021). Additionally, variations in the setup, among others, such as the characteristics of the cameras and the distance ratio between the camera and the surface area of the markers, probably contributed to this observed difference in performance. Moreover, their system relies heavily on a top-down configuration, while our introduction of a cubic marker offers increased visibility in the camera's field of view.

4.1.6. F1-score

Our automated annotation yielded an F1-score of 95.28 %, implying that the system correctly detected presences and excluded non-presences. F1-score represents a harmonious balance between precision and sensitivity. Such a value means that out of 100 detections, 95 are expected to be correctly identified, attesting to the reliability of the proposed automated annotation method. No parallel could be established with previous literature related to ArUco-like tracking using this metric.

In sum, the robustness and reliability of our system can be confirmed given the high performance (mostly > 95 %) demonstrated by the multiple indicators in our analysis. Each of these indicators, contributing its unique perspective, converges to validate the system's ability to annotate animal presence within a region of interest with high reliability.

4.2. Misreadings

Although not significantly affecting overall performance indicators, we observed that in our study, about 5 % of the performance decreases in our annotation system were attributed to misreadings. These errors can be categorised into two groups, namely reading failures (i.e., False Negatives) and false readings (i.e., False Positives), each having distinct causes. Some suggestions and practical arrangements to minimise misreadings when implementing 3D-ArUco markers for annotating animal presence within a region of interest are formulated.

4.2.1. Reading failures (i.e., false negatives)

For ArUco detection to function correctly, the black and white grids composing the marker and the surrounding white buffer zones must be completely visible and free of any occlusions to ensure precise decoding. Visibility issues with ArUco markers as causes of reading failures have often been mentioned in previous studies (e.g., Alarcón-Nieto et al., 2018; Eagan et al., 2022; Wolf et al., 2023). In our study, the highest rate of reading failures was observed with video recordings about the metal perch, followed by the indoor litter area, enrichment and pophole. Three major reasons for reading failures can be highlighted.

Firstly, except for the metal perch, occasional blurriness was observed, particularly in the focus region around most of these resources, where the camera distances were the greatest, ranging from 2 to 2.5 m, compromising image clarity. For the metal perch, there was notably poor camera arrangement with an oblique angle of nearly 50 degrees, limiting visibility from above while allowing only frontal visibility of one side of the cubic marker when the chickens were perching.

Secondly, physical interferences also led to false negatives. These reading failures occurred when chickens adopted stationary positions where the marker became partially or completely obstructed. These obstructions occurred when the birds' feathers masked the marker, e.g., during (night-) daytime perching or preening; they also happened when any other housing component or animal obstructed the camera's view.

A final factor contributing to visibility issues could be related to variable lighting conditions and or direct exposure of the markers, causing reflections that hindered the precise detection of the pattern during processing (Alarcón-Nieto et al., 2018; Crall et al., 2015; Sclocco et al., 2021). We experienced such cases, which are problematic when the animal remains stationary, especially in the indoor litter, pophole or enrichment areas, which are the most exposed to daylight.

4.2.2. False readings (i.e., false positives)

The false readings, referred to as false positives, are misreadings that can result from confusion between the random patterns in the environment and those of the markers (Hurník et al., 2021). In our study, we observed such erroneous readings mainly during the processing of recordings from the drinker, feeder, and wooden perch, and this was anticipated, as these resources were placed on slatted plastic with white edges and hollow patterns that naturally mimic ArUco marker. False readings only happened on rare occasions and only lasted for the duration of a sudden lighting variation, light reflections, glare, motion blur in environment components, and other unidentifiable causes. Despite the implementation of temporal filtering to discard short-lasting false detections, as well as the deliberate selection and reading restriction of the tags on which our developed algorithm should operate, eliminating these errors completely remained seemingly unattainable in those areas of the poultry barn.

4.3. Other limitations of the study

Three limitations, particularly about the methodology, can be noted in this study. One is the loss of the absolute scale of the classification outcomes (i.e., elements of the confusion matrix); these were transformed into ratios relative to the duration of presence. We opted for standardised values rather than absolute counts to allow fair comparison

across resources, as chicken presence is over-represented in some resources compared to others. Besides the loss of absolute scale, standardisation assumes a uniform visit of resources (i.e., a homogeneous distribution across all standardised segments) and focuses on average behaviour, assuming a constant level of chicken activity at the resources. Given that the main objective of our study was to validate the use of the ArUco marker and not to focus on the behaviours per se, the assumption of a uniform distribution of resource visits did not affect the achievement of our aim.

The second limitation lies in the risk of inaccurately estimating metrics such as accuracy and specificity. It is possible to observe artificial inflation of these metrics because, in their calculation formulas, negative cases — situations where the chickens are not at the resource — are a determining factor influencing the result, although our standardisation can reduce this influence to a certain extent. Adrion et al. (2020) and Alindekon et al. (2024) highlighted that the number of negatives in animal behaviours is generally very high, with animals spending more time absent than present at the resource. The other performance metrics, such as Precision, Sensitivity, and the F1-Score, are nevertheless less susceptible to such an imbalance in the dataset.

The third limitation of our proposed automated annotation method lies in its inability to distinguish actual resource use from mere presence in the region of interest. Our marker-based tracking system, as we propose it, does not allow us to determine for certain if an animal is genuinely using a resource, but it can confirm with more than 95 % certainty if the animal is in the defined region of interest around the resource. This constraint is similar to that of RFID technology, which also indicates if an animal is in an area of interest but cannot confirm if the animal is actually using the resource (Alindekon et al., 2023). To annotate with certainty genuine resource usage, one must use equipment capable of doing so. For example, for drinker use, a device equipped with water flow meters to measure each animal's water consumption, as in Maselyne et al. (2016); for perch use in chickens, a system that measures the use of perches using a load cell module recording the weight of the animals as in Wang et al. (2019). Advanced technologies, such as those incorporating machine learning algorithms (e.g., Guo et al., 2022; Liu et al., 2023), may also be employed to gain insights into actual resource use.

4.4. Practical insights on applying the 3D-ArUco system for validation

4.4.1. Minimising misreads

Several practical adjustments are required to minimise reading failures and improve the readability of ArUco markers. Instead of laminated paper, non-reflective, 3D-printed supports can be used for better effectiveness. Regular cleaning and maintenance are necessary to ensure optimal performance over time. Camera placement should also be tailored to the behaviours of interest. For static behaviours like resting or sleeping, an overhead camera works best, while in areas with dynamic movements, such as near feeders or drinkers, cameras should be angled at 35 degrees and placed about 1.6 m high.

To prevent false readings, it is crucial to use distinct marker patterns that are not easily confused with the surrounding environment. Conducting a preliminary analysis without markers can help identify potential environmental patterns that might cause algorithmic errors, allowing researchers to choose the most suitable markers for their study.

4.4.2. Cross-validation tool applicable to various technologies

Our 3D-ArUco system can be especially valuable for validating other wearable tracking technologies, provided it does not interfere with their functionality. For example, this could apply to body-worn trackers like RFID, which, like the proposed system, tracks animals and generates spatiotemporal data on their movements. By cross-referencing data from the 3D-ArUco system, researchers can verify the accuracy of RFID outputs.

However, the use of 3D-ArUco markers may present certain

challenges, particularly when validating technologies that integrate vision-based tracking systems. These systems often use object detectors (e.g., bounding boxes; [Siriani et al., 2022](#)) to locate individual animals and associate their identities across frames. Problems can arise when two 3D-ArUco markers fall within the same bounding box, potentially leading to identity confusion or tracking inaccuracies. This limitation can also occur in segmentation-based and keypoint-based tracking systems, where overlapping markers or animals may complicate identity association.

4.4.3. Adapting the 3D-ArUco system to different species and environments

While this study focuses on poultry, the adaptability of the 3D-ArUco system makes it suitable for various species and environments. Researchers must customise the system based on species-specific factors like marker placement, material durability, and environmental complexity. For example, robust materials are essential for pigs, known for biting and potentially damaging markers. For cattle, which may dislodge vests, using multiple identical ArUco markers on waterproof supports attached to collars, as demonstrated in [Sadrazadeh et al. \(2024\)](#), can be a viable alternative to vests or 3D markers. The successful use of body-worn devices, such as accelerometers and GPS trackers, enduringly fixed to animals like sheep and waterbirds ([Kölzsch et al., 2016](#); [Ikuriot et al., 2021](#)), suggests that 3D markers could similarly be securely affixed for these species. Since the 3D-ArUco system is intended for short-term validation, it offers flexibility and can be tailored to meet the needs of different species and research objectives effectively.

5. Conclusions

This paper has detailed the implementation and demonstrated the effectiveness of a computer vision method employing cubic ArUco markers for validating animal tracking technologies. The method showed high validation performance in automated annotation of presence within regions of interest and significant time savings for humans, offering a precise and reliable alternative to visual observation. This can reduce observer fatigue, bias and physical discomfort in validating other emerging technologies. Furthermore, the method utilises pre-existing open-source pattern recognition techniques, simplifying its use. This eliminates the need to develop complex new algorithms, making it accessible to a broad range of researchers. This paper demonstrates that introducing reliable technological solutions can support and significantly improve animal behaviour research.

CRediT authorship contribution statement

Serge Alindekon: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jana Deutsch:** Writing – review & editing, Methodology, Investigation. **T. Bas Rodenburg:** Writing – review & editing. **Jan Langbein:** Writing – review & editing. **Birger Puppe:** Writing – review & editing, Resources. **Helen Louton:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendices and Supplementary Data

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Data availability

Data will be made available on request.

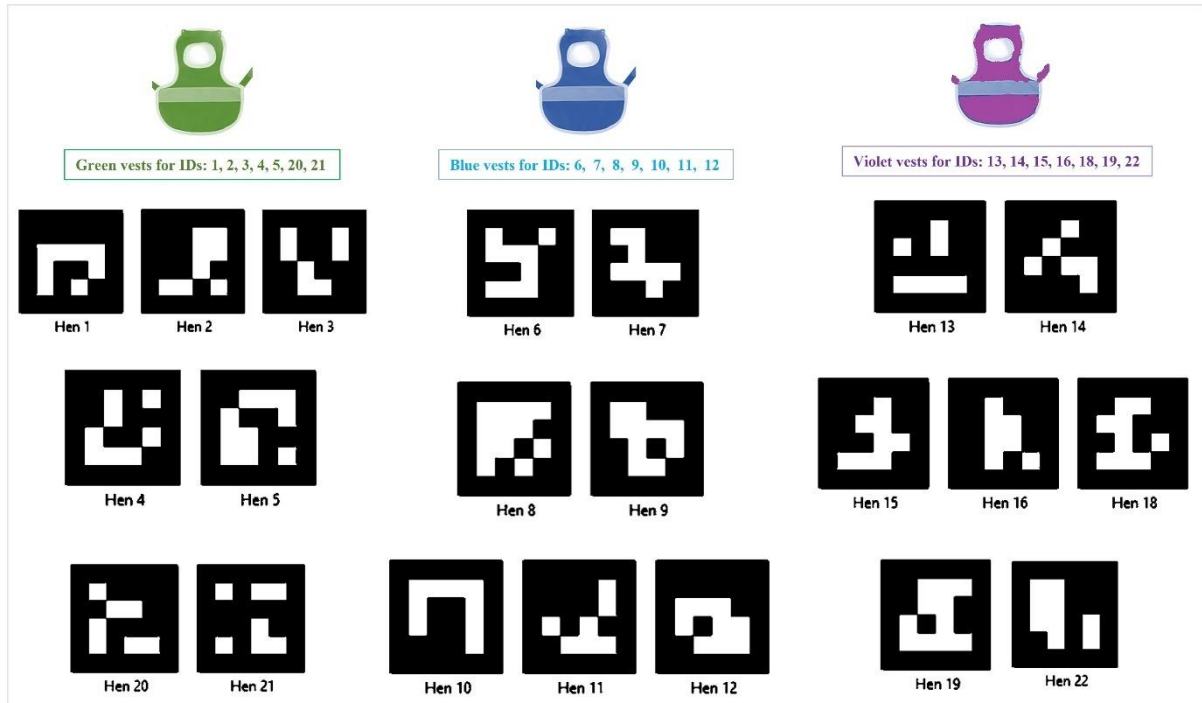
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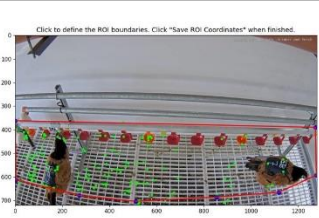
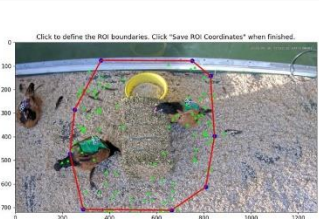
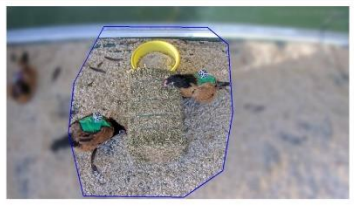
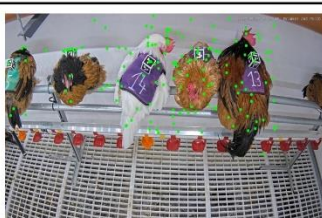
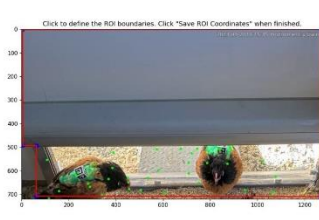
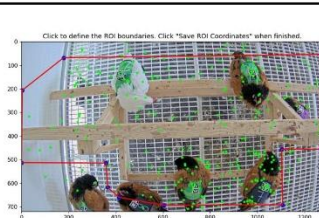
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APPENDICES

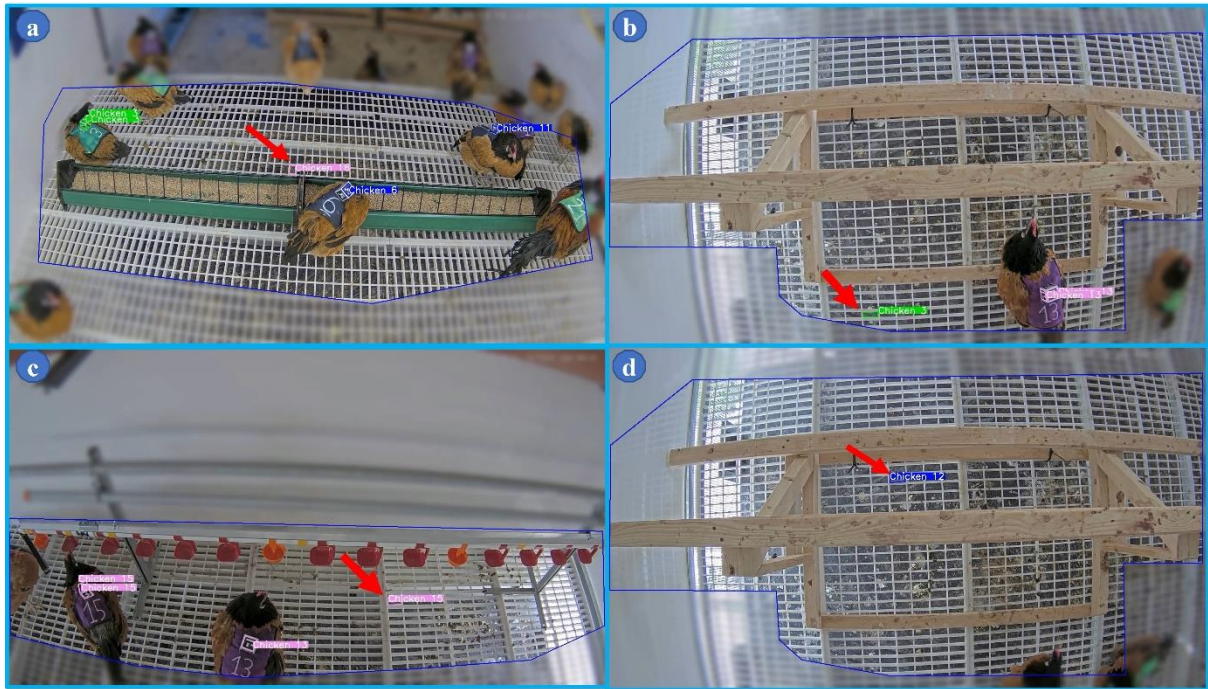
Appendix A: detailed overview of ArUco markers with corresponding IDs and the associated coloured vests worn by chickens in the validation study. Markers can be downloaded from <https://chev.me/arucogen/>



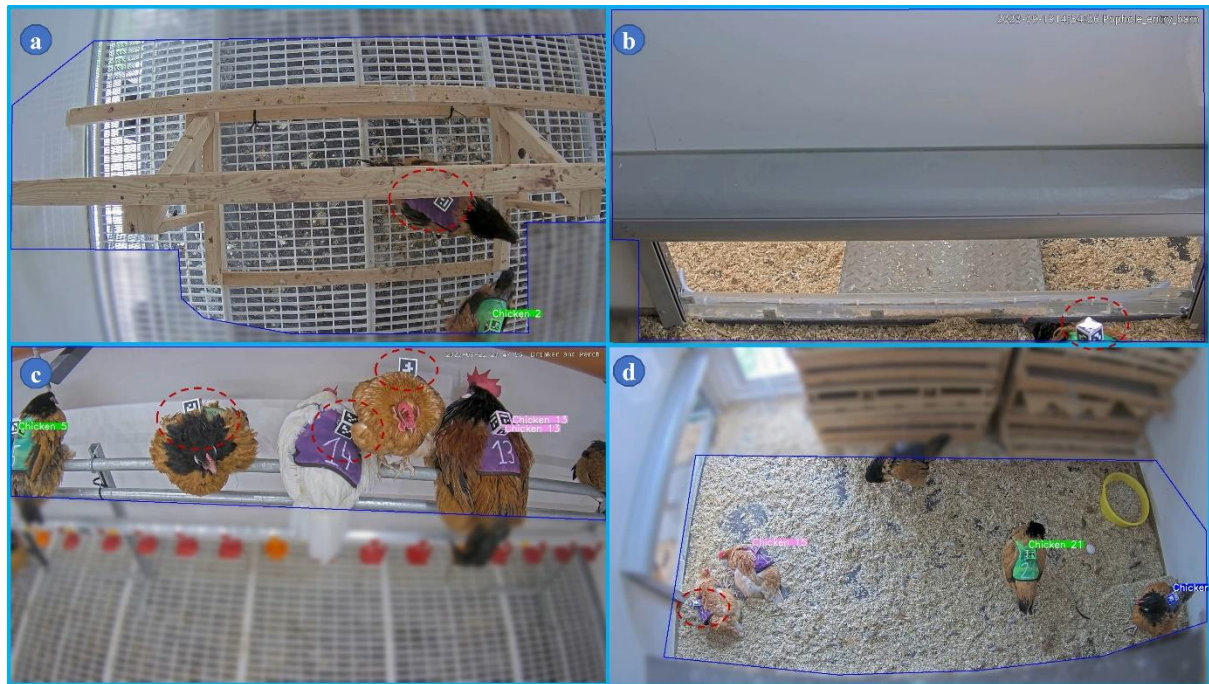
Appendix B: Images illustrating the outcome of each step in defining the regions of interest (ROI) for resources, excluding the feeder

	1 Marking of the area mainly occupied by animals using the resources	2 Outlining manually a Region of Interest (ROI) that covers most of the marked area	3 Blurring peripheral areas on video sequences to be annotated
Drinker			
Enrichment			
Indoor litter area			
Metal perch			
Pophole			
Wooden perch			

Appendix C: Examples of False Positive cases — although rare and lasting only 1.30 ± 2.17 seconds ($N = 164$ cases). The red arrows indicate those cases in the figure. Case (a) demonstrates such an occurrence in an annotated video sequence at the feeder, cases (b) and (d) in videos of the wooden perch, and case (c) in an annotated video sequence related to the drinker's region of interest.

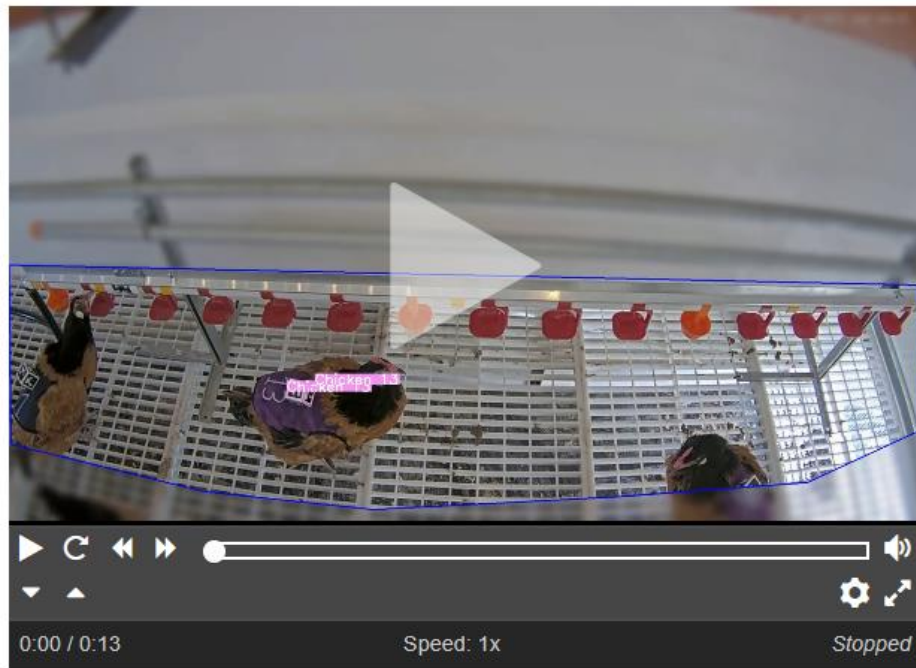


Appendix D: Examples of False Negative cases, highlighted by red dashed circles. Case (a) indicates such an occurrence in an annotated video sequence of the wooden perch, Case (b) in one video of the pophole, and Case (c) in a video sequence related to the metal perch. Case (d) shows such an occurrence within the indoor litter area.



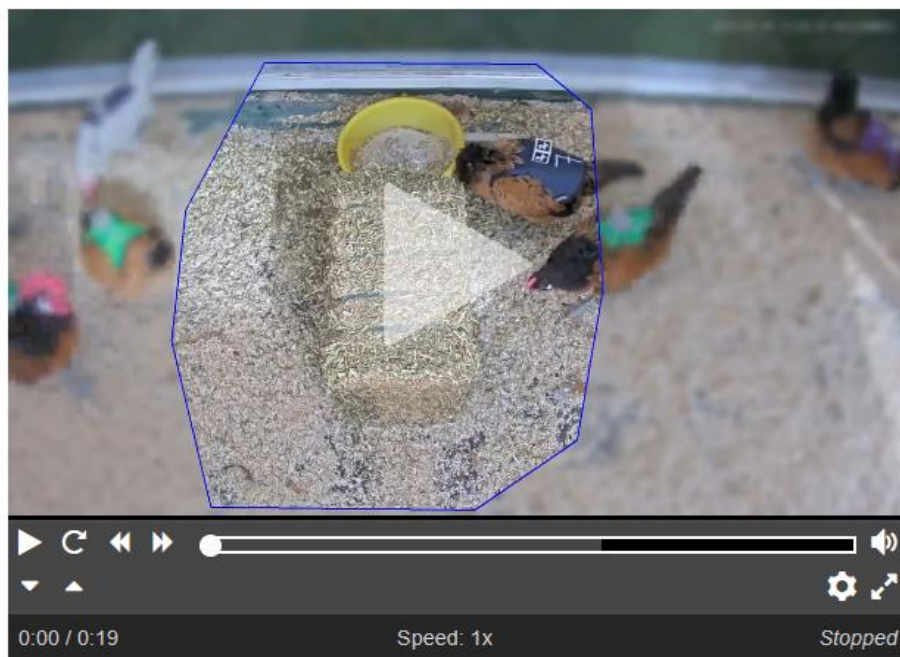
SUPPLEMENTARY DATA

1. Supplementary video 1.



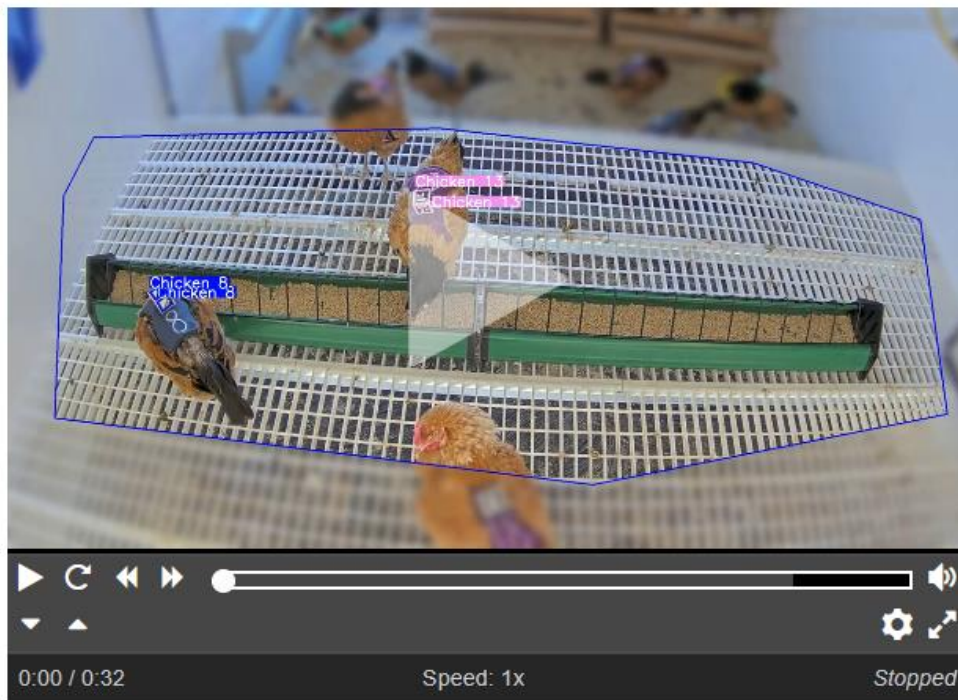
[Download video \(1MB\)](#)

2. Supplementary video 2.



[Download video \(6MB\)](#)

3. Supplementary video 3.



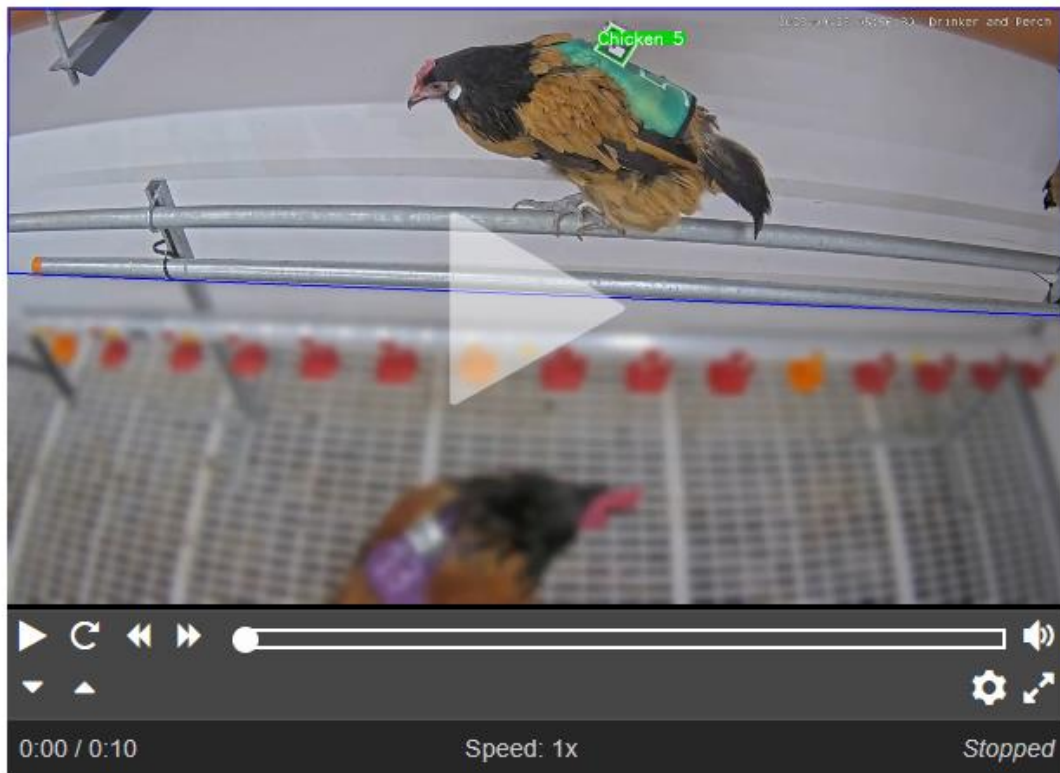
[Download video \(4MB\)](#)

4. Supplementary video 4



[Download video \(2MB\)](#)

5. Supplementary video 5.



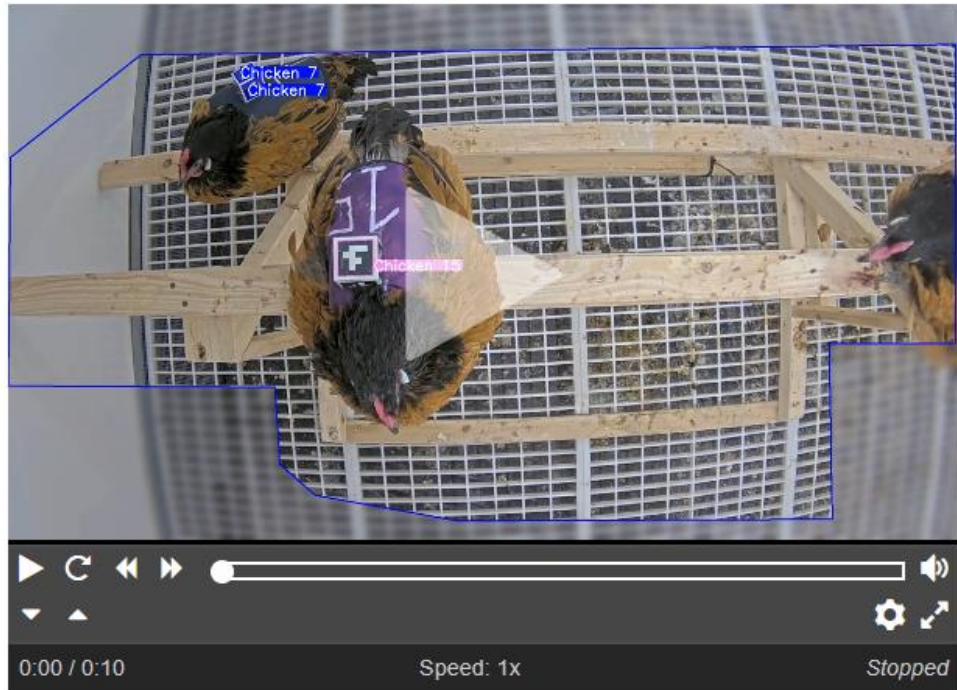
[Download video \(2MB\)](#)

6. Supplementary video 6.



[Download video \(1MB\)](#)

7. Supplementary video 7.



[Download video \(1MB\)](#)

V. Paper 4: Development and Computer-Assisted Validation of a Radio Frequency Identification System for Tracking Individual Chicken Visits to Functional Areas

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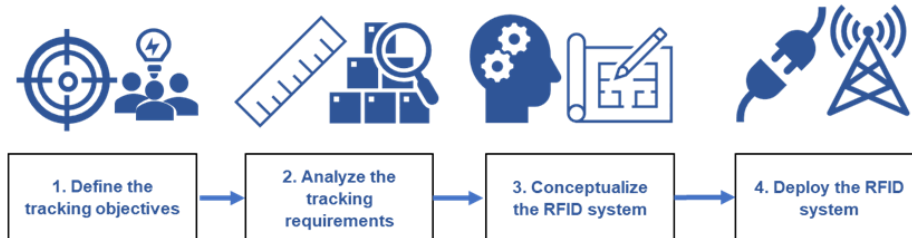
DOI: <https://doi.org/10.1016/j.psj.2025.105627>

Submitted, 23.04. 2025/ Accepted July 30, 2025 / Published: July 31, 2025

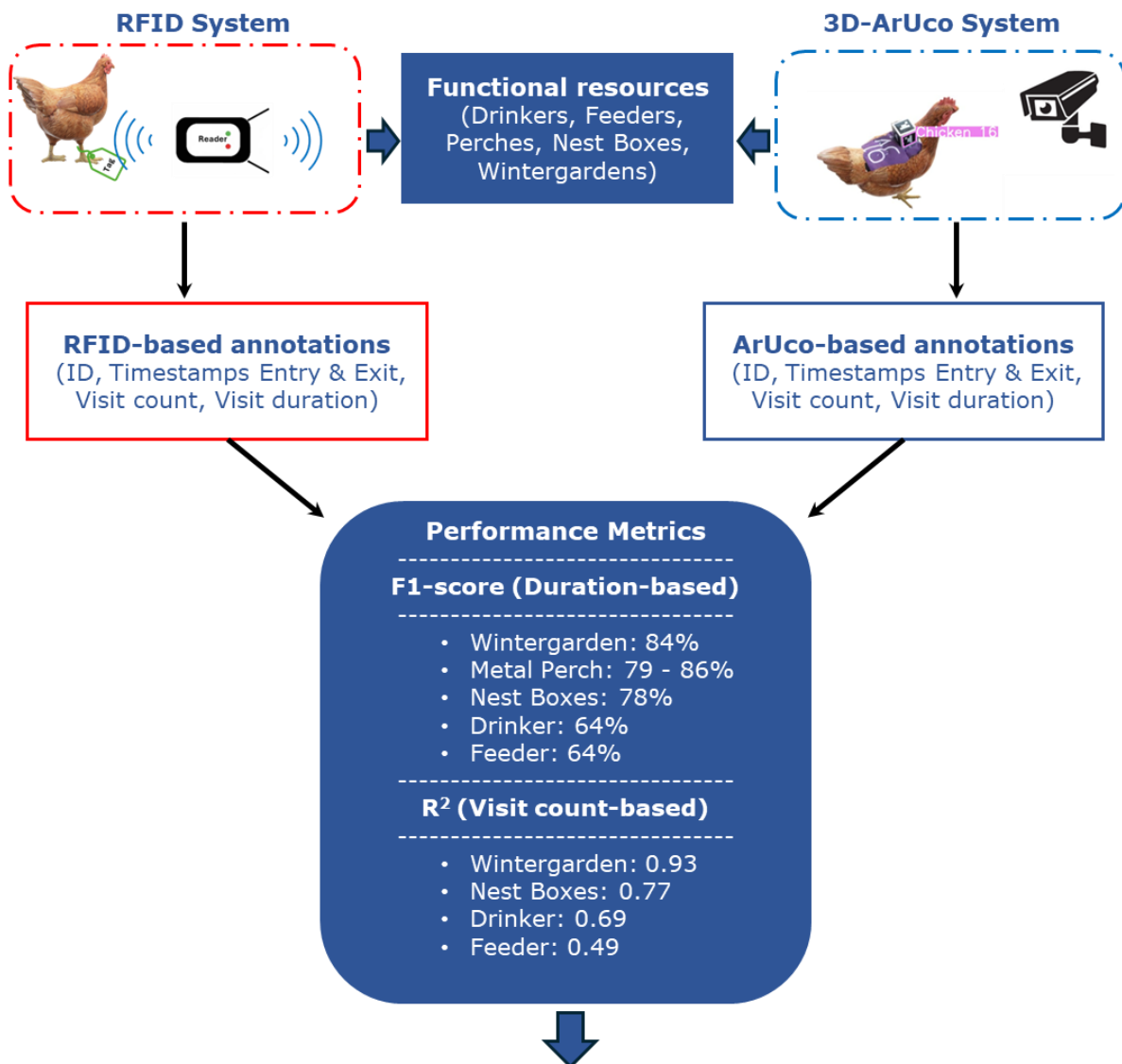
Graphical Abstract Paper 4

Development and Computer-Assisted Validation of an RFID System for Tracking Chicken Visits Within Functional Areas

I. RFID System development and implementation



II. Automated RFID Validation via ArUco-Based Benchmarking



Conclusion:

- Ready-for-use RFID: Wintergarden, Metal Perch, Nest Box
- RFID requiring improvements: Drinker, Feeder



Development and computer-assisted validation of a radio frequency identification system for tracking individual chicken visits to functional areas

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ABSTRACT

Understanding how laying hens interact with functional resources—such as drinkers, feeders, perches, nest boxes, and wintergardens—is essential for meeting their physiological needs and enabling species-specific behaviors. This knowledge is crucial for poultry welfare assessments and precision livestock management. However, traditional ethological data collection methods, including direct observation and manual video analysis, are labor-intensive, prone to observer bias, and impractical for individual-level tracking. To overcome these challenges, we developed and validated an RFID-based system for automated, non-invasive tracking of individual hens' visits to key resources, using an established ArUco-based video annotation system as the reference standard. For validation, twenty-one laying hens were fitted with RFID leg bands and 3D-ArUco markers and monitored over five days in a mobile barn setup equipped with ultra-high-frequency RFID antennas. Alignment between data from the RFID and 3D-ArUco systems allowed calculation of performance metrics such as the F1-score—defined as the harmonic mean of precision and sensitivity—for visit durations and event detections (i.e., entries and exits), and the coefficient of determination (r^2) for visit counts. Wintergarden showed the highest performance (84 % F1-score, 93 % r^2). Metal perch achieved F1-scores of 79 % and 86 % for access and leaving events. Nest boxes showed intermediate performance (78 % F1-score, 77 % r^2), while drinkers and feeders were lower (64 % F1-score each; r^2 values of 69 % and 49 %). These findings confirm RFID's potential for tracking visits to wintergardens, perches, and nest boxes—demonstrating sufficient performance for practical use, though further optimization through antenna positioning remains possible. For feeders and drinkers, however, accurate tracking remains challenging, and complementary technologies may be required, as rapid movements reduce tag dwell time, overcrowding causes signal interference, and open areas increase misreads from nearby surrounding movement. This study highlights RFID's value for behavioral research at the individual level in poultry and supports research-driven innovation in housing equipment design. It also demonstrates how a computer-assisted approach can facilitate validation across diverse behavioral contexts.

Introduction

The interactions of chickens with functional resources—such as drinkers, feeders, perches, nests, and enriched outdoor runs—are crucial

for the expression of their natural behaviors and for meeting their physiological needs. These interactions are fundamental for ensuring chickens' welfare, as they directly impact both their affective states and physical health (Lay et al., 2011). Optimizing access to these resources

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also benefits farmers by reducing the incidence of health and behavioral problems (e.g., feather pecking), and decreasing associated veterinary costs, thus improving poultry productivity and farm profitability (Europe Food Safety Authority, 2005; Nicol et al., 2013).

However, studying individuals' resource use remains a significant challenge. Traditional monitoring methods primarily rely on direct human observation and onscreen video analysis. While these approaches are common, they present significant limitations: they are not only time-consuming but also subject to potential errors due to human fatigue (Girard and Cohn, 2016; Alarcón-Nieto et al., 2018), and they are impractical for long-term continuous monitoring in complex environments such as poultry barns. To overcome these limitations, Radio Frequency Identification (RFID) technology offers a promising and effective solution for research applications. RFID provides several advantages over traditional behavioral tracking methods. These include remote monitoring, independence from lighting conditions and line-of-sight, as well as the long lifespan of passive RFID tags, which do not require batteries (Brown-Brandl et al., 2019). Furthermore, RFID has been successfully used to study not only resource visits and health conditions but also social interactions within flocks. For instance, Richards et al. (2012) demonstrated, using RFID tracking, that hens with keel bone fractures had reduced access to outdoor resources, while Gómez et al. (2022) used movement data derived from RFID tracking to model social associations among hens.

The adoption of new animal behavior tracking technologies is crucial for advancing research and practice, but it is essential to ensure that the information they provide is valid and reliable (Martin and Bateson, 2007). Validation is a necessary step in the implementation of any new system; however, it presents several significant methodological challenges.

Traditionally, systems are validated against human-coded observations, a process that is not only time- and labor-consuming—as illustrated by Sales et al. (2015), who logged over 256 h of manual video annotation, describing it as “time-intensive”—but also vulnerable to fatigue and inconsistency (Anderson and Perona, 2014). Achieving reliable coding often requires expert training, repeated calibration, and high inter-rater agreement (Bakeman and Quera, 2011; Hallgren, 2012). Even trained observers may struggle due to the ambiguous or context-dependent nature of behaviors (Altmann, 1974; Martin and Bateson, 2007).

Another major challenge lies in the lack of universally accepted validation frameworks. In the absence of standardized metrics or methodological guidelines, validation reports vary widely in how they assess the performance of animal behavior tracking technologies—some relying on quantitative indices, others on qualitative assessments. The metrics themselves are often calculated differently across studies, which renders cross-study comparisons difficult, if not meaningless (Alindekon et al., 2023).

In response to these challenges, computer-assisted, video-based tools—such as those using spatial tracking or automated annotation—offer a promising alternative by reducing the burden of human coding. Yet, these tools face limitations. Many remain costly (Dell et al., 2014), require technical customization, and continue to struggle with reliable individual identification under markerless conditions—a task complicated by frequent identity switches, complex environments, and large group sizes. While some progress has been made (e.g., Pérez-Escudero et al., 2014; Walter and Couzin, 2021), robust, markerless individual identification in field conditions remains underdeveloped.

Taken together, these challenges may explain why nearly half of RFID-based studies in poultry behavior did not mention independent validation (Alindekon et al., 2023).

The primary goal of this study is to develop and validate an RFID system for tracking chickens in a controlled environment by monitoring their interactions with key functional resources. Here, we present the design and implementation of a novel RFID system, developed based on

general guidelines described by Alindekon et al. (2023), followed by a comparison of the collected data with ArUco annotations to evaluate the system's validity and reliability. ArUco-based annotation, implemented as a computer-assisted video tracking system, was chosen as the validation benchmark. This method is both robust and well-established, and it closely aligns with human observations (95 % consistency), as shown in our previous study (Alindekon et al., 2025). That study was conducted on laying hens using the same mobile barn, the same camera setup, and the same body-worn markers. In addition to its accuracy, the ArUco system enabled annotation that was approximately 5.4 times faster than manual scoring. Crucially, it also allowed for consistent identification of individuals over time, further supporting its use as a practical and efficient validation reference.

Materials and methods

Animals, housing and husbandry

Animals and ethical considerations

The study was approved by the Animal Experimentation Ethics Committee of Mecklenburg-Pomerania, under the number AZ 7221.3-18196_23. Twenty-one 45-week-old Lohmann Brown laying hens, acquired from a commercial farm, were used.

Characteristics of barn and resources

The hens were housed from 4 September 2024 to 30 October 2024 in a mobile poultry barn (ROWAv 200 V 4.0) at the Friedrich Harms Animal Experimentation Station, University of Rostock, in Dummerstorf, Germany. The barn consisted of two compartments: a 16 m² main barn and a 14 m² wintergarden, which provided a semi-open space for scratching and dust bathing. The pophole to the Wintergarden measured 120 cm in width and 35 cm in height, with a 10 cm wide plastic barrier for the chickens to cross. In the main barn, a wooden nest box system was arranged in two rows of six individual nests. Each nest measured 28 × 38 × 25 × 50 cm, with a sloped plastic bottom that allowed eggs to roll forward into a collection area. The bottom of each nest was lined with wood shavings. The same material was used as litter to cover the floor in front of the nest area. A raised plastic slatted area in the main barn also housed various resources. Two linear plastic feed troughs, each 0.5 meters long and accessible from both sides, were used. The drinking system included 2 cups and 12 nipple drinkers along a 3-meter drinker line, positioned 50 cm above the plastic slatted flooring. Two types of perches were available: a 2.25-meter cylindrical metal perch and an L-shaped wooden perch, with two lines.

Animal management

The hens were fed 3 to 5 kg feed (PANTO® LMK Legemehl Korn, Germany), split into two portions: one at 7:30 AM and the other at 5:30 PM. The hens were visually inspected twice daily, with a focus on the group's overall behavior (e.g., general activity levels, signs of excessive aggression or abnormal pecking, vocalizations) and appearance (e.g., plumage, signs of injury). The barn was lit for 16 h per day, from 5:30 AM to 9:30 PM. The wintergarden, where the hens had exclusive access to a box containing a mixture of zeolite and fine sand for dustbathing, as well as fresh air, direct sunlight, and ample space for foraging, was automatically opened from 9:30 AM to 8:30 PM. The chickens were acclimated to this environment for six weeks before data collection began.

RFID development and deployment

The development and deployment steps follow the guidelines of Alindekon et al. (2023), which detail the procedures.

Tracking objectives

We intended to develop an RFID system that collects data on the

presence of individual chickens within the functional areas of key resources (feeder, drinker, perch, nest boxes, and the wintergarden), referred to as areas of interest (AOIs). The RFID system should record timestamps for entry and exit—and for some resources, continuously register presence on a second-by-second basis—enabling the calculation of frequency, duration, and resource visits over time.

Tracking requirements and equipment selection

Selecting RFID components required careful consideration of technical constraints, operational conditions, and compatibility with our tracking objectives. To ensure technical robustness and optimal system integration, we adopted a cross-disciplinary approach, as recommended by Siegford et al. (2023). We collaborated with specialized RFID engineers (Wilmsmeier Solutions, Germany) to ensure full alignment between technical requirements and our research objectives. The details of the selected equipment and the rationale behind each choice are presented below.

- **Antennas.** Due to the linear nature of most resources, a linear antenna was required to cover the length of these resources. The antennas needed to be flexible, allowing them to be easily shaped and installed along the linear structures of the barn. Therefore, LOC-FIELD® cable-like antennas (FEIG Electronic, Germany) were chosen. These cable-like antennas generate a cylindrical, localized electromagnetic field, adjustable from 1 cm to 2.5 meters, with an active length of at least 2 meters, providing an omnidirectional reading profile. The antenna models were selected based on the length of the resource: ID ANT.U LOCFIELD® Type: 200-250-28-EU used for the interior of the nests, along the underside of the drinker line, and along the popholes leading to the wintergarden; ID ANT.U LOCFIELD® Type: 200-300-50-EU used for the feeders and perch lines.
- **Readers.** The system needed to manage simultaneous tag detection when multiple chickens were within the read range, and also to accommodate high-speed reading in areas such as the pophole and nest entry. This required selecting readers capable of handling mass readings and equipped with an anti-collision protocol—a mechanism that ensures signals from different RFID tags do not interfere with each other, allowing each tag to be accurately identified even in high-crowding zones. Additionally, the system required a reading range extending from close contact to a distance corresponding to the body length of a chicken, estimated at around 30 cm. To meet these requirements, the FEIG LRU1002 Fixed Long Range UHF RFID Reader (FEIG Electronic, Germany), operating in the 865–868 MHz range as required by European ETSI (European Telecommunications Standards Institute) regulations, was selected.
- **Tags.** Given the challenging environment, including the presence of manure and dust, and compatibility with the selected reader, the tracking conditions required finding tags designed to operate in the UHF range. These tags needed to be made of materials that provide elasticity and resistance to poultry manure and pecking, as well as be lightweight to avoid compromising the chickens' well-being. The FLEXTAG 62 × 12-TPU-YE (HellermannTyton, Germany), made of thermoplastic polyurethane and weighing only 3 g, was selected to meet the needs of our study.
- **Coaxial Cables.** The criteria were to ensure a uniform cable type and length to guarantee consistent signal transmission and power settings across the system, minimizing signal loss. All antennas were connected to the readers using ID ISC.ANT.C6-A UHF-Antennenkabel SMA/SMA 6 m coaxial cables (FEIG Electronic, Germany).

Tag 'N track RFID software solution for data management

As with equipment selection, developing RFID data management software involves challenges that require technical competencies not typically found in animal behavior specialists. To address these challenges, we partnered with software engineers (Wille Engineering,

Germany) to create a custom solution called Tag 'N Track. This program, built using an API and integrated with middleware from the RFID reader manufacturer, tackles key technical issues such as time synchronization across all readers, data buffering, and the implementation of a core algorithm for detecting enter/exit events (with each RFID tag generating an event every 1000 ms).

From the outset, the software was designed to use the system clock on the host computer for timestamping. This allows the RFID system to be integrated with, and synchronised to, any other tracking system installed in parallel (e.g., a camera system for validation purposes).

The software provides real-time data visualization, efficient storage via InfluxDB's UI Data Explorer, hardware monitoring to prevent data loss, and up-time monitoring with alerts. [Supplementary Material A](#) provides an overview of the query interface in InfluxDB's UI Data Explorer, with a screenshot showing RFID event data visualization as an example.

System architecture

The goal was to have an architecture that allowed for efficient and synchronized data collection across all functional areas in the barn. In total, three readers were used. Each reader operated as a standalone subsystem and was connected to a certain number of antennas linked to resources of focus. The data from the various readers were then centralized through a network to a hosting computer for storage and analysis ([Fig. 1](#)).

Tag attachment to chickens

The RFID tags were glued to a standard chicken identification ring and fitted to the chickens' ankles in a flag-like position. Positioning the tags in this way was intended to maximize the contact surface with the cylindrical electromagnetic field generated by the antennas and help minimize interference from body tissue ([Fig. 2](#)).

Antenna placement on focal resources

The antennas were deployed to optimize the electromagnetic field coverage, ensure reliable readings, and minimize the risk of missed RFID tag readings in the functional areas of the resources where the chickens position themselves for use ([Fig. 3](#)).

- **Metal Perch.** An antenna was installed beneath the metal perch line at a distance of 7.5 cm from the metal. It was fixed on a wooden slat attached below the perch and enclosed in a plastic tube to prevent direct contact with both the metal and chicken droppings, thereby reducing potential signal interference.
- **Feeding Area.** Two antennas were placed along the feed troughs, fixed directly to the slatted floor on both sides, at the chickens' foot level, ensuring independent coverage on each side, regardless of which side the chickens approach.
- **Pophole Passage (Barn to Wintergarden and vice versa).** Two antennas were installed to register the passages between the barn and the wintergarden. The first antenna is placed on the upper edge of the barrier that the chickens must cross, while the second antenna is positioned on the other side of the wintergarden, arranged in a serpentine antenna layout, in order to extend the electromagnetic field coverage and ensure more effective capture of the chickens' passage.
- **Drinking Area.** An antenna was installed beneath the drinker line on the slatted floor, protected by PVC to shield it from moisture and water runoff.
- **Nest boxes.** A single RFID antenna was placed at the interior entrance of the nest, positioned near the bottom and extending through each row to detect entry and exit timestamps as chickens moved in and out. The antenna was arranged in a sinusoidal pattern, vertically aligned on a single level, to ensure coverage of all individual nest boxes.

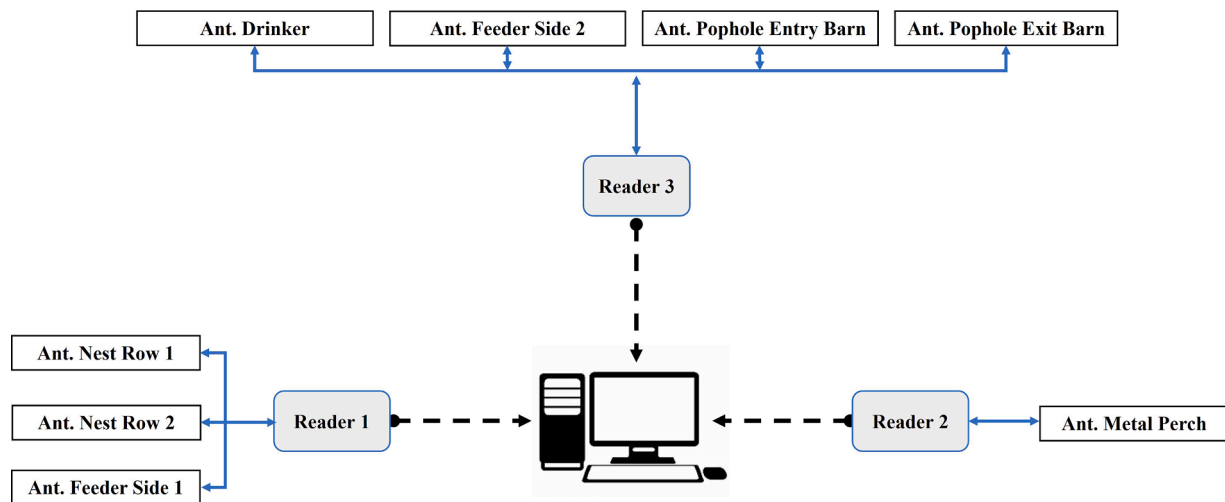


Fig. 1. System architecture of the RFID system to monitor individual chickens' presence within functional areas of key resources. The blue line with two-headed arrows represents the coaxial cables connecting the reader and the antennas (Ant.). The dashed arrows correspond to the ethernet cable connecting the reader and the host computer.

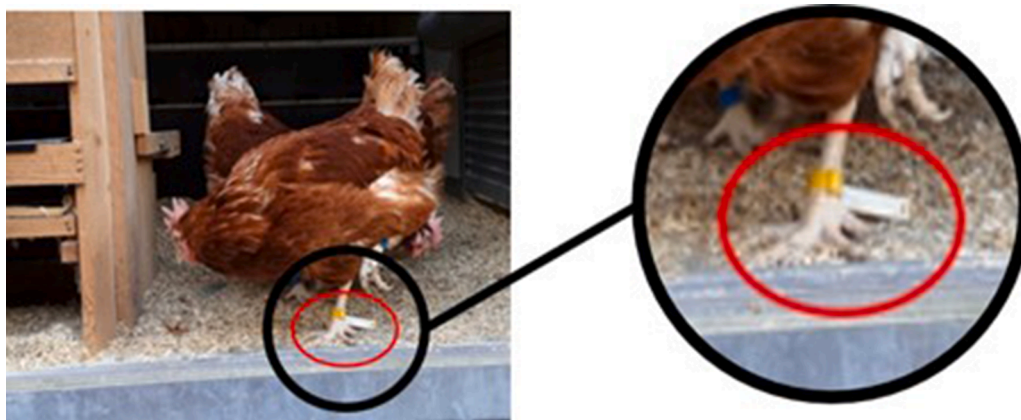


Fig. 2. An RFID tag is attached to a standard chicken leg ring and fixed to the ankle in a flag-like position, perpendicular to the tarsus. The RFID tag and attachment point are marked with a red circle.

Operating parameters of the readers

The power settings applied were informed by preliminary installation and simulation tests conducted without animals, using the FEIG ISOStart+ software (Version 11.05.00), a configuration and testing tool provided by FEIG Electronic for their RFID readers. The tests involved 10 tags placed simultaneously at 10 cm intervals along a linear axis, repeated twice over five iterations.

For feeders, the drinker, and nest boxes, RFID readers were configured at 0.1 W, which is also their minimum allowable power setting. At this level, tag detection rate was consistently achieved at 100 % up to approximately 10 cm, began to decline around 15 cm, and dropped to zero beyond 30 cm. This range was considered sufficient for the intended applications, and 0.1 W was therefore maintained in these areas.

In contrast, for specific locations such as the metal perch and the pophole leading to the wintergarden, the power level was increased to 0.2 W in order to achieve 100 % tag detection. This adjustment was made to account for potential signal interference caused by metal structures (in the case of the perch), the positioning of the readers, and the need to capture rapid animal movement. The physical layout of these zones—such as elevated perches and pophole crossing points—helped minimize the risk of unintended tag detection, even at the slightly higher power level.

The readers operated at a fixed read rate of one read per second, and

the EPC Gen 2 anti-collision protocol in dense mode was enabled to ensure reliable identification in shared-use areas where multiple animals could be present simultaneously.

Processing raw data of RFID annotations

Since the nature of raw RFID data varied across resources, the processing approach was adapted accordingly to ensure accurate identification and quantification of visit events.

- **Feeder and Drinker.** The raw data from the RFID data management system for these resources were highly fragmented, with visit sequences characterized by numerous short and isolated events, separated by momentary interruptions. To achieve a more reliable and consistent quantification of visits, we applied a bout-merging criterion for each resource—a temporal threshold used to link consecutive RFID detections into a single visit event. These thresholds were defined based on the average presence durations in the feeder and drinker areas, as measured using the gold standard video system.

After applying the bout-merging criteria, the first timestamp within each merged detection sequence was assigned as the entry time, and the last timestamp as the exit time for that visit. This

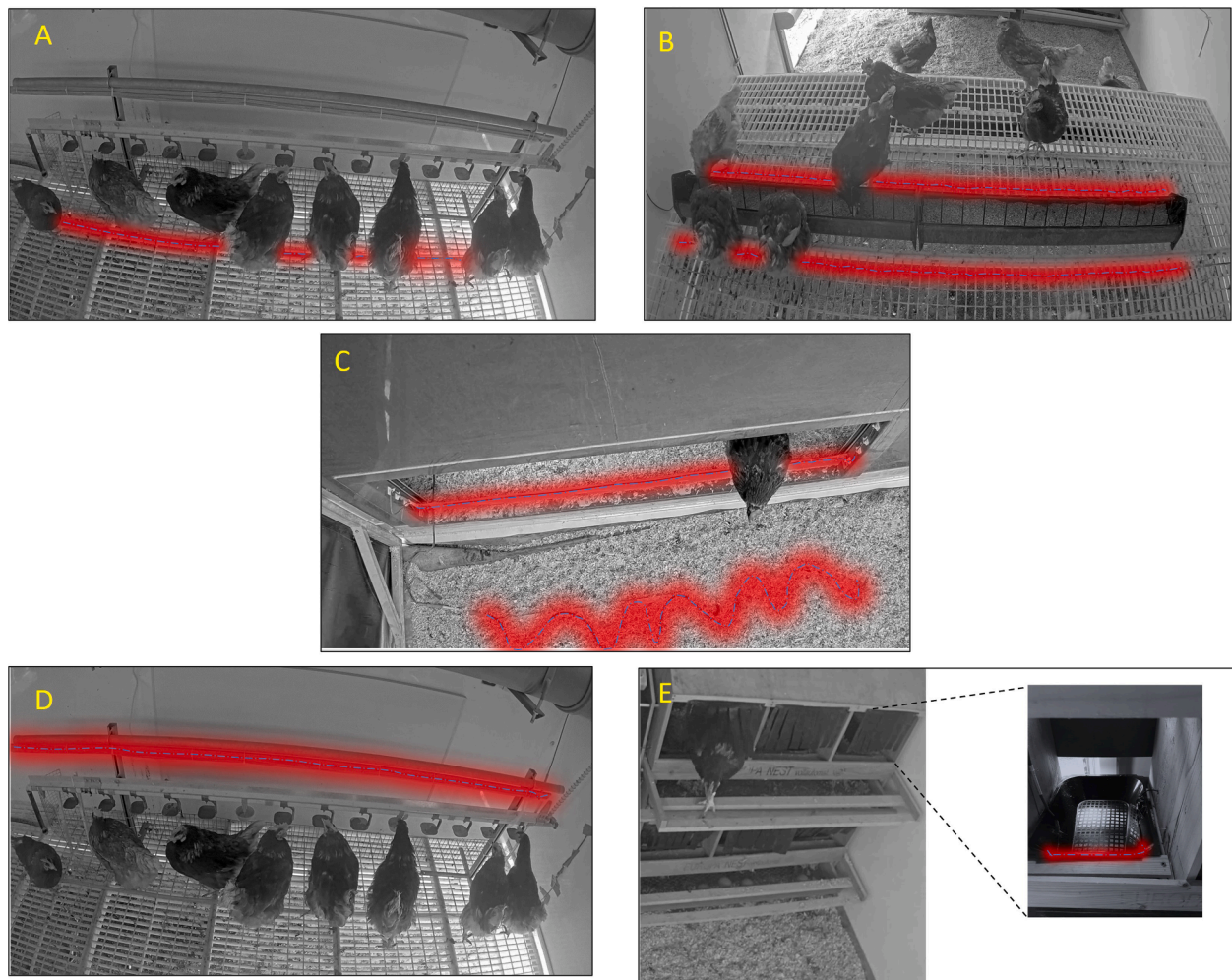


Fig. 3. Placement of antennas and expected signal distribution (marked in red) along them to register chickens in the resources' functional areas. The figure shows in A: Antenna covering the drinking line; B: Two antennas in the feeding area; C: Two antennas for registering Barn-to-Wintergarden and vice versa; D: Antenna for the metal perch line; E: Antenna deployed across nest boxes.

approach allowed us to extract coherent visit events from otherwise fragmented RFID readings.

After applying the bout-merging criteria, the first timestamp within each merged detection sequence was assigned as the entry time, and the last timestamp as the exit time for that visit. This approach allowed us to extract coherent visit events from otherwise fragmented RFID readings.

- **Nest box.** Raw data from RFID antennas placed at the interior entrance of the nest boxes were used to determine visit events. For each individual, the first detection within the morning time window was considered the entry timestamp, and the last detection before exiting the nest was used as the exit timestamp. The visit duration was calculated as the time difference between these two points. Since the antennas were located inside the nest, intermittent signal loss during occupancy was expected and intermediate detections were disregarded.
- **Wintergarden.** We adopted an indirect approach based on the sequential order of RFID detections to identify visit events and calculate their count and duration. First, we distinguished between the RFID antennas covering the barn interior and the single RFID antenna positioned inside the Wintergarden, right at its entrance. To define a complete visit, we applied a double-transition logic: when a chicken was first detected by an antenna inside the barn and then at the Wintergarden RFID point, this indicated a "Barn to Wintergarden" transition. Conversely, if the first detection occurred in the

Wintergarden and was followed by one in the barn, this signified a "Wintergarden to Barn" transition. Only sequences including both transitions were used to determine visit metrics. By calculating the time difference between each Wintergarden entry timestamp and the subsequent exit transition, we determined both the total number of visits and the duration of each visit.

- **Metal Perch.** To determine nighttime perch occupancy, we first identified the time of access (i.e., entry) using the timestamp of the first RFID detection on the perch after the evening feeding period (served at 5:30 PM). This moment typically corresponds to hens jumping onto the perch while still in a standing position, which would be expected to allow more reliable detection. Once perched in a sitting position, however, RFID tracking becomes less effective: the hens' body posture and weight press the leg-mounted tag against the perch and may block the antenna's magnetic field, leading to signal loss or gaps in detection. The time of perch leaving (i.e., exit) was defined as the last RFID detection recorded before the next morning feeding period. Since hens usually stand up before leaving the perch, this posture would be expected to permit tag detection again. This method assumes that hens remain perched through the night, making the first detection after evening feeding and the final detection before morning feeding useful indicators of nighttime perch access and leaving.

Benchmarking video recordings and 3D-ArUco marker annotations

To facilitate the validation of the RFID system during deployment, a computer-assisted, video-based method involving 3D-ArUco Marker Annotations was used. The technical details regarding the cameras used, installation procedures, and 3D-ArUco marker tracking are provided in our previously published paper (Alindekon et al., 2025), where we also detail the high reliability of this benchmarking system, which is used here as the gold standard. The practical recommendations outlined in that paper have also been applied in the present study.

Camera installation and video recording

The cameras were positioned to focus on each key resource used by the chickens, and continuous video recording was conducted throughout the entire lighted period of the day (5:30 AM to 9:30 PM) from 15 October 2024 to 19 October 2024. For the analysis, however, we extracted specific video segments corresponding to fixed time windows, applied consistently across days for each resource.

The selection of these time windows—considered as peak activity periods—was guided by preliminary observations during pilot recordings, daily management and intervention routines within the barn, and supported by findings from the poultry behavior literature, notably the review by Appleby et al. (2004). This approach allowed us to focus on periods of high activity and reduce the inclusion of low-interest events (negatives).

The selected hours for each resource were as follows:

- **Drinker:** 8:00 AM – 10:00 AM and 2:00 PM – 4:00 PM
- **Feeder:** 6:00 AM – 7:00 AM, 8:00 AM – 10:00 AM, and 6:00 PM – 7:00 PM
- **Nest:** 6:30 AM – 10:30 AM
- **Metal Perch:** 5:30 AM – 8:30 AM and 7:00 PM – 9:30 PM
- **Pophole:** 9:00 AM – 1:00 PM

In total, 16 h of video recordings per resource were obtained (four hours each day), except for the metal perch, which, due to the lighting schedule, was recorded for 2.5 h for the evening entry and 3 h for the morning exit events.

Video timestamps were generated using the system clock of the host computer, which also handled RFID data collection, ensuring a shared time base between both systems, so that any discrepancy would reflect RFID reading performance rather than a time misalignment.

Definition of areas of interest (AOIs) around resources for video annotations

The AOIs referred to functional areas defined in the videos, representing the spaces where the chickens actively engaged with the resources (i.e., effectively used them). This process involved marking the resource users from multiple selected frames, outlining the functional areas, and blurring non-relevant areas. The resource-specific overlay was then applied to the corresponding videos, ensuring that only the hens within the designated areas of interest were visible for presence tracking. Details on the procedure for defining AOIs are provided in Alindekon et al. (2024; 2025).

3D ArUco marker tracking and visit event identification

During the video recording, the chickens wore vests equipped with 3D ArUco markers, similar to QR codes, each associated with a unique numeric identifier. These markers were automatically detected in each frame of the videos using OpenCV version 4.10.0, run in Python (version: 3.11.4). With the frame rate of each video known, it was possible to calculate the duration of presence for each unique ID in the designated areas of interest (AOIs). This allowed the automated collection of timestamps for entries and exits, as well as the duration of presence within the AOIs, which was saved in a CSV file. To infer resource visits based on raw data from ArUco detection within the AOIs, specific considerations were made according to the nature of the

resources:

- **Feeder and Drinker.** For these resources, the visit duration of each individual was determined based on their presence within the AOI assigned to each resource.
- **Wintergarden.** Access to this resource was through a pophole, with AOIs defined on the barn side and on the Wintergarden side. The direction of passage was inferred from the sequence of detections: if the first detection occurred on the barn side and the next on the Wintergarden side, movement was considered from barn to Wintergarden. Conversely, if detection began on the Wintergarden side and was followed by one on the barn side, the movement was considered from Wintergarden to barn. Using this directional information, and applying the same double-transition logic as in the RFID data, we inferred visits as sequences involving both an entry and a subsequent exit. This allowed us to calculate the number and duration of visits to the Wintergarden.
- **Nest.** Due to space constraints and the wide camera angle, our setup was limited to monitoring the nest entrances rather than the interiors of the nest boxes. We inferred visits indirectly based on the presence of chickens at the nest entrance. Since the 3D ArUco-based system only captured presence on the nest access ramp without specifying the direction of movement, it was unclear whether a chicken disappearing from view had entered the nest or jumped onto the floor. Therefore, minimal human intervention was used to verify all exit events flagged by the ArUco-based system, ensuring that only confirmed nest entries were retained. Verification consisted of manually checking 128 flagged exit events; of these, 79 were confirmed as true nest entries and 49 were excluded as birds descending from the ramp. This verification step was quick (completed in a few minutes), objective, and required no behavioral interpretation—only the use of timestamps to locate the flagged event in the video and confirm the direction of movement.
- **Metal Perch.** Tracking perching over time was not feasible due to suboptimal camera installation. The camera's view was limited to a side angle, and a top-down perspective of the perching animals was not possible. Due to the sloped roof of the barn and the height of the perch, it was challenging to capture the full body of an animal once it was perched. When the chickens settled for the night, their feathers obscured the front part of the marker, further complicating continuous tracking. Moreover, the absence of light during the night made the video recordings unusable for monitoring night perching. As a result, tracking on the perch was limited to detecting timestamps for access in the evening and exit in the morning, with no continuous tracking of presence or duration during perching.

Fig. 4 presents video snapshots showing defined AOIs, representing the functional areas for each resource, along with the marker IDs displayed after the automated detection of presence at each resource. The ArUco-based tracking tool used for automated detection of presence served as a benchmark for validating the RFID outputs.

Alignment of processed RFID and ArUco-based data and metrics calculation

Since the nature of data varies across resources (entry and exit timestamps for the perch and visit durations for other resources), different approaches for alignment and metrics calculation were applied based on each specific case.

Duration-based metric calculations

This approach was applied to the feeder, drinker, nest box, and wintergarden. Alignment between RFID and ArUco data was performed on a second-by-second basis by matching timestamps, allowing the computation of confusion matrix elements for each individual and each resource at every time point. These elements included True Positives



Fig. 4. The area of interest (AOI, non-blurred zone) for 3D-ArUco annotations used as the ground truth for validating RFID annotations. Markers on the chickens present are automatically identified, and their IDs are displayed from the moment they enter and throughout their presence in the AOI. The figure shows in A: AOI defined for the drinking line; B: Two AOIs in the feeding area; C and D: AOIs covering Barn-to-Wintergarden and vice versa, respectively; E: AOI defined for the metal perch line; F: AOI defined for nest boxes.

(TP), False Negatives (FN), False Positives (FP), and True Negatives (TN).

From these, standard performance metrics can be derived. However, we selectively focused on those most relevant for evaluating detection quality. We computed Precision ($TP / [TP + FP]$), Sensitivity ($TP / [TP + FN]$), and their harmonic mean, the F1-score ($2 \times [Precision \times Sensitivity] / [Precision + Sensitivity]$). The F1-score was chosen because it combines both precision and sensitivity into a single value, providing a balanced measure of the system's ability to correctly detect actual events while minimizing both missed detections and false positives. Its calculation excludes true negatives and therefore offers a more meaningful evaluation of detection performance in the context of animal behavior.

Accuracy, by contrast, reflects the overall proportion of correct decisions—whether detecting a presence or correctly identifying an absence. Unlike the F1-score, it includes true negatives in its calculation and was therefore not used in our analysis. In behavioral monitoring, where animals often spend long periods away from a focal resource, true negatives can dominate the data. This can lead to misleadingly high accuracy values, even when the detection of actual events is poor (Adrian et al., 2020; Alindekon et al., 2023). For this reason, accuracy was deemed inappropriate for our validation goals.

Visit count-based metrics calculation

This approach was also applied to the feeder, drinker, nest box, and wintergarden. Here, the total number of visits per individual were compared between the two methods to quantify how well they agreed for each resource. This involved identifying the correlation between RFID-based and ArUco-based outputs and then computing the coefficient of determination, which was used as an indicator of agreement and reliability.

Timestamps difference-based metric calculation

This approach was applied exclusively to the Metal Perch. Based on the validation method described by Gebhardt-Henrich et al. (2023), we adopted a 60-second matching window between RFID and gold standard timestamps. If the time difference between the two timestamps was ≤ 60 s, the event was considered a match. Events were classified as follows: TP (True Positive, when the time difference met the threshold), FP (False Positive, when RFID detections had no corresponding ArUco match), and FN (False Negative, when ArUco detections had no corresponding RFID match). Finally, Precision, Sensitivity, and F1-score were computed separately for both entry and exit events.

Results

Overview of collected data

Since the data-collection approach differed by resource, we broadly grouped the resources into three categories.

Feeder and drinker

Without bout merging, the RFID-based visits for the feeder and drinker were highly fragmented, resulting in numerous short-duration visits—unlike the ArUco-based annotations, which produced fewer, more continuous visits. However, when we applied bout-merging criteria to the RFID data (153 s for the feeder and 73 s for the drinker, derived from ArUco detections), these previously fragmented RFID visits were consolidated, yielding visit counts and durations comparable to

those recorded by ArUco. Specifically, bout merging increased the mean visit duration for the drinker from 5.10 s to 71.99 s, closely matching the ArUco-based average of 72.33 s. For the feeder, the mean duration rose from 8.83 s to 205.71 s, approaching the ArUco-estimated average of 152.44 s (Fig. 5). These adjustments also significantly reduced the number of RFID-detected visits, enhancing consistency between the two systems. RFID data, incorporating these bout criteria, were used for both resources throughout the rest of the study.

Nest boxes and wintergarden

For these resources, the RFID- and ArUco-based visit counts were similar, indicating no need for additional bout-merging criteria. Table 1 summarizes the results for these two resources.

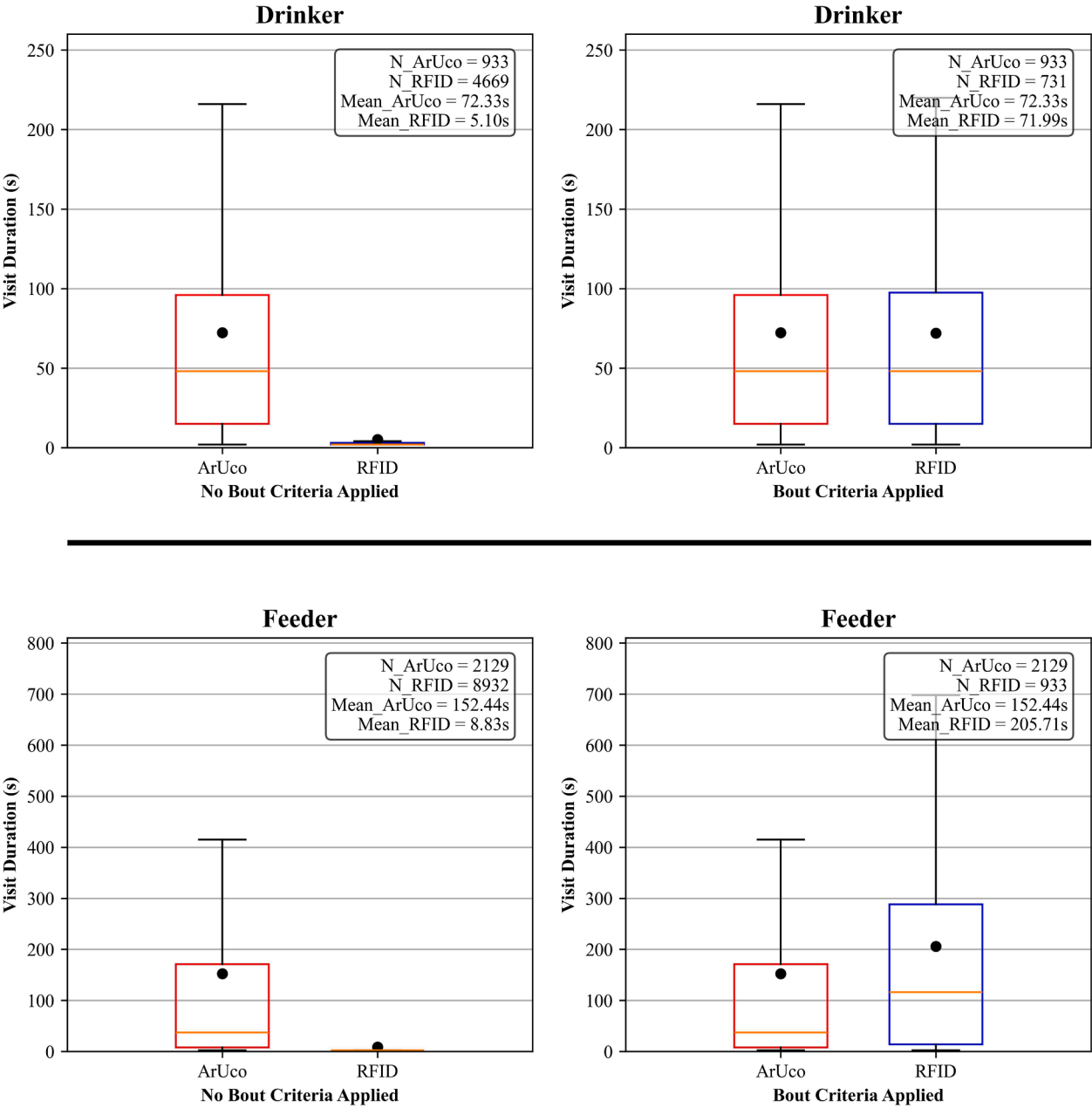


Fig. 5. Effectiveness of bout criteria application on RFID visit data. The average presence duration according to the ArUco-based annotation (73 seconds for the feeder, 153 s for the drinker) was used to reduce fragmented, short-duration visits interspersed with RFID gaps. N_ArUco and N_RFID refer to the number of visits recorded by each approach, while mean_ArUco and mean_RFID represent their respective average durations (in seconds).

Table 1

Overview of Nest box and Wintergarden visits. The data presented are the mean duration per visit in seconds (mean $\pm$ SD) and the total number of visits recorded by both RFID and ArUco across all individuals and days of data collection. No bout-merging criteria were applied.

Resources	Mean duration (s)		Total Visits	
	ArUco	RFID	ArUco	RFID
Wintergarden	2227.65 $\pm$ 2230.88	1637.90 $\pm$ 1888.05	176	180
Nest boxes	1900.92 $\pm$ 960.73	1789.94 $\pm$ 1638.55	77	79

Metal perch (access and leaving)

For the metal perch, the differences between the RFID and ArUco timestamps at the occurrence of each event were 125.32 ± 410.40 s for entry events and 25.61 ± 37.18 s for exit events.

The [supplementary material B](#) provides a visual comparison of RFID- and ArUco-based resource visit events, using a single chicken as a representative example.

Performance metrics

Performance metrics based on visit durations or occurrence timestamps

Based on the F1-score, which balances precision and sensitivity, the performance metrics revealed notable differences among the resources ([Table 2](#)). Wintergarden usage exhibited the highest performance with an F1-score of 0.84. For the metal perch, the system achieved high F1-scores: 0.79 for perch access and 0.86 for morning perch leaving, with the matching window between RFID and ArUco timestamps set to 60 s. Nest boxes followed with an F1-score of 0.78. Lastly, both the drinker and feeder displayed relatively lowest F1-scores of 0.64 ([Table 1](#)), with high precision values (0.86 and 0.73, respectively) but low sensitivity (0.51 for the drinker and 0.57 for the feeder).

Visit count-based performance metrics

All resources exhibited correlations between ArUco and RFID visit counts above 0.70, but across resources, the correlations varied, with the strongest agreement observed in Wintergarden ([Fig. 6](#)). The coefficients of determination indicated that, for most of these resources, a substantial portion of the variance in RFID visits was explained by video visits. In Wintergarden, 93 % of the variance in RFID visits was explained by video visits. In Nest boxes and Drinker, 77 % and 69 % of the variance were explained, respectively. Feeder exhibited the weakest alignment between the two recording methods, with video visits accounting for only 49 % of the variance in RFID visits.

Discussion

This study investigated the reliability and validity of an RFID system in capturing the interactions of chickens housed in a mobile barn with several key functional resources, such as drinker, feeder, perch, nest boxes, and wintergarden. The RFID system was benchmarked against a computer-assisted, automated 3D ArUco-based video annotation system. Our findings revealed significant differences in RFID performance among the resources. Overall, the monitoring of visits with RFID to the wintergarden, metal perch (within the defined temporal threshold), and nest boxes can be considered acceptable, as these provide a reliable balance between precision and sensitivity. However, for drinkers and feeders, RFID requires adjustments to improve accuracy.

Table 2

Performance metrics (Precision, Sensitivity, and F1-Score) of RFID-based tracking for different key resources, benchmarked against 3D-ArUco annotations.

Metrics	Wintergarden	Perch leaving	Perch access	Nest boxes	Drinker	Feeder
Precision	0.98	0.85	0.82	0.80	0.86	0.73
Sensitivity	0.74	0.87	0.75	0.77	0.51	0.57
F1-score	0.84	0.86	0.79	0.78	0.64	0.64

Transitions and visits in wintergarden monitoring with RFID

Using the proposed setup, the RFID accurately identified 9 out of 10 wintergarden visits (93 % coefficient of determination). In terms of visit duration, for the 100 s indicated by the gold standard as an effective visit, the RFID approach successfully captured 74 of those seconds (sensitivity = 74 %), while 98 % of the RFID-detected seconds were correct (precision = 98 %). These results confirm the efficacy of the RFID system for monitoring wintergarden visits and align with findings in the literature. For instance, [Thurner et al. \(2010\)](#) reported a 98 % identification rate for outdoor range usage when comparing RFID with video recordings of pophole passages, while [Gebhardt-Henrich et al. \(2014\)](#) achieved 94 % accuracy in detecting entries relative to exits.

The successful monitoring of wintergarden visits using only RFID can be attributed to two main factors. First, the integration of multiple RFID antennas provides redundancy, which, as demonstrated in [Gebhardt-Henrich et al. \(2023\)](#), significantly improves sensitivity. In their study, deploying multiple antennas in transition zones resulted in RFID data matching the observational data at a rate of 93 %, compared to only 39 % for an isolated antenna. Second, implementing a double-transition principle—where a passage is validated only if identification occurs sequentially at an entry and an exit—minimizes false readings ([Siegford et al., 2016](#)).

RFID-based monitoring of wintergarden visits offers a valuable tool for optimizing management practices and enhancing poultry welfare. First, RFID facilitates the development and evaluation of enrichment strategies in the wintergarden by assessing individual usage frequency and enrichment preferences—such as monitoring the provision of insects as supplementary feed ([Ruhnke et al., 2018](#)). Next, it identifies key factors—such as the season (e.g., winter or summer) and the time of day ([Taylor et al., 2017](#))—that significantly influence outdoor use, thereby enabling managers to fine-tune resource access. Finally, RFID-based monitoring provides essential behavioral insights for fundamental poultry studies by revealing both intra- and inter-individual behavior patterns ([Larsen et al., 2017](#); [Campbell et al., 2018](#)) and elucidating how chickens form social associations under commercial farming conditions ([Gómez et al., 2022](#)).

RFID for monitoring nighttime perch access and leaving

Using a 60-second matching window between RFID and ArUco timestamps, the RFID system showed satisfactory performance in detecting metal perch use during the night. Out of 100 events indicated by the gold standard, the system successfully identified 75 perch access events and 87 leaving events. Exit events were detected more reliably than entries, as reflected by the much lower standard deviation in timestamp differences (37.18 s vs. 410.40 s). A plausible explanation is that birds tend to exhibit more variable and unstable movements when accessing the perch—such as shifting posture, bending their legs, or adjusting balance with the body leaning close to the perch—making initial detection more prone to error. In contrast, leaving the perch generally involves a clearer and more direct movement, which likely contributes to more consistent detection. While the exact reason for this difference in detection sensitivity is unclear, further investigation would be beneficial. Nevertheless, sensitivities of 75 % and 87 %, respectively, can already be considered satisfactory for both event types.

These results are comparable to those reported by [Wang et al. \(2019\)](#), who achieved higher accuracies (97.77 % and 99.88 %) using

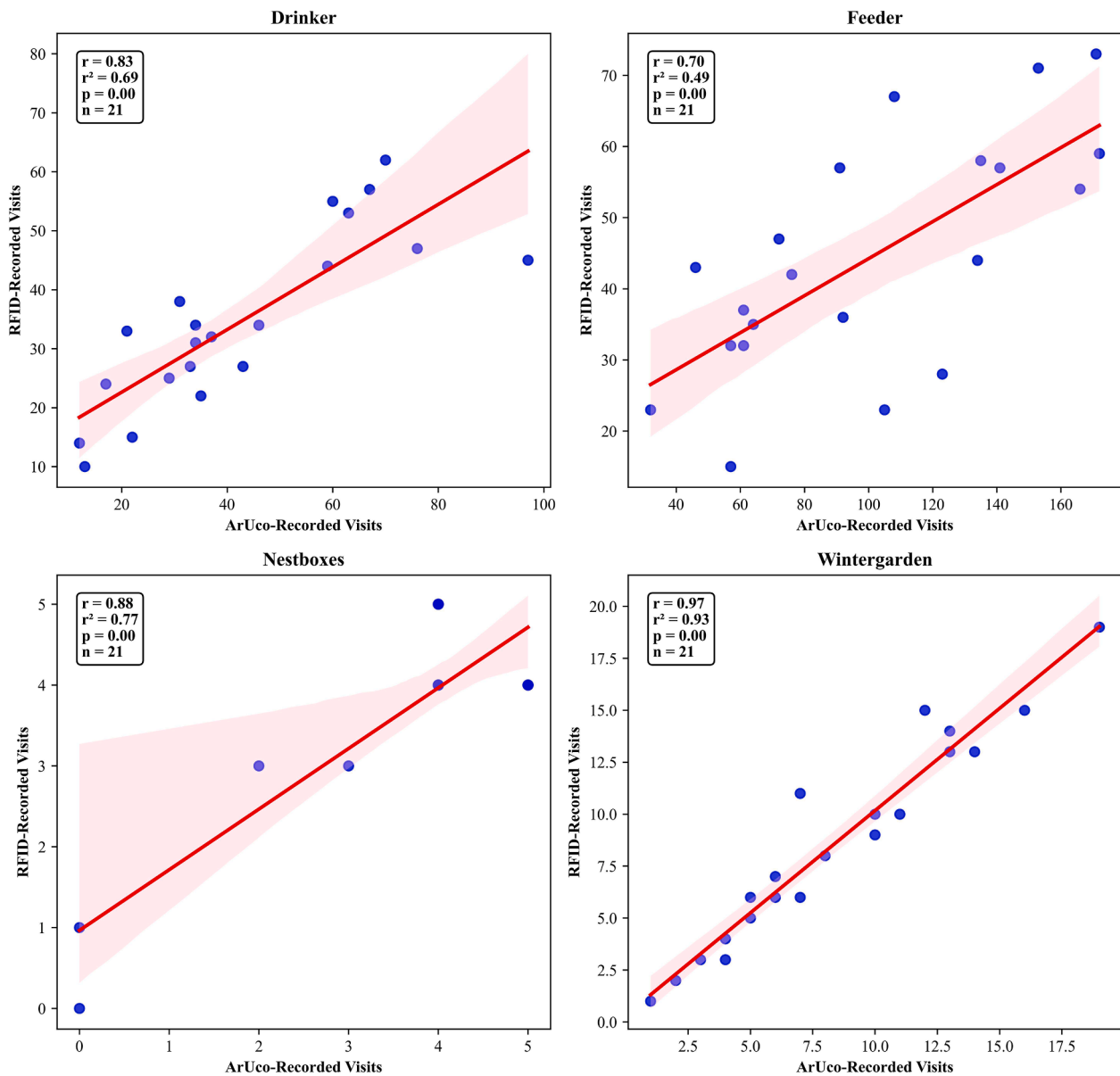


Fig. 6. Correlation between total ArUco and RFID visit counts per chicken over the entire data collection period. “r” represents Pearson’s correlation coefficient and “r-squared” is the coefficient of determination.

two RFID tags per bird—an approach that may partly explain their superior performance. Our findings are also in line with Gebhardt-Henrich et al. (2023), who reported 93 % sensitivity in detecting tier transitions. Together, these metrics indicate that RFID can reliably monitor nighttime perching behavior in chickens under certain conditions.

The ability to track nighttime perching behavior reliably with the RFID setup used here stems from two key factors. First, the elevated perch position minimizes false readings by isolating the perch from other resources and interference. Second, precise timestamps capture only brief standing moments—just before settling onto the perch in the evening and immediately before leaving it in the morning—which helps avoid errors associated with sitting postures. Continuous, second-by-second RFID detection would likely be less effective in this context due to intermittent signal obstruction caused by the hen’s body tissue (Brown-Brandl et al., 2019). The inability to clearly distinguish actual perch movements from signal interruptions makes the current RFID setup unsuitable for continuous second-by-second monitoring of daytime perch use.

Nonetheless, a second-by-second approach could potentially be

achieved through other means. For instance, integrating RFID with complementary technologies such as load cells installed under the perch (Wang et al., 2019) may enable continuous and precise occupancy tracking, even during signal loss. Similarly, low-frequency RFID systems could theoretically support second-by-second detection if technical limitations related to multi-animal detection are addressed. In parallel, alternative tagging strategies—such as attaching tags to body areas that would be less affected by signal blockage in the context of nighttime perching posture (e.g., the neck or wings; Toth et al., 2013; Li et al., 2017)—could also help mitigate signal loss, though such approaches require further validation.

Even in such cases, we would not expect significantly different reliability outcomes between a second-by-second and an event-based approach—at least for nighttime perching, which is the specific context here. Unlike other resources such as feeders or drinkers, where birds interact briefly and irregularly, nighttime perch occupancy is prolonged and stable. Therefore, the nature of the behavior itself supports the validity of using only the first and last detection points as reliable indicators of presence.

Precisely monitoring perch access and leaving timestamps with RFID technology offers numerous research opportunities in ethology and poultry welfare. Social hierarchy significantly influences perch access, with dominant birds often monopolizing elevated perching spaces and displacing subordinate individuals (Cordiner and Savory, 2001; Wang et al., 2022). Since RFID technology accurately identifies the order and timing of successful and unsuccessful perch-use events, it could provide deeper insights into social hierarchy compared to traditional visual observation methods.

Additionally, RFID technology could further support investigations into the links between individual health, welfare conditions, and perch utilization. Precise monitoring can identify individuals that consistently hesitate or take longer to perch or descend, signaling underlying welfare issues such as social stress (e.g., intimidation or exclusion) or health issues (e.g., open wounds, keel bone fractures, foot pad dermatitis).

Nest box visit monitoring with RFID

The proposed RFID system proved to be generally reliable for monitoring nest box usage, although some errors were observed in recording both usage duration and visit counts. Our results indicate that over three-quarters of the nest box usage durations and visit counts were accurately recorded (F1-Score: 78 % and 77 %).

Compared to the ground truth, our RFID system showed discrepancies in detecting nest visits, as it could not distinguish brief nest inspections from prolonged visits indicative of actual nest use. As long as the chicken remained within the same nest row, the system merged successive detections into a single extended visit, even when the bird briefly exited between detections. In contrast, the video validation could differentiate short inspections from true visits based on movement between rows and detection by another antenna. This limitation stems from the use of a single antenna per row, which prevents separate detection of entry and exit events.

Compared to the literature, our findings are similar to those reported by Sibanda et al. (2020), who documented 80 % sensitivity for detecting chickens in nest box areas, with a coefficient of determination ranging between 53 % and 66 %. However, our performance remains lower than that of Li et al. (2017), who achieved a precision of 91.4 % in bird detection (calculated as the percentage of chickens detected by the RFID system relative to those observed in video images) and a coefficient of determination of 98 %. This discrepancy is likely due to differences in setup. In their study, the authors positioned their antennas beneath the nests and equipped each chicken with two tags—one on the neck and another on the leg. This arrangement likely contributed to their superior performance. In contrast, our study used a single tag attached to the ankle and a single antenna arranged at the interior entrance of the nest.

To improve nest box monitoring, it may be beneficial to increase the number of RFID antennas and strategically place them around the nest boxes. For example, installing an antenna under the access ramp, one at the entrance (as currently implemented), and another beneath the nest box—as demonstrated by Thurner et al. (2008); Zaninelli et al. (2015) and Li et al. (2017)—could significantly enhance system performance.

Additionally, monitoring can be further optimized by integrating weight measurement technology to track individual oviposition events based on body weight fluctuations. Heinrich et al. (2013) successfully recorded oviposition events by combining an RFID system with a weighing system, allowing for precise individual identification and automated oviposition detection.

In addition to hardware improvements, machine learning trained on video-validated data could improve the classification of nest visits. Hens often perform brief, repeated inspections before selecting a nest, particularly during the pre-oviposition period, and these short visits differ in duration and pattern from actual laying events (Cooper and Appleby, 1996). A trained model could use these features to distinguish between exploratory behavior and effective nest occupancy for oviposition. This would improve biological accuracy and reduce

misclassification caused by hesitation or nest exploration.

RFID for feeder and drinker visit monitoring

RFID performance remained suboptimal when tracking both the feeder and the drinker. Considering the drinker, if we look at 100 s identified by the gold standard as an effective visit, the RFID system successfully captured 51 s (sensitivity = 51 %), while 86 % of the RFID-detected seconds were correct (precision = 86 %). For the feeder, out of 100 gold-standard seconds, 57 s were correctly captured (sensitivity = 57 %), and 73 % of the RFID-detected seconds were correct (precision = 73 %). Regarding visit counts, one-third of drinker visits and half of feeder visits went undetected (correlation coefficients: 69 % for drinkers, 49 % for feeders), despite efforts to merge closely spaced registrations and reduce interference from physical objects, metals, liquids, and electrical noise—factors known to degrade UHF RFID signals (Brown-Brandl et al., 2019).

Three main factors contribute to RFID misreads in these areas. First, feeding and drinking behaviors involve short-duration visits (Shynkaruk et al., 2019) and rapid, high-intensity, intermittent movements (Li et al., 2020; Yang et al., 2020), accompanied by frequent positional shifts—leading to detection gaps (Alindekon et al., 2023, 2024). This issue is further compounded by the open and freely accessible layout of these areas, which allows birds to pass through casually without necessarily engaging with the resource—a similar observation was made in pigs by Maselyne et al. (2016). Second, partial positioning of birds can affect signal capture, as individuals may not remain fully within the RFID read range. Third, signal interference from overlapping RFID tags—caused by multiple birds in close proximity—can further hinder consistent detection, particularly in high-crowding zones such as around feeders and drinkers (Alindekon et al., 2023).

Several solutions can be considered to address the limitations of RFID-based detection. One practical approach involves monitoring water and feed intake using individual stations equipped with RFID. As demonstrated by Maselyne et al. (2016) in pigs, this method employs physical partitions to guide animals into well-defined detection zones, reducing ambiguity caused by unstructured access. However, modifying the environment to physically constrain animal movement may disrupt natural behavioral patterns, reducing the practical relevance of RFID-generated data and limiting its applicability in commercial poultry settings.

Another promising strategy is the integration of RFID with vision-based tracking technologies. Although this fusion was not pilot-tested in the present study, it represents a promising direction for future work. For instance, combining YOLO object detection with multi-object tracking algorithms—such as SORT, DeepSORT, or BoT-SORT—can provide continuous and robust animal identification through visual means. These systems have already shown strong performance in animal monitoring contexts (Siriani et al., 2023; Yang et al., 2024; Triyanto et al., 2024). If combined with RFID, they can offer a complementary data stream that may improve the overall accuracy and reliability of behavior tracking. Additionally, visual marker tracking using technologies such as ArUco markers, AprilTags, or QR codes—as applied by Alarcón-Nieto et al. (2018), van Putten et al. (2025), and Alindekon et al. (2025)—can further support individual identification, especially in cases where RFID signal detection is unreliable or incomplete. Future research should validate the feasibility of combining these technologies with RFID to develop an optimized system suitable for commercial poultry farming.

General limitations of the study

Several constraints related to RFID tracking, reproducibility, and the ArUco-based reference method should be considered as general limitations of this study.

RFID-related limitations

One of the primary limitations of RFID tracking is that it detects presence near a resource but does not confirm actual engagement. This is particularly relevant for feeders, drinkers, and nest boxes, where mere presence does not necessarily indicate usage (Doornweerd et al., 2023). For instance, in nest boxes, RFID cannot distinguish between oviposition and non-oviposition visits, such as when a hen merely enters and exits without laying an egg. Similarly, for feeders and drinkers, some RFID detections may reflect hens merely passing by or standing near the resource without actual consumption. Based on our previous study (Alindekon et al., 2024), the proportion of RFID-detected visits that correspond to confirmed usage was estimated at approximately 60 % for feeders and 50 % for drinkers.

Practical considerations for commercial applications must be taken into account. While RFID technology is promising for behavioral monitoring, its implementation is currently more feasible in controlled research environments than in large-scale commercial settings. Several factors influence RFID efficiency in real-world applications. In this study, the system was deployed in a specific experimental barn, where customized materials (e.g., wooden nest boxes instead of metallic ones) were used to minimize interference and ensure accurate RFID detection. Furthermore, the small flock size and low animal density provided better tracking conditions. In contrast, commercial farms typically have higher stocking densities and limited space per animal (Leone and Estévez, 2008), which could significantly impact RFID tracking performance.

Methodological considerations

Beyond RFID system constraints, certain methodological aspects of the study should also be acknowledged. One key factor is the variability in the criteria definition and performance metric calculations across studies. The bout criterion varies significantly in the literature (Turner et al., 2008; Li et al., 2017, 2019; Wang et al., 2019), affecting comparability. Some studies test multiple thresholds and select the one that maximizes performance metrics, while others rely on fixed values based on prior research or an error minimization approach. In this study, we applied a bout threshold derived from the average visit duration obtained from ArUco annotations. Similarly, the calculation of performance metrics (e.g., precision, sensitivity, and F1-score) differs across studies, further complicating comparisons (Alindekon et al., 2023). Future research should carefully consider the bout criteria selection and performance metric calculations to enhance reproducibility and comparability with our findings.

Another methodological limitation arises from the ArUco-based validation approach, which has two major constraints. First, ArUco tracking relies on line-of-sight visibility and sufficient lighting, making it sensitive to obstructions and lighting conditions. If a bird's marker is obscured or the lighting is inadequate, tracking accuracy is compromised (Alarcón-Nieto et al., 2018; Eagan et al., 2022; Wolf et al., 2023). Unlike RFID, which functions even in complete darkness, ArUco tracking became ineffective after lights were turned off. This limitation prevented us from benchmarking perch use throughout the night. Instead, we were only able to monitor evening access and morning departure times, when lighting conditions allowed reliable detection.

Second, ArUco tracking primarily provides positional data but does not inherently capture directional movement. This limitation was particularly evident in nest box monitoring, where we could not confirm whether a hen remained inside, was perching, or was descending when it disappeared from detection. Consequently, human observation was occasionally required to resolve ambiguities, though this was minimal. To address this issue in future studies, researchers could incorporate orientation vectors or install cameras inside each nest box to automatically identify entry and exit behaviors. Each approach presents distinct challenges: reliable algorithm development for the former, and the need for additional equipment and data infrastructure (e.g., multiple cameras, storage) for the latter, which may pose logistical and funding constraints. When considering camera installation, care should be taken

to ensure that devices do not compromise the internal space required by ethical guidelines, thereby avoiding disruption of the hens' natural nesting behavior.

Understanding these limitations is essential for interpreting the results accurately and guiding future improvements in automated tracking systems.

Conclusion

This study developed and automatically validated an RFID-based tracking system to monitor laying hens' interactions with functional resources in a controlled environment. The system reliably tracked several behaviors, including access to the wintergarden, night-time perching, and, to some extent, visits to the nest boxes. However, further refinement is needed to accurately capture interactions with the feeders and drinkers. These findings underscore the importance of rigorous validation for behavioral monitoring methods in poultry ethology, housing, and welfare research. Future improvements to RFID-based systems should involve a hybrid approach, combining hardware upgrades with advancements in software, as well as downstream data handling and analysis. Moreover, this study demonstrates the use of computer-assisted validation through 3D-ArUco tracking, which facilitates the evaluation of behavioral monitoring tools and supports the development and adoption of new tracking technologies in poultry science.

Disclosures

The authors wish to confirm that there are no known conflicts of interest associated with this publication. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary materials

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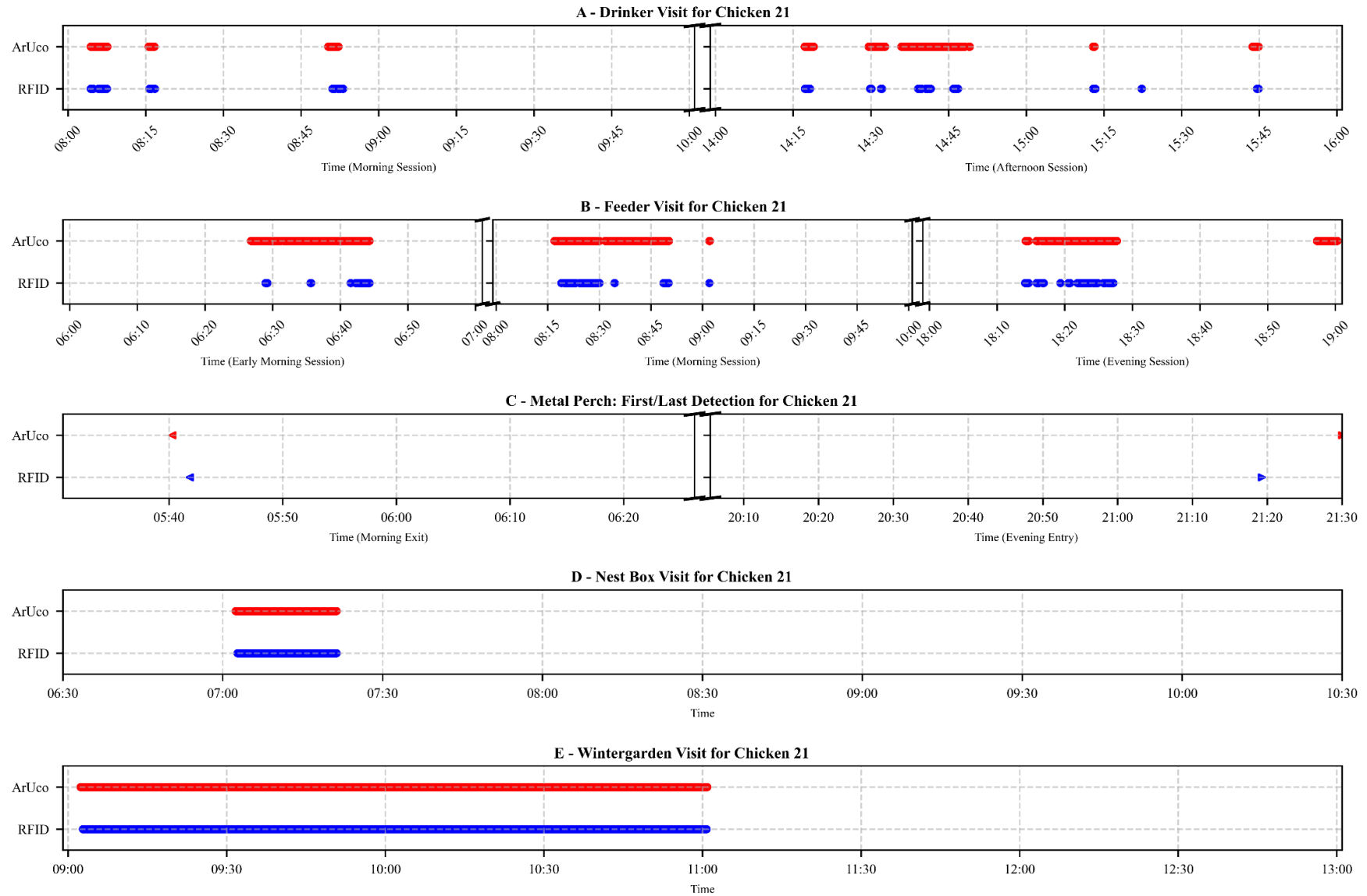
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Supplementary Materials



Supplemental Material A: Screenshot of the query interface in InfluxDB's UI Data Explorer, showcasing a real-time time series visualization of RFID event data. The example displays visit durations for a single hen and a specific resource, with options to filter by RFID tag ID and timestamp range, based on automatically detected entry and exit events.



Supplementary material B: Visualization showing reconstructed visit events (post hoc) based on data recorded by the ArUco and RFID systems—Case of Chicken ID 21 on Day 3. This example was selected because the chicken was present across all resources on the same day, making it representative. Panels A

and B respectively show the raw visit data for the feeder and drinker, without applying any bout criteria, based on the two detection methods. Panels D and E present the inferred visit data for the nest and wintergarden, where indirect inference approaches were applied. For these resources, blue lines (continuous or dashed) represent presence detected by RFID annotations, while red dots indicate ArUco detections, which serve as the gold standard. Panel C displays timestamps for access to and exit from the metal perch, according to both methods. Left-pointing arrows mark exit times, while right-pointing arrows indicate evening access. Red arrows represent ArUco annotations, and blue arrows correspond to RFID data.

VI. Synthesis and General Discussion

This thesis aimed to promote the effective adoption of RFID technology in poultry behaviour research by providing both a diagnostic perspective, via a systematic review of its current use and empirical limitations, and a constructive contribution, through the proposal of a roadmap and practical tools to facilitate its implementation. The thesis highlights key gaps in reporting, validation, and data interpretation, and proposes solutions to enhance the accuracy, consistency, and reproducibility of RFID-based studies in poultry behaviour research.

6.1. Findings — Synthesis, Interpretation and Contribution to the Field

Paper 1 (Alindekon et al., 2023) lays the groundwork by conducting a comprehensive review of RFID deployment in poultry behaviour research; it defines foundational problems and proposes directions for future work. In particular, it highlights severe deficiencies in the reporting and validation of RFID systems, with 43% of studies failing to report system validity and 26% omitting technical details. It identifies these deficiencies—both technical and practical—as the result of three major classes of challenges: the lack of guidelines for deployment, validation, and reporting. In part, Paper 1 addresses these gaps by proposing a multi-phase roadmap for system deployment, by reviewing how key studies have conducted validation (including discussion of relevant terminologies and metrics), and by suggesting a checklist for improving reporting practices. All these efforts are directed towards greater standardisation, uncovering key factors necessary for reliable and reproducible RFID use in poultry research.

Building on these foundational insights, Paper 2 (Alindekon et al., 2024) deepens the understanding of a critical interpretational issue, tackling a non-technical challenge raised but not resolved by Paper 1: the assumption that RFID proximity data reliably indicates actual resource use. Through empirical analysis, Paper 2 demonstrates that proximity is not always a strong proxy for resource use, revealing considerable variability depending on the resource (e.g., high for perches, low for popholes). This finding underscores the need to refine data interpretation protocols—a refinement that directly responds to the broader interpretational and validation concerns raised in Paper 1.

Addressing another critical gap identified in Paper 1—namely, the inconsistent and labour-intensive nature of RFID data validation—Paper 3 (Alindekon et al., 2025) offers a technical solution: an automated validation tool based on 3D ArUco markers. Designed to support the verification of RFID-based presence detection within functionally defined areas, the tool

significantly improves validation efficiency (reducing annotation time by 82.7%) and enhances reliability (with a Spearman correlation of 0.96 and sensitivity exceeding 92%). Paper 3 thus operationalises a practical solution to a validation challenge diagnosed in Paper 1, while building on the functional zone concept introduced in Paper 2.

Finally, Paper 4 (Alindekon et al., 2025a) tests and validates the integrated approach in a practical setting. It brings together the advancements of all preceding papers, implementing the RFID deployment roadmap proposed in Paper 1, applying the interpretational caution urged by Paper 2, and utilising the automated validation tool developed in Paper 3. In a mobile poultry barn setup, Paper 4 evaluates RFID tracking across various resources, showing strong performance for some (e.g., metal perch and wintergarden) but weaker results for others (e.g., feeder and drinker). This practical demonstration not only validates the proposed framework and tools but also highlights the remaining technological limitations, suggesting areas where complementary methods may be needed in future research.

Together, the thesis forms a cohesive, progressive synthesis aimed at advancing RFID methodology for poultry behaviour research, leading to greater accuracy, efficiency, and biological relevance.

6.2. Comparison with Existing Literature

6.2.1. Supporting the Need for RFID Validation in Animal Tracking

Despite its critical role, the rigorous evaluation of tracking technologies—ensuring devices perform as intended with accurate, reliable, and reproducible results—remains notably insufficient across species. Our findings in poultry research (Paper 1, Alindekon et al., 2023) confirmed that validation practices are often limited or inconsistently reported, reflecting a broader problem across the livestock sector. For instance, studies in pigs have shown that only 5% of commercially available Precision Livestock Farming (PLF) technologies have undergone independent validation (Gómez et al., 2021), while in dairy cows, merely 14% of over 128 available technologies have been externally tested (Stygar et al., 2021). This systemic gap means that many deployed technologies lack empirical verification, increasing the risk of biased data and unreliable outcomes.

As also highlighted in our review (Paper 1), several studies corroborate the urgent need for robust validation. Proper validation is fundamental to ensuring research quality; without it, conclusions may rest on unreliable data. Iserbyt et al. (2018) raised similar concerns,

particularly regarding RFID data used without ground-truth comparison, warning about misestimations due to system misreads. Furthermore, Schmeling et al. (2021) demonstrated that system accuracy varied significantly between indoor and outdoor environments, underscoring the need for context-specific validation. Weary and von Keyserlingk (2023) likewise stress the need for independent validation and caution against generalising findings beyond tested contexts.

Validation is also essential for safeguarding animal welfare by confirming that tracking devices do not adversely impact behaviour or health. This ethical dimension, emphasised in our work (Paper 1), is critical before large-scale deployment. Previous studies have shown that improperly fitted devices, such as leg tags or collars, can cause feather loss and skin irritation (Kölzsch et al., 2016; Stadig et al., 2018), highlighting the necessity of empirical proof that technologies do not disrupt natural behaviours.

Additionally, validation helps to detect technical limitations and enables corrective action. Problems such as signal interference from metal or water in barns (Brown-Brandl et al., 2019) or performance shifts due to subtle environmental variations (Gebhardt-Henrich et al., 2014; Maselyne et al., 2014, 2016) highlight the necessity for rigorous system testing, a point reinforced by our synthesis (Paper 1). These insights support essential refinements, such as antenna repositioning (Adrion et al., 2020) or sensor recalibration (Li et al., 2017).

In summary, as emphasised throughout our work (Paper 1, Alindekon et al., 2023), validation is crucial not only for enabling immediate corrective measures but also for ensuring reliable data interpretation within the limitations of system performance. Given fluctuating farm conditions (e.g., structural changes, seasonal behaviours), ongoing validation and periodic re-testing are necessary—much like the routine recalibration of traditional measurement tools—to maintain sustained data accuracy in both research and practical farming applications.

6.2.2. Challenging the Assumption that Proximity Equals Resource Use

Our findings challenge the common assumption that proximity data—such as RFID-detected presence near resources—directly translates into actual resource use (Paper 2, Alindekon et al., 2024). While RFID effectively indicates an animal’s presence in a designated area (e.g., near a feeder), it does not confirm functional utilisation, as we demonstrated in our study.

Reflecting this concern, Doornweerd et al. (2023) referred to RFID data on chickens as "apparent feeding" and "apparent drinking", recognising that proximity alone cannot confirm specific actions such as ingestion. Similarly, Abecia et al. (2024), investigating sheep drinking behaviour via proximity loggers, emphasised that simply being close to water troughs often fails to correlate with actual drinking events. These findings support our own results (Paper 2), where we observed that proximity data sometimes substantially overestimated actual resource use, depending on the resource type.

Moreover, these limitations extend beyond resource use to broader behavioural contexts, such as social interactions. Proximity loggers are sometimes employed to investigate contact patterns among animals; however, they face similar challenges in distinguishing genuine affiliative encounters from coincidental spatial overlap (Ryder et al., 2012; Camargo et al., 2025).

Taken together, these findings highlight a critical gap in relying solely on proximity-based technologies: they capture where an animal is, but not what it is doing. As demonstrated in our work (Paper 2, Alindekon et al., 2024), researchers should remain cautious and complement proximity data with additional validation methods—such as direct observations or video recordings—to accurately interpret animal behaviour and resource use.

6.2.3. Advancing Validation Methodologies with Automated Cross-Referencing Tools

In our study (Paper 3, Alindekon et al., 2025), we developed an automated tool for detecting animal presence and tracking, aiming to replace or complement manual annotation in validation workflows. Although other automated tracking solutions have been developed to support similar goals, important differences distinguish our approach from existing methods.

For example, EthoVision XT is well-regarded for reducing the burden of manual observation (Ross et al., 2019; van Leuffen et al., 2023), offering features such as persistent individual tracking and robust handling of occlusion and group dynamics, and has already been validated in poultry behaviour studies. However, its commercial nature and associated costs can limit accessibility, particularly for large-scale or resource-constrained research settings.

Free tools such as BehaviourDEPOT also show promise but are currently optimised for single-animal analysis (Gabriel et al., 2022), making them unsuitable for tracking groups of chickens, especially when persistent individual identification is required. To the best of our knowledge, BehaviourDEPOT has not been validated in poultry environments and may struggle with occlusion and vertical movement—common challenges in poultry housing systems.

SLEAP, another promising tool, supports multi-animal tracking and assigns persistent IDs based on temporal motion models or appearance-based classification (Pereira et al., 2022). Nevertheless, its reliance on visually distinguishable features is problematic in commercial poultry breeds, where uniformity is high. In addition, to the best of our knowledge, no published studies have applied SLEAP in poultry settings.

Similarly, while ToxTrac is a free tool capable of tracking multiple individuals and assigning IDs (Rodriguez et al., 2018), its performance diminishes with larger groups (>20 animals) and it has not been validated under poultry housing conditions, where crowded, visually complex environments present significant tracking challenges.

By contrast, the method we propose (Paper 3, Alindekon et al., 2025) is open-source, user-friendly, and does not require specialised coding expertise. This accessibility is supported by robust reading algorithms and the public availability of ArUco markers (Garrido-Jurado et al., 2014, 2016). Importantly, we validated our system directly in poultry-specific environments, considering real-world rearing conditions and behavioural patterns around key resources such as feeders and drinkers. As a result, our solution offers a cost-effective, practical, and reproducible alternative for researchers seeking an automated tool to support RFID validation in poultry behaviour studies.

6.3. Identified Limitations and Suggested Improvements

6.3.1. Challenges for Large-Scale Commercial Adoption

A key limitation of this study is its primary focus on scientific researchers rather than on commercial poultry producers. While the RFID-based approach described here can effectively monitor how birds utilise different functional areas within a barn, it is most practical in smaller, research-oriented flocks where costs, logistics, and control measures are more manageable. Consequently, its direct transferability to large-scale commercial operations remains uncertain. Producers may question whether the added expense and complexity of new technology—particularly in high-density settings—would be offset by tangible benefits (Liu and Liu, 2024; Abdul-Majid et al., 2024; Akinyemi et al., 2025).

Future investigations should therefore examine the feasibility and acceptability of RFID systems for commercial producers. It would be valuable to conduct cost-benefit analyses that consider per-bird expenses, potential labour savings, and any measurable improvements in animal management or welfare. Surveys or interviews with poultry farmers could also shed

light on how open they might be to adopting RFID or alternative technologies such as video-based tracking systems, which may be more familiar or less costly to implement. Ultimately, identifying and addressing producers' needs, budget constraints, and the practical challenges of scaling up will be crucial to determining whether RFID or other tracking methods gain wider acceptance in commercial poultry settings.

6.3.2. Limited Flock Size and Housing Configuration

In our studies (Papers 2–4, Alindekon et al., 2024, 2025, 2025a), we used a relatively small flock and a specific type of mobile housing, which may restrict the generalisability of the results. The resource usage and presence data collected are therefore tied to this particular setup and cannot be directly applied to other production systems or housing conditions. However, it is important to note that mobile poultry housing is becoming increasingly popular, particularly in Europe. In Germany, for example, the Federal Association of Mobile Poultry Farming (BVMG) reported that by early 2021, around 300,000 poultry places in mobile housing systems were registered under the association, with approximately 98% dedicated to egg production. These systems supply around 500,000 consumers with shell eggs (Adler et al., 2021).

This growing popularity reflects the increasing interest in regional, welfare-friendly production systems, where mobile barns offer both improved opportunities for species-specific behaviours and new market opportunities for small and medium-sized farms (Biju, 2019; EU CAP Network, 2022).

Given this growing relevance, our findings contribute valuable insights for an expanding sector of poultry production. Nevertheless, further research is needed to explore whether the behavioural patterns observed (Papers 2 and 4) hold across larger flock sizes and a broader range of housing systems, including more intensive commercial environments. Future studies should also aim to validate the robustness of RFID-based behavioural tracking methods (Paper 4, Alindekon et al., 2025a) under different environmental and management conditions. Such research would provide a deeper understanding of how flock size, housing design, and management practices influence poultry behaviour and resource use across diverse contexts.

6.3.3. Challenges of RFID in Complex Barn Areas

Tracking the presence of chickens in specific zones of poultry housing presents several challenges for RFID-based monitoring systems, as previously highlighted in our review (Paper

1, Alindekon et al., 2023) and further confirmed by our experimental findings (Paper 4, Alindekon et al., 2025a). Our results (Paper 4) showed that these difficulties arise—particularly at feeders and drinkers—from a combination of behavioural specificities, technical limitations, and the open spatial design of certain resource areas. Chickens often exhibit rapid, burst-like movements in these zones, adjusting their positions within milliseconds and engaging in feeding bouts that may last only a few seconds (Shynkaruk et al., 2019). Such brief and transient behaviours can exceed the temporal resolution of standard RFID systems, particularly those not employing high-frequency technologies (Brown-Brandl et al., 2019). As a result, birds that quickly enter and exit the detection range may go unrecorded, an issue we also observed in our validation tests (Paper 4).

Moreover, our data (Paper 4) indicated that high bird density further complicates detection accuracy. Movement intensity around feeders and drinkers is known to be particularly high (Li et al., 2020; Yang et al., 2020), and in densely populated settings, overlapping bodies and erratic access to shared resources can create blind spots for RFID antennas. This challenge becomes especially pronounced when bird movement exceeds the system's read rate, leading to both missed detections and false positives (Gebhardt-Henrich et al., 2014; Campbell et al., 2017b). Furthermore, unlike more spatially structured areas—such as perches, nest boxes, or wintergardens—feeders and drinkers are typically open and accessible from multiple directions. As our work highlighted (Paper 4), this lack of physical constraints allows birds to move through these zones casually or briefly, sometimes triggering unwanted readings without true engagement with the resource.

Similar challenges have been reported in other species. Maselyne et al. (2016a), for instance, observed inconsistent RFID detection of pig visits to drinkers, largely due to short presence durations and unstructured access routes, supporting the interpretation of our findings.

One practical workaround is to employ individual feeding or drinking stations equipped with RFID technology, allowing for more accurate monitoring of water and feed intake. As demonstrated by Maselyne et al. (2016a) in pigs, the use of physical partitions can guide animals into well-defined detection zones and reduce ambiguity. Alternatively, integrating RFID with camera-based tracking—such as YOLO object detection combined with multi-object tracking algorithms—has been proposed to improve monitoring continuity (Siriani et al., 2023; Yang et al., 2024; Triyanto et al., 2024).

Furthermore, as discussed in our work (Paper 4, Alindekon et al., 2025a), RFID tracking becomes less effective when chickens are perched, since their body weight and posture press the foot-mounted tag against the perch and cover it with body tissue, shielding it from the magnetic field. Metallic components of the perch can additionally disrupt signal transmission. Combining RFID with load cells placed beneath the perch, as demonstrated by Wang et al. (2019), offers a potential solution. Future research should continue to explore the feasibility of implementing these combined strategies to enhance the accuracy and consistency of poultry behaviour monitoring across various housing contexts.

6.3.4. ArUco Marker Visibility Constraints and Limited Behavioural Insight

Our work (Paper 3, Alindekon et al., 2025) identified two main limitations associated with the proposed 3D ArUco marker-based animal tracking cross-referencing tool. The first limitation pertains to challenges related to marker visibility and detection. Our findings showed that the tool is highly dependent on a clear line of sight for accurate decoding of the marker's black-and-white grid and its white buffer zone. Previous studies have similarly noted that any obstruction—whether from environmental factors or from the animals themselves—can disrupt detection (Alarcón-Nieto et al., 2018; Eagan et al., 2022; Wolf et al., 2023). In our experiments (Paper 3), we observed instances where birds occluded markers with their bodies or feathers, impairing detection reliability.

Environmental conditions also contributed significantly to marker detection challenges, as observed in our study (Paper 3). In low-light settings, such as during nighttime operations, the reduced contrast between the marker and the background hampered accurate decoding, consistent with previous findings (Berral-Soler et al., 2024). Similarly, reflective surfaces were found to introduce confusion by distorting the appearance of the marker (Garrido-Jurado et al., 2022), while complex background textures occasionally led to false positive identifications (Hurník et al., 2021). These observations underline the critical importance of considering environmental design factors when deploying marker-based tracking systems.

To mitigate these challenges, several practical measures have been identified in our work (Paper 3). These include regular cleaning of camera lenses and marker surfaces to prevent dust and debris accumulation, mounting markers on non-reflective surfaces, and ensuring consistent lighting conditions to maintain adequate contrast. Moreover, we recommend conducting preliminary environmental scans—capturing images without markers present—to detect recurring background patterns and to select marker designs with sufficient internal complexity

to minimise misidentification. Future studies could also explore integrating deep learning-based frameworks, such as DeepArUco++ (Berral-Soler et al., 2024), into the detection pipeline, which may enhance robustness under extreme lighting and reflective conditions.

The second major limitation relates to the lack of behavioural insight provided by marker-based systems. As discussed in our study (Paper 3), while ArUco markers can confirm that an animal is within a tracking zone, they cannot directly infer the specific behaviours being performed, mirroring the interpretational challenges observed in RFID-based tracking systems (Le et al., 2024). To address this, future research could adopt integrated sensor approaches. For instance, Doeke et al. (2024) successfully embedded accelerometers alongside ArUco markers in sheep, enabling the correlation of location data with activity types such as feeding, resting, or illness-related lethargy.

Similarly, complementary device integration could enrich behavioural interpretation. Water flowmeters, for example, could measure individual drinking behaviour at troughs, as demonstrated by Maselyne et al. (2016), while load cell modules placed under perches could monitor perch use (Wang et al., 2019). Furthermore, recent advances in machine learning (Guo et al., 2022; Liu et al., 2023) could be leveraged to classify and predict complex behaviours based on combined spatial and sensor data, ultimately providing a more comprehensive understanding of resource use and welfare indicators in poultry.

In summary, although 3D ArUco marker-based tracking provides an accessible and robust method for animal localisation, as shown in our work (Paper 3, Alindekon et al., 2025), addressing its visibility constraints and complementing it with behavioural sensors or intelligent algorithms remains essential for advancing its application in detailed poultry behaviour monitoring.

VII. Outlook and Perspectives for Future Research and Development

Beyond the scope of this thesis, three main perspectives emerge as promising avenues for future development and research. These include methodological refinement and follow-up studies, development opportunities for industry and R&D, and a research outlook in poultry ethology and welfare science.

The first avenue concerns the direct improvement of the methodological framework proposed in this thesis. This has already been discussed in detail in the previous section, where specific limitations of the current approach were identified and matched with practical recommendations for future studies. These suggestions include, for instance, expanding the applicability of the framework to a wider range of housing systems (e.g., fixed barns, aviaries), and integrating RFID with complementary sensors—such as accelerometers, flowmeters, load cells, or computer vision systems—to overcome known blind spots in behavioural detection. These adjustments would strengthen future implementations, particularly in studies similar to this one, while mitigating current constraints.

The second outlook relates to innovation opportunities for industry stakeholders—particularly those engaged in sensor development, animal behaviour monitoring technologies, and precision livestock farming solutions—who could leverage this work to improve commercial behaviour-tracking systems. The outcomes of this thesis may assist R&D teams in better understanding the capacities and limitations of RFID for behavioural monitoring in poultry and in adapting their systems accordingly, including applying the proposed model to facilitate validation efforts. Potential applications include, for example, using the deployment guidelines established for poultry species (e.g., Paper 1), incorporating the behavioural interpretation framework (e.g., Paper 2) to design more informative system dashboards or alerts, or adopting the automated validation benchmarks proposed in this work (e.g., Paper 3) to test and improve commercial tracking solutions—whether RFID-based or compatible alternatives—more efficiently before deployment on the market.

The third perspective addresses fundamental and applied research in poultry ethology, animal welfare science, and behavioural phenotyping. Building on the RFID tracking approaches validated in this thesis—which have proven reliable for assessing use of certain key resources—future studies could investigate individual-level behavioural traits such as personality, coping strategies, or circadian activity rhythms. By linking proximity-based behavioural data with

intrinsic factors—such as age, genotype, or early-life experience—researchers may gain new insights into the biological significance and heritability of behavioural patterns.

Such approaches could be particularly valuable for welfare-oriented breeding programs. For example, selection could focus on animals that make more consistent or efficient use of key resource areas, such as outdoor ranges, nest boxes, or perches. Understanding which individuals utilise these areas more frequently or appropriately could help identify behavioural profiles associated with improved adaptability, spatial competence, or positive welfare states. This could ultimately contribute to the development of precision selection strategies that align behavioural traits with both animal welfare and productivity outcomes.

Additionally, behavioural tracking may serve as a non-invasive tool for detecting early signs of health or welfare issues. Altered movement patterns or reduced visits to specific areas might indicate pain or discomfort, such as that caused by keel bone fractures or lameness. Combining RFID-based tracking with physiological measurements or machine learning models could therefore support the development of refined, data-driven welfare monitoring systems in poultry science.

VIII. General Conclusion

This thesis aimed to promote the effective adoption of RFID technology in poultry behaviour research by providing both a diagnostic perspective and a constructive contribution. It comprises four research papers that together offer a comprehensive review of the current literature and document several technical and conceptual challenges associated with using RFID in dynamic poultry environments. These challenges include empirical limitations in reporting, validation, and data interpretation, all of which can affect the accuracy and reproducibility of RFID-based studies. Moreover, the thesis outlines a clear roadmap and presents practical tools to address these issues and enhance the reliability of behavioural tracking in poultry. It also provides evidence-based recommendations for system design and validation, and suggests areas for further investigation—such as integrating complementary tracking methods (e.g., camera-based systems). Ultimately, this research contributes to the field by offering insights and practical approaches that may support future studies and assist both academic researchers and industry practitioners in developing more accurate, consistent, and reproducible RFID applications in animal behaviour research.

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Resume and Curriculum Vitae



Serge Alindekon joined the Faculty of Agronomic Sciences (FSA-UAC) in Benin in 2011, where he earned a Bachelor's degree in Animal Production. For his final dissertation, he focused on local poultry. He then pursued his first Master's degree in Animal Production and Management at the University of Ibadan, Nigeria, during which he conducted research on quail performance and welfare in various production systems.

After completing his first Master's, Serge worked as a technical coordinator at Production Animal Conseils in Benin, where he supervised the breeding of Hyla parental rabbits and managed incubation activities for the dissemination of broiler chickens and guinea fowl. Two years later, he enrolled in a specialised Master's programme in Animal Welfare, Physiology and Behaviour at Wageningen University, the Netherlands. During this programme, he completed two complementary research projects. In his first dissertation, conducted within the Animal Physiology and Adaptation group, he investigated the effects of manipulating incubation conditions and providing early access to feed on animal resilience against *E. coli* infections. In his second dissertation, within the Applied Animal Behaviour group, he examined the impact of specific lighting conditions and early-life incubation environments on stress reactivity in chickens. Additionally, he participated in collaborative projects, including the EU Project "Chicken Stress ", and completed the Research Master Cluster Track, which prepared him for a scientific career.

Deeply interested in animal tracking technologies, Serge developed a research proposal that was accepted for funding his PhD project (2021–2025) by the German Academic Exchange Service (DAAD). The research project was hosted by the University of Rostock, where he is currently finalising his doctorate on behavioural indicators of animal welfare by utilising longitudinal tracking technologies to monitor individual chickens within large flocks.

Driven by a passion for scientific communication, Serge regularly shares his work and commitment to animal welfare at international conferences. His research interests encompass animal physiology, behaviour, and welfare through innovations in data science and technology,

such as video analysis, acceleration measurements, feed intake records, and the study of auditory and olfactory signals. With extensive experience in machine learning, time series forecasting, pattern recognition, computer vision, and data integration, he excels at using and validating sensors relevant to animal health, welfare, and resilience—key themes in his doctoral research. In addition, Serge remains deeply invested in promoting animal welfare research and improving production practices in developing countries, future farming systems, and genetics—not only in poultry but across all animal species.

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I am a dedicated researcher focused on advancing animal welfare through technological solutions such as RFID and computer vision. I draw upon my professional experience and research background in animal behaviour, adaptation, health, and management to contribute to progress in animal agriculture.

Education

PhD in Animal Behaviour and Welfare Science (*Summa cum laude*)

University of Rostock, Germany (Oct 2021 – Nov 2025). Research Focus: Investigating chicken behaviour using RFID and computer vision technologies.

MSc in Animal Science

Wageningen University & Research, Netherlands (Oct 2019 - Aug 2021). Specialisation: Animal Adaptation, Health, and Behaviour.

MSc in Animal Science

University of Ibadan, Nigeria (Mar 2016 - Aug 2017). Specialisation: Animal Production and Management

BSc in Agricultural Science (Animal Production)

University of Abomey-Calavi, Benin (2011 - 2014)

Research and Professional Experience

Research Associate

Ludwig Maximilian University (LMU Munich), Germany (Sept 2025- present). Chair of Animal Welfare, Ethology, Animal Hygiene and Husbandry

- Development and evaluation of smart technologies for monitoring animal behaviour and welfare
- Data collection, processing, and behavioural analysis in animal research and production environments
- Contribution to interdisciplinary projects combining ethology, AI, and digital animal welfare solutions

Research Intern

Wageningen University & Research, Netherlands (Feb 2021 - Jun 2021). Specialisation: Animal Welfare, Physiology and Behaviour

- Conducted research on mid-incubation eggshell temperature and hatch-feeding strategies.

- Utilised ingestible electronic pill loggers to monitor broiler resilience to Avian Pathogenic E. coli.

Research Intern

Utrecht University, Netherlands (Aug 2020 - Jan 2021)

- Implemented research protocols and performed behavioural tests to assess chicken welfare.
- Measured physiological indicators using brain, blood, and feather collections for stress hormone analysis.

Technical Coordinator

Productions Animales Conseils SARL, Benin (Feb 2017 - Aug 2019)

- Managed welfare and reproduction of broiler breeders and hatchery operations.
- Supervised HYLA rabbit multiplication from GP stocks and traditional poultry breeding programmes.

Technical Sales and Production Assistant

Productions Animales Conseils SARL, Benin (Jun 2015 - Dec 2015).

- Advised producers on best production practices, including alternative nutritional strategies to replace antibiotics.
- Delivered field training and provided techno-economic management support to enhance overall production efficiency.

Research Assistant

University of Abomey-Calavi, Benin (Jun 2014 - Dec 2015).

- Conducted nutritional experiments using local feedstuffs (e.g., Moringa, water hyacinth).
- Assessed management challenges—including health, housing, and predation constraints—in extensively reared local chickens through collaboration with village vaccinators, gaining practical insights into semi-natural poultry husbandry.

Additional Training and Coursework

- **PhD Seminar: Automated Tracking of Animal Location and Movement** (Utrecht University, The Netherlands, 2025)
- **Professional Certificate in Animal Breeding and Genetics (MOOC)** (Wageningen University & Research, The Netherlands, 2024)
- **Certificate in Laboratory Animal Science (FELASA Category B)** (Humboldt University, Germany, 2022)
- **Workshop: Rapid Diagnosis of IBD and Avian Influenza in Poultry Farms** (CECAV, Spain, 2021)
- **The Role of Livestock in Future Food Systems, Extra MSc course** (Wageningen University & Research, The Netherlands, 2020)

- **Ecophysiology, Extra MSc course** (Wageningen University & Research, The Netherlands, 2019)
- **Certificate in Chicken Behaviour and Welfare (MOOC)** (University of Edinburgh, United Kingdom, 2016)

Publications

1. Alindekon, S., Rodenburg, T. B., Langbein, J., Puppe, B., Wilmsmeier, O., Wille, S., & Louton, H. (2025). Development and computer-assisted validation of a radio frequency identification system for tracking individual chicken visits to functional areas. *Poultry Science*, 104, 105627.
2. Alindekon, S., Deutsch, J., Rodenburg, T. B., Langbein, J., Puppe, B., & Louton, H. (2025). Tag “n” Track: Tackling the validation challenge in animal behaviour studies through automated referencing with ArUco markers. *Computers and Electronics in Agriculture*, 229, 109812.
3. Alindekon, S., Deutsch, J., Langbein, J., Rodenburg, T. B., Puppe, B., Homeier-Bachmann, T., & Louton, H. (2024). Inferring resource use from functional area presence in a small, single-flock of chickens in a mobile barn. *Poultry Science*, 103, 104123.
4. Alindekon, S., Rodenburg, T. B., Langbein, J., Puppe, B., Wilmsmeier, O., & Louton, H. (2023). Setting the stage to tag “n” track: A guideline for implementing, validating and reporting a radio frequency identification system for monitoring resource visit behavior in poultry. *Poultry Science*, 102, 102799.
5. Alindekon, S., Abu, O. A., Babayemi, J. O., Agblo, P., & Adjovi-boco, Y. M. (2018). Growth performance of Japanese quail (*Coturnix coturnix japonica*) reared on deep litter and cages in the hot humid tropics of Southern Bénin Republic. *Animal Production*, 20, 191–198.
6. Alindekon, S. (2015). *Dispositif de santé animale de proximité mis en place par le Projet d'Appui au Développement de l'Aviculture Villageoise (PADAV2) dans la commune de Bopa*. Edilivre.

Conferences

• Oral Presentations

1. **Alindekon, S.**, Rodenburg, T. B., Langbein, J., Puppe, B., Wilmsmeier, O., Wille, S. & Louton, H. (2025). Development and computer-assisted validation of an RFID system for

monitoring night-time perching behaviour in chickens. In *Proceedings of the 58th International Society for Applied Ethology Congress (ISAE)*, Utrecht, The Netherlands.

2. **Alindekon, S.,** Deutsch, J., Rodenburg, T. B., Langbein, J., Puppe, B., & Louton, H. (2024). Tag 'n' Track: Facilitating the validation of recent animal behaviour monitoring tools with automated annotation. In *Proceedings of the 11th European Conference on Precision Livestock Farming (ECPLF)*, Bologna, Italy.
3. **Alindekon, S.,** Deutsch, J., Rodenburg, T. B., Langbein, J., Puppe, B., & Louton, H. (2024). Tag 'n' Track: Facilitating validation of animal behaviour tracking technologies with automated annotation. In *Proceedings of the 57th International Society for Applied Ethology Congress (ISAE)*, Curitiba, Brazil.
4. **Alindekon, S.,** Deutsch, J., Rodenburg, T. B., Langbein, J., Puppe, B., & Louton, H. (2024). Tag 'n' Track: Simplifying the validation of animal behaviour tracking technologies with automated annotation. In *Proceedings of the 16th European Poultry Congress (EPC)*, Valencia, Spain.

- **Poster Presentations**

1. Ahozonlin M. C., **Alindekon, S.,** & Dossa, L. H. (2025). Preliminary assessment of the physical welfare of tethered and free-ranging Lagune cattle in the Oueme Valley region of southeast Benin, West Africa. In *Proceedings of the 58th International Society for Applied Ethology Congress (ISAE)*, Utrecht, The Netherlands.
2. **Alindekon, S.,** Rodenburg, T. B., Langbein, J., Puppe, B., Wilmsmeier, O., & Louton, H. (2023). RFID system for poultry behaviour research: How to set the stage to “Tag 'n' Track”. In *Proceedings of the 56th International Society for Applied Ethology Congress (ISAE)*, Tallinn, Estonia.
3. **Alindekon, S.,** Rodenburg, T. B., Langbein, J., Puppe, B., Wilmsmeier, O., & Louton, H. (2023). RFID system for poultry behaviour research: How to set the stage to “Tag 'n' Track”. In *Proceedings of the XI European Symposium on Poultry Welfare*, Prague, Czech Republic.

Invited talk/lecture/presentation

- **Presentation at the Applied Ethology Webinar** – “Tag 'n' Track: Automating Annotation for Easy Validation of Animal-Tracking Technologies”, June 17, 2024

- **Lecture for MSc Students of the Faculty of Agronomic Sciences (FSA), Benin** – Precision Livestock Farming and How It Can Be Used to Study and Improve the Welfare of Farm Animals, November 5 and November 9, 2024
 - **Invited Speaker at FBN, Dummerstorf** – “Implementation and Automated Validation of RFID Systems for Poultry Behaviour Monitoring”, October 7, 2014
 - **Colloquium Presentation at FLI, Celle** – “RFID for Monitoring Poultry Behaviour”, August 22, 2022
-

Skills

- **Software Skills:** Python, RStudio, Power Query, Power Pivot, BORIS, Audacity
 - **Research Skills:** Experimental design, data analysis, statistical modelling, automated program development, animal handling
 - **Communication Skills:** Public speaking, scientific writing, active listening, creative problem-solving
-

Professional Memberships

- Member of the World's Poultry Science Association (WPSA)
 - Member of the International Society for Applied Ethology (ISAE)
-

Languages

- **English:** Full Professional Proficiency | **German:** Elementary Proficiency | **French:** Native Proficiency
-

Hobbies and Interests

- Documentary Enthusiast and Pigeon Fancier
 - Reading and Tinkering
-

I certify that all the provided information is sincere and exact.

Rostock, 12.01.2026

Serge Alindekon

Declaration of Academic Honesty

I hereby declare that I have written this dissertation independently and without unauthorised assistance. All sources and aids used have been clearly indicated and cited accordingly.

I confirm that this dissertation has not been submitted, in the same or a similar form, to any other institution or examination board as part of an academic degree.

I am aware that false declarations may have legal consequences and can lead to the revocation of the academic degree awarded.

Rostock, 12.01.2026

Serge Alindekon