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EU-FORA SERIES 8

Ensuring ethical production of beef: A comprehensive risk assessment of animal welfare during transportation and slaughter processes

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts.

Abstract

Animal welfare is a recognised and important issue within the European Union, addressed through comprehensive regulations such as Regulations 1/2005 and 1099/2009, which emphasise the necessity to respect and protect the welfare of animals, particularly during transport and slaughter procedures. These legislative measures are part of an ongoing European commitment to ensure ethical treatment and minimise suffering for food-producing animals, aligning with broader food safety and public health goals. This project aimed to create a prototype of an updated cattle path designed to minimise stress and reduce reliance on coercive means such as electric prods, while also decreasing the risk of injury due to trauma or falls. The approach focused on implementing evidence-based methods and integrating preventative design features that support both animal behaviour and safety in handling environments. The ultimate goal was to guarantee the welfare and protection of animals as specified in European guidelines, while also achieving improvements in meat quality. By advancing these objectives, the project demonstrates how compliance with animal welfare standards can be harmonised with operational efficiency and product quality, benefitting both animals and consumers across the European Union.

KEYWORDS

animal welfare, beef production, cattle, slaughter process, transport of animal

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SUMMARY

The European Union has developed a comprehensive and stringent regulatory framework to ensure both food safety and animal welfare throughout the food supply chain. Beginning with Regulation (EC) No 178/2002, the EU established core principles for food law, created the European Food Safety Authority (EFSA) and outlined essential procedures for protecting public health. This foundation was expanded by the 2004 'hygiene package', which harmonised hygiene and control standards from animal farming through food distribution.

Animal welfare is now an integral part of food safety legislation, notably emphasised in Regulations 853/2004, 625/2017 and 1099/2009. These laws mandate coordinated, transparent and science-based actions across all food chain actors and require continual updates to incorporate new scientific knowledge. Official veterinarians play a key role in enforcing compliance, taking corrective action and upholding welfare standards, especially during animal transport and slaughter.

To enhance animal welfare and reduce stress during handling, an innovative prototype cattle path made from multilayer pressed cardboard was developed. This eco-friendly, recyclable material was tested in real slaughterhouse conditions for resistance to compression, tearing, humidity and trampling. Cattle using the prototype path exhibited fewer slips, stops and required no electric prods. Biochemical measurements further showed lower levels of cortisol and beta-endorphins – markers of stress – in these animals, indicating improved welfare outcomes.

The results demonstrate that the integration of proper handling practices and sustainable materials can effectively reduce animal stress, improve welfare and enhance both meat quality and operational safety in abattoirs. This project aligns with EU legal requirements, scientific best practices and sustainability objectives, offering a valuable model for modernising livestock management across Europe.

1 | INTRODUCTION

The European Union has established a robust regulatory framework to ensure food safety and animal welfare throughout all stages of the food supply chain, beginning with Regulation (EC) No 178/2002. This regulation laid down the core principles of food legislation, created the European Food Safety Authority (EFSA) and defined procedures to safeguard public health. The 'hygiene package' adopted in 2004 reorganised regulations, taking effect from 1 January, 2006, and ensuring safety in animal farming, production and distribution.

EFSA was founded to protect consumer health and restore confidence after public health crises in the 1990s, such as BSE and dioxin contamination events. Its mandate is clear: provide scientific opinions and risk assessments for European institutions, thereby guiding laws affecting consumer safety. EFSA's impartial advice underpins regulatory acts – an example being Regulation 854/2004, which simplified rules for official controls on animal-origin products based on EFSA's guidance.

Animal welfare has become increasingly central to EU food legislation. Regulation 882/2004 asserted that animal health and welfare significantly influence food quality and safety, disease prevention and humane treatment. European law requires communication between all actors in the food supply chain to ensure coordinated, science-based practices. Regulation 853/2004 reiterates the need for efficient communication across food business operators, while Regulation 625/2017 and 1099/2009 further stress adaptable controls, transparency and continual updates reflecting new scientific knowledge (Anonymus, 2002, 2004a, 2004b, 2017, 2019). Official veterinarians, under these laws, ensure immediate corrective action for non-compliance, enforce proportionate responses and cooperate with other authorities to uphold welfare standards, especially during transport and slaughter (Čobanović et al., 2024; Ferri et al., 2023; García-Díez et al., 2023; Harley et al., 2012; Kautto et al., 2023; Valkova et al., 2022).

Protecting animals at the time of slaughter is legislatively framed as minimising anxiety and suffering. Regulation 1099/2009 acknowledges that even under optimal conditions, slaughter can cause pain or fear. Thus, personnel must implement advanced techniques and comply with sector best practices to reduce suffering. Continuous improvement is encouraged, recognising that better animal protection also leads to improved meat quality and safer working conditions for slaughterhouse staff (Anonymus, 2013; Arzoomand et al., 2019; Guardone et al., 2020; Stärk et al., 2014; Vidal et al., 2016).

Despite regulatory advances, there is no full scientific consensus on the best parameters for assessing unconsciousness in slaughtered animals. Regulation 1099/2009 recommends using indicators such as loss of posture, lack of voluntary movement and absence of reflexes or responses to stimuli as practical measures of unconsciousness and insensitivity. Ongoing research aims to refine these benchmarks and improve the effectiveness of stunning methods.

Large-scale European studies – such as the Dialrel project and BoRest – have tackled these complex issues. Dialrel, funded by the European Commission, brought together academics, industry stakeholders and public authorities to examine religious, legislative and welfare standards, striving to harmonise divergent views and practices. BoRest evaluated containment systems and practices across EU and third countries, assessing welfare impacts of slaughter with and without stunning. Key findings have informed updated recommendations and equipment designs, balancing animal welfare, meat quality, work safety and socio-economic constraints (https://www.dialrel.net, https://op.europa.eu/en/publication-detail/-/publication/02969f61-ca0a-11e9-992f-01aa75ed71a1#).

Practical guidelines have emerged from EU research and field observations to guide slaughterhouse design and operation (Anonymus, 2013, 2016, 2020; Antunović et al., 2021; Bonardi et al., 2021; Ferri et al., 2023). Key principles include:

- Layout, construction and equipment: Well-designed fences, corridors, ramps and flooring reduce animal stress and risk of injury. Non-slip, well-drained floors and gentle slopes enable animals to move independently and calmly. Corridors should allow group movement, avoid dead ends and sharp turns and use solid sides to prevent distractions.
- Lighting and noise: Animals prefer moving from dark to lighter areas but are frightened by sudden or intense lights and loud noises. Uniform lighting and minimising sudden noises help prevent panic.
- Water, ventilation, pens: Ready access to clean water, ample space to turn and separation of different animal types reduces aggression and distress. Effective ventilation counters heat and gas build-up.
- Equipment for containment and handling: Mechanical restraints should be tailored to species and used only by trained staff. Unacceptable practices include suspending, immobilising with electric shocks or causing pain intentionally – such actions are strictly prohibited.
- Handling best practices: Calm, confident and gentle handling avoids causing bruising or distress. Handlers should use natural animal behaviours and avoid obstructing or frightening animals. Injured animals unable to move should be euthanised where they are to avoid further suffering.

The correct application of stunning must always be verified. After stunning, operators check for immediate collapse, lack of regular breathing, absence of righting reflex and other signs to confirm unconsciousness. EFSA recommends specific criteria for monitoring and, if necessary, immediate corrective procedures.

The project also explored the stress response in cattle, noting that both short- and long-term stress (measured biochemically by cortisol and beta-endorphins) affects welfare and meat quality. Chronic stress disrupts immunity, growth and behaviour, while acute pain is moderated by neuropeptides such as beta-endorphin. Good design and handling reduce negative stress effects and improve outcomes.

A particularly innovative aspect of this research was the prototyping of cattle paths and traps using eco-sustainable materials such as corrugated cardboard. This material, commonly used for packaging, proved to be 100% recyclable,

biodegradable and customisable. It was tested in two thicknesses (1.5 and 3 cm) as flooring and side panels. Repeated tests with increasing numbers of cattle evaluated resistance to compression, tearing, humidity and trampling. Observation showed that, after trial runs with groups of 5, 15 and 25 animals, the material maintained functionality, though some damage was evident – providing valuable data for future refinements and confirming the promise of sustainable solutions in the improvement of animal handling environments.

In conclusion, the body of EU legislation, supported by scientific research and practical innovation, continues to drive improvements in animal welfare and food safety. Projects like these underscore the value of combining legal requirements, evidence-based best practices and sustainable design to ensure the ethical and efficient management of livestock in the European food industry.

2 | BACKGROUND

This project aimed to create a prototype of an updated cattle path that minimizes stress and reduces the use of coercive means such as electric prods, while also decreasing the risk of injury due to trauma or falls. The ultimate goal was to ensure the welfare and protection of the animals, as outlined by European guidelines, while simultaneously improving the quality of the meat produced.

3 | DATA AND METHODOLOGIES

3.1 | Data

Stress, especially long-term, can negatively affect the welfare of an animal. Stress is a widely used term in human society and may be defined as 'the non-specific response of the body to any demand for change'. In general, stress is a change in the psychological, physiological and/or physical characteristics of an organism. During stress, the animal's behaviour is altered to increase alertness, increase cardiac output, respiration and catabolism, as well as to divert blood flow to provide full perfusion of the brain, heart and muscles. In response to a stressor, the hypothalamic–pituitary–adrenal axis is activated, resulting in the secretion of its terminal hormones, the glucocorticoids. One of these glucocorticoids – and the most common stress hormone in mammals – cortisol, plays a key role in the release of energy, immune and mental activity, development and growth, as well as reproductive functions. In its important role in the stress response, cortisol is sensitive to both physical and emotional stimuli; its release is based on a circadian rhythm. Under physiological stress, cortisol modulates the immune system and mobilises energy stores, making more resources available to respond to a particular stressor. While glucocorticoids are useful for short-term survival, prolonged/increased release (chronic stress) can lead to metabolic, immune and physiological dysfunctions. Therefore, the cumulative occurrence of stressors could lead to changes in the welfare of an animal, changes in its social behaviour and the development of pathologies. It has been shown that chronic stress in domestic animals leads to altered levels of cortisol, reduced growth and reduced play behaviour in the young. High levels of cortisol are also linked to many diseases and behavioural, physiological, nutritional disorders, as well as obesity and diabetes caused by increased plasma glucose concentrations due to cortisol (Andoni et al., 2023; Ferri et al., 2023; García-Díez et al., 2023; Stella et al., 2022; Valadez-Noriega et al., 2022; Valkova et al., 2022).

Beta-endorphin is an endogenous opioid neuropeptide and peptide hormone produced in certain neurons within the central and peripheral nervous systems. Beta-endorphin's function is known to be associated with hunger, fear and pain, as well as maternal care, sexual behaviour and reward cognition. Broadly speaking, beta-endorphin is mainly used in the body to reduce stress and maintain homeostasis. In behavioural research, studies have shown that beta-endorphin is released in response to a wide variety of stimuli. Beta-endorphins have been studied primarily for their influence on nociception (i.e. pain perception). Beta-endorphins modulate pain perception in both the central and peripheral nervous systems. When pain is perceived, pain receptors (nociceptors) send signals to the dorsal horn of the spinal cord and then up to the hypothalamus, via the release of a neuropeptide called substance P. In the peripheral nervous system, this signal causes T lymphocytes – white blood cells from the immune system – to be recruited to the area where pain was perceived. The T lymphocytes release beta-endorphin in this localised region, allowing it to bind to opioid receptors, causing direct inhibition of substance P. In the central nervous system, beta-endorphins bind to opioid receptors in the dorsal root and inhibit the release of substance P in the spinal cord, reducing the number of excitatory pain signals sent to the brain. The hypothalamus responds to the pain signal by releasing beta-endorphins through the periaqueductal grey network, which acts primarily to inhibit the release of GABA, a neurotransmitter that prevents dopamine release. Therefore, inhibition of GABA release by beta-endorphin allows for greater dopamine release, contributing in part to the analgesic effect of beta-endorphin itself. The combination of these pathways reduces the sensation of pain, allowing the body to stop a painful impulse once it has been sent (Čobanović et al., 2024; Earley et al., 2017; García-Díez et al., 2023; Guardone et al., 2020; Hartung, 2003; Padalino & Raidal, 2020; Valadez-Noriega et al., 2022; Valkova et al., 2022).

Corrugated cardboard is the material mainly used for packaging. It consists of two flat paper surfaces, known as liners, and a corrugated sheet, all held together by natural adhesives. The combined action of the liners and the inner wave gives rigidity and strength to the cardboard and, as a result, to the packaging. There are many types of cardboard that differ based on the papers used, weights, wave heights (single, double or triple) and the coefficient of corrugation. Corrugated

cardboard is 100% recyclable and biodegradable and allows versatile protection and transport of the products it wraps. The different types of waves that can be combined give rise to an extremely versatile product, making infinite uses, development and customisation possible thanks to the use of state-of-the-art digital printing and cutting technologies, ongoing research into materials and finishes and the design and creativity of designers.

3.2 | Methodologies

The resistance to compression, tearing, humidity and trampling were evaluated both in the single-layer version and with multiple layers coupled together for repeated tests with a high number of animals.

3.3 | Resistance test to compression, tearing, humidity and trampling

The chosen product was delivered by the manufacturing company in two types: thickness 1.5 cm and thickness 3 cm. In short, the test consisted of placing the flooring along the corridor that leads the animals from the resting stalls to the trap and a panel at the curve leading to the final section of the path. The condition of the material, pending more precise laboratory assessments, is illustrated in the following images (Photograph 1–15).



PHOTOGRAPH 1 Folding floor of pressed cardboard, thickness 3 cm in three layers.



PHOTOGRAPH 2 Folding floor of pressed cardboard, thickness 1.5 cm in two layers.



PHOTOGRAPH 3 Side panel for prototyping the curved path.



PHOTOGRAPH 4 Installation of supports for side panels.



PHOTOGRAPH 5 Cardboard floor in situ.



PHOTOGRAPH 6 Side panel in situ.



PHOTOGRAPH 7 Condition of the floor after the passage of 5 animals.



PHOTOGRAPH 8 Condition of the floor after the passage of 15 animals.



PHOTOGRAPH 9 Condition of the floor after the passage of 25 animals.



PHOTOGRAPH 10 Condition of the side panel after the passage of 25 animals.



PHOTOGRAPH 11 Details of damage to the side panel after the passage of 25 animals.



PHOTOGRAPH 12 Side panel still intact and able to perform the function after the passage of 25 animals.



PHOTOGRAPH 13 Condition of the materials at the end of the resistance test.

3.4 | Material

The material is beige and is well tolerated by the animals (cf. the indications from the Fear Free colour palette association: (https://www.dvm360.com) (Figure 1).



FIGURE 1 Palette of colours that do not frighten animals, with indication of the colour of the material used in our prototype.

After the transit of 25 animals, with wet concrete underneath, the material did not experience damage that would render it unusable or hinder the advancement of the animals. The same can be said for the side panel, positioned at the point of greatest contact and pressure from the animals (it is a narrowing, also a critical point in the corridor where two animals often present themselves simultaneously).

The uniformity of the material ensures the animals proceed linearly and without hesitation. It should be noted here that the current configuration of the concrete floor has a grill that, alternating dark and light areas, makes animal movement uncertain.

Another important note is the difficulty animals have walking on the existing slippery floor. The cardboard prototype instead allows animals to move forward without stress, to the point that after contacting the surface their gait becomes more secure and brisk.

Similar considerations apply to the panelling, which makes the wall uniform and limits distractions for the animals (Ceccarelli et al., 2018; García-Díez et al., 2023; Guardone et al., 2020).



PHOTOGRAPH 14 Brisk and safe gait on the floor covered by the prototype.

The material can be used successfully in the creation of the definitive prototype, which should then be evaluated by measuring animal welfare and meat quality parameters with reference to control animals (see PSR project filed at the time of the call) (Figure 2).

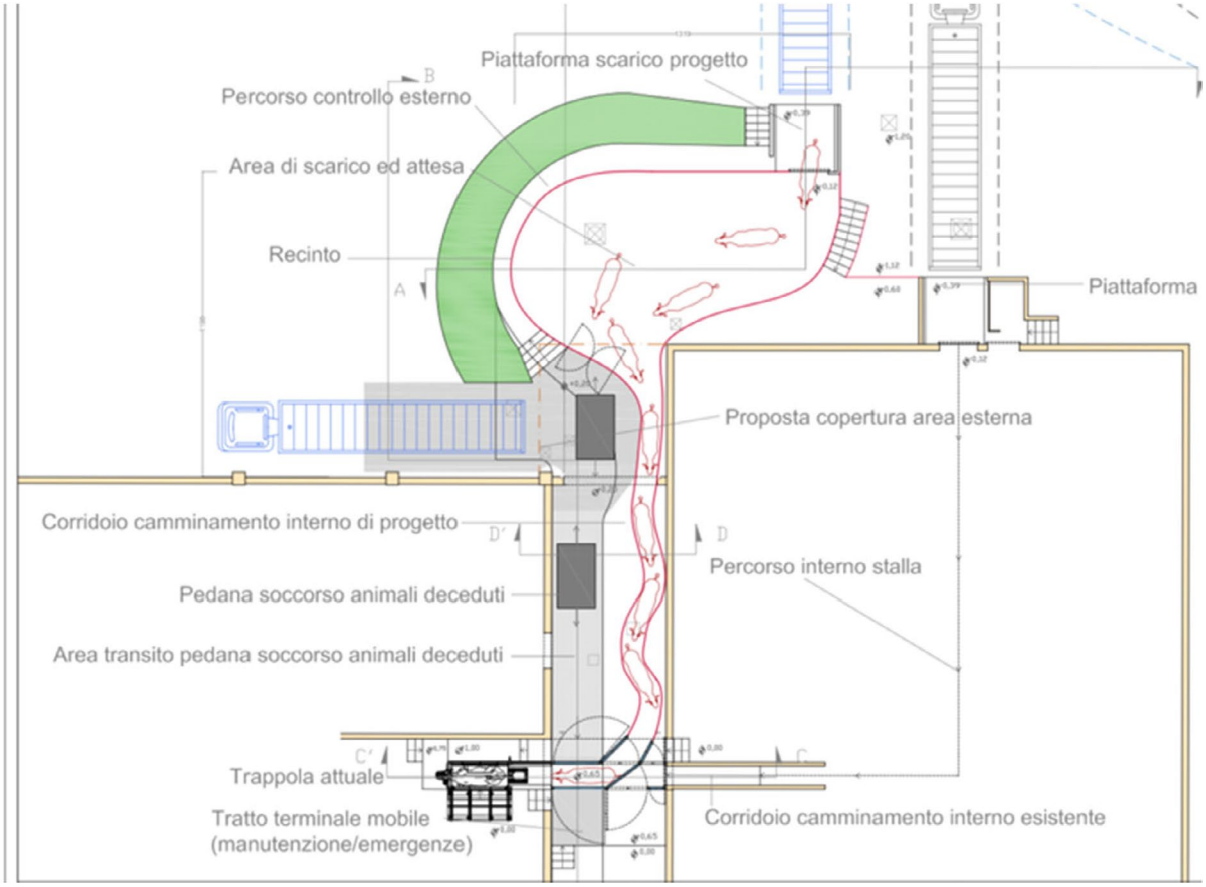
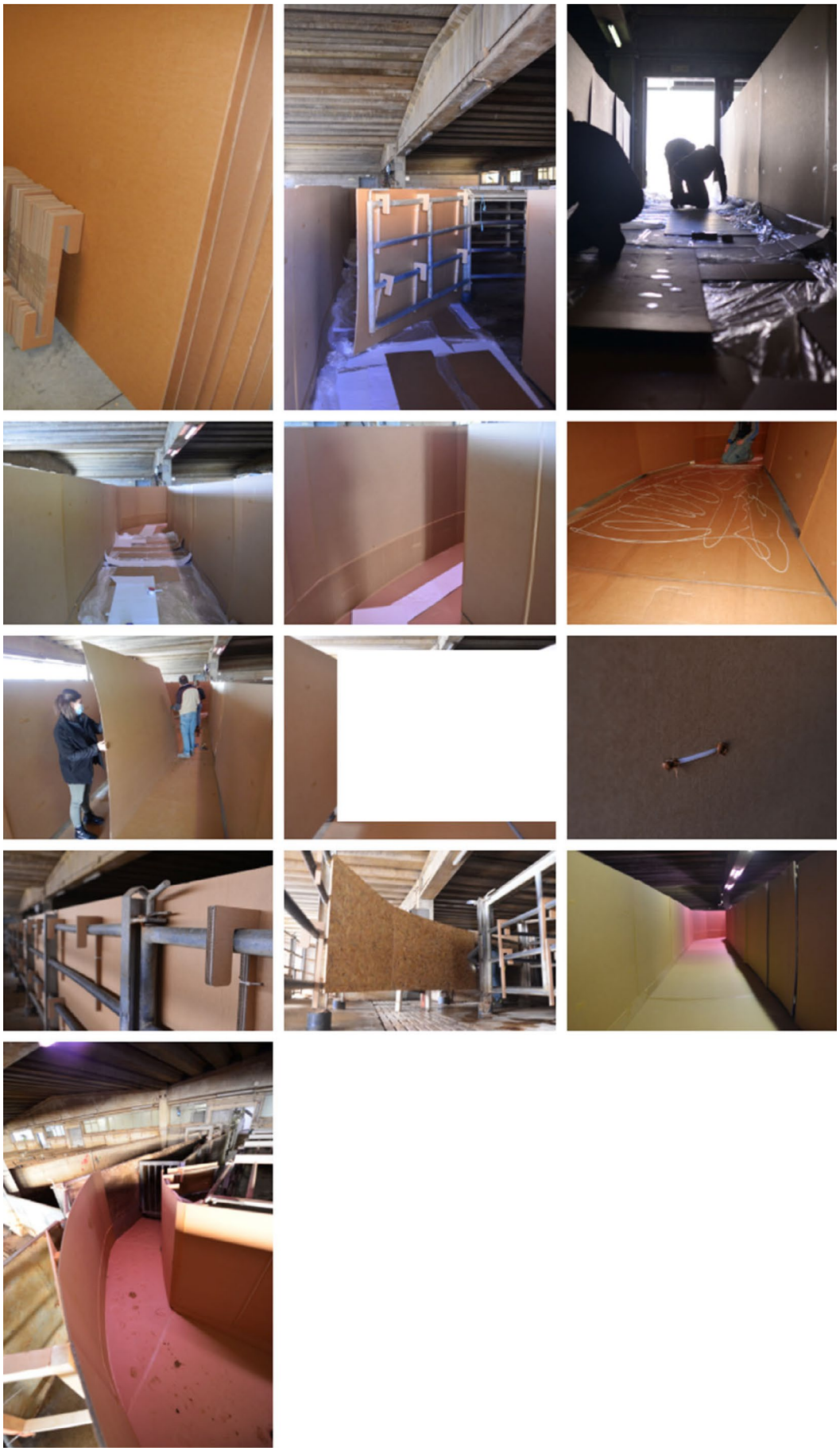


FIGURE 2 The path with a curved layout.



PHOTOGRAPH 15 Photos showing the construction of the prototype. The utmost care was taken to ensure uniformity of the structure and to limit light infiltration through the use of adhesives of the same colour as the panels.

3.5 | Statistical analysis

Data Analysis Software. All statistical analyses were performed using StatView (version X, Abacus Concepts Inc., Berkeley, CA, USA) for Mac OS. StatView is an integrated statistical analysis and graphics package offering comprehensive capabilities for descriptive and inferential statistics, including ANOVA and post-hoc comparisons. StatView was selected for its intuitively clear interface and low learning curve, which are especially advantageous for teaching purposes, despite being an older software package designed to run exclusively on PowerPC Macintosh systems with Mac OS 9.

Statistical Analysis. Descriptive statistics (mean and standard deviation) were calculated for each group. Group comparisons for serum cortisol and beta-endorphin concentrations were conducted using a one-way analysis of variance (ANOVA), provided parametric assumptions (normality and homogeneity of variance) were met. Whenever ANOVA indicated a significant overall effect ($p < 0.05$), pairwise group differences were further assessed using Tukey's Honestly significant difference (HSD) post-hoc test to control for multiple comparisons. The significance threshold for all statistical tests was set at $p < 0.05$. All reported p-values correspond to two-tailed tests unless otherwise specified.

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4 | ASSESSMENT

4.1 | Observed welfare parameters

Number of activities/behaviours indicative of non-compliance with animal welfare for each animal; mean of 25 animals per group (Table 1).

TABLE 1 Observational parameters.

	Prod	Falls/slips	Stoppage in march
Control group ($n = 25$)	0.56	0.75	0.50
Prototype group ($n = 25$)	0.00	0.00	0.04

Analysis of the table shows that in the control group, the operators had to resort to using the electric prod once every two animals (0.56 times per animal) and the animals often suffered falls or slips (0.75 times per animal) or stopped (0.50 times per animal).

In the group of animals examined with the prototype installed, it was never necessary to use the electric prod and no animal ever slipped. Only in one case did an animal make a 'U-turn', but this is attributable to the excessive width of the pre-existing structure, which with the prototype could only be partially reduced.

4.2 | Blood chemistry parameters

The animals under study were divided into three groups. The first group (A), made up of 17 animals, used the prototype path to reach the capture trap. The second (B), made up of 24 animals, used the normal path on a day characterised by low stress levels. The third (C), made up of 30 animals, used the normal path on a day characterised by high stress levels. The animals in these groups were heterogeneous in breed, age, sex and origin. Blood samples were taken during jugulation using tubes without anticoagulant. Samples were transported to the laboratory and centrifuged at 3000 rpm for 10 minutes. The serum thus obtained was collected and placed in sterile Eppendorf tubes for subsequent analyses. Quantification of cortisol and beta-endorphins was carried out on the serum using commercial ELISA kits (Abbexa LTD, Cambridge, UK). These are kits based on a competitive binding technology between enzyme and antibody. Standards and samples to be analysed are introduced into the available wells. At the end of the procedure, the optical density of the individual wells was measured spectrophotometrically at a wavelength of 450 nm and the analyte concentration was calculated.

As regards cortisol, group A (prototype) had the lowest average value (375.96 ng/mL), followed by group B and group C (controls) with average values of 397.71 ng/mL and 1029.26 ng/mL respectively. The difference in cortisol concentration

in the serum was very large and statistically significant between groups A and B compared to group C, with a *p*-value < 0.0001. As for beta-endorphins, the lowest serum values were those of group A (prototype) averaging 8.03 pg/mL, followed by groups B and C (controls) with mean values of 9.35 pg/mL and 31.14 pg/mL respectively. Again, the difference between control group C and the other two groups was statistically significant with a *p*-value < 0.05.

Cortisol is considered an important blood chemistry indicator of stress. In stressful conditions, the animal's behaviour is altered to increase alertness, cardiac output, respiration and catabolism, as well as to divert blood flow to ensure full perfusion of the brain, heart and muscles. In response to a stressor, the hypothalamic–pituitary–adrenal axis is activated, resulting in the secretion of its terminal hormones, the glucocorticoids, among which is cortisol. Beta-endorphins are one of three types of endogenous opioid neuropeptides produced by mammals. Beta-endorphins are mainly used by the body to reduce stress and maintain homeostasis. The results obtained show that proper management of animals aimed at minimising stress factors in the phases prior to slaughter leads to significant reductions in serum levels of these analytes and that the prototype path under study excellently fulfils this function (Figures 3 and 4).

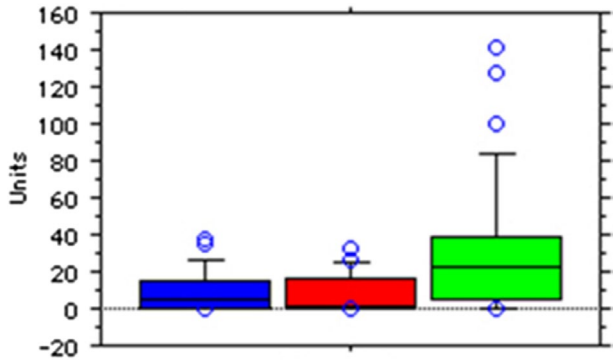


FIGURE 3 Beta-endorphins level in serum samples, expressed in pg. mL^{-1}. Blue: Normal path with correct handling; red: Innovative path; green: Normal path with non-correct handling.

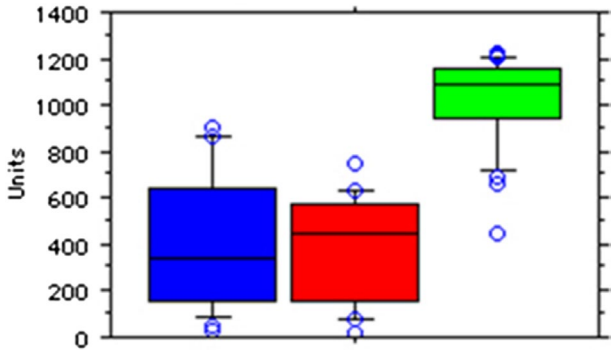


FIGURE 4 Cortisol level in serum samples, expressed in ng mL^{-1}. Blue: Normal path with correct handling; red: Innovative path; green: Normal path with non-correct handling.

5 | CONCLUSION

For the prototype's construction, different materials were evaluated. In the end, the choice fell on multilayer pressed cardboard which guaranteed the right balance between functionality, cost and resilience. The group managed using the innovative prototype path and correct handling procedures exhibited a reduced need for coercive intervention by the operators. Additionally, the animals displayed improved ethological indicators of stress, and their blood levels of cortisol and beta-endorphins were lower.

The results obtained in this study demonstrate that the prototype path enables cattle to move forward more easily, thereby enhancing their welfare and minimising risks for both the animals and operators. The application of the prototype path developed in this study has the potential to significantly improve animal protection conditions and meat quality in numerous abattoirs.

6 | RECOMMENDATIONS

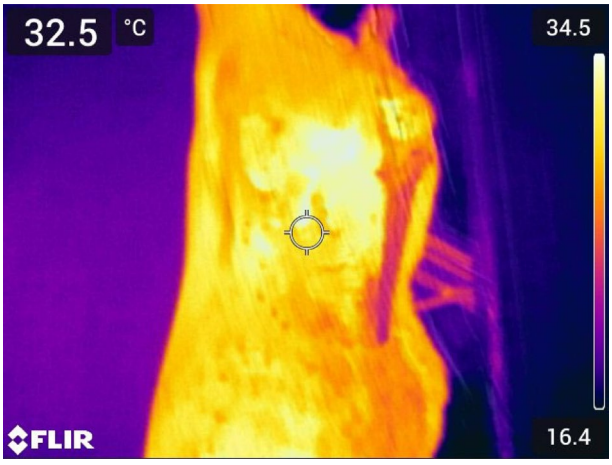
1. Ensure that animal handling pathways are designed with non-slip flooring, gentle slopes and curved layouts to facilitate calm, efficient cattle movement and minimise stress and injury.

2. Replace or cover traditional concrete floors and barriers with eco-sustainable, shock-absorbing materials such as multi-layer pressed cardboard to reduce noise, visual distractions and slips.
3. Avoid the use of electric prods and coercive handling devices whenever possible; instead, rely on environmental and behavioural design to promote voluntary animal movement.
4. Provide uniform, diffused lighting in all areas used for animal holding and transfer, while minimising noise and shadows, to prevent animal agitation and hesitation.
5. Regularly train slaughterhouse staff in humane handling techniques, including recognising indicators of animal stress and unconsciousness, and responding promptly to welfare issues.
6. Maintain ready access to clean water, adequate ventilation and appropriate space for different animal groups to reduce aggression and dehydration during lairage.
7. Continuously monitor animal welfare indicators and blood biochemistry (such as cortisol and beta-endorphin levels) to evaluate the effectiveness of welfare interventions.
8. Adapt controls and procedures based on ongoing scientific research and regulatory updates, ensuring that best practices are routinely implemented and revised.
9. Encourage collaboration and transparent communication among all food chain stakeholders, including veterinarians, operators and competent authorities, to ensure coordinated animal welfare management.
10. Prioritise the use of recyclable, biodegradable and animal-friendly materials in the construction of new abattoir infrastructure to advance both welfare and sustainability goals.

7 | OTHER ACTIVITIES DURING THE EU-FORA FELLOWSHIP

During the fellowship, the fellow had the opportunity to participate to various scientific activities. These included:

a. contact with stakeholders
b. learning about specifics of the Italian meat industry during meetings of the research team and visit to processing plants and collect the data in the industry condition
c. laboratory work in ongoing projects, including: conducting microbiological and biochemical analyses and cultures; preparing microbiological media; and performing minor laboratory work
d. working in a cell culture laboratory
e. weekly meetings with state veterinary services on RA and best practices
f. meetings with the scientists and specialists from the hosting site
g. meeting and training with the Inspectors from Polish National Food Quality Inspection organised by sending site
h. g. active participation to the activities of the master in official control of food, https://www.ispezioneperugia.it and of the PhD program in veterinary medicine, https://medvet.unipg.it/didattica/dottorati-diricerca/sanita-e-scienze-sperimentali-veterinarie that are coordinated by the local supervisor Prof. Cenci Goga)
i. desk work and data analysis
j. participation in classes with students at the slaughterhouse during meat quality evaluation and veterinary inspection
k. an attempt to use a thermal imaging camera FLIR to assess animal welfare.



ABBREVIATIONS

ANOVA analysis of variance
HSD Honestly significant difference

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