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Opinion Paper: Working on alternative methods in experimental animal sciences. Experience from the case of fistulated ruminants.

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Highlights

- Public concern in France led to ending fistulated ruminant experimental model.

- No alternative method fully meets current scientific standards or ethical expectations.

- This case illustrates shifting animal sciences with evolution of social criticism and public policies.

- Developing alternatives needs a systemic approach and revised research goals.

- Authors urge broader dialogue to guide the transition toward new research models.

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Keywords: animal experimentation, animal welfare, responsible research, ruminant nutrition, systemic approach

Introduction

In France, in 2019, the public disclosure of an activist group's investigation into fistulated cows in animal research heightened political concern over the use of experimental animals especially for livestock sector. Following this episode, illustrating increasingly prominent public debate on animal welfare concerns (Carrié et al., 2023), and given the general trend in Europe towards reducing animal testing (European Directives 86/609/CEE and 2010/63/UE), the French National Research Institute for Agriculture, Food and Environment (INRAE) has made a political decision to stop after 2025 the use of fistulated ruminants, a model historically central to ruminant nutrition research. According to the available information, this is the first case in public animal research to create a precedent in the international scientific community. Furthermore, the international congress of European Federation of Animal Science (EAAP) in 2023 showed a large concern among scientists with the question of animal experimentation. Thus, we, as an interdisciplinary team of specialists in physiology, microbiology, ethology and sociology working together on implementation of alternative practices in the physiology of ruminant nutrition, have decided to share our experience and reflections with colleagues who might be concerned with similar challenges.

Overview of the fistulated ruminant model

A fistulated animal is one that has been equipped through a surgical procedure with a permanent fistula or cannula which ensures direct access to a specific internal organ for experimental purposes. This model has been especially developed in the 1950's in farm ruminants to study their complex digestive system and their productive capacities. The cannulation in ruminants targets primarily the rumen (Laflin and Gnad, 2008) and duodenum. Thus, the research conducted in close to real conditions has aimed to predict the multiple effects of diet on livestock production, the quality of final products (milk and meat), feed efficiency, emissions in the environment (methane, nitrogen, phosphorus), and animal health. Until recently, the fistulated ruminant model was widely accepted by the scientific community in physiology as it was scientifically and ethically justified (Doreau 2008). The surgical insertion of a cannula has been framed by professional veterinary norms and know-how, including pain reduction. Yet, whether the moral and regulatory application of the "3R" (Replacement, Reduction, Refinement) ethical principles (Russel et Burch 1959) helped to optimize the number of animals and to minimize their suffering, increasing controversies are driving researchers to reconsider the "Replacement" dimension with a more serious perspective.

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Alternative methods

Our five-year work explored a range of alternative methods: non-animal, animal non-invasive or refined invasive (see Table 1 in the annex). No one alternative approach alone is sufficient to replace the reference model of fistulated animals and to answer the whole panel of research questions. Furthermore, the considered methods produce results with overall a lower level of data quality and have diverse levels of compliance with 3R principles and with societal expectations. For example, a combination of *in vitro* and *in silico* approaches allows drastic reduction in animal involvement in the experimental process, but their reliability is still problematic. Also, gastroesophageal sampling allows rumen fluid collection without surgery, which is useful for *in vitro* digestion studies (Boudon et al. 2025), but still involves animal restraint or discomfort. Even if alternative *in vivo* practices can be refined through training for better experimenter-animal cooperation (De Boyer Des Roches et al. 2025) or through veterinary know-how improvements (Durand et al., 2021), their societal acceptability cannot be guaranteed.

Our prospective vision

Our results and debates have strengthened our belief that we have to change our research logic. Rather than focusing on finding an ideal substitute for the contested experimental animal model, we have to understand more systemically how evolving societal issues shape our research objectives and questions.

In the future, due to climate change, the quality and availability of feed resources will constantly evolve, as will the characteristics of the animals used to valorize them. Furthermore, the diversification of societal demands on the livestock sector regarding the nature and quality of human food products, fair farmer incomes, animal welfare standards, etc. heightens sociopolitical tensions and directly impacts productive and research strategies and practices. Thus, we as scientists and our stakeholders (farmers, animal feed companies and the animals themselves) will need a more adaptable conceptual framework to quickly adapt to fast-changing environmental, economic and sociopolitical conditions.

Our future research will very probably rely on a flexible approach combining high-throughput analysis of in-farm collected data and *in vitro* measurements on feed. Modeling and AI tools will rely on past research results and already constituted databases. If these methods meet societal concerns for more respectful and reduced use of experimental animals, they will likely also shape our scientific standards of data quality (precision, accuracy, reliability) and our communication practices (enhanced transparency and openness with publication of papers with a high level of meta data description, a more interdisciplinary approach, etc.).

This collective shift can be supported by means of suitable cooperative tools. We have used such one tool called ASIRPA Real Time (https://asirpa.hub.inrae.fr). Based on the notion of responsible research and innovation (Owen, Bessant, and Heintz 2013), this approach helps to assess and adjust our research trajectories for a better societal impact in a constantly evolving context.

Conclusion

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The case of the fistulated ruminant model in France is illustrative of more general ongoing developments in animal research. Societal concerns regarding animal welfare shape research practices, not only through the evolution of normative regulation of animal testing, but also through increasing sociopolitical scrutiny of the livestock sector. Whether or not animal testing may be necessary in particular situations such as health crises, we have to make our research less dependent on invasive *in vivo* methods.

If this engages animal scientists directly in the search for alternatives, this calls for an approach that is systemic as possible. We have identified (at least) three challenges this shift raises for multilevel discussions and actions:

- Revision of the research questions and objectives we are able to address without (or with less) invasive animal methods. This can be achieved through a disciplinary/sectorial approach between scientists and the stakeholders directly concerned.

- Evolution of norms and standards of the data produced and of the results cited in scientific communication. An enlarged, international scientific community including science journals and publishers has to be involved.

- Identification and dynamic analysis of interconnected societal issues. This presumes a transdisciplinary dialogue with an enlarged panel of stakeholders including civil society and policymakers.

As it is a long-term commitment, we suggest pursuing an exchange of ideas and collaborations with colleagues from different disciplines interested in the alternative and systemic approaches of experimental animal science.

Ethics approval

Not applicable.

Data and model availability statement

Not applicable. Information can be made available from the authors upon request.

Declaration of generative AI and AI-assisted technologies in the writing process

Not applicable.

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Declaration of interest

There is no potential conflict of interest.

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References

Boudon, A., Kwiatkowski, D., Niderkorn, V., Forano, E., Nozière, P., Silberberg, M., 2025. Oral-stomach sampling to replace rumen-fistulated animals in ruminant nutrition research - a case study. bioRxiv. doi: 10.1101/2025.01.17.633359. Posted January 22, 2025

Carrié, F., Doré, A., and Michalon, J., 2023. Sociologie de la cause animale. Repères. Éditions La Découverte, Paris, France

Journal Pre-proofs

De Boyer Des Roches, A., Dumoulin, E., Roux, E., Guinotte, J., Brunet, V., Otz, P., 2025. Medical Training in Dairy Heifers – A Pilot Study. Applied Animal Behaviour Science 286, 106624. doi: 10.1016/j.applanim.2025.106624

Doreau, M., 2008. Apports et limites des techniques alternatives à la chirurgie expérimentale du tractus digestif des herbivores. Bulletin de l'Académie Vétérinaire de France 161, 425-433. doi:10.4267/2042/48167

Durand, D., Faure, M., Lamberton, P., Lemosquet, S., De Boyer Des Roches, A., 2021. A Multiparametric Approach to Assessing Residual Pain Experienced by Dairy Cows Undergoing Digestive Tract Surgery under Multimodal Analgesia. Animal 15, 100338. doi:10.1016/j.animal.2021.100338

Laflin, S.L. and Gnad, D.P., 2008. Rumen cannulation: Procedure and use of a cannulated bovine. The Veterinary Clinics of North America. Food Animal Practice 24, 335-340. doi: 10.1016/j.cvfa.2008.02.007

Owen, R., Bessant, J., and Heintz, M., éd., 2013. Responsible Innovation: Managing the Responsible Emergence of Science and Innovation in Society. Wiley, Chichester, UK.

Russell, W.M.S. and Burch, R.L., 1959. The Principles of Humane Experimental Technique. Methuen & Co. Ltd, London, UK.

Table 1. Comprehensive approach of alternative methods to permanently fistulated ruminants to address overall challenges of agroecological transition, adaptation to climate change, and "One Health" targets in livestock production.

Method	Severity of procedure	Research questions			Advantages	Limits	Animal welfare concern
		Feed evaluation[1]	Animal needs / efficiency[2]	Rumen microbiota[3]			
Reference animal invasive method							
Permanent rumen/ duode	Moderate for surgery with	X	X	X	Reference method	Surgery, irreversibility,	Pain and stress during and just after the surgery,

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nal cannulation	anesthesia/ analgesia[4]				controversy	even though limited by anesthesia/analgesia
Alternative non-animal methods						
Enzymatic methods without rumen fluid	N/A	X		Non-animal; good correlation with *in situ* measurement	Would require *in vivo* validation for new or uncommon feed resources	N/A
In vitro fermentation with preserved rumen fluid withdrawn at the slaughterhouse	N/A	X		Non-animal; good correlation with *in vivo* measurement of degradation	Analysis of methanogenesis and N utilization requires continuous fermentation devices	N/A
Modeling	N/A	X		Non-animal	Potential loss in precision or predictability with new or uncommon feeding practices	N/A
Alternative animal non-invasive methods						
Fecal excretion kinetics of an	N/A	X	X	Non-invasive	Lack of precision	N/A

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indigestible plant component[5]							
Analysis of volatile compounds in expired air	N/A		X		Non-invasive	Still at proof-of-concept stage	N/A
Alternative animal methods with limited invasiveness							
Rumen boluses[6]	N/A		X		Very limited invasiveness, dynamic measurements	Limited number of measured parameters	Very transient sensation during the introduction of the bolus
Buccal swabbing[7]	N/A			X	Very limited invasiveness; proxy of ruminal acidosis	Samples not representative of all ruminal functions	Limited discomfort during sampling
Alternative animal invasive methods - Rumen							
Non-surgical: Gastro-esophageal sampling	Light to moderate	(X)[8]	(X)[9]	X	Allows rumen sampling in large populations; provides suitable inocula for *in vitro* fermentation	Needs good technical expertise to avoid both salivary contamination and injuries to the animal; possible controversy	Discomfort during sampling, depending on technical skill and possibly limited by training the animals

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Refined surgery: Removable mini cannula	Moderate for surgery with anesthesia/ analgesia; reversibility by tissue regeneration	X	Allows perfusion for nutritional studies and fluid and digesta sampling	Surgery, cauterization after removing the cannula is more or less rapid; possible controversy	Pain and stress during and just after the surgery, even though limited by anesthesia/analgesia.

Alternative animal invasive methods - Post-rumen (abomasum or duodenum)[10]

Removable duodenal mini cannula	Moderate for surgery with anesthesia/ analgesia; reversibility by tissue regeneration	X	Allows perfusion for nutritional studies and fluid and digesta sampling	Surgery; cauterization after removing the cannula is rapid; possible controversy	Pain and stress during and just after the surgery, even though limited by anesthesia/analgesia. Almost no pain and stress at removal
Refined surgery: Small temporary catheters in abomasum[11]	Moderate for surgery with anesthesia/ analgesia; reversibility by tissue regeneration	X	Allows perfusion for nutritional studies	Surgery; long cauterization after removing the catheter; possible controversy	Pain and stress during and just after the surgery, even though limited by anesthesia/analgesia. No pain and stress at removal

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[1] Evaluation of feed and diet as source of nutrients, micronutrients, methane, nitrogen or phosphorous waste…

[2] Animal needs (nutrients, micronutrients) or efficiency (i.e. ability to ingest and valorize feed resources)

[3] Rumen microbiota as mediator of multiple responses of animals to diet (feed efficiency, waste, health, quality of products)

[4] References: https://doi.org/10.1111/vsu.14182; https://doi.org/10.1016/j.animal.2021.100338

[5] As a proxy of transit rate of digesta in the rumen

[6] Reference: https://doi.org/10.1017/S1751731117001677

[7] Reference: https://doi.org/10.1038/s41598-025-18063-1

[8] Gastro-esophageal sampling can be a way to obtain inoculum for *in vitro* fermentation in controlled conditions

[9] *In vivo* fermentation profiles in the rumen can add information about animal (ruminal) needs in (micro)nutrients or digestive health and efficiency

[10] Concerns only alternative to permanent cannulation of duodenum

[11] Reference: https://doi.org/doi: 10.3168/jds.S0022-0302(06)72585-1

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