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MEASURING ON-FARM WELFARE IN RABBITS: A REVIEW WITH EMPHASIS ON ANIMAL-BASED INDICATORS

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Abstract: Based on current definitions, animal welfare has to be linked to a life worth living, as perceived by animals, thanks to positive experiences rather than to the mere absence of negative ones. The measure of on-farm welfare of livestock is crucial to improve farming systems, identify critical points and compare different farming systems in view of welfare labelling protocols. To this end, species-specific protocols are necessary, which should use different types of indicators, i.e. resources-based indicators, management-based indicators and, especially, animal-based indicators. These indicators should work under different farming systems and for different animal categories and can be used to assess welfare in the short term or during the productive life of the animal. Last but not least, indicators should be able to measure the affective state of animals in terms of positive emotions. In this scenario, rabbits are quite unique, as little information is available about i) their behavioural needs under farming conditions; ii) the degree of suffering associated with the behavioural restrictions that can occur under farming conditions; iii) the indicators to be used in the very different housing and management conditions in which rabbits can be farmed; and iv) the relationships between emotions and affective states of animals as well as the effect on resiliency of rabbits under different conditions. In this context, the present review is aimed at summarising the state of the art and designing a road map for assessing on-farm rabbit welfare based on the most recent knowledge and approaches with special emphasis on candidate animal-based indicators for measuring both negative and positive affective states of rabbits. The identification of positive welfare indicators is a big challenge, given the biological and behavioural characteristics of rabbits. Accordingly, a comprehensive and robust assessment of rabbit welfare on farms cannot do without structure- and management-based indicators, which should be included in validated and standardised protocols using a multi-indicator approach.

Key Words: welfare consequences, protocols, indicators, positive welfare, rabbit.

INTRODUCTION

Definitions and measures for animal welfare have been widely discussed and perspectives have been modified over the years. While new challenges have been identified for more animal welfare on farms (Paulović *et al.*, 2024), there is common agreement on the fact that welfare is the quality of life as perceived by the animal, which can range from a life worth living, characterised by positive emotions, optimal health and social relationships, to a life not worth living, which is full of negative emotions, illness, pain and frustration (Mellor *et al.*, 2015; Mellor, 2016; LIFT, 2024).

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In this panorama, previous studies (as reviewed by Verga *et al*., 2007, 2009; Trocino and Xiccato, 2006; Szendrő and Mc Nitt, 2012; Szendrő *et al*., 2019) and EFSA Scientific Opinions (2005; 2020) largely focused on welfare and health of farmed rabbits, with special emphasis on welfare consequences, i.e. issues related to negative experiences. In detail, EFSA (2020) identified several behavioural restrictions (restriction of movement; resting problems; inability to perform maternal behaviour; inability to express social positive behaviour; inability to perform gnawing behaviour; abnormal behaviours; fear) and other problems including health issues (prolonged hunger; prolonged thirst; pododermatitis; locomotory disorders; skin lesions; respiratory disorders; gastroenteric disorders; skin disorders; reproductive disorders; mastitis; neonatal disorders; thermal stress). In absence of scientific information, the severity of these welfare consequences for each animal category was scored based on an EKE exercise (Expert Knowledge Elicitation) from 0 (no welfare impairment at all) to 10 (highest possible suffering for a rabbit), with different criteria for behaviour-related (unfulfilled essential behaviour, from high to low motivation; pathological/physiological consequences and acute stress reaction) and health-related welfare consequences (unfulfilled essential behaviour, e.g. feeding, pain and discomfort).

Nevertheless, while the degree of suffering associated with health issues can be more objective to score, little scientific information is available about the degree of suffering associated with the behavioural restrictions that can occur under farming conditions, as the behavioural needs of rabbits under these conditions have not been fully elucidated (EFSA, 2020). In fact, rabbits are a recently domesticated species and there is less scientific information on their needs and welfare on farms than for other animals.

In this context, EFSA (2012) proposed a common framework for animal welfare risk assessment or animal welfare assessment in practice, which is based on the Welfare Quality project, including four principles and twelve animal-based criteria (Table 1) for which species-specific and validated indicators to be used on farms should be identified.

A change in the perspective for assessing animal welfare can be identified based on the revision of the Five Domains Model, which initially focused only on the negative impacts on animal welfare: 1) Nutrition (water and food deprivation; malnutrition); 2) Environment (physical and atmospheric challenges); 3) Health (disease, injuries and functional impairment); 4) Behaviour (behavioural and/or interactive movement restrictions); 5) Mental domain (thirst/hunger, anxiety, fear, pain and distress) (Mellor and Reid, 1994). The revision of the Five Domains Model has highlighted a range of factors generating specific negative or positive responses in the animal per each of the first three domains (Nutrition, Physical Environment and Health) and has redesigned the forth domain (Behavioural Interactions) subdividing it according to the nature of animal interactions with their environment, other non-human animals and humans, including consideration of the grading of negative and positive welfare impacts (Mellor *et al.*, 2020).

Whatever the approach to animal welfare assessment, species-specific protocols are necessary, which should use different types of indicators based on resources, management and, especially, animal-based measures (ABMs). In fact, these latter are measurements of physiological or behavioural responses of an animal or an effect of the external environment on it that can be used to assess its welfare (EFSA, 2012).

Table 1: Principles and animal-based criteria used as guidelines for animal welfare assessment according to the Welfare Quality® project (modified from EFSA, 2012).

Principles	Criteria
1. Good feeding	1. Absence of prolonged hunger (i.e. they should have a suitable and appropriate diet)
	2. Absence of prolonged thirst (i.e. a sufficient and accessible water supply)
2. Good housing	3. Comfort around resting
	4. Thermal comfort (not too hot not too cold)
	5. Ease of movement (enough space to move around freely)
3. Good health	6. Absence of injuries (e.g. skin damage and locomotory disorders)
	7. Absence of disease (i.e. high standards of hygiene and care)
	8. Absence of pain induced by management practice
4. Appropriate behaviour	9. Expression of social behaviours (normal, non-harmful, e.g. grooming)
	10. Expression of other behaviours (species-specific behaviours)
	11. Good human-animal relationship
	12. Positive emotional state (fear, distress, frustration and apathy to be avoided)

For rabbits, there are only three structured examples of protocols including different types of indicators and structured for systematic use on farms. However, to the best of the authors' knowledge, none of these protocols has yet been fully validated considering the very different housing conditions under which rabbits may be farmed. These protocols have been proposed and applied at farm level by private individuals/bodies or are going to be used as part of official veterinary controls.

In Spain, to develop an external welfare certification (Certificado Welfair®, visit https://www.animalwelfair.com/es/certificado-welfair/), the protocols formulated by the Institute of Agrifood Research and Technology (IRTA, Spain) in collaboration with the Basque Institute for Agricultural Research and Development (NEIKER) are based on the Welfare Quality® (Blockhuis *et al*., 2010) and AWIN® and differentiate between reproducing does with litters and males (Dalmau *et al*., 2020) and growing rabbits (Botelho *et al*., 2020). The protocols define sampling methods and time (first week after parturition, at artificial insemination and after weaning for the breeding sector), as well as the scoring systems for each indicator. The scores for each indicator are then weighted and used to calculate an overall welfare score whereby threshold criteria for welfare assessment are identified.

In France, the farming industry has proposed the EBENE protocol (ITAVI, 2018) for welfare assessment in reproducing does and growing rabbits based on Welfare Quality® principles and criteria. An app (EBENE®) is also available for use by farmers, technicians and veterinarians. The result of each indicator and the overall result are graphically represented and compared with regional results. The feasibility and repeatability of the protocol have been validated in the field, but improvements are requested (Warin *et al*., 2021).

In Italy, the Ministry of Health and the National Reference Centre for Animal Welfare (CReNBa) have developed a check list as part of the Classyfarm system available to official veterinarians for assessing rabbit welfare on farms (https://www.classyfarm.it/index.php/what-it) within the framework of official controls related to the annex to Legislative Decree 146/2001 (Implementation of Directive 98/58/EC on the protection of animals kept for farming purposes) and in the "Guidelines for Rabbit Farming" (Circular of the Ministry of Health, 01/09/2021) (Ministero della Salute, 2023). The check list is based on measures and data related to the hazards arising from environmental conditions (management, facilities, equipment and microclimatic conditions) and derived from the detection of the most important ABMs. The end result of the Classyfarm assessment is the classification of farms by an overall welfare score that expresses the level of risk of the farm, besides the identification of legal non-compliances.

Since field data were not yet available and scientific information was not sufficient, EFSA Scientific Opinion, published in 2020, assessed the condition of rabbits kept in different husbandry systems (conventional and alternative) by consulting experts in the field. Specifically, EFSA used an index developed by considering the impact of different farming systems on the behavioural and health welfare consequences in the main rabbit categories obtained through expert consultation.

Overall, based on this brief introduction, information on rabbit welfare and their needs under farming conditions is still scarce. Indicators have not been validated under all farming conditions and the use of ABMs is jeopardised; few protocols have been applied in the field and scarce reference data are available about the on-farm welfare of rabbits in Europe, apart from the EFSA results of the EKE. In this context, the present review aims at summarising the state of the art and designing a road map for assessing on-farm rabbit welfare based on the most recent knowledge and approaches with special emphasis on candidate ABMs for measuring both negative and positive affective states of rabbits.

RABBIT BEHAVIOURAL AND WELFARE NEEDS

As with other species, the study of behaviour is crucial for understanding the rabbit's needs and assessing its welfare under the different husbandry and housing conditions within the framework of principles identified by EFSA (2012). In the case of rabbits, we must refer to their behaviour in the wild and/or under natural and semi-natural conditions. Domestication of the rabbit is in fact rather recent and has not produced substantial changes in its behavioural repertoire except for the intensity and frequency of certain behaviours, such as the greater daytime activity of the domestic rabbit compared to the wild rabbit (Trocino and Xiccato, 2006; EFSA, 2020; Rödel, 2022; González-Mariscal *et al*., 2022). Accordingly, behavioural needs of farmed rabbits are presented here with reference to existing knowledge under wild and semi-wild conditions, specifying when known the motivation for the different behaviours with reference both to the principles identified by EFSA (2012) and the Five Domains (Mellors *et al*., 2020).

Good feeding and nutrition

Rabbits are herbivores; they select concentrates, they are characterised by the physiological mechanism of caecotrophy and the diets fed under commercial conditions must supply a suitable quantity and quality of fibre besides nutrients according to their physiological states and nutritional requirements. In the wild, rabbits spend from 30 to 70% of their activity time outside the burrow grooming, searching for feed and eating, with a variability depending on the age, season and availability of feed. Grazing behaviour is performed mainly during late afternoon and night, whereas during daylight rabbits tend to stay in warrens. However, this behavioural pattern can change depending on the presence of predators in their environment (Delibes-Mateos *et al.*, 2021).

Usually under farming conditions, rabbits are fed *ad libitum* with balanced diets. Sometimes, specific restriction programmes can be implemented to reduce digestive problems in growing rabbits and/or to manage feed intake in young and reproducing does.

Good housing and behavioural interactions with the environment

Under wild conditions, rabbit social behaviour, reproduction and survival are based on their capability of creating underground burrows for their housing. These underground areas are fundamental for rabbits to escape predators and to give birth to their blind, deaf and hairless young kits (Delibes-Mateos *et al.*, 2021), besides keeping them away from adverse climatic conditions. Under farming conditions, burrows/warrens can play a role in outdoor systems, whereas under conventional conditions different types of cages/pens work.

In the wild, when they have satisfied their nutritional requirements, rabbits spend most of their time resting in groups, close to each other, showing a complex social activity as discussed below (see Appropriate behaviour). Under farming conditions, this means that suitable surfaces/floors for resting must be provided, as well as clean space with sufficient dimensions. In fact, rabbits increase self-grooming when kept in dirty soils/litters (Dal Bosco *et al.*, 2002). Moreover, as age increases, growing rabbits reduce the time spent resting with the body stretched in favour of resting with the body crouched (Trocino *et al.*, 2018; Birolo *et al.*, 2022), and the motivations behind this latter behaviour have not been fully elucidated.

Then, for locomotor activities, rabbits usually move on the ground by small jumps; they can use longer jumps to overcome obstacles and reach elevated positions. Their requirements for the time to be spent in this activity have not yet been defined, whereas time spent moving has always been found to be very limited under farming conditions, when free and easy access to feed and water is provided. Accordingly, space requirements for movement have not yet been set for rabbits under farming conditions. Nevertheless, a movement restriction has been identified as the inability to perform three consecutive steps in a linear direction (EFSA, 2020).

Regarding relationships between environment/housing and species-specific behaviours, exploratory activities of rabbits include digging under wild conditions and sniffing their surroundings, often associated with gnawing. This latter behaviour is highly motivated and must also be satisfied under farming conditions by the provision of suitable materials.

Other behaviours, which may entail changes/adaptation in the housing systems and could be taken into account in alternative systems with outdoor access, include anti-predator behaviour, i.e., the alert posture on the hind legs and with erect ears, the rapid flight towards a hiding place and immobility that the rabbit uses to confuse and escape predators. In addition, a rabbit can often be found guarding the entrance to the communal burrow and alerting the group about the presence of danger by tapping a paw on the ground. Finally, under natural conditions, rabbits prefer a terrain in which they can easily dig and an environment with abundant vegetation, especially bushes to be used for feeding, but also to hide from predators. These issues can be considered as requirements when designing alternative farming systems with outdoor access.

Appropriate behaviour and behavioural interactions with other animals

Rabbits are known to be social animals. The minimum group size is one adult male, one female and her litter, but the social unit may include one to four males and one to nine females. Group size changes depending on the availability of natural resources and the environment (Szendrő *et al.*, 2019; Rödel, 2022). In the group, fights are sporadic because

hierarchies are defined early on. Males are usually tolerant towards females and younger rabbits; females sometimes show competition for the choice of the nest site for giving birth and caring for the litter. Hierarchies are separated by sex (von Holst, 2002) and this regulates social relations, access to resources and, therefore, the longevity of the animals (González-Mariscal *et al.*, 2022). To establish these hierarchies, under natural conditions, rabbits fight among themselves but usually without causing each other serious injuries. In commercial farms, conditions do not always allow physical contact among adult rabbits (e.g., reproducing does kept in individual cages) and, when they are kept in groups (e.g., growing and fattening rabbits or reproducing does in collective systems), the conditions under which the group is formed and the social behaviours/activities may substantially differ from what happens under wild conditions. Positive social interactions between adults persist over time between dominant males and females of their groups, which probably helps to establish and maintain social bonds (Von Holst *et al.*, 1999).

Regarding sexual behaviour, wild rabbits mate almost exclusively in the first hours after parturition and reproductive activity is regulated by the increased number of daylight hours in spring. In commercial farms, rabbits are inseminated 12-18 d after parturition, and less after weaning the litter, to avoid an over-exploitation of the female and a high replacement rate, which are associated with fast reproductive rhythms and early mating. Reproductive performance remains high throughout the year with a constant photoperiod of 14-16 h of light. In commercial farms, the use of artificial insemination prevents the expression of the pre-mating sexual behaviour characteristic of the wild rabbit and still present in domestic rabbits. Nevertheless, the effect of this restriction on the affective state of rabbits is not known.

Reproducing does have specific physiological requirements concerning their maternal behaviour. This latter consists of i) nest construction before parturition; ii) a single, rapid daily nursing session; and iii) weaning of the litter. Maternal behaviour is controlled by hormonal factors that regulate nest construction, and non-hormonal factors such as the rabbit social position. Both these factors influence litter care and milk production (González-Mariscal *et al.*, 2022). Under natural conditions, 3-4 d before giving birth the doe leaves the communal burrow to prepare the nest in a different site, burrowing into the ground, covering the bottom with plant material and, before giving birth, with her fur torn off from her abdomen and thorax. After parturition, once the first suckling is completed, the doe closes the nest and leaves it to return to her kits only once a day, usually after sunset and for the few minutes (2-5) she needs for lactation. During this short time, thanks to the high protein and energy content of the doe milk, kits ingest the amount of nutrients and energy they need for their rapid growth. The doe definitively opens the nest when the kits reach the age of 18-20 d; at this age, they have already begun to ingest the hard faeces pellets left by the mother in the nest, thus facilitating the start of the microbial colonisation of the caecum. If the doe has been mated immediately after birth, as usual in the wild, milk production decreases abruptly 20 d after birth and around 24-25 d the doe leaves the nest to prepare for the next birth. If the doe is not pregnant, weaning of the litter can take place some days later.

As for other species-specific behaviours, rabbits perform various comfort activities, including those directed at their own bodies (self-grooming) and those directed at conspecifics (allo-grooming) which can be satisfied only when they are reared in a group.

Finally, play behaviour is often registered in weanling kits and in young rabbits up to about 2 mo. This behaviour includes active movements such as leaping, frisking together in circles and half-circles and more passive ones such as nuzzling and licking each other gently while resting side by side (Lockley, 1961).

Mental state and behavioural interactions with humans

Rabbits as prey animals modify their behaviour and activity depending on the presence of predators (Delibes-Mateos *et al.*, 2021). This means that the rabbit-human relationship is largely affected by how this is managed under farming conditions. The presence of and contact with conspecifics evidently play a role, since solitary animals seem to be more fearful of exploring the surroundings in search of feed, whereas rabbits in groups tend to move further away from the scrubland (Villafuerte and Moreno, 1997). Rabbits that also interact positively more frequently with conspecifics as juveniles are less stressed and are more active in presence of fearful stimuli such as a predator scent, showing a higher scanning activity (Rödel *et al.*, 2006). A positive effect of the presence of conspecifics on the reduction of the fear level has also been proven in reactivity tests in farmed rabbits kept in individual, bicellular, and collective cages (Trocino *et al.*, 2013).

ON-FARM WELFARE ASSESSMENT: ANIMAL-BASED INDICATORS

Assessment of animal welfare based on the above-identified principle/criteria or domain entails the use of indicators, which could be based on animals (ABMs), resources, or management and should be valid (in capturing animal welfare information), feasible (in terms of adaptability to different housing systems and types of environments) and reliable (in providing the same results when the same observer repeats the assessments or when there is agreement between two or more observers after receiving sufficient training). Based on the most recent outcomes, ABMs criteria should be preferred to assess on-farm welfare (EFSA, 2012). In rabbits, there is wide variability and lack of standardisation and information with respect to the indicators that can be used under the different farming conditions, besides the scale of measurement, the use or non-use of thresholds, the way in which results are aggregated to assess the welfare situation in the farm and/or for comparison between different situations. In fact, while ABMs have been validated for other species, in the case of the farmed rabbit the scientific literature and field data are scarce (EFSA, 2020). The EFSA Scientific Opinion (2020) proposed possible ABMs for rabbits that could be included in a protocol developed on the basis of the Welfare Quality Project schemes and criteria (de Jong *et al.*, 2011) (Table 2), which, however, has not yet been validated and/or applied in the field.

More recently, on the basis of the literature published in the last 10 yr (2013-2023), different indicators used under different conditions in protocols for assessing the welfare of rabbits on commercial farms have been identified (Paulović *et al.*, 2024) based on the principles identified by the Welfare Quality project and by EFSA for the assessment of

Table 2: Animal-based measures (ABMs) proposed by de Jong *et al.* (2011) (modified from EFSA, 2020).

	ABMs for reproducing does and growing rabbits
Good feeding	
Absence of prolonged hunger	Body condition score
Absence of prolonged thirst	Resource-based measures
Good housing	
Comfort around resting	Fully stretched lying in the pen or at the elevated platform or shelter
	Simultaneous resting in group housing
Thermal comfort	Respiration rate
	Red ears
Ease of movement	Hopping (number of consecutive hops), jumping, turning, running
	Number of lame rabbits
Good health	
Absence of injuries	Skin injuries/wounds
	Pododermatitis (only for reproducing does)
	Toe and ear damage figures (only for growing rabbits)
	Trichophagy (only for growing rabbits)
Absence of diseases	Percentage mortality and selection
	Clinical scoring of rabbits, consisting of symptoms listed
	Technical performance
Absence of pain induced by management procedures	Which mutilations are used (for identification)
	Presence of tissue growth when using ear marks.
Appropriate behaviour	
Expression of social behaviour	Scoring of injuries and wounds
	Scoring social behaviour
Expression of other behaviours	Abnormal behaviours
	Coat condition
	Kit mortality
Good human-animal relationship	Human approach test
Positive emotional state	Fear for novel objects
	Description of behaviour of a group
	Hopping behaviour in young rabbits

Table 3: Indicators used in protocols for assessing the welfare of farmed rabbits (modified from Paulović *et al.*, 2024).

Category	Indicator[1]	Description	Welfare consequence[1]
Behaviour	Abnormal behaviours	Stereotypic and abnormal behaviour	Inability to perform exploratory or foraging behaviour
	Social behaviour	Agonistic behaviour (grouped as well as individual behaviours such as frequency of displacements), negative, positive social behaviour	Group stress
	Isolated animals	Presence of isolated housed rabbits	Isolation stress
Environment	Cage/pen design	Size, presence of resources (e.g., elevated platform, foot rest) and behaviour related to this (e.g. standing upright, lying fully stretched)	Movement restriction
	Thermal stress	Panting, shivering and climate conditions (e.g., temperature)	Heat/cold stress
	Cleanliness	Clean or dirty body (parts), including wet body (parts)	Resting problem
Health	Skin lesions, wounds and dermatitis	On all body parts, including dermatitis, abscesses, dermatomycosis but not on hocks/ feet	Soft tissue lesions and integument damage
	Ocular and nasal discharge	Ocular and nasal discharge	Respiratory problems
	Pododermatitis	All degrees of pododermatitis and wet and dirty feet and including heel and middle foot	Soft tissue lesions and integument damage
	Mortality	Total mortality including culls, mortality per category (kits, does)	
Mental state	Animal-human relationship	Includes tests (avoidance distance, touch test), measures such as % or number of animals that can be approached, and aspects of handling by caretakers	Handling stress
Feeding	Water provision	Number of drinkers, cleanliness, water flow, etc.	Prolonged thirst
	Body condition	Includes scoring of body condition, proportions of lean or fat animals	Prolonged hunger
	Feed provision	Feeder space, cleanliness, type of feed, etc.	Prolonged hunger

[1]According to the definitions in EFSA (2022).

animal welfare (Good Behaviour, Good Housing, Good Health, Good feeding) supplemented with the Mental State criterion, according to the Five Domains Model (Table 3). These included both resource-based and ABMs where the welfare consequence intended to be measured are specified in Table 3.

On the other hand, the European Reference Centre for Animal Welfare for Poultry and Other Small Farmed Animals (EURCAW-Poultry SFA, 2023) has previously reviewed indicators used in the different protocols. Based on the available information and an EKE process, EURCAW-Poultry SFA assessed and scored the validity, feasibility and reliability of the different indicators, ranging from 1 (low validity/reliability based on literature or expert opinion; low feasibility, high cost/high execution time/high handling of animals is required) to 3 (high validity/reliability based on literature or expert opinion; high feasibility, low cost equipment is required. The following discussion resumes the outcomes of the evaluation by EURCAW-Poultry SFA (2023) with reference to ABMs for the different domains.

Health

The ABMs related to health are those with the highest validity, feasibility and validity over all animal categories and different protocols (Table 4). Skin alterations or injuries are mostly assessed by visual inspection considering the position, extent and severity of the lesions with different scoring methods. These latter can be more or less simple,

Table 4: Indicators for assessing Good Health under farming conditions: validity, feasibility and reliability (modified from EURCAW-Poultry SFA, 2023).

Animal category	Welfare consequence	Animal-based measures	Validity	Feasibility	Reliability
All	Skin lesions and wounds	Number of animals with wounds on the body and scoring by severity (e.g. size)	XX	XXX	XXX
Growing rabbits	Skin lesions and wounds	Number of animals with skin lesions (abscesses, ulcers etc.) and wounds and severity scoring Number of animals with wounds and scoring by severity Presence of skin lesions (abscesses) and wounds (no scoring) on eyes, ears, body (except ventral part)	XX	XX	XX
All	Skin lesions and wounds	Animals with fallen ears	XXX	XXX	XX
All	Skin problems	Animals with hairless areas	XXX	XXX	XXX
All	Skin problems	Animals with mange	XXX	XXX	XXX
All	Skin problems	Animals with dermatophytosis	XXX	XXX	XXX
All	Skin problems	Hair sampling for the detection of dermatophytes	XXX	X	XXX
Breeding rabbits	Pododermatitis	Number of animals with pododermatitis and severity scoring Number of animals with hyperkeratosis or ulceration (presence/absence)	XXX	XX	XXX
Breeding rabbits	Mastitis	Number of animals with mastitis and scoring by severity (mild/severe) (requires palpation)	XXX	XXX	XXX
All	Mortality	Mortality percentage on farm (period varying according to the protocol)	XXX	XXX	XXX
All	Culling rate	Culling rate on farm (period varying according to the protocol)	XXX	XXX	XXX
All	Respiratory disorders	Animals with nasal discharge (visually)	XX	XXX	XX
All	Respiratory disorders	Nasal swabs for detection of pathogenic respiratory bacteria and quantification	XXX	X	XXX
All	Respiratory disorders	Animals with ocular discharge (visually)	XX	XXX	XXX
All	Respiratory disorders	Animal showing coughing and sneezing (minimum observation time: 2 min)	XX	XXX	XXX
Breeding rabbits	Gastroenteric disorders	Number of animals with a hard abdomen (Enteropathy)	X	XX	XX
All	Gastroenteric disorders	Number of animals with liquid faeces around the perianal area (diarrhoea)	X	XX	XXX
All	Gastroenteric disorders	Rectal swabs for detection of pathogenic intestinal bacteria and quantification	XXX	X	XXX
All	Locomotory disorders	Number of animals with torticollis (scoring: moderate/severe)	XXX	XXX	XXX
All	Locomotory disorders	Lameness (Gait score)	XXX	XX	XXX

X: low; XX: medium; XXX: high.

based on the need of facilitating inspections and increasing the feasibility of measurements under commercial and field conditions. Mycosis is assessed as presence/absence, whereas isolation and culture of hair samples have reduced feasibility, despite high validity and reliability. Dermatophytosis must be diagnosed differentially from mite lesions, which are also evaluated as presence/absence.

Both pododermatitis and mastitis are evaluated as presence/absence and/or with different scoring that consider the severity and extent of the lesions in reproducing animals and, when possible, take into account the parity order and/or propose aggregations on overall indices referring to entire group in the farm.

The presence of torticollis, often associated with *Pasteurella multocida* middle ear infection and *Encephalitozoon cuniculi* infection, is defined as an abnormal position of the head and problems maintaining balance. It could be assessed in both reproducing females and fattening rabbits considering: no torticollis; a moderate problem, when the animal has a twisted neck but can eat and drink without difficulty; and a severe problem, when the twisted neck makes access to food and water difficult for the animal.

Respiratory disorders are assessed as presence/absence of animals with nasal discharge and/or eye discharge, whereas the presence of coughing and/or sneezing is not the best indicator for these disorders in terms of reliability/repeatability. Gastroenteric disorders can be assessed as animals presenting liquid faeces around the anus and/or considering the presence of pathogenic bacteria isolated from rectal swabs by non-rapid methods.

Mortality and/or culling rates finally represent overall indicators of animal health, being a measure of health problems, inadequate animal management and overall poor welfare.

Measures can refer to different production categories and consider the age of the animals (pre-weaning; post-weaning), if aggregating several cycles and/or referring to average, median and/or minimum values.

Good feeding and nutrition

The indicators identified for prolonged hunger are characterised by medium-high validity, feasibility and reliability (Table 5), where the body condition score (BCS) is a measure of inadequate feeding (unbalanced diet, insufficient quantity of diet) and presence of diseases. An impairment of the body condition has been linked with the onset of diseases such as mastitis, pododermatitis and rhinitis. The BCS can be assessed by visual inspection or by palpation, where visual assessment may reduce the validity and reliability of the indicator, but allows for increased feasibility in terms of time and reduced stress from handling animals. A further indicator, body symmetry, has recently been proposed (Cohen and Ho, 2023), for which information about validity, feasibility and reliability is not yet available.

Good housing and behavioural interactions with the environment

The welfare consequences that were largely intended to be measured by specific ABMs in the literature first refer to behavioural restrictions (resting, movement) and then to physiological alterations of the animal due to inadequate environmental conditions (Table 6).

Generally speaking, the corresponding measures usually have a low to medium degree of validity, with the exception of animal cleanliness, which is a measure recognised as highly valid. This measure is related to possible resting difficulties (associated with physical discomfort, cold stress, injuries, pain) and indirectly measures the hygienic

Table 5: Indicators for assessing Good Feeding under farming conditions: validity, feasibility and reliability (modified from EURCAW-Poultry SFA, 2023).

Animal category	Welfare consequence	Animal-based measures	Validity	Feasibility	Reliability
Reproducing does	Prolonged hunger	BCS assessed with palpation	XXX	XX	XX
Reproducing does	Prolonged hunger	BCS visually assessed	XX	XX	XX
Growing rabbits	Prolonged hunger	Number of small rabbits: twice as small as the others or very thin animals	XXX	XXX	XXX

X: low; XX: medium; XXX: high.

Table 6: Indicators for assessing Good Housing under farming conditions: validity, feasibility and reliability (modified from EURCAW-Poultry SFA, 2023).

Animal category	Welfare consequence	Animal-based measures	Validity	Feasibility	Reliability
Growing rabbits	Resting problem	Animals lying fully stretched	XX	X	X
All	Resting problem	Number of dirty and wet animals (scoring)	XXX	XX	XX
Growing rabbits	Resting problem	Number of animals resting in a group	*Lack of knowledge*		
Reproducing does	Movement restriction	Number of animals performing at least 2 jumps in the same direction or to/from the platform. Number of animals jumping and moving freely (%)	X	XX	X
Growing rabbits	Movement restriction	Number of animals making 1 jump or 2 jumps in different directions (minimum observation time: 2 min)	X	X	X
All	Movement restriction	Animals in upright position	Not assessed by EURCAW		
Reproducing does	Thermal stress	Panting animals	X	XX	X
All	Thermal stress	Lying fully stretched hyperventilating animals with red ears	XX	X	XX
Reproducing does	Thermal stress	Shivering animals (cold stress)	X	XX	X

X: low; XX: medium; XXX: high.

state of the housing or cages, besides the suitability of the facilities (type of flooring) and/or management (density of animals, frequency of cleaning interventions). As for the feasibility and reliability of ABMs intended to measure defects in the design and management of the environment, none of the proposed measures can be considered robust (Table 6).

Behavioural interactions with other animals and humans and mental health

The measure of aggression among rabbits in terms of skin lesions of the different categories can be related to lesions when aggression occurs repeatedly and the animals do not have the possibility of retreating or hiding. With respect to the ABMs referring to the Good Behaviour criteria, in most cases they were proposed to detect abnormal behaviours that may be associated with a condition of stress and frustration, sometimes due to a lack of resources necessary to display species-specific behaviours and/or fear (Table 7).

In general, the validity, feasibility and reliability of these ABMs are usually low or not measurable as they have never been tested under practical conditions (Table 7). The low validity of these ABMs for measuring stress/frustration confirms that information on the affective state (negative or positive) of rabbits is rather scarce.

POSITIVE WELFARE

As described above, the welfare assessment schemes applied up to now for rabbits have always referred to criteria and indicators that could measure the absence of negative experiences in the context of different principles or domains. Nevertheless, as previously introduced, the approach with respect to animal welfare is shifting: providing animals with opportunities for rewarding experiences and situations in which they feel satisfied is recognised as key for their welfare, beyond the alleviation of any suffering. Positive feelings could outweigh negative ones in order to achieve good overall welfare, even if how this could be achieved is still up for debate (Rault *et al.*, 2023). Importantly, the ability of animals to cope with different stimuli and environments for reaching positive emotional states and being resilient under different farming conditions can greatly contribute to their welfare status (Rault *et al.*, 2023). Nevertheless, several factors (e.g. genetics, pre-birth and early experiences, etc.) account for individual differences

Table 7: Indicators for assessing Behavioural interactions with other animals and humans and Mental state under farming conditions: validity, feasibility and reliability (modified from EURCAW-Poultry SFA, 2023).

Animal category	Welfare consequence	Animal-based measures	Validity	Feasibility	Reliability
Reproducing does	Skin lesions and wounds	Number of animals biting other adults or kits (minimum observ. time: 2 min)	XX	X	XX
Growing rabbits	Skin lesions and wounds	Number of animals biting or fighting with conspecifics (minimum observ. time: 2 min)	X	XX	XX
Reproducing does	Inability to perform positive social interaction	Number of allo-grooming events (minimum observ. time: 2 min)	X	XX	XX
Growing rabbits	Inability to perform positive social interaction	Number of allo-grooming events (minimum observ. time: 2 min)	XX	X	XX
All	Inability to perform gnawing behaviour	Number of animals biting or digging the cage for more than 3 sec.	XX	X	XX
	Abnormal behaviour	Animals showing head shaking, swaying, cage gnawing, empty digging, obsessive cleaning (minimum observ. time: 2 min)			
Reproducing does	Abnormal behaviour	Number of nervous and restless animals (minimum observ. time: 2 min)	X	XX	XX
Growing rabbits	Abnormal behaviour	Number of nervous and restless animals (minimum observ. time: 2 min)	X	X	XX
Reproducing does	Abnormal behaviour	Number of animals performing self-grooming (minimum observ. time: 2 min)	*Lack of knowledge*	XX	XX
Growing rabbits	Abnormal behaviour	Number of animals performing self-grooming (minimum observ. time: 2 min)	*Lack of knowledge*	X	XX
Reproducing does	Fear	Good human-animal relationship: human approach test with a 10 cm stick (for 30 sec)	X	XX	XXX
All	Pain	Squeal loudly and grind the teeth	*Lack of knowledge*		
All	Pain	Assessment of facial expressions (Rabbit Grimace Scale)	XXX	*Lack of knowledge*	

X: low; XX: medium; XXX: high.

among animals (LIFT, 2024). In other words, the previous (positive or negative) experience of animals can affect their cognitive ability and finally their ability to cope with the environment. Definitively, this is a more comprehensive approach to animal welfare that takes into account both physical and emotional aspects (Turner, 2019; Paulović *et al.*, 2023).

Although the behavioural needs of rabbits are fairly well-known, affective states are poorly understood. In other words, we do not clearly and fully know the situations that are rewarding and positively stimulate the affective status of rabbits. We have not yet identified indicators of a positive affective status that could help us evaluate rabbit welfare and/or compare different farming practices and systems. Information about how the cognitive ability of rabbits can be affected by positive or negative experiences, and how this can influence farm welfare, are not yet available. On the other hand, some information is available in laboratory rabbits (Jirkof *et al.*, 2019; Cohen and Ho, 2023).

Compared to other animals, rabbits are even more sensitive and difficult to deal with regarding this topic. In fact, they are very sensitive animals. As prey animals, they are constantly vigilant and mentally occupied with the potential threat of predators, and can exhibit a range of emotional states in a relatively short period. In general, the study of animal behaviour is considered to be more functional for the assessment of affective state than the measurement of physiological and neuroendocrine variables (Jirkof *et al.*, 2019; Turner, 2019). On the other hand, even behaviours for

Table 8: Putative candidate behaviours for assessing affective status and positive welfare in rabbits (modified from Cohen and Ho, 2023).

Category	Description
Natural behaviours	Binkying or frolicking (jumping rapidly while shaking head and flinging hindlimbs to the side)
	Grooming (self-grooming, allo-grooming, mutual grooming)
	Nocturnal/crepuscular behaviour
	Nesting (for breeding does)
	Regular eating with occasional drinking
	Coprophagia
Territorial and hierarchical behaviours	Scent marking by chinning objects
	Cage guarding*
	Marking territory with urine or faeces (spraying): May be due to frustrated sexual behaviours of entire rabbits*
Social and exploratory behaviours	Foraging
	Investigative behaviour
	Rearing or peri-scoping
	Digging or burrowing
	"Tooth purring" or "teeth chattering": different from tooth grinding (bruxism)
Resting behaviours	Sprawling or stretching out[1]
	Laying down or "flopped" on their side[1]

[1]Could be also negative/neutral

which a relation with a positive emotional situation is widely recognised (e.g. playing) could in practice give different information both related to positive or non-positive well-being depending on the situation (e.g. different age of the animals, duration and time of expression, context) (Jirkof *et al.*, 2019).

Thus, some behaviours have been identified as possible candidates for measuring a positive affective status and identifying positive indicators in rabbits, with special reference to natural behaviours, territorial and hierarchical behaviours, social and exploratory behaviours and resting behaviours (Table 8). While some of these behaviours are clearly associated with the good status of the animals (e.g., nesting for breeding does, regular eating with occasional drinking and coprophagia for all categories), the validity of others for evaluating positive welfare needs to be based on the knowledge of the behavioural requirements of rabbits under farming conditions.

More specifically, research would be needed into the relationship between certain behaviours (with special reference to spontaneous behaviours, playing, movement, social and exploratory behaviour, maternal behaviour, body and ear position, facial expressions and vocalisations) and the positive affective state in different categories of rabbits. The relative importance of these behaviours should evaluate the rabbit response when offering rewarding materials and/or when allowing expressing species-specific behaviours with strong motivation (gnawing materials as environmental enrichment; group resting and allo-grooming; the possibility of moving away from conspecifics; nest construction and access). Indeed, if agency is referring to what animals "want", i.e. motivated behaviours, which could be driven by the associated/expected positive emotions, agency domain and behavioural interactions have been proposed as the framework under which positive animal welfare can be assessed within the Five Domains Model (Littlewood *et al.*, 2023).

In laboratory rodents as in other species, different tests have been used to assess their biological needs. These tests are also referred to as apparatus-based behavioural test paradigms, including anxiety-related tests, preference tests, strengths of preferences and cognitive judgement bias tests (Table 9).

Observations under different conditions (i.e. different apparatus-based behavioural test paradigms) will reveal behaviours associated with affective state. In detail, in laboratory rodents, the following behaviours have been recorded to evaluate their affective states, which could also be tested in rabbits, i.e. spontaneous behaviour, play behaviour, vocalisation, facial expression, nest building, burrowing and grooming. These behaviours also have the potential to be used in definition of the affective states of rabbits.

Table 9: Main Apparatus-Based Behavioural Test Paradigms used in laboratory rodents to assess their affective state (modified from Cohen and Ho, 2023).

Anxiety-related tests	Elevated plus-maze (EPM) Elevated zero maze (EZM) Black/white box (B/W box or *Dark-light exploration test*) Open-field test (OF) Free exploration tests (FET)
Preference test	Test the preference between two or more items
Strengths of preferences	Test the willingness to pay for the chosen item
Cognitive judgement bias	Affective states are measured indirectly testing cognitive abilities

These tests can also be used for evaluating any differentiated responses in animals previously offered materials and/or situations that are rewarding with respect to the possibility of expressing species-specific behaviour. Based on the same principle, cognitive tests evaluate the animal response with respect to the ability to make positive/negative judgements on ambiguous stimuli based on their emotional state.

Out of the different tests (Table 10), the open-field and the novel-object tests on one side and the human approach and tonic immobility tests have largely been used to evaluate the level of anxiety or fear of farmed rabbits with respect to a novel environment/object and humans, respectively. Although these tests aim to describe the level of anxiety/fear in animals subjected to different experimental protocols, they can themselves elicit a state of anxiety and, accordingly, they must be associated with other tests and measurements in order to provide robust results for

Table 10: Apparatus-Based Behavioural Test Paradigms used in welfare studies on farmed rabbits.

Test type	Tested factors	Reference
Open-field test	Presence of gnawing hay blocks	Birolo *et al.*, 2022
	Environmental enrichments; age	Trocino *et al.*, 2019
	Floor type; stocking density; age	Trocino *et al.*, 2018
	Litter size; age	Gümüş *et al.*, 2018
	Pre-natal and post-natal effects of semi-group housing on rabbit behaviour	Buijis and Tuyttens, 2015
	Cages vs pens	Trocino *et al.*, 2014
Novel-Object Test	Gnawing objects	Birolo *et al.*, 2022
	Semi-group vs. single housing	Buijis and Tuyttens, 2015
	Environmental enrichments; age	Trocino *et al.*, 2018
Dark-light box test	Litter size; age	Gümüş *et al.*, 2018
Human approach testing	Presence of gnawing objects	Birolo *et al.*, 2022
	Floor type; stocking density; age	Trocino *et al.*, 2018
Preference test	Social contact vs seclusion of does	Dal Bosco *et al.*, 2020
	Cage size	Mikó *et al.*, 2012
	Nesting material for does	Farkas *et al.*, 2018
	Floor type; gnawing material	Princz *et al.*, 2008
	Presence of mirrors	Dalle Zotte *et al.*, 2009
	Floor type	Morisse *et al.*, 1999
Tonic immobility	Cages vs pens	Trocino *et al.*, 2014
	Floor type; stocking density; age	Trocino *et al.*, 2018
	Housing system; age	Trocino *et al.*, 2013
Object recognition task Object location task Olfactory object recognition task	Type of litter; age	Gümüş *et al.*, 2018
Social runway test	Semi-group vs. single housing	Buijis and Tuyttens, 2015

comparisons (Jirkof *et al.*, 2019). In fact, Buijs and Tuyttens (2015) hypothesised that the results they obtained with rabbits during the open-field test more likely described the motivation of rabbits to look for conspecifics rather than their fear level towards the new environment. They concluded that this test could not be considered appropriate in terms of (negative) rabbit welfare assessment. On the other hand, its potential for evaluating (positive) exploratory behaviours needs further investigation.

In farmed rabbits, several studies have also used preference tests to gather information on the most preferred situation out of different cage sizes, nesting material, enrichment types and/or floor type. These tests, however, have not yet been calibrated and validated in rabbits in the perspective of evaluating their on-farm welfare through behavioural needs and emotions.

CONCLUSIONS

In the absence of fundamental basic knowledge about behavioural needs and emotions in rabbits, the optimisation of their on-farm welfare is particularly challenging. This fact is even more impacting in view of the current transition towards collective and cage-free housing systems as foreseen by the European Resolution that followed the Initiative of the European Citizens "End the cage age". In these systems, some major behavioural needs may be challenged, such as social relationships and maternal behaviours of reproducing does, as in farms the conditions under which the group is formed and the social activities or behaviours may differ substantially from those in natural or semi-natural environments.

To date, the assessment of welfare in farmed rabbits under different housing and management conditions has been based on animal-based indicators related to health concerns and behavioural restrictions have been used. In perspective, the assessment of welfare in farmed rabbits should in the future also include positive welfare indicators, considering that animals should be provided with opportunities for positive experiences under the assumption that no pain or suffering is inflicted on an animal. In this context, the identification of positive welfare indicators is even more challenging, given the biological and behavioural characteristics of this species, as well as the lack of validated protocols and methods for other species. Accordingly, a comprehensive and robust assessment of on-farm rabbit welfare cannot do without structure- and management-based indicators, which should be included in validated and standardised protocols using a multi-indicator approach.

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