

Issues of Declining Livestock Breeds: Revisiting Domestic Animal Diversity in Pastoral Systems

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Abstract

Concerns for the disappearance of local breeds go back to the beginning of scientific breeding and the early national policies of agriculture intensification in Europe at the time of the industrial revolution. That initial Eurocentric framing of domestic animal diversity as 'local' breeds, largely the result of natural selection and potentially a useful source of 'raw' genetic material, remains dominant. Today, however, the debate around domestic animal diversity has been globalized, and so includes livestock breeding populations and livestock systems that developed *outside* the European experience. This paper looks at domestic animal diversity from the vantage point of one of such cases: cattle breeding among the Wodaabe pastoralists in Niger. The research is based on a combination of qualitative methodologies standard in social anthropology and quantitative analysis of memorized herd genealogies over a 20-year period. Results show that a competent herder can control cattle mating in over 90 percent of cases. Complex learned behaviour in cattle, particularly related to feeding competence, is a major selection criterion. The Wodaabe specialize in using the short-lived and unpredictable grazing opportunities, which is characteristic of Sahelian rangelands. To successfully interface the unpredictable variability in potential inputs, they breed herds with exceptional levels of within-breed diversity, crucially including epigenetic traits. The common practice of conflating Domestic Animal Diversity (DAD) with Animal Genetic Resources (AnGR), therefore, falls short of adequately representing the relationship between 'local breeds' and livelihood in pastoral systems.

Keywords

Domestic animal diversity; Breeding; Wodaabe; Pastoralism; Animal genetic resources

1. Introduction

A concern for Domestic Animal Diversity (from now on referred to as DAD) formally entered the international arena in 1946, when the then newly created FAO was assigned the 3 aspects of 1 task of cataloguing, maintaining, and utilizing livestock

biodiversity (Phillips, 1981)¹. Today, the FAO publishes the *State of the World's Report on Animal Genetic Resources for Food and Agriculture*, in collaboration with some 150 scientists around the world (Rischkowsky & Pilling, 2007; Scherf & Pilling, 2015). According to the most recent of these reports, 17% of known livestock breeds are classified as 'at risk of extinction'. The actual proportion could be higher, as for about half of the known breeds there is no sufficient information to assess the risk level. The risk of loss of domestic animal diversity is mainly seen as triggered by changes within the livestock sector, driven by economic, social, demographic and political factors. Domestic animal diversity is included in the Convention on Biological Diversity, which outlined its roots in 'traditional knowledge, innovations and practices', emphasizing the necessity of conserving domestic animal species 'in the surroundings where they have developed their distinctive properties' (UN, 1992: Art. 2).

Concerns about the disappearance of local breeds go back to the early days of animal science, concomitantly with the industrial revolution and the launch of national policies of agricultural intensification. The first comprehensive description of livestock breeds in the British islands (Low, 1842) worried about the preservation of 'centuries old environmental fitness'. This was seen as a 'raw material', resulting from centuries of natural selection, difficult to reproduce, and yet a potentially useful base for the new practice of scientific breeding in the context of the new practices of agricultural intensification (Hall & Clutton-Brook, 1989). While wrapped in scientific universality, this was an entirely Eurocentric view at a very particular moment of history.

That early framing has never stopped dominating the debate. Today, it is at the root of the common practice of using the concepts of 'Domestic Animal Diversity' (DAD) and 'Animal Genetic Resources' (AnGR) interchangeably. An argument often used for promoting the conservation of domestic animal genetic resources is that many livelihood systems depend on it. While sound in its intentions, this argument remains captive of the initial Eurocentric view of domestic animal diversity as breed-based, as genetic material and as a product of adaptation to a *local* natural environment understood as a constraint to production.

Conservation strategies focusing on AnGR target a representative sample of genotype-environment adaptation, with priority given to breeds presenting particularly desirable and rare traits — for example, a resistance to certain diseases (FAO-CGRFA, 2004; Reist-Marti et al., 2003). But what about the livestock-based livelihood systems that developed *outside* the European experience? What if the livelihood system specializes in engaging *positively* with the variable natural environment rather than trying to shelter from it (FAO, 2021)? And what if the system is mobile, and, therefore, 'local conditions' are not stable but variable, and largely the outcome of management, as in pastoral systems?

Thus, this paper looks at the domestic animal diversity from the vantage point of the study of cattle breeding among Peul Wodaabe pastoralists in Niger. As evident, the importance of domestic animal diversity for livelihoods can go well beyond the European experience and the current AnGR focus.

2. Methodology

This paper is based on *i.* research among the Wodaabe in Niger carried out in 2000-2005 (19 months of fieldwork) and 2008 (3 months); *ii.* qualitative data on cattle breeding and feeding selectivity using semi-structured interviews among Turkana and Karamojong pastoralists in Kenya and Uganda, Arab pastoralists in Chad and

¹ A series of seminal reference works followed, on cattle breeds in India and Pakistan (Joshi & Philips, 1953), Africa (Joshi et al., 1957), Europe (French et al., 1966), and on Mediterranean sheep breeds (Mason, 1967).

Sudan, and Somali, Boran, and Dassenetch pastoralists in Ethiopia between 1999 and 2020; and *iii.* ten years of work (since 2013) on pastoralism and pastoral development in relation to the role of mobility in environments characterized by high levels of unpredictable variability.

The study of the cattle breeding system among the Wodaabe used standard methods from social anthropology² and a method for analyzing herd genealogies and herders' breeding decisions. The latter was specifically developed for handling memorized cattle genealogies and, therefore, embedding multiple cross-checking mechanisms. Data collection included the year of birth, sale or death — and, where relevant, the year of borrowing or lending — for each animal in the herd (males and females) over a 20-year period (1985-2005). It also included the name of the mother and father of each animal; the name of the owner of the father; the reason for selling the animal; and, in case of animals, whether had been borrowed or lent, the nature of the herder's relationship with the owner or receiver. Two herds, for a total of 101 cattle as of 2005, were analyzed with this genealogical method. Building a 'map' of the herd in this way allowed for real-time crosschecking of new data against those already collected. Crosschecking was also done by asking the same questions a second time, about random animals, during interviews weeks apart from one another. A quantitative analysis of this data using a commercial database, later enabled the production of 'snapshots' showing all the animals in the herd, their age and gender and their kinship relations, and whether they were sold, lent, returned or died, in each of the 20 years covered by the study. Finally, herders' breeding decisions and their explanations were analyzed in light of scientific literature from a range of disciplines including animal science, rangeland management, animal behaviour science, evolutionary biology and social anthropology.

The bulk of findings in the next section are from Krätli (2008a)³. When the information presented was already in the public domain at the time of the study, references are provided.

3. Results: Domestic Animal Diversity and Pastoralism

3.1 What is pastoralism?

On about 40 per cent of land on Earth, rainfall is highly unpredictable. For food producers who specialize in being in the right place at the right time, these unpredictable environments offer important opportunities. Mobile pastoralists are such producers (FAO, 2021; Kaufmann et al., 2018).

Pastoral systems all over the world are highly diverse, but they all share the same adaptive approach: working closely with livestock interacting with rangelands, and making decisions based on learning from such interaction (Sharifian et al., 2022). From the Sahel to the Arctic, pastoral systems developed as an integral part of their natural environment, not by trying to separate from it. They manage livestock's grazing itineraries so that the animals feed better than they would without the herder, and this is done at a variety of spatial and temporal scales depending on the level of specialization (Krätli & Schareika, 2010; Meuret & Provenza, 2014; Molnár et al., 2020).

Pastoral systems specialize in making use of highly unpredictable environments, using livestock to take advantage of the important, but scattered and short-lived opportunities offered by the rangelands. When the functional processes in the pastoral system can be kept variable enough to match the pace of unpredictable

² Participatory observation, focus group discussions, semi-structured interviews, and Participatory Rural Appraisal (PRA) techniques.

³ Published as synthesis in Krätli (2008b).

change in the environment, the system's outputs are relatively stable even in highly variable conditions (FAO, 2021).

In the face of the uncertainty associated with making use of a highly unpredictable environment, the logic of pastoralism is to keep options open: flexibility of options compensates for lack of certainty (Krätli & Schareika, 2010). Promoting and maintaining high levels of domestic animal diversity in the herds is part of this logic. For example, with a few exceptions (especially in the Arctic region), pastoralists typically keep several species in their herds — for example cattle, camels, sheep, goats and donkeys — and even different lineages within the same cattle breed. This helps them match the diversity of opportunities in their environment. But the most important strategy in this regard is by fostering variability within the breeding population itself.

Hall (2004) finds that some of the highest levels of within-breed diversity are found in pastoral systems. In his description, 'within-breed variation provides the flexibility that the breeds need to have if they are to respond to changing conditions'. We will see that pastoralists foster within-breed diversity in their own way. A livestock breeding population developed to perform under pastoral management conditions is a different entity compared to a breed developed to maximize a single trait. Despite the long interest in local breeds by the international community, livestock breeding in the most specialized 'local' contexts — pastoral systems — has so far received surprisingly little attention.

3.2 Wodaabe Pastoralism in Niger

The Wodaabe in Niger one of the largest cattle-breed in Sub-Saharan Africa, the long-horned *na'i bodeeji* ('red cows' in Fulfulde, Wodaabe's vernacular language). These animals were formally characterized as 'Red Bororo zebu' in the early 20th century, during the French colonial administration (Krätli, 2009; Mornet & Koné, 1941). The cattle of the Wodaabe have been recorded to feed on more than 60 varieties of plants, including bushes, trees and grasses — often thorny and and/or toxic at certain stages of their life cycle (Bonfiglioli, 1981). The Wodaabe and their livestock operate in a natural environment where the temperature reaches 50°C, with precipitations between 0 and 400 mm in one rainy season stretching over a maximum of four months. By comparison, in 2021, livestock experts in the United Kingdom warned that "with 'temperatures now regularly reaching 20°C and beyond... [our] cows are extremely susceptible to heat stress"⁴.

The Wodaabe's herd management strategy is equally remarkable. In order to keep their animals on the best possible pasture also during the nine-month-long dry season, they can camp as far as 30 kilometers away from the water point. Watering is done every other day during most of the dry season, and every second day in the last couple of months. This strategy also exploits the ability in cattle to keep them in a positive energy balance when a low-protein roughage diet is combined with a degree of water restriction (Granier, 1968; Rogerson, 1963).

The Wodaabe literally live with their livestock. Their camp includes a space for the herd where a fire is lit every evening only for animals and they gather around it. The herd gathers around the fire. No fence is used. At night, the cattle leave the camp at their own and return after a few hours of grazing. When moving with their herders, these animals *follow* the herder rather than being herded from behind, and are able to respond to numerous commands. All this contributes to optimizing feeding efficiency and facilitating management in conditions where even the smallest

⁴ Dairy Global, <https://www.dairyglobal.net/health-and-nutrition/health/a-greater-issue-in-uk-heat-stress-impact-on-dmi/>

advantages can make an important difference (Breman & De Witt, 1983; Krätli & Schareika, 2010).

3.3 Engaging with the natural environment: variability in nutrients over time and space

In the Sahel, the rain falls in itinerant showers. In most of these rangelands, it is impossible to predict from one year to the next where pasture will grow. Nutrients for livestock are distributed unevenly and unpredictably. Variability rules at all scales, both in time and space. Variations between seasons (time) and between macro-ecological zones (space) are the most obvious examples. Following the rains, the concentration of nutrients in pasture also *increases* as one moves north towards the Sahara — drier areas have less pasture biomass but of higher quality (Breman & De Wit, 1983). The concentration of nutrients also varies between plants and between the parts of a plant (Breman & de Ridder, 1991). In terms of variability in time, during the life cycle of a plant, nutrients first peak and then decrease as the plant uses them for its own reproduction (Alimaev, 2003; Ball et al., 2001; Ronga et al., 2020). Nutrient content in pasture also changes between day and night, peaking in the evening after a day of photosynthesis (Ball et al., 2001; Burns et al., 2005; Scialdone & Howard, 2015).

Livestock feeding opportunities also expand and contract at unpredictable intervals between years, often dramatically. In order to keep productivity as high as possible on highly variable resources, livestock need to feed in the right place at the right time, and be able to select the best bites. For all that to happen, herders need the right herd: animals capable of moving quickly even over long distances, and capable of feeding discerningly and efficiently once presented with the opportunity. In other words, they need herds highly skilled in interacting successfully with the natural environment. So, what makes a pastoral herd 'right'?

3.4 Breeding Cattle among the Wodaabe

Wodaabe's pastoralism rests on a sophisticated cattle breeding system supported by several customary institutions — most importantly, a matrilineal cattle naming system shared among all Wodaabe clans and Peul livestock-keepers more broadly, from Senegal to Sudan. New born calves, males and females, are named after the mother. This naming system organizes the herd into matrilineal families and makes it possible to track the outputs of breeding, both across herds and across human generations. A name refers to a cattle lineage as well as each one of its members. For example, in talking about a cow currently in herd, named 'Guddel', a herder might say 'Guddel was already in the herd of my grandfather, when my father was a child'— although at that time the actual cow, or even her mother, had not yet been born (interview with Jiima bi Ardi, March 2004).

Cattle genealogies are systematically memorized, although of course this is not achieved by all herders to the same degree. Close monitoring and a period of heat (oestrus) in these cattle that lasts only a few hours⁵, allow for a strict control of reproduction. In 2005, the analysis of herd genealogy indicated that a competent herder can control mating in over 90 percent of cases (Krätli, 2009).

Non-productive animals are systematically sold. Breeders borrow reproductive bulls and lend cows across open networks including tens of herds. Lending of heifers between friends and relatives is institutionalized. These animals remain in the receiving herd for the time it takes to deliver one or two calves, and are returned pregnant. Crossbreeding is a traditional practice, used to expand the range of

⁵ This information from the herders matches general descriptions of oestrus cycle in zebu. Cuq (1973) reports a much wider difference between extremes compared to *Bos Taurus*, with an average time of acceptance of a male spanning between 4 and 8 hours.

production strategies available to the household, or to adapt the herd when moving into entirely new areas or when facing new socio-economic conditions (Boutrais, 2007).

3.5 A Particular Attention to Learned Behaviour

Animals in a pastoral system need to be productive and sufficiently robust to reproduce under demanding conditions of environment and management. To date, Wodaabe's livestock achieve this with minimal inputs of feed supplement or veterinary services. Besides matching the basic requirements for survival and reproduction, selective mating is aimed at building and maintaining diversity within the herd. In order to thrive in the highly variable environmental conditions of the Sahel, building a capacity for variability into the herd takes priority for the Wodaabe over maximizing a single trait. The following statement is enlightening in this context:

'We have preferred lineages but do not maximise their number in the herd. If we did that, the entire herd would be made of similar animals and we don't want that. We need different lineages with a variety of functional skills' (focus group discussion with herders, May 2004).

A major criterion for selection is *behaviour*, including complex learned behaviours passed from mothers to calves and between peers. Feeding competence is crucial. Social behaviour within the herd, and an animal's attachment to the herders, are also important as they can have positive outcomes on the animals' health, learning ability and feeding efficiency, for example by reducing antagonism and stress. Social interactions can jeopardise or favour cattle feeding performance in various ways (Bouissou et al., 2001; Dumont & Boissy, 1999; Waiblinger et al., 2006). The mere proximity of dominants can cause subordinates to slow down their bite rate and even stop feeding (Bennet & Holmes, 1987). Work on African buffalo revealed that an animal's physical condition is heavily affected by the herd's social organisation and its position within it (Prins, 1996).

In the herds of the Wodaabe, antagonistic and aggressive behaviours are minimized by removing most of the males above a certain age, and by actively promoting bonding relationships between the animals. For example, at night the calves are tethered to both sides of the 'calf-rope' stretched north-south across the camp; they are always attached in the same position relative to one another. The herders explain that this is in order to favour the creation of bonds between the animals (called 'preferential relationship' by animal behaviour specialists) at a time when they most need reassurance as the mothers (dams) leave for night grazing.

Having a high number of preferential relationships within the herd results into reduced aggressiveness, increased tolerance in competitive situations, and enhanced positive interactions, such as grooming through licking. Research from applied animal behaviour science highlights the potential economic return of management strategies that minimize negative social interaction within the herd, and recommends measures apt to stabilize dominance and favour preferential relationships (Boissy et al., 2001; Bouissou et al. 2001). Preferential relationships between animals are described by Wodaabe herders with the same vernacular word they also use to talk about friendship between people. Their cattle management system also enhances calf-dam bonds. Calves are allowed to spend several hours per day with their mothers, both around the camp in the evening and during the morning grazing — also a critical learning opportunity.

Wodaabe herds are complex social organizations not dissimilar to the herds of wild herbivores but with lower levels of internal antagonism. Favouring the number of bonds within the herd works towards improving herd nutrition: more even feeding patterns across ranks without cost for the high-ranking ones, and, therefore, a better feeding performance of the herd as a whole. Here below are some other examples of

learned behaviour, the functionality of which Wodaabe herders appreciate and strive to build into their herds through their breeding practices:

- *noppina*: feeding on new grass when it is still young, ingesting only minimum sand by pinching the short grass with the muzzle as sheep would — gives extra days feeding on green pasture at the beginning of the rainy season.
- *geeti*: being very attached to the household's members and ferociously mistrustful of strangers and unfamiliar practices — helps managing the herd and makes it hard to steal.
- *dikku* (also *halhonge*): to have 'character', for example a cow that has collapsed on the ground out of exhaustion yet that when helped to stand up does not collapse again but starts feeding;
- *gamtudi*: on the range, these animals are always some distance from the group, exploring for better pasture — when managed by the herder, they can improve overall feeding performance of the herd.

Herders welcome specific behavioural patterns to a higher degree, but lineages showing exceptional levels of such traits are not maximised within the herd at the expenses of the other lineages.

4. Discussion: Breeding for Variability

Working on camel breeding among the Rendille in Kenya, Brigitte Kaufmann found that the animals producing most milk during the rainy season — those that animal science would see as the best milk producers in the herd — were considered by the herders to be the 'weakest animals' (Kaufmann, 2007). This was because they were barely able to feed their calves during the dry season. On the other hand, animals that did not perform particularly well during the rainy season were the best milk producers during the dry season. The Rendille herders valued multiple *types* of performance — to match variability of conditions — over absolute best performance in best conditions (Kaufmann, 2007).

In the case of cattle breeding among the Wodaabe, multiple *types* of performance are actively pursued and maintained. The role of within-breed genetic variation is important, but clearly within-breed diversity used in pastoralism stretches well beyond genetic traits. This is a breeding system aimed at embedding not only variability of genetic resources, but also epigenetic gene-expression that complements complex learned behaviour functional to interfacing with the environment (Day et al., 2003; Jablonka & Lamb, 2006; Lewontin, 2000). In other words, breeding practices are aimed at introducing and maintaining particular learned behaviours. The focus of the breeders is more on the herd and its lineages than on individual animals. We know from decades of research on ruminants' feeding behaviour that the mother's influence begins in the womb (as flavors of foods she eats reach her amniotic fluid), continues after birth (through flavors in her milk). When offspring begin to forage, the mother is a model for what and what not to eat, and where and where not to go. Learned behaviors and abilities involve anatomical and physiological changes in organ systems, including the microbiome (Landau & Provenza, 2020). Many of such changes are inheritable, in epigenetic ways, and, therefore, subject to breeding strategies.

Besides feeding competence, animals' attachment to the herders and social organization within the herd are very important. Other examples of sought-after behavioural traits include knowledge of the territory and orientation and experience in managing difficult terrains or high temperatures. There are many more, and combinations of these learned behaviours create the pastoral herds adapted to successful interaction with the multiple, biodiverse and variable landscapes they inhabit.

The primary objective of breeding under these variable conditions is not to maximise a trait or set of traits towards some absolute optimum with the right combination of genes. The primary objective is to keep as high as possible the capacity of a given herd to function as a matching interface with ever-changing landscapes. Pastoral breeds are constantly in the making; they are developed to interface production with landscapes that are also constantly in the making; they are *defined* by their variability.

This peculiar approach to breeding — *breeding for variability* in interacting with a variable environment — seems to offer an important lesson now particularly important also in the face of climate change (FAO, 2021). In this light, livestock breeding in pastoral systems, and the different meaning it gives to domestic animal diversity beyond the current focus on genetic material, carries new relevance well beyond pastoralism.

5. Conclusion

The analysis of Wodaabe cattle breeding shows high levels of systematic monitoring and control of mating and routine culling. It also shows a strong emphasis on the generation and maintenance of domestic animal diversity, especially within-breed diversity including both genetic *and* epigenetic traits. Of particular importance to the herders are multiple types of complex learned behaviour that are functional to the animals' interaction with the natural environment. Both the reproduction and the effectiveness of these learned behaviours depend on the social organization within the herd: mother-calf and peer-to-peer learning; and as low-stress feeding conditions. Within this approach, the breeding unit is the herd rather than the individual animal.

The 'domestic animal diversity' which is *economically relevant* in pastoralism — and most likely in many traditional livestock breeding systems in family-farming contexts (Provenza, 2008) — is, therefore, not limited to genetic resources and to individual animals' performance. In fact, this particular kind of DAD found in pastoral systems remains largely outside the conventional genetic notion of domestic animal diversity — which for the time being continues to focus at the level of the individual animal in isolation from the environment or with the environment merely seen as a constraint⁶.

The common practice of conflating DAD with AnGR with its narrow focus on 'adaptation to local conditions' is inadequate to represent the relationship between livestock breeds and livelihood in pastoral systems.

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⁶ In the face of climate change, this model of food production based on externalizing nature has itself become a critical distinguishing trait in the current call to rethink agricultural systems before it is too late (Pretty, 2002; Scherr & McNeeley, 2007; Shiva et al., 2019; UNEP, 2021; FAO, 2021).

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Author's Declarations and Essential Ethical Compliances

Author's Contributions (in accordance with ICMJE criteria for authorship)

This article is 100% contributed by the sole author. He conceived and designed the research or analysis, collected the data, contributed to data analysis & interpretation, wrote the article, performed critical revision of the article/paper, edited the article, and supervised and administered the field work.

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Research involving human bodies or organs or tissues (Helsinki Declaration)

The author(s) solemnly declare(s) that this research has not involved any human subject (body or organs) for experimentation. It was not a clinical research. The contexts of human population/participation were only indirectly covered through literature review. Therefore, an Ethical Clearance (from a Committee or Authority) or ethical obligation of Helsinki Declaration does not apply in cases of this study or written work.

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The author(s) solemnly declare(s) that this research has not involved any animal subject (body or organs) for experimentation. The research was not based on laboratory experiment involving any kind of animal. Some contexts of animals are also indirectly covered through literature review. Therefore, an Ethical Clearance (from a Committee or Authority) does not apply in cases of this study or written work. It is because I believe it is also not necessary as I did not 'conduct research on animals', but simply the livestock breeding system of a particular community of pastoralists. As clearly mentioned in the description of methodology, no animal was touched or directly affected by my research in anyway. I asked questions to herders, and that's all. Yet, I have filled in ARRIVE Checklist and been appending it.

Research on Indigenous Peoples and/or Traditional Knowledge

The author(s) solemnly declare(s) that this research has not involved Indigenous Peoples as participants or respondents, with the documentation of their Indigenous Knowledge. Some other contexts of Indigenous Peoples or Indigenous Knowledge are only indirectly covered through literature review. An Ethical Clearance 'to conduct research on indigenous peoples' Indigenous knowledge is also not relevant, because Wodaabe people are not registered or recognised as an 'Indigenous People' in Niger, in Africa, or by the UN. Therefore, an Ethical Clearance (from a Committee or Authority) or prior informed consent (PIC) of the respondents or Self-Declaration in this regard does not apply in cases of this study or written work.

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The author(s) solemnly declare(s) that this research has not involved the plants for experiment or field studies. The contexts of plants were only indirectly covered through literature review. Thus, during this research the author(s) obeyed the principles of the Convention on Biological Diversity and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

(Optional) Research Involving Local Community Participants (Non-Indigenous)

The author(s) solemnly declare(s) that this research has involved local community participants or respondents belonging to non-Indigenous peoples. Yet, this study did not involve any child in any form directly. The contexts of different humans, people, populations, men/women/children and ethnic people are also indirectly covered

through literature review. Besides, my research focussed on herders' perception and practices, without resulting in any information of value for marketing purposes (e.g., I did not collect or even investigated animal genetic material). Therefore, an Ethical Clearance (from a Committee or Authority) or prior informed consent (PIC) of the respondents or Self-Declaration in this regard does not apply in cases of this study or written work. A permit issued by Niger for conducting field/social research was obtained.

(Optional) PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)

The author(s) has/have NOT complied with PRISMA standards. It is not relevant in case of this study or written work.

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The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item	Recommendation	Section/line number, or reason for not reporting
Study design	1 For each experiment, provide brief details of study design including: <ol style="list-style-type: none"> The groups being compared, including control groups. If no control group has been used, the rationale should be stated. The experimental unit (e.g. a single animal, litter, or cage of animals). 	not relevant
Sample size	2 <ol style="list-style-type: none"> Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. Explain how the sample size was decided. Provide details of any <i>a priori</i> sample size calculation, if done. 	2, p2, line 16
Inclusion and exclusion criteria	3 <ol style="list-style-type: none"> Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established <i>a priori</i>. If no criteria were set, state this explicitly. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. For each analysis, report the exact value of <i>n</i> in each experimental group. 	2, p2, lines
Randomisation	4 <ol style="list-style-type: none"> State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly. 	not relevant
Blinding	5 Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	not relevant
Outcome measures	6 <ol style="list-style-type: none"> Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size. 	not relevant
Statistical methods	7 <ol style="list-style-type: none"> Provide details of the statistical methods used for each analysis, including software used. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met. 	not relevant
Experimental animals	8 <ol style="list-style-type: none"> Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures. 	not relevant
Experimental procedures	9 For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: <ol style="list-style-type: none"> What was done, how it was done and what was used. When and how often. Where (including detail of any acclimatisation periods). Why (provide rationale for procedures). 	section 2
Results	10 For each experiment conducted, including independent replications, report: <ol style="list-style-type: none"> Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). If applicable, the effect size with a confidence interval. 	not relevant